THE ROLE OF THE QUEEN IN WAX SECRETION AND COMB BUILDING IN THE CAPE HONEYBEE, *APIS MELLIFERA CAPENSIS* (Escholtz).

Dissertation submitted in fulfilment of the requirements of the degree of

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by

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Errata

For all Tables referring to components of the mandibular gland, read decenoic, not decanoic, acid.

BEES AND WOMEN COMPARED.

Out of the experience of ruling Bees may be learned how to rule most women: for there is some resemblance between them.

1. Bees are very sensible and apprehensive of any good or evill that is for them or against them.
2. Bees are very teachy and passionate.
3. Bees if they be well governed, and kept in good order, are very industrious, but let them be out of order, or ill handled, and there comes no good of them, but hurt and trouble.

} So are women

1. Therefore to answer their senses which are so quick & apprehensive, let them not apprehend or see any evill from you by your example, but alwaies good.

2. For their passions, overcome them by reasons and love: But some are so passionate, that reason cannot rule; others so sottish and sluttish, that they cannot bee ordered, or altred from what they are: for the will is more froward than the minde is ignorant. But if you can win a woman thus, she may be both profitable and a comfort.

Remnant 1637

TABLE OF CONTENTS

List of tables

CHAPTER 7:

List of figures

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Abstract

The role of the queen in wax secretion and comb building was studied in the the Cape honeybee, *Apis mellifera capensis* (Escholtz). The percentage of bees bearing wax and the amount of wax borne by these bees did not differ between the experiments. This meant that the queenless and queenright colonies had the potential to construct equal amounts of comb as the amounts of wax available for comb building was the same.

Contrary to this prediction, queenright colonies constructed 8 times more comb than their queenless counterparts. Queenright *Apis mellifera scutellata* colonies constructed 4 times more comb than their queenless counterparts. The increased amount of 9-oxo-2-decanoic acid (9ODA) in the *A.m.capensis* mandibular gland secretions could not alone account for this difference. In fact, *A.m.capensis* and *A.m.scutellata* colonies constructed similar amounts of comb when they were given their own queens or queens from the other race.

Worker bees need to have direct contact with their queen for comb building to be enhanced. Even when the queen had her mandibular glands extirpated and tergite glands occluded large amounts of comb were constructed than when access to the queen was limited. Direct

access to the head of a mated queen proved to be the stimulus enhancing comb building. No comb was constructed when the workers had access to the abdomen of the queen. Virgin queens did not stimulate comb building. The relatively large amounts of 9ODA and 9HDA from the mandibular glands of Cape virgin queens had not influenced comb building.

Worker sized cells were generally constructed. These cells were slightly smaller than those constructed by European honeybees, but were indicative of African bees. A few queenless colonies constructed cells that were of an intermediate drone and worker size.

Four mandibular gland pheromones were measured by gas chromatography. No correlations between these pheromones and the comb construction measurements were found. It is unlikely that the mandibular gland pheromones are the only pheromones that stimulate comb building. Pheromones from other glands on the head may contribute towards the enhancement of comb building, and they are not present in virgin queens.

Chapter 1

General Introduction

Wax is secreted from 4 pairs of wax glands situated on the ventral abdominal surface of worker bees (Hornbostel 1744, Hunter 1792 and Huber 1814). Young adult bees aged 6-15 days have well developed wax glands that secrete wax (Rosch 1927 and Hepburn 1986). These bees hang in chains (festoons) in areas where comb is to be constructed (Huber, 1814). The festoon bee removes a wax scale (that is of an appropriate size, Hepburn 1986) from her abdomen with her hind leg. The wax scale is then brought to the mouth where it is chewed and manipulated for comb building (Kurstjens et al. 1985). Whether festoon bees or house bees (performing other duties) directly contribute wax scales for comb building is uncertain and is currently under investigation (Magnuson and Muller, personal comm).

In honeybee colonies, comb is essential for brood rearing and food storage. Both queenright and queenless colonies construct comb but the former is usually 3-4 times greater than that of the latter (Dreischer 1956, Darchen 1956 and 1957, Rajashekharappa and Channabasavanna 1979 and Jay and Jay 1983). Both Huber (1814) and Darchen (1956 and 1957) noted that comb building was significantly reduced when the worker bees did not have direct contact with

their queen. Darchen (1968) also showed that the bees must have access to the queen head for comb building to be enhanced. Virgin queens were found to be ineffective in enhancing comb construction (Rajashekharappa and Channabasavanna, 1979). This led Hepburn (1986) to hypothesize that the head and hence the mandibular gland secretions (and particularly the pheromone 9-oxo-2-decanoic acid, (9ODA)) were necessary for enhancing comb building.

Pain (1955) and Butler (1954) provided evidence that a "queen substance" inhibits worker ovarian development and emergency queen cell construction. The major component of the mandibular gland secretion was found to be 9ODA (Callow and Johnston 1960 and Barbier and Lederer 1960) and was regarded as the pheromone responsible for these inhibitory responses (Pain 1961). Subsequently, other components of the mandibular gland secretion were identified (Callow et al. 1964) of which 9-hydroxy-2-decanoic acid (9HDA) was shown to have an inhibitory response in emergency queen cell construction (Butler and Callow 1968). Many laboratory experiments have been conducted to identify these inhibitory effects exerted by the queen (Free, 1987). It would seem that 9ODA and 9HDA play important roles, but that other pheromones from the mandibular and/or tergite and/or tarsal glands also have inhibitory effects (Gary and Morse 1962, Velthuis 1970 and Lensky and Slabezki 1981). It is likely that a combination of pheromones (including 9ODA and 9HDA) act together to cause these inhibitory effects (Slessor et al. 1988).

When this study was started, there was uncertainty concerning the race of the bees in the Eastern Cape. It was soon realized that the Eastern Cape honeybee was behaviourally different from the Transvaal honeybee (*Apis mellifera scutellata*) and it was discovered that these Eastern Cape honeybees were thelytokous (Hepburn et al. 1988). This laid the basis for a thorough investigation concerning the distribution of *Apis mellifera capensis* throughout Southern Africa (Hepburn and Crewe in press). Despite the controversies surrounding the distribution of the Cape honeybee (Ruttner 1977 and 1986 and Moritz and Kauhausen 1984), Hepburn and Crewe (in press) have found that it probably follows the distribution of the Cape flora as suggested by Tribe (1983). There was no particular reason as to why *A.m.capensis* were used in this study other than that these honeybees are indigenous to Grahamstown.

The Cape honeybee, *A.m.capensis* (Escholtz), is unique among honeybee races in that laying workers are thelytokous (Onions 1912, Jack 1916, Anderson 1963 and Hepburn and Crewe in press). This means that queenless and broodless colonies of *A.m.capensis* can rear a new queen and do not necessarily become hopelessly queenless as their African counterpart, *A.m.scutellata* (Hepburn et al. 1988). *A.m.capensis* laying workers are of particular interest because their mandibular gland secretions (particularly 9ODA) are abundant and are often comparable to those found in mated *A.m.capensis* queens (Ruttner et al. 1976, Hemmling et al. 1979, Crewe and Velthuis 1980 and Velthuis et al. 1990). Ideas that 9ODA and thelytoky may be

related or that ovarian development may be enhanced because Cape laying workers are dominant have been investigated but remain unresolved (Allsopp 1988). These arguments are interesting but extend beyond the scope of this study.

Hepburn (1986) showed that a number of stimuli influence comb building, these include nectar and pollen gathering, the presence of brood and a queen and the space and density of a nesting cavity. This work focusses on the role of the queen in comb building and addresses the following questions: 1) does the queen influence wax secretion ? 2) is comb building enhanced in queenright *A.m.capensis* colonies and how does this differ from *A.m.scutellata* ? 3) do workers need to have direct contact with their queen for comb building to occur ? 4) do the mandibular and/or tergite glands secretions play any role in comb building ? 5) is comb building enhanced when the workers have access to the head or abdomen of the queen ? and 6) do virgin queens enhance comb building ?

Chapter 2

Does the queen enhance comb building in *A.m.capensis* and *A.m.scutellata* colonies ?

Summary

A.m.capensis and *A.m.scutellata* worker bees in queenless and queenright colonies and colonies given an *A.m.capensis* virgin queen secreted equal amounts of wax for comb building. However, queenright *A.m.scutellata* and *A.m.capensis* colonies constructed 3 and 8 times more comb than their respective queenless counterparts. This difference was not correlated with the increased percentage amount of queen substance (9ODA) present in the mandibular glands of the *A.m.capensis* queens. The queenright colonies of both races constructed similar amounts of comb. *A.m.capensis* colonies headed by an *A.m.scutellata* queen and *A.m.scutellata* colonies headed by an *A.m.capensis* queen also constructed similar amounts of comb. There were no correlations between any of the queen pheromones and the comb construction measurements. Colonies given an *A.m.capensis* virgin queen constructed significantly less comb than any of the colonies given a mated queen. When the colonies given an *A.m.capensis* virgin queen and the queenless colonies were compared, no differences were found. The

presence of a mated queen is necessary for enhancing comb construction. 9ODA on its own is an unlikely candidate but it may act in conjunction with other pheromone(s).

Introduction

Colony survival in honeybees is dependent on the secretion of wax and its manipulation into comb for food storage and brood rearing. Wax secretion and comb construction are two separate activities that are independently controlled (Hepburn, 1986). Wax secreting bees from queenright and queenless colonies have similar histological developments of the wax glands (Dreischer 1956 and Goetze and Bessling 1959). However, the wax secreting bees from queenright colonies bare a 40% greater mass of wax than the wax secreting bees from queenless colonies (Goetze and Bessling 1959). Queenright colonies construct 3-4 times more comb than queenless colonies even when the colonies are of similar size (Goetze and Bessling 1959, Rajashekarappa and Channabasavanna 1979 and Jay and Jay 1983). Clearly, the presence of the queen somehow enhances wax secretion and comb construction as the potential to secrete equal amounts of wax and construct equal amounts of comb in these colonies is evident from the development of the wax glands.

Darchen (1968) showed that the head of the queen was necessary for stimulating comb building. Hepburn (1986) proposed that 9ODA secreted

from the mandibular glands of mated queens is responsible for enhancing wax secretion and comb construction. Since, *A.m.capensis* queens have 20% more 9ODA in their mandibular gland secretions than *A.m.scutellata* queens (Crewe 1982), these differences were compared to investigate the effects of queen substance on wax secretion and comb construction in *A.m.capensis* and *A.m.scutellata* colonies.

Young virgin queens have relatively small amounts of 9ODA in their mandibular glands (Butler and Paton 1962 and Pain et al. 1967). As the virgin queen becomes older, so her mandibular gland secretion becomes more queenlike (Butler and Paton 1962, Pain et al. 1967 and Crewe 1982). When Rajashekarappa and Channabasavanna (1979) compared queenless colonies, colonies given a virgin or mated queen for comb construction in *A.cerana*, they found that the colonies given a mated queen constructed four times more comb than the queenless colonies and the colonies given a virgin queen. Since *A.m.capensis* virgin and mated queens have comparable amounts of 9ODA in their mandibular glands (Crewe 1988), wax secretion and comb construction were also measured in colonies given an *A.m.capensis* virgin queen.

Methods and materials

A. Experimental set-up and procedure

A group of 8 five-framed queenright *A.m.capensis* colonies from the Grahamstown area and another group of 13 five-framed queenright *A.m.scutellata* colonies from Pretoria were placed in an apiary in Grahamstown in late summer 1987. The two races were placed 800 metres apart from each other at either end of the apiary (Fig 1). During the experiment the bees were fed a 40% sugar solution twice a day from numerous feeders in the apiary. The bees had equal access to the food and the amount available to each hive was 2 litres/day. All the queens were mated and had been laying eggs for some time.

The experiment began by dequeening all the colonies in the morning. Ten hours later, each colony was given a "queen" treatment. Each queen was introduced into a colony with 6 workers in a haircurler which was closed at either end with cork. The cork was removed the morning after the introduction. This allowed the colony to become accustomed to the new queen (and her odour) without stinging her. At the same time that the queen introductions were made, each colony was given 2 dummy frames (frames that had wooden blocks inserted to fill the inside of the frame instead of comb foundation), 1 frame with sealed brood, honey and pollen stores and 2 empty frames (with no comb foundation) on which to construct comb.

The virgin queens were collected from queenless *A.m.capensis* colonies that were requeening at another apiary site. These queens were collected on emergence and kept in an incubator at 32^{o}C with a few worker bees. These virgin queens were not more than 4 days old at the time they were introduced into the experimental colonies. Queen excluders were inserted at the entrance of each colony to prevent the queen from mating.

Figure 1. The apiary site. *A.m.scutellata* colonies were in the furthermost field while the *A.m.capensis* colonies are seen in the foreground. The 2 races were separated to prevent drifting between them.

In the first experiment (4 May), 4 *A.m.capensis* colonies were each given a conspecific but different *A.m.capensis* queen while 4 colonies were not given a queen. Nine *A.m.scutellata* colonies were each given a conspecific but different *A.m.scutellata* queen while 4 *A.m.scutellata* colonies remained queenless. On 10 May (6 days later), the empty frames containing the newly constructed comb were collected (Fig. 2). At the same time, a sample of 100 bees were collected from festoons in each colony for wax scale recovery. The remaining frames were taken out of the hive and the hive with bees was weighed. Colony size was determined by subtracting the empty hive weight from the weight of the hive and bees. One thousand bees were killed and weighed so that colony size could be calculated.

The next morning (11 May), all of the colonies were dequeened and reciprocal crosses between the races were performed: 4 *A.m.capensis* colonies were each given an *A.m.scutellata* queen, and 4 *A.m.scutellata* colonies were each given an *A.m.capensis* queen. The frames were distributed and the queen was introduced as previously described. After 6 days (17 May), the frames of newly constructed comb were harvested, wax festoon bees were collected and colonies were weighed to determine colony size. The next morning, all the colonies were dequeened and the queen heads were each placed in 1ml dichloromethane and stored in a refridgerator at -20°C for gas chromatography analysis. The *A.m.scutellata* colonies colonies were killed to prevent contamination of the *A.m.capensis* colonies.

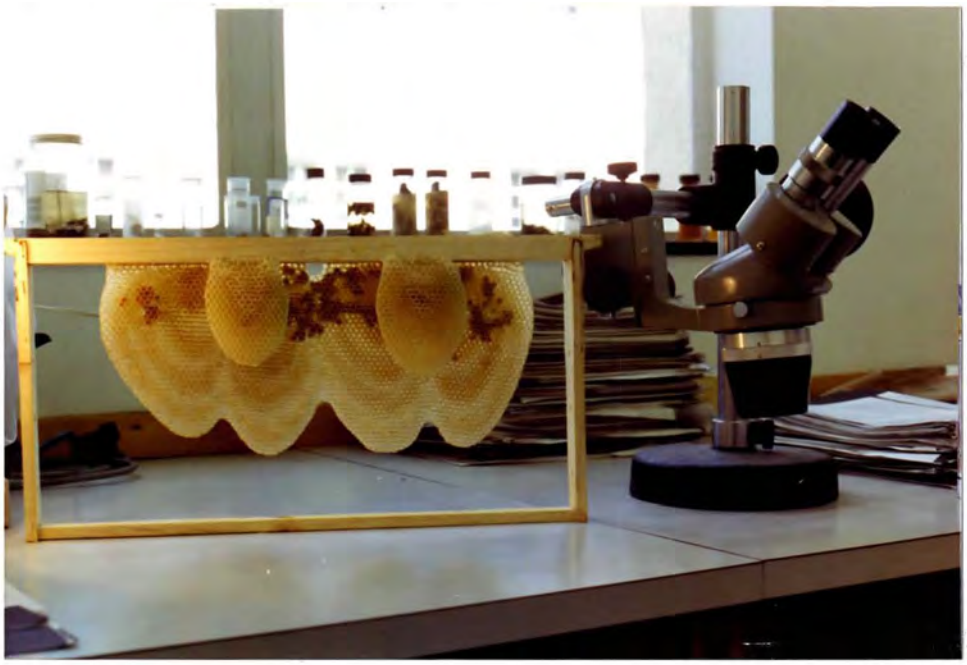

Figure 2. An example of newly constructed comb from a queenright *A.m.capensis* colony.

On the 18 May, 4 *A.m.capensis* colonies were each given a virgin queen. Queen introduction and the distribution of frames occurred as previously mentioned. Six days later (24 May), the newly constructed comb was harvested, wax festoon bees collected and colonies weighed to determine colony size. These queens remained in the colonies after the experiment and were allowed to mate.

B. Data collection and analysis

The samples of festoon bees were frozen soon after collection. The percentage of festoon bees bearing wax were recorded and the wax borne by these bees was removed and weighed. The surface area of the newly constructed comb was measured by photocopying the comb and then weighing the photocopied area of comb. The surface area relative to the weight of a cm^2 piece of blank photocopying paper was calculated. The comb was then melted at 90 ^{o}C for 12 hours in a beaker of water to separate the wax from the honey and pollen. The melted comb was then cooled and weighed. The cell type was recorded and diameter of 25 cells from the centre, left and right sides of the photocopied comb were measured with an ocular micrometer. The cell diameter was measured from one mid-wall to the mid-wall on the opposite side of the cell (Hepburn 1983).

Each gas chromatography sample was derivatized with BSFTA

(Bis(trimethylsilyl)-trifluroracetamid) and then concentrated with nitrogen gas before being analyzed with a Hewlett Packard 5890A Gas Chromatograph fitted with a fused silica capillary column, diameter 0.2mm x 25m. The gas chromatographic program started at 60°C and increased at a rate of 3°C/min to 70°C. This temperature was held for half a minute and then increased at a rate of 20°C/min to 250°C. This final temperature was held for 10 minutes. The signals from the flame ionization detectors were quantified with a Hewlett Packard 3393A integrator. Identification of the compounds of the secretions was by comparison of the retention times with those of authentic standards.

Results

A. Wax secretion

The mean colony size, percentage of festoon bees bearing wax and the weight of the wax scales borne by these bees under different conditions are shown in Table 1 (See Apendix A.1 for raw data). There were no significant differences in the percentage of festoon bees bearing wax and the amount of wax secreted per festoon bee under the various queenright and queenless conditions (Tables 4 and 5). The only statistical difference in the amount of wax borne by the festoon bees occurred when the *A.m.capensis* colonies given an *A.m.scutellata* queen were compared to the *A.m.scutellata* colonies given an *A.m.capensis* queen (Table 4). This result is not perturbing as the *A.m.capensis* workers

Table 1. The mean ± (Standard Deviation) colony size, percentage of festoon bees bearing wax and the weights of the wax scales borne by these bees in experiment 1. *

Treatment	n	Colony size	% Bees bearing wax	Amount of wax secreted /bee (µg/1000 bees)
MQ1	4	9355	67	473.2
		3622	15	65.7
QL1	4	3793	55	575.9
		1481	14	250.0
MQS1	9	9205	75	575.5
		3441	13	128.5
QLS1	4	7399	73	621.5
		3842	5	70.2
CWSQ1	4	6371	61	470.0
		3025	18	68.4
SWCQ1	4	6992	77	638.6
		3058	3	51.7
VQ1	4	6464	47	358.2
		4664	1	74.6

*

MQ1 - *A.m.capensis* colonies given a mated *A.m.capensis* queen
MQS1 - *A.m.scutellata* colonies given a mated *A.m.scutellata* queen
QL1 - queenless *A.m.capensis* colonies
QLS1 - queenless *A.m.scutellata* colonies
CWSQ1 - *A.m.capensis* colonies given a mated *A.m.scutellata* queen
SWCQ1 - *A.m.scutellata* colonies given a mated *A.m.capensis* queen
VQ1 - *A.m.capensis* colonies given a virgin *A.m.capensis* queen
The number after the colony description indicates the experiment number.

headed by the *A.m.scutellata* queen could have donated more of their wax scales for the construction of comb than the *A.m.scutellata* workers headed by the *A.m.capensis* queen (Hepburn 1986). It would appear that the queenright and queenless colonies have the potential to construct equal amounts of comb as the amount of wax available was more or less the same.

B. Comb building

Table 2 shows the mean surface area, weight and cell diameters of comb constructed under the various queen conditions. (The comparisons in Tables 4 and 5 were made from per bee measurements; this was to standardize the data taking colony size effects into account.) The queenless colonies always constructed considerably less comb than their queenright counterparts (Tables 2 and 4). The colonies given an *A.m.capensis* virgin queen also constructed significantly less comb than the queenright colonies (Table 5). There were no significant differences when the colonies given a virgin queen were compared to the queenless colonies (Table 5). The *A.m.capensis* and *A.m.scutellata* queenless colonies showed no differences in the amounts of comb which they constructed (Tables 2 and 4).

The *A.m.capensis* and *A.m.scutellata* queenright colonies constructed 8 and 3 times

Table 2. The mean ± (S.D.) surface area, weight and cell diameters of the comb constructed by worker honeybees in experiment 1. *

Treatment	n	Surface area (mm^2)/bee	Weight (μg)/ bee	Cell diame-ter
MQ1	4	48.6 8.9	2745.0 591.2	4.2 0.3
QL1	4	6.0 7.5	325.6 402.4	4.1 0.2
MQS1	9	55.8 24.2	3494.0 1561.8	4.2 0.2
QLS1	4	18.9 12.2	1129.4 721.3	4.6 0.3
CWSQ1	4	62.6 21.7	3240.0 1061.3	3.7 0.1
SWCQ1	4	77.6 8.5	3925.0 668.1	4.0 0.4
VQ1	4	16.5 13.6	1038.8 869.9	3.8 0.1

*
Abbreviations for the different experimental conditions are described in Table 1.

Table 4. A comparison of statistically significant differences between the different experimental conditions (except for the colonies given an *A.m.capensis* virgin queen) for wax secretion, comb construction and queen pheromones in experiment 1. *

	%B	WR	SA	WE	DIA	9ODA	10HDA	9HDA
MQ1 vs QL1	-	-	+	+	-			
MQS1 vs QLS 1	-	-	+	+	+			
MQ1vs MQS1	-	-	-	-	-	-	-	-
QL1 vs QLS1	-	-	-	-	+			
MQ1 vs CWSQ1	-	-	-	-	+	-	-	-
MQS1 vs SWCQ1	-	-	-	-	-	-	-	-
QLS1 vs SWCQ1	-	-	+	+	+			
QL1 vs CWSQ1	-	-	+	+	-			
CWSQ1 vs SWCQ1	-	+	-	-	-			

* Kruskal- Wallis one-way analysis of variance test. Significance is defined as $P < 0.05$. No significance and significance are denoted as - and + respectively. The abbreviations for the experimental conditions are described in Table 1.

%B - percentage of festoon bees bearing wax
WR - amount of wax removed from 100 bees divided by the number of bees bearing wax in the festoon
SA - surface area (mm^2) of comb constructed /1000 bees
WE - weight (μg) of comb constructed /1000 bees
DIA - diameters of the cells that were constructed (mm)
9ODA - the percentage amount of 9-oxo-2-decanoic acid
10HDA - the percentage amount of 10-hydroxy-2-decanoic acid
9HDA - the percentage amount of 9-hydroxy-2-decanoic acid

Table 5. A comparison of statistical differences between the different experimental conditions including the colonies given an *A.m.capensis* virgin queen for the wax secretion and comb construction measurements in experiment 1. *

	%B	WR	SA	WE	DIA
VQ1 vs MQ1	-	-	+	+	+
VQ1 vs QL1	-	-	-	-	-
VQ1 vs MQS1	-	-	+	+	+
VQ1 vs QLS1	-	-	-	-	+
VQ1 vs CWSQ1	-	-	+	+	-
VQ1 vs SWCQ1	-	-	+	+	-

* Kruskal-Wallis one-way analysis of variance test. Significance is defined as $P < 0.05$. No significance and significance are denoted by - and + respectively.

Abbreviations are explained in Table 1. The number after the colony description indicates the experiment number.

%B - percentage of festoon bees bearing wax
WR - amount of wax removed from 100 bees divided by the number of bees bearing bees bearing wax in the festoon
SA - surface area (mm^2) of comb constructed /1000 bees
WE - weight (μg) of comb constructed /1000 bees
DIA - diameters of the cells that were constructed (mm)

more comb than their respective queenless counterparts (Table 2). Since the *A.m.capensis* queens had 20% more 9ODA in their mandibular gland secretions, the increased amount of comb constructed by these colonies could be attributed to 9ODA (Table 3). However, the *A.m.capensis* given an *A.m.capensis* queen and *A.m.scutellata* colonies given an *A.m.scutellata* queen constructed similar amounts of comb (Table 4).

When the queenless *A.m.scutellata* colonies and colonies given an *A.m.capensis* virgin queen were compared to the *A.m.scutellata* colonies headed by an *A.m.capensis* queen, significant differences in the comb building measurments were found (Tables 4 and 5). Similarly, queenless *A.m.capensis* colonies and colonies given an *A.m.capensis* virgin queen constructed significantly less comb than *A.m.capensis* colonies headed by an *A.m.scutellata* queen (Tables 4 and 5). There were no differences in the amount of comb constructed by the colonies of both races given either an *A.m.capensis* or an *A.m.scutellata* queen (Tables 2 and 4). These results suggest that it is the presence of a mated queen (regardless of race) that enhances comb construction.

Similar sized cells were constructed in the queenright and queenless *A.m.capensis* colonies (Table 2). The queenless *A.m.scutellata* colonies constructed significantly larger cells than their queenright counterparts and queenright *A.m.capensis* colonies (Tables 2 and 4). The diameters of the

cells constructed by the queenless colonies of both races also differed significantly (Table 4). However, in the comparisons between the queenright colonies of both races, no differences were found (Tables 2 and 4). *A.m.capensis* workers headed by an *A.m.scutellata* queen constructed smaller cells than when headed by their own queen (Tables 2 and 4). No significant differences were found when the *A.m.scutellata* colonies were headed by an *A.m.capensis* queen than their own *A.m.scutellata* queen (Tables 2 and 4). Colonies given an *A.m.capensis* virgin queen constructed relatively small cells which differed significantly from the queenright *A.m.capensis* and *A.m.scutellata* and queenless *A.m.scutellata* cells (Tables 2 and 4).

C. Pheromones

Figure 3 shows a gas chromatogram of an extract of a mated *A.m.capensis* queen head. The *A.m.capensis* queens had on average 20% more 9ODA in their mandibular glands than the *A.m.scutellata* queens (Table 3). However, this difference was not significant (Table 4). The amounts of 9HDA and 10HDA in the mandibular gland secretions were also measured and found not to differ between the 2 races (Tables 3 and 4). No correlations between any of these mandibular gland secretions and any of the comb construction measurements were found.

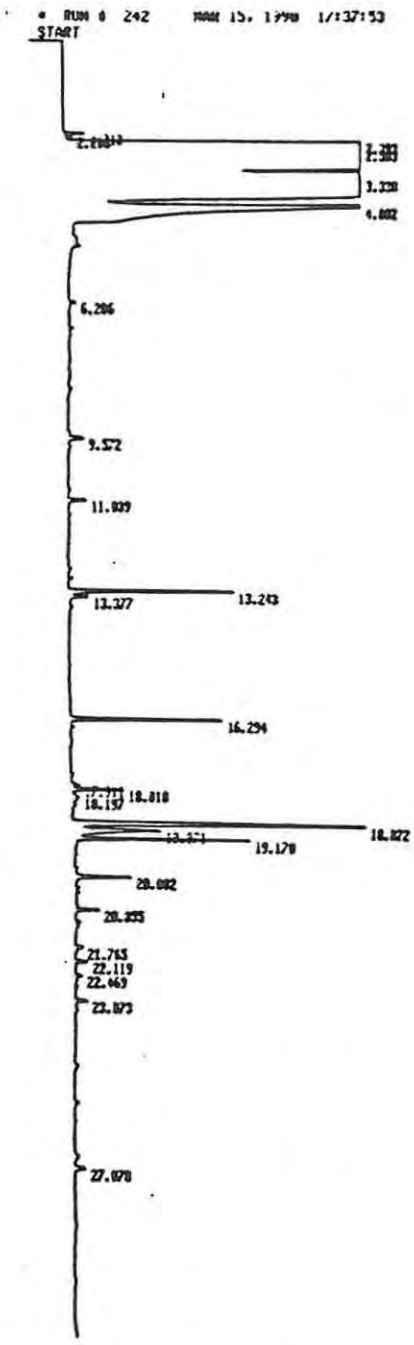

Figure 3. A gas chromatogram of an extract of a mated *A.m.capensis* queen head. The identity of the peaks were as follows: a) 13.243 minutes - octanoic acid (internal standard) b) 18.822 minutes - 9ODA c) 19.170 minutes - 9HDA and d) 20.855 minutes - 10HDA.

Table 3. The mean ± (S.D.) percentage composition of the major components of the mandibular gland secretions of mated *A.m.scutellata* and *A.m.capensis* queens. *

Queen	% Composition of the components present			
	9ODA	9HDA	1OHDA	Total acids (ug/head)
A.m.capensis (n = 4)				
$\bar{x}$	72.6	16.1	11.3	70.3
±	26.4	27.4	21.0	12.0
A.m.scutellata (n = 9)				
$\bar{x}$	51.2	42.9	14.9	142.6
±	49.2	55.4	18.6	67.5

*

9ODA - the percentage amount of 9-oxo-2-decanoic acid
1OHDA - the percentage amount of 10-hydroxy-2-decanoic acid
9HDA - the percentage amount of 9-hydroxy-2-decanoic acid

Discussion

It would appear that the queen holds little influence over the ability of worker bees to secrete wax (Dreischer 1956, Darchen 1957 and Goetze and Bessling 1959). Hepburn et al (1984) showed that queenless bees reared away from queenright ones secreted less wax than queenless bees reared in the vicinity of queenright ones. Goetze and Bessling (1959) also showed that queenright colonies bore 40 % more wax than queenless colonies. However, these results show that queenright (mated and virgin queens) and queenless colonies have similar amounts of wax available for the construction of comb (Hastings personal comm). The latter experiments were field experiments while the former were conducted under laboratory conditions. This may account for the discrepencies in the results. "... that bees reduced to small numbers lose their industry, their activity, and imperfectly continue their ordinary labors. Thus their instinct is modified by each operation that reduces them to too small a number. To render such an experiment truly conclusive, it should then be made in a populous hive ..." (Huber 1814).

The queenright *A.m.capensis* and *A.m.scutellata* colonies constructed 8 and 4 times more comb than their respective queenless counterparts (Table 2). No significant differences in the amount of comb constructed between the queenright colonies of both races were found (Tables 2 and 4). It was anticipated that the *A.m.scutellata* colonies headed by an *A.m.capensis* queen

anticipated that the *A.m.scutellata* colonies headed by an *A.m.capensis* queen would have constructed more comb than when headed by their own queen and the *A.m.capensis* colonies headed by an *A.m.scutellata* queen would have constructed less comb than when headed by their own *A.m.capensis* queen. These predictions failed (Tables 2 and 4) and similar amounts of comb were constructed in all the queenright colonies. Although there was a difference in the amount of 9ODA in the mandibular glands of the queens of both races, this difference was not likely to play any role in enhancing comb construction.

A.m.capensis virgin queens secrete comparable amounts of 9ODA from their mandibular glands as mated *A.m.capensis* queens (Crewe 1988). However, the colonies given a mated *A.m.capensis* or *A.m.scutellata* queen always constructed significantly more comb than the colonies given an *A.m.capensis* virgin queen (Table 5). Although the virgin queen mandibular glands were not analyzed in this experiment, it is evident that 9ODA (alone) cannot influence the amount of comb constructed in queenright colonies. No significant differences between the colonies given a virgin queen and the queenless colonies were found for any of the comb construction measurements (Tables 2 and 4, Verheijen-Voogd 1959 and Rasjashekarappa and Channabasavanna 1979). Once again there is evidence to suggest that 9ODA from the queen mandibular glands does not enhance comb construction; although it may act synergistically with other pheromone(s).

Free (1987), extended the work of Taranov (1959), showing that as colony size increased, so the amount of comb built decreased. This relationship was derived by dividing the amount of wax produced by the total number of young bees in the colony. This relationship did not hold in this experiment and is difficult to interpret as it is not known whether all the young bees in a colony construct comb. Whether Taranov (1959) took the wax secreted or comb constructed as wax produced is uncertain (and confusing). Furthermore, this relationship was tested for both wax secretion and comb building (using per bee measurements) in all experiments. It was not found to hold for any of the data sets and it is not discussed again.

The diameters of the cells that were constructed were somewhat smaller when compared to the measurements of Garin (1926) and Smith (1961). The queenright colonies of both races constructed similar sized cells although *A.m.scutellata* workers are generally known to construct larger cells (Smith 1961). The largest cells were constructed by the queenless *A.m.scutellata* colonies (Table 2). These cells were an intermediate drone and worker cell size (Vogt 1911 and Owens and Taber 1973). The differences in cell diameters of the cells constructed by the queenless *A.m.capensis* and *A.m.scutellata* colonies maybe attributed to the lytoky and arrhenotoky respectively (Onions 1912 and Hepburn and Crewe in press). The smallest cells were constructed by the *A.m.capensis* colonies headed by an *A.m.scutellata* queen (Table 2). The workers in the colonies given a virgin queen also constructed

relatively small cells (Table 2). The differences in the diameters of the cells could not be readily accounted for and whether the queen is able to control the size of the cell that is constructed is uncertain (Free 1987).

There were also no significant differences in the amounts of queen pheromones measured between the two races (Table 4). No correlations between any of the queen pheromones and comb construction measurements were found. Clearly, it is the presence of a mated queen that enhances comb construction, and not a particular pheromone.

Butler (1954), Verheijen-Voogd (1959) and Pain (1961) showed that the physical as well as the chemical presence of the queen is important for regulating "normal" colony behaviour. It is likely that the physical and chemical presence of the queen act together in enhancing comb construction and that queen substance alone is not sufficient. These ideas are developed in the next chapter.

Chapter 3

Contact chemoreception - the key to enchancing comb construction in *A.m.capensis* honeybees.

Summary

The percentage of festoon bees bearing wax and the amount of wax borne by these bees was the same for colonies given a "free" queen, a queen in a single or double cage, a dead queen or queenless colonies. The colonies given a queen in a single or double cage, a dead queen or no queen constructed significantly less comb than their controls (colonies given a "free" queen). Colonies given a queen in a single or double cage constructed similar amounts of comb. The colonies given a dead queen constructed significantly more comb than the colonies given a caged queen (single and double together). The colonies given a caged queen (single and double cages) constructed similar amounts of comb when compared independantly and together with the queenless colonies. No correlations between 9ODA, 10HDA and 9HDA and any of the comb construction measurements were found. The results showed that worker bees needed to have direct contact with a mated queen to obtain pheromone(s) that enhanced comb building in queenright

colonies.

Introduction

That the presence of a mated queen enhances comb construction in honeybee colonies is certainly apparent from the work of Darchen (1956 and 1957), Verheijen-Voogd (1959), Rajashekharappa and Channabasavanna (1979), Jay and Jay (1983) and Chapter 2. The amount of comb constructed in queenright *A.m.capensis* and *A.m.scutellata* colonies was not correlated with the amount of 9ODA in the heads of these queens (Chapter 2). Comb was never readily constructed in colonies given a virgin queen (Rajashekarappa and Channabasavanna 1979 and Chapter 2). These experiments all showed that a mated queen somehow enhanced comb building.

Worker bees need to have direct contact with their queen to obtain pheromones that maintain and regulate the colony (Butler 1954, Verheijen-Voogd 1959, Seeley 1979, Ferguson and Free 1980, Seeley 1985 and Free 1987). The sources of these pheromones are the mandibular (Callow et al. 1964) and tergite glands (Velthuis 1970) and are obtained from the queen during retinue behaviour (Seeley 1979, Ferguson and Free 1980). These pheromones inhibit worker ovarian development and queen cell construction amongst other things (Butler 1954, Pain 1955 and Verheijen-Voogd 1959). However, their mode

of action is not understood as queens with extirpated mandibular glands are still able to maintain control over their colonies (Gary and Morse 1962, Velthuis 1970 and Butler et al. 1974). Whether the mandibular and/or tergite glands secrete the pheromone(s) responsible for maintaining colony cohesion is a moot point, but the worker bees need to have direct contact with their queen to obtain these pheromones. Whether the worker bees need to have direct contact with their queen for comb building to be enhanced was investigated.

Methods and Materials

A. Experimental set-up and procedure

Twenty five-framed queenright *A.m.capensis* colonies were placed in an apiary in Grahamstown in mid-December 1987. The queens were all mated and had been laying eggs for some time. During the experiment the bees were fed a 40% sugar solution once a day from various feeders in the apiary. The bees had equal access to the food and the amount available to each hive was a litre/day.

The single and double cages were made from wooden strips joined to cover an area 12.5cm x 11cm and were covered with a wire mesh 0.2mm x 0.2mm. The cages were each waxed onto the frame containing the sealed brood and food. Two single cages mounted on each other

constituted a double cage. The worker bees given a queen in a single cage could both smell and touch her through the wire mesh, whereas the worker bees given a queen in a double cage could only smell their queen. The dead queens that were used had been stored in ethanol for 4 months and were air-dried 2 weeks prior to the experiment.

On 15 December, all the colonies were dequeened in the morning and 10 hours later the queens were placed back into their colonies such that: 3 colonies were each given their queen with a few workers in a single cage, 4 colonies were each given their queen and a few worker bees in a double cage, 1 colony was given a dead queen, 2 colonies remained queenless and 10 colonies were each given their own queen who was allowed to move freely across the frames ("free" queens). The "free" and dead queens were introduced with a few workers into their colonies in a haircurler that had been sealed at both ends with cork. The cork from one end of the haircurler was removed the following morning (Chapter 2). The frames in each colony were distributed so that each one had 2 dummy frames (frame having a wooden block inserted to fill the area where comb foundation is normally placed in an empty frame), 1 frame of sealed brood and honey and 2 empty frames on which to construct comb (Chapter 2).

Six days later (21 December), the empty frames containing the newly constructed comb were collected. One hundred festoon bees were collected from each colony for wax scale recovery and were immediately frozen on returning to the laboratory. Each hive was weighed after all the frames had been removed so that the weight of the hive and bees could be used to estimate colony size (Chapter 2).

The next morning (22 December), all of the colonies were dequeened once again. This time the 4 colonies that were each given a " free" queen the previous week, were given the same queen but in a single cage. Three colonies each given a queen in a single cage on 15 Dec, were given the same queens who now moved freely across the frames. Three colonies that were each given a "free" queen were now given the same queen in a double cage. The colonies given queens that were previously in a double cage were each given the same queen who was now freely able to move across the frames. Two of the colonies given a "free" queen were now each given a dead queen while the colony that had been given a dead queen was now given a "free" queen. The remaining colony that had been given a "free" queen was now made queenless (the 2 queenless colonies were excluded from the experiment). The frames in each colony were distributed and the queens were introduced as previously described.

Six days later (28 December), the empty frames containing the newly

constructed comb were collected. One hundred festoon bees were collected from each colony for wax scale recovery and were frozen on returning to the laboratory. The colonies were weighed (as previously mentioned) to obtain an estimate of colony size. All the queens were captured and decapitated. Their heads were each placed in 1ml of dichloromethane and stored in a refridgerator at -20°C to be analyzed gas chromatographically at a later date.

B. Data collection and analysis

From the samples of festoon bees, the percentage of bees bearing wax was determined and the amount of wax borne by these bees was removed and weighed. The surface area of the newly constructed comb was measured by calculating the surface area of photocopies of the comb (Chapter 2). The comb was then melted over water to separate the wax from any honey and pollen (Chapter 2). The melted comb was then cooled and weighed. The amount of wax secreted/bee and the surface area and weight of the comb constructed/bee were calculated so that the data could be standardized to make comparisons. The cell type (worker or drone) was determined and the diameter of 25 cells from the centre, left and right sides of the photocopied comb were measured with an ocular micrometer. The cell diameter was taken from the one mid-wall to the mid-wall on the opposite side of the cell (Hepburn 1983).

The gas chromatographic samples were analyzed as described in Chapter 2.

Four queens in a double cage, 2 in a single cage and a colony given a "free" queen died during the experiment. These colonies and their controls were excluded from the results. It is not known how long after their introduction that they died as the hives were not opened during the experiment.

Results

A. Wax secretion

Table 1 shows the mean colony size, percentage of festoon bees bearing wax and the amount of wax borne by these bees under the different experimental conditions. (The raw data is shown in Appendix B.1) The percentage of festoon bees bearing wax and the amount of wax borne by these bees did not differ when the various experimental conditions were compared (Tables 1 and 4). These results support the hypothesis that the queen has little influence over the ability of the bees to secrete wax (Hastings personnal comm and Chapter 2).

Table 1. The mean ± (Standard Deviation) colony size, percentage of festoon bees bearing wax and the amount of wax secreted by these bees under the different experimental conditions. *

Treatment	n	Colony size	% Bees bearing wax	Amount of wax secreted /bee (µg/1000 bees)
DO2	3	5255	78	695.6
		2188	18	462.0
MQ2	3	4142	54	818.8
		6165	10	264.5
SI2	5	3876	64	636.0
		2307	20	245.2
MQ2	5	4946	73	491.0
		2992	17	210.3
DEAD2	3	3571	54	869.0
		1803	11	102.5
MQ2	3	1761	58	801.5
		1655	10	462.7
QL2	3	4487	63	563.7
		2757	25	329.1

*
MQ2 - colonies given a "free" queen (queen was able to move freely across the comb)
DO2 - colonies given a queen in a double cage
SI2 - colonies given a queen in a single cage
DEAD2 - colonies given a dead queen
QL2 - queenless colonies
The number after each experimental condition refers to the experiment number

B. Comb building

Table 2 shows the mean surface area, weight and diameters of the cells of the newly constructed comb. The colonies given a caged queen (single and and double cage), constructed significantly less comb than when the colonies had access their "free" queen (Tables 2 and 4). Colonies having direct access to a dead queen and queenless colonies also constructed significantly less comb than when the colonies were given a "free" queen (Tables 2 and 4). No significant differences for these comb construction measurements were found when all of the colonies given a "free" queen were compared (Table 4).

Similar amounts of comb were constructed in the colonies given a single or double caged queen (Table 4). The colonies given a queen in a single and double cage together constructed significantly less comb than both of their controls (Table 4). Furthermore, these colonies given the caged queens constructed significantly less comb than the colonies given a dead queen (Table 4). Yet, the colonies given a dead queen constructed significantly less comb than their controls (Table 4). These results suggest the worker bees need to have direct contact with their queen in order for comb building to be enhanced (cf. Butler 1954 and 1960, Verheijen-Voogd 1959 and Velthuis 1970).

Table 2. The mean ± (S.D.) surface area, weight and cell diameters of the newly constructed comb under the different experimental conditions. *

Treatment	n	Surface area (mm^2)/1000 bees	Weight (μg/1000 bees)	Cell diameter (mm)
DO2	3	0 -	0 -	-
MQ2	3	4.0 3.4	311.8 448.0	4.8 0.3
SI2	5	0.008 0.02	0.26 0.6	-
MQ2	5	5.4 6.8	188.9 336.0	4.9 0.5
DEAD2	3	1.6 1.5	125.3 108.5	5.5 1.0
MQ2	3	9.0 5.4	571.9 188.5	4.9 0.5
QL2	3	0 -	0 -	-

*
The abbreviations for the experimental conditions are described in Table 1.

However, these results are complicated by the fact that no significant differences were found when the colonies given single or double caged queens were independantly compared to the colonies given a dead queen (Table 4). There were also no significant differences when the colonies given a dead queen were compared to the queenless colonies (Table 4). Furthermore, the colonies given a single or double caged queen constructed similar amounts of comb to the queenless colonies (Table 4). There were no significant differences when the colonies given a single or double caged queen, a a dead queen or no queen were all compared together (Table 4).

The colonies given a dead queen constructed intermediate worker and drone sized cells (Table 2, Vogt 1911 and Owens and Taber 1973). These cells were significantly larger than the cells constructed by the worker bees when they were given a "free" queen (Table 4). The colonies given a "free" queen constructed worker sized cells similar to those measured by Smith (1961). No cell diameters for the colonies given a queen in a single or double cage and queenless colonies were measured as too few cells were constructed.

C. Pheromones

Table 3 shows the mean percentage composition of the major components of

Table 3. The mean ± (S.D.) percentage composition of the major components of the mandibular gland secretions of *A.m.capensis* queens. *

Queen	n	% Composition of the components present 9ODA	9HDA	10HDA	Total acids (µg/head)
Double caged & "free" queens	3	45.5 3.0	54.0 3.3	0.5 0.1	60.7 9.5
Single caged & "free" queens	5	58.6 14.6	29.0 27.9	12.4 17.2	103.4 124.1
"Free" queens given to colonies given a dead queen	3	55.3 1.0	44.0 14.1	0.8 0.5	59.9 11.2

*

9ODA - the percentage amount of 9-oxo-2-decanoic acid
9HDA - the percentage amount of 9-hydroxy-2-decanoic acid
10HDA - the percentage amount of 10-hydroxy-2-decanoic acid

Table 4. A comparison of statistically significant differences between the different experimental conditions for wax secretion, comb construction and pheromone values. *

	%B	WR	SA	WE	DIA	9ODA	10HDA	9HDA
All MQ2 compared	-	-	-	-	-	-	-	-
DO2 vs MQ2	-	-	+	+	+			
SI2 vs MQ2	-	-	+	+	+			
DEAD2 vs MQ2	-	-	+	+	+			
QL2 vs all MQ2	-	-	+	+	+			
SI2 vs DO2	-	-	-	-	-	-	-	-
SI2, DO2 vs MQ2	-	-	+	+	+	-	-	-
SI2, DO2 vs DEAD2	-	-	+	+	+			
DO2, SI2 vs QL2	-	-	-	-				
DO2 vs DEAD2	-	-	-	-				
SI2 vs DEAD2	-	-	-	-				
DEAD2 vs QL2	-	-	-	-				
DO2 vs QL2	-	-						
SI2 vs QL2	-	-						
DO2 vs SI2 vs DEAD2 vs QL2	-	-	-	-	+	-	-	-

* Kruskal-Wallis one way analysis of variance test. Significance is defined as $P < 0.05$. No significance and significance are - and + respectively.

The abbreviations for the experimental conditions are given in Table 1.

%B - percentage of festoon bees bearing wax
WR - amount of wax (μg/1000 bees) removed from 100 bees divided by the number of bees bearing wax in a festoon
SA - surface area (mm^2) of comb constructed /1000 bees
WE - weight (μg) of comb constructed /1000 bees
DIA - diameters (mm) of cells constructed
9ODA - the percentage amount of 9-oxo-2-decanoic acid
10HDA - the percentage amount of 10-hydroxy-2-decanoic acid
9HDA - the percentage amount of 9-hydroxy-2-decanoic acid

the mandibular gland secretions of the various queens. No secretions from the dead queen mandibular glands were measured. Although the colonies given a queen in a single cage had more acids in their mandibular gland secretions, this difference was not significant when compared to the other mandibular gland secretions (Tables 3 and 4). There were no significant differences in the percentage compositions of 9ODA, 9HDA and 1OHDA between the various queens (Table 4). No correlations between 9ODA, 9HDA and 1OHDA and the comb construction variables were found. This result indicates that naturally occurring variations in these queen pheromones plays a limited role in comb building (Chapter 2).

Discussion

It appears the queen has little influence over the control of wax secretion in honeybee colonies (Goetze and Bessling 1959, Hastings personnal comm and Chapter 2). Whether the queen was present or absent or whether the worker bees could touch and/or smell her did not affect the percentage of festoon bees bearing wax and the amount of wax borne by these bees. Wax secretion seems to occur independantly of the queen and must be influenced by other factor(s).

There is sufficient evidence showing that direct access to a living queen is essential for enhancing comb construction (Tables 2 and 4). Direct access to the queen without a pheromonal secretion (dead queen) takes preference to colonies given access to the odour of a mated queen but no direct contact (Table 4). However, this result is not altogether clear and it seems that the workers need to have direct access to the queen who is secreting pheromone(s) that stimulate and enhance comb building.

The diameters of the cells that were constructed are of a similar size to those measured by Anderson (1960) and Smith (1961). The colonies given a dead queen constructed significantly larger cells which were of an intermediate worker and drone size (Vogt 1911 and Owens and Taber 1973). These results suggest that direct contact with the queen does not determine the size of the cell that is constructed. It is not known whether the queen plays any role in determining the size of cell that is to be constructed (Free 1987 and Chapter 2).

The mean percentage composition of the major components of the queen mandibular gland secretions were slightly less than those found by Crewe (1982) and Chapter 2. The differences in the total number of acids may be attributed to loss during collection or seasonal variation (Free 1987). There were no statistical differences in the percentage compositions of 9ODA, 9HDA and 1OHDA between the various queens.

These pheromones are unlikely to enhance comb building independantly, but they may act synergistically with other pheromones (Chapter 2).

The part of the queen most frequently licked and palpated during retinue behaviour is the abdomen although the head is thought to be more attractive (Velthuis 1970 and Free 1987). The workers obtain queen pheromone(s) by touching the queen head or abdomen with their antennae during retinue behaviour (Seeley 1979 and Ferguson and Free 1980). Beetsma and Schoonhoven (1966) and Kaissling and Renner (1968) have shown that worker bees possess cells on their antannae that specifically react to the odours of the queen. These pheromone(s) is/are passed throughout the colony during worker-worker interactions (Allen 1955, Korst and Velthuis 1982 and Free 1987). This experiment shows that the workers need to have direct contact with their queen to obtain pheromone(s) that enhance comb building. Whether direct contact with the head, thorax or abdomen any combination of these is necessary for enhancing comb building has yet to be determined (Chapters 4, 5 and 6).

Contact chemoreception plays an important role in inhibiting worker ovarian development (Verheijen-Voogd 1959 and Velthuis 1970) and queen cell construction (Butler 1954 and 1960). In both instances, if the worker bees did not have direct contact with their queen, the

inhibition of queen cell construction and worker ovarian development was lost. Similarly, the workers needed to have direct contact with a mated queen for comb building to readily occur. It is possible that pheromone(s) picked up by the workers during retinue behaviour also enhance comb building. When the workers were not able to touch their queen, comb building was significantly reduced. Whether the workers need to have direct contact with a queen with/without mandibular and/or tergite glands was tested in the next experiment.

Chapter 4

An introduction: Do mandibular and/or tergite gland secretions of queen honeybees enhance comb building in *A.m.capensis* colonies ?

Summary

Wax secretion and comb construction were investigated in colonies given a queen with/without mandibular and/or tergite glands or a dead queen. The percentage of festoon bees bearing wax and the amount of wax borne by these bees was the same for each of the colonies. However, colonies given a queen without mandibular glands and tergite glands occluded or a dead queen constructed considerably less comb than colonies given a queen without mandibular glands but tergite glands intact or a queen with mandibular glands but tergite glands occluded. 9ODA, 9HDA and 10HDA from the queen heads were not correlated with the wax secretion or comb construction measurements. These results suggest that pheromone(s) from either the head or abdomen of the queen enhances comb building in honeybee colonies.

Introduction

Queenright and queenless *A.m.capensis* honeybee colonies secrete equal amounts of wax which is available for the construction of comb (Chapters 2 and 3). However, queenright colonies construct significantly more comb than the queenless colonies, colonies given a virgin queen, a queen in a single or double cage or a dead queen (Darchen 1956 and 1957, Rajashekharappa and Channabasavanna 1979 and Chapters 2 and 3). Worker bees need to have direct contact with their queen for comb building to proceed (Chapter 3). If the worker bees have limited contact with their queen (by caging her), then comb construction in these colonies is greatly reduced.

The worker bees obtain various pheromones from their queen during retinue behaviour (Seeley 1979, Ferguson and Free 1980 and Free 1987). Some of these pheromones are known to inhibit worker ovarian development and queen cell construction (Butler 1954 and 1956, de Groot and Voogd 1954, Verheijen-Voogd 1959, Pain 1961 and Free 1987). If the queen is confined so that the workers have limited access to her, these inhibitory responses are lost or reduced (Hess 1942, Butler 1954, de Groot and Voogd 1954, Verheijen-Voogd 1959). If the mandibular glands of the queen are removed, then queen cell construction and worker ovarian development are still inhibited (Gary and Morse 1962, Morse and Gary 1963, Velthuis 1970 and 1972). Clearly, secretions from the abdomen

(tergite and/or Koschevnikov glands) and head other than the mandibular glands (Gary and Morse, 1962) secrete these inhibitory pheromones. Whether these pheromones play a role in enhancing comb building in queenright colonies is unknown. Hence, wax secretion and comb construction were investigated in colonies given a queen with/without mandibular and/or tergite glands.

Methods and Materials

A. Experimental set-up and procedure

Thirty five-framed queenright *A.m.capensis* colonies were collected in early April 1988 from Kraaifontein near Cape Town and were placed in an apiary in Grahamstown. These bees were not disturbed for 3 weeks and were fed a 50% sugar solution (approximately 1 litre/colony) 3 times a week. A week before the start of the experiment, all of the colonies were examined and found to have a mated laying queen. During the experiment, the colonies were fed a 50 % sugar solution from various feeders in the apiary. The amount available to each hive was approximately 1 litre/colony/day.

On the 7 May, all the colonies were dequeened in the morning and the queens were taken back to the laboratory where they were placed in an incubator at 32^{o}C until the operation. The queens were anaesthetized on

ice for 2 minutes prior to the operations. Five queens were not operated on (but were anaesthetized) and had intact mandibular and tergite glands (control queens). Five queens had their mandibular glands removed using Gary's techinque (Gary 1961). There were 5 sham queens who had an incision made on each gena but did not have their mandibular glands removed. The tergite glands of 5 queens were occluded by painting several layers of nail varnish over the dorsal surface of the abdomen (Velthuis 1970). Five queens had their mandibular glands extirpated and tergite glands occluded. Three weeks prior to the experiment, 5 mated queens (with intact mandibular and tergite glands) that had been dead for a year were removed from the alcohol in which they were stored.

Ten hours after dequeening, each queen and 6 worker bees in a haircurler that had been sealed at either end with cork were placed back into the hive from which it came (Chapters 2 and 3). At the same time the frames in each colony were rearranged so that each had: 1 frame containing honey and pollen, 2 frames of eggs, larvae and sealed brood and 2 empty frames on which to construct comb. The following morning (8 May), the cork at one end of each haircurler was removed so that the queen was now able to move freely in the hive.

Six days later (13 May), 100 festoon bees and the newly constructed comb

were collected. Each colony was weighed so that an estimate of colony size could be obtained (Chapter 2). The next morning (14 May) all of the colonies were dequeened. The queens were decapitated and each head was placed in 1ml of dichloromethane and stored in a refridgerator at -20°C for gas chromatographic analysis.

B. Data collection and analysis

The percentage of festoon bees bearing wax was determined and the wax scales borne by these bees were removed and weighed. The surface area of the newly constructed comb was measured by photocopying the comb and then weighing the photocopied area of the comb (Chapter 2). The comb was then melted in a beaker of water to separate the honey and pollen from the wax (Chapter 2). The melted comb was then cooled and weighed. The diameter of 25 cells from the centre, left and right sides of the photocopied comb were measured with an ocular micrometer. The cell diameter was taken from the one mid-wall to the mid-wall on the opposite side of the cell (Hepburn 1983).

The gas chromatographic samples were analysed as mentioned in Chapter 2.

During the experiment 10 colonies absconded. These included: 3 colonies given a sham operated queen, 3 colonies given a dead queen, 2 colonies

given a queen without mandibular glands and tergite glands occluded, 1 control colony and 1 colony given a queen without mandibular glands but tergite glands intact. This reduced the sample sizes considerably and may account for some of the anomalous results obtained in this experiment.

Results

A. Wax secretion

The mean colony size, percentage of festoon bees bearing wax and the amount of wax borne by these bees under the different experimental conditions are shown in Table 1. (Appendix C.1 shows the raw data set.) No significant differences in the percentage of festoon bees bearing wax and the amount of wax secreted by these bees were found (Table 4). This supports the hypothesis that the queen plays no role in wax secretion (Chapter 2 and 3). Wax secretion seems to be a physiological process that occurs within the division of labour, much like the development of the hypopharangeal glands (Free 1987).

B. Comb building

The greatest amount of comb was constructed in colonies given a queen

Table 1. The mean ± (Standard Deviation) colony size, percentage of festoon bees bearing and the amount of wax borne by these bees under the different experimental conditions. *

Treatment	n	Colony size	% Bees bearing wax	Amount of wax secreted /bee (µg/1000 bees)
SH3	2	8159	47	433.5
		3295	1	35.6
MG3	4	9820	52	447.4
		2654	14	98.4
T3	5	6337	53	530.9
		5535	24	243.7
MQ3	4	10069	62	361.5
		6822	13	245.0
MT3	3	1152	80	526.5
		639	9	91.7
DEAD3	2	5537	53	226.7
		701	7	-

*
SH3 - colonies given a sham operated queen
MG3 - colonies given a queen without mandibular glands but tergite glands intact
T3 - colonies given a queen with mandibular glands but tergite glands occluded
MQ3 - colonies given a queen with intact mandibular and tergite glands
MT3 - colonies given a queen without mandibular glands and tergite glands occluded
DEAD3 - colonies given a dead queen
The numbers after each experimental condition refers to the experiment number.

without mandibular glands but tergite glands intact and a queen with mandibular glands but tergite glands occluded (Table 2). The colonies given a sham operated queen constructed more comb than the control colonies (Table 2). No comb was constructed in the colonies given a queen without mandibular glands and tergite glands occluded or a dead queen (Table 2).

When comparisons between these colonies for the surface area and weight of comb constructed were made, no significant differences were found; except that the colonies given a sham operated queen constructed significantly more comb than the colonies given a queen without mandibular glands and tergite glands occluded (Table 4). One would also have expected to have found a significant difference between the colonies given a sham operated queen and the colonies given a dead queen. However, the results in Table 4 show no such difference. This result is most likely to be the effect of the small sample sizes and the large amounts of variation (Appendix C.1). In fact, one would also have expected significant differences between the control colonies and each of the colonies given a queen lacking mandibular and/or tergite glands to have occurred. These results were somewhat anomalous and it was decided that the experiment should be repeated (Chapter 5).

Although the worker bees constructed larger cells in this experiment

Table 2. The mean ± (S.D.) surface area, weight and diameters of the cells of the comb constructed by worker honeybees under the different experimental conditions. *

Treatment	n	Surface area (mm^2/bee)	Weight (μg/bee)	Cell diameter (mm)
SH3	2	1.9 2.1	164.4 179.9	5.1 -
MG3	4	12.4 17.0	977.2 1465.0	5.2 0.3
T3	5	12.2 12.3	960.2 931.3	5.1 0.4
MQ3	4	0.9 1.8	17.8 35.6	5.1 -
MT3	3	0 -	0 -	- -
DEAD3	2	0 -	0 -	- -

*
The abbreviations for the experimental conditions are found in Table 1.

than in the previous experiments (Chapters 2 and 3, Appendix C.1), the cells were of a similar size to those constructed by other honeybee races (Smith 1958, Tribe and Fletcher 1976 and Ruttner 1986). The diameters of the cells constructed did not differ significantly between the various experimental conditions (Tables 2 and 4).

C. Pheromones

The mean percentage composition of the major components of the mandibular gland secretions of the various queens are shown in Table 3. Although the percentage amount of each pheromone is similar to that found by Crewe (1982), the total number of acids was considerably less than that found by Butler and Paton (1962), Pain et al. (1967) and Shearer et al. (1970). Seasonal variation in the secretion of these pheromones may have accounted for these differences (Free 1987).

Queens without mandibular glands but tergite glands intact had significantly more 9ODA on their heads than queens with mandibular glands but tergite glands occluded (Table 4). The converse was found when the percentage amounts of 9HDA were compared between these colonies (Table 4). The queens without mandibular glands and tergite glands occluded also had significantly less 9ODA than the queens with mandibular glands but tergite glands occluded (Table 4). No other differences in the percentage amounts of 9ODA, 9HDA and 10HDA

Table 3. The mean ± (S.D.) percentage composition of the major components of the mandibular gland secretions of the various queens. *

Queen	% Composition of the components present in the head				
	n	10HDA	9HDA	9ODA	Total acids (µg/head)
SH3	2	25.0	25.9	49.1	21.1
		23.6	17.0	40.5	7.1
MG3	4	16.1	11.0	72.9	17.8
		14.5	16.3	19.7	8.5
T3	5	14.1	48.7	37.2	48.9
		13.0	19.7	20.5	31.9
MT3	3	31.1	6.1	62.6	8.2
		43.9	10.5	54.3	6.3
MQ3	4	25.5	24.6	49.9	31.1
		28.9	21.6	37.8	25.3

*
The abbreviations for the different experimental conditions are shown in Table 1.

9ODA - the percentage amount of 9-oxo-2-decanoic acid
9HDA - the percentage amount of 9-hydroxy-2-decanoic acid
10HDA - the percentage amount of 10-hydroxy-2-decanoic acid

Table 4. A comparison of statistically significant differences between the different experimental conditions for wax secretion, comb construction and pheromone measurements. *

	%B	WR	SA	WE	DIA	9ODA	9HDA	1OHDA
SH3 vs MG3	-	-	-	-	-	-	-	-
SH3 vs T3	-	-	-	-	-	-	-	-
SH3 vs MQ3	-	-	-	-	-	-	-	-
SH3 vs MT3	-	-	+	+	-	-	-	-
SH3 vs DEAD3	-	-	-	-				
MG3 vs T3	-	-	-	-	-	+	+	-
MG3 vs MQ3	-	-	-	-	-	-	-	-
MG3 vs MT3	-	-	-	-	-	-	-	-
MG3 vs DEAD3	-	-	-	-				
T3 vs MQ3	-	-	-	-	-	-	-	-
T3 vs MT3	-	-	-	-	-	-	+	-
T3 vs DEAD3	-	-	-	-				
MQ3 vs MT3	-	-	-	-	-	-	-	-
MQ3 vs DEAD3	-	-	-	-				

* Kruskal-Wallis one way analysis of variance test. Significance is defined as $P < 0.05$. No significance and significance are given as - and + respectively. The abbreviations for the different

experimental conditions are shown in Table 1.

%B - percentage of festoon bees bearing wax
WR - the amount of wax (μg /1000) removed from 100 bees divided by the number of bees bearing wax in a festoon
SA - the surface area (mm^2) of comb constructed /1000 bees
WE - the weight (μg) of comb constructed /1000 bees
DIA - the diameter (mm) of the cells constructed
9ODA - the percentage amount of 9-oxo-2-decanoic acid
9HDA - the percentage amount of 9-hydroxy-2-decanoic acid
10HDA - the percentage amount of 10-hydroxy-2-decanoic acid

between the various queens were found. No correlations between any of the pheromones and the comb construction measurements were found.

Discussion

This experiment shows that a pheromone or pheromones from either the mandibular or tergite glands is/are necessary for enhancing comb building. Furthermore, these results are difficult to interpret as statistical analyses show that variation within each treatment was large (Tables 2 and 4 and Appendix C.1). Despite these somewhat anomalous results, a few interesting facts have come to light which may present themselves in a repeat of this experiment (Chapter 5).

The percentage of festoon bees bearing wax and the amount of wax available for the construction of comb in the various experimental colonies was the same (Tables 1 and 4 and Chapters 2 and 3). It seems that a queen plays no role in controlling wax secretion and that housebees will secrete wax regardless of intrinsic hive conditions.

Honeybee queens lacking mandibular and/or tergite glands and dead queens must obviously have reduced amounts of "queen pheromone(s)" present on their on their body surfaces than queens with intact mandibular and tergite glands (Tables 3 and 4 and Butler et al. 1973). This lack of

"queen substance" may have accounted for no comb being constructed in colonies given a queen without mandibular glands and tergite glands occluded or a dead queen (Table 2). These results suggest that the presence of either the mandibular and/or tergite glands in honeybee queens is necessary for enhancing comb building (Table 2).

However, this idea is not easily explained in this data set (Tables 2 and 4 and Appendix C.1). No significant differences for the comb construction measurements were found when the colonies given a queen with intact mandibular and tergite glands and a queen without mandibular glands but tergite glands intact or a queen with mandibular glands but tergite glands occluded or a queen without mandibular glands and tergite glands occluded were compared (Table 4). Furthermore, colonies given a queen without mandibular glands but tergite glands intact or a queen with mandibular glands but tergite glands occluded constructed similar amounts of comb as the colonies given a queen without mandibular glands and tergite glands occluded (Table 5). These results are difficult to explain especially since the control colonies constructed such small amounts of comb. The cooler weather conditions experienced in May 1988 as compared to May 1987 may have affected comb building.

Although larger cells that were constructed in this experiment (Table 2), they were within the upper range of those reported by Smith (1961)

and Tribe and Fletcher (1976). It is likely that a lack of "queen pheromone(s)" may have induced the workers to construct slightly larger cells. This idea will be tested in a repeat of this experiment (Chapter 5).

Although fewer fatty acids were present in the mandibular gland secretions (Table 3), no correlations between the amounts of 9ODA, 9HDA and 10HDA on the queen heads for the comb construction measurements were found. It is likely that two or more of these pheromones and/or others may be necessary for enhancing comb construction (cf. Butler 1959 and Pain 1961). Because of the dubious nature of these results, this possibility has not been explored.

Worker bees need to have direct contact with their queen for comb building to be enhanced (Chapter 3). This experiment suggests that the worker bees construct more comb when they have direct access to their queen which must possess either mandibular or tergite glands (Table 2). Because of the difficulties of this experiment, a repeat will be conducted and at the same time the bees will have direct access to either a head or an abdomen of a queen.

Chapter 5

Mandibular gland extirpation, tergite gland occlusion; the head or the abdomen of a mated queen - which of these enhances comb building ?

Summary

Colonies given a queen with/without mandibular and/or tergite glands, a queen head or abdomen or a dead queen secreted similar amounts of wax. However, colonies that had access to a whole queen or a queen head (with/without mandibular glands) constructed significantly more comb than the colonies having access to a queen abdomen (with/without tergite glands) or a dead queen (the whole queen, head or abdomen). Colonies given a queen with intact mandibular glands tended to construct more comb than those given a queen without mandibular glands. The colonies given access to the head of a queen (with/without mandibular glands) in a division board constructed similar amounts of comb to the colonies that had unrestricted access to their queen (with/without mandibular glands). The pheromones measured could not be correlated to any of the comb construction measurements. Whether increased or decreased

amounts of these pheromones were present, similar amounts of comb were constructed in the colonies having direct access to a queen head. Thus, comb construction in honeybee colonies is enhanced when the worker bees have direct access to the queen head whether the mandibular glands are present or not.

Introduction

Queenright honeybee colonies construct significantly more comb than queenless ones although both produce the same amount of wax/bee (Chapters 2, 3 and 4). Yet despite its significance in suppressing worker ovarian development and queen cell construction (Butler 1954 and Pain 1955), the pheromone 9ODA is not sufficient to account for enhanced comb building in queenright colonies (Chapters 2, 3 and 4), although it may act in concert with other phermone(s) in doing so (Slessor et al. 1988 and 1990). Moreover, queens whose mandibular glands have been surgically removed are just as effective in maintaining "normal" colony behaviours (such as maintaining these inhibitory responses) as queens with intact mandibular glands (Gary and Morse 1962, Velthuis and van Es 1964, Velthuis 1970 and Butler et al. 1974).

Worker bees must have direct contact with their queen for comb building to be enhanced (Chapter 3). When queenless colonies were

head and then the abdomen were most effective in maintaining these inhibitory responses of the queen (Butler 1954, Verheijen-Voogd 1959 and Free 1987). Since comb building is enhanced in colonies given direct contact to their queen (Chapter 3), the requirements for the suppression of the development of the worker ovaries and emergency queen cell construction invite experiments to pinpoint the source(s) of active material(s) related to comb building. Thus queens with or without mandibular glands and with or without tergite glands were tested for their ability to enhance comb building in *A.m.capensis* honeybee colonies. Furthermore, comb building in colonies given access to the head (with/without mandibular glands) or the abdomen (with/without tergite glands occluded) of the queen were also investigated.

Materials and methods

A. Experimental set-up and procedure

Forty-five five-framed queenright *A.m.capensis* colonies were placed in an apiary in Grahamstown in early November 1988. The queens were all mated and had been laying eggs for some time. During the first part of the experiment (20-26 December) the bees were housed in nucleus hives and were placed in pairs such that the entrances of each hive in the pair faced opposite directions (Fig 4). A week later (27 December - 2

Figure 4. Nucleus hives arranged in pairs with entrances facing opposite directions.

Figure 5. A Langstroth hive that has been divided by a division board so that a nucleus hive pair could be accommodated on one side. The other nucleus hive was accommodated on the other half of the division board.

January) the bees from each pair of nucleus hive were separately transferred into one half of a Langstroth hive that had been divided by a division board (Fig 5). The separate entrances of each half of the Langstroth hive faced opposite directions (matching that of the previously occupied nucleus hives) and each half had its own lid. Each division board had a small hole (5mm diameter) made in the centre about one third from the top of the board. Small pieces of wood were glued just below the hole so that the queen could set her feet down on one side and her abdomen on the other (Fig 6 and 7). The dead queens that were used had been stored in ethanol for about 9 months and were air-dried 2 weeks prior to the experiment.

The mandibular glands were extirpated using Gary's technique (Gary 1961), while the dorsal surface of the queen's abdomen was occluded by painting several layers of nail varnish onto the dorsal surface (Velthuis 1970). The queens were anaesthetized on ice for 2 minutes before any operations were performed (Chapter 4). Control queens with intact mandibular and tergite glands were also anaesthetized. For the division board part of the experiment, the queens were anaethetized on ice before being placed through a small hole in a piece of a surgical rubber glove. The queen's head, thorax and legs were on one side of the piece of the surgical glove while the abdomen was on the other. The queen in the piece of the

Figure 6. A queen being attended to by the worker bees in the division board.

Figure 7. The abdomen of a queen in the division board.

surgical glove was then placed through the hole in the division board (Fig 6 and 7). During each experiment the bees were fed a 40% sugar solution once a day from various feeders in the apiary. The bees had equal access to the food and the amount available to each hive was 1 litre/day.

On 20 December, all colonies were dequeened in the morning and 10 hours later the queens were placed back into their colonies of origin such that 9 colonies (4 paired colonies and 1 single colony) were each given their queen with: 1) intact mandibular and tergite glands, 2) mandibular glands extirpated but tergite glands intact, 3) mandibular glands intact but tergite glands occluded, 4) mandibular glands extirpated and tergite glands occluded and 5) a dead queen (with mandibular and tergite glands intact). Each queen (and a few workers) were introduced into each colony in a haircurler that had been sealed at either end with cork (Chapters 2, 3 and 4). The cork at one end of the haircurler was removed the following morning. At the same time that the queen introductions were made, the frames in each (nucleus) hive were distributed so that there were 2 honey and pollen frames, 1 frame of sealed brood and larvae and 2 empty frames for comb building.

Six days later (26 December), the empty frames now containing the newly constructed comb were collected from each colony. One hundred festoon bees were collected from each colony for wax scale recovery. The colonies were weighed so that an estimate of colony size could be obtained (Chapter 2).

The next morning (27 December), all of the colonies were dequeened once again. The queens were inserted into the division boards as previously mentioned. The queens in their division boards were each placed in a Langstroth hive 10 hours after dequeening. One pair of 4 sets of colonies each had access to a queen head with intact mandibular glands while each of the other colonies in the 4 pairs had access to the abdomen with intact tergite glands (on the other side of the division board). Similarly, one pair of another set of 4 colonies had access to a queen head without mandibular glands while each of the other colonies in the 4 pairs had access to the queen abdomen with intact tergite glands. In another set of 4 colony pairs, each had access to a queen head with intact mandibular glands while the other 4 colonies in the pair had access to the abdomen with occluded tergite glands. Similarly, one pair of another set of 4 colonies had access to a queen head without mandibular glands while each of the other colonies in the 4 pairs had access to the queen abdomen with occluded tergite glands. One colony in each of 3 pairs had access to a dead queen head while the others in the pairs each had access to the dead queen abdomen. Four colonies were each given a queen in a control division board (the division board only extended downwards one-third of the distance between the top and bottom of the hive) in a nucleus hive, so that the worker bees had access to both the head and abdomen of the queen. The frames were distributed so that each

colony (each half of the Langstroth hive) had 2 frames of pollen and honey, 1 frame of sealed brood and larvae and 1 empty frame on which to construct comb.

Six days later (2 January), the frames containing the newly constructed comb were collected. One hundred festoon bees were collected from each colony for wax scale recovery. The colonies were weighed so that an estimate of colony size could be obtained (Chapter 2). All the queens were captured and decapitated. Their heads were each placed in 1ml of dichloromethane and stored in a refrigerator at -20°C for gas chromatographic analysis.

B. Data collection and analysis

From the samples of festoon bees, the percentage of bees bearing wax was determined and the amount of wax borne by these bees was removed and weighed. The surface area of the newly constructed comb was calculated by measuring the surface area of photocopies the comb (Chapter 2). The comb was then melted over water to separate the wax from any honey and pollen (Chapter 2). The melted comb was then cooled and weighed. The surface area and weight of the comb were calculated in "per bee" terms to standardize the data. The cell type (worker or drone) and diameters of 25 cells from the centre, left and right sides of the photocopied comb were measured with an ocular

micrometer. The cell diameter was taken from the one mid-wall to the mid-wall on the opposite side of the cell (Hepburn 1983).

The gas chromatographic samples were analyzed as described in Chapter 2.

During the first week of the experiment, 2 colonies given a queen without mandibular glands but with intact tergite glands, 2 colonies given a queen with tergite glands occluded but with intact mandibular glands, 3 colonies given a queen without mandibular glands and tergite glands occluded and 5 colonies given a dead queen absconded. A queen from each treatment in the division board experiment (except for the dead queen colonies) died during the experiment. Data from all these colonies were excluded from the data set.

Results

A. Wax secretion

The colonies given a whole queen with intact mandibular and tergite glands and those having a queen with mandibular glands but tergite glands occluded had more festoon bees bearing wax than all the other colonies (Table 1). (Raw data is shown in Appendix D.1). The percentage of festoon bees bearing wax was significantly greater when the colonies given a queen with intact mandibular glands but

Table 1. The mean ± (Standard Deviation) percentage of festoon bees bearing wax under the various experimental conditions where the bees had access to the whole queen (head and abdomen) and only to the head or abdomen. *

Treatment	"Free moving" queens		Queens in division boards			
	n	Whole queen	n	Head of queen	n	Abdomen of queen
MQ4	9	68 16	3	57 15	3	61 6
MG4	7	62 11	3	53 13	3	65 6
T4	7	78 11	3	68 13	3	65 1
MT4	6	64 11	3	63 1	3	62 21
DEAD4	4	63 10	3	64 16	3	80 4
C04	3	64 6				

*
MQ4 - colonies given a queen with intact mandibular and tergite glands
MG4 - colonies given a queen without mandibular glands but tergite glands intact
T4 - colonies given a queen with mandibular glands but tergite glands occluded
MT4 - colonies given a queen without mandibular glands and tergite glands occluded
DEAD4 - colonies given a dead queen with intact mandibular and tergite glands
C04 - colonies given a queen with intact mandibular and tergite glands in a division board control
The number after the experimental condition indicates the experiment number.

tergite glands occluded were compared to colonies given a queen without mandibular glands and/or tergite glands occluded or a dead queen (Table 7). In all the other comparisons, no significant differences were found (Table 7). In the division board experiments the percentage of festoon bees bearing wax did not differ among colonies that had access to a whole queen, her head or abdomen (Tables 1 and 8). Only those colonies having access to a dead queen abdomen had significantly more bees bearing wax than the colonies given a division board control or colonies having access to the whole body of a dead queen (Tables 1 and 8).

Similar amounts of wax/bee were found in all the colonies given a whole queen (Tables 2 and 7). Interestingly, colonies given a whole queen or the head of a queen in a divsion board with intact mandibular glands (and/or tergite glands occluded) tended to have more wax/bee than those colonies given a queen (a whole queen or a queen in a division board) without mandibular glands (Table 2); however, these differences were not significant (Table 8). In the division board experiments, colonies having access to the head of a living queen with mandibular glands (with occluded tergite glands) had more wax/bee than those having access to the head of a dead queen (Tables 2 and 8). When all of the colonies given access to all of the queen heads in the division board were compared, a significant

Table 2. The mean ± (S.D.) weight of wax scales removed from the festoon bees under the various experimental conditions where the bees had access to a whole queen (head and abdomen) and only to the head or abdomen of the queen. *

Treatment	"Free moving" queens		Queens in division boards			
	n	Whole queen	n	Head of queen	n	Abdomen of queen
MQ4	9	357.4 213.2	3	553.5 245.3	3	278.3 151.3
MG4	7	273.0 97.1	3	241.7 113.7	3	381.5 190.4
T4	7	376.0 125.2	3	478.0 207.4	3	344.3 95.5
MT4	6	356.6 70.9	3	371.0 161.0	3	493.8 443.4
DEAD4	4	321.7 167.0	3	235.9 72.1	3	420.2 68.1
C04	3	360.9 178.6				

*
The abbreviations for the experimental conditions are shown in Table 1.

difference in the amount of wax/bee was found (Table 8). That the colonies having access to the head of a queen with mandibular glands bore more wax than colonies whose queen lacked mandibular glands or colonies given a dead queen may have accounted for these differences (Table 8). Festoon bees having access to the abdomen of a queen (whether tergite glands were present or not) had as much wax/bee as those having access to either the whole queen or to the head of a queen (Tables 2 and 8).

B. Comb building

The largest amount of comb was constructed when the colonies had access to a whole queen, particularly those with intact mandibular glands (Tables 3 and 4). Colonies having access to a live queen with mandibular and/or tergite glands constructed significantly more comb than those colonies given a dead queen or a queen without mandibular glands and/or tergite glands (Tables 3, 4, and 7). Although colonies given a queen with mandibular glands tended to construct more comb than colonies given a queen without mandibular glands, these differences were not always significant (Table 7).

Colonies having access to a queen abdomen with/without tergite glands

Table 3. The mean ± (S.D.) surface area (mm^2/bee) of comb constructed under the various experimental conditions where the worker bees had access to the whole queen (head and abdomen) and only to the head or abdomen of the queen. *

Treatment	"Free moving" queens		Queens in division boards			
	n	Whole queen	n	Head of queen	n	Abdomen of queen
MQ4	9	15.3 12.2	3	0.5 0.9	3	0
MG4	7	4.1 7.8	3	3.8 4.1	3	0
T4	7	12.4 9.3	3	13.6 13.3	3	0
MT4	6	10.3 17.2	3	8.3 14.4	3	0
DEAD4	4	1.6 3.5	3	0	3	0
CO4	3	4.2 3.5				

*
The abbreviations for the experimental conditions are given in Table 1.

Table 4. The mean ± (S.D.) weight (μg/bee) of the comb constructed under the various experimental conditions where the bees had access to the whole queen (head and abdomen) and only the head or abdomen of the queen. *

Treatment	"Free moving" queens		Queens in division boards			
	n	Whole queen	n	Head of queen	n	Abdomen of queen
MQ4	9	1303.9 1241.1	3	116.9 202.5	3	0
MG4	7	380.0 615.4	3	408.6 376.1	3	0
T4	7	1334.3 1111.8	3	1022.9 977.6	3	0
MT4	6	1235.5 1804.7	3	581.7 1007.5	3	0
DEAD4	4	264.1 590.5	3	0	3	0
CO4	3	439.3 359.9				

*
The abbreviations for the experimental conditions are given in Table 1.

never constructed comb (Tables 3 and 4). Similar amounts of comb were constructed by the colonies given a division board control and the colonies having access to the queen head (Table 8). However, the colonies having access to the abdomen with/without tergite always constructed significantly less comb than the colonies given a division board control (Table 8). The colonies given a division board control constructed significantly more comb than the colonies having access to the head or abdomen of a dead queen (Tables 3, 4 and 8).

Colonies having access to the head side of a queen with intact mandibular glands (and tergite glands) and a queen without mandibular glands (and tergite glands occluded) constructed less comb than when they had access to the same "whole" queen (head and abdomen); but these differences were not significant (Tables 3, 4 and 8). Colonies having access to the head side of a queen without mandibular glands (but tergite glands intact) and a queen with mandibular glands (but tergite glands occluded) constructed similar amounts of comb when these colonies had access to their whole queen (Tables 3, 4 and 8). Comb construction did not differ significantly among the colonies where the worker bees had access to their whole queen and then to the head of the same queen with/without mandibular glands (Tables 3, 4 and 8).

Significantly more comb was constructed when the colonies had access to the head of a queen with mandibular glands (and/or tergite glands) were compared to the abdomen sides of these queens (Table 8). Interestingly, no differences in the amount of comb constructed were found when the colonies having access to the queen head without mandibular glands (and/or tergite glands) were compared to the colonies having access to the abdomens of these queens (Table 8). No comb was constructed when the colonies had access to a dead queen, whether it was the whole queen, the head or abdomen (Table 8).

No significant differences were found when the colonies having access to a queen head with intact mandibular glands (with/without tergite glands occluded) were each compared to each of the colonies given access to a queen head without mandibular glands (with/without tergite glands occluded) and dead queens (Table 8). This comparison remained unchanged when the colonies given a dead queen were removed from the analysis (Table 8). There were no significant differences among the diameters of the cells that were constructed under the various experimental conditions (Tables 5, 7 and 8).

C. Pheromones

Table 5. The mean ± (S.D.) diameters of the cells constructed under the various experimental conditions where the worker bees had access to the whole queen (head and abdomen) and only the head or abdomen of the queen. *

Treatment	"Free moving" queens		Queens in division boards			
	n	Whole queen	n	Head of queen	n	Abdomen of queen
MQ4	9	4.9 0.1	3	0	3	0
MG4	7	5.1 0.1	3	5.1 0.4	3	0
T4	7	5.1 0.2	3	5.1 -	3	0
MT4	6	4.9 0.1	3	5.1 -	3	0
DEAD4	4	5.1 -	3	0	3	0
CO4	3	5.0 -				

*
The abbreviations of the experimental conditions are given in Table 1.

Table 6. The mean ± (S.D.) percentage compostion of the major components of the mandibular gland secretions of the queens from the various experimental conditions. *

Treatment	Percentage composition of the compounds measured					
	n	9ODA	9HDA	10HDA	10HHDA	Total acids (µg)
"Free queens"						
MQ4	9	68.6	22.7	6.4	2.3	149.9
		31.7	27.7	10.3	5.2	376.5
MG4	7	28.3	32.9	14.0	24.8	6.0
		20.0	33.7	15.7	28.8	7.1
T4	7	66.4	20.8	10.0	2.8	14.0
		30.7	19.3	17.0	3.9	13.7
MT4	6	78.3	3.5	11.1	7.1	0.1
		4.7	5.0	8.0	1.8	0.1
Queens in division boards						
CO4	3	76.3	22.7	1.0	0	374.6
		18.9	18.2	1.1		612.0
FH4	3	72.2	25.6	1.0	1.2	11.9
		48.2	44.6	1.4	2.3	18.2
MGH4	3	32.4	25.7	2.4	39.5	4.7
		29.1	44.6	4.2	33.0	6.5
TH4	3	83.4	8.8	2.4	5.4	9.6
		11.5	14.4	2.3	4.3	11.8
MTH4	3	70.6	8.9	15.8	4.7	1.7
		13.8	9.9	10.0	4.3	2.8
DEH4	3	30.5	5.4	13.2	50.9	1.4
		43.1	7.6	18.8	69.4	0.6

*

FH4 - colonies given access to a queen head with mandibular glands (tergite glands intact) in a division board

MGH4 - colonies given access to a queen head without mandibular glands (tergite glands intact) in a division board

TH4 - colonies given access to a queen head with mandibular glands (tergite glands occluded) in a division board

MTH4 - colonies given access to a queen head without mandibular glands (tergite glands occluded) in a division board

DEA4 - colonies given access to a dead queen head with mandibular glands (tergite glands intact) in a division board

The other experimental conditions are given in Table 1.

9ODA - the percentage amount of 9-oxo-2-decanoic acid

9HDA - the percentage amount of 9-hydroxy-2-decanoic acid

1OHDA - the percentage amount of 10-hydroxy-2-decanoic acid

1OHHDA - the percentage amount of 10-hydroxy-decanoic acid

Table 7. A comparison of statistical differences between the different experimental conditions for the wax secretion, comb construction and pheromone values for the colonies that were given a queen who "moved freely" across the comb. *

	%B	WR	SA	WE	DIA	9ODA	9HDA	10HDA	10HDAA
MQ4 vs MG4	-	-	-	-	-	+	-	-	-
MQ4 vs T4	-	-	-	-	-	-	-	-	-
MQ4 vs MT4	-	-	-	-	-	-	-	-	-
MQ4 vs DEAD4	-	-	+	+	-				
MG4 vs T4	+	-	-	-	-	-	-	-	+
MG4 vs MT4	-	-	-	-	-	+	-	-	-
MG4 vs DEAD4	-	-	-	-	-				
T4 VS MT4	+	-	-	-	-	-	-	-	-
T4 vs DEAD4	+	-	+	+	-				
MT4 vs DEAD4	-	-	-	-	-				
MQ4 vs MG4 vs T4 vs MT4	-	-	-	-	-	-	-	-	+
MQ4 vs T4 vs MG4	-	-	-	-	-	-	-	-	+
MQ4 vs T4 vs MT4	-	-	-	-	-	-	-	-	-
MQ4 vs T4 vs DEAD4	-	-	+	+	-	-	-	-	-
MQ4 vs MT4 vs DEAD4	-	-	+	+	-	-	-	-	-

	%B	WR	SA	WE	DIA	9ODA	9HDA	1OHDA	1OHDAA
MQ4 vs MG4 vs DEAD4	-	-	+	+	-	-	-	-	+
MQ4 & T4 vs MG4 & MT4	-	-	+	+	-	-	-	-	+
MQ4 & T4 vs MT4 & DEAD4	-	-	+	+	-	-	-	-	-
MQ4 & T4 vs MG4 & DEAD4	-	-	+	+	-	+	-	-	+
MQ4 & T4 vs MG4, MT4 & DEAD4	-	-	+	+	-	-	-	-	-

* Kruskal-Wallis one way analysis of variance test. Significance is defined as $P < 0.05$. No significance and significance are defined as - and + respectively.

The abbreviations for the experimental conditions are given in Table 1.

%B - percentage of festoon bees bearing wax
WR - amount of wax (μg/1000 bees) removed from 100 festoon bees divided by the number of festoon bees bearing wax
SA - surface area (mm^2) of comb constructed /1000 bees
WE - weight (μg) of comb constructed /1000 bees
DIA - diameter (mm) of cells constructed
9ODA - the percentage amount of 9-oxo-2-decanoic acid
9HDA - the percentage amount of 9-hydroxy-2-decanoic acid
1OHDA - the percentage amount of 10-hydroxy -2-decanoic acid
1OHHDA - the percentage amount of 10-hydroxy-decanoic acid

Table 8. A comparison of statistically significant differences between the different experimental conditions for the wax secretion, comb building and pheromone values when the bees had access to a queen in a division board. *

	%B	WR	SA	WE	DIA	9ODA	9HDA	10HDA	10HHDA
CO4 vs FH4	-	-	-	-		-	-	-	-
CO4 vs FA4	-	-	+	+					
CO4 vs MGH4	-	-	-	-	-	-	-	-	+
CO4 vs MGA4	-	-	+	+					
CO4 vs TH4	-	-	-	-	-	-	-	-	-
CO4 vs TA4	-	-	+	+					
CO4 vs MTH4	-	-	-	-	-	-	-	+	-
CO4 vs MTA4	-	-	+	+					
CO4 vs DEH4	-	-	+	+					
CO4 vs DEA4	+	-	+	+					
FH4, MGH4, TH4, MTH4 & DEH4 all compared	-	-	-	-	-	-	-	-	-
FH4, MGH4, TH4 & MTH4 compared	-	-	-	-	-	-	-	-	-
FH4, MGH4, TH4 & MTH4 vs MQ4, MG4,T4 & MT4 all compared	-	-	-	-	-	-	-	-	-

	%B	WR	SA	WE	DIA	9ODA	9HDA	1OHDA	1OHHDA
FA4, MGA4, TA4, MTA4 & DEA4 all compared	-	-	-	-		-	-	-	-
MQ4 vs FH4 vs FA4	-	-	+	+					
MQ4 vs FH4	-	-	-	-		-	-	-	-
MQ4 vs FA4	-	-	+	+					
MG4 vs MGH4 vs MGA4	-	-	-	-	-				
MG4 vs MGH4	-	-	-	-	-	-	-	-	-
MG4 VS MGA4	-	-	-	-					
T4 vs TH4 vs TA4	-	-	-	-	-				
T4 vs TH4	-	-	-	-	-	-	-	-	-
T4 vs TA4	-	-	+	+					
MT4 vs MTH4 vs MTA4	-	-	-	-	-				
MT4 vs MTH4	-	-	-	-	-	-	-	-	-
MT4 vs MTA4	-	-	-	-					
DEAD4 vs DEH4 vs DEA4	-	-	-	-					
DEAD4 vs DEH4	-	-	-	-	-	-	-	-	-
DEAD4 vs DEA4	+	-	-	-					
FH4 vs MGH4 vs TH4	-	-	-	-		-	-	-	+
FH4 vs TH4	-	-	-	-		-	-	-	-
FH4 vs MTH4	-	-	-	-		-	-	+	-

	%B	WR	SA	WE	DIA	9ODA	9HDA	1OHDA	1OHHDA
FH4 vs DEH4	-	-	-	-		-	-	-	-
MGH4 vs TH4	-	-	-	-	-	+	-	-	-
MGH4 vs MTH4	-	-	-	-	-	-	-	-	+
MGH4 vs DEH4	-	-	-	-		-	-	-	-
TH4 vs MTH4	-	-	-	-	-	-	-	+	-
TH4 vs DEH4	-	+	-	-		-	-	-	-
MTH4 vs DEH4	-	-	-	-		-	-	-	-
FH4 vs TH4 vs MGH4	-	-	-	-	-	-	-	-	-
FH4 vs TH4 vs MTH4	-	-	-	-	-	-	-	+	-
FH4 vs TH4 vs DEH4	-	-	-	-		-	-	-	-
FH4 vs MTH4 vs DEH4	-	-	-	-		-	-	-	-
FH4 vs MGH4 vs DEH4	-	-	-	-	-	-	-	-	-
TH4 vs MTH4 vs DEH4	-	-	-	-	-	-	-	-	-
TH4 vs MGH4 vs DEH4	-	-	-	-	-	-	-	-	-
FH4 & TH4 vs MGH4 & MTH4	-	-	-	-	-	-	-	-	-
FH4 & TH4 vs MGH4 & DEH4	-	-	-	-		-	-	+	-

	%B	WR	SA	WE	DIA	9ODA	9HDA	1OHDA	1OHHDA
FH4 & TH4 vs MTH4 & DEH4	-	+	-	-		+	-	-	+
FH4 & TH4 vs MGH4, MTH4 & DEH4	-	+	-	-	-	-	-	-	-

* Kruskal-Wallis one way analysis of variance test. Significance is defined as $P < 0.05$. No significance and significance are denoted by - and + respectively.

CO4 - colonies given a queen in a control division board (mandibular and tergite glands intact)
FH4 - colonies given a queen head with intact mandibular glands (tergite glands intact) in a division board
FA4 - colonies given a queen abdomen with intact tergite glands (mandibular glands intact) in a division board
MGH4 - colonies given a queen head without mandibular glands (tergite glands intact) in a division board
MGA4 - colonies given a queen abdomen with intact tergite glands (mandibular glands extirpated) in a division board
TH4 - colonies given a queen head with mandibular glands (tergite glands occluded) in a division board
TA4 - colonies given a queen abdomen with tergite glands occluded (mandibular glands intact) in a division board
MTH4 - colonies given a queen head without mandibular glands (tergite glands occluded) in a division board
MTA4 - colonies given a queen abdomen with tergite glands occluded (mandibular glands extirpated) in a division board
DEH4 - colonies given a dead queen head with mandibular glands (tergite glands intact) in a division board
DEA4 - colonies given a dead queen abdomen with tergite glands (mandibular glands intact) in a division board
The number after each experimental condition refers to the experiment number.

%B - percentage of festoon bees bearing wax
WR - amount of wax (μg/1000 bees) removed from 100 festoon bees divided by divided by the number of festoon bees bearing wax
SA - surface area (mm^2) of comb constructed /1000 bees
WE - weight (μg) of comb constructed /1000 bees

DIA - diameter (mm) of cells constructed
9ODA - percentage amount of 9-oxo-2-decanoic acid
9HDA - percentage amount of 9-hydroxy-2-decanoic acid
10HDA - percentage amount of 10-hydroxy-2-decanoic acid
10HHDA - percentage amount of 10-hydroxy-decanoic acid

Table 6 shows the percentage amounts of 9ODA, 9HDA, 1OHDA and 1OHDAA measured from the heads of the queens. Queens with intact mandibular glands tended to have more 9ODA than queens from which the mandibular glands had been extirpated (Table 6). However, when statistically compared, these differences were not always significant (Table 7). The whole queens and division board control queens with intact mandibular and tergite glands had more total acids than queens with either or both mandibular and tergite glands missing (Table 6). Although the queens lacking both mandibular and tergite glands tended to have a relatively high percentage of 9ODA than queens with intact glands, but the individual titres are actually quite small in terms of total acids recovered (Table 6). The amounts 9HDA and 1OHDA did not differ significantly among the various queens.

In the division board experiments, queens with intact mandibular glands also tended to have more 9ODA on their heads than queens with extirpated mandibular glands (Table 6). The queens in the division board without mandibular glands (and tergite glands) had significantly larger amounts of 1OHDA on their heads than the division board queens with mandibular glands (and tergite glands) and queens without mandibular glands (but tergite glands intact) (Tables 6 and 8). The amount of 9HDA did not differ between any of the queens (Tables 6 and 8). The only time that 9ODA differed

significantly between the queens was when the queens with intact mandibular glands with/without tergite glands were compared to the queens without mandibular glands and the dead queens (Tables 6 and 8). No correlations between any of the pheromones and the comb construction measurements were found.

Discussion

That the queen has little influence over the secretion of wax (Chapters 2, 3 and 4) is further supported by this experiment. Festoon bees bore more or less equal amounts of wax whether they had access to the whole queen or only part of the queen (head or abdomen) (Tables 1,2, 7 and 8). Similarly, mandibular gland extirpation or tergite gland occlusion did not affect this process. Most of the colonies given access to queens lacking functional tergite glands had a greater percentage of festoon bees bearing wax (Tables 2, 7 and 8). These colonies absconded soon after the experiment. Because bees tend to have more wax on hand prior to absconding than do settled bees (Hepburn 1988) and because the percentage of bees bearing wax is higher in swarms than in settled bees (Hepburn and Whiffler 1988), this may have accounted for the increased percentage of festoon bees bearing wax in these colonies.

Colonies that had access to a living queen (with/without mandibular

and/or tergite glands) always constructed more comb than colonies given a dead queen (Tables 3 and 4, Verheijen-Voogd 1959). No significant differences in the various permutations of the colonies given a queen with/without mandibular and/or tergite glands were found (Table 7). But, significantly more comb was constructed when all the colonies given access to whole queens with intact mandibular glands (tergite glands present/absent) were compared to all the colonies given access to the whole queens without mandibular glands (tergite glands present/absent) or dead queens (Table 7). This suggests that the presence of mandibular glands in living queens is necessary for comb building to be enhanced.

However, that the mandibular glands are necessary for enhancing comb building was not completely supported by the division board experiment (Table 8). Here, worker bees only having access to a living queen head with or without mandibular glands constructed similar amounts of comb (Tables 3 and 4). No significant differences in the amounts comb constructed were found when colonies had access to the head or the whole queen (Tables 3, 4 and 8). Thus, comb building in colonies given queens with intact mandibular glands may in fact be a property of the head and not of the mandibular glands as such. This interpretation would be consistent with the observation that colonies given queens without mandibular glands were still able to maintain "normal" queenright colony behaviours (Gary and Morse

1962, Velthuis and van Es 1964, Velthuis 1970, Butler et al. 1974 and Free 1987).

It may be that the same pheromones responsible for maintaining "normal" colony behaviours in colonies given a queen lacking mandibular glands also enhance comb building. It is unlikely that the queen abdomen contributes to enhancing comb building because no comb was constructed when the bees had access to the abdomens of queens offered in the various permutations (Tables 3 and 4, Verheijen-Voogd 1959 and Darchen 1968). The lack of any differences in cell sizes across the full spectrum of results suggests that the various queen conditions employed here do not directly influence this aspect of comb building (Chapters 2 and 3).

The amounts of 9ODA, 9HDA, 1OHDA and 1OHHDA recovered from the queens are comparable to those previously reported for the Cape honeybee (Crewe 1982). It is unlikely that these pheromones enhance comb building as more comb should have then been constructed by colonies given a queen with intact mandibular glands than those whose given a queen without mandibular glands (Table 8). That there were no differences in comb building between the colonies given a queen with/without mandibular glands and the lack of correlations between the pheromones and comb construction variables further suggests that any one of these pheromones are unlikely to enhance comb

building (Chapters 2, 3 and 4).

It is possible that the secretions of other glands on the head of a queen may enhance comb building. Since colonies given a queen with mandibular glands tended to construct more comb than colonies given a queen without mandibular glands, mandibular gland pheromones may also act together with other glandular secretions to enhance comb building. A queen with extirpated mandibular glands can still inhibit emergency queen cell construction (Gary and Morse 1962), worker ovarian development (Velthuis 1970) and promote comb building (Tables 7 and 8 and Chapter 4). That 9ODA from the mandibular glands is responsible for these behaviours is unlikely, but a complex system probably involving 9ODA and other glandular secretions (from the head or legs ?) is likely (Slessor et al. 1988 and 1990). It would seem that "Specificity is the blend" (Blum 1985).

Chapter 6

Do virgin queens influence wax secretion and comb building in honeybee colonies ?

Summary

Worker bees in queenless colonies, colonies given a mated or virgin queen (with/without mandibular and/or tergite glands), a dead virgin queen or a virgin queen in a division board secreted equal amounts of wax. However, the colonies given a mated queen always constructed significantly more comb than the other colonies. No differences in the amounts of comb constructed by colonies given a virgin queen (with/without mandibular and/or tergite glands), or a dead virgin queen, or a queen in a division board or no queen were found. Although colonies given a virgin queen in summer tended to construct more comb than in autumn, these differences were not always significant. The amounts of 9ODA, 9HDA and 1OHDA extracted from the heads of the mated and virgin queens did not differ. These pheromones could not account for the enhanced comb building in colonies given a mated queen.

Introduction

The presence or absence of the queen in a honeybee colony does not affect the amount of wax secreted by the worker bees (Chapters 2-5). Wax secretion seems to be a physiological process that occurs independently of the queen. Comb building on the other hand is greatly influenced by the presence of a queen. Queenright colonies always construct more comb than queenless ones (Chapters 2-5) and comb building is enhanced when worker bees have direct physical contact with their queen (Chapter 3). In fact, the worker bees need to have direct contact with the head of the queen for comb building to be enhanced (Chapter 5). Similar amounts of comb are constructed in colonies given queens with or without mandibular glands (Chapter 5).

Virgin queens do not stimulate comb building (Chapter 2, Verheijen-Voogd 1959 and Rajashekharappa and Channabasavanna 1979). The reduced amounts of pheromones present in their mandibular glands *vis-a-vis* those of mated queens (Crewe 1982) have been hypothesized as factor(s) responsible for this reduced comb building activity (Hepburn 1986). Since *A.m.capensis* virgin queens secrete similar amounts of 9ODA, 9HDA and 10HDA as do mated *A.m.capensis* queens (Crewe 1988); virgin queens with/without mandibular and/or tergite glands were tested to see if these queens could stimulate comb building. Virgin queens were also

placed in a division board to see if a virgin queen head could stimulate comb building.

Methods and Materials

A. Experimental set-up and procedure

Forty-five and fifty five-framed nucleus hives containing queenright *A.m.capensis* colonies were placed in an apiary in Grahamstown in March 1989 (experiment 1) and in November 1989 (experiment 2), respectively. The queens were of an unknown age, but they had been mated and were observed to be egg laying. A queen excluder was fixed at the entrance of each nucleus hive to prevent the virgin queens from mating.

Two weeks prior to the experiments, 8 Langstroth colonies from another apiary were dequeened. Ten days later, the frames containing the newly constructed queen cells were collected and placed in an incubator at 34°C in the laboratory. The incubator was cleared every 6 hours and any queens that had emerged were placed in queen cages with a few worker bees and food (queen candy and water).

Experiment 1 began by dequeening 15 nucleus hives/day on 3 consecutive mornings (26-28 April). The queens were brought back to the laboratory where they were placed in queen cages with a few

worker bees in an incubator at 34°C. All of the queens that were to be used in the experiment on that day were each anaesthetized on ice for 2 minutes (Chapters 4 and 5). The mandibular glands were extirpated (Gary 1961) and the tergite glands were occluded (Velthuis 1970) during this time. Each queen and a few worker bees were then placed in a haircurler that had been sealed with cork at either end. The newly emerged (virgin) queens that were used in the experiments were not more than 4 days old when they were introduced into the colonies. The dead virgin queens were queens that died (naturally) shortly after emergence.

Ten hours after dequeening, 3 mated queens with intact mandibular and tergite glands, 2 virgin queens with intact mandibular and tergite glands, 2 virgin queens without mandibular glands but with tergite glands intact, 2 virgin queens with mandibular glands but tergite glands occluded, 2 virgin queens without mandibular glands and tergite glands occluded and 2 dead virgin queens with intact mandibular and tergite glands were each introduced into a colony (in a haircurler) that had been dequeened on that day (day 1, 26 April). Similarly, on day 2 (27 April), 2 mated queens with intact mandibular and tergite glands, 3 virgin queens with intact mandibular and tergite glands, 2 virgin queens without mandibular glands but tergite glands intact, 2 virgin queens with mandibular glands but tergite glands occluded, 2 virgin queens without mandibular

glands and tergite glands occluded and 2 dead virgin queens were each introduced (in a haircurler) into a colony (10 hours after dequeening) that had been dequeened that day. On day 3 (28 April), 1 mated queen with intact mandibular and tergite glands, 3 virgin queens with intact mandibular and tergite glands, 2 virgin queens without mandibular glands but tergite glands intact, 2 virgin queens with mandibular glands but tergite glands occluded, 2 virgin queens without mandibular glands and tergite glands occluded and 4 dead virgin queens with intact mandibular and tergite glands were each placed (10 hours after dequeening) in a colony that had been dequeened that day. Two colonies were queenless on the first two days, while only 1 colony was queenless on day 3.

In experiment 2, 8 colonies were arranged in pairs so that the entrances of each hive in the pair faced opposite directions (Chapter 5 and Fig 4). These pairs of colonies were used for the division board part of the experiment. In this experiment, 10 colonies/day were dequeened on 5 consecutive days (31 December, 1-4 January). The queens were brought back to the laboratory where they were placed in queen cages with a few worker bees in an incubator at 34^{o}C. In the laboratory, all the queens that were to be used in the experiment on that day were anaesthetized on ice for 2 minutes. Mandibular extirpation and tergite gland occlusion techniques were

performed. Each queen with a few worker bees was placed in a haircurler that had been sealed with cork at either end. The 4 virgin queens used in the division board experiments were placed through a small hole in a piece of surgical rubber glove. The queen head, thorax, legs and wings were on one side of the rubber membrane while the abdomen was on the other (Chapter 5 and Figs 6 and 7). The queen was then placed into a division board that had a 5mm diameter hole about a third of the way from the top of the board. The dead virgin queens were queens that died (naturally) shortly after emergence.

Ten hours after dequeening on day 1 (31 December), 2 mated queens with intact mandibular and tergite glands, 2 virgin queens with intact mandibular and tergite glands, 2 virgin queens without mandibular glands but tergite glands intact, 2 virgin queens with mandibular glands but tergite glands occluded and 2 virgin queens without mandibular glands and tergite glands occluded were each placed (in a haircurler) in a colony that had been dequeened on that day. Similarly, 10 hours after after dequeening on days 2, 3, 4, and 5 (1 January, 2 January, 3 January and 4 January) a queen was placed (in a haircurler) in a colony that had been dequeened that day. The following queens were each placed in a colony: 1) Day 2, 2 mated queens with intact mandibular and tergite glands, 2 virgin queens with intact mandibular and tergite glands, 2 dead virgin queens with intact

mandibular and tergite glands, 1 division board queen with intact mandibular and tergite glands and 2 colonies remained queenless 2) Day 3, 2 virgin queens without mandibular glands but tergite glands intact, 2 virgin queens with mandibular glands but tergite glands occluded, 2 virgin queens without mandibular glands and tergite glands occluded, 2 dead virgin queens with intact mandibular and tergite glands and 1 division board queen with intact mandibular and tergite glands 3) Day 4, 2 mated queens with intact mandibular and tergite glands, 2 virgin queens with intact mandibular and tergite glands, 2 virgin queens without mandibular glands but tergite glands intact, 2 virgin queens with mandibular glands but tergite gland occluded and 2 colonies remained queenless 4) Day 5, 2 virgin queens without mandibular glands and tergite glands occluded, 2 dead virgin queens with intact mandibular and tergite glands, 2 division board queens with intact mandibular and tergite glands and 2 colonies remained queenless.

The paired colonies that were given a queen in the division board were transfered into a Langstroth hive (Chapter 5). The division board divided the hive and each half had its own entrance (facing opposite directions, Fig 7) and lid. The bees on one side of the division board had access to the queen head/thorax, while the bees on the other side had access to the abdomen. There was no access or communication between the 2 divided colonies.

In both experiments, after a queen had been placed in a hive, the frames were rearranged so that each hive had 1 frame of brood (eggs, larvae and sealed brood), 2 frames of honey and pollen and 2 empty frames on which to construct comb. The division board colonies were only given 1 empty frame for comb building. The queens were released the morning after introduction by removing the cork from one end of the haircurler. During the experiments, the bees were fed daily and were given a 40 % sugar solution in open feeders throughout the apiary. The bees had equal access to the food and the amount available to each colony was 1 litre/day.

Six days after the queens had been introduced into the colonies (eg. in experiment 1, day 1, 6 days later it is 3 May; in experiment 2, day 1, 6 days later it is 7 January etc.), the frames of newly constructed comb were removed and a sample of 50 bees were collected from the festoons in each colony. All of the frames were taken out of each colony and the hive with bees was weighed. An estimate of colony size was then calculated by subtracting the empty hive weight from the weight of the hive and bees (Chapter 2). The colonies were dequeened the next morning. The queens were decapitated and each head was placed in 1ml of dichloromethane and stored in a refridergator at -20^{o}C for gas chromatographic analysis.

B. Data collection and analysis

The samples of festoon bees were frozen soon after collection. The percentage of bees bearing wax was recorded and the wax borne by these bees was removed and weighed. The surface area of the newly constructed comb was measured by calculating the surface areas of photocopies of the comb (Chapter 2). The comb was melted over water for wax separation (Chapter 2). The melted comb was then cooled and weighed. The amount of comb constructed was calculated from per bee measurments to standardize the data in terms of colony size. The diameters of 25 cells (worker and drone) were measured with an ocular micrometer from the centre, left and right sides of the photocopied comb. A cell diameter measurement was taken from the one mid-wall to the mid-wall on the opposite side of the cell (Hepburn 1983).

The gas chromatographic samples were analyzed as described in Chapter 2.

In experiment 1, 3 colonies with mated queens, 1 colony with a virgin queen without mandibular glands but tergite glands intact and 3 colonies with virgin queens with mandibular glands but tergite glands occluded absconded, leaving their queens behind. Two virgin queens without mandibular glands but tergite glands intact, 3 virgin queens with mandibular glands but tergite glands occluded and 3 virgin queens without mandibular glands and tergite glands occluded

were found dead in the haircurlers the morning after they were introduced. These colonies were now redesignated as having been given dead queens.

In experiment 2, 1 colony with a virgin queen without mandibular glands but tergite glands intact, 2 colonies with virgin queens with mandibular glands but tergite glands occluded and 4 queenless colonies absconded. Once again, all these swarms absonded leaving their queens behind. One virgin queen with mandibular glands but tergite glands occluded and 6 queens without mandibular glands and tergite glands occluded died on introduction into the colony. These colonies were redesignated as having been given dead virgin queens.

Results

A. Wax secretion

In experiment 1, the percentages of festoon bees bearing wax and the amount of wax borne by these bees were not significantly different between the various treatments with one exception (Tables 1 and 4 and Appendix E.1). Festoon bees in colonies given virgin queens with intact mandibular and tergite glands and colonies given virgin queens without mandibular glands but tergite glands intact had more wax/bee than the festoon bees from the colonies given a dead virgin queen (Table 4), but

Table 1. The mean ± (Standard Deviation) colony size, percentage of festoon bees bearing wax scales and the weights of the scales borne by these bees for the various treatments. *

	Experiment 1				Experiment 2			
Treatment	n	CS	%B	WR	n	CS	%B	WR
Mated queen with intact mandibular and tergite glands	3	8693 2607	68 4.2	292.9 157.5	6	10909 4103	70 18.8	426.4 130.1
Virgin queen with intact mandibular and tergite glands	8	7089 5364	78 3.7	441.7 87.8	6	9221 5316	80 10.2	378.9 98.9
Virgin queen without mandibular glands but with tergite glands	3	6512 3945	76 5.3	446.1 123.5	5	7574 3619	81 13.9	480.3 88.6
Virgin queen with mandibular glands but tergite glands occluded					3	5489 3631	81 9.5	391.8 135.6
Virgin queen without mandibular glands and tergite glands occluded	3	9443 8100	-	-				
Dead virgin queen	16	6192 3574	75.2 12.8	258.8 92.2	13	8479 3902	70.5 4.7	298.4 50.1
Queenless colonies	5	7514 3575	68.3 22	277.9 106	2	10541 8512	70 -	351.4 -
Virgin queen in a division board, access to the head with mandibular glands					4	3379 2823	73 24	2540 44.9

	n	CS	%B	WR	n	CS	%B	WR
Virgin queen in a division board, access to the abdomen with tergite glands					4	3777 -	64 -	493.8 -

* n is the number of colonies used in each treatment
- no festoons present on the empty frames at collection

CS - colony size (the number of workers)
%B - the percentage bees bearing wax in a festoon
WR - the weight of the wax scales removed from 50 bees divided by the number of bees bearing wax (μg/1000 bees)

the percentage of bees bearing wax in these festoons was the same.

A similar result was found in experiment 2. The festoon bees from the colonies given a virgin queen without mandibular glands but tergite glands intact had significantly more wax/bee than the colonies given a dead virgin queen (Tables 1 and 5 and Appendix E.2). The colonies given a virgin queen with mandibular glands but tergite glands occluded had significantly more bees bearing wax than the colonies given a dead virgin queen (Tables 1 and 5). There were no significant differences between the other treatments (Table 5). No seasonal differences (comparisons between experiments 1 and 2) for the percentage of bees bearing wax and the amount of wax/bee were found (Table 6). This suggests that the amount of wax/bee available for the construction of comb in these 2 experiments was more or less the same (Chapters 2-5).

B. Comb building

In experiment 1, the amount of comb constructed by the colonies with mated queens was significantly greater than that constructed by all the other colonies (Tables 2 and 4 and Appendix E.1). The colonies given virgin queens with intact mandibular and tergite glands constructed more comb than those colonies whose virgin queens lacked mandibular glands but whose tergite glands were intact or colonies given a virgin queen

Table 2. The mean ± (S.D.) surface area, weight and cell diameters of the cells of the comb constructed by the worker honeybees for the various treatments. *

	Experiment 1				Experiment 2		
Treatment	n	SA	WE	DIA	n SA	WE	DIA
Mated queen with intact mandibular and tergite glands	3	12.7 9.8	962.3 541.4	5.0 0.1	6 49.2 39.5	3946.6 2752.6	4.6 0.2
Virgin queen with intact mandibular and tergite glands	8	3.2 6.9	241.4 510.8	4.9 0.1	6 17.3 18.0	1653.4 1659.6	4.6 0.1
Virgin queen without mandibular glands but tergite glands are intact	3	0 -	0 -	-	5 32.5 44.1	3210.8 4645.9	4.6 0.1
Virgin queen with mandibular glands but tergite glands occluded					3 7.4 12.9	958.0 1659.3	4.7 -
Virgin queen without mandibular glands and tergite glands occluded	3	0 -	0 -	-			
Dead virgin queen	16	0.1 0.5	11.4 45.6	5.1 -	13 3.9 8.5	290.6 665.4	5.5 0.9
Queenless colonies	5	0 -	0 -	-	2 0.5 0.6	33.2 46.9	-
Virgin queen in a division board, access to the head with mandibular glands					4 0 -	0 -	-

	n	SA	WE	DIA	n	SA	WE	DIA
Virgin queen in a division board, access to the abdomen with tergite glands					4	0 -	0 -	-

* n is the number of colonies in each treatment
- did not measure as no or very little comb was constructed

SA - the surface area (mm^2) of comb constructed per 1000 bees
WE - the weight (μg) of comb constructed per 1000 bees
DIA - cell diameter (mm) of the constructed comb

without mandibular glands and tergite glands occluded (Table 2); however, these differences were not significant (Table 4). Colonies given dead virgin queens constructed a little comb, but this did not differ from that of queenless colonies or those virgin queens (with/without mandibular and/or tergite glands) (Tables 2 and 4).

In experiment 2, all of the colonies constructed comb except those only having access to the head/thorax or abdomen of a virgin queen (Table 2). The amounts of comb constructed by colonies with mated queens did not differ from that of colonies given virgin queens (with intact mandibular and tergite glands; without mandibular glands but with tergite glands intact and without mandibular glands and tergite glands occluded) (Tables 2 and 5). The queenless colonies, those with dead virgin queens and colonies having access to either the head/thorax or abdomen of a virgin queen constructed significantly less comb than those given mated queens (Table 5). Colonies given virgin queens with intact mandibular and tergite glands constructed similar amounts of comb as did those given access to a virgin queen without mandibular glands but tergite glands intact, a virgin queen with intact mandibular glands but tergite glands occluded and queenless colonies (Table 5).

The colonies given a dead virgin queen or a virgin queen in a division board (the bees having access to the head/thorax or abdomen) constructed

significantly less comb than the colonies given a virgin queen with intact mandibular and tergite glands (Table 5). Interestingly, no significant differences were found when the colonies given a virgin queen without mandibular glands but tergite glands intact, a virgin queen with mandibular glands but tergite glands occluded, a dead virgin queen, a virgin queen in a division board (access to the head/thorax or abdomen) or queenless colonies were compared (Table 5). This suggests that colonies headed by a virgin queen with intact mandibular and tergite glands construct more comb than colonies given a virgin queen with/without mandibular and/or tergite glands. However, these differences were not significant (Table 5). Furthermore, when all the colonies given a virgin queen were compared to the queenless colonies and colonies given a dead virgin queen, no significant differences were found (Table 5). This result is similar to that of experiment 1.

Initially there appear to be large differences in the amounts of comb constructed by the colonies with virgin queens in experiments 1 and 2 (Table 2). Although colonies given a virgin queen (with intact mandibular and tergite glands) construct more comb in summer than in autumn, this difference is not significant (Table 6). The colonies given a dead virgin queen constructed significantly more comb in summer than in autumn (Table 6). No differences in the amount of comb constructed were found between the queenless colonies, the

colonies given a virgin queen without mandibular glands but tergite glands intact and colonies given a mated queen (Table 6). Since season could affect comb building in some instances (colonies given a virgin queen with intact mandibular and tergite glands and colonies given a dead virgin queen), no further comparisons between the experiments were made.

The diameters of the cells that were constructed in experiment 1 were similar in size to those constructed in other experiments (Chapters 3-5). No significant differences were found between the treatments (Table 4). In experiment 2, the cell diameters were slightly smaller than those constructed in experiment 1 (Table 2, Anderson 1960 and Garin 1926). No significant differences were found between the colonies even though the colonies given a dead virgin queen constructed lightly larger cells (Tables 2 and 5). When comparisons were made between the two experiments, no significant differences were found except that the colonies given a mated queen in experiment 1 constructed significantly larger cells than the colonies given a mated queen in experiment 2 (Table 6).

C. Pheromones

In experiment 1, the queens with intact mandibular glands (mated, virgin or dead) had larger amounts of pheromones present than queens without

Table 3. The mean ± (S.D.) percentage composition of the major components of the mandibular gland secretions of the queens from the various experiments. *

	Experiment 1				Experiment 2			
Treatment	n	9ODA	9HDA	10HDA	TOT	n	9ODA	9HDA	10HDA	TOT
Mated queen with mandibular and tergite glands	3	96.6 2.8	2.8 2.0	0.6 0.8	177.8 76.4	6	71.0 28.5	28.3 28.0	0.8 1.0	33.6 32.6
Virgin queen with mandibular and tergite glands	8	84.2 27.2	1.5 3.1	14.3 27.7	205.4 218.0	6	80.9 20.9	0 -	19.1 20.9	70.4 110
Virgin queen without mandibular glands but tergite glands intact	3	99.1 0.1	0.1 0.1	0 -	28.7 40.4	5	87.4 21.8	0 -	12.6 21.8	7.7 4.3
Virgin queen with mandibular glands, tergite glands occluded						3	65.2 4.6	13.6 16.1	21.3 11.5	116 153
Virgin queen without mandibular glands, tergite glands occluded	3	63.7 31.5	0 -	36.3 31.5	2.1 1.7					
Dead virgin	16	92.1 10.5	3.1 3.6	4.8 10.5	308.9 180.6	13	71.8 24.6	0.9 1.6	27.3 23.7	10.0 8.8

	n	9ODA	9HDA	10HDA	TOT	n	9ODA	9HDA	10HDA	TOT
Virgin queen in a division board, access to the head with with mandibular glands						4	43.1 51.4	0 -	23.5 40.8	4.1 3.6

* n is the number of colonies used in each treatment

9ODA - the percentage amount of 9ODA on the queen heads
9HDA - the percentage amount of 9HDA on the queen heads
10HDA - the percentage amounts of 10HDA on the queen heads
TOT - total amount of acids present in the head (μg/head)

Table 4. Comparisons between the different treatments for the wax secretion measurements, comb construction measurements and the mandibular gland pheromones in experiment 1. *

	%B	WR	SA	WE	DIA	TOT	9ODA	9HDA	10HDA
MQ5 vs VQ5	-	-	+	-	-	-	-	-	-
MQ5 vs MGV5	-	-	+	+		-	-	-	-
MQ5 vs MTV5	-	-	+	+		-	-	-	-
MQ5 vs DEAD5	-	-	+	+	-	-	-	-	-
MQ5 vs QL5	-	-	+	+					
VQ5 vs MGV5	-	-	-	-		-	-	-	-
VQ5 vs MTV5	-	-	-	-		-	-	-	-
VQ5 vs DEAD5	-	+	-	-		-	-	-	-
VQ5 vs QL5	-	-	-	-					
MGV5 vs MTV5	-	-	-	-		-	-	-	-
MGV5 vs DEAD5	-	+	-	-		-	-	-	-
MGV5 vs QL5	-	-	-	-					
MTV5 vs DEAD5	-	-	-	-		+	-	-	-
MTV5 vs QL5	-	-	-	-					
DEAD5 vs QL5	-	-	-	-					
VQ5 vs MGV5 vs MTV5	-	-	-	-		-	-	-	-
MQ5 vs VQ5 vs MGV5 vs MTV5	-	-	+	+	-	-	-	-	-

	%B	WR	SA	WE	DIA	TOT	9ODA	9HDA	1OHDA
DEAD5 vs QL5 vs VQ5 vs MGV5 vs MTV5	+	-	-	-	-	-	-	-	-
DEAD5 vs QL5 vs MQ5	-	-	+	+	-	-	-	-	-
DEAD5 vs QL5 vs MQ5 vs VQ5 vs MGV5 vs MTV5	-	-	+	+	-	+	-	-	-

* Kruskal-Wallis one-way analysis of variance test. Significance is defined as $P < 0.05$. No signficant difference is shown as -, while a significant difference is shown as +.

MQ5 - mated queens with intact mandibular and tergite glands
VQ5 - virgin queens with intact mandibular and tergite glands
MGV5 - virgins queen without mandibular glands but with tergite glands
MTV5 - virgins queen without mandibular glands and tergite glands occluded
DEAD5 - dead virgin queens
QL5 - queenless colonies
The number after each experimental condition refers to the experiment number.

%B - percentage of bees bearing wax in a festoon
WR - the weight of the wax scales removed from 50 bees in a festoon divided by the number of bees bearing wax (μg/1000 bees)
SA - the surface area (mm^2) of comb constructed per 1000 bees
WE - the weight (μg) of comb constructed per 1000 bees
DIA - cell diameter of the cells constructed (mm)
TOT - the total amount of acids found on the queen head (μg/head)
9ODA - the percentage amount of 9ODA found on the queen head
9HDA - the percentage amount of 9HDA found on the queen head
1OHDA - the percentage amount of 1OHDA found on the queen head

Table 5. Comparisons between the different treatments for the wax secretion measurements, comb building measurements and the queen pheromones in experiment 2. *

	%B	WR	SA	WE	DIA	TOT	9ODA	9HDA	10HDA
MQ6 vs VQ6	-	-	-	-	-	-	-	-	-
MQ6 vs MGV6	-	-	-	-	-	-	-	-	-
MQ6 vs TV6	-	-	-	-	-	-	-	-	+
MQ6 vs DEAD6	-	-	+	+	-	-	-	-	-
MQ6 vs QL6	-	-	+	+					
MQ6 vs FHV6	-	-	+	+		-	-	-	-
MQ6 vs FAV6	-	-	+	+					
VQ6 vs MGV6	-	-	-	-	-	-	-	-	-
VQ6 vs TV6	-	-	-	-	-	-	-	-	-
VQ6 vs DEAD6	-	-	+	+	-	-	-	-	-
VQ6 vs QL6	-	-	-	-					
VQ6 vs FHV6	-	-	+	+		-	-	-	-
VQ6 vs FAV6	-	-	+	+					
MGV6 vs TV6	-	-	-	-	-	-	-	-	-
MGV6 vs DEAD6	-	+	-	-	-	-	-	-	-
MGV6 vs QL6	-	-	-	-	-				
MGV6 vs FHV6	-	-	-	-		-	-	-	-
MGV6 vs FAV6	-	-	-	-					

	%B	WR	SA	WE	DIA	TOT	90DA	9HDA	10HDA
TV6 vs DEAD6	+	-	-	-	-	-	-	-	-
TV6 vs QL6	-	-	-	-					
TV6 vs FHV6	-	-	-	-		-	-	-	-
TV6 vs FAV6	-	-	-	-					
DEAD6 vs QL6	-	-	-	-					
DEAD6 vs FHV6	-	-	-	-		-	-	-	-
DEAD6 vs FAV6	-	-	-	-					
QL6 vs FHV6	-	-	-	-					
QL6 vs FAV6	-	-	-	-					
FHV6 vs FAV6	-	-	-	-					
VQ6 vs MGV6 vs TV6	-	-	-	-	-	-	-	+	-
VQ6 vs MGV6 vs TV6 vs FHV6 vs FAV6	-	-	-	-	-	-	-	+	-
VQ6 vs MGV6 vs TV6 vs FHV6	-	-	-	-	-	-	-	+	-
VQ6 vs MGV6 vs TV6 vs FAV6	-	-	-	-					
VQ6 vs MGV6 vs TV6 vs MQ6	-	-	-	-	-	-	-	+	-
VQ6 vs MGVQ6 vs TV6 vs MQ6 vs FH6V vs FAV6	-	-	+	+		-	-	+	-
VQ6 vs MGV6 vs TV6 vs MQ6 vs FHV6	-	-	-	-		-	-	+	-
VQ6 vs MGV6 vs TV6 vs MQ6 vs FAV6	-	-	-	-					

	%B	WR	SA	WE	DIA	TOT	9ODA	9HDA	10HDA
DEAD6 vs QL6 vs FHV6 vs FAV6	-	-	-	-		-	-	-	-
DEAD6 vs QL6 vs FHV6	-	-	-	-		-	-	-	-
DEAD6 vs QL6 vs FAV6	-	-	-	-					
DEAD6 vs QL6 vs VQ6 vs MGV6 vs TV6	-	-	-	-	-	-	-	-	-
DEAD6 vs QL6 vs VQ6 vs MGV6 vs TV6 vs FHV6 vs FAV6	-	-	-	-	-	-	-	-	-
DEAD6 vs QL6 vs VQ6 vs MGV6 vs TV6 vs FHV6	-	-	-	-	-	-	-	-	-
DEAD6 vs QL6 vs VQ6 vs MGV6 vs TV6 vs FAV6	-	-	-	-	-				
DEAD6 vs QL6 vs MQ6	-	-	+	+	-	-	-	-	-
DEAD6 vs QL6 vs MQ6 vs FHV6 vs FAV6	-	-	+	+	-	-	-	-	-
DEAD6 vs QL6 vs MQ6 vs FHV6	-	-	+	+	-	-	-	-	-
DEAD6 vs QL6 vs MQ6 vs FAV6	-	-	+	+					
DEAD6 vs QL6 vs MQ6 vs VQ6 vs MG6V vs TVQ6 vs FHV6 vs FAV6	-	-	+	+	-	-	-	+	-

* Kruskal-Wallis one-way analysis of variance test. Significance is defined as $P < 0.05$. No significant differences are shown by -, while

significant differences are shown by +.

MQ6 - mated queens with intact mandibular and tergite glands
VQ6 - virgin queens with intact mandibular and tergite glands
MGV6 - virgin queens without mandibular glands but tergite glands intact
TV6 - virgin queens with mandibular glands but tergite glands occluded
DEAD6 - dead virgin queens
QL6 - queenless colonies
FHV6 - virgin queens in a division board, access to the head with intact mandibular glands
FAV6 - virgin queens in a division board, access to the abdomen with tergite glands intact
The number after each experimental conditions refers to the experiment number.

%B - the percentage bees bearing wax in a festoon
WR - the weight of the wax scales removed from 50 bees in a festoon divided the number of bees bearing wax (μg/1000 bees)
SA - the surface area (mm^2) of comb constructed per 1000 bees
WE - the weight (μg) of comb constructed per 1000 bees
DIA - the cell diameter of the cells constructed (mm)
TOT - the total amount of queen pheromones found in the head of the queen (μg/head)
9ODA - the percentage amount of 9ODA found on the queen head
9HDA - the percentage amount of 9HDA found on the queen head
1OHDA - the percentage amount of 1OHDA found on the queen head

Table 6. Comparisons between the different treatments for the wax secretion measurements, comb construction measurements and the queen pheromones in experiments 1 and 2. *

	%B	WR	SA	WE	DIA	TOT	9ODA	9HDA	1OHDA
MQ5vs MQ6	-	-	-	-	+	-	-	-	-
VQ5 vs VQ6	-	-	-	+	-	-	-	-	-
MGV5 vs MGV6	-	-	-	-		-	-	-	-
DEAD5 vs DEAD6	-	-	+	+	-	+	-	-	-
QL5 vs QL6	-	-	-	-					
MQ5 vs MQ6 vs VQ5 vs VQ6 vs MG5 vs MG6 vs TV6 vs MTV5 vs DEAD5 vs DEAD6 vs QL5 vs QL6 vs FHV6 vs FAV6	-	-	-	-	-	+	-	+	-

* Kruskal-Wallis one-way analysis of variance. Significance is defined as $P < 0.05$. No significant differences are shown by -, while significant differences are shown by +.

The abbreviations for the different experimental conditions are given in Tables 4 and 5.

mandibular glands (Table 3 and Crewe 1988). The dead virgin queens had significantly more pheromones than the virgin queens without mandibular glands and tergite glands occluded (Table 4). No significant differences in the percentages of 9ODA, 9HDA and 10HDA were found between the various queens (Table 4). The amounts of pheromone present on the queens in experiment 2 were less than that found in experiment 1 (Table 3). These differences were not significant except for the dead virgin queens (Table 6). In experiment 2, the queens with intact mandibular glands also tended to have more pheromones present than queens without mandibular glands (Table 2). There were no significant differences between the amounts of pheromone and their relative percentages (Table 5). However, the percentage of 9HDA differed amoung virgin queens and when they were compared to mated queens and the virgin queen in the division board (Table 5).

Discussion

The presence or absence of a queen in a honeybee colony does not appear to affect the secretion of wax by worker bees (Chapters 2-5). The percentage of bees bearing wax in a festoon and the amount of wax borne by these bees was the same for most of the treatments (Tables 4, 5 and 6). Whether the queen was mated or not, or whether the mandibular glands were extirpated and/or tergite glands occluded; the amount of wax/bee was essentially the same in all colonies. No seasonal

differences in the percentage of bees bearing wax or the amount of wax borne/bee were detected. This supports the hypothesis that the secretion of wax is a physiological process that occurs independently of the queen in young adult honeybees (Chapters 2-5).

In both experiments, the colonies given a mated queen constructed 3-4 times more comb than the colonies given a virgin queen with intact mandibular and tergite glands (Table 2). This result is consistent with others (Chapter 2 and Rajashekharappa and Channabasavanna 1979). Although more comb was constructed in the summer experiment (Table 2), this did not differ significantly from the autumn experiment (Tables 2 and 6). Colonies given a mated queen always constructed significantly more comb than the queenless colonies, colonies given a dead virgin queen and colonies having access to the virgin queen in a division board (Tables 2, 4 and 5). Similar amounts of comb were constructed by the colonies given a mated or virgin queen (with/without mandibular and/or tergite glands) in experiment 2 (Table 5).

In experiment 1, the colonies given a mated queen constructed significantly more comb than the colonies given a virgin queen (with/without mandibular and/or tergite glands) (Table 4, Chapter 2). Although the colonies given a virgin queen with intact mandibular and tergite glands constructed significantly more comb than the colonies

given a dead virgin queen or a virgin queen in a division board (in experiment 2), no significant differences were found when all the other colonies given a virgin queen (mandibular glands but tergite glands occluded or a queen without mandibular glands but tergite glands intact) were compared to the latter (Table 5). No significant differences were found when all the colonies given a virgin queen were compared together (Table 5). This suggests that colonies headed by a virgin queen with intact mandibular and tergite glands in summer contruct more comb than a colony headed by a similar type of queen in autumn (Table 6).

Comparisons in Tables 4 and 5, show that colonies with mated queens always constructed significantly more comb than all of the colonies headed by a virgin queen (with/without mandibular and/or tergite glands) all compared together. No significant differences were found when the colonies given a virgin queen (with/without mandibular and/or tergite glands) were compared to the queenless colonies, colonies given a dead virgin queen and colonies where the bees had access to either the virgin queen head or abdomen (Tables 4 and 5). The queenless colonies, colonies given a dead virgin queen and colonies having access to either the virgin queen head or abdomen always constructed significantly less comb than the colonies headed by a mated queen (Tables 4 and 5). Thus, there appears to be a comb construction hierarchy in which (1) colonies with mated queens are most stimulated to

build comb, followed by (2) those with virgin queens with intact mandibular and tergite glands, then (3) the colonies headed by a virgin queen with/without mandibular and/or tergite glands and finally (4) the queenless colonies, colonies given a dead virgin queen and colonies having access to a virgin queen in the division board (cf. Verheijen-Voogd, 1959).

Cells that were of an intermediate worker and drone size (Vogt 1911 and Owens and Taber 1973) were constructed by the colonies given a dead virgin queen in experiment 2 (Table 2). These cells were also constructed by the colonies given a dead mated queen (Chapter 3). Thus, it would seem that the absence of the queen would allow the workers to construct these cells (Langstroth 1853). However, this result has not always been consistent as the queenless worker bees usually constructed normal worker-sized cells (Chapters 3-5). Whether the queen plays a decisive role in determining the type of cell that is to be constructed is uncertain and cannot be determined from the available data.

The diameters of the worker cells that were constructed in experiment 2 were similar to those measured by Garin (1926). Slightly larger cells were constructed in experiment 1 (Table 2, Chapters 3-5). Interestingly, the colonies given a mated queen and colonies given a virgin queen (with/without mandibular and/or tergite glands)

constructed cells having similar diameters (Tables 2, 4 and 5). When the diameters of the cells were compared between the two experiments, only those in the colonies given a mated queen differed (Table 6).

The amounts of pheromone found on the heads of the queens with intact mandibular glands were similar to those reported by Crewe and Velthuis (1980). No significant differences were found in the amounts of pheromone extracted from the heads of the mated and virgin queens (with intact mandibular glands) (Tables 4 and 5 and Crewe 1988). Small amounts of pheromone were found on the virgin queens whose mandibular glands had been extripated (Table 3). Similar percentages 9ODA, 9HDA and 10HDA were recovered from the mated and virgin queen heads (regardless of the presence of the mandibular glands) in both experiments (Table 3). No correlations between any of the pheromones and the amounts of comb constructed were found.

If comb building is to be enhanced by pheromones of the queen (Hepburn 1986), then equal amounts of comb should have been constructed by the colonies given mated or virgin queens because the amounts of pheromones recoverable from these queen heads were the same (Table 3 and Crewe 1988). However, the colonies with virgin queens always constructed less comb than the colonies given a mated queen (Tables 2, 4 and 5, Chapter 2 and Rajashekharappa and Channabasavanna 1979). Thus, these pheromones acting alone are unlikely to be responsible for

enhancing comb building in queenright colonies. That the presence of a mated queen head enhances comb building has already been established (Chapter 5). The presence of a virgin queen head seems to play no role in comb construction (Tables 2 and 5). Whether 9ODA, 9HDA or 1OHDA act together with other pheromones to enhance comb building remains to be seen.

Chapter 7

Queen cell construction - the number of queen cells constructed at the end of each experiment.

Summary

The worker bees in queenless colonies, colonies given a dead queen or a queen that died during the experiment constructed significantly more queen cells than colonies given a living mated or virgin queen; it did not matter whether the queen was in a division board or had mandibular and/or tergite and/or tarsal glands missing. Similar numbers of queen cells were constructed when the workers had access to either the head or abdomen of a mated, virgin or dead queen (with/without mandibular and/or tergite glands). Colonies given a queen without mandibular, tergite and tarsal glands constructed more queen cells than colonies given a queen with intact mandibular, tergite and tarsal glands. Significantly more queen cells were constructed in colonies given a mated queen without mandibular glands than colonies given a mated queen with mandibular glands. The number of queen cells constructed in colonies given a virgin queen with/without mandibular and/or tergite

glands was the same. Colonies given a queen in a single or double cage constructed more queen cells than colonies given a queen who was able to move "freely" across the comb. Mated or virgin queens with intact mandibular, tergite and tarsal glands were "most effective" out of all the treatments in inhibiting queen cell construction. Colony size was not correlated to the number of queen cells constructed for any of the treatments.

Introduction

Queen cell construction in queenright honeybee colonies is inhibited by the presence of a living queen (Huber 1814, Mussbichler 1952, Butler 1954, Fell and Morse 1984 and Free et al. 1985). If a queen is accidently lost from a colony (through predation or death), the worker bees rear a new one from a young larva or an egg (Fell and Morse 1984, Seeley 1985 and Free 1987).

The contents of the mandibular glands of a mated queen smeared onto the body of a dead (extracted) queen, inhibited emergency queen cell construction in small queenless colonies (Butler and Simpson 1958). Synthetic 9ODA on a dead extracted queen decreased the tendency of small groups of queenless bees to build queen cells. However, neither the odour of 9ODA alone nor 9HDA alone were as effective as when both of these pheromones were presented together (Butler and

Callow 1968). Thus, these 2 pheromones secreted from the mandibular glands of a mated queen were considered to be responsible for inhibiting queen cell construction. This hypothesis was further supported when virgin queen extracts were found to be less effective in inhibiting queen cell construction than the extracts of a mated queen (Butler 1960). Since virgin queens tend to secrete less 9ODA and 9HDA from their mandibular glands than mated queens (Butler and Paton 1962 and Pain et al. 1967), this idea seemed plausible.

However, colonies given a mated queen with or without mandibular glands were equally effective in inhibiting queen cell construction (Gary and Morse 1962). Worker bees having access to the head or abdomen of a queen were both able to inhibit queen cell construction (Butler 1954). Furthermore, Free et al. (1985) found that virgin queens were as effective as mated queens in inhibiting queen cell construction. Although all these combined results are contradictory, it is generally agreed that a "scent" from a living queen inhibits queen cell construction (Free 1987).

The number of queen cells constructed in each colony at the end of each of the previously described experiments were recorded. (The number of queen cells recorded from failed experiments were also included in the analysis.) These results were analyzed in hope

that some information might be gained concerning queen cell construction.

Methods and Materials

The number of emergency queen cells that were constructed in each colony (a total of 299 colonies were used in 7 experiments) at the end of each experiment were recorded. These experiments were conducted in various apiaries in Grahamstown between December and May from 1987-1990. Although these experiments occurred at different times of the year, queen cell construction was always inhibited (except during swarming) in well established queenright colonies, even though the signal(s) for queen recognition could possibly have changed with season (Butler 1954, Fell and Morse 1984 and Free 1987). Hence, colonies that were given similar queens in all the experiments were grouped together. Each experiment has been described in detail in Chapters 2-6.

Experiment 7 has not been written up as no comb was constructed in these colonies. The number of queen cells constructed at the end of the experiment were counted. This experiment was designed to reinvestigate the effect of a virgin queen on comb building as well as the presence or absence of the tarsal glands (Lensky and Slabezki 1981) on mated queens. The

experiment was conducted in February 1990 and followed the same experimental procedure that has been described for each experiment.

Results

Table 1 shows the mean colony size and the number of emergency queen cells that were constructed under the various experimental conditions. (The raw data is shown in Appendix F.1). When all of the colonies were compared, there were significant differences between them regarding the numbers of emergency queen cells constructed (Table 2). The queenless colonies, colonies given a dead queen or a queen that died during the experiment constructed significantly more queen cells than the colonies given a mated or virgin queen with intact mandibular and with tergite glands or a virgin queen without mandibular glands but tergite glands intact (Table 2). This result was also found when the colonies given a mated or virgin queen with/without mandibular and/or tergite glands were compared to the queenless colonies, the colonies given a dead queen or a queen that died during the experiment (Table 2). No significant differences were found when the queenless colonies, colonies given a dead queen or a queen that died during the experiment were compared to each other and to any of the colonies given a queen in

Table 1. Colony size and the mean number of queen cells constructed under each experimental condition. The means and þ Standard Deviations (S.D.) are given. *

Queen	n	Colony size	Mean number of queen cells constructed
MQ	37	8033 4715	0.3 1.0
VQ	17	7042 5262	0.4 1.2
MGM	11	9488 2724	3.5 4.1
MGV	11	5819 3872	0.4 1.2
TM	12	9371 6136	2.4 6.3
TV	8	5683 2892	0.4 1.1
MTM	10	8647 5980	4.0 5.5
MTV	4	8720 6770	0 0
DEAD	39	7259 4082	4.8 3.4
DIED	29	5838 3801	5.6 6.5
QL	11	6334 4861	7.2 5.0
DO	3	5255 2188	0.3 0.6

Queen	n	Colony size	Mean number of queen cells constructed
SI	5	3876	3.4
		2117	4.8
SH	2	8159	0
		3295	0
CO	3	9038	1.3
		6089	0.6
FH	9	5289	5.6
		2576	7.2
FA	9	4056	4.9
		1965	3.3
MGH	3	4992	0
		1784	0
MGA	3	6067	1.7
		2068	2.9
TH	3	6176	1.0
		4504	1.7
TA	3	7289	5.7
		2065	9.0
MTH	3	5473	2.0
		3725	1.7
MTA	3	6750	0
		5689	0
DEH	3	4468	1.3
		2831	2.3
DEA	3	7810	0.7
		4236	1.2
DIEH	7	5902	5.3
		2421	4.2

Queen	n	Colony size	Mean number of queen cells constructed
DIEA	6	4763	2.3
		2450	2.3
FVH	4	4483	4.8
		2113	5.3
FVA	4	4053	1.0
		5520	1.4
T1	4	4113	0.3
		940	0.5
T2	4	6661	0
		3607	0
T3	4	2519	1.8
		1238	1.5
T1M	4	4805	0.3
		1962	0.5
T2M	4	4759	0.3
		2696	0.5
T3M	3	6860	0.8
		2276	1.0
T1MT	4	4020	5.0
		2006	4.7
T2MT	4	5016	5.0
		2820	2.9
T3MT	3	2479	1.5
		2261	0.7

*

MQ - colonies given a mated queen with intact mandibular and tergite glands

VQ - colonies given a virgin queen with intact mandibular and tergite glands

MGM - colonies given a mated queen without mandibular glands but tergite glands intact
MGV - colonies given a virgin queen without mandibular glands but tergite glands intact
TM - colonies given a mated queen with intact mandibular glands but tergite glands occluded
TV - colonies given a virgin queen with intact mandibular glands but tergite glands occluded
MTM - colonies given a mated queen without mandibular glands and tergite glands occluded
MTV - colonies given a virgin queen without mandibular glands and tergite glands occluded
DEAD - colonies given a dead mated or virgin queen (with/without mandibular and/or tergite glands
DIED - colonies given a mated or virgin queen, but the queen died during the week of the experiment
QL - queenless colonies
DO - colonies given a mated queen with intact mandibular and tergite glands but the queen was in a double cage
SI - colonies given a mated queen with intact mandibular and tergite glands but the queen was in a single cage
SH - colonies given a mated queen with intact mandibular and tergite glands, but the genae had an incision made in them
CO - colonies given a queen in a division board, the bees had access to the head and abdomen of the queen with intact mandibular and tergite glands
FH - colonies given a mated queen in a division board, the bees had access to the head with intact mandibular glands
FA - colonies given a mated queen in a division board, the bees had access to the abdomen with intact tergite glands
MGH - colonies given a mated queen in a division board, the bees had access to the head without mandibular glands
MGA - colonies given a mated queen in a division board, the bees had access to the abdomen with intact tergite glands
TH - colonies given a mated queen in a division board, the bees had access to the head with intact mandibular glands
TA - colonies given a mated queen in a division board, the bees had access to the abdomen with tergite glands occluded
MTH - colonies given a mated queen in a division board, the bees had access to the head without mandibular glands
MTA - colonies given a mated queen in a division board, the bees had access to the abdomen with tergite glands occluded
DEH - colonies given a dead mated queen in a division board, the bees had access to the head with intact mandibular glands
DEA - colonies given a dead mated queen in a division board, the bees had access to the abdomen with intact tergite glands
DIEH - colonies given a mated queen in a division board who died during the week of the experiment, the bees had access to the head with intact mandibular glands

DIEA - colonies given a mated queen in a division board who died during the week of the experiment, the bees had access to the abdomen with intact tergite glands
FVH - colonies given a virgin queen in a division board, the bees had access to the head with intact mandibular glands
FVA - colonies given a virgin queen in a division board, the bees had access to the abdomen with intact tergite glands
T1 - colonies given a mated queen with intact mandibular and tergite glands, but 1 pair of tarsal glands removed
T2 - colonies given a mated queen with intact mandibular and tergite glands, but 2 pairs of tarsal glands removed
T3 - colonies given a mated queen with intact mandibular and tergite glands, but all tarsal glands removed
T1M - colonies given a mated queen without mandibular glands, tergite glands intact and 1 pair of tarsal glands removed
T2M - colonies given a mated queen without mandibular glands, tergite glands intact and 2 pairs of tarsal glands removed
T3M - colonies given a mated queen without mandibular glands, tergite glands intact and all tarsal glands removed
T1MT - colonies given a mated queen without mandibular glands, tergite glands occluded and 1 pair of tarsal glands removed
T2MT - colonies given a mated queen without mandibular glands, tergite glands occluded and 2 pairs of tarsal glands removed
T3MT - colonies given a mated queen without mandibular glands, tergite glands occluded and all tarsal glands removed

Table 2. One-way anaylsis of variance (square-root transformation) was used to analyse the differences between the queen treatments. Tukey's Studentized Range Method and Scheffe's Method were used to group the data. *

Comparison	F value	P value	Tukey's Studentised Range Method	Sheffe's Method
MQ, VQ, MGV, TV, MTV, DEAD, QL, DO, SI, DIED, SH, MGM, TM, MTM, CO, FH, FA, MGH, MGA, TH, TA, MTH, MTA, DEH, DEA, DIH, DIA, FVH, FVA, T1, T2, T3, T1M, T2M, T3M, T1MT, T2MT, T3MT	3.49	0.000	DEAD grouped from MQ, VQ, & MGV QL grouped from MQ, VQ & MGV DIED grouped from MQ, VQ & MGV FA grouped from MQ	none
MQ, VQ, MGV, TV, MTV, DEAD, DIED, QL, MGM, TM, MTM	9.13	0.000	DEAD grouped from MQ, VQ, MGV & TV QL grouped from MQ, VQ, MGV & TV DIED grouped from MQ, VQ, MGV, TV	DEAD grouped from MQ & VQ QL grouped from MQ & VQ DIED grouped from MQ & VQ
MQ, MGM, TM, MTM, DEAD, QL, DIED	8.06	0.0000	DEAD grouped from MQ, QL grouped from MQ DIED grouped from MQ	DEAD grouped from MQ QL grouped from MQ DIED grouped from MQ

Comparisons	F value	P value	Tukey's Studentized Range Method	Scheffe's Method
DEAD, QL, DIED, CO, FH, FA, MGH, MGA, TH, TA, MTH, MTA, DEH, DEA, DIH, DIA, FVH, FVA	1.51	0.101	none	none
DEAD, QL, DIED, T1, T2, T3, T1M, T2M, T3M, T1MT, T2MT, T3MT	2.6	0.006	none	none
DEAD, QL, DIED, MGM, TM, MTM, CO, FH, FA, MGH, MGA, TH, TA, MTH,MTA, DEH, DEA, DIH, DIA, FHV, FAV	1.44	0.111	none	none
MGV, TV, MTV, DEAD, QL, DIED, MGM, TM, MTM, CO, FH, FA, MGH, MGA, TH, TA, MTH, MTA, DEH, DEA, DIH, DIA, FHV, FHA	2.28	0.0014	none	none
DEAD, QL, DIED, DO, SI	1.35	0.258	none	none
DEAD, QL, DIED	0.29	0.75	none	none
MQ, VQ, MGV, TV, MTV, SH, MGM, TM, MTM	4.02	0.0003	MGM grouped from MQ & VQ MTM grouped from MQ & VQ	none
MQ, VQ, MGM, MT, MTM	5.95	0.0003	MGM grouped from MQ MTM grouped from MQ	MGM grouped from MQ MTM grouped from MQ

Comparisons	F value	P value	Tukey's Studentized Range Method	Scheffe's Method
MQ, MGM, TM, MTM	5.87	0.001	MGM grouped from MQ MTM grouped from MQ	MGM grouped from MQ MTM grouped from MQ
VQ, MGV, MTV, TV	0.14	0.938	none	none
MQ, VQ, MGV, MTV, TV	0.12	0.976	none	none
MGV, MTM, MGM, MTM	3.54	0.02	none	none
MQ, VQ, MT, TV	1.83	0.149	none	none
CO, FH, FA, MGH, MGA, TH, TA, MTH, MTA, DEH, DEA, DIH, DIA, FVH, FVA	1.18	0.32	none	none
FH, MGH, TH, MTH, DEH, DIH, FVH	1.01	0.443	none	none
FA, MGA, TA, MTA, DEA, DIA, FVA	1.88	0.125	none	none
T1, T2, T3, T1M, T2M, T3M, T1MT, T2MT, T3MT	2.82	0.022	none	none
MQ, VQ, MGV, TV, MTV, DEAD, QL, DIED, FHV, FAV	11.21	0.001	DEAD grouped from MQ, VQ, MGV, MTV & TV QL grouped from MQ, VQ, MTV, & TV DIED grouped from MQ, VQ, MGV, TV & MTV	DEAD grouped from MQ, VQ & MTV QL grouped from MQ, VQ & MGV DIED grouped from MQ, VQ & MGV

Comparison	F value	P value	Tukey's Studentized Range Method	Scheffe's Method
MQ, MGM, TM, MTM, DEAD, QL, DIED, CO, FH, FA, MGH, MGA, TH, TA, MTH, MTA, DEH, DEA, DIH, DIA	3.37	0.0000	DEAD grouped from MQ QL grouped from MQ DIED grouped from MQ	DEAD grouped from MQ
VQ, MGV, MTV, TV, DEAD, QL, DIED, CO, FH, FA, MGH, MGA, TH, TA, MTH, MTA, DEH, DEA, DIH, DIA	3.74	0.000	DEAD grouped from VQ & MGV QL grouped from VQ & MGV DIED grouped from VQ & MGV	none
MQ, VQ, MGV, TV, MTV, MGM, TM, MTM, T1, T2, T3, T1M, T2M, T3M, T1MT, T2MT, T3MT	3.33	0.0001	MGM grouped from MQ MTM grouped from MQ	none
MQ, VQ, T1, T2, T3, T1M, T2M, T3M, T1MT, T2MT, T3MT	5.85	0.000	T1MT grouped from MQ, VQ, T1, T2, T1M & T2M T2MT grouped from MQ, VQ, T2 & T2M T3MT grouped from MQ & VQ	T1MT grouped from MQ & VQ
MQ, VQ, MGV, TV, MTV, T1, T2, T3, T1M, T2M, T3M, T1MT, T2MT, T3MT	5.07	0.000	T1MT grouped from MQ, VQ, MGV, TV, MTV, T1, T2, T1M & T2M T2MT grouped from MQ, VQ, MGV, TV, MTV, T2 & T2M T3MT grouped from MQ & VQ	T1MT grouped from MQ & VQ
MQ, VQ, DO, SI	4.36	0.008	SI grouped from MQ & VQ	SI grouped from MQ & VQ
VQ, MGV, TV, MTV, DO, SI	2.11	0.08	none	none

Comparison	F value	P value	Tukey's Studentized Range Method	Scheffe's Method
MQ, MGM, TM, MTM, DO, SI	4.0	0.003	MGM grouped from MQ MTM grouped from MQ	none
DO, SI, T1, T2, T3, T1M, T2M, T3M, T1MT, T2MT, T3MT	1.88	0.086	none	none
DO, SI, CO, FH, FA, MGH, MGA, TH, TA, MTH, MTA, DEH, DEA, DIH, DIA, FVH, FVA	1.16	0.325	none	none

*

none - none of the treatments grouped
Queen treatment abbreviations, see Table 1.

a division board (whether the bees had access to the head or abdomen of a mated, virgin or dead queen, with/without mandibular and/or tergite glands), the colonies given a mated or virgin queen with/without mandibular or tergite glands, the colonies given a mated queen with/without mandibular and tergite glands and without 1, 2 or 3 pairs of tarsal glands or the colonies given a mated queen in a single or double cage (Table 2). These results show that the worker bees must have direct access to a mated or virgin queen with intact mandibular and tergite glands for emergency queen cell construction to be "completely" inhibited.

When the colonies given a mated or virgin queen with/without mandibular and/or tergite glands were compared, the colonies given a mated queen without mandibular and/or tergite glands constructed significantly more queen cells than the colonies given a mated or virgin queen with intact mandibular and tergite glands (Table 2). A similar result was found when the colonies given a mated queen with/without mandibular and/or tergite glands were all compared (Table 2). However, no significant differences were found when the colonies given a virgin queen with/without mandibular and/or tergite glands were compared. These results suggest that the colonies given a virgin queen with/without mandibular and/or tergite glands and colonies given a mated queen with intact mandibular glands and/or tergite glands were most effective in

inhibiting emergency queen cell construction (than a mated queen without mandibular glands) (Table 2).

No significant differences were found when comparisons between all the following treatments were made: 1) all the colonies given access to a mated queen head with/without mandibular glands 2) all the colonies given access to a mated queen abdomen with/without tergite glands occluded 3) colonies given access to a mated, virgin or dead queen in a division board, the bees either had access to the head (with/without mandibular glands) or to the abdomen (with/without tergite glands occluded) 4) colonies given a mated queen with/without mandibular and tergite glands and without 1, 2 or 3 pairs of tarsal glands (Table 2).

When the colonies given a mated or virgin queen with/without mandibular and/or tergite glands were compared to the colonies given a mated queen with/without mandibular and tergite glands and with 1, 2 or 3 pairs of tarsal glands missing, the colonies given a mated or virgin queen with intact mandibular and tergite glands constructed significantly fewer queen cells than the colonies given a mated queen without mandibular glands, tergite glands and 1, 2 or 3 pairs of tarsal glands (Table 2). These results again show that the presence of a mated or virgin queen with intact mandibular, tergite and tarsal glands in a colony is necessary for the "complete"

suppression of emergency queen cell construction.

Colonies given a queen in a single cage constructed significantly more queen cells than colonies given a mated or virgin queen with intact mandibular and tergite glands (Table 2, Butler 1954). No differences in the number of queen cells constructed were found when the colonies given a mated or virgin queen with/without mandibular and/or tergite glands, colonies given a mated queen with/without mandibular and tergite glands and without 1, 2 or 3 pairs of tarsal glands and colonies given a mated, virgin or dead queen in a division board when the bees had access to either the head (with/without mandibular glands) or the abdomen (with/without tergite glands) were compared to the colonies given a queen in a single or double cage (Table 2). These results all showed that the worker bees needed to have direct contact with a mated or virgin queen with intact mandibular, tergite and tarsal glands for queen cell construction to be inhibited. Queen cell construction was inhibited to some extent in all of the other colonies given a living queen regardless of whether the queen had mandibular, tergite and/or tarsal glands missing, or if the queen was in a division board or caged (Table 2).

No correlations between colony size and the number of queen cells constructed were found. The number of emergency queen cells constructed at queenloss appears to depend on the number of young

larvae and eggs available to rear new queens (Butler 1954 and Free 1987). Since the number of young larvae and eggs in a colony varies with season and within colonies, the number of queen cells constructed in any group of colonies is likely to vary (Appendix F.1 and Fell and Morse 1984). The number of queen cells constructed in a queenless colony must be determined by some other factor(s).

Discussion

Most of the emergency queen cells that were constructed in these experiments occurred in the queenless colonies, the colonies given a dead queen or a queen that died during the experiment (Tables 1 and 2). Although the workers had direct contact with the body of a dead queen or a queen that died, emergency queen cell construction in these colonies was not inhibited (Tables 1 and 2). Colonies given a mated queen in a single or double cage were unable to inhibit the construction of queen cells (Tables 1 and 2, Mussbichler 1952 and Butler 1954). That the worker bees need to have direct contact with a living queen for queen cell construction to be inhibited, is readily apparent.

The colonies given a mated queen without mandibular glands (and/or tergite glands) constructed significantly more queen cells than the

colonies given a mated queen with intact mandibular glands (and/or tergite glands) (Table 2). However, Butler (1961) and Gary and Morse (1962) found that queen cell construction was as effectively inhibited by mated queens with or without mandibular glands. The differences between these results are not easily explained. It is likely that the pheromone(s) secreted from the mandibular glands (particularly 9ODA and 9HDA) are not responsible for inhibiting queen cell construction (Butler and Callow 1968 and Free 1987).

Virgin queens with or without mandibular glands (and/or tergite glands) also inhibited queen cell construction (Table 2, Free et al. 1985). Cape virgin and mated queens secrete more or less equal amounts of 9ODA in their mandibular glands (Crewe 1988). Clearly, the mandibular gland secretion alone cannot account for the inhibition of queen cell contruction. Secretions from other glands could possibly contribute to the inhibition of queen cell construction. The tergite glands now appear to be an unlikely source of such pheromones, as mated or virgin queens with occluded tergite glands inhibited queen cell construction (Table 2).

The tarsal gland secretion could play some role in inhibiting emergency queen cell construction (Table 2, Butler 1957 and Simpson 1960). Colonies given a queen without mandibular, tergite and tarsal glands constructed more queen cells than colonies given a mated or virgin

queen with intact mandibular, tergite and tarsal glands (Tables 1 and 2). However, colonies given a mated queen with/without mandibular and tergite glands and without 1, 2 or 3 pairs of tarsal glands were compared to each other, no significant differences were found (Table 2).

Similar numbers of queen cells were constructed when the workers had access to either head (with/without mandibular glands) or abdomen (with/without tergite glands) of a mated, virgin or dead queen (Table 2 and Butler 1954). Although these colonies constructed slightly more queen cells than colonies given a mated or virgin queen with intact mandibular and tergite glands, these differences were not significant (Tables 1 and 2). These results support Butler's idea that a "scent" inhibiting queen cell construction is found on all parts of the queen's body (Butler 1961).

Fell (1979 cited in Fell and Morse 1984) and Free (1987) has suggested that the number of queen cells constructed in a colony is related to colony size. Such a relationship did not hold in these experiments (Table 1 and Appendix F.1). Variation in the number of queen cells constructed by the workers in colonies given similar treatments were also noted by Fell and Morse (1984). These authors suggested that the number of queen cells constructed is a "function of the number required for the inhibition under the feedback control system and

losses due to mortality". This hypothesis is difficult to test and it is not certain that these results support it.

The scent of a living mated or virgin queen (with intact mandibular, tergite and tarsal glands) inhibits queen cell construction in honeybee colonies. The origin(s) of this scent is/are unknown. The mandibular and tarsal glands may secrete some substance(s) that contribute to the inhibiting scent. It is also possible that the scent is a part of the waxy covering of the living queen's body (Butler 1954). This idea requires investigation.

Chapter 8

General conclusion

The amount of wax/bee and the percentage of festoon bees bearing wax was more or less the same in all experiments. It did not matter whether the colonies were queenless or queenright or whether they were given a mated or virgin queen with/without mandibular and/or tergite glands. Even when the queen was caged or in a division board, the amount of wax available for the construction of comb was essentially the same. This suggests that wax secretion is an involuntary process in which young adult honeybees aged 6-15 days secrete wax depending on the colony needs (Hepburn 1986). This process appears to be very different from that of colonies preparing to swarm (Hepburn 1988 and Hepburn and Whiffler 1988).

In contrast, there were substantial differences in the amounts of comb constructed under the various experimental conditions. Queenright *A.m.capensis* colonies constructed 8 times more comb than their queenless counterparts, while in other honeybee races this difference is usually 3-4 times greater in queenright colonies. The larger amounts of 9ODA secreted by *A.m.capensis* queens could not alone account for this difference. In fact, no differences were found when the

queenright *A.m.capensis* and *A.m.scutellata* colonies were compared. *A.m.capensis* and colonies given an *A.m.scutellata* queen and *A.m.scutellata* colonies given an *A.m.capensis* queen constructed similar amounts of comb when compared to each other. Colonies given a virgin queen constructed significantly less comb than queenright colonies.

When the bees had limited or no access to their queen, only small amounts of comb were constructed. More comb was constructed when these colonies had direct access to a whole dead queen. It did not matter whether mandibular glands were extirpated or present and or tergite glands occluded or not, similar amounts of comb were constructed when the bees had direct access to their (living) queens. In fact, the bees only needed direct access to the head of their queen (with/without mandibular glands) for comb building to be enhanced. No comb was constructed when the colonies had access to the abdomen of a queen (with/without tergite glands).

Colonies having access to a virgin queen with/without mandibular and/or tergite glands always constructed less comb than colonies given a mated queen. Although *A.m.capensis* virgin and mated queens secrete similar amounts of 9ODA and 9HDA from their mandibular glands (Crewe 1988), the differences in the amount of comb constructed could not be attributed to either one of these pheromones. The amounts of 9ODA, 9HDA and 10HDA extracted from the heads of the queens were measured by gas

chromatography. The amounts of these pheromones were similar to those measured by Crewe (1982 and 1988). No correlations between these pheromones and the comb building measurements were found.

The diameters of the cells that were constructed varied between the experiments. The smallest cells were constructed in experiment 1, while the diameters of the cells constructed in the other experiments were similar to those measured by Garin (1926), Anderson (1960) and Smith (1961). Some of the queenless colonies tended to construct slightly larger cells which were of an intermediate drone and worker size (Vogt 1911 and Owens and Taber 1973). Whether the queen plays a role in determining the size of cell constructed is uncertain. Since some queenless colonies and colonies given a dead queen were able to construct "normal" sized worker cells, the extent to which laying workers and/or pseudoqueens mimic the queen are unknown.

The number of queen cells constructed at the end of each experiment were counted. Queenless colonies, colonies given a dead queen or a queen that died during the experiment constructed significantly more queen cells than the colonies given a living mated or virgin queen. More queen cells were constructed when the queens were caged than when they were "freely" moving across the comb. Colonies given queens without mandibular and/or tergite and/or tarsal glands were not as effective as a queen with intact mandibular, tergite and tarsal glands in inhibiting emergency

queen cell construction. Similar numbers of queen cells were constructed when the bees had access to the head or abdomen of the queen. Queen cell construction was "effectively" inhibited when the colony was headed by a mated or virgin queen with intact mandibular, tergite and tarsal glands.

The queen in a honeybee colony plays an important role in suppressing worker reproduction and maintaining worker behaviours that ensure colony survival (Seeley 1985). The physical presence of the queen and access to her pheromonal secretions are essential to her role as "queen" in the colony (Butler 1973). When access to the queen and/or her pheromonal secretions is/are inhibited, the colonies begin to behave as though they were queenless (Free 1987). Worker bees obtain these pheromones from their queen by direct contact chemoreception during retinue behaviour (Seeley 1979 and Ferguson and Free 1980). These pheromones are then passed onto other workers during worker-worker intractions such as trophollaxis (Korst and Velthuis 1982 and Free 1987).

The worker bees need to have direct contact with the head of a mated queen for comb building to be enhanced. It is likely that the worker bees obtain pheromone(s) from the queen head during retinue behaviour. The mandibular glands are unlikely to be the sole source of this/these pheromone(s) as similar amounts of comb were

constructed in colonies that had access to the head of a queen with/without mandibular glands. Futhermore, none of the mandibular gland pheromones measured here were correlated with the amounts of comb constructed. The labial, tarsal or salivary glands may secreted pheromone(s) that contribute to enhancing comb building in queenright colonies. It is possible that the cuticular hydrocarbons may also play a role (Butler 1954, Crewe 1988). It is not known whether these glands secrete pheromone(s) or how the cuticular hydrocarbons of a queen honeybee affect colony behaviour. Both these areas require investigation. As our knowledge of honeybee pheromones and behaviour expands, so the precise role of the queen in comb building may be determined.

References

Allen MD (1955) Observations of honeybees attending their queen. Brit J Animal Behav 17:112-116

Allsopp MH (1988) Mandibular gland açids and laying workers in African honeybees. In: GR Needham, RE Page, M Delfinado-Baker, CE Bowman (eds) Africanized honey bees and bee mites. Ellis Horwood Limited, Chichester

Anderson RH (1960) African bee cell size problem. Bee World 40:125-126

Anderson RH (1963) The laying worker in the Cape honeybee, *Apis mellifera capensis*. J Apic Res 2:85-92

Barbier J, Lederer E (1960) Structure chimique de la substance royale de la reine d'abeille (*Apis mellifica* L.). C R Sèanes acad D Sci Nat 251:1131-1135

Beetsma J, Schoonhoven LM (1966) Some chemosensory aspects of the social relations between the queen and the worker in the honeybee (*Apis mellifera* L.). Proc Konink Nederlandse Akade Wetenschappen 69:645-

647

Blum MS (1985) Exocrine systems. In: MS Blum (ed) Fundamentals of Insect Physiology. John Wiley and Sons, New York, Chichester, Brisbane, Toronto, Singapore

Butler CG (1954) The method and importance of the recognition by a colony of honey-bees (*A.mellifera* L.) of the presence of its queen. Trans R Entmol Soc Lond 105:11-29

Butler CG (1956) The source of the substance produced by a queen honeybee (*Apis mellifera*) which inhibits development of the ovaries of the workers of her colony. Proc R Entomol Soc Lond 34:137-138

Butler CG (1957) The process of queen supersedure in colonies of honeybees (*Apis mellifera* Linn.). Insectes Sociaux 4:211-223

Butler CG (1959) The source of the substance produced by a queen honeybee (*Apis mellifera*) which inhibits development of the ovaries of the workers of her colony. Proc R Entomol Soc Lond 34:137-138

Butler CG (1960) Queen recognition by worker honeybees (*Apis mellifera* L.). Experientia 16:424-426

Butler CG (1960) Queen substance production by virgin queen honeybees, (*Apis mellifera*). Proc R Entomol Soc Lond 35:170-171

Butler CG (1961) The scent of queen honeybees (*A.mellifera*) that causes partial inhibition of queen rearing. J Insect Physiol 7:258-264

Butler CG (1973) The queen and the "Spirit of the Hive". Proc Entomol Soc Lond 48:62-65

Butler CG, Callow RK (1968) Pheromones of the honeybee (*Apis mellifera* L.): the "inhibitory scent" of the queen. Proc R Entomol Soc Lond 43:62-65

Butler CG, Paton PN (1962) Inhibition of queen rearing by queen honeybees (*Apis mellifera* L.) of different ages. Proc R Entmol Soc Lond A 37:114-116

Butler CG, Simpson J (1958) The source of queen substance of the honeybee (*Apis mellifera* L.). Proc R Entomol Soc Lond 33:120-122

Butler CG, Callow RK, Koster CG, Simpson J (1973) Perception of the queens by workers in the honeybee colony. J Apic Res 12:159-166

Butler CG, Callow RK, Greenway AR, Simpson J (1974) Movement of the

pheromone, 9-oxodec-2-enoic acid, applied to the body surfaces of honeybees (*Apis mellifera*). Entomol et experimentalis et applicata 17:112-116

Callow RK, Johnston NC (1960) The chemical constitution and synthesis of queen substances of honeybees (*Apis mellifera* L.). Bee World 41:152-153

Callow RK, Chapman JR, Paton PN (1964) Pheromones of the honeybee: Chemical studies of the mandibular gland secretion of the queen. J Apic Res 3:77-89

Crewe RM (1982) Compositional variability: The key to the social signals produced by honeybee mandibular glands. In: MD Breed, CD Michener, HE Evans (eds) The biology of social insects. Westview, Boulder.

Crewe RM (1988) Natural history of honey-bee mandibular gland secretions: development of analytical techniques and the emergence of complexity. In: GR Needham, RE Page, M Delfinado-Baker, CE Bowman (eds) Africanized honey bees and bee mites. Ellis Horwood Limited, Chichester.

Crewe RM, Velthuis HHW (1980) False queens: a consequence of mandibular gland signals in worker honeybees. Naturwissenschaften 67:467

Darchen R (1956) La reine d' *Apis mellifica* et les constructions cirières. C R Hebd Seances Acad Sci D Sci Nat 243:439-441

Darchen R (1957) La reine d' *Apis mellifica*, les ouvrières pondeuses et les constructions cirières. Insectes Sociaux 4:321-325

Darchen R (1968) Le travail de la cire et la construction dans la ruche. In: R Chauvin (ed) Traitè de biologie de l'abeille. Masson, Paris.

de Groot AP, Voogt S (1954) On the ovary development in queenless worker bees (*Apis mellifera* L.). Experientia 10:384-385

Dreischer H (1956) Untersuchungen über die Arbeitstätigkeit und Drüsenentwicklung alterbestimmer Bienen im weisellosen Volk. Zool Jhrb Abt Allg Zool Physiol Tiere 66:429-472

Fell RD (1979) The production, recognition and treatment of queen cells in the honey bee (*Apis mellifera* L.) colony. Ph.D Thesis, Cornell University, Ithaca, New York U.S.A.

Fell RD, Morse RA (1984) Emergency queen cell construction in the honey bee colony. Insectes Sociaux 31:221-237

Ferguson AW, Free JB (1980) Queen pheromone transfer within honeybee colonies. Physiol Entomol 5:359-366

Free JB (1987) Pheromones of Social Bees. Chapman and Hall, London

Free JB, Ferguson AW, Simkins JR (1985) Influence of virgin honeybees (*Apis mellifera*) on queen rearing and foraging. Physiol Entomol 10:271-274

Garin H (1926) Observations on differences in size of cells in old and new combs. SABJ 3:39-40, 3:53-56

Gary NE (1961) Mandibular gland extirpation in living queen and worker honey bees (*Apis mellifera*). Ann Entomol Soc Am 54:529-531

Gary NE, Morse RA (1962) Queen cell construction in the honey bee (*Apis mellifera* L.) colonies headed by queens without mandibular glands. Proc R Entomol Soc Lond 37:76-78

Goetze G, Bessling BK (1959) Die Wirkung verschiedener Futterung der Honigbiene auf Wachserzeugung und Bautatigkeit. Z Bienenforsch 4:202-209

Hastings H (1990) Personal communication

Hemmling C, Koeniger N, Ruttner F (1979) Quantitative Bestimmung der 9-oxodecensäure im Lebenszyklus der Kapbiene (*Apis mellifera capensis* Escholtz.). Apidologie 10:227-240

Hepburn HR (1983) Comb construction by the African honeybee, *Apis mellifera adansonii* J Entomol Soc S Afr 46:87-101

Hepburn HR (1986) Honeybees and wax: An experimental natural history. Springer-Verlag, Berlin.

Hepburn HR (1988) Absconding in the African honeybee: the queen, engorgement and wax secretion. J Apic Res 27:95-102

Hepburn HR, Crewe RM (1990) Defining the Cape honeybee: reproductive traits of queenless workers. S Afr J Sci (in press).

Hepburn HR, Whiffler LA (1988) Dwarfs and queenballs in swarms of the Cape honeybee. S Afr J Sci 80:363-368

Hepburn HR, Hugo JJ, Mitchell D, Nijland MJM, Scrimgeour AG (1984) On the energetic costs of wax production by the African honeybee, *Apis mellifera adanonsii*. S Afr J Sci 80:363-368

Hepburn HR, Nefdt RJC, Whiffler LA (1988) Queenloss in the Cape honeybee: the interactions of brood, laying workers (false queens ?) and queen cells. S Afr J Sci 84:778-780

Hess G (1942) Über den Einfluss der Weisellösigkeit des fruchbarkkeitsvitamins E auf die Ovarian der Beinenarbeiterin. Schweiz Bienen-Ztg 1:33-109

Hornbostel HC (orig Melittophilus Theosebastus) (1744) Neue Entdeckung, wie das Wachs von Bienen Komt. Hamburg Vermis Bibliothek 2:45-62

Huber F (1814) Nouvelles observations sur les abeilles, 2. Translated (1926) Dadant, Hamilton.

Hunter J (1792) Observations on bees. Philos Trans R Soc 82:128-196

Jack RW (1916) Parthenogensis amongst the workers of the Cape Honey-bee. R Trans Entomol Soc Lond 61:396-403

Jay SC, Jay DM (1983) Wax production by aged worker honeybees of European and tropical African origin. J Apic Res 22:229-231

Kaissling KE, Renner M (1968) Antennale Rezeptoren für Queen Substance und Steizelduft bei der Honigbeine. Z Vergl Physiol 59:357-361

Korst PJAM, Velthuis HHW (1982) The nature of trophallaxis in honeybees. Insectes Sociaux 29:209-221

Kurstjens SP, Hepburn HR, Schoening FRL, Davidson BC (1985) The conversion of wax scales into comb wax by African honeybees. J Comp Physiol B 156:95-102

Langstroth LL (1853) Langstroth on the hive and the honeybee. (facsimile, 1977) Root, Medina

Lensky Y, Slabezki Y (1981) The inhibiting effect of the queen (*Apis mellifera* L.) foot-print pheromone on the construction of swarming queen cups. J Insect Physiol 27:313-323

Magnuson PC (1990) Personal communication

Moritz RFA, Kauhausen D (1984) Hybridization between *Apis mellifera capensis* and adjacent races of *Apis mellifera*. Apidologie 15:211-222

Morse RA, Gary NE (1963) Further studies of the responses of honeybee (*Apis mellifera* L.) colonies to queens with extirpated mandibular glands. Ann Entomol Soc Am 56:372-374

Muller WJ (1990) Personal communication.

Mussbichler A (1952) Die Beteutung ausserer Einflusse und der Corpora Allata bei der Afterweisel-entstehung von *Apis mellifera*. Zeit vergl Physiol 34:207-221

Onions GW (1912) South African "fertile worker bees". Agric J Un S Afr 3:720-728

Owens CD, Taber S (1973) Size and shape of comb construction by honey bees in a 1.2 m^3 box during one season. J Econ Entomol 66:1234-1236

Pain J (1955) Dosage biolgique et spectrographic de l'ectohormone des d'abeilles. C R Seaces acad D Sci Nat 240:670-672

Pain J (1961) Sur la phèromone des reines d'Abeilles et ses effets physiologiques. Ann Abeille 4:73-152

Pain J, Barbier M, Roger B (1967) Dosages individuels des acides cèto-9-dècene-2-oique et hydroxy-10-dècene-2-oique dans les tètes des reines et des ouvrières d'abeilles. Ann Abeille 10:45-52

Rajashekharappa BJ, Channabasavanna GP (1979) Importance of mated queen in comb expansion of the honeybee *Apis cerana*. Mysore J Agric Sci

13:51-54

Remnant R (1637) A discourse or historie of bees. Young, London (Republished in 1982 by the International Bee Research Association, U.K.)

Rösch GA (1927) Über die Bautätigkeit im Bienevolk und das Alter der Baubienen. Weiterer Beitrag zur Frage nach der Abeitsteilung im Bienenstaat. Z Vgl Physiol 6:265-298

Ruttner F (1977) The problem of the Cape bee (*A.m.capensisi* Escholtz): parthenogensis - size population - evolution. Apidologie 8:281-294

Ruttner F (1986) Geographical variabilty and classification. In: TE Rinderer (ed) Bee genetics and Breeding. Academic Press.

Ruttner F, Koeniger N, Veith HJ (1976) Queen substance bei eier legenden Arbeiterinnen der Honigbiene (*Apis mellifica* L.) Naturwissenschaften 9:434

Seeley TD (1979) Queen substance dispersal by messenger workers in honeybee colonies. Behav Ecol Sociobiol 5:391-415

Seeley TD (1985) Honeybee Ecology: A Study of Adapation in Social Life.

Princeton University Press, Princeton, New Jersey.

Shearer DA, Boch R, Morse RA, Laigo FM (1970) Occurence of 9-oxodec-trans-2-enoic acid in queens of *Apis dorsata*, *Apis cerana* and *Apis mellifera*. J Insect Physiol 16:1437-1441

Simpson J (1960) Induction of queen rearing in honeybee colonies by amputation of their queens' front legs. Bee World 4:286-287

Slessor KN, Kaminski L-A, King GGS, Borden JH, Winston ML (1988) Semiochemical basis of the retinue response to queen honeybees. Nature 332:354-356

Slessor KN, Kaminski L-A, King GGS, Winston ML (1990) Semiochemicals of the honeybee queen mandibular glands. J Chem Ecol 16:851-860

Smith FG (1958) Beekeeping observations in Tanganyika. Bee World 39:29-36

Smith FG (1961) The races of honeybees in Africa. Bee World 42:255-260

Taranov GF (1959) The production of wax in the honeybee colony. Bee World 40:113-121

Tribe GD (1983) What is the Cape bee ? SABJ 77-87

Tribe GD, Fletcher DJC (1976) Rate of development of the workers of *Apis mellifera adansoniiL.* In: Fletcher DJC (ed) African Bees, Taxonomy, Biology and Economic use. Apimondia.

Velthuis HHW (1970) Queen substance from the abdomen of the honeybee queen. Zeit vergl Physiol 70:210-222

Velthuis HHW (1972) Observations on the transmission of queen substance in the honeybee colony by the attendants of the queen. Behav 41:105-129

Velthuis HHW, van Es J (1964) Some functional aspects of mandibular glands of the queen honeybee. J Apic Res 3:11-16

Velthuis HHW, Ruttner F, Crewe RM (1990) Differentiation in Reproductive Physiology and Behaviour During the Development of Laying Worker Honey Bees. In: W Engels (ed) Social Insects: an evolutionary approach to Castes and Reproduction. Springer-Verlag

Verheijen-Voogd C (1959) How worker bees perceive the presence of their queens. Z Vgl Physiol 41:527-582

Vogt H (1911) Geometrie und Ökonomie der Bienenzelle. Trewend Granier, Breslau.

Appendices

Appendix A.1. The raw data from experiment 1 (Chapter 2).

Queen	CS	%B	WR	SA	WE	DIA	9ODA	9HDA	10HDA
MQ1	14642	79	549.7	47.2	2188.2	3.9	95.5	0.7	3.8
MQ1	6719	47	480.9	56.8	3406.8	4.5	98.3	0.7	1.0
MQ1	8720	77	472.7	53.8	3078.0	4.3	96.6	1.1	2.3
MQ1	7340	66	389.4	36.7	2309.3	4.1	0	42.9	57.1
QL1	3652	67	392.5	15.6	827.0	3.8			
QL1	1918	40	507.5	0	0	-			
QL1	5514	67	944.8	8.3	475.2	-			
QL1	4087	46	458.7	0	0	-			
MQS1	7201	73	472.7	66.0	3630.1	4.3	0	40	60
MQS1	14318	70	595.7	68.0	4745.8	4.2	75.4	1.1	23.5
MQS1	10806	69	669.6	32.5	1960.0	4.2	0	18	82
MQS1	6468	78	679.5	65.3	3755.4	4.3	99.1	0.4	0.5
MQS1	10092	87	469.0	105.5	6802.4	3.9	90.7	1.7	7.6
MQS1	9879	91	579.1	59.2	3434.6	4.6	*	*	*
MQS1	2641	85	416.5	34.3	1957.6	4.0	93.2	0.8	6
MQS1	9048	77	813.0	40.4	3099.0	4.0	*	*	*
MQS1	12393	46	484.8	30.9	2064.9	4.5	*	*	*
QLS1	12409	66	669.7	2.1	87.8	4.2			
QLS1	3083	73	701.4	18.8	1229.3	4.7			
QLS1	7442	76	593.4	24.4	1494.2	4.5			
QLS1	6663	78	*	30.3	1706.4	4.8			
CWSQ1	10898	82	536.6	46.3	2550.0	3.7	93.2	0.8	6.0
CWSQ1	4921	60	400.0	76.8	3792.0	3.9	0	40	60
CWSQ1	5069	64	473.4	41.8	2172.0	3.7	75.4	1.1	23.5
CWSQ1	4596	39	*	85.3	4447.3	3.6	0	18	82
SWCQ1	4949	77	676.6	76.5	3610.8	3.8	0	42.9	57.1
SWCQ1	11195	79	579.7	71.7	4109.9	3.7	95.5	0.7	3.8
SWCQ1	7303	*	*	89.9	4765.2	3.8	98.3	0.7	1

Queen	CS	%B	WR	SA	WE	DIA	9ODA	9HDA	1OHDA
SWCQ1	4522	74	659.5	72.3	3217.6	4.5	96.6	1.1	2.3
VQ1	3170	13	649.3	26.2	56.8	*			
VQ1	13437	46	415.4	26.2	1775.7	3.7			
VQ1	4096	48	273.9	0.2	22.0	*			
VQ1	4615	*	385.4	10.4	606.7	3.8			

* - missing data

MQ1 - *A.m.capensis* colonies given a mated *A.m.capensis* queen
MQS1 - *A.m.scutellata* colonies given a mated *A.m.scutellata* queen
QL1 - queenless *A.m.capensis* colonies
QLS1 - queenless *A.m.scutellata* colonies
CWSQ1 - *A.m.capensis* colonies given an *A.m.scutellata* queen
SWCQ1 - *A.m.scutellata* colonies given an *A.m.capensis* queen
VQ1 - colonies given a virgin *A.m.capensis* queen

CS - colony size
%B - the percentage of festoon bees bearing wax
WR - the amount of wax removed from 100 festoon bees divided by the number of bees bearing wax
SA - surface area (mm^2) of comb constructed /1000 bees
WE - weight (µg) of comb constructed /1000 bees
DIA - diameters of the cells that were constructed (mm)
9ODA - percentage of 9-oxo-2-decanoic acid
9HDA- percentage of 9-hydroxy-2-decanoic acid
1OHDA - percentage of 10-hydroxy-2-decanoic acid

The number after the colony description refers to the experiment number.

Appendix B.1. The raw data from experiment 2 (Chapter 3).

Queen	CS	%B	WR	SA	WE	DIA	9ODA	9HDA	10HDA
DO2	7618	58	682.8	0	0	-	45.5	53.9	0.8
DO2	3299	81	498.8	0	0	-	*	*	*
DO2	4847	94	905.3	0	0	-	45.4	54.2	0.4
MQ2	3642	47	389.4	5.1	499.7	5.0	45.5	53.9	0.8
MQ2	528	65	1543.1	6.7	429.9	4.6	*	*	*
MQ2	8257	50	524.0	0.1	5.9	4.9	45.4	54.2	0.4
SI2	6515	46	308.7	0.04	1.3	-	65.1	34.0	0.9
SI2	1158	81	871.6	0	0	-	42.5	56.7	0.8
SI2	4847	69	714.5	0	0	-	45.4	54.2	0.4
SI2	4485	41	78.0	0	0	-	61.5	0	38.5
SI2	2373	85	1207.1	0	0	-	78.3	0	21.7
MQ2	1112	61	472.1	15.2	786.0	5.1	65.1	34.0	0.9
MQ2	4087	81	335.8	0	0	-	42.5	56.7	0.8
MQ2	8257	50	524.0	9.9	5.9	4.9	45.4	54.2	0.4
MQ2	7692	84	411.9	1.4	98.9	4.8	61.5	0	38.5
MQ2	3581	91	711.0	0.7	50.9	4.8	78.3	0	21.7
DEAD2	5375	41	409.8	1.8	189.0	6.2	*	*	*
DEAD2	1770	60	1725.0	0	0	-	*	*	*
DEAD2	3568	61	472.1	2.9	186.9	4.8	*	*	*
MQ2	3642	47	389.4	5.1	499.7	5.0	45.5	53.9	0.8
MQ2	528	65	1543.1	6.7	429.9	4.6	*	*	*
MQ2	1112	61	472.1	15.2	786.0	5.1	65.1	34.0	0.9
QL2	4285	56	308.9	0	0	-			
QL2	233	42	526.2	0	0	-			
QL2	8943	91	856.0	0	0	-			

DO2 - colonies given a queen in a double cage
MQ2 - colonies given a "free" queen (the queen was able to move freely across the comb
SI2 - colonies given a queen in a single cage
DEAD2 - colonies given a dead queen
QL2 - queenless colonies

* - missing values
- - no measurements made because of a lack of data

%B - percentage of festoon bees bearing wax
WR - amount of wax (μg/1000 bees) removed from 100 bees divided by the number of bees bearing wax in a festoon
SA - surface area (mm^2) of comb constructed /1000 bees
WE - weight (μg) of comb constructed /1000 bees
DIA - diameters (mm) of cells constructed
9ODA - the percentage amount of 9-oxo-2-decanoic acid
9HDA - the percentage amount of 9-hydroxy-2-decanoic acid
10HDA - the percentage amount of 10-hydroxy-2-decanoic acid

Appendix C.1. Raw data from experiment 3 (Chapter 4). *

Queen	CS	%B	WR	SA	WE	DIA	10HDA	9HDA	9ODA
SH3	10489	46	458.7	0.4	37.2	-	41.6	37.9	20.5
SH3	5829	48	408.3	3.3	291.6	5.1	8.3	13.9	77.8
MG3	13706	49	420.4	5.3	336.3	4.9	14.9	35.1	50.0
MG3	8078	44	593.2	37.5	3158.0	5.4	37.0	0	63.0
MG3	8155	73	387.7	0	0	-	6.5	2.9	90.6
MG3	9340	42	414.3	6.8	414.3	5.4	6.1	6.0	87.9
T3	10926	22	409.1	15.1	1340.8	5.0	3.6	71.1	25.3
T3	13400	-	-	17.3	1346.3	4.8	27.5	49.0	23.5
T3	2474	59	489.8	28.7	2114.0	5.5	30.9	40.9	28.2
T3	658	79	884.8	0	0	-	22.0	72.6	6.8
T3	4228	50	340.0	0	0	-	8.1	65.3	41.9
MQ3	4800	66	569.7	0	0	-	*	*	*
MQ3	1487	73	423.3	0	0	-	2.0	40.6	57.4
MQ3	3644	-	-	0	0	-	16.7	0	83.3
MQ3	16959	47	91.5	3.6	71.2	5.1	57.8	33.3	8.9
MT3	556	89	424.7	0	0	-	3.2	0	96.8
MT3	1075	81	551.9	0	0	-	81.8	18.2	0
MT3	1826	71	602.8	0	0	-	8.8	0	91.2
DEAD3	6033	48	226.7	0	0	-			
DEAD3	5041	58	*	0	0	-			

*

- - no measurement made as there was either insufficient data or data was not available for collection

* - data was missing

SH3 - colonies given a sham operated queen
MG3 - colonies given a queen without mandibular glands but tergite glands intact
T3 - colonies given a queen with mandibular glands but tergite glands occluded
MQ3 - colonies given a queen with intact mandibular and tergite glands
MT3 - colonies given a queen without mandibular glands and tergite glands occluded
DEAD3 - colonies given a dead queen
The number after the experimental condition refers to the experiment number.

CS - colony size
%B - the percentage of festoon bees bearing wax
WR - the amount of wax (μg/1000 bees) removed from 100 bees divided by the number of festoon bees bearing wax
SA - surface area (mm^2) of comb constructed /1000 bees
WE - weight (μg) of comb constructed /1000 bees
DIA - diameters (mm) of the cells constructed
10HDA - percentage amount of 10-hydroxy-2-decanoic acid
9HDA - percentage amount of 9-hydroxy-2-decanoic acid
9ODA - percentage amount of 9-oxo-2-decanoic acid

Appendix D.1. Raw data from experiment 4 (Chapter 5). *

Queen	CS	%B	WR	SA	WE	DIA	9ODA	9HDA	1OHDA	1OHHDA
MQ4	8600	74	212.2	7.1	582.6	4.9	*	*	*	*
MQ4	14901	89	856.2	21.4	1712.0	5.0	16.5	77.2	2.4	3.9
MQ4	8312	66	315.2	21.5	1031.0	4.8	100	0	0	0
MQ4	7710	53	264.2	0	0	-	83.7	4.9	11.4	0
MQ4	5292	61	224.6	0	0	-	100	0	0	0
MQ4	1558	49	320.4	29.9	3655.0	4.7	36.6	18.8	29.9	16.1
MQ4	1470	49	273.5	33.2	2739.5	4.9	52.2	43.3	4.5	0
MQ4	10991	76	206.6	8.4	562.3	4.8	97.7	2.1	0.2	0
MQ4	1733	92	543.5	16.2	1452.4	5.0	61.4	36.5	2.1	0
MG4	12195	63	330.2	0	0	-	24.0	19.0	33.4	23.6
MG4	8897	54	231.5	0.8	124.8	-	7.0	77.2	7.3	8.5
MG4	12205	67	429.9	0.2	19.8	-	23.3	59.6	1.1	16.0
MG4	7553	65	193.8	2.4	131.1	5.0	61.3	8.8	28.4	1.5
MG4	4643	45	140.0	0	0	-	25.8	0	0	74.2
MG4	12047	61	259.0	21.4	1643.6	*	*	*	*	*
MG4	7544	80	326.3	4.2	741.0	5.1	*	*	*	*
T4	4578	83	392.8	15.9	1151.2	4.9	*	*	*	*
T4	11676	79	331.6	17.9	1359.2	5.2	83.3	13.8	2.9	0
T4	4040	86	539.5	0	0	-	54.3	39.5	5.3	0.9
T4	12316	86	445.3	10.2	822.5	5.0	70.2	25.4	2.6	1.8
T4	13020	54	214.8	7.3	932.4	5.0	89.8	0	0	10.2
T4	13530	78	483.3	28.5	3606.1	4.9	10.2	45.3	44.5	0
T4	21602	80	225.0	6.7	1468.4	5.4	90.3	0.8	4.6	4.3
MT4	6987	65	332.3	2.8	246.2	4.9	*	*	*	*
MT4	15958	74	316.2	0	0	-	*	*	*	*
MT4	12622	79	403.8	0.4	43.7	5.0	*	*	*	*
MT4	9916	48	400.0	43.4	3638.9	*	*	*	*	*
MT4	16718	58	439.7	15.3	3484.1	4.9	81.7	7.1	5.4	5.8
MT4	12346	59	247.5	0	0	-	75.0	0	16.7	8.3
DEAD4	13604	68	233.8	0	0	-				
DEAD4	9601	47	121.3	0	0	-				
DEAD4	1854	72	448.6	0	0	-				

Queen	CS	%B	WR	SA	WE	DIA	9ODA	9HDA	1OHDA	1OHHDA
DEAD4	10732	69	271.0	7.8	1320.4	5.1				
C04	4355	61	159.0	4.9	725.6	*	70.3	29.7	0	0
C04	6839	71	425.4	0.4	35.3	*	97.7	2.1	0.2	0
C04	15921	60	498.3	7.4	557.1	5.0	61.4	36.5	2.1	0
FH4	5569	67	735.8	0	0	*	16.5	77.2	2.4	3.9
FH4	6157	40	650.0	1.5	350.7	*	100	0	0	0
FH4	2385	63	274.6	0	0	*	100	0	0	0
FA4	5499	68	433.8	0	0	-				
FA4	6157	57	269.5	0	0	-				
FA4	2543	59	131.6	0	0	-				
MGH4	4181	63	234.9	3.3	485.5	5.3	7.0	77.2	7.3	8.5
MGH4	7037	58	358.6	8.2	740.3	4.8	64.1	0	0	35.9
MGH4	3757	38	131.6	0	0	-	25.8	0	0	74.2
MGA4	6700	72	558.3	0	0	-				
MGA4	7744	64	406.3	0	0	-				
MGA4	3757	60	180.0	0	0	-				
TH4	980	59	372.9	0	0	-	70.2	25.4	2.6	1.8
TH4	8574	61	344.3	26.6	1947.7	5.1	89.8	0	0	10.2
TH4	8974	83	716.9	14.2	1121.0	5.1	90.3	0.8	4.6	4.3
TA4	6461	66	390.9	0	0	-				
TA4	5767	64	234.4	0	0	-				
TA4	9639	66	407.6	0	0	-				
MTH4	9771	63	504.8	25.0	1745.0	5.1	55.2	19.5	25.3	0
MTH4	3173	63	192.3	0	0	-	81.7	7.1	5.4	5.8
MTH4	3475	63	415.9	0	0	-	75	0	16.7	8.3
MTA4	1275	38	181.6	0	0	-				
MTA4	12632	76	1001	0	0	-				
MTA4	6342	72	298.6	0	0	-				
DEH4	1203	50	268.0	0	0	-				
DEH4	5962	60	153.3	0	0	-				
DEH4	6240	81	286.4	0	0	-				

Queen	CS	%B	WR	SA	WE	DIA	9ODA	9HDA	1OHDA	1OHDAA
DEA4	6048	76	377.6	0	0	-				
DEA4	4739	81	498.8	0	0	-				
DEA4	6240	83	384.3	0	0	-				

* "Free queens" (colonies given a whole queen)

MQ4 - colonies given a "free queen" with intact mandibular and tergite glands
MG4 - colonies given a "free queen" without mandibular glands but tergite glands intact
T4 - colonies given a "free queen" with mandibular glands but tergite glands occluded
MT4 - colonies given a "free queen" without mandibular glands and tergite glands occluded
DEAD4 - colonies given a dead queen (whole queen) with intact mandibular and tergite glands

Queens in a division board

CO4 - colonies given a queen with intact mandibular and tergite glands in a division board control
FH4 - colonies given access to the head of a queen with intact mandibular glands (tergite glands intact) in a division board
FA4 - colonies given access to a queen abdomen with intact tergite glands (mandibular glands intact) in a division board
MGH4 - colonies given access to the head of a queen without mandibular glands (tergite glands intact) in a division board
MGA4 - colonies given access to the abdomen of a queen with intact tergite glands (mandibular glands occluded) in a division board
TH4 - colonies given access to the head of a queen with mandibular glands (tergite glands occluded) in a division board
TA4 - colonies given access to the abdomen of a queen with tergite glands occluded (mandibular glands intact) in a division board
MTH4 - colonies given access to the head of a queen without mandibular glands (tergite glands occluded) in a division board
MTA4 - colonies given access to the abdomen of a queen with tergite glands occluded (mandibular glands extirpated) in a division board
DEH4 - colonies given access to head of a dead queen with mandibular glands (tergite glands intact)in a division board
DEA4 - colonies given access to the abdomen of a dead queen with tergite glands intact (mandibular glands intact) in a division board

The number after each experimental condition refers to the experiment number.

%B - percentage of festoon bees bearing wax
WR - amount of wax (μg/1000 bees) removed from 100 festoon bees divided by the number of festoon bees bearing wax
SA - surface area (mm^2) of comb constructed /1000 bees
WE - weight (μg) of comb constructed /1000 bees
DIA - diameters (mm) of the cells constructed
9ODA - percentage amount of 9-oxo-2-decanoic acid
9HDA - percentage amount of 9-hydroxy-2-decanoic acid
10HDA - percentage amount of 10-hydroxy-2-decanoic acid
10HHDA - percentage amount of 10-hydroxy-decanoic acid

- - no data or not sufficient data collected for the condition
* - missing data

Appendix E.1. The raw data from experiment 1 (April-May 1989) *

QUEEN	CS	%B	WR	SA	WE	DIA	HTOT	9ODA	9HDA	10HDA
MQ5	7470	71	404.2	21.1	1364.3	5.1	*	*	*	*
MQ5	11686	-	-	15.0	1175.8	4.9	123.7	94.6	4.2	1.2
MQ5	6922	65	181.5	1.9	346.7	4.9	231.8	98.6	1.4	0
VQ5	8025	74	494.6	0	0	-	*	*	*	*
VQ5	1548	76	373.7	0	0	-	*	*	*	*
VQ5	18552	80	537.5	5.9	502.9	5.0	558.1	36.1	0.3	63.6
VQ5	1789	-	-	0	0	-	*	*	*	*
VQ5	7192	-	-	0	0	-	240.2	94.7	0	5.3
VQ5	6533	-	-	19.6	1428.1	4.8	169.1	90.5	7.0	2.5
VQ5	8720	-	-	0	0	-	2.3	100	0	0
VQ5	4356	82	361.0	0	0	-	57.2	99.8	0.2	0
MGV5	3725	74	574.3	0	0	-	*	*	*	*
MGV5	11028	82	436.3	0	0	-	0.1	100	0	0
MGV5	4782	72	327.8	0	0	-	57.2	99.8	0.2	0
MTV5	18312	-	-	0	0	-	1.1	45.5	0	54.5
MTV5	2437	-	-	0	0	-	4.0	100	0	0
MTV5	7580	-	-	0	0	-	1.1	45.5	0	54.5
DEAD5	5810	77	210.4	0	0	-	*	*	*	*
DEAD5	12714	70	254.3	0	0	-	575.0	96.8	2.8	0.4
DEAD5	2975	51	143.1	0	0	-	*	*	*	*
DEAD5	7200	74	245.9	0	0	-	*	*	*	*
DEAD5	9184	88	352.3	0	0	-	*	*	*	*
DEAD5	10249	89	411.2	0	0	-	241.3	74.5	1.9	23.6
DEAD5	6997	-	-	0	0	-	*	*	*	*
DEAD5	3160	-	-	0	0	-	*	*	*	*
DEAD5	6654	58	271.8	0	0	-	*	*	*	*
DEAD5	1038	-	-	0	0	-	*	*	*	*
DEAD5	3123	81	125.9	0	0	-	296.7	100	0	0
DEAD5	10787	76	218.4	0	0	-	*	*	*	*
DEAD5	1492	-	-	0	0	-	353.0	90.7	9.3	0
DEAD5	2678	-	-	0						
DEAD5	9387	-	-	0	0	-	*	*	*	*
DEAD5	5618	88	354.5	2.1	182.2	5.1	78.6	98.7	1.3	0

QUEEN	CS	%B	WR	SA	WE	DIA	HTOT	9ODA	9HDA	1OHDA
QL5	4976	43	209.3	0	0	-				
QL5	10657	-	-	0	0	-				
QL5	2558	80	400.0	0	0	-				
QL5	9063	82	224.4	0	0	-				
QL5	10314	-	-	0	0	-				

*

- - no festoons were present in the colony at the time of sampling
* - the queen head was not analyzed

MQ5 - colonies given a mated queen with intact mandibular and tergite glands
VQ5 - colonies given a virgin queen with intact mandibular and tergite glands
MGV5 - colonies given a virgin queen without mandibular glands but tergite glands intact
MTV5 - colonies given a virgin queen without mandibular glands and tergite glands occluded
DEAD5 - colonies given a dead virgin queen
QL5 - queenless colonies

QUEEN - the type of queen given to each colony
CS - colony size
%B - the percentage bees bearing wax in a festoon
WR - the weight of the wax scales removed from 50 bees divided by the number of bees bearing wax (μg/1000 bees)
SA - the surface area (mm^2) of comb constructed per 1000 bees
WE - the weight (μg) of comb constructed per 1000 bees
DIA - cell diameter (mm) of the constructed comb
HTOT - total amount of acids present on the queen head (μg/head)
9ODA - the percentage amount of 9ODA on the queen head
9HDA - the percentage amount of 9HDA on the queen head
1OHDA - the percentage amount of 1OHDA on the queen head

Appendix E.2. The raw data from experiment 2 (December 1989 - January 1990) *

QUEEN	CS	%B	WR	SA	WE	DIA	HTOT	9ODA	9HDA	10HDA
MQ6	18080	-	-	19.4	2477.9	4.8	20.7	83.6	14.5	1.9
MQ6	8683	78	466.7	30.7	2936.8	4.5	*	*	*	*
MQ6	11232	-	-	0	0	-	5.8	100	0	0
MQ6	6607	82	492.7	98.0	6114.7	4.4	85.8	24.6	73.5	1.9
MQ6	8340	42	233.3	56.5	4436.5	4.6	11.7	66.7	33.3	0
MQ6	12510	78	512.8	90.6	7713.8	4.5	44.1	80	20	0
VQ6	8173	-	-	17.8	1590.6	4.5	*	*	*	*
VQ6	8118	-	-	51.6	4742.5	4.6	6.9	100	0	0
VQ6	19322	80	457.5	13.3	998.9	-	7.0	58.6	0	41.4
VQ6	3466	70	280.0	0	0	-	*	*	*	*
VQ6	7276	76	307.9	5.8	659.7	4.7	197.4	84.2	0	15.8
VQ6	8970	94	470.2	17.3	1928.7	4.5	*	*	*	*
MGV6	11241	-	-	0	0	-	7.1	100	0	0
MGV6	4726	-	-	107.5	11236	4.7	12.2	100	0	0
MGV6	11357	90	513.3	26.4	2288.3	4.6	*	*	*	*
MGV6	3549	65	380	0	0	-	3.7	62.2	0	37.8
MGV6	6997	88	547.7	28.5	2529.7	4.5	*	*	*	*
TV6	9638	76	513.2	22.3	2874.0	4.7	224.0	68.4	2.2	29.4
TV6	2891	75	416.7	0	0	-	8.4	61.9	25.0	13.1
TV6	3939	92	245.5	0	0	-	*	*	*	*
DEAD6	6765	-	-	0	0	-	*	*	*	*
DEAD6	8739	-	-	0	0	-	*	*	*	*
DEAD6	9489	-	-	0	0	-	*	*	*	*
DEAD6	6932	-	-	1.8	187.5	6.1	11.6	100	0	0
DEAD6	4050	-	-	7.9	444.4	4.9	*	*	*	*
DEAD6	14531	-	-	0	0	-	0.5	60	0	40
DEAD6	6070	-	-	1.7	428.3	6.4	*	*	*	*
DEAD6	4328	70	237.1	8.2	237.1	4.5	*	*	*	*
DEAD6	12047	-	-	0.3	33.2	-	*	*	*	*
DEAD6	6320	74	343.2	30.4	2436.7	-	*	*	*	*
DEAD6	4624	64	278.1	0	0	-	*	*	*	*
DEAD6	9934	74	335.1	0.1	10.1	-	*	*	*	*
DEAD6	16393	-	-	0	0	-	17.9	55.3	2.8	41.9

QUEEN	CS	%B	WR	SA	WE	DIA	HTOT	9ODA	9HDA	1OHDA
FHV6	4096	90	222.2	0	0	-	5.4	100	0	0
FHV6	*	-	-	0	0	-	0	0	0	0
FHV6	5774	-	-	0	0	-	*	*	*	*
FHV6	266	56	285.7	0	0	-	6.8	29.4	0	70.6
FVA6	973	-	-	0	0	-				
FVA6	*	64	493.8	0	0	-				
FVA6	760	-	-	0	0	-				
FVA6	9599	-	-	0	0	-				
QL6	16560	70	351.4	0	0	-				
QL6	4522	-	-	0.9	66.3	-				

*

- - no festoons were present at the time of sampling or the colonies were not weighed

* - queen heads were not analyzed

MQ6 - colonies given a mated queen with intact mandibular and tergite glands

VQ6 - colonies given a virgin queen with intact mandibular and tergite glands

MGV6 - colonies given a virgin queen without mandibular glands but tergite glands intact

TV6 - colonies given a virgin queen with mandibular glands but tergite glands occluded

DEAD6 - colonies given a dead virgin queen

FHV6 - colonies having access to the head of a virgin queen with intact mandibular glands in a division board

FAV6 - colonies having access to the abdomen of a virgin queen with intact tergite glands in a division board

QL6 - queenless colonies

QUEEN - type of queen given to a colony

CS - colony size

%B - the percentage bees bearing wax in a festoon

WR - the weight of the wax scales removed from 50 bees divided by the number of bees bearing wax (μg/1000 bees)

SA - the surface area (mm^2)of comb constructed per 1000 bees

WE - the weight (μg) of comb constructed per 1000 bees

DIA - cell diameters (mm) of the constructed comb

HTOT - total amount of acids present in the queen head (μg/head)

9ODA - the percentage amount of 9ODA present on the queen head

9HDA - the percentage amount of 9HDA present on the queen head

1OHDA - the percentage amount of 1OHDA present on the queen head

Appendix F.1. The colony size and number of emergency queen cells constructed by each colony from experiments 2-7. *

Queen	Colony size	Number of queen cells constructed
MQ2	3642	0
MQ2	528	0
MQ2	8257	0
MQ2	1112	0
MQ2	4087	0
MQ2	8257	0
MQ2	7692	0
MQ2	3581	0
MQ2	3642	0
MQ3	4800	2
MQ3	1487	2
MQ3	3644	0
MQ3	16959	0
MQ4	8600	0
MQ4	14901	0
MQ4	8312	0
MQ4	7710	0
MQ4	5292	0
MQ4	15587	0
MQ4	14707	0
MQ4	10991	0
MQ4	17330	5
MQ5	7469	0
MQ5	11686	0
MQ5	6922	0
MQ6	18080	1
MQ6	8683	0
MQ6	11232	2
MQ6	6607	0
MQ6	8340	0
MQ6	12510	0
MQ7	5551	0
MQ7	2428	0

Queen	Colony size	Number of queen cells constructed
MQ7	9453	0
MQ7	8683	0
MQ7	4180	0
MQ7	4263	0
VQ5	8025	0
VQ5	1548	0
VQ5	18552	0
VQ5	1789	0
VQ5	7191	0
VQ5	6533	0
VQ5	8720	0
VQ5	4356	0
VQ6	8173	1
VQ6	8118	0
VQ6	19322	0
VQ6	3466	0
VQ6	7276	0
VQ6	8970	0
VQ7	5051	5
VQ7	2336	0
VQ7	287	0
MGM3	13706	1
MGM3	8078	1
MGM3	8155	5
MGM3	9340	4
MGM4	12195	5
MGM4	8897	0
MGM4	12205	5
MGM4	7553	0
MGM4	4643	0
MGM4	12047	3
MGM4	7544	14
MGV5	3725	0
MGV5	11028	4
MGV5	4782	0
MGV6	11241	0
MGV6	4726	0

Queen	Colony size	Number of queen cells constructed
MGV6	11537	0
MGV6	3549	0
MGV6	6997	0
MGV7	955	0
MGV7	1186	0
MGV7	4281	0
TM3	10926	0
TM3	13400	5
TM3	2474	0
TM3	658	0
TM3	4228	0
TM4	4578	0
TM4	11676	0
TM4	4040	1
TM4	12316	0
TM4	13020	22
TM4	13530	0
TM4	21602	1
TV6	9638	0
TV6	2891	0
TV6	3939	0
TV7	8322	0
TV7	1196	3
TV7	6849	0
TV7	7497	0
TV7	5134	0
MTM3	556	0
MTM3	1075	0
MTM3	1826	2
MTM4	8461	3
MTM4	6987	1
MTM4	15958	17
MTM4	12622	1
MTM4	9916	0
MTM4	16718	7
MTM4	12346	9

Queen	Colony size	Number of queen cells constructed
MTV5	18312	0
MTV5	2437	0
MTV5	7580	0
MTV7	6552	0
DEAD2	5375	0
DEAD2	1770	0
DEAD2	3568	8
DEAD3	6033	1
DEAD3	5041	6
DEAD4	13604	6
DEAD4	9601	10
DEAD4	1854	6
DEAD4	10732	0
DEAD4	16245	0
DEAD5	5810	7
DEAD5	12714	0
DEAD5	2975	2
DEAD5	7200	5
DEAD5	9184	8
DEAD5	10249	1
DEAD5	6997	5
DEAD5	3160	4
DEAD5	6654	11
DEAD5	1038	0
DEAD5	3123	4
DEAD5	10787	3
DEAD5	1492	0
DEAD5	2679	4
DEAD5	9387	7
DEAD5	5616	3
DEAD6	6765	2
DEAD6	8739	4
DEAD6	9489	6
DEAD6	6932	9
DEAD6	4050	6
DEAD6	14531	11
DEAD6	6070	3
DEAD6	4328	6

Queen	Colony size	Number of queen cells constructed
DEAD6	12047	7
DEAD6	6320	9
DEAD6	4624	8
DEAD6	9934	10
DEAD6	16393	6
DIED2	927	0
DIED2	2039	0
DIED2	1743	0
DIED2	1242	0
DIED2	*	4
DIED2	2474	0
DIED4	13419	17
DIED4	11399	20
DIED4	4884	16
DIED4	8368	4
DIED4	10732	4
DIED4	2595	6
DIED4	6719	20
DIED4	9823	8
DIED7	3337	2
DIED7	5551	5
DIED7	11769	0
DIED7	1492	6
DIED7	5607	9
DIED7	11695	3
DIED7	*	0
DIED7	7859	6
DIED7	*	0
DIED7	6580	13
DIED7	4940	0
DIED7	983	3
DIED7	4180	0
DIED7	5709	15
DIED7	5718	0
QL4	6245	14
QL5	4876	8
QL5	10657	9
QL5	2558	2
QL5	9063	7

Queen	Colony size	Number of queen cells constructed
QL5	10314	15
QL6	16560	12
QL6	4522	5
QL7	1066	2
QL7	742	0
QL7	2966	5
DO2	7618	0
DO2	3299	1
DO2	4847	0
SI2	6515	10
SI2	1158	0
SI2	4847	0
SI2	4485	7
SI2	2373	0
SH3	10489	0
SH3	5829	0
CO4	4355	2
CO4	6839	1
CO4	15921	1
FH4	5569	17
FH4	6157	4
FH4	2385	0
FH7	3234	18
FH7	10870	0
FH7	6747	8
FH7	4958	0
FH7	2836	1
FH7	4847	2
FA4	5499	8
FA4	1546	9
FA4	2543	8
FA7	*	6
FA7	*	4
FA7	6181	1

Queen	Colony size	Number of queen cells constructed
FA7	3364	6
FA7	6478	0
FA7	2781	2
TH4	980	0
TH4	8574	0
TH4	8974	3
TA4	6461	16
TA4	5767	1
TA4	9639	0
MTH4	9771	3
MTH4	3173	3
MTH4	3475	0
MTA4	1275	0
MTA4	12632	0
MTA4	6342	0
DEH4	1203	4
DEH4	5962	0
DEH4	6240	0
DEA4	6048	2
DEA4	4739	0
DEA4	12642	0
DIH4	4959	4
DIH4	5944	11
DIH4	7714	5
DIH4	8779	1
DIH4	2563	6
DIH4	3284	0
DIH7	8072	10
VH6	4096	5
VH6	6394	2
VH6	5774	0
VH6	1668	12
VA6	973	1
VA6	*	3

Queen	Colony size	Number of queen cells constructed
VA6	760	0
VA6	10425	0
T17	2781	1
T17	4124	0
T17	4819	0
T17	4726	0
T27	1946	0
T27	6209	0
T27	10518	0
T27	7970	0
T37	2178	3
T37	4309	3
T37	2133	1
T37	1455	0
T1M7	3689	0
TIM7	3077	1
T1M7	4949	0
T1M7	7506	0
T2M7	6441	0
T2M7	6682	0
T2M7	862	0
T2M7	5051	1
T3M7	3939	0
T3M7	6950	2
T3M7	9499	1
T1MT7	7053	0
T1MT7	1761	8
T1MT7	3337	1
T1MT7	4448	10
T2MT7	6534	1
T2MT7	2039	2
T2MT7	4217	3
T2MT7	8804	8
T3MT7	5004	7
T3MT7	4077	2

Queen	Colony size	Number of queen cells constructed
T3MT7	880	1

*

* - missing data
MQ - colonies given a mated queen with intact mandibular and tergite glands
VQ - colonies given a virgin queen with intact mandibular and tergite glands
MGM - colonies given a mated queen without mandibular glands but tergite glands intact
MGV - colonies given a virgin queen without mandibular glands but tergite glands intact
TM - colonies given a mated queen with mandibular glands but tergite glands occluded
TV - colonies given a virgin queen with mandibular glands but tergite glands occluded
MTM - colonies given a mated queen without mandibular glands and tergite glands occluded
MTV - colonies given a virgin queen without mandibular glands and tergite glands occluded
DEAD - colonies given a dead mated or virgin queen (with/without mandibular and/or tergite glands)
DIED - colonies given a mated or virgin queen, but the queen died during the week of the experiment
QL - queenless colonies
DO - colonies given a mated queen with intact mandibular and tergite glands but the queen was in a double cage
SI - colonies given a mated queen with intact mandibular and tergite glands but the queen was in a single cage
SH - colonies given a mated queen with intact mandibular and tergite glands, but the genae had an incision made in them
CO - colonies given a queen in a division board, the bees had access to the head and abdomen of the queen with intact mandibular and tergite glands
FH - colonies given a mated queen in a division board, the bees had access to the head with intact mandibular glands
FA - colonies given a mated queen in a division board, the bees had access to the abdomen with intact tergite glands
MGH - colonies given a mated queen in a division board, the bees had access to the head without mandibular glands
MGA - colonies given a mated queen in a division board, the bees had access to the abdomen with intact tergite glands
TH - colonies given a mated queen in a division board, the bees had access to the head with intact mandibular glands
TA - colonies given a mated queen in a division board, the bees had

access to the abdomen with tergite glands occluded
MTH - colonies given a mated queen in a division board, the bees had access to the head without mandibular glands
MTA - colonies given a mated queen in a division board, the bees had access to the abdomen with tergite glands occluded
DEH - colonies given a dead mated queen in a division board, the bees had access to the head with intact mandibular glands
DEA - colonies given a dead mated queen in a division board, the bees had access to the abdomen with intact tergite glands
DIH - colonies given a mated queen in a division board who died during the week of the experiment, the bees had access to the head with inatct mandibular glands
DIA - colonies given a mated queen in a division board who died during the week of the experiment, the bees had access to the abdomen with intact tergite glands
FVH - colonies given a virgin queen in a division board, the bees had access to the head with intact mandibular glands
FVA - colonies given a virgin queen in a division board, the bees had access to the abdomen with intact tergite glands
T1 - colonies given a mated queen with intact mandibular and tergite glands, but 1 pair of tarsal glands removed
T2 - colonies given a mated queen with intact mandibular and tergite glands, but 2 pairs of tarsal glands removed
T3 - colonies given a mated queen with intact mandibular and tergite glands, but all tarsal glands removed
T1M - colonies given a mated queen without mandibular glands but tergite glands intact and 1 pair of tarsal glands removed
T2M - colonies given a mated queen without mandibular glands but tergite glands intact and 2 pairs of tarsal glands removed
T3M - colonies given a mated queen without mandibular glands but tergite glands intact and all tarsal glands removed
T1MT - colonies given a mated queen without mandibular glands and tergite glands occluded and 1 pair of tarsal glands removed
T2MT - colonies given a mated queen without mandibular glands and tergite glands occluded and 2 pairs of tarsal glands removed
T3MT - colonies given a mated queen without mandibular glands and tergite glands occluded and all tarsal glands removed
The number after each experimental condition refers to the experiment number.