THE MANDIBULAR GLAND SECRETIONS OF THE CAPE HONEYBEE (*APIS MELLIFERA CAPENSIS* ESCH.): FACTORS AFFECTING THE PRODUCTION OF THE CHEMICAL SIGNAL AND IMPLICATIONS FOR FURTHER DEVELOPMENT OF BEEKEEPING IN SOUTH AFRICA

Dissertation submitted in fulfilment of the
requirements for the degree of

DOCTOR OF PHILOSOPHY

of

RHODES UNIVERSITY

by

GEORGINA ELIZABETH JONES

February 2000

ABSTRACT

The chemical composition of the mandibular gland extracts of *Apis mellifera capensis* virgin queens was analysed by gas chromatography-mass spectroscopy. Thirty-seven compounds from various chemical groups including aliphatic and aromatic acids and diacids, phenols, alkanes, amino acids and sugars were identified. Among the identified compounds were the queen mandibular pheromone components 9ODA, 9HDA, HVA and HOB and the other aliphatic acids and phenols considered to be the major components of *A.m. capensis* mandibular glands.

Ontogenetic changes in the concentration of the mandibular gland secretions of virgin queens were largely quantitative in nature with the total volume and that of most of the compounds increasing with queen age. The final level of 9ODA is reached at the premating stage, approximately three days after emergence, when it comprises approximately 87% of the major constituents of the mandibular gland signal. Hostile reactions by workers towards introduced virgin queens can be correlated to the relative proportion of 9ODA present in the mandibular gland secretions. This seems to indicate that it is the complete spectrum of the signal and not individual compounds that determine worker reaction towards introduced queens.

Keeping queens singly, with or without workers, in an incubator and in small mating nucleus hives proved to be the most successful methods of queen rearing in respect to survival rate in *A.m. capensis*. The presence of workers during the ageing of virgin queens was found to significantly affect the chemical composition of the mandibular gland secretions of queens. The reaction of workers towards introduced virgin queens reared under different holding conditions varied, with queens reared with workers eliciting significantly less hostile reactions from workers than those reared without workers.

Mated queens from five localities in the Eastern Cape were characterised on the basis of the chemical composition of their mandibular gland secretions and the ratio of 9ODA:10HDA. No significant differences were detected and none of the queens sampled could be considered to be *A.m. capensis* based on their mandibular gland signal.

The findings of this study provide baseline data for the development of a queen-rearing program tailored to the specific requirements of *A.m. capensis.*

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

APPENDIX 1

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to the following, without whose help this thesis would not have been possible:

Professor Randall Hepburn for his tireless supervision and guidance throughout this study; and for funding the work;

Mike Allsopp for providing queen samples, and especially for his participation in endless discussions which provided the foundation for many of ideas and concepts examined in this study;

Aubrey Sonnemann for his untiring assistance and advice with the GC and GC-MS analysis;

Terry Butterworth for constructing the observation hives and his many valuable suggestions for overcoming technical difficulties;

Professor Robin Crewe of the University of Pretoria for providing some of the mandibular gland standards;

Professor Mike Davies-Coleman and Kerry McPhail of the Rhodes University Chemistry Department for their assistance with the chemical classification and structures of the compounds identified in the samples;

Professor Sarah Radloff of the Rhodes University Statistics Department for her advice on the statistical analyses of the data;

Dr Gay Youthed, Sally Guye and Janet Longman for their diligent proof-reading;

My fellow "bee-slaves", especially Sacha Reece, Shiresh Ranchhod, Pete Illgner, Gavin Johnston and Rob van Hille, who proved that "slave-life wasn't all bad";

Terry and Janet Longman and the chinchillas for their support and providing me with a home in Grahamstown; and

My parents, Ann and Tim Jones, for their unquestioning support and encouragement throughout my academic years.

The National Research Foundation (Foundation for Research Development) and Rhodes University are gratefully acknowledged for their financial assistance.

To kindred spirits with whom I have shared many memorable experiences, thank you and continue to live by the philosophy:

"Life is not worth living unless you risk everything
Unless you risk everything you don't have a life"

CHAPTER ONE

GENERAL INTRODUCTION
CHEMICAL COMMUNICATION, THE CAPE HONEYBEE *Apis mellifera capensis* AND QUEEN PRODUCTION

Chemical communication by pheromones, defined by Free (1987) as chemicals produced in exocrine glands that elicit behavioural and/or physiological responses in individuals of the same species thereby acting as a chemical message, is a much studied aspect of social insect biology (c.f. Free 1987; Van der Meer *et al* 1998). It is the well developed exocrine systems of social insects that distinguishes them from solitary insects. Many specialised exocrine glands capable of producing pheromones have been identified in social insects, including the mandibular, tergal, venom and pretarsal glands of honeybees (Wilson 1965; Billen and Morgan 1998; Hefetz 1998). The exocrine secretions produced by these glands are often characterised by complex mixtures of natural products with diverse functions, including reproductive control, colony defence and control of age polyethism (Blum and Brand 1972; Pain 1973; Howard and Blomquist 1982; Blum 1987; Robinson and Huang 1998).

PHEROMONES AND PHEROMONAL SOURCES OF HONEYBEES

Mandibular Gland Secretions

Considered to be the major source of pheromone control in honeybees, the mandibular glands and their secretions have been the focus of much research. The structure (Lensky *et al* 1985b; Vallet *et al* 1991; De Hazan *et al* 1989b), chemical profiles (Crewe and Velthuis 1980; Crewe 1982, 1988; Allsopp 1988; Crewe *et al* 1990a,b; Engels *et al* 1997b) and behavioural and physiological effects (Hemmling *et al* 1979; Saiovici 1983; Velthuis 1985, 1990; Slessor *et al* 1988, 1990; Winston *et al* 1989, 1991; Velthuis *et al* 1990) of the mandibular gland secretions have been investigated for all three castes and in many of the honeybee races.

Queen mandibular gland secretions have been shown to function as both releaser and primer pheromones (Pankiw *et al* 1996; Winston and Slessor 1998). According to Winston and Slessor (1998) releaser pheromones elicit specific behaviours such as alarm, orientation and

mate attraction while primer pheromones exercise a more fundamental level of control by influencing worker physiology, thereby mediating colony reproduction and influencing aspects of colony organisation, caste structure and division of labour. The releaser pheromone functions of queen mandibular secretions include: the attraction of workers to form a retinue around the queen (Slessor *et al* 1988; Kaminski *et al* 1990); attraction of workers to swarms and swarm cohesion (Morse 1963; Butler *et al* 1964; Butler and Simpson 1967; Winston *et al* 1989); attracting drones to virgin queens by acting as an aphrodisiac (Butler 1967; Butler *et al* 1967; Loper *et al* 1996); and, stimulating foraging by workers (Jaycox 1970; Free *et al* 1985; Higo *et al* 1992). The diverse actions of queen mandibular gland secretions as a primer pheromone include: inhibiting queen rearing (Butler and Callow 1968; Doolittle *et al* 1970; Melathopoulos *et al* 1996; Winston *et al* 1991); functioning in association with other pheromones to suppress ovary development and egg-laying in workers (Plettner *et al* 1993); inhibiting juvenile hormone biosynthesis in workers (Hilderbrandt and Kaatz 1990; Kaatz *et al* 1992); delaying the onset of swarming (Winston *et al* 1991); and, delaying the foraging age of workers (Pankiw *et al* 1996; Winston and Slessor 1998).

Tergal Gland Secretions

Renner/Baumann or tergal glands, located on tergites II-VI, work in conjunction, perhaps synergistically, with the mandibular glands in inhibiting ovarial development in workers, eliciting retinue behaviour and in the maintenance of colony cohesion (Velthuis 1970; Free 1987; Billen *et* al 1986). While mandibular gland secretions are attractive to workers from a distance, tergal gland secretions are detected by workers only upon contact (Velthuis 1970; Vierling and Renner 1977; Billen *et al* 1986). In addition tergal gland secretions function as an aphrodisiac to mounting drones (Butler 1971; Vierling and Renner 1977) and could be a possible source of kin recognition labels (Moritz and Crewe 1988b). Espelie *et al* (1990) identified decyl decanoate and decanoic acid esters with longer chain-lengths as the major components of tergal gland secretions. Wossler and Crewe (1999) determined that the tergal gland secretions of the southern African honeybees, *A.m. capensis* and *A.m. scutellata*, are composed of long-chain fatty acids, long-chain esters and a linear series of unsaturated hydrocarbons with (*Z*)-9-octadecenoic acid identified as the major compound in the secretion.

Footprint Pheromone

The tarsal glands, or Arnhart glands, located on the fifth tarsomere of honeybee legs secrete an oily exudate which is deposited by the footpads on comb surface (Chauvin 1962; Juška 1978; Lensky *et al* 1985a; Lensky and Cassier 1992). Lensky and Slabezki (1981) demonstrated that the footprint pheromone in association with mandibular gland excretions functions in the inhibition of queen cell construction. Chemical secretions by the tarsal glands are not restricted to honeybees having been identified in several hymenopteran species including bumblebees and wasps (Pouvreau 1991).

Queen Fecal Pheromones

During hostile confrontations with workers virgin queens release a pheromone in their feces which repels workers and releases autogrooming behaviour (Post *et al* 1987; Blum and Fales 1988). Page *et al* (1988) identified *o*-aminoacetophenone as the compound responsible for terminating antagonistic reactions of workers towards queens. Although the complete function of the complex mixture of constituents in the fecal exudate of queens remains to be established, Breed *et al* (1992) suggests that it may also be used by workers to discriminate among queens and by queens to scent-mark their colonies.

Queen Koschevnikov Gland Pheromone

Queen sting glands or Koschevnikov glands probably function in eliciting aggressive and balling behaviour by workers and in mutual queen recognition (Blum *et al* 1983; Lensky *et al* 1991). It is likely that the queen Koschevnikov gland pheromone acts synergistically with other pheromones such as those produced in the mandibular and tergal glands (Lensky *et al* 1991; Pettis *et al* 1998).

Alarm Pheromones

With the exception of 2-heptanone, produced in the mandibular glands, alarm pheromones are produced in the sting apparatus and because of their importance in colony defence are particularly well studied pheromonal secretions (Boch and Shearer 1967; Blum 1969; Crewe 1976; Lensky and Cassier 1995; Schmidt 1998). The release of alarm pheromones by a worker during a hostile encounter with an intruder evokes an alarm response amongst nestmates, attracting them to the intruder (Free 1960, 1987; Cassier *et al* 1994). Production of alarm pheromones and response thresholds display ontogenetic changes and are at a maximum at the age at which workers perform guarding and defence tasks (Boch and Shearer 1971; Crewe and Hastings 1976; Collins 1980; Whiffler *et al* 1988; Paxton *et al* 1994).

Nasonov Gland Pheromones

The nasonov gland pheromone, an abdominal gland secretion produced exclusively by worker honeybees, acts as a long-range pheromonal cue that functions in co-ordinating swarm behaviour (Morse and Boch 1971; Mautz *et al* 1972; Boch and Morse 1974; Avitabile *et al* 1975; Schmidt *et al* 1993). In addition the nasonov pheromone is used to provide orientation cues, attracting nestmates to water or rich food sources and in guiding workers to the hive entrance (Williams *et al* 1982; Melksham *et al* 1988). Nasonov glands secrete volatile terpenoids and protein compounds, the later functioning to enhance the attractiveness of the volatile fraction (Pickett *et al* 1980; Pickett *et al* 1981; Cassier and Lensky 1994). The attractiveness of synthetic nasonov mixture is enhanced by the presence of worker footprint pheromone (Williams *et al* 1981), further highlighting the synergistic relationship of honeybee pheromones.

Brood Pheromones

The ability of workers to identify brood and determine its sex, caste and developmental stage is essential for ensuring the necessary feeding and care of the larvae (Free and Winder 1983). Le Conte and collegues (1990, 1994, 1994/1995, 1995) identified a brood pheromone comprising ten fatty acids, on larvae cuticle, that provides the chemical signal enabling workers to identify brood and administer the appropriate care. In addition to the pheromonal role attributed to these compounds, two of them have been demonstrated to act as kairomones that enable the parasitic mite, *Varroa jacobsoni*, to recognise receptive larvae (Le Conte *et al* 1989; Trouiller *et al* 1991).

Variations in Pheromone Production

Pheromone production has been demonstrated to be affected by age (Allsopp 1988; Whiffler *et al* 1988; Crewe and Moritz 1989; Slessor *et al* 1990; Engels *et al* 1997b), caste (Crewe and Velthuis 1980; Free and Winder 1983; Crewe *et al* 1990a,b; Plettner *et al* 1993, 1997; Gadagkar 1996, 1997) and race (Crewe 1982; Plettner *et al* 1997; Wossler and Crewe 1999). The variation in pheromone production exhibited by the different races of honeybees provides researchers with an additional probe for distinguishing the different races of *Apis mellifera*. This is of particular importance to South African beekeeping because of the consequences of the interactions and subsequent hybridisation between the two southern African races of honeybees, *Apis mellifera capensis* and *Apis mellifera scutellata*. Variations in the production of worker sting pheromones have been used to determine the distribution of *A.m. capensis* and *A.m. scutellata* as well as the extent of the hybrid zone (Hepburn *et al* 1994, 1998). Crewe (1984, 1988) found significant differences in the mandibular gland secretions of the two races of southern African honeybees.

THE CAPE HONEYBEE, *Apis mellifera capensis*, AND THE "*CAPENSIS* INVASION"

The Cape honeybee, *A.m. capensis*, is a small, relatively docile, black bee which naturally occurs in the fynbos biome along the south-west and south coast of South Africa (Hepburn and Crewe 1990, 1991; Hepburn and Jacot-Guillarmod 1991). *A.m. capensis* is unique in a number of well documented characteristics which distinguishes it from the other races of *Apis mellifera* (c.f. Hepburn and Guye 1991). *A.m. capensis* workers have the ability to lay diploid eggs by parthenogenesis and consequently produce female offspring (Onions 1912, 1914; Lundie 1954; Hepburn and Crewe 1990; Hepburn *et al* 1991; Allsopp and Crewe 1993; Hepburn 1994). In addition *A.m. capensis* workers produce a pheromonal bouquet in the absence of a queen which resembles a queen-like signal, the result of which is that *A.m. capensis* workers placed in the company of other races develop chemically and assume a "false-queen" or pseudo-queen" status (Hemmling *et al* 1979; Hepburn 1992; Allsopp and Crewe 1993). It is these unique characteristics which are responsible for the usurpation of *A.m. scutellata* colonies by *A.m. capensis* workers and accounts for the invasive

characteristics of *A.m. capensis* introduced into areas traditionally considered the domain of *A.m. scutellata* (Cooke 1992; Allsopp and Crewe 1993; Hepburn and Allsopp 1994).

Traditionally *A.m. capensis* is essentially a coastal race of the fynbos with the greater part of South Africa constituting the domain of *A.m. scutellata* (Hepburn and Crewe 1990). The two races hybridise along a relatively stable fragmented and elongated zone roughly between two ranges of mountains which define the boundaries of the two races (Allsopp and Crewe 1993; Hepburn *et al* 1994, 1998). *A.m. capensis* appears to be specifically adapted to the physical and biological peculiarities of the fynbos biome in which it naturally occurs (Hepburn and Jacot-Guillarmod 1991) and seems to enjoy climatic, metabolic and reproductive advantages over *A.m. scutellata* throughout the hybrid zone (Hepburn *et al* 1993). It is thought that the presumed superior colony reproduction and performance of *A.m. scutellata* prevents any significant intrusion of *A.m. capensis* on its boundary by sheer force of numbers (Allsopp and Crewe 1993). The current situation of the "*capensis* invasion" is partially due to the action of beekeepers and possibly also to invasive characteristics of *A.m. capensis*. It is therefore likely that the problem will only be alleviated through decisive action on the part of South African beekeepers. Management programmes developed specifically for the two races of southern African honeybees based on an understanding of their biology and that of their hybrids is essential if the current situation is to be managed effectively.

QUEEN PRODUCTION

The production of queens is an integral component of any beekeeping management programme. The development of successful queen production programmes relies on an understanding of natural queen production and baseline data of the race for which the programme is being developed. Queen rearing in honeybee colonies occurs naturally upon loss of the queen or for colony reproduction by swarming or supersedure. Reproductive swarming is the principal means of colony reproduction and involves one or more queens being reared from eggs laid by the queen in specially constructed queen cells (Allen 1965). In European honeybees the old queen then leaves the nest with a swarm, leaving one of the new queens to head the mother colony (Free 1987). Supersedure involves the rearing of a new queen in the presence of the old queen and the subsequent replacement of the queen and generally involves no new colony formation (Winston 1987). In the Cape honeybee, *Apis mellifera capensis*, colony reproduction occurs by swarming and is correlated with an influx of pollen and increased brood production, while queen replacement is by supersedure correlated to declining pollen availability and decreased brood production (Allsopp and Hepburn 1997).

The desire to replace old failing queens with young queens selectively bred to enhance desirable characteristics has led to queen rearing comprising an integral part of commercial beekeeping (Morse 1979). Consequently many methods of queen rearing have been

suggested all with the objective of rearing and introducing young vigorous queens from healthy, productive stock for the purpose of increasing the average production of honey per colony and improving the pollination potential and efficiency of colonies (McGregor 1992). According to Anderson (1983) systematic replacement of queens together with good management enables beekeepers to double their honey production. Queen rearing has developed into a specialised industry in many countries, with beekeepers and researchers obtaining their queens from commercial sources. Advances in queen rearing techniques have enabled researchers to acquire large numbers of queens of known heritage and age which have been reared and maintained under controlled conditions. This has proved particularly useful in ontogenetic studies and has led to a greater understanding of queen development (Engels *et al* 1997b).

The unique biology of *A.m. capensis* makes queen rearing with this race difficult and has resulted in the development of specialised queen-breeding techniques (Cooke 1986). *A.m. capensis* queens can be successfully raised only in queenright colonies thus precluding the use of traditional methods of queen rearing in which queenless colonies are used for rearing queens (Morse 1979; Anderson *et al* 1983; McGregor 1992). A South African beekeeper, who rears *A.m. capensis* queens, advocates the rearing of queens singly in cages in an incubator and determined that they could remain caged for up to a week without any risk of jeopardising their chances of becoming good layers (Cooke 1986). The introduction of artificial insemination (AI) in queen production has further complicated the process of queen rearing. Buys (1988) recommends that for the best AI results with *A.m. capensis*, queens be maintained without workers before and after AI which should be done when the queens are 4-5 days old.

SCOPE AND AIMS

Chemical communication is an integral component of social insect societies with the secretions of the mandibular glands providing particularly important signals for queen-drone (Gary 1962; Loper *et al* 1996) and queen-worker interactions (Moritz and Crewe 1988a; Slessor *et al* 1988; Kaminski *et al* 1990; Pettis *et al* 1998). Changes in the composition of mandibular gland secretions affect the behaviour of worker bees towards queens (Engels *et al* 1997b). The success of any queen production technique relies on queen acceptance by the workers (Cooke 1986). Consequently the chemical composition of the mandibular gland secretions of introduced queens contributes to their acceptance by workers thus determining the success of queen introduction. It is therefore likely that an understanding of the changes which occur in mandibular gland secretions and the consequent reactions of workers to queens will assist in the development of a queen production programme that ensures optimal queen acceptance by workers.

The quantity and quality of the mandibular gland secretions of honeybee queens when sampled are a complex function of social conditions, environmental factors and the rate of production in the gland (Free 1987; Crewe and Moritz 1989). In the existing literature, there has been little or no distinction made as regards the specific conditions pertaining to virgin queen honeybee mandibular gland signals. Insufficient attention has been paid to the age of the queens sampled, or the holding conditions of sampled queens prior to GC analysis, thus comparisons of findings from different studies are not possible.

The aim of this study was twofold. Firstly it aimed to determine the chemical composition of the mandibular gland secretions of *Apis mellifera capensis* queens. Secondly, this study investigated the impact of the age of honeybee virgin queens, and their immediate holding conditions prior to sampling on the nature of the chemical signal recovered. The implications of these findings on queen production and the further development of beekeeping in South Africa with *Apis mellifera capensis* will be considered.

CHAPTER TWO

IDENTIFICATION OF THE COMPOUNDS FOUND IN MANDIBULAR GLAND EXTRACTS OF THE CAPE HONEYBEE QUEEN, *Apis mellifera capensis*

SUMMARY

The mandibular gland secretions of *Apis mellifera capensis* virgin queens were analysed by gas chromatography-mass spectroscopy. The chemical identity of 37 compounds belonging to various chemical groups, including aliphatic and aromatic acids and diacids, phenols, alkanes, amino acids and sugars, was confirmed. Among the identified compounds were the 10-carbon fatty acids, functionalised at the last (ω) and penultimate (ω-1) positions, which are characteristic of honeybee mandibular secretions and which have important functions in the colony in respect of caste discrimination. These included 9ODA and 9HDA the major constituents of the queen mandibular pheromone (QMP). Also present were the other two components of the QMP, HVA and HOB, as well as the other aliphatic acids and phenols considered to be the major components of the mandibular gland secretions of *A.m. capensis*.

INTRODUCTION

The chemical nature of the compounds produced in the mandibular glands of honeybee queens has been the subject of much investigation since it was determined that the contents of these glands were capable of inhibiting queen production in queenless workers (Butler and Simpson 1958). This extract, first referred to as “queen substance” by Butler (1954) has been found to encompass a complex blend of chemicals, for which the function of many of the compounds identified still elude us.

The first studies involved the extraction and concentration of “queen substance” which resulted in the production of a crystalline material of high activity. Analyses indicated that the crystalline material was an αβ-unsaturated carboxylic acid, with an unconjugated carboxylic group and a molecular weight of approximately 190 (Butler *et al* 1959). This acid was thought to be closely related to 10-hydroxy-2-decenoic acid (10HDA) previously identified in secretions

of worker mandibular glands (Barker *et al* 1959b; Callow *et al* 1959) and in royal jelly along with another 10-hydroxy acid, 10-hydroxydecanoic acid (10HDAA; Barker *et al* 1959a; Weaver and Law 1960).

(*E*)-9-ketodec-2-enoic acid (9ODA), the major chemical compound of the queen's mandibular gland, was the first compound identified from mandibular gland extracts (Barbier and Lederer 1960; Callow and Johnston 1960), its identity being confirmed by synthesis (Butler *et al* 1961). Brown and Felauer (1961) detected trace amounts of 9ODA in royal jelly.

Phenols considered important components of the mandibular gland extracts of queens and workers (Crewe and Velthuis 1980; Crewe and Moritz 1989) were first identified in queen extracts by Pain and her co-workers (1962). C_6-C_{10} aliphatic diacids were also detected in honeybees and their secretions by these authors.

An additional 13 components were identified by Callow *et al* (1964) from extracts of crushed queen heads. They include methyl 9-oxodecanoate, methyl 9-oxodec-2-enoate, methyl 9-hydroxydecanoate, methyl 9-hydroxydec-2-enoate, methyl *p*-hydroxybenzoate, nonoic acid, decanoic acid, 2-decenoic acid, 9-oxodecanoic acid, 9-hydroxydecanoic acid, 9-hydroxy-2-decenoic acid, 10-hydroxy-2-decenoic acid and *p*-methoxybenzoic acid. Among the identified compounds were a number of C_{10} aliphatic compounds, the prominence of which suggested a special significance in the biochemistry of honeybees (Callow *et al* 1964). Eighteen other substances were indicated but not identified.

Following the identification of this array of chemical compounds workers concentrated on establishing the biological function of these compounds. 9ODA was found to prevent queen rearing and worker ovarial development (Butler *et al* 1961). It functions as a sex attractant of the queen (Gary 1962) and as an aphrodisiac (Butler 1967). 9-Hydroxydecanoic acid (9HDAA) enables workers to find the queen when swarming (Butler and Simpson 1967) while 9-hydroxydec-2-enoic acid (9HDA) was found to be responsible for stabilising swarm clusters (Butler *et al* 1964).

The availability of 19 analogues and homologues of 9ODA enabled Blum *et al* (1971) to critically analyse the effect on attractancy of all major modifications of the functionality of the honeybee sex attractant. They found that 9ODA is the only one of the 20 compounds that possesses detectable activity. The relative rigidity of this compound supports the presence of a complementary olfactory receptor on the antennae of drone antennae (Kaissling and Renner 1968; Adler *et al* 1973).

The improvement of specialised analytical techniques (Tumlinson and Heath 1976; Blomquist *et al* 1980; Howard *et al* 1980; Pomonis *et al* 1980; Tumlinson 1988; Bagnères and Morgan

1990) and gas chromatographic accessories (Crewe 1988) ensured advances in the determination of the chemical nature of the mandibular gland secretions. The introduction of a new derivatization technique (Crewe and Velthuis 1980) which allowed for the rapid analysis of a single mandibular gland did much to advance the chemical analysis of the extracts of these glands. This has assisted in increasing our understanding of the roles of these compounds in mediating insect behaviour (Crewe 1988).

Later studies identified additional compounds in mandibular glands and queens heads. Hexenoic acid, octanoic acid and (E)-oct-2-enoic acid were identified by Boch *et al* (1979). The percentage compositions of odd–numbered hydrocarbons, of chain lengths C23 – C35, were identified in African races of honeybees (Sellers *et al* 1987).

The compounds considered to be the major components of the mandibular gland secretions of southern African honeybees include 9ODA, the 9 and 10-hydroxy acids (9HDAA, 9HDA, 10HDAA, 10HDA), 2-methoxy-4-hydroxy phenylethanol, 7- and 8-hydroxyoctanoic acid (7HOA and 8HOA) and methyl *p*-hydroxybenzoate (HOB; Crewe and Velthuis 1980; Crewe 1982,1988; Crewe *et al* 1990a,b).

Engels and his co-workers (1997b) investigated the complete spectrum of mandibular gland volatiles of *A.m. carnica*. They identified more than 100 compounds, many of which had not previously been described as constituents of mandibular gland secretions of queen honeybees. Similarly, comprehensive lists of the compounds identified in the prominent exocrine glands, including mandibular glands, of *Bombus terrestris* have been compiled (Hefetz *et al* 1996).

The objective of this study was to investigate the volatiles in the mandibular gland secretions of the Cape honeybee, *Apis mellifera capensis* and to produce a composite “map” of the chemical compounds of the mandibular gland secretions of Cape honeybees.

MATERIALS AND METHODS

Sample Source and Preparation

Mandibular glands from *Apis mellifera capensis* virgin queens aged one, three, seven, 14 and 28 days-old reared alone or with 50 workers in an incubator or reared in small nucleus hives were used to identify the chemical compounds present in the mandibular gland secretions of such queens. The mandibular glands of mated queens collected from five localities in the Eastern Cape Province of South Africa were assayed.

Individual queen samples were prepared by removing the mandibles and their attached mandibular glands and placing the pair in 100µl dichloromethane for spectroscopy (Merck, Uvasol). The samples were stored at -5°C until required.

Chemical Analysis

The mandibular gland extracts were prepared and analysed according to methods similar to those of Crewe and Moritz (1989) described below. The mandibles were removed from the solvent, which was then evaporated just to dryness under a stream of N_2. The sample residue was redissolved in 25µl of the derivitising agent bis-(trimethylsilyl)trifluoroacetamide (BSTFA) for gas chromatography (Merck) and 25µl of the internal standard solution and allowed to react at room temperature for at least 10 minutes before being analysed.

Internal standard solutions were made by accurately weighing approximately 1mg of *n*-octanoic acid (Sigma) and 1 mg of *n*-tetradecane (Sigma) on a Sartorius MCI microbalance. The solution was prepared with 4ml of dichloromethane for spectroscopy. The resultant solution of known composition had a tetradecane concentration of approximately 0.250µg/µl.

Relative mass ratios (RMR) were calculated using a standard solution consisting of accurately weighed amounts (± 1mg) of the following compounds: octanoic acid, tetradecane, methyl 4-hydroxybenzoic acid, 4-hydroxybenzoic acid, dodecanoic acid, (E)-9-ketodec-2-enoic acid, 2-(3-methoxy-4-hydroxyphenyl)ethanol, 10-hydroxydecanoic acid, palmitoleic acid and cis-9-octodecenoic acid (oleic acid) in a 4ml solution of dichloromethane. Relative mass ratios (RMR) for each of the compounds were calculated relative to tetradecane. The resultant RMRs were used to calculate the mass of each of the compounds in the queen extracts. For those compounds for which standards were not available the RMR of a compound of similar structure was used to estimate the amounts of these compounds present in the sample.

Relative mass ratios and the mass of individual compounds were calculated using the following formulae:

$$\text{RMR} = \frac{\text{Area (std 1)} / \text{Area (tetradecane)}}{\text{Mass (std 1)} / \text{Mass (tetradecane)}}$$

$$\text{Mass (cmp 1)} = \frac{\text{Area (cmp 1)} / \text{Area (tetradecane)} \times \text{Mass (tetradecane)}}{\text{RMR}}$$

Analysis of samples was accomplished using a Hewlett-Packard 5890 gas chromatograph (GC) and the compounds identified using a Finnigan GCQ mass spectrometer interfaced with Finnigan MAT gas chromatograph (GC-MS). The GC and GC-MS were equipped with a *HP Ultra 1* 25m x 0.32mm x 0.52µm film thickness methyl silicone coated fused silica capillary column.

Hydrogen was used as a carrier gas for the GC which was programmed as follows: 60 °C for one minute, then 50 °C/minute to 100 °C and then programmed at 3 °C/minute from 100 °C to 220 °C and held at this temperature for 10 minutes. The inlet temperature was 230 °C while the detector temperature was 280 °C. For each sample, chromatograms were recorded and peak areas quantified with a HP3396 Series II Integrator. Compound peaks were identified using their retention times relative to the two internal standards. Identification of compounds was confirmed using GC-MS. The GC-MS used helium as its carrier gas and was programmed as follows: 70 °C for one minute, then programmed at 40 °C/minute to 120 °C, followed by a 3 °C/minute program from 120 °C to 220 °C and then held at the final temperature for 10 minutes.

RESULTS

Gas chromatographic and mass spectroscopic analyses of the mandibular glands of *Apis mellifera capensis* queens reveal an abundance of compounds (Figure 2.1). The chemical identification of 37 compounds was confirmed in this analysis. According to their chemical structures the identified compounds may be grouped into several chemical classes including aliphatic and aromatic acids and diacids, phenols, alkanes, amino acids, sugars and indole.

The identification of 18 aliphatic acids was confirmed (Table 2.1) from the extracts of mandibular glands of *Apis mellifera capensis* queens. This group of chemicals include (E)-9-ketodec-2-enoic acid (9ODA) and 9-hydroxydec-2-enoic acid (9HDA), two of the compounds which constitute the queen mandibular gland pheromone (QMP; Winston and Slessor 1998). In addition, the fatty acids normally associated with queen mandibular gland secretions (Crewe 1988) and those considered to be the major compounds identified in the head extracts of *A.m. capensis* workers (Crewe *et al* 1990a) are included in this group of compounds. Included in the group are the C_{16} and C_{18} saturated and unsaturated fatty acids.

The phenols identified (Table 2.2) are considered to be major compounds of the mandibular gland secretions (Crewe and Moritz 1989). Methyl *p*-hydroxybenzoate (HOB) and 4-hydroxy-3-methoxyphenylethanol (HVA) are the other compounds which constitute the QMP (Winston and Slessor 1998).

Quantities of aliphatic diacids (Table 2.3), straight chain hydrocarbons (Table 2.4) and aromatic acids and diacids (Table 2.5) were detected in the mandibular gland extracts of the queens analysed.

In addition, a variety of miscellaneous complex molecules were identified from mandibular gland extracts of *A.m. capensis* queens (Table 2.6). These included amino acids, sugars and the heterocyclic amine indole.

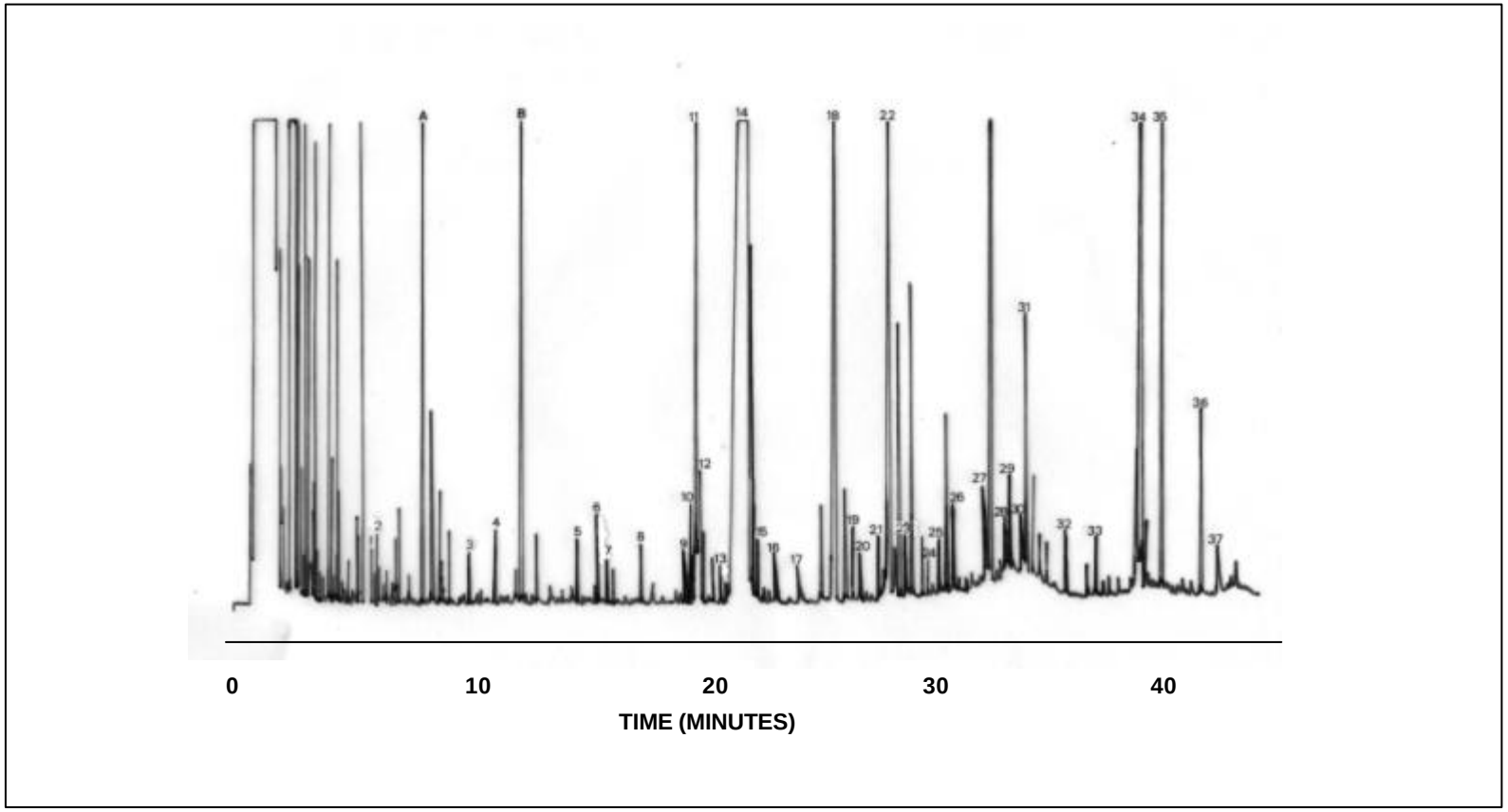


Figure 2.1: Representative mandibular gland extract of an *Apis mellifera capensis* virgin queen: (1) 2-Hexanoic acid, (2) Indole, (A) Internal Standard – Octanoic acid, (3) Propanoic acid, (4) L-Serine, (B) Internal Standard – n-Tetradecane, (5) HOB, (6) L-Proline, (7) 2-Propenoic acid, (8) 7HOA, (9) Eicosane, (10) 4HBA, (11) 8HOA, (12) Glutamic acid, (13) Dodecanoic acid, (14) 9ODA,, (15) HVA , (16) 9HDAA, (17) Benzoic acid, (18) 9HDA, (19) 10HDAA, (20) Decanoic acid, (21) Suberic acid, (22)10HDA, (23) Vanillylpropionic acid, (24) Azelaic acid, (25) Cinnamic acid, (26) D-Glucose, (27) Palmitoleic acid, (28) Pamitelaidic acid, (29) 10-Undecenoic acid, (30) L-Altrose, (31) Palmitic acid, (32) Heneicosane, (33) Undecandioic acid, (34) Oleic acid, (35) Stearic acid, (36) Docosane, (37) Hexacosane.

Table 2.1: The chemical structures of the aliphatic acids identified from extracts of the mandibular glands of *Apis mellifera capensis* queens.

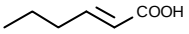
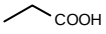
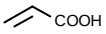
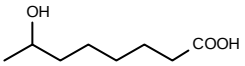
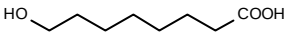
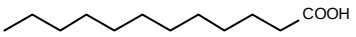
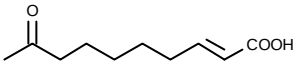
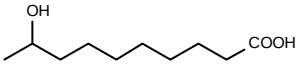
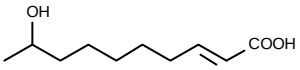
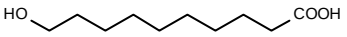
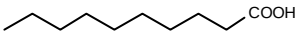
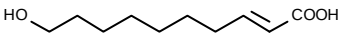
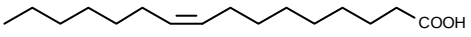
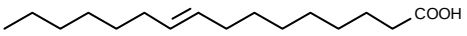
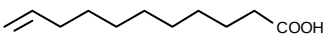
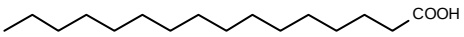
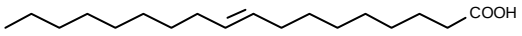
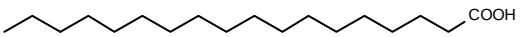
Compound	**Chemical Structure**
2-Hexenoic acid	
Propanoic acid	
2-Propenoic acid	
7-Hydroxyoctanoic acid (7HOA)	
8-Hydroxyoctanoic acid (8HOA)	
Dodecanoic acid	
(*E*)-9-ketodec-2-enoic acid (9ODA)	
9-Hydroxydecanoic acid (9HDAA)	
(*E*)-9-hydroxydec-2-enoic acid (9HDA)	
10-Hydroxydecanoic acid (10HDAA)	
Decanoic acid	
(*E*)-10-hydroxydec-2-enoic acid (10HDA)	
Palmitoleic acid (*Z*-9-hexadecenoic acid)	
Palmitelaidic acid (*E*-9-hexadecenoic acid)	
10-Undecenoic acid	
Palmitic acid (Hexadecanoic acid)	
Oleic acid (9-octadecenoic acid)	
Stearic acid (Octadecanoic acid)	

Table 2.2: The chemical structures of the phenols identified from extracts of the mandibular glands of *Apis mellifera capensis* queens.

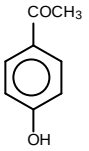
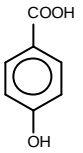
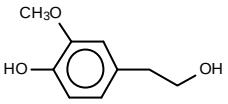
Compound	Chemical Structure
Methyl-*p*-hydroxybenzoate (HOB)	
4-Hydroxybenzoic acid (4HBA)	
4-Hydroxy-3-methoxyphenyl ethanol (HVA)	

Table 2.3: The chemical structures of the aliphatic diacids identified from extracts of the mandibular glands of *Apis mellifera capensis* queens.

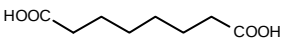
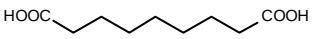
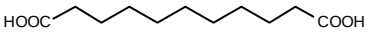
Compound	Chemical Structure
Suberic acid (Octanedioic acid)	
Azelaic acid	
Undecandioic acid	

Table 2.4: The chemical structures of the alkanes identified from extracts of the mandibular glands of *Apis mellifera capensis* queens.

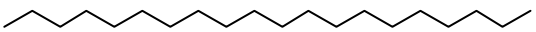
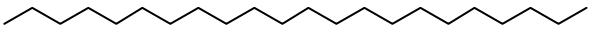
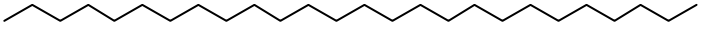
Compound	Chemical Structure
Eicosane	
Heneicosane	
Docosane	
Hexacosane	

Table 2.5: The chemical structures of the aromatic acids and diacids identified from extracts of the mandibular glands of *Apis mellifera capensis* queens.

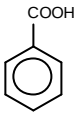
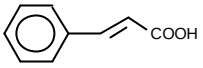
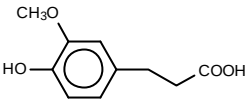
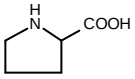
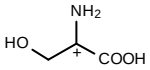
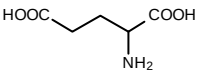
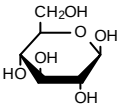
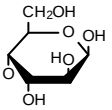
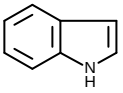
Compound	Chemical Structure
Benzoic acid	
Cinnamic acid	
Vanillylpropionic acid	

Table 2.6: The chemical structures of the amino acids, sugars and indole identified from extracts of the mandibular glands of *Apis mellifera capensis* queens.

Compound	Chemical Structure
L-Proline	
L-Serine	
Glutamic acid	
D-Glucose	
L-Altrose	
Indole	

DISCUSSION

The mandibular glands of *Apis mellifera capensis* queens proved to be an opulent source of a multitude of volatile compounds. These compounds serve in chemical communication and are likely to function in other biological activities in honeybee colonies (Hefetz *et al* 1996).

The 37 compounds listed in Tables 2.1 - 2.6 represent just less than half of the volatiles present in the mandibular gland extracts of *A.m. capensis* queens. The other volatiles are present in varying amounts but the chemical identity of the compounds could not be confirmed. For this reason, analyses in this study were restricted to those compounds for which the chemical identity was certain.

Many aliphatic acids and phenols have been isolated and identified from extracts of the mandibular glands of honeybees of the various *Apis* species. Although the compounds present are often the same, the proportion of the various compounds in relation to one another are species- and even race-specific as well as caste-specific. The aliphatic acids 9ODA, 9HDA, 10HDA and 10HDAA have been identified from the mandibular gland extracts of queens and workers of the Asian open-nesting species, *A. dorsata*, *A. florea* and *A. andreniformis* (Butler *et al* 1967; Shearer *et al* 1970; Plettner *et al* 1997). Plettner *et al* (1997) found that in addition to the four aliphatic acids the glands of the Asian and European/African cavity-nesting species, *A. cerana* and *A. mellifera* respectively, were characterised by the presence of the phenols, HOB and HVA. On a subspecies level, differences in the composition of the chemicals (9ODA, 9HDA, HOB and HVA) constituting the queen mandibular pheromone (QMP) as defined by Slessor *et al* (1988, 1990, 1998) have been observed in European and Africanized queens (Pankiw *et al* 1996). Variations in some of the compounds identified from queen mandibular gland extracts of the southern African races of honeybees, *A.m. capensis*, *A.m. intermissa* and *A.m. scutellata*, have been detected (Crewe 1982, 1988; Crewe and Moritz 1989).

Of the aliphatic acids identified from the queens analysed in the present study many have been previously identified in *A.m. capensis* queens. 8HOA, 9ODA, 9HDA, 10HDAA and 10HDA were initially identified in the mandibular gland secretions of *A.m. capensis* queens (Crewe 1982, 1988). More recently 7HOA, decanoic acid, dodecanoic acid, 9HDAA and 10HDA were included in the list of compounds considered to be the major components of the head extracts of *A.m. capensis* (Crewe *et al* 1990a). These authors mentioned the presence of fatty acids with chain lengths greater than twelve carbons but did not identify specific compounds. C_{16} and C_{18} acids were found to be prominent components of the queens analysed in this study and are represented by a variety of isomers. The same isomers were identified in head extracts of *A.m.* capensis queens (Moritz and Crewe 1991), the mandibular gland extracts of A.*m. carnica* queens (Engels *et al* 1997b), the developmental stages of *A.m.*

fasciata and *A.m. carnica* (Atallah *et al* 1982) and in the bumblebee *Bombus terrestris* (Hefetz *et al* 1996).

Recent studies investigating the biosynthesis of the hydroxy acids in workers and queens have identified precursors and by-products of these biosynthetic pathways. Decanoic, hexadecanoic and octadecanoic acids were identified as precursors (Plettner *et al* 1995). Following the incorporation of deuterated potential intermediates and precursors into mandibular blend components using GC-MS these authors provided evidence that octadecanoic acid (stearic acid) was converted into 10HDA with higher efficiency than the other two precursors. Their results suggested that unfunctionalised fatty acids with less than 18 carbons are lengthened to $C_{18:0}$ before functionalization thereby confirming that stearic acid is the entry point of the biosynthetic pathway (Plettner *et al* 1996). In queens stearic acid undergoes hydroxylation at the ω-1position, followed by β-oxidation to produce 9HDA, oxidation of this ω-1-hydroxy group produces 9ODA (Plettner *et al* 1996, 1997). 8HOA is produced as an end point by-product in the hydroxy acid biosynthetic pathway (Plettner *et al* 1995).

The three phenols identified in these analyses were identified in head extracts of *A.m. intermissa* (Crewe and Moritz 1989) and *A.m. carnica* (Engels *et al* 1997b). These results further suggest that the mandibular gland signals of honeybee queens, and possibly other hymenopteran species, do not differ in their general chemical composition. It is therefore not the presence or absence of compounds but rather their concentration and relative proportions which distinguishes one race of honeybee from another.

Aliphatic diacids, C_6 – $C_{10,}$ have been previously identified in honeybees (Pain *et al* 1962). They identified suberic acid in royal jelly and queen larvae while azelaic acid was identified in queens.

Insect hydrocarbons have been well researched as they are omnipresent in insect exocrine secretions. Many semiochemical and behavioural functions have been attributed to hydrocarbons in a wide variety of insect groups. They comprise a significant portion of the cuticular lipids which prevent desiccation (Howard and Blomquist 1982; Blomquist *et al* 1998). They form components of the sex pheromones of many dipteran species (Blomquist *et al* 1987), Coleoptera (rove beetle *Aleochara curtula* - Peschke and Metzler 1986) and Lepidoptera (geometrid moths - Li *et al* 1993). In Hymenoptera hydrocarbons are produced in a variety of glands and serve various biological functions. In the ants undecane is produced in the Dufour's gland and functions as a sex pheromone (Walter *et al* 1993). A variety of hydrocarbons are produced in the postpharyngeal gland (Bagnères and Morgan 1991). In bumblebees hydrocarbons produced in the tarsal glands have been attributed to flower marking (Hefetz *et al* 1996) while other sites are a source of recognition cues (Oldham *et al*

1994). Hefetz and his co-workers (1996) postulate that due to the quantities and variety of hydrocarbons present in the glandular exudates of *Bombus terrestis* it is unlikely that they are simply lining the cuticular intima of the glands but rather serve an as yet unidentified specific function. In termites cuticular hydrocarbons serve as species- and caste-recognition cues (Howard *et al* 1982). In honeybees the same function has been attributed to the hydrocarbons they produce which allows for subfamily recognition (Arnold *et al* 1996).

Of the four alkanes identified in these analyses of *A.m. capensis* mandibular gland extracts the C_{21}, C_{22} and C_{26} hydrocarbons have been identified in mandibular gland extracts of *A.m. carnica* (Engels *et al* 1997b). Amongst the hydrocarbons identified in mandibular gland extracts of *Bombus terrestris* were the four found in this study (Heftz *et al* 1996). In addition these authors identified these hydrocarbons in extracts of the other cephalic glands, including hypopharyngeal glands and labial glands, as well as the tarsal and Dufour's glands. All alkanes between C_{19} – C_{33} hydrocarbons and uneven-numbered alkanes C_{35} – C_{41} are present in cuticular extracts of newly-emerged *A.m. capensis* workers (Francis *et al* 1985). The hydrocarbons identified in this study were identified in the cuticular lipids of all three honeybee castes and in hemolymph of workers and drones, queen hemolymph was not analysed (Francis *et al* 1989). The presence of straight chain hydrocarbons in a wide variety of honeybee extracts suggests that those identified in this study and others may not be glandular in origin but rather cuticular hydrocarbons originating from the cuticle of associated body parts. In the case of mandibular glands the straight-chain hydrocarbons may be contaminants from the attached mandibles.

The extracts of *A.m. capensis* mandibular glands include a group of aromatic compounds whose function is unknown. Given the importance of the phenols HOB, 4HBA and HVA in the mandibular gland complex one may speculate that the additional aromatic compounds may also be of importance in the chemical signal of queen honeybees. Worth considering is the presence of aromatic acids and their derivatives (including benzoic, cinnamic and vanillic acids) in propolis resin (Greenway *et al* 1987; Marcucci 1995). These compounds exhibit antibacterial and antiviral activity and are reportedly responsible for some of the biological and pharmacological properties attributed to propolis (Ivanovska *et al* 1995; Marcucci 1995). The significance of these compounds is worth investigating in order to determine whether possible biological significance can be attributed to them. Antibacterial and antifungal activity has been attributed to 10HDA, hexanoic and octanoic acids identified in worker mandibular glands and royal jelly (Boch *et al* 1979).

Amino acids and sugars have been identified as components of honeybee food sources. Royal jelly, the principal food of queen honeybees produced by the cephalic glandular system of worker honeybees (Haydak 1970); constituents include amino acids and sugars (Palma 1992; Klaudiny *et al* 1994). Amino acids and sugars have been identified in honey (Sancho *et*

al 1991; Bogdanov *et al* 1996) and pollen samples (Singh and Singh 1996). Indole, a fused-ring heterocycle, occurs commonly in natural products, having pronounced biological activity (McMurry 1988) but its function in honeybees is unknown. It is possible that these miscellaneous compounds are not products of the mandibular glands of queens but rather contaminants from other sources, including food.

Despite the significant amount of research that has been undertaken on the compounds produced in the mandibular glands of queen honeybees many questions still remain unanswered. The biological significance of many of the identified compounds which constitute the complex of the mandibular gland extract of *A.m. capensis* queens remains to be established. In addition the identity of other compounds still needs confirmation. Studies using synthetic QMP indicate that the aliphatic acids 9ODA and 9HDA and the aromatic compounds HOB and HVA which comprise the QMP are the only pheromonal substances produced in the mandibular glands of honeybee queens (Winston and Slessor 1998). This has been deduced because the effect of synthetic QMP and queen mandibular gland extracts on the behaviour of workers are not significantly different ($P < 0.05$ - Slessor *et al* 1988, 1998). Thus, according to Winston and Slessor (1998) the array of other compounds is unlikely to function as pheromones although they could have other semiochemical and biological functions. While some of these compounds may be found to function in the chemical communication of these organisms, many may as, suggested by Engels *et al* (1997b) be solvents or biosynthetic pathway by-products without, semantic significance for the royal message.

CHAPTER THREE

THE INFLUENCE OF AGE ON THE PRODUCTION OF THE CHEMICAL COMPOUNDS IDENTIFIED IN THE MANDIBULAR GLANDS OF *Apis mellifera capensis* VIRGIN QUEENS

SUMMARY

Changes in the patterns of the mandibular gland volatiles of *Apis mellifera capensis* virgin queens were followed from emergence until 28-days old. Ontogenetic changes in the mandibular gland secretions were largely quantitative in nature, with the total volume and that of most of the compounds increasing with queen age. The mandibular gland extracts are characterised by the predominance of 9ODA three days after emergence. The final level of 9ODA content is reached at the premating stage, about three days after emergence, when it comprises approximately 87% of the major components of the mandibular gland secretion.

INTRODUCTION

Age-related changes in the development of honeybees are well documented. Anatomical, behavioural, physiological and chemical changes have been observed in all three castes which constitute a honeybee colony. Changes in the chemical composition of the extracts of exocrine glands are particularly well documented and occur frequently in conjunction with other ontogenetic changes. The ultrastructure of the mandibular glands of queens has been found to develop in conjunction with pheromonal activity during ontogenetic development (De Hazan *et al* 1989b). Similar age-related changes in the fine structure and secretory activity of the mandibular glands of honeybee drones have been demonstrated (Lensky *et al* 1985b). In workers these changes are often associated with age polyethism. The changes in the composition and quantity of the defence pheromones produced by workers in their sting and mandibular glands are a case in point. Optimal amounts are produced by workers at the time in their development when they are most likely to serve as guard bees in the colony (Whiffler *et al* 1988).

The attractiveness of queens to workers and drones increases as the queens age and are mated. Gary (1961) observed that recently eclosed virgin queens attracted few, if any, workers, while aged virgins (4 weeks or older) are as attractive as laying queens. According to Gary (1961) chemical mediation rather than other communicative media, such as auditory or visual, is indicated in the attractiveness of queens to workers, with the mandibular gland secretion being primarily responsible for queen attractiveness. Butler (1960) determined that the quantities of queen substance obtained from virgin queens increased with age, with little or none detectable in 1-day old virgins, increased amounts occurring in 1-week old virgins and still greater quantities in 3-week old virgins. In addition, the amount of queen substance produced in laying queens is greatest. He attributed the inability of virgin queens to inhibit queen rearing and difficulties in replacing virgin queens with mated queens to be a consequence of the small amount of queen substance produced in colonies headed by virgin queens.

With the identification of 9ODA as the principal queen substance (Barbier and Lederer 1960; Callow and Johnston 1960) ontogenetic changes in the production of this compound were determined. Butler and Paton (1962) found that the quantity of 9ODA in queen heads increased considerably (7.20µg to 132.50µg) as queens aged from 1-2 days to 5-10 days. Increased production in 9ODA with age in queens was found not to be restricted to *A. mellifera,* with *A. dorsata* exhibiting similar changes (Shearer *et al* 1970).

Detailed analyses of the mandibular gland extracts of honeybee queens have revealed that ontogenetic changes in the chemical composition of these glands are more complex than a simple increase in quantity. Ontogenetic changes in mandibular gland signals have been found to differ for the various races of *A. mellifera*. The secretions of young virgin *A.m. mellifera* and *A.m. scutellata* queens (1 day-old) are dominated by 10HDA giving the signal a worker-like characteristic (Crewe and Velthuis 1980; Crewe 1982). These authors found that the relative proportion of 10HDA in the mandibular gland signal decreases, while that of the queen mandibular pheromones (QMP), 9ODA and 9HDA, increases as the queens age and become mated. *A.m. intermissa* 1-day old queen head extracts are dominated by 9HDA with a significant contribution of 9ODA, in 4 day-old queens the contribution of these two dominant components are reversed with 9ODA dominating the signal (Crewe and Moritz 1989).

Of the compounds comprising the QMP complex as defined by Slessor *et al* (1988) only 9HDA is present in the extracts of queens of California stock eclosed within the previous 24 hours (Slessor *et al* 1990). These authors found that the relative proportion of 9HDA decreased, to comprise approximately 12% of the complex in older unmated Californian queens, with 9ODA making up approximately 86% of the QMP complex. Mated queens laying for five weeks have extracts that comprise approximately 53% 9ODA and 39% 9HDA. Small quantities of the aromatic HOB were detected in the older unmated queens while HVA was only detected in

mated queens (Slessor *et al* 1990). The ontogenetic patterns of *A.m. carnica* resemble those of Africanized and European queens (Slessor *et al* 1990; Pankiw *et al* 1996) with the main differences being related to the smaller quantities of 9HDA found in *A.m. carnica* mated queens (Engels *et al* 1997b). In addition Engels *et al* (1997b) found oleic acid to be the main component of recently emerged queens and suggested that the persistent levels of this acid in young queens, which decreases only with egg-laying activity, could label gynes.

Comparisons of the composition of the queen mandibular gland secretions of the southern African races of honeybees have revealed significant differences. *A.m. scutellata* virgin queen mandibular gland secretions contained similar quantities of 9ODA and 9HDA (approximately 39% and 32% respectively) while those of mated queens consist of approximated 65% 9ODA, 14% 9HDA and 8% 10HDA (Crewe 1988). In comparison Crewe (1988) found the mandibular gland secretion of virgin and mated *A.m. capensis* queens to be similar in composition with 9ODA constituting in excess of 80% and 9HDA approximately 10% of the major compounds of the signal.

Changing ontogenetic patterns of queen pheromones is not restricted to honeybees having been recently described for other social bees. Analyses of Dufour's gland secretion of the primitively eusocial halictine bees (Ayasse *et al* 1990, 1993) and the head extracts of the highly eusocial stingless bee *Scaptotrigona postica* (Engels *et al* 1997a) revealed that receptive virgin queens have a distinct label. Engels *et al* (1997b) suggested that a similar developmental profile of queen pheromones could be expected in honeybees which could assist in maintaining harmony within colonies during the transient period of the non-monogynous phase when new queens are reared.

The objective of this study was to investigate the possible occurrence of ontogenetic patterns in the mandibular gland secretions of the Cape honeybee, *Apis mellifera capensis*.

MATERIALS AND METHODS

Samples and Sample Preparation

Queens were reared from established *Apis mellifera capensis* colonies in Stellenbosch, South Africa (33° 56' S, 18° 52' E). Upon emergence the virgin queens were placed in 40ml bottles in an incubator at 30-32ºC. The queens were supplied with honeywater and water *ad libitum*. They were maintained under these conditions until they were used in the behavioural observations discussed in chapter four and the mandibular glands removed and prepared as described in chapter two.

Data were obtained from the following: one, three, seven, 14 and 28 day-old virgin queens.

Chemical Analysis

Chemical analysis of the mandibular gland extracts of the virgin queens was performed using gas chromatography and gas chromatography – mass spectroscopy as described in chapter two.

Statistical Analysis

Statistical analyses were performed using the program SigmaStat™ Statistical Software for Windows Version 1.0 (Jandel Scientific). Each compound was tested using either a parametric or non-parametric test depending upon whether the normality test and equal variance test were passed. A One Way ANOVA using the Student-Newman-Keuls Test was the parametric test performed to test whether the difference between the groups (virgin queen age) was greater than would be expected by chance ($P < 0.05$). For comparisons which failed the normality or equal variance test an ANOVA on Ranks using the Kruskal-Wallis test was performed.

RESULTS

Quantities of the compounds in the mandibular gland extracts of *Apis mellifera capensis* virgin queens generally increased as the age of the queens sampled increased, with significant differences ($P < 0.05$) being detected between queens of different ages. The extracts are characterised by the predominance of 9ODA three days after eclosion (Tables 3.1-3.7). Significantly increased levels of 9ODA, 9HDA and HVA, components of the queen mandibular pheromone (QMP) complex, were found in virgin queens aged seven and 14 days. At 28 days the volumes had receded to levels equivalent to those detected in one- and three-day old virgin queens (Tables 3.1 and 3.2). HOB, the fourth component of the QMP complex was generally detected in trace amounts and showed no significant difference with queen age (Table 3.2; $P = 0.135$). The observed increase in 9ODA was found to correspond with an increase in 8HOA and a decrease in palmitic and stearic acids (Tables 3.1 and 3.3; Figure 3.1).

Oleic acid was found to be the dominant compound in the mandibular gland signals of one-day old *A.m. capensis* queens and although present in smaller quantities continued to make a significant contribution to the signal of older queens (Table 3.3). Several other C_{16} and C_{18} aliphatic acids were present in significant quantities in queens of all ages (Table 3.3).

The aliphatic diacids, alkanes, aromatic acids and diacids and the various other miscellaneous compounds identified in the extracts of the *A.m. capensis* queens sampled were present in relatively small quantities (Tables 3.4-3.7). Ontogenetic changes were observed for some of these compounds and were consistent with the general age-dependent increase in quantity of the mandibular gland secretions.

Of the compounds considered to be the major constituents of mandibular gland signals (Table 3.8) 9ODA predominated in the signal of all *A.m. capensis* queens sampled. 9ODA comprised approximately 72% of these compounds in one-day old queens, significantly less than the approximate 87% it comprised in older queens (Table 3.8).

Table 3.1: Amounts (µg; mean [a] ± SE) of the major aliphatic acids present in *Apis mellifera capensis* virgin queens of different ages.

Compound	Age (days)				
	1	**3**	**7**	**14**	**28**
	n = 10	n = 9	n = 10	n = 10	n = 3
7HOA	0.206 [a] ± 0.069	0.815 [b] ± 0.495	0.487 [ab] ± 0.080	1.119 [b] ± 0.168	2.182 [b] ± 0.976
8HOA	1.20 [a] ± 0.069	1.74 [ab] ± 0.350	2.93 [ab] ± 0.681	8.89 [b] ± 1.750	1.55 [ab] ± 0.402
9ODA	24.7 [a] ± 6.95	158.5 [ab] ± 25.81	421.6 [b] ± 53.58	496.5 [b] ± 56.63	194.3 [ab] ± 56.63
9HDAA	0.169 [a] ± 0.093	0.626 [ab] ± 0.093	1.189 [b] ± 0.139	1.199 [b] ± 0.233	0.463 [ab] ± 0.236
9HDA	3.44 [a] ± 0.514	19.46 [ab] ± 5.378	35.22 [b] ± 5.900	32.31 [b] ± 4.513	22.19 [ab] ± 15.891
10HDAA	1.341 [a] ± 0.330	0.235 [b] ± 0.071	0.536 [ab] ± 0.075	0.472 [ab] ± 0.093	0.490 [ab] ± 0.075
10HDA	1.04 [a] ± 0.221	2.01 [b] ± 0.844	12.38 [b] ± 4.540	8.94 [ab] ± 2.508	3.45 [ab] ± 2.083

[a] Means within one row followed by the same letter are not significantly different (P > 0.05, Kruskal-Wallis). For rows in which no statistically significant difference was detected (P > 0.05, Kruskal-Wallis) no letters are included.

Abbreviations for the compounds are as follows: 7HOA = 7-Hydroxyoctanoic acid; 8HOA = 8-Hydroxyoctanoic acid; 9ODA = (*E*)-9-ketodec-2-enoic acid; 9HDAA = 9-Hydroxydecanoic acid; 9HDA = (*E*)-9-Hydroxydec-2-enoic acid; 10HDAA = 10-Hydroxydecanoic acid; 10HDA = (*E*)-10-Hydroxydec-2-enoic acid.

Table 3.2: Amounts (µg; mean [a] ± SE) of the major phenols present in *Apis mellifera capensis* virgin queens of different ages.

Compound	Age (days)				
	1	**3**	**7**	**14**	**28**
	n = 10	n = 9	n = 10	n = 10	n = 3
HOB	0.08687 ± 0.05677	0.00098 ± 0.00037	0.00196 ± 0.00039	0.00142 ± 0.00035	0.00023 ± 0.00003
4HBA	0.00307 [acd] ± 0.00117	0.00169 [ad] ± 0.00007	0.01657 [b] ± 0.00181	0.02390 [bc] ± 0.01041	0.00778 [bd] ± 0.00600
HVA	0.494 [a] ± 0.136	1.383 [ab] ± 0.297	3.339 [b] ± 0.570	5.575 [b] ± 2.001	0.708 [ab] ± 0.099

[a] Means within one row followed by the same letter are not significantly different (P > 0.05, Kruskal-Wallis). For rows in which no statistically significant difference was detected (P > 0.05, Kruskal-Wallis) no letters are included.

Abbreviations for the compounds are as follows: HOB = Methyl-*p*-hydroxybenzoate; 4HBA = 4-Hydroxybenzoic acid; HVA = 4-Hydroxy-3-methoxyphenyl ethanol

Table 3.3: Amounts (µg; mean [a] ± SE) of the minor aliphatic acids present in *Apis mellifera capensis* virgin queens of different ages.

Compound	Age (days)				
	1	**3**	**7**	**14**	**28**
	n = 10	n = 9	n = 10	n = 10	n = 3
2-Hexenoic acid	0.379 ± 0.089	1.026 ± 0.284	0.349 ± 0.050	0.315 ± 0.032	2.006 ± 0.701
Propanoic acid	0.097 ± 0.030	0.129 ± 0.036	0.099 ± 0.025	0.087 ± 0.020	0.110 ± 0.064
2-Propenoic acid	0.00055 ± 0.00034	0.03387 ± 0.03370	0.01781 ± 0.01759	0.16365 ± 0.13293	0.00009 ± 0.00009
Dodecanoic acid	0.329 [a] ± 0.046	0.104 [b] ± 0.031	0.128 [ab] ± 0.035	0.110 [b] ± 0.036	0.002 [b] ± 0.001
Decanoic acid	0.093 [a] ± 0.092	0.391 [ab] ± 0.088	0.613 [b] ± 0.083	0.911 [b] ± 0.177	0.643 [ab] ± 0.200
Palmitoleic acid	0.002 [a] ± 0.00049	0.622 [ab] ± 0.16174	1.122 [b] ± 0.22261	1.396 [b] ± 0.26062	0.009 [ab] ± 0.00738
Palmitelaidic acid	1.684 [a] ± 0.223	0.872 [b] ± 0.134	0.877 [b] ± 0.136	1.011 [b] ± 0.106	0.386 [b] ± 0.192
10-Undecenoic acid	2.950 ± 0.612	3.099 ± 0.428	2.765 ± 0.406	3.396 ± 0.392	0.935 ± 0.562
Palmitic acid	17.31 [a] ± 3.212	11.49 [ab] ± 1.650	0.409 [b] ± 0.077	0.326 [b] ± 0.044	3.74 [b] ± 1.891
Oleic acid	131.1 [a] ± 18.35	70.7 [b] ± 12.25	81.2 [b] ± 9.97	72.1 [b] ± 7.34	13.4 [b] ± 10.93
Stearic acid	31.22 [a] ± 4.13	17.28 [b] ± 2.59	21.42 [b] ± 2.33	17.41 [b] ± 1.24	9.69 [b] ± 8.97

[a] Means within one row followed by the same letter are not significantly different ($P > 0.05$, Kruskal-Wallis). For rows in which no statistically significant difference was detected ($P > 0.05$, Kruskal-Wallis) no letters are included.

Table 3.4: Amounts (µg; mean [a] ± SE) of the aliphatic diacids present in *Apis mellifera capensis* virgin queens of different ages.

Compound	Age (days)				
	1	**3**	**7**	**14**	**28**
	n = 10	n = 9	n = 10	n = 10	n = 3
Suberic acid	0.864 ± 0.109	0.833 ± 0.171	0.853 ± 0.204	0.884 ± 0.419	1.049 ± 0.789
Azelaic acid	0.002 ± 0.00046	0.002 ± 0.00039	0.002 ± 0.00041	0.195 ± 0.15386	0.002 ± 0.00086
Undecandioic acid	1.242 [ab] ± 0.613	1.128 [b] ± 0.718	0.187 [ab] ± 0.093	0.835 [b] ± 0.747	7.215 [b] ± 1.798

[a] Means within one row followed by the same letter are not significantly different (P > 0.05, Kruskal-Wallis). For rows in which no statistically significant difference was detected (P > 0.05, Kruskal-Wallis) no letters are included.

Table 3.5: Amounts (µg; mean [a] ± SE) of the alkanes present in *Apis mellifera capensis* virgin queens of different ages.

Compound	Age (days)				
	1	**3**	**7**	**14**	**28**
	n = 10	n = 9	n = 10	n = 10	n = 3
Eicosane	0.012 [a] ± 0.01079	0.159 [ab] ± 0.04143	0.467 [b] ± 0.06666	0.551 [b] ± 0.14304	0.002 [ab] ± 0.00021
Heneicosane	2.037 [a] ± 0.346	2.091 [ab] ± 1.650	0.409 [b] ± 0.077	0.326 [b] ± 0.044	19.258 [a] ± 7.856
Docosane	6.70 [a] ± 1.143	1.20 [b] ± 0.154	1.61 [b] ± 0.198	1.63 [b] ± 0.378	1.24 [b] ± 0.360
Hexacosane	0.006 ± 0.003	0.698 ± 0.416	4.951 ± 1.731	6.821 ± 2.106	5.164 ± 2.675

[a] Means within one row followed by the same letter are not significantly different (P > 0.05, Kruskal-Wallis). For rows in which no statistically significant difference was detected (P > 0.05, Kruskal-Wallis) no letters are included.

Table 3.6: Amounts (µg; mean [a] ± SE) of the aromatic acids and diacids present in *Apis mellifera capensis* virgin queens of different ages.

Compound	Age (days)				
	1	**3**	**7**	**14**	**28**
	n = 10	n = 9	n = 10	n = 10	n = 3
Aromatic acids					
Benzoic acid	0.001 [a] ± 0.00035	0.478 [ab] ± 0.14066	0.602 [b] ± 0.15074	0.771 [b] ± 0.09217	2.712 [b] ± 1.12110
Cinnamic acid	0.257 ± 0.113	0.346 ± 0.097	0.178 ± 0.073	0.840 ± 0.261	1.064 ± 0.238
Aromatic diacids					
Vanillylpropionic acid	0.338 ± 0.074	0.733 ± 0.273	0.481 ± 0.323	1.058 ± 0.745	0.136 ± 0.134

[a] Means within one row followed by the same letter are not significantly different (P > 0.05, Kruskal-Wallis). For rows in which no statistically significant difference was detected (P > 0.05, Kruskal-Wallis) no letters are included.

Table 3.7: Amounts (µg; mean [a] ± SE) of the amino acids, sugars, indole and an unknown compound present in *Apis mellifera capensis* virgin queens of different ages.

Compound	Age (days)				
	1	**3**	**7**	**14**	**28**
	n = 10	n = 9	n = 10	n = 10	n = 3
Amino acids					
L-Proline	0.091 [a] ± 0.040	0.182 [ab] ± 0.050	0.567 [b] ± 0.143	0.474 [b] ± 0.062	0.217 [ab] ± 0.137
Glutamic acid	0.051 [a] ± 0.019	0.093 [a] ± 0.014	0.179 [c] ± 0.021	0.242 [b] ± 0.025	0.107 [ac] ± 0.013
L-Serine	0.074 ± 0.033	0.021 ± 0.017	0.064 ± 0.026	0.028 ± 0.019	0.001 ± 0.001
Sugars					
D-Glucose	0.973 [ac] ± 0.209	0.670 [bc] ± 0.228	0.830 [ac] ± 0.107	1.273 [ab] ± 0.301	2.742 [a] ± 0.195
L-Altrose	0.004 [a] ± 0.00166	0.002 [a] ± 0.00023	0.002 [a] ± 0.00029	0.323 [b] ± 0.04521	0.136 [ab] ± 0.13430
Indole	0.547 ± 0.077	0.510 ± 0.082	0.447 ± 0.067	0.384 ± 0.029	0.878 ± 0.280

[a] Means within one row followed by the same letter are not significantly different (P > 0.05, Kruskal-Wallis). For rows in which no statistically significant difference was detected (P > 0.05, Kruskal-Wallis) no letters are included.

Table 3.8: Percentage (mean [a] ± SE) composition of the head extracts of *Apis mellifera capensis* virgin queens of different ages.

Compound	Age (days)				
	1	**3**	**7**	**14**	**28**
	n = 10	n = 9	n = 10	n = 10	n = 3
HOB	0.1723 [a] ± 0.1230	0.00695 [b] ± 0.00388	0.00078 [b] ± 0.00035	0.00027 [b] ± 0.00007	0.00018 [b] ± 0.00008
7HOA	0.748 ± 0.269	0.568 ± 0.269	0.147 ± 0.050	0.205 ± 0.019	1.883 ± 1.213
4HBA	0.01156 [a] ± 0.00341	0.00112 [b] ± 0.00019	0.00369 [a] ± 0.00052	0.00407 [a] ± 0.00151	0.00264 [a] ± 0.00114
8HOA	4.405 [a] ± 0.798	0.992 [ab] ± 0.212	0.785 [b] ± 0.223	1.602 [ab] ± 0.216	0.818 [ab] ± 0.160
9ODA	72.0 [a] ± 3.031	86.6 [b] ± 1.891	86.5 [b] ± 2.251	89.3 [b] ± 0.993	87.0 [b] ± 2.683
HVA	1.442 ± 0.377	0.744 ± 0.113	0.805 ± 0.141	1.056 ± 0.382	0.459 ± 0.214
9HDAA	0.363 ± 0.200	0.393 ± 0.067	0.327 ± 0.085	0.205 ± 0.039	0.181 ± 0.103
9HDA	11.99 [a] ± 1.161	9.45 [ab] ± 1.604	8.52 [ab] ± 1.448	5.90 [b] ± 0.415	7.85 [ab] ± 2.729
10HDAA	5.319 [a] ± 1.587	0.145 [b] ± 0.046	0.164 [b] ± 0.055	0.107 [b] ± 0.032	0.302 [b] ± 0.125
10HDA	3.56 ± 0.694	1.15 ± 0.429	2.79 ± 0.816	1.57 ± 0.364	1.47 ± 0.465

[a] Means within one row followed by the same letter are not significantly different (P > 0.05, Kruskal-Wallis). For rows in which no statistically significant difference was detected (P > 0.05, Kruskal-Wallis) no letters are included.

Abbreviations for the compounds are as follows: HOB = Methyl-*p*-hydroxybenzoate; 7HOA = 7-Hydroxyoctanoic acid; 4HBA = 4-Hydroxybenzoic acid; 8HOA = 8-Hydroxyoctanoic acid; 9ODA = (*E*)-9-ketodec-2-enoic acid; HVA = 4-Hydroxy-3-methoxyphenyl ethanol; 9HDAA = 9-Hydroxydecanoic acid; 9HDA = (*E*)-9-Hydroxydec-2-enoic acid; 10HDAA = 10-Hydroxydecanoic acid; 10HDA = (*E*)-10-Hydroxydec-2-enoic acid.

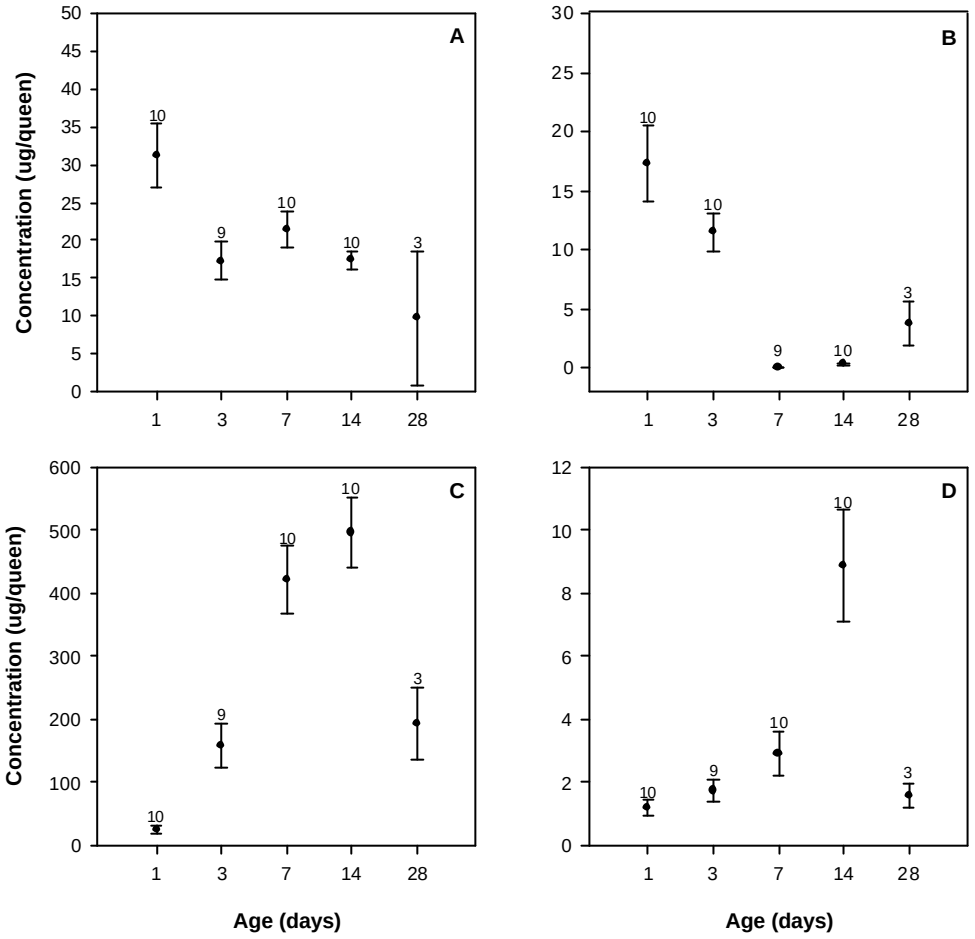


Figure 3.1: Ontogenetic changes in the volume of four compounds implicated in the biosynthesis of 9ODA **(C)**. Stearic acid **(A)** and palmitic acid **(B)** are precursors of the biosynthetic pathway while 8HOA **(D)** is thought to be an end-point by-product (Plettner *et al* 1995).

DISCUSSION

The mandibular gland secretions of *Apis mellifera capensis* queens exhibit some changes related to age. These changes are largely quantitative in nature, with the amounts of most of the compounds increasing with age. Qualitative changes are not as pronounced as those presented for other species of *Apis* (Plettner *et al* 1997) and other races of *A. mellifera* (Crewe 1982, Pankiw *et al* 1996).

Consistent with the finding of Engels *et al* (1997b), recently emerged *A.m. capensis* queens have a signal dominated by oleic acid. The one-day old queens analysed in this study had a signal in which all of the identified volatiles, albeit in small or trace quantities, are present. *A.m. capensis* appears to produce a mandibular gland signal in young virgin queens which is unique to the race. In comparison to the Californian stock queens analysed by Slessor *et al* (1990), the ontogenetic composition of the QMP compounds of *A.m. capensis* is more complex. 9HDA is the only compound of the QMP complex present in young Californian queens (<24 hours old - Slessor *et al* 1990) while detectable quantities of 9ODA, 9HDA and HVA were found in all one-day old queens analysed in this study. HOB was detected in minute or trace quantities in the queens analysed. Although the signal of *A.m. capensis* queens more closely resembles that of the other southern African honeybee, *A.m. scutellata,* than it does that of Africanized and European queens, significant differences in the percentage composition of the major compounds, as identified by Crewe (1982), are apparent. Young (1-2 day old) *A.m. scutellata* queen signals are dominated by 10HDA with a significant contribution by 9ODA (Crewe 1982). In comparison, when considering the percentage of the major compounds of the mandibular gland secretions (Table 3.8), the one-day old queens analysed in this study had a signal in which 9ODA predominated (72%) with 10HDA only making a small contribution (3.56%) to the signal. It has been suggested that the significance of the uniqueness of the signal of young *A.m. capensis* queens is necessary for the establishment of appropriate queen/worker relationships in a race where workers readily produce queen-like signals (Crewe 1988).

A progressive increase in the quantities of the major compounds (Table 3.1 and 3.2) for the first few days, followed by a relatively stable period between one and two weeks after emergence, characterises the ontogenetic patterns of the mandibular gland secretion of *A.m. capensis* queens. This corresponds with the period in which virgin queens are most likely to leave the nest in search of drones (Anderson *et al* 1983). The mandibular gland signals of these receptive queens are characterised by large quantities of 9ODA comprising in excess of 80% of the major compounds of the secretion (Table 3.8). Secretions of mated *A.m. capensis* were found to be dominated by 9ODA in the same relative proportion as that observed for the receptive virgin queens in this study, with 9HDA making a similar contribution to the signal in both receptive virgins and mated queens (Crewe 1982).

Comparisons of the relative proportions of 9ODA in the mandibular gland signals of the receptive virgins of the different races is difficult because many of the studies only provide data for the four compounds comprising the QMP complex (Slessor *et al* 1990; Pankiw *et al* 1996; Engels *et al* 1997b). These studies all found that 9ODA predominated in the QMP complex of receptive Africanized and European virgin queens. Data provided by Crewe (1982) and Plettner *et al* (1997) suggest that the data of these other studies are not a true reflection of the signal of receptive queens. Crewe (1982) and Plettner *et al* (1997) data indicates that when the "worker substance", 10HDA, is incorporated into relative calculations of the contribution of the major compounds in mandibular gland extracts, a different pattern emerges. They found that receptive virgins of *A. mellifera* (Plettner *et al* 1997) and *A.m. mellifera* (Crewe 1982) had a signal dominated by 10HDA with a significant contribution of 9ODA. Receptive *A.m. intermissa* queens have a signal dominated by 9ODA with a significant contribution of 9HDA (Crewe and Moritz 1989). The receptive queen data presented in this study further illustrates the uniqueness of the *A.m. capensis* mandibular gland signal.

The observed increase in 9ODA production as *A.m. capensis* queens age corresponds with an increase in 8HOA and a decrease in palmitic and stearic acids (Figure 3.1). These results concur with the biosynthetic pathways proposed by Plettner *et al* (1998) for the synthesis of 9ODA in queens. Palmitic and stearic acids are identified as precursors of the pathway, while 8HOA is considered a by-product in the synthesis of 9ODA (Plettner *et al* 1995). Plettner *et al* (1998) suggested that the stearic acid required for the biosynthesis of 9ODA is produced in the mandibular gland. The data presented here provides quantitative evidence to support this suggestion. The elevated levels of the precursors and their associated isomers found in one-day old queens (which decreases as 9ODA production increases as the queens age) indicates that these compounds are produced in the mandibular gland and are utilised in 9ODA synthesis, resulting in the decreased levels observed in older queens producing increased levels of 9ODA.

It has been suggested that the ontogenetic changes may be involved in premating, mating and postmating dominance status of queens, being of particular significance during the period of temporary polygyny before swarming (Crewe 1988; Engels *et al* 1997b). Characteristic signals may also function to label receptive queens as reported in other social bees (Ayasse *et al* 1990, 1993; Engels *et al* 1997a). It has been proposed that race-specific characteristics in the chemical composition of mandibular gland extracts are related to differences in the natural history and social organisation of the various races (Crewe 1988).

The functional significance of the ontogenetic changes and race-specific composition reported in the mandibular gland signals has yet to be fully understood. Suggestions have been hypothesised with the available data lending support to the various proposals, further

emphasising the complexity of the mandibular gland signal and the need for additional studies to provide a comprehensive understanding of its biological significance.

CHAPTER FIVE

THE EFFECT OF HOLDING CONDITIONS ON QUEEN SURVIVAL AND THE PRODUCTION OF THE CHEMICAL COMPOUNDS IDENTIFIED IN THE MANDIBULAR GLANDS OF *Apis mellifera capensis* VIRGIN QUEENS

SUMMARY

The effect of various holding conditions on the survival of virgin queens was investigated by rearing queens in different holding conditions. The results demonstrate that queen banking is not a viable method of rearing *Apis mellifera capensis* virgin queens. Keeping queens singly, with or without small groups of workers in an incubator and in small mating nucleus hives, proved to be the most successful methods of queen rearing in *A.m. capensis*, with high survival rates being achieved using these techniques. Queens kept for 12 days under the holding conditions which proved to be the most successful in terms of queen survival were used to determine the effect of holding condition on the chemical composition of virgin queen mandibular gland secretions. It was determined that in addition to age, caste and race which have previously been shown to affect the chemical composition of mandibular gland secretions, the presence of workers during the ageing of virgin queens significantly influences the mandibular gland signal.

INTRODUCTION

Ontogenetic studies require a series of samples of individuals of known age. Studies investigating the ontogenetic development of the chemical composition of the mandibular glands of honeybee queens have used virgin queens ranging in age from newly emerged queens less than 24-hours old to ones that are 40-days old (Crewe 1982; Slessor *et al* 1990). Various methods of maintaining queens are available to the researcher, enabling him to produce virgin queens of the desired age. The holding conditions selected to age queens for experiments has largely been dependent on the individual preference of the queen rearer and

may be influenced by the race of queen being reared (this chapter). Queens are most frequently aged by queen banking, where large numbers of virgin queens are caged in a single hive without worker attendants (Yadava and Smith 1971a; Lensky *et al* 1991) or in individual cages, with or without attendant workers, in an incubator (Post *et al* 1987; Moritz and Crewe 1991; Pham-Delègue *et al* 1993). Alternatively, virgin queens are placed in small (three-frame) colonies with worker bees until they reach the desired age (Crewe and Moritz 1989).

The high mortality rate amongst older (particularly 28-day old) *Apis mellifera capensis* virgin queens when rearing them for ontogenetic studies (chapter 3) prompted an experiment to test the effect of different holding conditions on queen production in this race. Knowledge of queen production in African honeybees, and the Cape honeybee in particular, is limited with information in the published literature restricted to a few reports on queen-rearing techniques (Beyleveld 1935; Anderson 1965; Cooke 1986; McGregor 1992). A successful rearing technique should not only ensure a high survival rate of queens but should also ensure a high rate of acceptance of virgin queens by workers (Ribbands 1953; Cooke 1986). The effect of queen holding conditions on the reaction of workers to introduced queens was investigated using behavioural bioassays (chapter 6). Antagonistic reactions by workers to introduced queens are generally attributed to the “alien odour” emitted by the new queen (Free 1987).

In the existing literature little or no distinction is made regarding the specific conditions pertaining to virgin queen mandibular gland secretions with insufficient detail being paid to the holding conditions of queens before processing. The chemical composition of mandibular gland extracts has previously been demonstrated to be affected by age, caste and race (c.f. Free 1987). The objective of this study was to ascertain whether the holding conditions under which queens are kept to age them, had any affect on signal development. In particular, it investigated what effect the presence of workers during the ageing process of queens had on the chemical development of virgin *A.m. capensis* queens using the compounds identified from mandibular gland secretions in chapter two.

MATERIALS AND METHODS

Samples and Sample Preparation

Apis mellifera capensis queens were reared from colonies in Stellenbosch, South Africa (33° 56' S, 18° 52' E). Queen larvae from a single donor colony were reared in queen cells in four host colonies. Upon emergence, queens were kept for 12 days or until they died under one of six holding conditions described below:

A – Alone in bottles in an incubator

Upon emergence, 15 virgin queens were placed in individual bottles in an incubator. Each bottle contained an *ad libitum* supply of concentrated honey-water and water; no workers were present. Standard, 40ml screw-top, plastic bottles with holes drilled in the base were used. Incubator temperature was 30-32°C.

B – Singly in cages with 50 workers in an incubator

Fifteen small Liebefeld cages containing 50 newly emerged bees (all coming from the same frame) were given a virgin queen. The glass fronted, meshed floor, wooden cages measured approximately 100 x 85 x 130mm. A piece of empty honeycomb was fixed inside the cage. The bees were fed concentrated honey-water and water *ad libitum*.

C – Singly in queenless mating nucleus hives

Ten three-frame mating nucleus hives were set up. Each frame had a feeder, one frame of brood, one frame of honey and pollen and one to two frames of bees. The bees and frames were obtained from ten different colonies. The hives were moved to new sites to prevent drifting. Each hive was given two queen cells to emerge within 24 hours. Colonies were sealed with a queen excluder to prevent queen mating flights from taking place.

D – Multi-virgins in a queenless colony, worker access to queens

A colony which been queenless for six weeks and which had some laying worker brood was used. Three days prior to the introduction of 18 virgin queens, the colony was given two frames of emerging bees. Eighteen queen cages, with queen excluder on both sides, were placed in a single frame inserted between the two brood frames.

E – Multi-virgins in queenless colony, worker access restricted

A queenless colony as described above was used. Eighteen virgin queens were placed in wooden cages with gauze which allowed the workers to feed the queens but prevented them from getting through to the queen.

F – Multi-virgins in a queenright colony

The mated queen was locked between two frames thus achieving a queen compartment. Eighteen queen cages, with queen excluder on both sides, were placed in one frame which was placed two frames away from the queen compartment. Three days prior to the introduction of the virgin queens, the colony was given two frames of emerging bees. One queen was placed into each of the 18 cages.

Upon emergence, the number of queens alive in each group was checked after 24 hours, three days and five days. Twelve days after emergence the surviving virgin queens were removed from their holding conditions, used in the behavioural experiments discussed in

chapter six and then the mandibular glands removed and prepared as described in chapter two.

Chemical Analysis

Chemical analyses of the mandibular gland extracts of the virgin queens were performed using gas chromatography and gas chromatography–mass spectroscopy as described in chapter two.

Statistical Analysis

One-Way ANOVA and ANOVA on ranks were used as outlined in chapter two, to compare the amount of each compound identified from each group (holding condition).

RESULTS

The effect of holding conditions on the survival rate of virgin queens

Keeping virgin queens singly with a group of workers proved to be the most successful holding technique in terms of survival rates (Table 5.1). Holding condition B proved to be the most successful method for ageing queens, with all virgin queens kept in cages with small groups of workers under controlled conditions (incubator environment) still alive when the experiment was terminated after 12 days. Queen banking, in queenless and queenright colonies, proved to be an unsuccessful holding technique for ageing *Apis mellifera capensis* virgin queens, with all queens surviving less than five days.

Chemical analyses studies

A.m. capensis queens, kept under three holding conditions for 12 days, were used to determine the effect of holding condition on the chemical composition of virgin queen mandibular gland extracts.

The chemicals identified in this study from the mandibular gland extracts of *A.m. capensis* virgin queens can be broadly grouped into those which were found to be significantly affected by holding conditions ($P < 0.05$) and those that exhibited no significant difference in relation to holding condition ($P > 0.05$; Tables 5.2 - 5.8).

The volume of the aliphatic acids considered to be the major components of the mandibular gland extracts was significantly affected by the holding conditions under which virgin queens were reared; queens kept alone (holding condition A) produced significantly greater quantities than those kept with small groups of workers (B; $P < 0.05$; Table 5.2). Queens kept alone in bottles in an incubator (A) produced greater volumes of the major aliphatic acids (Table 5.2) and phenols (Table 5.3) than those kept with workers. Queens kept in mating nucleus hives

(C) with large numbers of workers produced greater quantities of the major aliphatic acids than those kept with small groups of workers in the incubator (B).

Holding conditions were found to have no significant effect on the quantity of the majority of the minor aliphatic acids and diacids, alkanes, aromatic acids and diacids and the various miscellaneous compounds identified in the mandibular gland extracts of the queens analysed in this study (Tables 5.4 - 5.8). For those compounds affected by holding condition, significant differences were detected between the amount of compounds identified in extracts of queens kept alone (A) and those kept with small groups of workers (C).

Table 5.1: The percentage of *Apis mellifera capensis* virgin queens surviving 24 hours, 3, 5 and 12 days after emergence for each of the holding conditions. The following holding conditions were tested: A = Alone in bottles in an incubator, B = Singly in cages with 50 workers in an incubator, C = Singly in queenless mating nucleus hives, D = Multi-virgins in a queenless colony, worker access to queens, E = Multi-virgins in queenless colony, worker access restricted and F = Multi-virgins in a queenright colony. n = number of queens reared in each condition.

Days	Holding Condition					
	A n = 15	**B** n = 15	**C** n = 10	**D** n = 18	**E** n = 18	**F** n = 18
24 hours	100	100	100	100	0	0
3 days	100	100	100	100	0	0
5 days	93.3	100	100	77.8	0	0
12 days	66.7	100	80.0	0	0	0

Table 5.2: Amounts (µg; mean [a] ± SE) of the major aliphatic acids present in 12 day-old *Apis mellifera capensis* virgin queens reared under each of the three holding conditions. Holding condition A = Alone in bottles in an incubator, B = Singly in cages with 50 workers in an incubator and C = Singly in queenless mating nucleus hives.

Compound	Holding Condition		
	A n = 9	**B** n = 12	**C** n = 6
7HOA	0.06153 [a] ± 0.04228	0.00003 [b] ± 0.00003	0.00694 [ab] ± 0.00673
8HOA	2.787 [a] ± 0.637	0.561 [b] ± 0.115	0.976 [ab] ± 0.260
9ODA	180.9 [a] ± 34.5	53.7 [b] ± 11.5	137.0 [ab] ± 35.6
9HDAA	0.09203 [a] ± 0.06047	0.00018 [b] ± 0.00007	0.00119 [ab] ± 0.00079
9HDA	8.12 [a] ± 3.131	1.12 [b] ± 0.215	4.06 [ab] ± 1.879
10HDAA	0.693 [a] ± 0.173	0.038 [b] ± 0.032	0.004 [b] ± 0.001
10HDA	7.951 [a] ± 4.332	0.728 [b] ± 0.414	3.062 [ab] ± 1.338

[a] Means within one row followed by the same letter are not significantly different (P > 0.05, Kruskal-Wallis). For rows in which no statistically significant difference was detected (P > 0.05, Kruskal-Wallis) no letters are included.

Abbreviations for the compounds are as follows: 7HOA = 7-Hydroxyoctanoic acid; 8HOA = 8-Hydroxyoctanoic acid; 9ODA = (*E*)-9-ketodec-2-enoic acid; 9HDAA = 9-Hydroxydecanoic acid; 9HDA = (*E*)-9-Hydroxydec-2-enoic acid; 10HDAA = 10-Hydroxydecanoic acid; 10HDA = (*E*)-10-Hydroxydec-2-enoic acid.

Table 5.3: Amounts (µg; mean [a] ± SE) of the major phenols present in *Apis mellifera* capensis virgin queens reared under each of the three holding conditions. Holding condition A = Alone in bottles in an incubator, B = Singly in cages with 50 workers in an incubator and C = Singly in queenless mating nucleus hives.

Compound	Holding Condition		
	A	**B**	**C**
	n = 9	n = 12	n = 6
HOB	0.25321 ± 0.16268	0.00040 ± 0.00002	0.00041 ± 0.00003
4HBA	0.00374 ± 0.00200	0.00157 ± 0.00030	0.00205 ± 0.00015
HVA	0.19579 [a] ± 0.08243	0.00062 [b] ± 0.00023	0.00228 [ab] ± 0.00089

[a] Means within one row followed by the same letter are not significantly different (P > 0.05, Kruskal-Wallis). For rows in which no statistically significant difference was detected (P > 0.05, Kruskal-Wallis) no letters are included.

Abbreviations for the compounds are as follows: HOB = Methyl-*p*-hydroxybenzoate; 4HBA = 4-Hydroxybenzoic acid; HVA = 4-Hydroxy-3-methoxyphenyl ethanol

Table 5.4: Amounts (µg; mean [a] ± SE) of the minor aliphatic acids present in *Apis mellifera capensis* virgin queens reared under each of the three holding conditions. Holding condition A = Alone in bottles in an incubator, B = Singly in cages with 50 workers in an incubator and C = Singly in queenless mating nucleus hives.

Compound	Holding Condition		
	A	**B**	**C**
	n = 9	n = 12	n = 6
2-Hexenoic acid	0.235 ± 0.005	0.239 ± 0.018	0.239 ± 0.010
Propanoic acid	0.105 ± 0.035	0.051± 0.033	0.036 ± 0.033
2-Propenoic acid	0.00097 ± 0.00042	0.00082 ± 0.00033	0.09312 ± 0.05841
Dodecanoic acid	0.213 ± 0.083	0.203 ± 0.046	0.109 ± 0.067
Decanoic acid	0.235 [a] ± 0.06011	0.001 [b] ± 0.00034	0.002 [ab] ± 0.00070
Palmitoleic acid	0.228 ± 0.11652	0.001 ± 0.00036	0.008 ± 0.00811
Palmitelaidic acid	0.404 ± 0.144	0.865 ± 0.233	0.651 ± 0.296
10-Undecenoic acid	2.04 ± 0.518	4.88 ± 0.983	3.96 ± 0.917
Palmitic acid	5.64 ± 0.841	9.48 ± 1.831	8.78 ± 2.160
Oleic acid	40.2 ± 11.3	67.0 ± 18.6	57.0 ± 18.5
Stearic acid	9.25 ± 2.13	15.08 ± 4.12	11.08 ± 2.27

[a] Means within one row followed by the same letter are not significantly different (P > 0.05, Kruskal-Wallis). For rows in which no statistically significant difference was detected (P > 0.05, Kruskal-Wallis) no letters are included.

Table 5.5: Amounts (µg; mean [a] ± SE) of the aliphatic diacids present in *Apis mellifera capensis* virgin queens reared under each of the three holding conditions. Holding condition A = Alone in bottles in an incubator, B = Singly in cages with 50 workers in an incubator and C = Singly in queenless mating nucleus hives.

Compound	Holding Condition		
	A	**B**	**C**
	n = 9	n = 12	n = 6
Suberic acid	0.436 ± 0.051	0.613 ± 0.076	0.543 ± 0.129
Azelaic acid	0.295 ± 0.073	0.492 ± 0.074	0.486 ± 0.100
Undecandioic acid	0.001 [a] ± 0.00036	0.175 [b] ± 0.12083	0.101 [b] ± 0.09055

[a] Means within one row followed by the same letter are not significantly different (P > 0.05, Kruskal-Wallis). For rows in which no statistically significant difference was detected (P > 0.05, Kruskal-Wallis) no letters are included.

Table 5.6: Amounts (µg; mean [a] ± SE) of the alkanes present in *Apis mellifera capensis* virgin queens reared under each of the three holding conditions. Holding condition A = Alone in bottles in an incubator, B = Singly in cages with 50 workers in an incubator and C = Singly in queenless mating nucleus hives.

Compound	Holding Condition		
	A	**B**	**C**
	n = 9	n = 12	N = 6
Eicosane	0.264 [a] ± 0.08432	0.001 [b] ± 0.00029	0.047 [ab] ± 0.04500
Heneicosane	0.138 ± 0.064	0.302 ± 0.099	0.195 ± 0.122
Docosane	1.33 ± 0.196	2.16 ± 0.278	2.03 ± 0.393
Hexacosane	1.29 ± 0.875	3.56 ± 0.775	2.60 ± 0.869

[a] Means within one row followed by the same letter are not significantly different (P > 0.05, Kruskal-Wallis). For rows in which no statistically significant difference was detected (P > 0.05, Kruskal-Wallis) no letters are included.

Table 5.7: Amounts (µg; mean [a] ± SE) of the aromatic acids and diacids present in *Apis mellifera capensis* virgin queens reared under each of the three holding conditions. Holding condition A = Alone in bottles in an incubator, B = Singly in cages with 50 workers in an incubator and C = Singly in queenless mating nucleus hives.

Compound	Holding Condition		
	A	**B**	**C**
	n = 9	n = 12	n = 6
Aromatic acids			
Benzoic acid	0.121 ± 0.07877	0.002 ± 0.000635	0.002 ± 0.00093
Cinnamic acid	0.07730 ± 0.07619	0.00008 ± 0.00005	0.00143 ± 0.00087
Aromatic diacids			
Vanillylpropionic acid	0.00155[a] ± 0.00057	0.00016[b] ± 0.00006	0.17854[ab] ± 0.17728

[a] Means within one row followed by the same letter are not significantly different (P > 0.05, Kruskal-Wallis). For rows in which no statistically significant difference was detected (P > 0.05, Kruskal-Wallis) no letters are included.

Table 5.8: Amounts (µg; mean [a] ± SE) of the amino acids, sugars, indole and an unknown compound present in *Apis mellifera capensis* virgin queens reared under each of the three rearing techniques. Holding condition A = Alone in bottles in an incubator, B = Singly in cages with 50 workers in an incubator and C = Singly in queenless mating nucleus hives.

Compound	Holding Condition		
	A	**B**	**C**
	n = 9	n = 12	n = 6
Amino acids			
L-Proline	0.231 [a] ± 0.07842	0.001 [b] ± 0.00032	0.176 [a] ± 0.08167
Glutamic acid	1.025 [a] ± 0.223	0.326 [b] ± 0.074	0.610 [ab] ± 0.096
L-Serine	0.181 ± 0.019	0.185 ± 0.020	0.128 ± 0.039
Sugars			
D-Glucose	0.227 [a] ± 0.117	0.133 [b] ± 0.075	0.158 [b] ± 0.142
L-Altrose	0.760 ± 0.122	1.459 ± 0.116	1.397 ± 0.135
Other			
Indole	0.362 ± 0.003	0.364 ± 0.026	0.355 ± 0.005

[a] Means within one row followed by the same letter are not significantly different (P > 0.05, Kruskal-Wallis). For rows in which no statistically significant difference was detected (P > 0.05, Kruskal-Wallis) no letters are included.

DISCUSSION

The results presented in this study clearly demonstrate that queen banking is not a viable method for keeping *Apis mellifera capensis* virgin queens and thus provide support for methods employed by beekeepers and researchers utilising this race of southern African honeybee. Keeping *A.m. capensis* queens singly in cages in an incubator was the method advocated by a Cape Town queen-rearer, who found that queens could be kept in this manner for up to a week without any risk of jeopardising their chances of becoming good layers (Cooke 1986). The virgin *A.m. capensis* queens used by Buys (1984) for his work on artificial insemination were kept in cages with about 200 workers. In contrast, queen banking is successfully used for ageing European and Africanized queens. Lensky *et al* (1991) kept *A.m. ligustica* virgin queens inside emerging cages in bee colonies until they were needed at 4-5 days old. Post *et al* (1987) kept queens individually in mesh cages in groups of eight for 2-3 weeks, while Yadava and Smith (1971a) used queen banks containing 52 standard Benton queen-mailing cages (see Snelgrove 1940 for cage description) per "queen holding" colony.

Queen banking may not work in *A.m. capensis* because of the large number of virgin queens emitting chemical signals simultaneously. The lack of a single dominant signal may result in confusion amongst the workers, who may not recognise the multitude of signals as originating from young queens. Also the ability of *A. m. capensis* laying workers or pseudoqueens to produce mandibular gland signals similar to those of queens (Crewe *et al* 1990a,b) may result in workers being unable to distinguish between virgin queens and the laying workers already present in queenless colonies (holding conditions D and E). This confusion could result in the queens not receiving the attention necessary to ensure their survival.

In addition to the effects on survival rate discussed above, holding condition during queen ageing was found to affect the chemical composition of the mandibular gland extracts of *A.m. capensis* virgin queens. Some compounds were affected by holding condition, with different quantities being produced by queens kept under different holding conditions. Other compounds were unaffected by holding condition, with similar quantities being produced by queens kept under all three holding conditions. The quantity of the aliphatic acids and phenols considered the major components of the mandibular gland signal (Crewe *et al* 1990a,b) varied significantly with holding condition, while most of the remaining identified compounds showed no difference between holding conditions.

The compounds exhibiting variations in quantity as a result of holding condition may represent the active components of the mandibular gland extract. These compounds may constitute the chemical signal as perceived by the workers, with its release being behaviourally mediated by queen-worker interactions. Evidence that queen-produced pheromones are removed from the queen and dispersed in the colony by queen attendants (Velthuis 1972, Butler *et al* 1974;

Seeley 1979, Juška *et al* 1981) was confirmed using tritium-labelled 9ODA (Webster and Prestwich 1988). Using this technique, Naumann and his co-workers (1991) were able to trace the movement of 9ODA within the colony by worker-queen and worker-worker interactions. If, as suggested, the chemical signal is mediated by queen-worker interactions, it is likely that the release of the signal is triggered by the presence of appropriate receivers. If this is true, it it is possible that in the absence of such receivers the levels of the chemicals constituting the chemical signal may build up in the mandibular glands, resulting in extracts with increased quantities of these compounds.

The three holding conditions used to age the virgin queens used in the chemical analyses represent different queen to worker ratios; holding condition A had no workers with the queens, B had 50 workers per queen while C had several thousand workers with the individual queens. Although no quantitative measure of queen-worker interactions were made, direct observations during the ageing of the virgin queens suggest that despite the fewer numbers of workers in the small cages (holding condition B) a greater amount of queen-worker interactions occur under these conditions than were observed to occur between queens and workers in the mating nucleus hives (holding condition C). Queens in nucleus hives were frequently observed in isolated regions of the hives, spending increasing periods in isolation probably resulting in decreased queen-worker interactions.

The data presented in these analyses support the suggestion proposed, in this study, of queen signal production being behaviourally mediated by queen-worker interactions. Virgin queens kept alone in bottles in the incubator had extracts containing significantly greater quantities of the compounds constituting the chemical signal than those kept with several thousand workers in mating nucleus hives. Queens kept with small groups of workers had mandibular gland extracts with intermediate quantities of the compounds. The findings of Naumann *et al* (1991), that the quantity of 9ODA detectable in the mandibular glands of mated queens increases with time spend in isolation from workers, lends support to this suggestion.

A variety of factors contribute to the composition of the chemical constituents of queen mandibular gland secretions with the quantity measured being a complex function of social and hive conditions. Workers, brood, wax, hive volume, hive temperature and rate of chemical production in the gland are some of the factors contributing to the development of the chemical siganl of queens prior to sampling. This study demonstrates that holding conditions and the resultant intensity of queen-worker interactions prior to sampling also contribute to the quantity of chemicals in queen mandibular gland samples. These findings have important consequences for comparisons between the quantities of chemical constituents measured in queen mandibular land secretions from different studies. It is likely that accurate and reliable comparisons are only possible if similar holding, social and environmental conditions have been used for ageing the virgin queens sampled.

CHAPTER SIX

BEHAVIOURAL REACTIONS OF WORKER HONEYBEES TO INTRODUCED *Apis mellifera capensis* VIRGIN QUEENS REARED UNDER DIFFERENT HOLDING CONDITIONS

SUMMARY

Small experimental cages containing approximately 100 workers were used to study worker reactions towards queens which were reared using different holding conditions. Worker responses towards introduced virgin queens included non-hostile or investigative behaviours which included licking and palpating of the queen by workers and hostile reactions which included the mounting, biting, pulling and stinging of the queens by the workers. The results of this investigation demonstrate that workers exhibit different levels of aggression to virgin queens reared under different conditions with queens reared with workers elicited significantly less ($P = 0.0140$) hostile reactions from workers than those reared alone.

INTRODUCTION

The contribution of the chemical compounds produced in the mandibular glands of honeybee queens in ensuring the optimal functioning of the colony has been well documented (c.f. Free 1987). Among the array of functions attributed to this glandular secretion is its role as an arrestant of workers within the nest, resulting in the production of a queen's court or retinue which almost always surrounds the queens (Naumann *et al* 1991). The queen retinue usually consists of eight or more worker attendants who are continually licking and palpating the queen with their antennae (Allen 1960; Free and Furguson 1982; Free 1987). Workers who have been in a queen's court move throughout the colony, making frequent reciprocal contacts with other workers, thereby facilitating the transport of the queen pheromones throughout the nest (Velthuis 1972; Seeley 1979; Ferguson and Free 1980;Naumann *et al* 1991, 1992, 1993). It has been demonstrated that on contact with a queen a worker acquires the queen pheromones on her anterior surfaces which may then be removed by her nestmates, thus facilitating the dispersal and dissipation of the pheromones (Juška *et al* 1981). According to

Juška *et al* (1981) a continual elimination of queen pheromones is essential for preventing saturation and the subsequent loss of control of pheromonally mediated behaviours.

While the importance of the transmission by workers of queen pheromones, and 9ODA in particular, in ensuring colony functioning has been established the consequences of such actions on the queen have not been considered. Having established that the presence of workers during ageing of virgin queens influenced the quantity of chemicals produced in the mandibular glands (chapter 5), this study aimed to ascertain what effect this had on worker behaviour towards queens. The purpose of this study, therefore, was to determine the effect of holding condition during the ageing of queens on worker responses upon queen introduction.

MATERIALS AND METHODS

Behavioural Observations

Behavioural observations were all carried out at the Department of Zoology and Entomology, Rhodes University, Grahamstown. Twelve day-old *Apis mellifera capensis* virgin queens, reared using three different holding conditions, were used to investigate the effect of holding condition on worker reactions to introduced virgin queens. The three holding conditions tested were: A – queens kept alone in bottles in an incubator, B – queens kept singly in cages with 50 workers in an incubator and C – queens kept singly in queenless mating nucleus hives. A comprehensive description of these holding conditions is provided in chapter five.

Groups of workers in small experimental cages were used to monitor worker behaviour towards introduced virgin queens. The cages had an internal diameter of 140 x 120 x 90 mm and were made of glass. One of the cage walls was mesh, while the opposite wall had a gap between the bottom of the cage and the wall, thus ensuring a good air supply and air flow in the cages. Each cage was supplied with ± 100 “test” workers. The groups of “test” workers were obtained from colonies maintained in Grahamstown. The colonies from which the “test” workers were obtained were different colonies to those from which the queen larvae and “holding” workers were taken. This ensured that nestmate recognition effects did not interfer with the experimental results. The “test” workers were collected off the frames of the donor colonies and introduced into the experimental cages. The cages were then placed in a constant environment room (28ºC) with continual red light. It was under these conditions that the experiments were conducted. Approximately four hours were allowed to lapse between populating the cages with workers and introducing the queens. According to Yadava and Smith (1971a) this ensured that the caged worker populations became accustomed to their new environment and that the general behavioural responses of the workers would return to normal before introduction of a queen. In addition, Yadava and Smith (1971a) considered that this would ensure the workers were in a queenless condition at the time of queen introduction.

Upon introduction, the reactions of the workers to the introduced queens were observed until such time as the workers were no longer responding to them. A voice-activated tape recorder was used by the observer to record the behavioural reactions. Due to the varying periods for which the queens were observed, the reactions of the workers to the introduced queens were analysed in terms of the reactions during the initial 15 minutes after introduction. Worker reactions to introduced queen were classified as either non-hostile investigative behaviours or hostile reactions.

Investigative behaviours included the licking and palpating of the queen on her abdomen by the workers, as well as antennal contacts between workers and the queen and other behaviours associated with food transfer (Free 1987). The hostile reactions observed were similar to those observed by Pettis *et al* (1998) and described in chapter four. They included the mounting, biting, pulling and stinging of the introduced queen by the workers.

Data Analysis

Worker reactions for the initial 15 minutes after introduction were analysed in terms of percentage of hostile and non-hostile reactions. One-way ANOVA were carried out on the data sets to determine whether rearing queens under different holding conditions affects the reaction of workers to introduced virgin queens.

RESULTS

Significant differences in the reactions of workers to *Apis mellifera capensis* virgin queens reared under different holding conditions were observed. Workers responded more aggressively to queens which had been reared alone (Holding condition A) than to those that had been raised with groups of workers (Holding conditions B and C; Table 6.1; P = 0.0140).

When queens reared alone (A) were introduced into groups of workers, they were immediately balled by the workers who surrounded them. Antagonistic behaviours towards introduced queens by the workers included stinging and biting, particularly of the legs and wings. These hostile reactions continued for an average of 32:06 ± 2.57 minutes whereupon the queen wandered around the cage, intermittently wiping her hind legs together, during this time she was occasionally investigated by workers who palpated and licked her.

In comparison, when queens reared with workers (B and C) were introduced into groups of workers, far fewer antagonistic behaviours by the workers to the introduced queen were observed. These behaviours ceased after an average of 15:36 ± 5:03 and 13:36 ± 4.37 minutes, respectively, to queens reared in cages with workers in an incubator (B) and those reared singly in mating nucleus hives (C). During the periods in which antagonistic behaviours were observed, a greater number of non-hostile reactions, including licking and palpating, were observed.

Table 6.1: Comparison of the reactions of worker honeybees to introduced virgin *Apis mellifera capensis* queens reared under three holding conditions. The data are presented as the percentage (mean [a] ± SE) of the hostile and non-hostile behaviours recorded for 15 minutes after the introduction of the queen into the group of workers. A – Alone in bottles in an incubator, B – Singly in cages with 50 workers in an incubator and C – Singly in queenless mating nucleus hives.

Reaction	Holding Condition		
	A	**B**	**C**
	n = 5	n = 5	n = 5
Hostile	56.0 [a] ± 11.90	29.6 [b] ± 7.03	14.8 [b] ± 4.37
Non-Hostile	44.0 [c] ± 11.90	70.4 [d] ± 7.03	83.8 [d] ± 4.37

[a] Means within one row followed by the same letter are not significantly different ($P > 0.05$, Kruskal-Wallis). For rows in which no statistically significant difference was detected ($P > 0.05$, Kruskal-Wallis) no letters are included.

DISCUSSION

The effect of queen age and the composition of queen exocrine gland secretions on workers' responses towards introduced queens is well documented (Gary 1962; Yadava and Smith 1971a,b; De Hazan *et al* 1989a; Lensky *et al* 1991; chapter 4). Other factors which have been found to influence worker reaction to introduced queens include: the queenright or queenless state of the colony (Yadava and Smith 1971c); the mated state of the queen (Butler and Simpson 1956); the degree of kinship between the workers and the queen (Page and Erickson 1986); and, the age of the of the receptive workers (Silva *et al* 1995). This study provides evidence that the presence of workers during the ageing of virgins queens also contributes to the reactions of workers to introduced queens.

The presence of workers during queen rearing impacts significantly on the reactions of workers to introduced queens. Workers reacted with increasing hostility and for longer periods to virgin queens which had been reared alone than they did to those which had been reared with workers. Virgin queens reared with small groups of workers in an incubator and ones reared singly in mating nucleus hives with several thousand workers exhibited no difference in the proportion of aggressive behaviours they received from workers.

Although this study only considers the impact of one of the factors (ie the presence of workers during queen rearing) that contributes to the reaction of workers to introduced queens, two hypotheses are advanced that could, in part, explain the mechanism whereby worker aggressive behaviour towards introduced queens is reduced when queens reared with groups of workers are introduced. The first suggests that queens reared alone may have a full complement of mandibular gland components, while those reared with workers have reduced levels of QMP and the other major compounds of mandibular gland extracts. This could increase their acceptance by colonies with the decreased levels of mandibular gland compounds emanating those of young virgin queens (Pettis *et al* 1998). The second hypothesis suggests that virgin queens reared with workers are exposed to workers through frequent interactions and, consequently, learn appropriate behaviours and actions towards workers, which could facilitate in their increased acceptance into groups of workers. Alternatively, worker aggression towards introduced queens could be mediated by a mechanism incorporating both hypotheses.

Evidence provided in this study suggests that the second hypothesis could contribute to the release of worker aggression towards introduced virgin queens. The less hostile reactions of workers to both groups of queens reared with workers, in contrast to the levels of aggression observed towards queens reared alone, supports this hypothesis. Workers reared alone may not learn appropriate behaviours and reactions towards workers and are therefore subjected to increased levels of hostility by workers. The increased opportunities of queen-worker interactions in small cages, in contrast to queens kept in mating nucleus hives, provide further

support for this hypothesis, with queens reared in small groups eliciting more hostile behaviours from workers upon introduction.

It can therefore be proposed that the presence of workers during the rearing of queens contributes to ensuring reduced risk of rejection and injury of queens subsequent to their introduction through aggressive encounters with workers. It is possible that queens learn appropriate behaviours through queen-worker interactions which contributes to facilitating their successful introduction into groups of workers.

CHAPTER SEVEN

REGIONAL VARIATIONS IN THE COMPOUNDS IDENTIFIED IN THE MANDIBULAR GLANDS OF MATED QUEENS FROM THE EASTERN CAPE PROVINCE, SOUTH AFRICA

SUMMARY

The feasibility of using queen mandibular gland extracts as an additional probe for defining the geographical distribution of *Apis mellifera capensis* and *Apis mellifera scutellata* and their hybrids was investigated. The mandibular gland secretions of mated queens from five localities in the Eastern Cape Province, South Africa, were compared and were found to exhibit no significant differences in the amount of compounds identified from the mandibular gland extracts. The ratio of 9ODA:10HDA, previously determined to differ qualitatively for the two races of southern African honeybees, was used to characterise the queens from the five localities. No significant difference (P = 0.131) was found in the 9ODA:10HDA ratio of the mated queens collected in the Eastern Cape Province and it was determined that none of the queens sampled could be characterised as *A.m. capensis* on the basis of their mandibular gland secretions. Consequently, mated queens from the five localities in the Eastern Cape Province could not be separated on their mandibular gland secretion profiles.

INTRODUCTION

Hybridisation between the two southern African races of honeybees, *Apis mellifera capensis* and *Apis mellifera scutellata*, is of considerable concern to the South African beekeeping industry. Introduction of *A.m. capensis* into areas previously regarded to be the domain of *A.m. scutellata* caused high losses to commercial beekeeping in the country as a result of what became known as the "*capensis* invasion" (Allsopp 1992; Cooke 1992; Allsopp and Crewe 1993). Consequently, research focused on defining the distribution of these two races and understanding the processes which had previously ensured the relative stability of the hybrid zone and the dominance of these two races in their respective habitats.

Traditionally *A.m. capensis* was thought to occupy a small region at the south-western tip of the country, centred around Cape Town, with *A.m. scutellata* occurring to the north (Ruttner 1977; Moritz and Kauhausen 1984). Detailed studies and improved techniques have resulted in revised distributions for these subspecies. The boundaries for the occurrence of the two races and that of their natural hybrid zone vary, depending upon the trait under investigation. Analyses of the reproductive biology, including the trait of "diploid eggs laid by workers" (DELW), ovariole number and spermatheca size, of bees from throughout Southern Africa provided a comprehensive data base providing a new assessment of the distribution of the Cape honeybee, *A.m. capensis* (Hepburn and Crewe 1990, 1991). Based on these traits, these authors consider *A.m. capensis* to occur along the south-west and south coast of South Africa extending inland as far as the first range of mountains. Further inland of this region hybridisation between *A.m. capensis* and *A.m. scutellata* occurs along a fragmented and elongated zone stretching approximately to a second range of mountains, beyond which is the domain of *A.m. scutellata*. Morphometric analyses to define the distribution of these races shifts the boundaries of *A.m. capensis* and the hybrid zone northwards in the Eastern Cape (Crewe *et al* 1994). The mitochondrial DNA haplotype, PoQQa is fixed in *A.m. capensis* and occurs with a 100% frequency to the north of the boundary of the hybrid zone (Moritz *et al* 1994). High variance in alarm pheromone production from the stings of guard bees was found to coincide with the hybridisation zones previously defined (Hepburn *et al* 1994).

The Eastern Cape presents an interesting situation for beekeepers and researchers alike because it represents a site of intense *A.m. capensis* and *A.m. scutellata* interactions. The boundaries of the hybrid zone are largely located in the Eastern Cape (Hepburn and Crewe 1991; Hepburn *et al* 1994,1998). This presents a problem for the beekeepers of the Eastern Cape because of the uncertainty of the characterisation of their bees, which results in a difficult management situation owing to the different management techniques required for the two races (Cooke 1986). Little it known about the biology of the hybrids of these two races (Crewe and Allsopp 1994), with no information available on the pheromonal properties of queens from colonies located on the edge of the hybrid zone. This study aims to address this deficit by providing baseline data on the mandibular gland secretions of mated queens collected from the fringes of the hybrid zone.

Coincident, but not concordant, zones of hybridisation between *A.m. capensis* and *A.m. scutellata* have been defined from analyses of different traits (Hepburn *et al* 1994, 1998). Consequently, additional probes are needed to further define the distribution of these races. The mandibular gland secretions of queens have been found to be relatively race specific, with queens of different species and races producing a unique combination of mandibular compounds (Crewe 1982, 1988; Plettner *et al* 1997). It is therefore possible that analyses of mandibular gland extracts may provide another probe to assist in defining the distribution of the southern African races of honeybee. One of the objectives of this study was to conduct a

preliminary investigation into the feasibility of queen mandibular gland extracts serving as a probe for defining geographical distribution of *A.m. capensis*, *A.m. scutellata* and their hybrids.

MATERIALS AND METHODS

Sample and Sample Preparation

Mated honeybee queens were obtained from colonies from five localities in the Eastern Cape province, South Africa. These localities are in the hybrid zone, or on the fringes of the hybrid zone, as previously defined (see Figure 4 Hepburn *et al* 1994). All queens were of unknown age. The reproductive status of the queens was confirmed by ovarial dissection. All queens used in the analyses were mated laying queens, with ovarioles containing mature sausage-shaped eggs (Allsopp 1988).

Queens were collected from Port Elizabeth (33°58'S 25°36'E), East London (33°00'S 27°54'E), Steynsburg (31°15'S 25°49'E), Cradock (32°08'S 25°36'E) and Grahamstown (33°18'S 26°32'E).

The mandibular glands were removed and prepared as described in chapter two.

Chemical Analysis

Chemical analysis of the mandibular gland extracts of the mated queens was performed using gas chromatography and gas chromatography–mass spectroscopy as described in chapter two.

Statistical Analysis

One-Way ANOVA and ANOVA on ranks were used as described in chapter two to compare the amount of each compound identified for each group (locality).

RESULTS

Mated queens, confirmed by ovarial dissection, from the five localities in the Eastern Cape Province of South Africa were found to exhibit no significant differences ($P > 0.05$) in the amount of the any of the 37 compounds identified from the mandibular glands extracts, with the exception of the hydrocarbon eicosane (Appendix I, Tables A-G). The interpretation of this data should however be treated with caution in light of the small number of samples available from each location. Due to the small sample sizes and consequent high variations, the data are better represented as relative percentage composition of the aliphatic acids and phenols considered to be the major constituents of mandibular gland extracts (Table 7.1).

The relative contribution of the major compounds of mandibular gland extracts were similar for the five localities, with no detectable significant differences between queens collected in the Eastern Cape for this study (Table 7.1; Figure 7.1; $P > 0.05$). 9ODA is the predominant

compound in queens from all localities (average of the means = 54.1%), the other aliphatic acid, 9HDA, comprising the queen mandibular pheromone (QMP) complex (Winston and Slessor 1998) is the second most predominant compound (17.0%). The phenols of the QMP complex, HOB and HVA, represent 7.99% and 4.52%, respectively, of the major compounds of the extract. The worker acid, 10HDA, comprised 8.06% and 10HDAA, 4.06% of the major compounds in mandibular gland extracts from mated queens collected in the Eastern Cape.

The 9ODA/10HDA ratio of the mated laying queens, previously determined to be qualitatively different for each race (Crewe 1988), collected from five Eastern Cape localities were found not to be significantly different (P = 0.139). The 9ODA/10HDA ratios of the queens from each locality are: Port Elizabeth = 11.14:1 ± 2.301, East London = 5.97:1 ± 4.308, Steynsburg = 12.65:1 ± 5.767, Cradock = 20.54 ± 5.24 and Grahamstown = 6.17 ± 0.939.

Table 7.1: Percentage (mean [a] ± SE) composition of the head extracts of mated queens from five localities in the Eastern Cape, South Africa.

Compound	Locality				
	Port Elizabeth	**East London**	**Steynsberg**	**Cradock**	**Grahamstown**
	n = 5	n = 2	n = 3	n = 2	N = 4
HOB	7.64 ± 1.56	6.01 ± 3.38	8.85 ± 2.71	7.64 ± 2.74	9.81 ± 2.79
7HOA	0.219 ± 0.089	0.407 ± 0.407	0.304 ± 0.192	0.276 ± 0.104	0.508 ± 0.134
4HBA	0.36 ± 0.25	0.00 ± 0	0.15 ± 0.14	0.50 ± 0.12	0.00 ± 0
8HOA	3.15 ± 0.234	5.09 ± 0.961	5.45 ± 2.287	2.62 ± 1.078	5.25 ± 0.445
9ODA	53.3 ± 3.30	42.9 ± 2.72	57.5 ± 4.96	65.1 ± 1.62	51.7 ± 3.22
HVA	8.68 ± 1.096	4.67 ± 4.637	3.89 ± 0.615	3.03 ± 1.880	2.31 ± 0.732
9HDAA	0.09 ± 0.06	0.00 ± 0	0.09 ± 0.04	0.06 ± 0.02	0.00 ± 0
9HDA	16.7 ± 2.134	21.0 ± 7.858	13.7 ± 3.134	13.6 ± 3.379	16.9 ± 0.971
10HDAA	4.22 ± 2.252	4.24 ± 2.753	3.19 ± 1.533	3.79 ± 2.643	4.85 ± 0.773
10HDA	5.59 ± 0.915	15.67 ± 11.762	6.87 ± 2.807	3.41 ± 0.949	8.75 ± 0.883

[a] Means within one row followed by the same letter are not significantly different (P > 0.05, Kruskal-Wallis). For rows in which no statistically significant difference was detected (P > 0.05, Kruskal-Wallis) no letters are included.

Abbreviations for the compounds are as follows: HOB = Methyl-*p*-hydroxybenzoate; 7HOA = 7-Hydroxyoctanoic acid; 4HBA = 4-Hydroxybenzoic acid; 8HOA = 8-Hydroxyoctanoic acid; 9ODA = (*E*)-9-ketodec-2-enoic acid; HVA = 4-Hydroxy-3-methoxyphenyl ethanol; 9HDAA = 9-Hydroxydecanoic acid; 9HDA = (*E*)-9-Hydroxydec-2-enoic acid; 10HDAA = 10-Hydroxydecanoic acid; 10HDA = (*E*)-10-Hydroxydec-2-enoic acid.

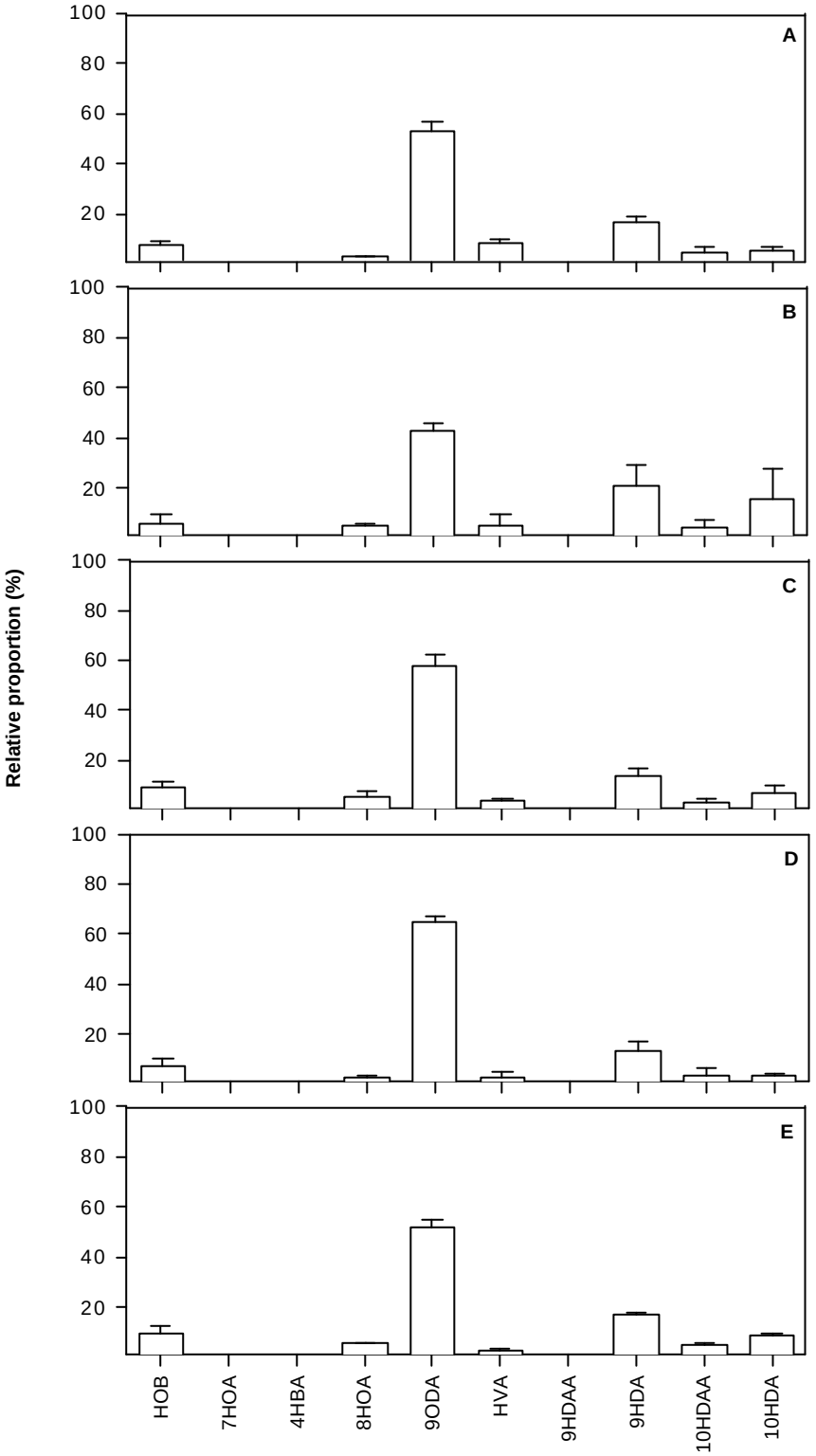


Figure 7.1: The relative proportions (%) of the major compounds of the mandibular gland extracts of mated queens from five localities in the Eastern Cape Province, South Africa; **A** = Port Elizabeth, **B** = East London, **C** = Steynsberg, **D** = Cradock and **E** = Grahamstown.

Abbreviations for the compounds are as follows: HOB = Methyl-*p*-hydroxybenzoate; 7HOA = 7-Hydroxyoctanoic acid; 4HBA = 4-Hydroxybenzoic acid; 8HOA = 8-Hydroxyoctanoic acid; 9ODA = (*E*)-9-ketodec-2-enoic acid; HVA = 4-Hydroxy-3-methoxyphenyl ethanol; 9HDAA = 9-Hydroxydecanoic acid; 9HDA = (*E*)-9-Hydroxydec-2-enoic acid; 10HDAA = 10-Hydroxydecanoic acid; 10HDA = (*E*)-10-Hydroxydec-2-enoic acid.

DISCUSSION

These analyses found no significant variation in queen pheromone production of queens sampled from five localities in the Eastern Cape. As the composition of the secretions of mated laying queens is unique for each honeybee race (Crewe 1988; Plettner *et al* 1997) it is unlikely the queens analysed in this study represent different races when classified using the trait queen mandibular gland extracts. This is contrary to the results expected when the distribution of *Apis mellifera capensis*, *Apis mellifera scutellata* and their hybrids, as defined in the literature is considered (Figure 4 in Hepburn *et al* 1994). Port Elizabeth is within the biologically defined distribution of "pure" *A.m. capensis* as determined by their reproductive biology (Hepburn and Crewe 1991) and morphometry (Crewe *et al* 1994). East London and Steynsburg are situated on the boundary of the hybrid zone and the southern limit of *A.m. scutellata* as defined by their reproductive biology (Hepburn and Crewe 1991) but within the hybrid zone as defined by morphometry (Crewe *et al* 1994). Cradock and Grahamstown are located within the hybridisation zone as defined by both reproductive biology and morphometrics (Hepburn and Crewe 1991; Crewe *et al* 1994).

Existing literature contains little comprehensive data detailing the mandibular gland secretions of African honeybee queens. No quantitative mass values of the chemical constituents of the mandibular gland secretions of "pure" *A.m. capensis* and *A.m. scutellata* mated queens have been published. The only published data is of a limited nature, providing percentage composition values for some of the compounds (Crewe 1982; Crewe *et al* 1990a,b). Consequently comparisons between the Eastern Cape queens analysed in this study and "pure" *A.m. capensis* and *A.m. scutellata* is only possible with the use of the ratio of 9ODA:10HDA, determined by Crewe (1988) to be quantitatively different for each race.

Comparing data obtained from samples of "pure" *A.m. capensis* and *A.m. scutellata* (collected from the Cape Town/Stellenbosh region and the greater Johannesburg area respectively, which represent localities well within the distribution range of the two races) with the results presented in this study provides new insight into the classification of honeybees from Eastern Cape localities as *A.m. capensis*, *A.m. scutellata* or hybrids. The ratio of 9ODA:10HDA in *A.m. capensis* has been found to be 84:1, while that of *A.m. scutellata* is 8.1:1 (Crewe 1984, 1988). The ratio of 9ODA:10HDA in Eastern Cape queens was found to be substantially lower than that of *A.m. capensis*, with Port Elizabeth queens exhibiting a ratio of only 11.14:1. Thus in terms of queen mandibular gland production Eastern Cape bees, including those from Port Elizabeth, can not be regarded as *A.m. capensis*. The queens from Grahamstown and East London were found to have a ratio of 9ODA:10HDA similar to that of *A.m. scutellata* while the those from Port Elizabeth, Steynsburg and Cradock were slightly higher. This suggests that the ratio of 9ODA/10HDA in hybrid queens may be similar or slightly higher than that of *A.m. scutellata.*

The results of these analyses further reflect the complexities of *A.m. capensis* and *A.m. scutellata* hybridisation and the difficulties associated with defining distribution ranges for these honeybee races. If queen mandibular gland extracts are to be used as a tool in defining the boundaries of the distribution of southern African honeybees, experimental data with hybrid queens needs to be undertaken to provide baseline data against which samples can be compared.

The findings of this study have important implications for queen production and beekeeping in South Africa. The results indicate that the Eastern Cape honeybee queens differ from *A.m. capensis* and *A.m. scutellata* queens and can probably be regarded as hybrid queens. It is likely that queen production programs and management techniques developed for the two races are unlikely to function effectively for these queens. As such, a queen production programme which considers the biology of Eastern Cape queens needs to be specially developed if beekeeping in the Eastern Cape is to function optimally.

CHAPTER EIGHT

DISCUSSION AND CONCLUSIONS

The findings of the foregoing chapters contribute to our knowledge of the biology of the Cape honeybee, *Apis mellifera capensis*, and have important implications for queen production and further development of beekeeping in South Africa.

MANDIBULAR GLAND SECRETIONS OF *Apis mellifera capensis*

Honeybee queens produce a multitude of volatile constituents in their mandibular glands (Crewe and Moritz 1989; Engels *et al* 1997b). The mandibular glands of *A.m. capensis* proved to be an opulent source of volatile compounds (chapters 2,3,5), with the chemical identity of 37 of the compounds present in mandibular gland extracts being confirmed. This is the most comprehensive composite "map" of the mandibular gland secretions of African honeybee queens produced to date. It provides the basis for the production of a complete composite "map" for honeybees similar to those that exist for the stingless bee *Scaptotrigona postica* (Engels *et al* 1997a) and the bumble bee *Bombus terrestris* (Hefetz *et al* 1996). Among the identified compounds are 9ODA, 9HDA, HVA and HOB which constitute the queen mandibular gland pheromone complex (QMP) as defined by Slessor *et al* (1988, 1990) and the other aliphatic acids and phenols considered to be the major components of the head extracts of *A.m. capensis* (Crewe *et al* 1990a,b).

Ontogenetic Changes

Ontogenetic changes in the components of the mandibular gland secretions of *A.m. capensis* are largely quantitative in nature, with the total volume and that of most of the individual compounds increasing with queen age (chapter 3). Qualitative changes in the composition of the mandibular gland secretions of *A.m. capensis* virgin queens were not as pronounced as those presented for other honeybee races (Crewe 1982; Crewe and Moritz 1989; Pankiw *et al* 1996).

The positive age-dependent responses identified in the quantities of mandibular gland extracts, and, in particular, the relative proportion of 9ODA to the other major compounds, of *A.m. capensis*, affected the reaction of queenright groups of workers to introduced virgin

queens. The proportion of antagonistic behaviours exhibited by workers towards introduced queens increased with the age of the queens (chapter 4). These findings provide partial support for the suggestion that the differential behaviour of worker bees towards an unknown queen are dependent on pheromonal cues and qualitative differences in mandibular gland volatiles (Engels *et al* 1997b; Pettis *et al* 1998).

The Effect of Holding Condition on Mandibular Gland Secretions

The chemical composition of the mandibular gland secretions of honeybee queens has been demonstrated to be affected by age (Crewe and Moritz 1989; Slessor *et al* 1990; Engels *et al* 1997b), caste (Crewe and Velthuis 1980; Crewe *et al* 1990a,b; Plettner *et al* 1993, 1997; Gadagkar 1996, 1997) and race (Crewe 1982; Plettner *et al* 1997. It becomes apparent that, in addition to these factors, the presence of workers during the ageing of virgin queens significantly influences the quantity of chemicals produced in the mandibular glands of honeybee queens (chapter 5). The reduction in the quantity of the compounds, considered to be the major constituents of the mandibular gland secretions, in queens reared with workers may to be a consequence of queen/worker interactions during the rearing process.

These findings have important consequences for studies comparing the quantities of the mandibular gland components of queens using previously published data. This study demonstrates that age of honeybee virgin queens, and more importantly their immediate holding conditions prior to processing, impact on the nature of the chemical signal recovered. Methodological differences in the methods used to rear and age queens make comparisons unreliable and may account for some of the differences reported in chemical profiles of queens of the same race (Engels *et al* 1997b). This indicates that more care needs to be taken in the collection of data used in such studies.

IMPLICATIONS FOR BEEKEEPING IN SOUTH AFRICA

South African beekeepers utilise the two southern African races of honeybee, the Highlands or African honeybee *Apis mellifera scutellata* and the Cape honeybee *Apis mellifera capensis*. Because of the pronounced biological differences between the two races, different management techniques are required for each race. While modifications are required, the basic management practices for *A.m. scutellata* are similar to those advocated for European and Africanized honeybees (c.f. Morse 1979; Anderson *et al* 1983; McGregor 1992). *A.m. capensis*, however, is a unique race among all the others due to its biological peculiarities (Ruttner 1977; Crewe 1981; Verma and Ruttner 1983; Velthuis *et al* 1990; Hepburn and Crewe 1991) thereby requiring the development of specialised management techniques particularly for queen production (Anderson 1965; Cooke 1986). The findings of the foregoing chapters provide some essential baseline data on *A.m. capensis*, required for the development of a sensible and effectual programme for queen production in this race.

Commercial Applications of Synthetic Honeybee Pheromones

Fundamental knowledge of honeybee pheromones has recently led to the use of synthetic pheromones and pheromone blends in research and management applications. The queen mandibular gland pheromone complex (QMP) as defined by Slessor *et al* (1988, 1990, 1998) and synthetic nasonov pheromone have been used in a variety of applications. Synthetic nasonov components have been demonstrated to attract swarms to bait hives (Villa and Schmidt 1992; Schmidt *et al* 1993) and to attract foragers in the field (Williams *et al* 1982). Williams *et al* (1981) determined that the presence of worker footprint pheromone enhanced the attractiveness of synthetic nasonov mixture and suggested that it may prove useful in attracting foragers to crops needing pollination. Synthetic QMP has been demonstrated to: inhibit the initiation of queen-rearing (Engels *et al* 1993; Melathopoulos *et al* 1996); initiate and maintain retinue behaviour in workers (Slessor *et al* 1988, 1990; Kaminski *et al* 1990); induce balling behaviour in workers (Pettis *et al* 1998); elicit approach and copulatory behaviour by drones (Boch *et al* 1975; Loper *et al* 1996); and, function in attracting workers to swarms and retarding swarm dispersal (Winston *et al* 1982, 1989).

Queen mandibular pheromone is being used commercially under the trade names “Bee Boost” for beekeeping use and “Fruit Boost” as an attractant for crop pollination (Winston and Slessor 1998). “Bee Boost” is used in commercial queen-rearing operations to enhance mating success and queen survival and as a queen substitute in shipping bulk packages of worker bees long distances (Naumann *et al* 1990; Winston and Slessor 1993, 1998). “Fruit Boost” is being sold as an attractant for use on varieties of blueberries, pears, apple and kiwifruit (Winston and Slessor 1998). Higo *et al* (1995) suggest that a combination of increased recruitment and greater time spent by foragers with increased flower visitation contribute to enhanced pollination of blooming crops treated with QMP.

The effectiveness of synthetic QMP in the form of “Bee Boost” and “Fruit Boost” in commercial queen rearing operations and in enhancing pollination, respectively, have been tested in America using European and Africanized honeybee stocks. Their potential in commercial applications using African honeybees has not been determined. The findings of mandibular gland secretion studies with *A.m. capensis* (Crewe 1982; Crewe *et al* 1990a,b; chapters 2, 3, 5) suggest that these synthetic blends of QMP are unlikely to be of commercial use for *A.m. capensis*. The relative proportions of 9ODA, 9HDA, HVA and HOB, the compounds which constitute the QMP, in *A.m. capensis* differ significantly from those found in European and Africanized honeybees. 9ODA and 9HDA comprise approximately 90% and 8%, respectively, of the QMP compounds in *A.m. capensis* queens. Compared with approximately 65% and 26% of 9ODA and 9HDA, respectively, that they constitute in European and Africanized mated queens (Pankiw *et al* 1996). Considering that all of the compounds are necessary to elicit the full range of worker responses to QMP (Winston *et al* 1989, 1991), and that the full blend of the major components of the queen mandibular gland extracts is necessary to elicit worker reactions in *A.m. capensis* (chapter 4), an effective synthetic blend should emulate the

composition of the natural blend. The effectiveness of "Bee Boost" and "Fruit Boost" with *A.m. capensis* needs to be determined experimentally. More rigorous investigation into their possible use in commercial beekeeping in southern Africa is beyond the scope of this work.

Queen Production with *Apis mellifera capensis*

Queen banking, successfully utilised for European and Africanized queens (Yadava and Smith 1971a; Post *et al* 1987; Lensky *et al* 1991), proved to be a non-viable method for rearing *A.m. capensis* virgin queens (chapter 5). High mortality rates were experienced amongst queens kept in queenbanks in queenless and queenright colonies. Keeping queens singly, with or without, small groups (50) of workers in an incubator and singly in mating nucleus hives proved to be the most successful methods of rearing *A.m. capensis* virgin queens, with high survival rates being achieved using these techniques.

A high survival rate of virgin queens is not the only requirement of a good queen rearing method; a high acceptance rate of introduced virgin queens by workers is of equal importance (Ribbands 1953; Cooke 1986). The presence of workers during queen rearing significantly affected the reactions of groups of workers towards introduced virgin queens (chapter 6). The results of these investigations provided support for the suggestion that virgin queens may learn appropriate behaviours towards workers during ageing, which facilitates an increased acceptance of introduced virgin queens by groups of workers. It is possible that queens may learn these appropriate behaviours through queen-worker interactions. Results of this study in which virgin queens were introduced into groups of workers suggests that the presence of workers during queen rearing could be necessary to ensure reduced risk of rejection and injury of queens through aggressive interactions with workers. This could facilitating their successful introduction into a colony and providing the beekeeper with a satisfactory queen rearing method.

Artificial or instrumental insemination (AI) is a technique which is becoming increasingly popular with queen breeders because of the opportunities it affords for controlled breeding, allowing a greater degree of genetic manipulation (Laidlaw 1987). Buys (1988), in his comprehensive investigation of AI in *A.m. capensis*, recommends that, for the best AI results, *A.m. capensis* queens should be maintained without workers in an incubator, as this ensures controlled environmental conditions and freedom from worker-borne diseases. In light of the results of the finding of the foregoing chapters (chapters 5, 6), in which increased levels of workers aggression towards introduced queens reared without workers were observed, it is suggested that this is not the best method of AI for *A.m. capensis*. Queens should rather be maintained with disease-free groups of workers in an incubator, a method which provides equally successful AI results in *A.m. capensis* (Buys 1984, 1988) and will ensure increased rates of acceptance of introduced queens by workers.

Beekeeping in South Africa

The South African beekeeping industry has recently undergone major changes in management practices in response to *A.m. capensis* and *A.m. scutellata* interactions that resulted in the "*capensis* invasion" (Allsopp 1992; Cooke 1992; Allsopp and Crewe 1993). Beekeepers experienced massive losses of *scutellata* colonies as a result of infestation by *capensis* workers. The occurrence of *capensis* workers in *scutellata* colonies manifest biologically as a "dwindling colony" syndrome, resulting in queen loss and a gradual decrease in the population of the colony, culminating in the death of the colony or the production of a *capensis* queen (Johannsmeier 1983; Allsopp 1992; Allsopp and Crewe 1993; Hepburn and Allsopp 1994). The impact on honey production and the pollination of crops such as lucerne, onions, sunflowers and tropical nuts is huge, with losses in excess of one million US dollars per annum having been experienced (Allsopp and Crewe 1993).

Allsopp and Crewe (1993) attributed uncontrolled migration of colonies, uncontrolled splitting of colonies, excessive stress as a result of pollination demands and generally poor management as having been responsible for the resulting situation and suggested decisive action to alleviate the disaster. Determining the distribution of these two races and their hybrids, understanding the processes that had previously ensured the relative stability of the hybrid zone and developing improved management techniques are necessary to ensure the future success of commercial beekeeping in South Africa. The movement of colonies by beekeepers has obscured the precise nature and extend of the hybrid zone (Allsopp and Crewe 1993), the distribution of which varies when different characteristic traits are considered (Hepburn and Crewe 1990, 1991; Hepburn *et al* 1994, 1998; chapter 7). Preliminary data on the honeybee queens of the Eastern Cape (chapter 7) indicates that Eastern Cape queens have mandibular gland secretions which differ from those of *A.m. capensis* queens but which may be similar to those of *A.m. scutellata* queens. Further investigations are required for the establishment of a comprehensive baseline data set. The findings of this study suggest that owing to the differences in the chemical signals produced by *A.m. capensis*, *A.m. scutellata* and Eastern Cape queens it is likely that specialised management practices may need to be developed for queen production in the Eastern Cape.

Improved management techniques are reliant on baseline data on aspects of the honeybee biology of the races for which the techniques are required. The study presented here focused on aspects of queen rearing and virgin queen development in *A.m. capensis* and investigated the use of queen mandibular gland extracts in classifying the honeybees of the Eastern Cape Province, South Africa. The results presented provide essential baseline data required for the development of a successful queen production programme and improved management practices for the Cape honeybee *A.m. capensis*. The future success of beekeeping in South Africa is dependent on the development of improved management techniques founded on data obtained from studies using the southern African honeybees, *A.m. capensis* and *A.m. scutellata*, and their hybrids.

REFERENCES

ADLER VE, DOOLITTLE RE, SHIMANUKI H and JACOBSON M (1973) Electrophysiological screening of queen substance and analogues for attraction to drone, queen, and worker honey bees. *J. Econ. Entomol.* **66**: 33-36.

ALLEN MD (1960) The honeybee queen and her attendants. *Anim. Behav.* **8**: 201-208.

ALLEN MD (1965) The role of the queen and males in the social organisation of insect communities. *Symp. Zool. Soc. Lond.* **14**: 133-157.

ALLSOPP MH (1988) Mandibular gland acids and laying workers in African honey bees. In: Needham GR, Page JR, Delfinado-Baker M and Bowman CE (eds) *Africanized honeybees and bee mites*. Ellis Horwood Ltd, Chichester p 72-79.

ALLSOPP MH (1992) The *capensis* calamity. *S. Afr. Bee J.* **64**: 52-55.

ALLSOPP MH and CREWE RM (1993) The Cape honeybee as a trojan horse rather than the hordes of genghis khan. *Amer. Bee J.* **133**: 121-123.

ALLSOPP MH and HEPBURN HR (1997) Swarming, supersedure and the mating system of a natural population of honey bees (Apis mellifera capensis). *J. Apic. Res.* **36**: 41-48.

AMBROSE JT (1975) Aggressive behaviour of honeybee workers towards foreign queens and an inconsistency with the stress-pheromone hypothesis. *Can. J. Zool.* **53**: 69-71.

ANDERSON D (1983) Queen rearing. *S. Afr. Bee J.* **55**: 14

ANDERSON RH (1965) A method for controlled queen-rearing in colonies of the Cape honeybee (*Apis mellifera capensis*). *S. Afr. J. Agric. Sci.* **8**: 1163-1164.

ANDERSON RH, BUYS B and JOHANNSMEIER MF (1983) *Beekeeping in South Africa*. Department of Agriculture, Pretoria.

ARNOLD G, QUENET B, CORNUET J-M, MASSON C, DE SCHEPPER B, ESTOUP A, and GASQUI P (1996) Kin recognition in honeybees. *Nature* **379**: 498.

ATALLAH MA, SOLIMAN MA and OSMAN F (1982) Long-chain fatty acids in different developmental stages of Egyptian and Carniolan honeybees and their local hybrid. *J. Apic. Res.* **21**: 185-187.

AVITABILE A, MORSE RA, and BOCH R (1975) Swarming honey bees guided by pheromones. *Ann. Entomol. Soc. Am.* **68**: 1079-1082.

AYASSE M, ENGELS W, HEFETZ A, LÜBKE G and FRANCKE W (1990) Ontogenetic patterns in amounts and proportions of Dufour's gland volatile secretions in virgin and nesting queens of *Lasioglossum malachurum* (Hymenoptera: Halicitidae). *Z. Naturforsch.* **45c**: 709-714.

AYASSE M, ENGELS W, HEFETZ A, TENGÖ J, LÜBKE G and FRANCKE W (1993) Ontogenetic patterns of volatiles identified in Dufour's gland extracts from queens and workers of the primitively eusocial halactine bee *Lasioglossum malachurum* (Hymenoptera: Halicitidae). *Insect Soc.* **40**: 41-58.

BAGNERES G and MORGAN ED (1990) A simple method for analysis of insect cuticular hydrocarbons. *J. Chem. Ecol.* **16**: 3263-3276.

BAGNERES AN and MORGAN ED (1991) The postpharyngeal glands and the cuticle of Formicidae contain the same characteristic hydrocarbons. *Experientia* **47**: 106-111.

BARBIER J and LEDERER E (1960) Structure chimique de la substance royale de la reine d'abeille (*Apis mellifera* L.). *Comptes Rendus des Séances de L'Académie de Science Paris* **251**: 1131-1135.

BARKER SA, FOSTER AB, LAMB DC and HODGSON N (1959a) Identification of 10-Hydroxy-Δ^2-decenoic acid in royal jelly. *Nature* **183**: 996-997.

BARKER SA, FOSTER AB, LAMB DC and HODGSON N (1959b) Biological origin and configuration of 10-Hydroxy-Δ^2-decenoic acid. *Nature* **184**: 634.

BEYLEVELD GP (1935) Queen-rearing in South Africa. *Glean. Bee Cult.* **63**: 528-533.

BILLEN J and MORGAN ED (1998) Pheromone communication in social insects: sources and secretions. In Van der Meer RK, Breed MD, Espelie KE and Winston ML (eds) *Pheromone communication in social insects*. Westview Press, Boulder Colorado, p 3-33.

BILLEN JPJ, DUMORTIER KTM and VELTHUIS HHW (1986) Plasticity of honeybee castes - occurrence of tergal glands in workers. *Naturwiss.* **73**: 332-333.

BLOMQUIST GJ, HOWARD RW, MCDANIEL CA, REMALEY S, DWYER LA, and NELSON DR (1980) Application of methoxymercuratin-demercuration followed by mass spectrometry as a convenient

microanalytical technique for double-bond location in insect-derived alkenes. *J. Chem. Ecol.* **6**: 257-269.

BLOMQUIST GJ, NELSON DR and DE RENOBLES V (1987) Chemistry, biochemistry, and physiology of insect cuticular lipids. *Arch. Insect Biochem. Physiol.* **6**: 227-265.

BLOMQUIST GJ, TILLMAN JA, MPURU S and SEYBOLD SJ (1998) The cuticle and cuticular hydrocarbons of insects: structure, function, and biochemistry. In Van der Meer RK, Breed MD, Espelie KE and Winston ML (eds) *Pheromone communication in social insects*. Westview Press, Boulder Colorado, p 34-54.

BLUM MS (1969) Alarm pheromones. *Ann. Rev. Entomol.* **14**: 57-80.

BLUM MS (1987) Biosynthesis of arthropod exocrine compounds. *Ann. Rev. Entomol.* **32**: 381-413.

BLUM MS and BRAND JM (1972) Social insect pheromones: their chemistry and function. *Am.Zoologist* **12**: 553-576.

BLUM MS and FALES HM (1988) Eclectic chemisociality of the honeybee: A wealth of behaviors, pheromones, and exocrine glands. *J. Chem. Ecol.* **14**: 2099-2107.

BLUM MS, BOCH R, DOOLITTLE RE, TRIBBLE MT, and TRAYNHAM JG (1971) Honey bee sex attractant: Conformational analysis, structural specificity, and lack of masking activity of congeners. *J. Insect Physiol.* **17**: 349-364.

BLUM MS, FALES HM, JONES TH, RINDERER TE, and TUCKER KW (1983) Caste-specific esters derived from the queen honey bee sting apparatus. *Comp. Biochemi. Physiol.* **75B**: 237-238.

BOCH R and MORSE RA (1974) Discrimination of familiar and foreign queens by honey bee swarms. *Ann. Entomol. Soc. Am.* **67**: 709-711.

BOCH R and SHEARER DA (1967) 2-Heptanone and 10-hydroxy-*trans*-dec-2-enoic acid in the mandibular glands of worker honey bees of different ages. *Z. Physiol.* **54**: 1-11.

BOCH R and SHEARER DA (1971) Chemical releases of alarm behaviour in the honey-bee*, Apis mellifera*. *J. Insect Physiol.* **17**: 2277-2285.

BOCH R, SHEARER DA, and YOUNG JC (1975) Honey bee pheromones: field tests of natural and artificial queen substance. *J. Chem. Ecol.* **1**: 133-148.

BOCH R, SHEARER DA, and SHUEL RW (1979) Octanoic and other volatile acids in the mandibular glands of the honeybee and in royal jelly. *J. Apic. Res.* **18**: 250-253.

BOGDANOV S, VIT P and KILCHENMANN (1996) Sugar profiles and conductivity of stingless bee honeys from Venezuela. *Apidologie*. **27**: 445-450.

BREED MD, STILLER TM, BLUM MS, and PAGE RE JR (1992) Honeybee nestmate recognition: Effects of queen fecal pheromones. *J. Chem. Ecol.* **18**: 1633-1640.

BROWN WH and FELAUER EE (1961) A new fatty acid from royal jelly. *Nature* **190**: 88.

BUTLER CG (1954) The method and importance of the recognition by a colony of honeybees (*A. mellifera*) of the presence of its queen. *Trans. R. Entomol. Soc. Lond.* **105**: 2-29.

BUTLER CG (1960) Queen substance production by virgin queen honey-bees (Apis mellifera L.). *Proc. R. Entomol. Soc. Lond.* **35A**: 170-171.

BUTLER CG (1967) A sex attractant as an aphrodisiac in the honeybee (*A. Mellifera* L.). *Proc. R. Entomol. Soc. Lond.* **42A**: 71-76.

BUTLER CG (1971) The mating behaviour of the honeybee (*Apis mellifera* L.). *J. Entomol.* **46**: 1-11.

BUTLER CG and CALLOW RK (1968) Pheromones of the honeybee (*Apis mellifera* L.): the "inhibitory scent" of the queen. *Proceedings of the Royal Entomological society of London* **43B**: 62-65.

BUTLER CG and PATON PN (1962) Inhibition of queen rearing by queen honey-*bees (Apis mellifera* L.) of different ages. *Proc. R. Entomol. Soc. Lond.* **37A**: 7-9.

BUTLER CG and SIMPSON J (1956) The introduction of virgin and mated queens, directly and in a simple cage. *Bee World* **37**: 105-114.

BUTLER CG and SIMPSON J (1958) The source of the queen substance of the honey-bee (*Apis mellifera* L.). *Proc. R. Entomol. Soc. Lond.* **33A**: 120-122.

BUTLER CG and SIMPSON J (1967) Pheromones of the queen honeybee (*Apis mellifera* L.) which enable her workers to follow her when swarming. *Proc. R. Entomol. Soc. Lond.* **42**: 149-154.

BUTLER CG, CALLOW RK, and JOHNSTON NC (1959) Extraction and purification of 'queen substance' from queen bees. *Nature* **184**: 1871.

BUTLER CG, CALLOW RK, and JOHNSTON NC (1961) The isolation and synthesis of queen substance, 9-oxodec-trans-2-enoic acid, a honeybee pheromone. *Proc.R. Soc.* **155B**: 417-432.

BUTLER CG, CALLOW RK, and CHAPMAN JR (1964) 9-Hydroxydec-trans-2-enoic acid, a pheromone stabalizing honeybee swarms. *Nature* **201**: 733.

BUTLER CG, CALAM DH and CALLOW RK (1967) Attraction of *Apis mellifera* drones by the odours of the queens of two other species of honeybees. *Nature* **213**: 423-424.

BUTLER CG, CALLOW RK, GREENWAY AR, and SIMPSON J (1974) Movement of the pheromone, 9-oxodec-2-enoic acid, applied to the body surfaces of the honeybees (Apis mellifera). *Ent.exp.& appl.* **17**: 112-116.

BUYS B (1984) Cape worker-bees dislike African virgin queens. *S. Afr. Bee J.* **56**: 63.

BUYS B (1988) Artificial insemination of the Cape honeybee *Apis mellifera capensis* Esch. and the influence of various factors on success. MScThesis, Stellenbosch University.

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

CALLOW RK, JOHNSTON NC, and SIMPSON J (1959) 10-Hydroxy-^{2}-decenoic acid in the honeybee (*Apis mellifera*). *Experentia* **15**: 421-422.

CALLOW RK, CHAPMAN JR, and PATON PN (1964) Pheromones of the honeybee: Chemical studies of the mandibular gland secretion of the queen. *J. Apic. Res.* **3**: 77-89.

CASSIER P and LENSKY Y (1994) The nassanov gland of the workers of the honeybee (*Apis mellifera* L.): ultrastructure and behavioural function of the terpenoid and protein components. *J. Insect Physiol.* **40**: 577-584.

CASSIER P, TEL-ZUR D, and LENSKY Y (1994) The sting sheaths of honeybee workers (*Apis mellifera* L.): Structure and alarm pheromone secretion. *J. Insect Physiol.* **40**: 23-32.

CHAUVIN R (1962) Sur l'épagine et sur les glandes tarsales d'Arnhart. *Insect. Soc.* **9**: 1-5.

COLLINS AM (1980) Effect of age on the response to alarm pheromones by caged honey bees. *Ann. Entomol. Soc. Am.* **73**: 307-309.

COOKE MJ (1986) Queen rearing – Ilse's way. *S. Afr. Bee J.* **58**: 54-60.

COOKE M (1992) Turnabout is fair play - Cape bee invades African bee territory. *Amer. Bee J.* **132**: 519-521.

CREWE RM (1976) Aggressiveness of honeybees and their pheromone production. *S. Afr. J. Sci.* **72**: 209-212.

CREWE RM (1981) Queens, false queens and *capensis*. *S. Afr. Bee J.* **53**: 18-21.

CREWE RM (1982) Compositional variability: The key to the social signals produced by honeybee mandibular glands. In: Breed MD, Michener CD and Evans HE (eds)*The biology of social insects*. Proceedings of the 9th Congress of IUSSI, Boulder, Colorado p 318-322.

CREWE RM (1984) Differences in behaviour and morphology between *capensis* and *adansonii*. *S. Afr. Bee J.* **56**: 16-20.

CREWE RM (1988) Natural history of honey-bee mandibular gland secretions: Development of analytical techniques and the emergence of complexity. In: Needham GR, Page JR, Delfinado-Baker M and Bowman CE (eds) *Africanized honeybees and bee mites*. Ellis Horwood Ltd, Chichester p 149-158.

CREWE RM and ALLSOPP MH (1994) Sex and the single queen: recent experiments with *capensis* and *scutellata* queens. *S.Afr. Bee J.* **66**: 58-62.

CREWE RM and HASTINGS H (1976) Production of pheromones by workers of *Apis mellifera adansonii*. *J. Apic. Res.* **15**: 149-154.

CREWE RM and MORITZ RFA (1989) Variation in the components of head extracts of workers and queens of *Apis mellifera intermissa* Buttel-Reepen. *Z. Naturfors.* **44c**: 590-596.

CREWE RM and VELTHUIS HHW (1980) False queens: A consequence of mandibular gland signals in worker honeybees. *Naturwiss.* **67**: 467-470.

CREWE RM, WOSSLER T and ALLSOPP MH (1990a) Workers in queen clothing: why *capensis* workers become pseudoqueens. In: Anderson RH and Buys B (eds) Bees and Beekeeping in Southern Africa. Proceedings of the International Beekeepers' symposium, Stellenbosch, South Africa. University Press. Stellenbosch p 83-89.

CREWE RM, WOSSLER T and ALLSOPP MH (1990b) Intercaste or mosaic, the worker mandibular gland secreations of Cape honeybees (*Apis mellifera capensis*). In: Veeresh GK, Mallik B and Viraktamath CA (eds) Social insects and the environment. Proceedings of the 11th International Congress IUSSI, Bangalore, India. Oxford and IHB Publishing Co. New Delhi p 415-416.

CREWE RM, HEPBURN HR, and MORITZ RFA (1994) Morphometric analysis of 2 southern African races of honeybee. *Apidologie* **25**: 61-70.

DE HAZAN M, LENSKY Y, and CASSIER P (1989a) Effects of queen honeybee (*Apis mellifera* L.) ageing on her attractiveness to workers. *Comp. Biochem. Physiol.* **93A**: 777-783.

DE HAZAN M, HYAMS J, LENSKY Y, and CASSIER P (1989b) Ultrastructure and ontogeny of the mandibular glands of the queen honey bee, *Apis mellifera* L. (Hymenoptera: Apidae). *Int. J. Insect Morph.Embr.* **18**: 311-320.

DOOLITTLE RE, BLUM MS, and BOCH R (1970) cis-9-Oxo-2-decenoic acid: Synthesis and evaluation as a honey bee pheromone and masking agent. *Ann. Entomol. Soc. Amer.* **63**: 1180-1185.

ENGELS W, ADLER A, ROSENKRANZ P, LÜBKE G, and FRANCKE W (1993) Dose-dependent inhibition of emergency queen rearing by syntheric 9-ODA in the honeybee, *Apis mellifera carnica*. *J. Comp. Physiol.* **163**: 363-366.

ENGELS W, ENGELS E and FRANCKE W (1997a) Ontogeny of cephalic volatile patterns in queens and mating biology of the neotropical stingless bee, *Scaptotrigona postica*. *Invert. Reprod. Devel.* **30**: 251-256.

ENGELS W, ROSENKRANZ P, ADLER A, TAGHIZADEH T, LÜBKE G and FRANCKE W. (1997b). Mandibular gland volatiles and their ontogenetic patterns in queen honey bees, *Apis mellifera carnica*. *J. Insect Physiol.* **43**: 307-313.

ESPELIE KE, BUTZ VM, and DIETZ A (1990) Decyl decanoate: a major component of the tergite glands of honeybee queens (*Apis mellifera* L.). *J. Apic. Res.* **29**: 15-19.

FERGUSON AW and FREE JB (1980) Queen transfer within honeybee colonies. *Physiol. Entomol.* **5**: 359-366.

FRANCIS BR, BLANTON WE and NUNAMAKER RA (1985) Extractable surface hydrocarbons of workers and drones of the genus *Apis*. *J. Apic. Res.* **24**: 13-26.

FRANCIS BR, BLANTON WE, LITTLEFIELD JL and NUNAMAKER RA (1989) Hydrocarbons of the cuticle and hemolymph of the adult honey bee (Hymenoptera: Apidae). *Ann. Entomol. Soc. Am.* **82**: 486-494.

FREE JB (1960) The stimuli releasing the stinging response of honeybees. *Anim. Behav.* **8**: 193-196.

FREE JB (1987) *Pheromones of Social Bees*. Cornell University Press, New York.

FREE JB and FERGUSON AW (1982) Transfer of pheromone from immature queen honeybees, *Apis mellifera*. *Physiol. Entomol.* **7**: 401-406.

FREE JB and WINDER ME (1983) Brood recognition by honeybee (*Apis mellifera*) workers. *Anim. Behav.* **31**: 539-545.

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

GADAGKAR R (1996) What's the essence of royalty – one keto group? *Current Science* **71**: 975-980.

GADAGKAR R (1997) The evolution of communication and the communication of evolution: The case of the honey bee queen pheromone. In: Lehrer M (ed) *Orientation and communication in arthropods*. Birkhäuser Verlag, Berlin. p 375-395.

GARY NE (1961) Queen honey bee attractiveness as related to mandibular gland secretion. *Science* **133**: 1479-1480.

GARY NE (1962) Chemical mating attractants in the queen honey bee. *Science* **136**: 773-774.

GREENWAY W, SCAYSBROOK T and WHATLEY FR (1987) The analyses of bud exudate of *Populus x euramericana*, and of propolis, by gas chromatography-mass spectrometry. *Proc. R. Soc. (Lond)* **232B**: 249-272.

HAYDAK MH (1970) Honey bee nutrition. *Ann. Rev. Entomol.* **15**: 143-156.

HEFETZ A (1998) Exocrine glands and their products in non-*Apis* bees: chemical, functional and evolutionary perspectives. In Van der Meer RK, Breed MD, Espelie KE and Winston ML (eds) *Pheromone communication in social insects*. Westview Press, Boulder Colorado, p 236-256.

HEFETZ A, TAGHIZADEH T, and FRANCKE W (1996) The exocrinology of the queen bumble bee Bombus terrestris (Hymenoptera: Apidae, Bombini). *Z. Naturfor.* **51c**: 409-422.

HEMMLING C, KOENIGER N and RUTTNER F (1979) Quantitative bestimmung der 9-oxodecensäure im lebenszyklus der Kapbiene (*Apis mellifera capensis* Escholtz). *Apidologie* **10**: 227-240.

HEPBURN HR (1992) Pheromonal and ovarial development covary in Cape worker honeybees, *Apis mellifera capensis*. *Naturwiss.* **79**: 523-524.

HEPBURN HR (1994) Reproductive cycling and hierarchical competition in Cape honeybees, *Apis mellifera capensis* Esch. *Apidologie*. **25**: 38-48.

HEPBURN HR and ALLSOPP MH (1994) Reproductive conflict between honeybees: usurpation of *Apis mellifera scutellata* colonies by *Apis mellifera capensis*. *S. Afr. J. Sci.* **90**: 247-249.

HEPBURN HR and CREWE RM (1990) Defining the Cape honeybee: reproductive traits of queenless workers. *S. Afr. J. Sci.* **86**: 524-527.

HEPBURN HR and CREWE RM (1991) Portrait of the Cape Honeybee, *Apis mellifera capensis*. *Apidologie* **22**: 567-580.

HEPBURN HR and GUYE SG (1993) An annotated bibliography of the Cape honeybee, *Apis mellifera capensis* Escho;tz (Hymenoptera: Apidae). *Afr. Entomol.* **1**: 235-252.

HEPBURN HR and JACOT GUILLARMOD A (1991) The Cape honeybee and the fynbos biome. *S. Afr. J. Sci.* **87**: 70-73.

HEPBURN HR, MAGNUSON P, HERBERT L, and WHIFFLER LA (1991) The development of laying workers in field colonies of the Cape honey bee. *J. Apic. Res.* **30**: 107-112.

HEPBURN HR, VILLET MH, JONES GE, CARTER AR, SIMON UI AND COETZER W (1993) Winter absconding as a dispersal mechanism of the Cape honeybee. *S. Afr. J. Sci.* **89**: 294-297.

HEPBURN HR, JONES GE and KIRBY R (1994) Introgression between *Apis mellifera capensis* Escholtz and *Apis mellifera scutellata* Lepeletier: the sting pheromones. *Apidologie* **25**: 557-565.

HEPBURN HR, RADLOFF SE and FUCHS S (1998) Population structure and the interface between *Apis mellifera capensis* and *Apis mellifera scutellata*. *Apidologie* **29**: 333-346

HIGO HA, COLLEY SJ, WINSTON ML and SLESSOR KN (1992) Effects of honey bee (*Apis mellifera* L.) queen mandibular gland pheromone on foraging and brood rearing. *Can. Entomol.* **124**: 409-418.

HIGO HA, WINSTON ML and SLESSOR KN (1995) Mechanisms by which honey bee (Hymenoptera: Apidae) queen pheromone sprays enhance pollination. *Ann. Entomol. Soc. Am.* **88**: 366-373.

HILDEBRANDT HH and KAATZ HH (1990) Impact of queen pheromone on the physiological status of worker honey bees (*Apis mellifera* L.). In: Veeresh GK, Mallik B and Viraktamath CA (eds) Social insects and the environment. Proceedings of the 11th International Congress IUSSI, Bangalore, India. Oxford and IHB Publishing Co. New Delhi p 740-741.

HOWARD RW and BLOMQUIST GJ (1982) Chemical Ecology and Biochemistry of Insect Hydrocarbons. *Ann. Rev. Entomol.* **27**: 149-172.

HOWARD RW, MCDANIEL CA, NELSON DR, and BLOMQUIST GJ (1980) Chemical ionization mass spectrometry - application to insect-derived cuticular alkanes. *J. Chem. Ecol.* **6**: 609-623.

HOWARD RW, MCDANIEL CA, NELSON DR, BLOMQUIST GJ, GELBAUM LT, and ZALKOW LH (1982) Cuticular hydrocarbons of *Reticulitermes virginicus* (Banks) and their role as potential species - and caste-recognition cues. *J. Chem. Ecol.* **8**: 1227-1239.

IVANOVSKA N, NEYCHEV H, STEFANOVA Z, BANKOVA V and POPOV S (1995) Influence of cinnamic acid on lymphocyte proliferation, cytokine release and *Klebsiella* infection in mice. *Apidologie* **26**: 73-81.

JAYCOX ER (1970) Honey bee queen pheromones and worker foraging behavior. *Ann. Entomol. Soc. Am.* **63**: 222-228.

JOHANNSMEIER MF (1983) Experiences with the Cape bee in the Transvaal. *S. Afr. Bee J.* **55**: 130-138.

JUSKA A (1978) Temporal decline in attractiveness of honeybee queen tracks. *Nature* **276**: 261.

JUSKA A, SEELEY TD, and VELTHUIS HHW (1981) How honeybee queen attendants become ordinary workers. *J. Insect Physiol.* **27**: 515-519.

KAATZ H-H, HILDEBRANDT H, and ENGELS W (1992) Primer effect of queen pheromone on juvenile hormone biosynthesis in adult worker honey bees. *J. Comp. Physiol.* **162B**: 588-592.

KAISSLING KE and RENNER M (1968) Antennale rezeptoren für queen substance und sterzelduft bei der honigbiene. *Z. Vergl. Physiol.* **59**: 357-361.

KAMINSKI L-A, SLESSOR KN, WINSTON ML, WAY NW, and BORDEN JH (1990) Honeybee response to queen mandibular pheromone in laboratory bioassays. *J. Chem. Ecol.* **16**: 841-850.

KLAUDINY J, HANES J, KULIFAJOVÁ, ALBERT Š and ŠIMÚTH J (1994) Molecular cloning of two cDNAs from the head of the nurse honey bee (*Apis mellifera* L.) for coding related proteins of royal jelly. *J. Apic. Res.* **33**: 105-111.

LAIDLAW JR (1987) Instrumental insemination of honeybee queens: its origin and development. *Bee World* **68**: 17-36, 71-88.

LE CONTE Y, ARNOLD G, TROUILLER J, MASSON C, CHAPPE B, and OURISSON G (1989) Attraction of the parasitic mite *Varroa* to the drone larvae of honey bees by simple aliphatic esters. *Science* **245**: 638-639.

LE CONTE Y, ARNOLD G, TROUILLER J and MASSON C (1990) Identification of a brood pheromone in honeybees. *Naturwiss.* **77**: 334-336.

LE CONTE Y, SRENG L and TROUILLER J (1994) The recognition of larvae by worker honeybees. *Naturwiss.* **81**: 462-465.

LE CONTE Y, SRENG L, SACHER N, TROUILLER J, DUSTICIER G and POITOUT H (1994/1995) Chemical recognition of queen cells by honey bee workers *Apis mellifera* (Hymenoptera: Apidae). *Chemoecology* **5/6**: 6-12.

LE CONTE Y, SRENG L and POITOUT H (1995) Brood pheromone can modulate the feeding behavior of *Apis mellifera* workers (Hymenoptera: Apidae). *J. Econ. Entomol.* **88**: 798-804.

LENSKY Y and CASSIER P (1992) Control of swarming by queen bee pheromones. *Bee Science* **2**: 7-11.

LENSKY Y and CASSIER P (1995) The alarm pheromones of queen and worker honeybees. *Bee World* **76(3)**: 119-129.

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

LENSKY Y, CASSIER P, FINKEL A, DELORME-JOULIE C, and LEVINSOHN M (1985a) The fine structure of the tarsal glands of the honeybee Apis mellifera L. (Hymenoptera). *Cell Tiss. Res.* **240**: 153-158.

LENSKY Y, CASSIER P, NOTKIN M, DELORME-JOULIE C, and LEVINSOHN M (1985b) Pheromonal activity and fine structure of the mandibular glands of honeybee drones (*Apis mellifera* L.) (Insecta, Hymenoptera, Apidae). *J. Insect Physiol.* **31**: 265-276.

LENSKY Y, CASSIER P, ROSA S and GRANDPERRIN D (1991) Induction of balling in worker honeybees (*Apis mellifera* L.) by "stress" pheromone from Koschewnikow glands of queen bees: behavioural, structural and chemical study. *Comp. Biochem. Physiol.* **100A**: 585-594.

LI J, GRIES G, GRIES R, BIKIE J and SLESSOR KN (1993) Chirality of synergistic sex pheromone of the western hemlock looper *Lambdina fiscellaria lugubrosa* (Hulst) (Lepidoptera: Geometridae). *J. Chem. Ecol.* **19**: 2547-2561.

LOPER GM, TAYLOR ORJR, FOSTER LJ, and KOCHANSKY J (1996) Relative Attractiveness of Queen Mandibular Pheromone Components to Honey Bee (Apis mellifera) Drones. *J. Apic. Res.* **35**: 122-123.

LUNDIE AE (1954) Laying worker bees produce worker bees. *S. Afr. Bee J.* **29**: 10-11.

MARCUCCI MC (1995) Propolis: chemical composition, biological properties and therapeutic activity. *Apidologie*. **26**: 83-99.

MAUTZ D, BOCH R, and MORSE RA (1972) Queen finding by swarming honey bees. *Ann. Entomol. Soc. Amer.* **65**: 440-773.

McGREGOR L (1992) Queen rearing with *Apis mellifera scutellata*. *S. Afr. Bee J.* **64**: 32-39.

McMURRY J (1988) *Organic Chemistry*. Brooks/Cole Publishing Company, Belmont California.

MELATHOPOULOS AP, WINSTON ML, PETTIS JS, and PANKIW T (1996) Effect of queen mandibular pheromone on initiation and maintenance of queen cells in the honey bee (Apis mellifera L.). *Can. Entomol.* **128**: 263-272.

MELKSHAM KJ, JACOBSEN N, and RHODES J (1988) Compounds which affect the behaviour of the honeybee, *Apis mellifera* L.: A review. *Bee World* **69**: 104-124.

MORITZ RFA and CREWE RM (1988a) Reaction of honeybee workers (*Apis mellifera* L.) to fatty acids in queen signals. *Apidologie* **19**: 333-342.

MORITZ RFA and CREWE RM (1988b) Chemical signals of queens in kin recognition of honeybees, Apis mellifera L. *J. Comp. Physiol.* **164A**: 83-89.

MORITZ RFA and CREWE RM (1991) The volatile emission of honeybee queens (*Apis mellifera* L.). *Apidologie* **22**: 205-212.

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

MORITZ RFA, CORNUET JM, KRYGER P, GARNERY L and HEPBURN HR (1994) Mitochondrial DNA variability in South African honeybees (*Apis mellifera* L.). *Apidologie* **25**: 61-70.

MORSE RA (1963) Swarm orientation in honeybees. *Science* **141**: 357-358.

MORSE RA (1979) *Rearing queen honey bees.* Wicwas Press, New York.

MORSE RA and BOCH R (1971) Pheromone concert in swarming honey bees (Hymenoptera: Apidae). *Ann. Entomol. Soc. Amer.* **64**: 1414-1417.

NAUMANN K, WINSTON ML, WYBORN MH and SLESSOR KN (1990) Effects of synthetic honey bee (Hymenoptera: Apidae) queen mandibular gland pheromone on workers in packages. *J. Econ. Entomol.* **83**: 1271-1275.

NAUMANN K, WINSTON ML, SLESSOR KN, PRESTWICH GD, and WEBSTER FX (1991) Production and transmission of honeybee queen (*Apis mellifera* L.) mandibular gland pheromone. *Behav. Ecol. Sociobiol.* **29**: 321-332.

NAUMANN K, WINSTON ML, SLESSOR KN, PRESTWICH GD, and LATLI B (1992) Intra-nest transmission of aromatic honey bee queen mandibular gland pheromone components: movement as a unit. *Can. Entomol.* **124**: 917-934.

NAUMANN K, WINSTON ML, and SLESSOR KN (1993) Movement of honey bee (*Apis mellifera* L.) queen mandibular gland pheromone in populous and unpopulous colonies. *J. Insect Behav.* **6**: 211-223.

OLDHAM NJ, BILLEN J and MORGAN ED (1994) On the similarity of Dufour's gland secretion and cuticular hydrocarbons of some bumble bees. *Physiol. Entomol.* **19**: 115-123.

ONIONS GW (1912) South African 'fertile' worker bees. *S. Afr. Agric. J.* **1**: 720-728.

ONIONS GW (1914) South African 'fertile' worker bees. *S. Afr. J. Union S. Afr.* **7**: 44-46.

PAGE RE JR and ERICKSON EHJR (1986) Kin recognition and virgin queen acceptance by worker honey bees (Apis mellifera L.). *Anim. Behav.* **34**: 1061-1069.

PAGE RE JR, BLUM MS, and FALES HM (1988) σ-Aminoacetophenone, a pheromone that repels honeybees (*Apis mellifera* L.). *Experientia* **44**: 270-271.

PAIN J (1973) Pheromones and hymenoptera. *Bee World* **54**: 11-24.

PAIN J, BARBIER M, BOGDANOVSKY D, and LEDERER E (1962) Chemistry and biological activity of the secretions of queen and worker honeybees (*Apis mellifera* L.). *Comp. Biochem. Physiol.* **6**: 233-241.

PALMA MS (1992) Composition of the freshly harvested Brazilian royal jelly: identification of carbohydrates from the sugar fraction. *J. Apic. Res.* **31**: 42-44.

PANKIW T, WINSTON ML, SLESSOR KN, PETTIS JS and TAYLOR OR (1996) Mandibular gland components of European and Africanized honey bee queens (*Apis mellifera* L.). *J. Chem. Ecol.* **22**: 605-615.

PAXTON RJ, SAKAMOTO CH, and RUGIGA FCN (1994) Modification of honey bee (*Apis mellifera* L.) stinging behaviour by within-colony environment and age. *J. Apic. Res.* **33**: 75-82.

PESCHKE K and METZLER M (1986) Cuticular hydrocarbons and female sex pheromones of the rove beetle, *Aleochara curtula* (Coleoptera, Staphylinidae). *Insect Biochem.* **17**: 167-178.

PETTIS JS, WESTCOTT LV and WINSTON ML (1998) Balling behaviour in the honey bee in response to exogenous queen mandibular gland pheromone. *J. Apic. Res.* **37**: 125-131.

PHAM-DELÈGUE MH, TROUILLER J, CAILLAUD CM, ROGER B, and MASSON C (1993) Effect of queen pheromone on worker bees of different ages: behavioural and electrophysiological responses. *Apidologie* **24**: 267-281.

PICKETT JA, WILLIAMS IH, MARTIN AP, and SMITH MC (1980) Nasonov pheromone of the honey bee, *Apis mellifera* L. (Hymenoptera: Apidae) Part I. Chemical characterization. *J. Chem. Ecol.* **6**: 425-434.

PICKETT JA, WILLIAMS IH, SMITH MC, and MARTIN AP (1981) Nasonov pheromone of the honey bee, *Apis mellifera* L. (Hymenoptera, Apidae). Part III. Regulation of pheromone composition and production. *J. Chem. Ecol.* **7**: 543-554.

PLETTNER E, SLESSOR KN, WINSTON ML, ROBINSON GE and PAGE RE (1993) Mandibular gland components and ovarial development as measures of caste differentiation in the honey bee (*Apis mellifera* L.). *J. Insect Physiol.* **39**: 235-240.

PLETTNER E, SUTHERLAND GRJ, SLESSOR KN and WINSTON ML (1995) Why not be a queen? Regioselectivity in mandibular secretions of honeybee castes. *J. Chem. Ecol.* **21**: 1017-1029.

PLETTNER E, SLESSOR KN, WINSTON ML AND OLIVER JE (1996) Caste-selective pheromone biosynthesis in honeybees. *Science* **271**: 1851-1853.

PLETTNER E, OTIS GW, WIMALARATNE PDC, WINSTON ML, SLESSOR KN, PANKIW T, and PUNCHIHEWA PWK (1997) Species- and caste-determined mandibular gland signals in honeybees (Apis). *J. Chem. Ecol.* **23**: 363-377.

PLETTNER E, SLESSOR KN and WINSTON ML (1998) Biosynthesis of mandibular acids in honey bees (*Apis mellifera*): *De nova* synthesis, route of fatty acid hydroxylation and caste selective β-oxidation. *Insect Biochem. Molec. Biol.* **28**: 31-42.

POMONIS JG, NELSON DR, and FATLAND CL (1980) Insect hydrocarbons 2. Mass spectra of dimethylalkanes and the effect of the number of methylene units between methyl groups on fragmentation. *J. Chem. Ecol.* **6**: 965-972.

POST DC, PAGE RE, and ERICKSON EH JR (1987) Honeybee (*Apis mellifera* L.) queen feces: source of a pheromone that repels worker bees. *J. Chem. Ecol.* **13**: 583-591.

POUVREAU A (1991) Morphology and histology of tarsal glands in bumble bees of the genera *Bombus, Pyrobombus*, and *Megabombus*. *Can. J. Zool.* **69**: 866-872.

RIBBANDS CR (1953) *The behaviour and social life of honeybees*. Dover Publications, Inc, New York.

ROBINSON GE and HUANG Z-H (1998) Colony integration in honey bees: genetic, endocrine and social control of division of labor. *Apidologie* **29**: 159-170.

RUTTNER F (1977) The problem of the Cape bee (*Apis mellifera capensis* Escholtz): parthenogenesis, size of population, evolution. *Apidologie* **8**: 281-294.

SAIOVICI M (1983) 9-Oxodecenoic acid and dominance in honeybees. *J. Apic. Res.* **22**: 27-32.

SANCHO MT, MUNIATEGUI S, HUIDOBRO JF and SIMAL-LOZANO J (1991) Provincial classification of Basque Country (northern Spain) honeys by their chemical composition. *J. Apic. Res.* **30**: 168-172.

SCHMIDT JO (1998) Mass action in honey bees: alarm, swarming and the role of releaser pheromones. In Van der Meer RK, Breed MD, Espelie KE and Winston ML (eds) *Pheromone communication in social insects*. Westview Press, Boulder Colorado, p 257-290.

SCHMIDT JO, SLESSOR KN, and WINSTON ML (1993) Roles of nasonov and queen pheromones in attraction of honeybee swarms. *Naturwiss.* **80**: 573-575.

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

SELLERS CS, MAR T, BERTSCH W, and CREWE RM (1987) Investigation of the African honeybees races. *J. High Res. Chrom. & Chrom. Comm.* **10**: 674-675.

SHEARER DA, BOCH R, MORSE RA, and LAIGO FM (1970) Occurrence of 9-oxodec-*trans*-2-enoic acid in queens of *Apis dorsata*, *Apis cerana*, and *Apis mellifera*. *J.Insect Physiol.* **16**: 1437-1441.

SILVA ECA DA, SILVA RMB DA, CHAUD-NETTO J, MORETI ACCC and OTSUK IP (1995) Influence of management and environmental factors on mating sucess of Africanized queen honey bees. *J. Apic. Res.* **34**: 169-175.

SINGH RP and SINGH PN (1996) Amino acid and lipid spectra of larvae of honey bee (*Apis cerana* Fabr) feeding on mustard pollen. *Apidologie*. **27**: 21-28.

SLESSOR KN, KAMINSKI L-A, KING GGS, BORDEN JH and WINSTON ML (1988) Semiochemical basis of the retinue response to queen honey bees. *Nature* **332**: 354-356.

SLESSOR KN, KAMINSKI L-A, KING GGS, and WINSTON ML (1990) Semiochemicals of the honeybee queen mandibular glands. *J. Chem. Ecol.* **16**: 851-860.

SLESSOR KN, FOSTER LJ AND WINSTON ML (1998) Royal flavours: Honey bee queen pheromones. In Van der Meer RK, Breed MD, Espelie KE and Winston ML (eds) *Pheromone communication in social insects*. Westview Press, Boulder Colorado, p 331-343.

SNELGROVE LE (1940) *The introduction of queen bees*. Purnell and Sons Ltd, London.

TROUILLER J, ARNOLD G, LE CONTE Y, MASSON C, and CHAPPE B (1991) Temporal pheromonal and kairomonal secretion in the brood of honeybees. *Naturwiss.* **78**: 368-370.

TUMLINSON JH (1988) Contemporary frontiers in insect semiochemical research. *J. Chem. Ecol.* **14**: 2109-2130.

TUMLINSON JH and HEATH RR (1976) Structure elucidation of insect pheromones by microanalytical methods. *J. Chem. Ecol.* **2**: 87-99.

VALLET A, CASSIER P, and LENSKY Y (1991) Ontogeny and the fine structure of the mandibular glands of the honeybee (*Apis mellifera* L.) workers and the pheromonal activity of 2-Heptanone. *J. Insect Physiol.* **37**: 789-804.

VAN DER MEER RK, BREED MD, ESPELIE KE AND WINSTON ML (1998) *Pheromone communication in social insects*. Westview Press, Boulder Colorado.

VELTHUIS HHW (1970) Queen substances from the abdomen of the honey bee queen. *Z. Physiol.* **70**: 210-222.

VELTHUIS HHW (1972) Observations on the transmission of queen substances in the honey bee colony by the attendants of the queen. *Behaviour* **41**: 105-129.

VELTHUIS HHW (1985) The honeybee queen and the social organization of her colony. *Exper. Behav. Ecol.* **31**: 543-557.

VELTHUIS HHW (1990) Chemical signals and dominance communication in the *honeybee Apis mellifera* [Hymenoptera: Apidae]. *Entomol.Gener.* **15**: 83-90.

VELTHUIS HHW, RUTTNER F and CREWE RM (1990) Differentiation in reproductive physiology and behaviour during the development of laying worker honey bees. In: Engles E (ed) *Social Insects – An evolutionary approach to castes and reproduction.* Springer-Verlag, Berlin p 231-243.

VERMA S and RUTTNER F (1983) Cytological analysis of the thelytokous parthenogenesis in the Cape honeybee (*Apis mellifera capensis* Escholtz. *Apidologie* **14**: 41-57.

VIERLING G and RENNER (1977) Die bedeutung des sekretes der tergittaschendrüsen für die attraktivität der bienenkönigin gegenüber jungen arbeiterinnen.(The secretion of the tergite glands and the attractiveness of the honey bee queen.) *Behav. Ecol. Sociobiol.* **2**: 185-200.

VILLA JD and SCHMIDT JO (1992) Does queen pheromone increase swarm capture in hives baited with nasonov pheromone? *J. Apic. Res.* **31**: 165-167.

WALTER F, FLETCHER DJC, CHAUTEMS D, CHERIX D, KELLER L, FRANCKE W, FORTELIUS W, ROSENGREN R and VARGO EL (1993) Identification of the sex pheromone of an ant, *Formica lugubris* (Hymenoptera, Formicidae). *Naturwiss.* **80**: 30-34.

WEAVER N and LAW JH (1960) Heterogeneity of fatty acids from royal jelly. *Nature* **188**: 938-939.

WEBSTER FX and PRESTWICH GD (1988) Synthesis of carrier-free tritium-labeled queen bee pheromone. *J. Chem. Ecol.* **14**: 957-962.

WHIFFLER LA, DRUSEDAU MUH, CREWE RM, and HEPBURN HR (1988) Defensive behaviour and division of labour in the African honeybee (*Apis mellifera scutellata*). *J. Comp. Physiol.* **163**: 401-411.

WILLIAMS IH, PICKETT JA, and MARTIN AP (1981) The nasonov pheromone of the honeybee *Apis mellifera* L. (Hymenoptera, Apidae). Part II. Bioassay of the components using foragers. *J. Chem. Ecol.* **7**: 225-237.

WILLIAMS IH, PICKETT JA, and MARTIN AP (1982) Nasonov pheromone of the honeybee. *Apis mellifera* L. (Hymenoptera,Apidae) Part IV. Comparative electoantennogram responses. *J. Chem. Ecol.* **8**: 567-574.

WILSON EO (1965) Chemical communication in the social insects. *Science* **149**: 1064-1071.

WINSTON ML (1987) *The biology of the honey bee*. Harvard University Press, Cambridge.

WINSTON ML and SLESSOR KN (1993) Applications of queen honey bee mandibular pheromone for beekeeping and crop pollination. *Bee World* **74**: 111-128.

WINSTON ML and SLESSOR KN (1998) Honey bee primer pheromones and colony organization: gaps in our knowledge. *Apidologie* **29**: 81-95.

WINSTON ML, SLESSOR KN, SMIRLE MJ and KANDIL AA (1982) The influence of a queen-produced substance, 9HDA, on swarm clustering behavior in the honeybee *Apis mellifera* L. *J. Chem. Ecol.* **8**: 1283-1288.

WINSTON ML, SLESSOR KN, WILLIS LG, NAUMANN K, HIGO HA, WYBORN MH, and KAMINSKI LA (1989) The influence of queen mandibular pheromones on workers attraction to swarm clusters and inhibition of queen rearing in the honey bee (*Apis mellifera* L.). *Insect. Soc.* **36**: 15-27.

WINSTON ML, HIGO HA, COLLEY SJ, PANKIW T and SLESSOR KN (1991) The role of queen mandibular pheromone and colony congestion in honey bee (*Apis mellifera* L.) reproductive swarming (Hymenoptera: Apidae). *J. Insect Behav.* **4**: 649-660.

WOSSLER TC and CREWE RM (1999) Mass spectral identification of the tergal gland secretions of female castes of two African honey bee races (*Apis mellifera*). *J. Apic. Res.* **38**: 137-148.

YADAVA RPS and SMITH MV (1971a) Aggressive behaviour of *Apis mellifera* L. workers towards introduced queens I. Behavioural mechanisms involved in the release of worker aggression. *Behaviour* **39**: 212-236.

YADAVA RPS and SMITH MV (1971b) Aggressive behavior of *Apis mellifera* L. workers towards introduced queens. II. Role of the mandibular gland contents of the queen in releasing aggressive behavior. *Can. J. Zool.* **49**: 1179-1183.

YADAVA RPS and SMITH MV (1971c) Aggressive behavior of *Apis mellifera* L. workers towards introduced queens. III. relationship between the attractiveness of the queen and worker aggression. *Can. J. Zool.* **49**: 1359-1362.

APPENDICES

APPENDIX I

Table A: Amounts (µg; mean [a] ± SE) of the major aliphatic acids present in mated queens from five localities in the Eastern Cape Province, South Africa.

Compound	Locality				
	Port Elizabeth	**East London**	**Steynsberg**	**Cradock**	**Grahamstown**
	n = 5	n = 2	n = 3	n = 2	n = 4
7HOA	0.537 ± 0.309	0.127 ± 0.127	2.105 ± 1.419	1.548 ± 0.621	1.452 ± 0.5837
8HOA	4.58 ± 2.550	1.05 ± 0.828	30.87 ± 21.877	14.72 ± 6.392	15.06 ± 6.290
9ODA	85.98 ± 45.98	7.50 ± 5.00	244.66 ± 119.87	361.35 ± 19.17	136.90 ± 44.66
9HDAA	0.32 ± 0.20	0.00 ± 0	0.58 ± 0.31	0.32 ± 0.11	0.00 ± 0
9HDA	22.84 ± 13.31	4.85 ± 4.13	58.08 ± 32.28	75.91 ± 20.88	48.21 ± 18.77
10HDAA	8.944 ± 7.619	0.422 ± 0.039	7.621 ± 3.776	20.593 ± 14.071	13.729 ± 4.827
10HDA	7.57 ± 4.152	1.30 ± 0.0145	35.40 ± 27.36	19.07 ± 5.797	25.16 ± 8.792

[a] Means within one row followed by the same letter are not significantly different (P > 0.05, Kruskal-Wallis). For rows in which no statistically significant difference was detected (P > 0.05, Kruskal-Wallis) no letters are included.

Abbreviations for the compounds are as follows: 7HOA = 7-Hydroxyoctanoic acid; 8HOA = 8-Hydroxyoctanoic acid; 9ODA = (*E*)-9-ketodec-2-enoic acid; 9HDAA = 9-Hydroxydecanoic acid; 9HDA = (*E*)-9-Hydroxydec-2-enoic acid; 10HDAA = 10-Hydroxydecanoic acid; 10HDA = (*E*)-10-Hydroxydec-2-enoic acid.

Table B: Amounts (µg; mean [a] ± SE) of the major phenols present in mated queens from five localities in the Eastern Cape Province, South Africa.

Compound	Locality				
	Port Elizabeth	**East London**	**Steynsberg**	**Craddock**	**Grahamstown**
	n = 5	n = 2	n = 3	n = 2	n = 4
HOB	13.30 ± 8.04	1.53 ± 1.39	46.77 ± 30.45	41.96 ± 14.03	30.82 ± 12.75
4HBA	1.23 ± 0.83	0.00 ± 0	0.87 ± 0.86	2.81 ± 0.77	0.01 ± 0.01
HVA	13.92 ± 8.27	1.45 ± 1.45	17.54 ± 10.23	16.52 ± 9.95	5.57 ± 2.60

[a] Means within one row followed by the same letter are not significantly different (P > 0.05, Kruskal-Wallis). For rows in which no statistically significant difference was detected (P > 0.05, Kruskal-Wallis) no letters are included.

Abbreviations for the compounds are as follows: HOB = Methyl-*p*-hydroxybenzoate; 4HBA = 4-Hydroxybenzoic acid; HVA = 4-Hydroxy-3-methoxyphenyl ethanol

Table C: Amounts (µg; mean [a] ± SE) of the minor aliphatic acids present in mated queens from five localities in the Eastern Cape Province, South Africa.

Compound	Locality				
	Port Elizabeth	**East London**	**Steynsberg**	**Cradock**	**Grahamstown**
	n = 5	n = 2	n = 3	n = 2	n = 4
2-Hexenoic acid	0.332 ± 0.155	0.123 ± 0.011	0.268 ± 0.143	0.328 ± 0.053	0.397 ± 1.094
Propanoic acid	0.10277 ± 0.07305	0.00013 ± 0.00013	0.19181 ± 0.11857	0.16954 ± 0.05252	0.00270 ± 0.00062
2-Propenoic acid	0.00005 ± 0.00005	0.00012 ± 0.00012	0.00018 ± 0.00009	0.00013 ± 0.00013	0.00963 ± 0.00940
Dodecanoic acid	0.418 ± 0.079	0.286 ± 0.021	0.412 ± 0.136	0.285 ± 0.112	0.110 ± 0.061
Decanoic acid	1.630 ± 0.870	0.617 ± 0.178	2.421 ± 1.429	2.917 ± 1.278	9.546 ± 8.693
Palmitoleic acid	1.283 ± 0.418	0.342 ± 0.342	4.371 ± 1.745	2.465 ± 0.036	2.486 ± 0.992
Palmitelaidic acid	0.787 ± 0.265	0.811 ± 0.292	1.060 ± 0.436	1.746 ± 0.418	1.178 ± 0.381
10-Undecenoic acid	2.274 ± 1.772	0.354 ± 0.035	2.075 ± 1.716	4.993 ± 0.175	3.526 ± 1.010
Palmitic acid	12.04 ± 6.891	2.85 ± 0.756	8.11 ± 6.014	18.87 ± 2.501	11.17 ± 3.184
Oleic acid	32.17 ± 17.24	3.46 ± 1.06	11.89 ± 7.74	94.65 ± 19.28	57.70 ± 19.15
Stearic acid	10.45 ± 4.54	4.63 ± 2.23	14.96 ± 11.28	35.88 ± 2.89	23.42 ± 6.75

[a] Means within one row followed by the same letter are not significantly different (P > 0.05, Kruskal-Wallis). For rows in which no statistically significant difference was detected (P > 0.05, Kruskal-Wallis) no letters are included.

Table D: Amounts (µg; mean [a] ± SE) of the aliphatic diacids present in mated queens from five localities in the Eastern Cape Province, South Africa.

Compound	Locality				
	Port Elizabeth	**East London**	**Steynsberg**	**Cradock**	**Grahamstown**
	n = 5	n = 2	n = 3	n = 2	N = 4
Suberic acid	0.863 ± 0.264	0.728 ± 0.159	0.601 ± 0.180	1.097 ± 0.296	0.661 ± 0.160
Azelaic acid	0.458 ± 0.227	0.326 ± 0.325	0.943 ± 0.837	1.210 ± 0.871	0.627 ± 0.576
Undecandioic acid	0.300 ± 0.194	0.002 ± 0.001	1.765 ± 1.416	0.630 ± 0.245	1.228 ± 0.394

[a] Means within one row followed by the same letter are not significantly different (P > 0.05, Kruskal-Wallis). For rows in which no statistically significant difference was detected (P > 0.05, Kruskal-Wallis) no letters are included.

Table E: Amounts (µg; mean [a] ± SE) of the alkanes present in mated queens from five localities in the Eastern Cape Province, South Africa.

Compound	Locality				
	Port Elizabeth	**East London**	**Steynsberg**	**Cradock**	**Grahamstown**
	n = 5	n = 2	n = 3	n = 2	n = 4
Eicosane	0.416 [ab] ± 0.241	0.067 [ab] ± 0.067	0.675 [ab] ± 0.234	1.081 [a] ± 0.024	0.022 [b] ± 0.019
Heneicosane	0.16196 ± 0.10304	0.00013 ± 0.00013	0.12494 ± 0.12349	0.50757 ± 0.25552	0.64851 ± 0.25079
Docosane	0.552 ± 0.175	0.354 ± 0.087	0.396 ± 0.164	1.909 ± 0.840	5.120 ± 1.816
Hexacosane	0.00019 ± 0.00007	0.00003 ± 0.000003	0.00032 ± 0.00003	0.00018 ± 0.00015	4.43615 ± 2.65581

[a] Means within one row followed by the same letter are not significantly different (P > 0.05, Kruskal-Wallis). For rows in which no statistically significant difference was detected (P > 0.05, Kruskal-Wallis) no letters are included.

Table F: Amounts (µg; mean [a] ± SE) of the aromatic acids and diacids present in mated queens from five localities in the Eastern Cape Province, South Africa.

Compound	Locality				
	Port Elizabeth	**East London**	**Steynsberg**	**Cradock**	**Grahamstown**
	n = 5	n = 2	n = 3	n = 2	n = 4
Aromatic acids					
Benzoic acid	1.046 ± 0.402	0.452 ± 0.452	5.765 ± 3.292	3.875 ± 0.713	2.963 ± 1.186
Cinnamic acid	0.417 ± 0.266	0.002 ± 0.001	0.539 ± 0.280	1.062 ± 0.023	0.012 ± 0.011
Aromatic diacids					
Vanillylpropionic acid	2.46 ± 1.166	1.65 ± 0.584	3.92 ± 1.806	5.84 ± 0.969	2.26 ± 0.652

[a] Means within one row followed by the same letter are not significantly different (P > 0.05, Kruskal-Wallis). For rows in which no statistically significant difference was detected (P > 0.05, Kruskal-Wallis) no letters are included.

Table G: Amounts (µg; mean [a] ± SE) of the amino acids, sugars and indole present in mated queens from five localities in the Eastern Cape Province, South Africa.

Compound	Locality				
	Port ELizabeth	**East London**	**Steynsberg**	**Cradock**	**Grahamstown**
	n = 5	n = 2	n = 3	n = 2	n = 4
Amino acids					
L-Proline	0.425 ± 0.145	0.257 ± 0.243	0.224 ± 0.095	0.363 ± 0.119	0.496 ± 0.169
Glutamic acid	0.779 ± 0.446	0.149 ± 0.149	3.140 ± 1.574	4.485 ± 1.363	0.168 ± 0.051
L-Serine	0.13970 ± 0.09860	0.00025 ± 0.00002	0.09734 ± 0.09709	0.14633 ± 0.14610	0.08694 ± 0.04895
Sugars					
D-Glucose	3.138 ± 2.312	0.649 ± 0.284	1.286 ± 0.707	1.610 ± 1.128	0.517 ± 0.494
L-Altrose	0.22 ± 0.15	0.00 ± 0	0.26 ± 0.15	0.50 ± 0.30	1.04 ± 0.27
Indole	0.07 ± 0.04	0.00 ± 0	0.42 ± 0.41	0.09 ± 0.08	0.31 ± 0.08

[a] Means within one row followed by the same letter are not significantly different (P > 0.05, Kruskal-Wallis). For rows in which no statistically significant difference was detected (P > 0.05, Kruskal-Wallis) no letters are included.