THE MANDIBULAR GLAND SECRETIONS AND OVARIAL DEVELOPMENT OF WORKER HONEYBEES (*APIS MELLIFERA*) IN THE EASTERN CAPE PROVINCE OF SOUTH AFRICA

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ABSTRACT

The Eastern Cape is an area in which *Apis mellifera capensis*, *A. m. scutellata* and their hybrid are known to naturally occur. I investigated the mandibular gland profiles and ovarial development of queenright workers from 4 localities. Their queens were then removed to determine how these aspects changed upon queen loss. In addition, drifted bees were analysed in the same way to determine how these factors changed once they had gained entry to a foreign hive. The queenright bees, form all 4 localities were found to have 9HDA as the most abundant of the 5 fatty acids measured and all localities had small percentages of 9ODA in their mandibular gland secretions. This resulted in relatively high queenright 9ODA:10HDA and 9HDA:10HDAA ratios. Despite this the percentage of bees with undeveloped ovaries was consistent with their queenright status. The mean values of these 2 ratios were significantly higher in the bees from East London and Cradock than those from Port Elizabeth. Steynsburg's bees were intermediate in this regard. Upon queen loss, the bees from all 4 localities had an increase in the percentage of 9ODA but the other compound changes in varying ways. East London's bees were the only ones not to become significantly more queen-like after queen loss. After 14 days without a queen, the mean values of these 2 ratios were much higher in the bees from Port Elizabeth than those from the other localities. Certain individuals from Port Elizabeth had values of these ratios that exceeded those found in *A. m. capensis* queens. Port Elizabeth was the only locality to display any surrogate queens and exhibited the highest increase in the number of bees with partially or fully developed ovaries. While certain individuals from the other localities had values of these ratios that exceeded these values reported in *A. m. scutellata* queens, Steynsburg's bees were the only ones that did increase in in terms of the number of bees with developed ovaries subsequent. The bees from Steynsburg were shown to suppress the mandibular gland and ovarial development of drifters from Port Elizabeth while bees from East London did not.

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CHAPTER 1

INTRODUCTION

A typical honeybee colony generally consists of 3 types of adults; a single queen who usually monopolises reproduction, a workforce of female honeybees, and at certain times of the year male sexuals, drones, raised entirely for the purpose of mating with virgin queens from other colonies. The production of haploid male offspring parthenogenically by worker honeybees (arrhenotoky) is a fail safe allowing the continuation of the queens genes through the drones in the event of queen loss and the absence of young queen derived female brood.

Prior to 1883 it was believed that this was the only form of reproduction worker honeybees were capable of but through the observations of De Villiers (1883, reproduced in Hepburn, 1990) and Onions (1912, 1914) it was suggested that worker honeybees in and around Cape Town were producing female brood in the honey supers, above the queen excluders. This alternative parthenogenic process was later scientifically verified by Jack (1916) and while the production of female brood by workers, had rarely been seen in subspecies of *Apis mellifera*, it was found to be extremely common in honeybees from this area of the Cape Province (Onions, 1912, 1914; Jack, 1916; Mackensen, 1943). These laying workers were present in all the colonies examined by Onions (1912) and were found to be tolerated by the queens and other workers. The subsequent dissection of laying workers revealed that they, like queens had spermathecae but that these never contained any sperm and therefore confirmed that the production of diploid female brood by workers was indeed parthenogenic (Onions, 1914; Anderson, 1962). This process, defined as thelytoky was suggested to be an automictic type of parthenogenesis (Ruttner, 1977) and this mechanism of automixis was later confirmed by Verma & Ruttner (1983).

That the production of females by workers being the rule as opposed to the exception, together with the fact that these bees showed other morphological and behavioural differences, led to the belief that there were two subspecies of *A. mellifera* within South Africa, *A. m. capensis* the black, docile bee (see Hepburn & Guye, 1993) and *A. m. scutellata,* the lighter more aggressive African bee (previously thought to be *A. m. adansonii*) (reviewed by Fletcher, 1978).

A. m. capensis queens were found to have spermathecae of greater volume and on average more sperm in their spermathecae than *A. m. scutellata* (Buys, 1990), implying that they mated with a greater number of drones in longer or more frequent mating flights. This combined with the high winds experienced on their native Cape Peninsula meant that they were more exposed to predation and less likely to return home. Thelytoky, possibly the result of a single recessive gene (Ruttner, 1988) or due to the effect of a microorganism (Allsopp, 1993b), is therefore thought to have evolved to compensate for this high frequency of queen loss in *A. m. capensis's* native area (Guy, 1976b; Tribe, 1983; Moritz & Kauhausen, 1984). Greeff (1996a), however, believes that thelytoky is the preferred mode of reproduction due to their life history.

While natural gene flow between the 2 subspecies is possible and does occur naturally, the interaction between the 2 subspecies was thought to be limited due to the fairly distinct habitats. *A. m. capensis's* lack of tolerance to the climatic conditions outside its natural range (Fletcher, 1975) and adaptations to their own local conditions (Tribe, 1983; Hepburn & Jacot Guillarmod, 1991) were thought to be the possible reason preventing *A. m. capensis* from moving into the area of *A. m. scutellata* distribution. *A. m. scutellata* on the other hand had been shown to be highly aggressive and invasive in South America (see Crewe, 1976) and this led to the belief that it was the docile *A. m. capensis* that was most at risk of being wiped out and that every attempt should be made to protect it (Ruttner, 1977; Moritz & Kauhausen, 1984).

Despite this belief, many beekeepers had commented on the invasiveness of *A. m. capensis* when kept in apiaries with foreign colonies (Onions, 1912,1914; Lundie, 1954; Johannsmeier, 1983). It should therefore not have been very surprising when reports of the invasion of *A. m. scutellata* colonies by *A. m. capensis* bees started to emerge by 1992, but the extent of these accounts was totally unexpected. This culminated in the loss of thousands of colonies and resulted in both huge monetary losses to beekeepers and more importantly immense setbacks to pollination services. The phenomenon became known as the '*capensis* calamity/problem' (Allsopp, 1992; Allsopp & Crewe, 1993) and has become highly publicised due to its potential threat, economically as well as agriculturally.

It has since been shown the migratory habits of bee keepers facilitated this problem. Through a series of hive movements for pollination and increasing purposes, colonies of *A. m. capensis* workers came in close contact with *A. m. scutellata* near Pretoria, well within its natural range (Cooke, 1992). Possibly due to differences in the mandibular gland secretions of the queens of the two subspecies, reported by Crewe (1982), an *A. m. capensis* worker in an *A. m. scutellata* colony is unaware of the presence of its queen. This *A. m. capensis* worker is therefore effectively in a queenless situation and consequently ovarial development takes place. The ability to lay female eggs enables the *A. m. capensis* worker's offspring to be raised as queens (Onions, 1912; Ruttner, 1977), but this is rare (Allsopp & Hepburn, 1997) and usually occurs only when a colony is depleted (Anderson, 1962). As is more often the case, the *A. m. capensis* larvae are able to manipulate their non-*capensis* hosts into providing them with more food that is slightly more similar to royal jelly, resulting in these females, as adults, having more queen like characteristics than those workers raised in *A. m. capensis* colonies (Beekman *et al.*, 2000). These unique abilities of *A. m. capensis*, among them the production of a queen-like mandibular gland signal in the absence of a true queen or in the presence of a foreign queen (Ruttner *et al.*, 1976; Hemmling *et al.*, 1979; Crewe, 1982) allow the *A. m. capensis* worker to be taken as the new 'queen' of the colony. The old queen is eventually killed or evicted (Lundie, 1954) and the colony can continue without a true queen for prolonged periods (Anderson, 1962). These workers, often appropriately called surrogate queens, do not have the egg laying potential of a true queen and the production of workers is dramatically reduced. The workers present in the colony perform poorly under these conditions (Allsopp & Crewe, 1993; Allsopp, 1993a, Greeff, 1997) and leads to what has become known as the dwindling colony syndrome (Hepburn & Allsopp, 1994; Allsopp & Crewe, 1993) the colony getting weaker and smaller, until only a handful of bees are left. These handfuls of bees can amalgamate with other colonies, thereby spreading the *A. m. capensis* workers to further colonies without human intervention (Hepburn *et al.*, 1999).

To date, the only practical solution to this problem has been the destruction of any colonies suspected of being infected by *A. m. capensis* and the legislative prohibition of the movement of colonies of *A. m. capensis* into the natural range of *A. m. scutellata* (Allsopp & Crewe, 1993; Johannsmeier, 1997). What exactly defines an *A. m. capensis* bee and the distribution of these 2 subspecies, is obviously vital to the implementation of these two preventative measures and has received much attention over the years.

Studies initially based on the darker colour of the Cape bee found *A. m. capensis* to be distributed throughout the south western part of the Cape Province, an area characterised by a Mediterranean climate with winter rainfall and a distinct macchia flora. Immediately to the north of this region was *A. m. scutellata* (misclassified as *A. m. adansonii*) (Guy, 1976a). When using 2 other characteristics of *A. m. capensis* (ie. the presence of a large spermatheca and a high number of ovarioles within the ovaries) its distribution was found to be dramatically smaller, consisting of approximately 10 000 colonies in a 50 km area around Cape Town (Ruttner, 1977). However, 7 years later Moritz and Kauhausen (1984) using 42 characters in a biometrical study, found no evidence of pure *A. m. capensis* and suggested that if not already extinct this subspecies was close to extinction. According to Ruttner (1988) *A. m. capensis,* defined by its unique trait of laying workers producing diploid eggs, was thought to be restricted to the Cape peninsula and immediate vicinity, a distribution rapidly shrinking. The appearance, however, of honeybees within the Eastern Cape that were *capensis*-like (Hepburn *et al.*, 1988; Hepburn, 1989) re-opened the matter and led researchers to use more characters of a more varied nature to pin down the distribution of *A. m. capensis*. By assessing the sex ratios of laying worker progeny and other biological characteristics Hepburn and Crewe (1990, 1991) defined 3 zones of honeybees within South Africa; the biologically distinct Cape honeybee (*A. m. capensis*) in the south and *A. m. scutellata* to the north, separated by a zone of hybridization. The hybrid zone was thought to be 200 km wide, lying between the Cape Fold Mountains in the south and the Nuweveldberge and Drakensberge in the north. This represented a dramatic increase in the distribution of *A. m. capensis*. Their range now extended to near East London, well into the Eastern Cape. A subsequent morphological analysis of these bees was in agreement with this pattern of distribution (Crewe *et al.*, 1994). This view became widely accepted and was instrumental in defining a boundary to migratory beekeeping which could then be implemented.

More recently, however, the frequency of mitochondrial and nuclear DNA profiles of these populations were found to be variable (Moritz *et al.*, 1994; 1998) and the analysis of alarm pheromone profiles also showed extensive variation (Hepburn *et al.*, 1994). This non concordance in the biological traits of these honeybee populations led to further scrutiny, this time with a suite of morphological and non-morphological traits. The distribution of these traits showed little compatibility and 4 distinct areas were proposed; an *A. m. capensis* and an *A. m. scutellata* morphocluster as well as 2 hybrid zones, either with or without thelytoky (Hepburn *et al.*, 1998). This demonstrated how different traits have moved different distances through a continuous population of honeybees. At this stage it is important to remember that while localities are referred to as 'thelytokous' or 'arrhenotokous', it is a generalisation. While the majority of workers in *A. m. capensis* colonies reproduce via thelytoky (Onions, 1912; Hepburn & Crewe, 1991) some of these colonies have been found to contain thelytoky and arrhenotokous workers (Hepburn & Radloff, 1998; Moritz *et al.*, 1999). The majority of workers from *A. m. scutellata* colonies on the other hand reproduce via arrhenotoky with only a few exceptions (Mackensen, 1943). Colonies within the hybrid zone have workers that reproduce via arrhenotoky and thelytoky, with the thelytokous laying workers having a significant reproductive dominance (Neumann *et al.*, 2000a). It is therefore not merely a matter of exhibiting either type of parthenogenic reproduction but the extent of this means of reproduction within the colony.

A finer spatial sampling with analysis of 12 morphometric characters led Steele *et al.* (1998) to question the existence of a hybrid zone, instead they proposed one population of bees showing morphometric variation in a smooth cline from north to south as a result of climatic variation. Their suggestion is that the extent of thelytoky is a more important issue than that of the *capensis* phenotype, with regard to solving the '*capensis* calamity'. Greeff (1997) had a similar view, believing that the fact that the bees are Cape bees is not important, it is the lack of communication that is the root of the problem.

Until recently it was thought that the invasion of *A. m. scutellata* colonies was purely accidental, with the orientation mistakes made by *A. m. capensis* resulting in them drifting into *A. m. scutellata* colonies. A case of being in the right place at the right time. Recently however, it was found that long-range dispersal of bees into foreign colonies occurred more often in *A. m. capensis* than in *A. m. scutellata* (Neumann *et al.* 2000b). They also found *A. m. capensis* workers to be significantly, more frequently invasive than either *A. m. scutellata* or their hybrid and disperses from queenright colonies preferentially go into queenless host colonies. These latest discoveries suggest that this dispersal is active rather than accidental.

It is documented that *A. m. capensis* show rapid and extreme development of their ovaries under queenless conditions (Crewe & Velthuis, 1980; Hepburn & Allsopp, 1994), and under these conditions have the unique ability to rapidly develop a mandibular gland secretion very similar to queens (Ruttner, 1976; Hemmling *et al.*, 1979; Crewe & Velthuis, 1980). However, these abilities have been demonstrated in only a few colonies from very few localities and subsequently inferred onto the morphometrically determined distribution. No data is presently available on the nature of these abilities in thelytokous hybrid bees, an important oversight as it has been suggested by many that the solution to the '*capensis* problem' lies in understanding the dynamics of this hybrid zone (Greef, 1997; Steele *et al.*, 1998).

Therefore in order to further shed some light on this phenomenon I set out to examine the mandibular gland secretions, ovarial development and reproductive status (a combination of the 2 former traits) of the bees from 4 localities within the Eastern Cape Province, an area of extensive *A. m. capensis* and *A. m. scutellata* interaction (Hepburn *et al.*, 1998).

Chapter 2 assess bees from these localities in the presence of a queen to determine, the percentage of 5 mandibular gland compounds present, the extent of ovarial development and the frequency of bees with various reproductive potentials. These data, while serving as a base line for subsequent chapters, also allow for regional comparisons.

Chapter 3 examines if and how these characteristics change when the queen is removed. Once again regional comparisons are done and I discuss these observations in terms of the ability to take over foreign colonies.

Chapter 4 examines how drifted bees differ, in respect of mandibular gland secretions, ovarial development and reproductive status, to bees of the same age that:

- were from the same locality but did not drift (non-drifters)
- were present in the colony that they drifted to (host colony)
- were from the same locality but drifted into colonies from different localities

Chapter 5 discusses the results of the thesis in their entirety.

CHAPTER 2

COMPOSITION OF MANDIBULAR GLAND SECRETIONS AND THE EXTENT OF OVARIAL DEVELOPMENT OF WORKERS UNDER QUEENRIGHT CONDITIONS

2.1 INTRODUCTION

Traditionally the role of the queen in a eusocial honeybee colony was seen as one of dominance over the reproductive subordinates, thereby keeping the colony performing sufficiently and maintaining colony structure. The most common view until recently was that in the absence of physical contact between the queen and all the workers within the colony, a form of chemical domination was achieved via pheromones released from numerous exocrine glands (Velthuis, 1985; Free, 1987; Blum & Fales, 1988; Velthuis, 1990).

The first major chemical compound to be identified in the mandibular glands of queens was (E)-9-oxo-2-decenoic acid (9ODA) (Callow & Johnston, 1960; Barbier & Lederer, 1960). It was later found to function as a sex pheromone (Gary, 1962; Butler, 1967) and inhibit queen rearing and ovarial development in workers (Butler, 1956; Butler *et al.*, 1961; Free, 1987). Hence it became known as the queen substance. (E)-9-hydroxy-2-decenoic acid (9HDA) is another of the more important fatty acids within the mandibular glands of queens which has been shown to have a stabilizing effect on swarm structure (Butler *et al.*, 1964).

The major tasks that worker honeybees perform in their lives are that of feeding the brood (by supplying the larvae with either worker or royal jelly), constructing and maintaining the nest, defending the colony and supplying it with food. The mandibular glands of workers were found to produce mainly (E)-10-hydroxy-2-decenoic acid (10HDA) (Barker *et al.*, 1959b; Callow *et al.*, 1959) a fatty acid that is found in royal jelly (Weaver

& Law, 1960) making up about 15% of it (Barker *et al.*, 1959a) and this compound consequently became know as the worker substance.

Studies concentrating on the differences between the mandibular gland secretions of workers and queens have shown that the fatty acids found in queen mandibular glands (eg 9ODA & 9HDA) are functionalised at the penultimate position (ω - 1) (Winston & Slessor, 1992), while the acids found in worker mandibular glands (eg 10HDA & 10-hydroxydecanoic acid (10HDAA)) are functionalised at the last position (ω) (Callow *et al.*, 1959).

While 10HDA had been found in small amounts in queens, 9ODA was not commonly found in European worker honeybee mandibular gland secretions but it was later discovered that *A. m. capensis* and *A. m. scutellata* workers were both capable of synthesizing 9ODA in their mandibular glands (see Crewe, 1988), blurring the previous distinction between the secretions of the different castes.

The disparity in the production of 9ODA led to the discovery that the biosynthetic capabilities of *A. mellifera* queens and workers are in fact similar and that their selectivity of which fatty acids to synthesise is largely determined by social position of an individual and not strictly related to caste (Plettner *et al.*, 1996). Arrhentotokous workers, whose role within the colony is fairly predetermined, therefore produce 10HDA and 10HDAA, which can be added to larval food to act as a preservative and increase its nutrient level. Thelytokous workers however, with the greater incentive to themselves reproduce under certain circumstances, can by selectively producing a greater range of fatty acids achieve varying levels of social position within the colony, ultimately producing mandibular gland secretions qualitatively similar to a queen (Ruttner *et al.*, 1976; Hemmling *et al.*, 1979; Crewe & Velthuis, 1980). While this does occasionally occur in arrhenotokous workers (Sakagami, 1958), it is observed much more frequently in thelytokous workers.

Not much research has been done on the profile of the mandibular gland secretions of *A. m. capensis* or *A. m. scutellata* in their queenright state, researches instead concentrating on these profiles in a queenless state. Hepburn and Allsopp (1994) and Jackson (1982) both examined the profiles of queenright *A. m. scutellata* mandibular gland secretions and determined the dominant notes.

The lack of expected worker reproduction in arrhenotokous colonies was previously explained by the presence of queen mandibular gland secretions, especially 9ODA, in terms of Butler's queen substance theory (1956). Now it is thought that the secretions of the queen from the tarsal and tergal glands together with those from the mandibular gland signal the presence of the queen (Seeley, 1995). While brood pheromones are partly able to inhibit the development of workers ovaries it seems the combination of these signals play a role in the lack of worker ovarial development.

One of the more recent explanations for the apparent lack of worker oviposition is worker policing (Ratnieks, 1988; Ratnieks & Visscher, 1989). It is based on the fact that high polyandry by the queen (Neumann *et al.*, 1998) results in workers not being equally related within a colony (super-sisters vs. half-sisters). Under these circumstances non-reproductive workers are on average more related to the drones produced by their mother than those produced by their laying worker sisters. The consequences are either aggressiveness to workers attempting to lay an egg or the ability to differentiate between worker and queen laid eggs and the adaptive behaviour of removing the former (Ratnieks & Visscher, 1989).

In thelytokous worker honeybees the situation is somewhat different as the lack of recombination (Moritz and Haberl, 1994) gives rise to clones. Under these circumstances the average relatedness of nestmates to the offspring of workers and queens is equal, eliminating the adaptiveness of worker policing (Moritz *et al.*, 1999) as predicted by Greeff (1996b). As a result worker reproduction is very common in queenright colonies and Moritz *et al.* (2000) conservatively estimated that 1/3 of the workers in a thelytokous *A. m. capensis* queenright colony, they examined, were worker offspring. Moritz *et al.* (1998) previously stated that the level of worker reproduction in *A. m. capensis* colonies is measurable at the population level. This shows that while the queen and brood pheromones have a role, at some level, on the development of workers ovaries, this role is not central and hence Moritz *et al.* (2000) concluded that the balance between conflict and cooperation is far more fragile in thelytokous than in arrhenotokous honeybees. Worker reproduction in *A. m. capensis* is therefore the rule as opposed to the exception. *A. m. capensis* workers have even been shown to be repelled by honeybee queens, a mechanism suggested to allow them to avoid suppression by the queen (Moritz *et al.* 2000).

Anderson (1962) and Hepburn *et al.* (1991) both measured the degree of ovarial development in queenright *A. m. capensis* colonies, while Jackson (1982), Hastings (1989) and Hepburn and Allsopp (1994) did the same for *A. m. scutellata* colonies.

Numerous studies have combined the degree of ovarial development and queen-like mandibular gland secretions and interpreted bees as false, pseudo or surrogate queens. The definition of these terms however varies from study to study and only Hepburn (1994) quantifies the frequency of these distinctive bees in a queenright state (in this case *A. m. capensis*).

The objective of this chapter is to document the relative percentages of certain fatty acids within the mandibular gland secretions, the extent of ovarial development and the frequency of various levels of reproductive status in queenright worker honeybees from various localities in the Eastern Cape Province, an area of intense interaction between *A. m. capensis* and *A. m. scutellata* (Hepburn *et al.*, 1998). The data was assessed for any regional variation and compared with known values for the 2 subspecies. It will later serve as a comparison for the subsequent chapter.

2.2 MATERIALS AND METHODS

2.2.1 Sample collection

In January 1998, during the peak honey flow, 6 honeybee colonies were collected simultaneously from each of 4 localities within the Eastern Cape Province of South Africa; Port Elizabeth, East London, Steynsburg and Cradock (Fig. 2.1). On collection each colony was screened in order to find the queen. Once found, the queen was placed with five (2 brood and 3 honey/pollen) of the ten frames into a small Langstroth-style 5-frame nucleus hive of 20 l capacity and marked as queenright, while the other five frames were placed in a second similar nucleus hive and marked as queenless. These colonies were then taken to an apiary in Grahamstown. The queen state of the queenless colonies was controlled by removing any developing queen cells. It is important to remember that the bees chosen represented natural populations as there is no bee breeding in these areas and native bees are caught in catch boxes to replenish beekeepers supply, as opposed to buying foreign colonies.

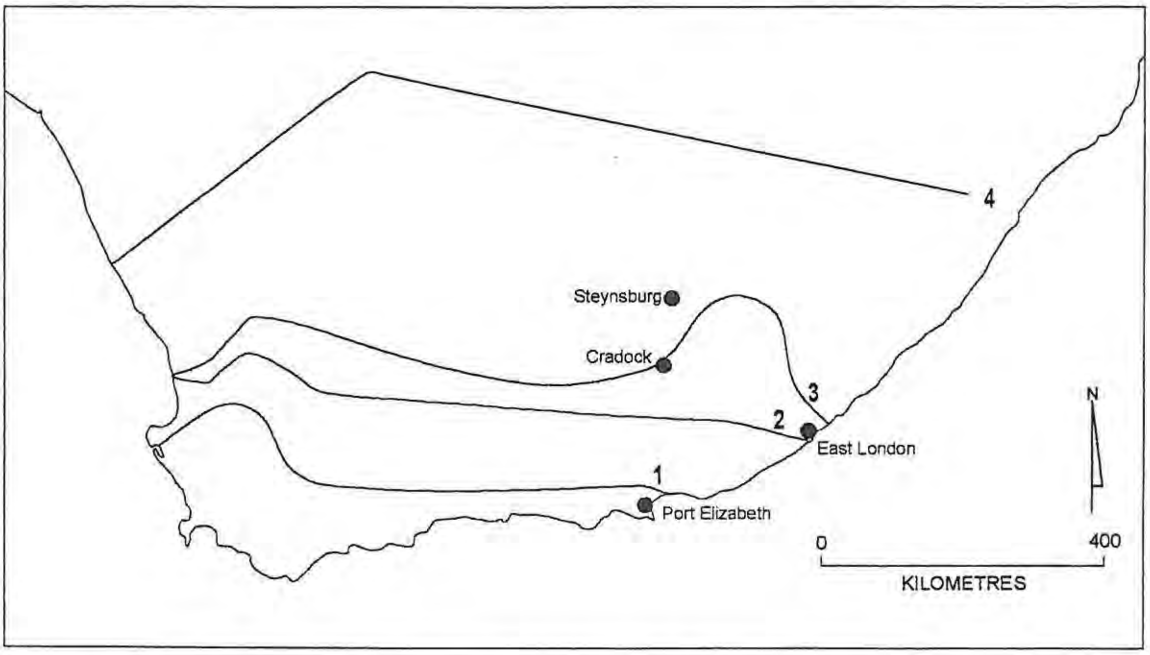

Fig. 2.1: Map of southern Africa illustrating the localities from which the bees were collected in relation to the distributions of morphometric and non-morphometric features of *A. m. capensis*, *A. m. scutellata* and the zone of introgression. Line 1 = northern limit of *capensis* morphocluster; line 2 = southern limit of *scutellata* morphocluster; line 3 = northern limit for thelytokous parthenogenesis; line 4 = northern limit for 100% frequency of the *capensis* haplotype P_0QQa (modified after Hepburn & Radloff, 1998).

During the initial manipulation of the experimental colonies (on collection from their respective localities) an initial sample (day 0) of ±12 bees was taken from a frame of each of the colonies. Subsequent samples were taken from the queenright colonies 9 and 14 days after collection. On returning to the laboratory these bees were dissected and their head's were placed in gas chromatographic vials with 1ml of dichloromethane (for gas chromatography, Merck). Each vial was numbered to ensure that individual bees could be distinguished. The vials were stored at -20 °C as were the matching abdomens in individually marked plastic Eppendorff vials.

2.2.2 Head extract analysis

2.2.2.1 Sample preparation

Each head sample was prepared a minimum of 3 months after collection . The bee's head was removed and the dichloromethane (DCM) evaporated off with nitrogen gas. 25μl of an internal standard, containing 1mg octanoic acid and 1mg tetradecane (both Sigma) in 4ml of DCM (for spectroscopy, Merck), was added immediately after full evaporation. The sample was then derivatised by adding 25μl of bis-(trimethylsilyl)-trifluoracetamide (BSTFA, for gas chromatography, Merck).

2.2.2.2 Gas chromatographic process

1μl of this prepared sample was then injected, on the same day as prepared, into an HP 5890 Series II Gas Chromatograph (GC) with an Ultra 1 crosslinked methyl silicone gum column (25mm x 0.32mm x 0.52μm film thickness) and analysed by a HP 3396 Series II integrator. Hydrogen was used as a carrier gas. The GC was programmed to remain at 60°C for 1 minute, then at a rate of 50°C/minute to increase to 100°C. The rate then decreased to 3°C/minute until the GC reached a temperature of 220°C and it remained at 220°C for a further 10 minutes. The temperature of the inlet and the detector were 230°C and 280°C respectively.

2.2.2.3 Data synthesis

Standard samples of known concentrations were run at intervals during the study. The areas under the peaks of the 5 compounds of interest (Table 2.1), in these standard samples were compared with areas of the tetradecane peaks in order to calculate relative mass ratios (RMR) for each of the fatty acids measured. The areas for each of the 5 fatty acids of interest, obtained from the samples of collected bees, were then converted into real amounts using their specific RMR's and the internal standard values.

Table 2.1: Fatty acids measured in the head extracts of *A. mellifera* workers.

Compound	Abbreviation
8-hydroxyoctanoic acid	8HOA
(E)-9-oxo-2-decenoic acid	9ODA
(E)-9-hydroxy-2-decenoic acid	9HDA
10-hydroxydecanoic acid	10HDAA
(E)-10-hydroxy-2-decenoic acid	10HDA

The absolute amounts of each of the compounds was then converted into a percentage relative to the other 4 compounds and the results from different colonies and different sampling days within a locality were pooled to obtain the queenright values.

2.2.2.4 Statistical analysis

The queenright samples for colonies within a locality were pooled to test for differences in the percentages of a compound or value of a ratio between localities. ANOVAs and Student-Newman-Keuls method of pairwise multiple comparison were used to test for significant differences in the percentage composition of the 5 mandibular gland compounds and various ratios of these compounds. Where data were not normally distributed the one way ANOVA on ranks and Dunn's method of pairwise multiple comparison were used.

2.2.3 Ovarial dissection

2.2.3.1 Dissection procedure

Each abdomen, after being removed from the freezer, was secured ventral side up, on a wax dissecting dish and submerged in water. By cutting laterally along the abdomen, from posterior to anterior the abdominal sternites (3^{rd}-6^{th}) could easily be detached. With a small amount of manipulation the left and right ovaries of the bee could be observed. The ovarial development was based on Velthuis (1970) with class 1 being inactive, class 2 those bees showing some development, from early signs to round or bean-shaped eggs and class 3 being typified by the presence of mature sausage-shaped eggs . The most developed ovary of the two was recorded as the ovarial development for that bee.

2.2.3.2 Data analysis

The results for colonies within a locality were pooled and the frequencies for each class of development calculated. These frequencies were then converted into percentages.

2.2.4 Reproductive status

2.2.4.1 Classifying reproductive status

Reproductive status is the term used here to signify the combination of an individual bee's mandibular gland profile and its ovarial development. The classification was based on that of Hepburn and Allsopp (1994) with a slight modification. From the GC analysis of the queens removed from the colonies in this study, it was found that the percentage composition of 9ODA in relation to the other 4 compounds ranged between 42.9% and 65.1% (Jones, 2000). Hepburn and Allsopp's (1994) level of 9ODA for surrogate queens (67%) seemed unreasonably high for this study. It was therefore decided to reduce the percentage to 45% of 9ODA and drop accordingly the lower percentage to 25%. The classes were therefore as follows:

1) normal workers = stage 1 ovaries, <25% 9ODA
2) intermediates = all bees not falling into other 4 classes
3) egg layers = stage 3 ovaries, <25% 9ODA
4) false queens = stage 1 or 2 ovaries, >45% 9ODA
5) surrogate queen = stage 3 ovaries, >45% 9ODA

2.2.4.2 Data analysis

The colonies within a locality were once again pooled and the frequencies for each class calculated. These frequencies were then converted into percentages.

2.3 RESULTS

2.3.1 Mandibular gland secretions

2.3.1.1 Relative abundance of compounds (within localities)

At all 4 localities 9ODA and 8-hydroxyoctanoic acid (8HOA) were the least abundant of the 5 compounds examined (Table 2.2). Of the two compounds 9ODA accounted for the lowest portion of the sample, making up on average only 3.7-5.6%. 8HOA was slightly more abundant than 9ODA, making up on average 6.9-8.0% of the sample. 9HDA was the most abundant compound at all 4 localities (36.9-50.3% of sample) (Table 2.2). The second most abundant compound was 10HDAA (22.5-31.4% of sample) and 10HDA was present in slightly lower percentages than 10HDAA, making up on average 14.4-21.0% of the samples.

2.3.1.2 Relative amounts of each compound between localities

There was no significant difference between the amounts of 8HOA or 9ODA at any of the localities (Table 2.2, 8HOA: $F=1.41$, $P=0.2377$ and 9ODA: $H=6.80$, $P=0.0784$). The bees from Port Elizabeth had significantly lower percentages of 9HDA and significantly higher percentages of 10HDA than the bees from East London or Cradock (9HDA: $H=32.9$, $P<0.0001$ and 10HDA: $H=24.5$, $P<0.0001$), and significantly higher percentages of 10HDAA than the bees from East London, Cradock or Steynsburg (Table 2.2, $H=28.5$, $P<0.0001$).

The patterns above are mirrored in the mean values of the 2 ratios measured, 9ODA:10HDA and 9HDA:10HDAA (Table 2.3). The bees from East London and Cradock had significantly higher values than Port Elizabeth's bees for the ratio of 9ODA:10HDA and

9HDA:10HDAA (H=19.9, P=0.0002; H=35.9, P<0.0001), approximately twice and 1.5 times more respectively.

Table 2.2: Percentage composition (mean ± SE) of 5 mandibular gland compounds[†] of workers from queenright colonies at 4 localities in the Eastern Cape Province.

		% Composition (mean ± SE)[*]				
Locality	n	8HOA	9ODA	9HDA	10HDAA	10HDA
Port Elizabeth	201	7.8 ± 0.4^{a}	3.7 ± 0.3^{a}	36.9 ± 1.6^{a}	31.4 ± 1.1^{a}	20.2 ± 1.2^{a}
East London	114	6.9 ± 0.6^{a}	5.6 ± 0.9^{a}	48.6 ± 2.0^{bc}	24.5 ± 1.4^{b}	14.4 ± 1.5^{b}
Steynsburg	92	8.0 ± 0.5^{a}	3.7 ± 0.6^{a}	41.1 ± 2.1^{ab}	26.2 ± 1.6^{b}	21.0 ± 2.0^{a}
Cradock	103	6.9 ± 0.5^{a}	4.5 ± 0.5^{a}	50.3 ± 2.3^{c}	22.5 ± 1.5^{b}	15.8 ± 2.1^{b}

[*] Similar letters within a column denote no significant difference (p>0.05).

[†] 8HOA = 8-hydroxyoctanoic acid, 9ODA = (E)-9-oxo-2-decenoic acid, 9HDA = (E)-9-hydroxy-2-decenoic acid, 10HDAA = 10-hydroxydecanoic acid & 10HDA = (E)-10-hydroxy-2-decenoic acid.

Table 2.3: 9ODA:10HDA and 9HDA:10HDAA ratios (mean ± SE) of workers from queenright colonies at 4 localities in the Eastern Cape Province and the 9ODA:10HDA ratio of queens from their respective localities.

		% Composition (mean ± SE)[*]		
Locality	n	9ODA / 10HDA		9HDA / 10HDAA
		Workers	Queens [†]	Workers
Port Elizabeth	201	1.1 ± 0.4^{a}	11.14 ± 2.3	3.7 ± 0.3^{a}
East London	114	2.3 ± 0.6^{c}	5.97 ± 4.3	5.6 ± 0.9^{b}
Steynsburg	92	0.6 ± 0.2^{ab}	12.65 ± 5.8	3.7 ± 0.6^{ab}
Cradock	103	2.8 ± 0.7^{bc}	20.54 ± 5.2	4.5 ± 0.5^{b}

[*] Similar letters within a column denote no significant difference (p>0.05).

8HOA = 8-hydroxyoctanoic acid, 9ODA = (E)-9-oxo-2-decenoic acid, 9HDA = (E)-9-hydroxy-2-decenoic acid, 10HDAA = 10-hydroxydecanoic acid & 10HDA = (E)-10-hydroxy-2-decenoic acid.

[†] Values for mated, laying queens of colonies used in this study from Jones (2000).

2.3.2 Degree of ovarial development

Table 2.4 shows that only 1 bee (0.9%) from East London had mature sausage shaped eggs in their natural queenright state. The majority of the bees (92.4-98.9%) at all 4 localities showed no sign of ovarial development while a small percentage (1.1-7.6%) at all 4 localities showed an intermediate level of ovarial development. Steynsburg and Cradock had higher proportions of class 2 bees than East London and Port Elizabeth.

2.3.3 Reproductive status

At all 4 localities the majority (89.1-97.9%) of the workers can be classed as having normal reproductive status under queenright circumstances and only a small percentage of the workers can be classed as intermediates, either ovarially or with respect to mandibular gland secretions (Table 2.5, 2.1-9.8%). While Steynsburg had the highest percentage of intermediate bees (9.8%) Port Elizabeth had the lowest percentage (2.1%). East London and Steynsburg were unusual as they were the only localities in which bees other than normal and intermediate were found. East London had very low percentages of egg layers and false queens under queenright conditions (0.9% of both) while Steynsburg had a higher percentage (1.1%) of false queens. No surrogate queens were found at any of the localities (Table 2.5).

Table 2.4: Ovarial development of workers from queenright colonies at 4 localities in the Eastern Cape Province. Previously published results for 2 subspecies are included for comparison.

		% Ovarial Development*		
Locality / subspecies	n	Class 1	Class 2	Class 3
Port Elizabeth	188	98.9	1.1	-
East London	114	97.4	1.7	0.9
Steynsburg	92	92.4	7.6	-
Cradock	103	93.2	6.8	-
Previously published results				
A. m. capensis (Anderson, 1962)		40-83	17-51	-
A. m. capensis (Hepburn *et al.*, 1991)		73.6-84.4	15.6-25.7	0-2.5
A. m. scutellata (Hepburn & Allsopp, 1994)		99	-	1
A. m. scutellata (Jackson, 1982 and Hastings, 1989)		100	-	-

[*] Class 1 = inactive, class 2 = some degree of development & class 3 = mature sausage shaped eggs.

Table 2.5: Reproductive status of workers from queenright colonies at 4 localities in the Eastern Cape Province.

Locality	n	% Reproductive status[*]				
		Normal	Intermediate	Egg layer	False queen	Surrogate queen
Port Elizabeth	188	97.9	2.1	-	-	-
East London	114	93.0	5.2	0.9	0.9	-
Steynsburg	92	89.1	9.8	-	1.1	-
Cradock	103	92.2	7.8	-	-	-

[*] Normal = class 1 ovaries & <25% 9ODA, intermediate = all bees not falling into any other class, egg layer = class 3 ovaries & <25% 9ODA, false queen =class 1 or 2 ovaries & >45% 9ODA, surrogate queen = class 3 ovaries & >45% 9ODA.

2.4 DISCUSSION

The dominance of 9HDA in the head extracts of queenright honeybees at all five localities examined, seems peculiar in light of the "worker substance" views that 10HDA is the dominant fatty acid in queenright workers and the fact that 9HDA is considered as one of the most important fatty acids in queen mandibular gland secretions (Velthuis, 1985; Free, 1987). However, there have been numerous recent accounts of fatty acids other than 10HDA being present in equal or larger amounts in workers. Hepburn and Allsopp (1994) found that the queenright head extracts of *A. m. scutellata* had 9HDA, 10HDA and 8HOA as the dominant compounds with 9HDA being present in the largest amounts. Jackson (1982) similarly found 9HDA, 10HDA, 8HOA and 10HDAA as the dominant notes of the *A. m. scutellata* workers he examined. The differences reported by various studies are increased due to the ages of the bees used, as changes in the bouquet of mandibular gland secretion occur as a worker bee ages (Boch & Shearer, 1967; Crewe & Hastings, 1976; Velthuis & van der Kerk, 1988). 9HDA and 8HOA occur in higher proportions in younger bees (Velthuis & van der Kerk, 1988) and as they age the amount of 10HDA increases (Boch & Shearer, 1967) and eventually predominates. As the bees used in this study were sampled directly from the brood frames, where the highest concentration of young bees is located, it can be deduced that the majority of them were relatively young in comparison to the rest of the work force and this could be the reason that the percentages of 9HDA were so high.

The presence of 9ODA, the 'queen substance' in small amounts relative to the 4 other compounds measured (3.7-5.6%), and therefore even smaller amounts relative to entire head extract, was not entirely surprising as 9ODA has been reported in queenright workers of *A. m. capensis* (Crewe *et al.*, 1990a&b) and *A. m. scutellata* (cf. Crewe, 1988). The percentage of 9ODA in the mandibular gland secretions (relative to the other 4 compounds) was very small in comparison to the percentage present in mated queens of *A. m. capensis* (84.83%) and *A. m. scutellata* (65.39%, both relative to the same 4 compounds; Crewe, 1982).

The amount of specific compound present, however, does not entirely effect how an individual is perceived by its colony mates, instead it is the mixture of these compounds that bees perceive and therefore the ratio of specific parts to one another (Crewe & Velthuis, 1980). For this reason we reported both the ratio's of 9ODA:10HDA and 9HDA:10HDAA which both give one an indication of the amounts of queen-like to worker-like acids. These results were surprising as while the 9ODA:10HDA ratio at the four localities was lower than the same value calculated for their respective queens (Jones, 2000) (Table 2.3) the values of this ratio were relatively high in respect to their queenright status. At 2 of the 4 localities (East London and Cradock) the queenright workers had average values of the 9ODA:10HDA ratio that were similar to those reported for *A. m. mellifera* queens (2.4) (Crewe, 1988). The high standard errors of these mean values indicate that certain individuals may even have exceeded this value. All mean values were however, lower than those reported for *A. m. scutellata* and *A. m. capensis* queens (8.1 and 84.0 respectively) (Crewe, 1988). The values for the 9HDA:10HDAA ratio also reflected higher percentages of queen-like acids than worker-like acids.

The queenright samples from the 4 localities in this study had higher proportions of undeveloped bees than either Anderson (1962) or Hepburn *et al.* (1991) found in *A. m. capensis* workers from colonies headed by mated, laying queens but slightly lower proportions of undeveloped bees than Hepburn and Allsopp (1994), Jackson (1982) and Hastings (1989) found in queenright *A. m. scutellata* colonies (Table 2.4).

As egg layers are found in queenright *A. m. capensis* colonies (Onions, 1912) and returning foraging bees have on occasion been found to have high amount of 9ODA relative to 10HDA (M. Allsopp pers. comm.) and neither of these occurrences seem to affect the reproductive capacity of queens within the colonies, it is of more interest to examine the number of bees with the combination of the two traits. Especially in the light that thelytokous surrogate queens (defined here as those smelling queen like and having mature sausage shaped eggs) in foreign colonies represent a potential threat. As was expected in a queenright state no surrogate queens were found within the samples from any of the colonies. The frequency of intermediate bees, those with minor ovarial and/or mandibular gland development, was below the 12% reported by Hepburn (1994) in *A. m. capensis* queenright colonies, and while he reported no egg layers or false queens (workers that smell queen like but do not lay eggs) in these colonies. Considering the number of bees sampled, the difference between this and the small percentages of egg layers and false queens found at East London and Steynsburg can be called insignificant.

Pheromones, both sting and mandibular gland have been used in the past as discriminators of subspecies (see Hepburn & Radloff, 1998). Most of these studies used high levels of intracolonial variation as indicators of hybridization, however, the qualitative and quantitative differences between the mandibular secretions is an alternative avenue of investigation. Examining the 4 localities with respect to the relative amounts of the 5 compounds measured was an attempt to identify any similarities or differences between them. The localities used in this study fall into an area of extreme interaction between the 2 subspecies. While these localities are geographically not very far apart they occur in an region where different morphometric clusters of the 2 species and their hybrid are found and colonies express varying degrees of different biological characteristics. The non-concordance between the morphometric and biological traits it this area demonstrates the dynamic and independent pattern of gene flow (Hepburn *et al.*, 1998). According to Hepburn *et al.* (1998) the bees from Port Elizabeth are morphometrically *A. m. capensis* and exhibit thelytoky. The bees from East London fall just within the boundary of the *A. m.*

scutellata morphocluster and Steynsburg's bees fall within this morphocluster but while East London's bees exhibit thelytokous parthenogenesis, Steynsburg's bees do not. Cradock's bees show high morphometric variance typical of hybrid bees and exhibit thelytoky (Hepburn *et al.*, 1998).

While there was a lack of significant differences between the percentage composition of 8HOA and 9ODA between localities Port Elizabeth's bees had significantly less 9HDA and more 10HDAA and 10HDA than either East London or Cradock. Due to this the ratios of 9ODA:10HDA and 9HDA:10HDAA of queenright worker bees from Port Elizabeth were significantly more worker-like than those from East London and Cradock, while these ratios for Steynsburg's bees were intermediate.

This difference between localities does not correspond to the similar investigation of the queens from these specific colonies (Jones, 2000), which showed no significant differences in the amount of the 10 compounds measured (which included the 5 used in this study). Her ratios of 9ODA:10HDA were found to be similar to slightly higher than that of *A. m. scutellata* queens and it was suggested that in terms of queen mandibular gland secretions the bees from these localities; Port Elizabeth, East London, Steynsburg and Cradock could be hybrids of *A. m. capensis* and *A. m. scutellata.*

It is surprising that Port Elizabeth's queenright bees are significantly less queen-like than those of East London and Cradock with regard to both ratios calculated as it is the only locality that lies within the *A. m. capensis* morphocluster. Port Elizabeth also represents the locality with the lowest frequency of bees with some degree of development or with other than normal reproductive status. The bees from Steynburg are intermediate with respect to the quality of their mandibular gland secretions and this is also surprising as it is the location that lies the furthest north, well within the *scutellata* morphocluster (Hepburn *et al.* 1998) and was the only locality sampled in this study that was not reported to exhibit thelytoky.

The workers used in this study were of unknown ages and this led to large amounts of variation in the samples, evident in the standard errors. As this was not sufficient enough to mask the differences between localities we can confidently say that the bees from the localities sampled represented different degrees of mandibular gland development under queenright conditions and that East London and Cradock's bees grouped together in this respect representing one extreme while Port Elizabeth's bees represented the other extreme while the bees from Steynsburg fell intermediately. Without sufficient mandibular gland secretion data on 'pure' *A. m. capensis* and *A. m. scutellata* from well within their areas of distribution and away from this region of high level interaction, it is impossible to determine who the bees from these Eastern Cape localities are more like with respect to mandibular gland secretions under queenright conditions. The level of ovarial development under queenright conditions, however, lay between that of *A. m. capensis* and *A. m. scutellata* and suggests that in terms of this trait the bees from these Eastern Cape localities could possibly be regarded as hybrids as suggested by the queen mandibular gland secretions (Jones, 2000). The frequency of different reproductive statuses also fell below that expected from *A. m. capensis*. With the independent pattern of gene flow, however, these facts could not be used to deduce anything about the other traits the bees exhibit.

What can be concluded from the data is that East London and Cradock's bees are significantly more queen-like with respect to their mandibular gland secretion than those from Port Elizabeth, and this may represent a possible head start in the development of a queen-like smell. The fact that they are known to exhibit thelytoky means that they have the reproductive ability to give rise to female offspring. Whether or not they develop their mandibular gland upon queen loss or in the presence of a foreign queen remains to be seen.

CHAPTER 3

CHANGES IN WORKER MANDIBULAR GLAND SECRETIONS AND OVARIAL DEVELOPMENT IN RESPONSE TO QUEEN LOSS

3.1 INTRODUCTION

The loss of honeybee queens occurs naturally on virgin mating flights and accidentally during colony manipulation by bee keepers. The subsequent absence of queen produced pheromones eliminates one of the many signals received by worker honeybees within a colony and results in a change in the dynamics of colony organisation (Free, 1987).

In queenless arrhenotokous colonies the lack of a queen and the presence of uncapped brood stimulates queen cell construction and partially inhibits the development of worker ovaries enabling the colony to rear a new queen (see Hepburn, 1994). In the absence of brood the development of worker ovaries is no longer inhibited and the workers produce drones. While in the latter the colony as a whole is doomed by the inevitable decrease in the work force, the genes of the colony persist through the mating of these drones with virgin queens.

In queenless *A. m. capensis* colonies the ability to lay female eggs opens up alternative pathways to the queenless bees. Among these options are rearing a queen from worker derived brood or continuing as a laying worker colony (Hepburn, 1994). The greater frequency at which *A. m. capensis* workers produce a queen-like smell plays an important role in enabling them to pursue the latter option. If some of the workers have developed to a sufficient level before a new queen emerges the rearing of the queens is abandoned (Hepburn *et al.*, 1988). This abandonment leads to the destruction of these queen cells and permits the further development of the workers ovaries and mandibular gland secretions (Hemmling *et al.*, 1979; Allsopp, 1988). The bees that show high levels of development, ovarially and/or pheromonally, inturn inhibit ovarial development (Crewe & Velthuis, 1980; Velthuis *et al.*, 1990) and changes in the pheromonal bouquet (Hemmling *et al.*, 1979) of less developed workers thereby prevent further queen rearing (Hepburn *et al.*, 1988; Whiffler & Hepburn, 1991b). These few developed workers are defined as either, surrogate/pseudo queens (mimicking a true queen in terms of mandibular gland secretions and the ability to lay eggs, (Ruttner *et al.*, 1976) or false queens (mimicking a true queen in terms of mandibular gland secretions without laying eggs, Velthuis, 1985). It has been shown that certain patrillines are more likely to produce offspring and become reproductively dominant (Moritz et al., 1996).

While the events following queen loss in a natural state are of importance it is in the context of the reproductive parasitism by *A. m. capensis* of foreign colonies that this situation gains its full significance. When a thelytokous *A. m. capensis* honeybee gains entry into a arrhenotokous *A. m. scutellata* hive, the situation it finds itself in mirrors that of the queenless environment. The inability of the *A. m. capensis* worker to acknowledge the presence of the foreign queen, possible due to lower percentages of 9ODA in non *A. m. capensis* queens mandibular gland secretions (Crewe, 1982), results in development equivalent to that in a queenless colonies. In an *A. m. scutellata* hive the development of *A. m. capensis* surrogate and false queens leads the colony to evict their own queen.

A. m. capensis workers developed ovarially and oviposit within 4-8 days after queen loss (Anderson, 1962; Ruttner & Hesse, 1981; Hepburn *et al.*, 1991), much quicker than *A. m. mellifera* (Anderson, 1962) and *A. m. scutellata* (Ruttner & Hesse, 1981; Velthuis & van der Kerk, 1988).

The development of a queen-like smell is partly due to the changes in the mandibular gland secretion of *A. m. capensis* workers when becoming queenless, and though the secretions of other glands that contribute to the queen-like smell play a role (tergal glands in *A. m. capensis* workers, Billen *et al.*, 1986, Wossler, 1993), it is the mandibular glands that have been the focus of the majority of work to date. The increase in the amounts of 9ODA relative to other mandibular gland components of *A. m. capensis* in queenless situations was demonstrated by Hemmling *et al.* (1979). While *A. m. scutellata* and *A. m. mellifera* rarely produce false queens they have only small percentages of 9ODA in their mandibular glands and 10HDA is dominant (Crewe & Velthuis, 1980; Crewe, 1981). *A. m. capensis,* however, readily produce false queens (Free, 1987) and in their mandibular glands 9ODA dominates (Crewe & Velthuis, 1980; Crewe, 1981; Crewe, 1988) after 2-6 days (Crewe, 1987; Crewe, 1988). The mandibular gland secretions of *A. m. capensis* false queens are queen like after 10 days (Crewe, 1987) with the percentage 9ODA exceeding the amount present in *A. m. mellifera* (Crewe & Velthuis, 1988) and *A. m. scutellata* mated queens (Crewe, 1984) and is similar to that of *A. m. capensis* mated queens (Crewe *et al.*, 1990a&b). It has been shown that the development of *A. m. capensis* mandibular gland secretions and ovaries occurs more rapidly and to a greater extent when they are in the minority (Saiovici, 1983) in the presence of a foreign subspecies (Crewe & Velthuis, 1980; Velthuis & Van der Kerk, 1988) as opposed to with members of the same subspecies.

By combining the degree of ovarial and mandibular gland development, Hepburn and Allsopp (1994) showed that a higher frequency of bees with various degrees of ovarial and mandibular gland development (intermediate bees, egg-layers, false queens and surrogate queens) occurred in queenless *A. m. capensis* than in *A. m. scutellata* colonies and that even higher frequencies of these bees were obtained when the *A. m. capensis* workers were introduced into queenless *A. m. scutellata* colonies.

All the data presented on *A. m. capensis* and *A. m. scutellata* are based on bees from very few localities, well within the suggested ranges of these bees. This has then been used to infer rapid ovarial development and development of a mandibular gland secretion dominated by 9ODA under queenless conditions, onto all other bees occurring within the debatable morphometrically defined capensis zone (Hepburn *et al.*, 1998). In addition, how the bees from the thelytokous hybrid zone respond to queen loss, with regard to these abilities, is not known.

In this chapter I de-queened numerous colonies from 4 localities within the Eastern Cape. I then assess to what degree these bees develop their mandibular gland secretions and/or ovaries. While the speed of development occurring in native queenless colonies is an underestimate of the speed of development in a foreign colony, it gives one an idea of the propensity of the bees to change. Having determined this I then compared the changes in the bees from these localities to reported levels of development in both queenless *A. m. capensis* and *A. m. scutellata* colonies.

3.2 MATERIALS AND METHODS

3.2.1 Sample collection

During the collection of the honeybee colonies they were split into equal-sized queenright and queenless halves, as described in chapter 2. Nine and 14 days after splitting, a sample of bees was taken from a frame of each of the queenless colonies. As the rate of absconding was high (Hepburn *et al.*, 2000) the number of colonies sampled differed between the different sampling days. The bees were processed and stored as described in chapter 2. The queen state of the colonies was controlled by removing any developing queen cells.

3.2.2 Mandibular gland secretions, ovarial development and reproductive status

The gas chromatographic and ovarial dissection processes are described in chapter 2 as are the classes of ovarial development and the grouping of reproductive status.

3.2.3 Statistical analysis

As for the results of the queenright data (chapter 2), the results from queenless colonies within a locality and from the same sampling day were pooled. The results obtained in chapter 2, for queenright colonies were included to assess the changes induced by queen loss. The changes between localities were also examined. ANOVAs and Student-Newman-Keuls method of pairwise multiple comparison were used to test for significant changes in the percentage composition of the 5 mandibular gland compounds and various ratios of these compounds. Where data were not normally distributed the one way ANOVA on ranks and Dunn's method of pairwise multiple comparison were used. The ovarial development and reproductive status data are presented as percentage distributions.

3.3 RESULTS

3.3.1 Mandibular gland secretions

3.3.1.1 How did the percentage compositions of the 5 mandibular gland compounds change in bees from within a locality, after being made queenless?

Port Elizabeth's bees showed no significant change in the percentage composition of 8HOA after becoming queenless (Table 3.1, F=2.09, P=0.1256). However 9 days after losing their queen the percentage composition of 9ODA and 9HDA in these bees had significantly increased and both remained significantly elevated 14 days after queen loss (Table 3.1, 9ODA: H=51.6, P<0.0001; 9HDA: H=22.7, P<0.0001). Nine days after the loss of the queen the percentage compositions of 10HDA and 10HDAA of these bees had decreased significantly and while the percentage composition of 10HDA had remained significantly lower after 14 days without a queen than in the queenright bees, that of 10HDAA increased to an intermediate level and no longer differed significantly from the queenright bees (Table 3.1, 10HDA: H=36.4, P<0.0001; 10HDAA: H=54.7, P<0.0001).

After being made queenless the bees from East London showed a significant increase in the percentage composition of 9ODA (Table 3.1, H=9.96, P=0.0069). The percentage compositions of the other 4 compounds measured did not change significantly after the colonies were made queenless (Table 3.1, 8HOA: H=3.35, P=0.1869; 9HDA: H=3.56, P=0.1682; 10HDAA: H=1.63, P=0.4431; 10HDA: H=5.29, P=0.0709).

After 9 queenless days the percentage compositions of 9ODA and 9HDA in the bees from Steynsburg, had increased significantly and the percentage composition of 9ODA remained significantly elevated 14 days after queen loss while that of 9HDA decreased to an intermediate level not significantly different from queenright bees (Table 3.1, 9ODA: H=19.6, P<0.0001; 9HDA: F=3.95, P=0.0213). Nine days after losing the queen the percentage composition of 10HDAA and 10HDA had decreased significantly but after 14 queenless days the percentage of 10HDAA was no longer significantly different from that

Table 3.1: Percentage composition (mean ± SE) of 5 mandibular gland compounds[†] of workers from queenright and queenless colonies at 4 localities in the Eastern Cape Province.

Locality	Number of days without a queen	n	Percentage Composition (mean ± SE)[*]				
			8HOA	9ODA	9HDA	10HDAA	10HDA
Port Elizabeth	0	201	7.8 ± 0.4^{a}	3.7 ± 0.3^{a}	36.9 ± 1.6^{a}	31.4 ± 1.1^{a}	20.2 ± 1.2^{a}
	9	54	6.5 ± 0.7^{a}	19.5 ± 3.2^{b}	52.4 ± 3.1^{b}	12.7 ± 1.9^{b}	8.9 ± 1.7^{b}
	14	14	5.4 ± 1.1^{a}	12.2 ± 3.9^{b}	53.6 ± 4.4^{b}	22.0 ± 4.6^{ab}	6.8 ± 2.1^{b}
East London	0	114	6.9 ± 0.6^{a}	5.6 ± 0.9^{a}	48.6 ± 2.0^{a}	24.5 ± 1.4^{a}	14.4 ± 1.5^{a}
	9	53	6.8 ± 0.7^{a}	12.0 ± 2.9^{b}	50.1 ± 2.8^{a}	21.7 ± 2.1^{a}	9.4 ± 1.1^{a}
	14	16	4.5 ± 0.6^{a}	7.4 ± 1.7^{b}	58.6 ± 5.5^{a}	20.6 ± 2.8^{a}	8.9 ± 3.6^{a}
Steynsburg	0	92	8.0 ± 0.5^{a}	3.7 ± 0.6^{a}	41.1 ± 2.1^{a}	26.2 ± 1.6^{a}	21.0 ± 2.0^{a}
	9	30	9.6 ± 1.1^{a}	12.1 ± 3.5^{b}	51.8 ± 3.6^{b}	15.1 ± 2.0^{b}	11.4 ± 1.7^{b}
	14	29	4.9 ± 0.6^{b}	12.1 ± 3.4^{b}	48.5 ± 3.6^{ab}	21.3 ± 3.2^{ab}	13.2 ± 2.7^{b}
Cradock	0	103	6.9 ± 0.5^{a}	4.5 ± 0.5^{a}	50.3 ± 2.3^{a}	22.5 ± 1.5^{a}	15.8 ± 2.1^{a}
	9	41	7.7 ± 0.8^{a}	8.5 ± 1.3^{b}	61.3 ± 3.5^{a}	15.6 ± 2.3^{b}	6.9 ± 1.6^{a}
	14	12	3.4 ± 0.7^{b}	6.2 ± 2.1^{ab}	64.7 ± 5.0^{a}	15.6 ± 3.0^{ab}	10.1 ± 2.9^{a}

[*] Similar letters within a column for a particular locality denote no significant difference ($p>0.05$).

[†] 8HOA = 8-hydroxyoctanoic acid, 9ODA = (E)-9-oxo-2-decenoic acid, 9HDA = (E)-9-hydroxy-2-decenoic acid, 10HDAA = 10-hydroxydecanoic acid & 10HDA = (E)-10-hydroxy-2-decenoic acid.

of the queenright bees while that of 10HDA was (Table 3.1, 10HDAA: H=12.3, P=0.0021; 10HDA: F=5.10, P=0.0072). The percentage composition of 8HOA had decreased significantly after 14 days of queenlessness (Table 3.1, 8HOA: H=13.6, P=0.0011).

The bees from Cradock showed no significant changes in the percentage compositions of 8HOA, 9HDA or 10HDA after being queenless for 9 days and for 9HDA and 10HDA these had not change significantly after being queenless for 14 days. The percentage composition of 8HOA, however, dropped significantly between 9 and 14 days (Table 3.1, 8HOA: H=8.33, P=0.0155; 9HDA: H=9.13, P=0.0104; 10HDA: H=8.99, P=0.0112). Nine days after the loss of their queen the percentage composition of 9ODA had increased significantly and that of 10HDAA had decreased significantly, however, 14 days after this loss the percentage compositions of both compounds were intermediate and no longer significantly different from their queenright values (Table 3.1, 9ODA: H=14.0, P=0.0009; 10HDAA: H=8.20, P=0.0166).

3.3.1.2 How did the ratios of certain mandibular gland compounds change in bees from within a locality, after queen loss?

The 9ODA:10HDA ratio had increased significantly 9 days after the removal of the queen in the bees from Port Elizabeth, Steynsburg and Cradock. They remained elevated 14 days after queen loss in the bees from Port Elizabeth and Steynsburg while the value of this ratio for Cradock's bees had decreased to an intermediate level no longer significantly different from the queenright bees (Fig. 3.1 & Table 3.2, Port Elizabeth: H=60.2, P<0.0001; Steynsburg: H=20.3, P<0.0001; Cradock: H=12.9, P=0.0016). The ratio of 9ODA:10HDA in the bees from East London did not change significantly after the queen was removed (F=1.94, P=0.1471).

The trends for the change in the 9HDA:10HDAA ratio were the same as those for the 9ODA:10HDA ratio. The only exception being that the 9HDA:10HDAA ratio, for the bees from Steynsburg 14 days after queen loss was no longer significantly different from the queenright bees (Fig. 3.1 & Table 3.2, Port Elizabeth: H=58.4, P<0.0001; East London: H=3.0, P=0.2233; Steynsburg: H=15.2, P=0.0005; Cradock: H=10.7, P=0.0048).

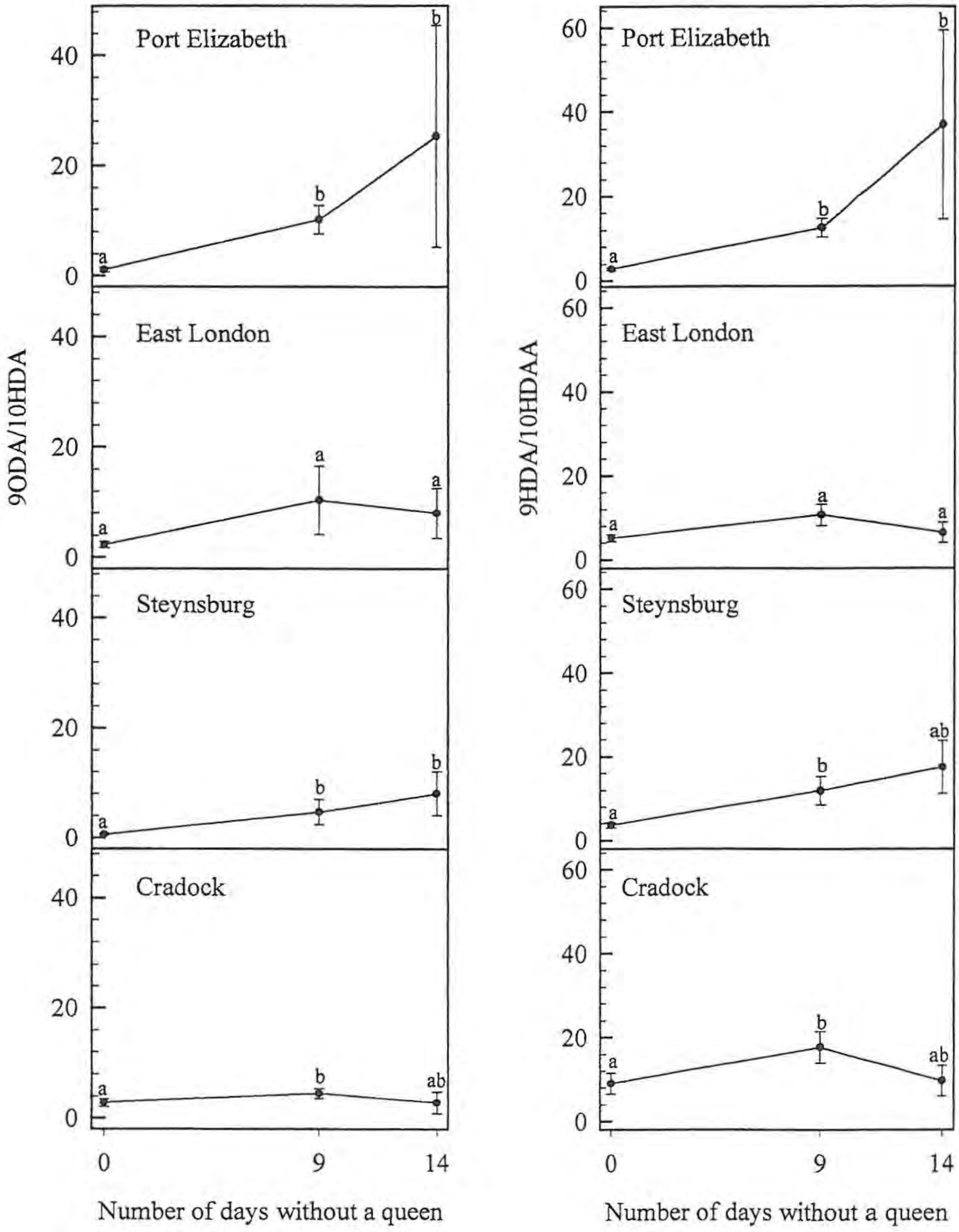

Fig. 3.1: Changes in the 9ODA:10HDA and 9HDA:10HDAA ratios (mean ± SE) of workers after queen loss at 4 localities in the Eastern Cape Province. Similar letters within a graph for a particular locality denote no significant difference ($p > 0.05$). 9ODA = (E)-9-oxo-2-decenoic acid, 9HDA = (E)-9-hydroxy-2-decenoic acid, 10HDAA = 10-hydroxydecanoic acid & 10HDA = (E)-10-hydroxy-2-decenoic acid.

Table 3.2: Percentage composition (mean ± SE) of 2 ratios of mandibular gland compounds of workers from queenright and queenless colonies at 4 localities in the Eastern Cape Province.

		% Composition (mean ± SE)[*]		
		Number of days without a queen		
Ratio of fatty acids[†]	Locality	0	9	14
9ODA/10HDA	Port Elizabeth	1.1 ± 0.4^{a}	10.2 ± 2.5^{a}	25.3 ± 20.1^{a}
	East London	2.3 ± 0.6^{c}	10.4 ± 6.2^{b}	8.0 ± 4.5^{a}
	Steynsburg	0.6 ± 0.2^{ab}	4.7 ± 2.3^{b}	8.1 ± 4.0^{a}
	Cradock	2.8 ± 0.7^{bc}	4.5 ± 0.9^{ab}	2.8 ± 2.0^{a}
9HDA/10HDAA	Port Elizabeth	2.8 ± 0.3^{a}	12.5 ± 2.2^{a}	36.9 ± 22.4^{a}
	East London	5.2 ± 0.9^{b}	10.7 ± 2.5^{a}	6.5 ± 2.4^{a}
	Steynsburg	3.7 ± 0.6^{ab}	11.9 ± 3.4^{a}	17.5 ± 6.3^{a}
	Cradock	9.0 ± 2.5^{b}	17.7 ± 3.8^{a}	9.7 ± 3.6^{a}

[*] Similar letters within a column for a particular ratio denote no significant difference ($p>0.05$).

[†] 9ODA = (E)-9-oxo-2-decenoic acid, 9HDA = (E)-9-hydroxy-2-decenoic acid, 10HDAA = 10-hydroxydecanoic acid & 10HDA = (E)-10-hydroxy-2-decenoic acid.

3.3.1.3 How did the changes in mandibular gland secretions differ between localities?

While in queenright colonies, East London and Cradock's bees had significantly higher 9ODA:10HDA ratios than Port Elizabeth's bees (chapter 2), after 9 days without a queen the 9ODA:10HDA ratio of Cradock's bees was no longer significantly different from that of Port Elizabeth's (Table 3.2, H=13.3, P=0.004). After 14 day without a queen there was no significant difference between the ratio of any of the localities (Table 3.2, H=2.3, P=0.512). Despite there being no significant difference the mean values were very different, with the mean value of the ratio 9ODA:10HDA for Port Elizabeth's bees being 3 times those of East London or Steynsburg's bees and 8 times that of Cradock's bees. The high standard error of the mean in the sample of bees from Port Elizabeth after 14 days without a queen, reflects the large range of values suggesting great variation in individuals within this locality.

Table 3.2: Percentage composition (mean ± SE) of 2 ratios of mandibular gland compounds of workers from queenright and queenless colonies at 4 localities in the Eastern Cape Province.

		% Composition (mean ± SE)*		
		Number of days without a queen		
Ratio of fatty acids †	Locality	0	9	14
9ODA/10HDA	Port Elizabeth	1.1 ± 0.4^{a}	10.2 ± 2.5^{a}	25.3 ± 20.1^{a}
	East London	2.3 ± 0.6^{c}	10.4 ± 6.2^{b}	8.0 ± 4.5^{a}
	Steynsburg	0.6 ± 0.2^{ab}	4.7 ± 2.3^{b}	8.1 ± 4.0^{a}
	Cradock	2.8 ± 0.7^{bc}	4.5 ± 0.9^{ab}	2.8 ± 2.0^{a}
9HDA/10HDAA	Port Elizabeth	2.8 ± 0.3^{a}	12.5 ± 2.2^{a}	36.9 ± 22.4^{a}
	East London	5.2 ± 0.9^{b}	10.7 ± 2.5^{a}	6.5 ± 2.4^{a}
	Steynsburg	3.7 ± 0.6^{ab}	11.9 ± 3.4^{a}	17.5 ± 6.3^{a}
	Cradock	9.0 ± 2.5^{b}	17.7 ± 3.8^{a}	9.7 ± 3.6^{a}

[*] Similar letters within a column for a particular ratio denote no significant difference ($p>0.05$).

[†] 9ODA = (E)-9-oxo-2-decenoic acid, 9HDA = (E)-9-hydroxy-2-decenoic acid, 10HDAA = 10-hydroxydecanoic acid & 10HDA = (E)-10-hydroxy-2-decenoic acid.

A similar pattern emerges from the 9HDA:10HDAA ratio. In the queenright state once again East London and Cradock's bees had significantly higher values than Port Elizabeth's bees (chapter 2). Nine days after becoming queenless however there was no significant difference between any of the localities (Table 3.2, H=7.72, P=0.0522) and neither was there after the bees had been queenless for 14 days (Table 3.2, H=1.1, P=0.7769). After 9 days, however, the mean values were similar and the standard error of the means comparable. In contrast, after 14 days without a queen the mean value of this ratio for Port Elizabeth's bees was approximately 4 times those of East London and Cradock's bees and twice that of Steynsburg's bees, with once again an exceptionally high standard error of the mean suggesting great variation.

3.3.2 The change in the degree of ovarial development after queen loss

Nine days after queen loss the proportion of bees with undeveloped ovaries had decreased at all localities while the percentage with ovaries exhibiting some level of development increased (Figure 3.2 & Table 3.3). The percentage of bees from East London with developed ovaries (any degree) increased by the largest amount (10.6%) while the proportion of bees from the other 3 localities increased by similar smaller amounts (2.7-3.0%). Surprisingly 14 days after queen loss, the percentage of bees from Steynsburg with partially or fully developed ovaries had decreased by 3.4% from the 9 day value and was equivalent to that found within queenright colonies from the same locality. In contrast the percentage of partially or fully developed bees from Port Elizabeth, East London and Cradock had further increased, in this time period. It was the colonies from Port Elizabeth that increased the most (24.8%) while those from East London and Cradock increased by smaller amounts (11.8% and 15.2% respectively). Over the entire 14 days without a queen, the percentage of bees from Port Elizabeth with partially or fully developed ovaries increased by the largest amount of all localities (27.5%) and went from being the locality with the largest percentage of undeveloped bees (initially) to the locality with the least after 14 days without a queen (71.4%). The percentage of bees from East London and Cradock with partially or fully developed ovaries over the entire 14 days without a queen had increased by 22.4% and 18.2% respectively.

After queen loss East London and Port Elizabeth were the only localities to have bees with mature sausage shaped eggs (Table 3.3) increase by 1% and 1.9% respectively within 9 days of queen removal.

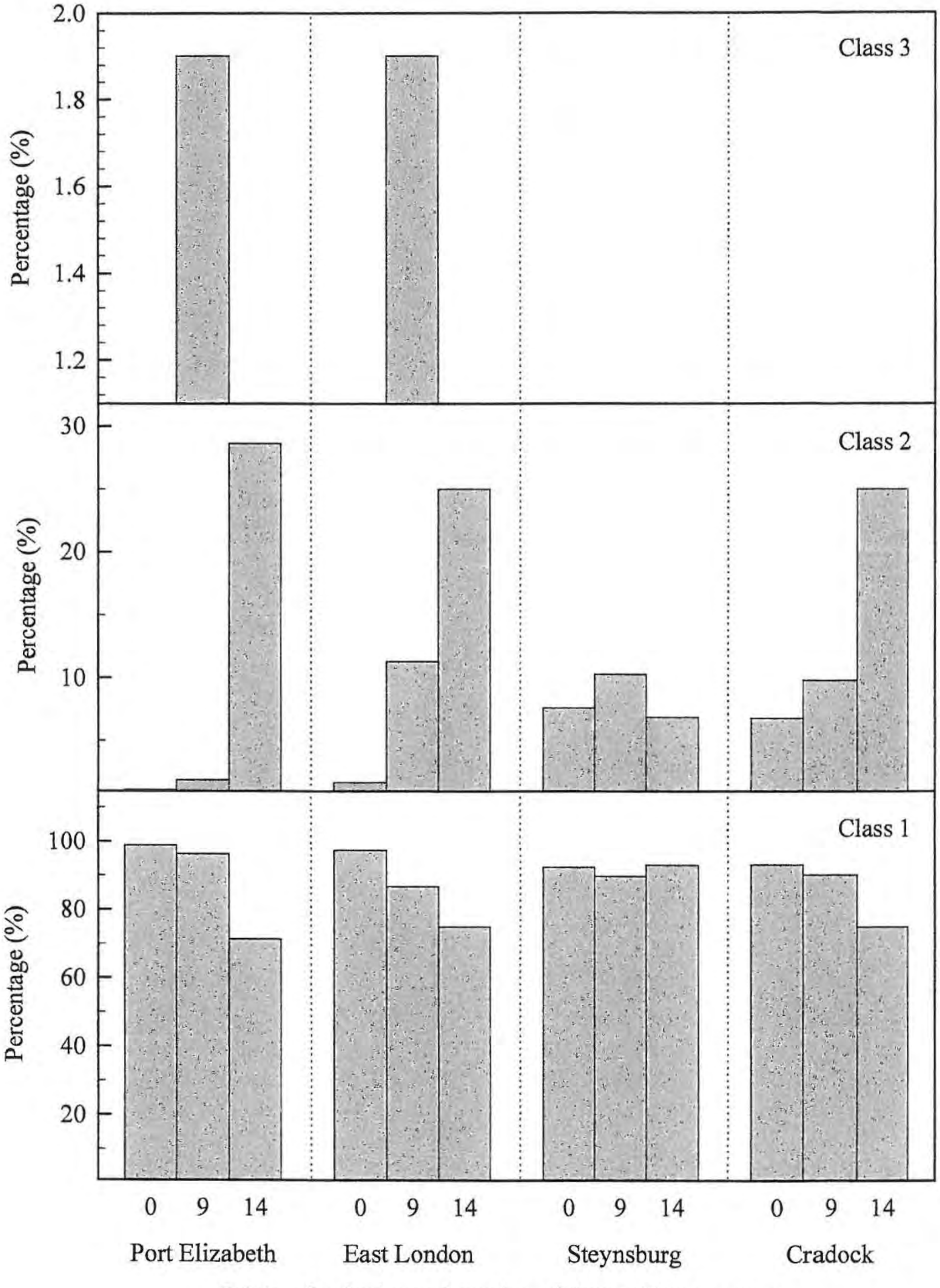

Fig. 3.2: Changes in ovarial development after queen loss, at 4 localities in the Eastern Cape Province. Class 1 = undeveloped, class 2 = some degree of development & class 3 = mature sausage shaped eggs.

Table 3.3: Ovarial development of workers from queenright and queenless colonies at 4 localities in the Eastern Cape Province.

Locality	Number of days without a queen	n	Ovarial development*		
			Class 1	Class 2	Class 3
Port Elizabeth	0	188	98.9	1.1	-
	9	54	96.2	1.9	1.9
	14	14	71.4	28.6	-
East London	0	114	97.4	1.7	0.9
	9	53	86.8	11.3	1.9
	14	16	75.0	25.0	-
Steynsburg	0	92	92.4	7.6	-
	9	29	89.7	10.3	-
	14	29	93.1	6.9	-
Cradock	0	103	93.2	6.8	-
	9	41	90.2	9.8	-
	14	12	75.0	25.0	-

* Class 1 = undeveloped, class 2 = some degree of development & class 3 = mature sausage shaped eggs.

3.3.3 The change in reproductive status after queen loss

After 9 days the proportion of intermediate bees in colonies from Port Elizabeth, East London and Cradock had increased (Fig. 3.3 & Table 3.4). Colonies from East London and Cradock increasing by the most (11.8% and 11.7% respectively) and Port Elizabeth by the least (3.4%). After 14 days without a queen the percentage of intermediate bee had again increased (15.9, 8.0% and 13.8% respectively). There were, however, less intermediate bees in colonies from Steynsburg after 9 queenless days (decreased by 2.9%) and this had not changed after 14 day without a queen. After 9 and 14 days without a queen colonies

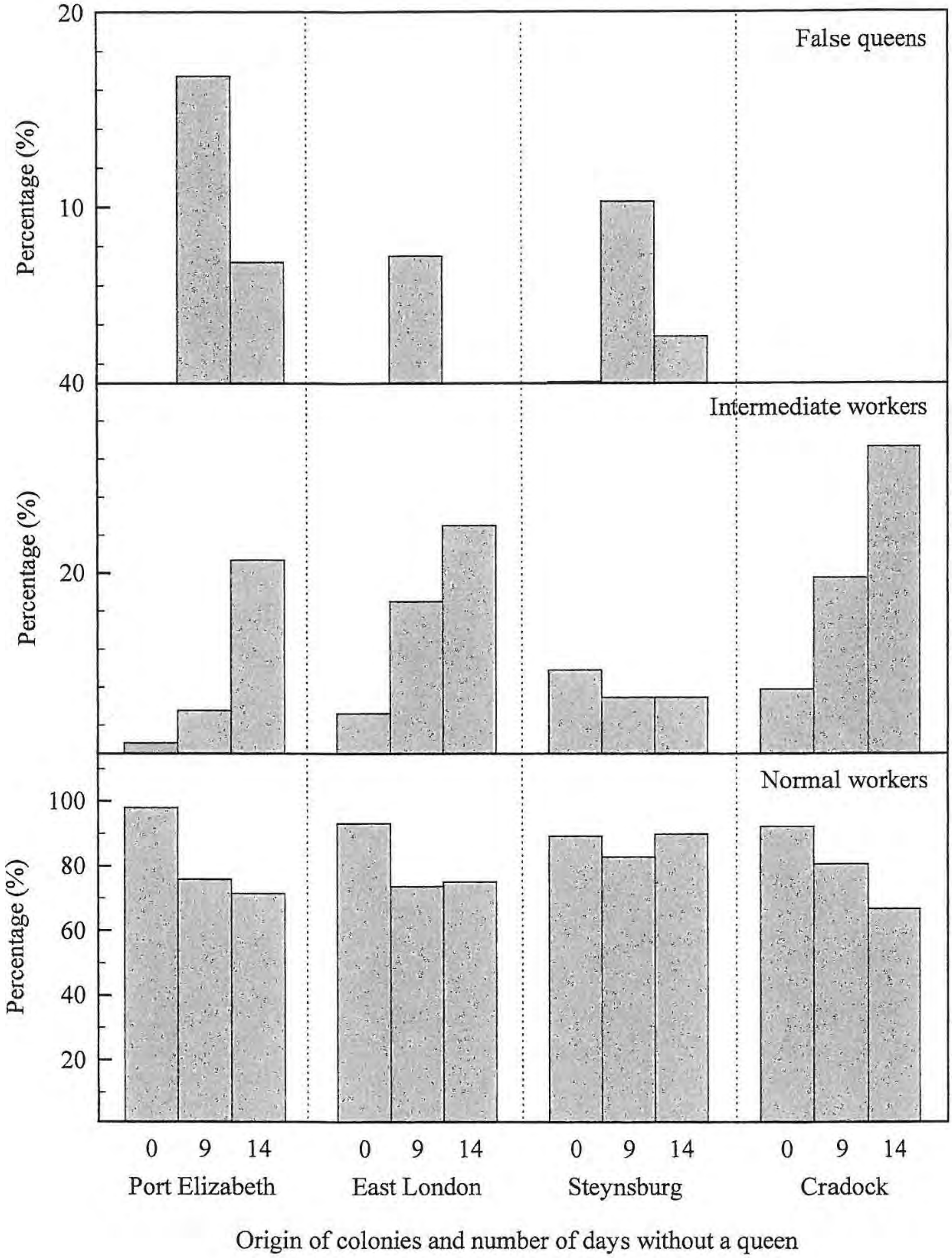

Fig. 3.3: Changes in reproductive status after queen loss, at 4 localities in the Eastern Cape Province. Normal = class 1 ovaries & <25% 9ODA, intermediate = all bees not falling into any other class, false queen =class 1 or 2 ovaries & >45% 9ODA.

from Cradock had the highest percentages of intermediate bees followed by those form East London then Port Elizabeth and finally Steynsburg (Fig. 3.3 & Table 3.4).

Table 3.4: Distribution of reproductive status of workers from queenright and queenless colonies at 4 localities in the Eastern Cape Province.

Locality	Number of days without a queen	n	Reproductive status* normal	intermediate	egg layer	false queen	surrogate queen
Port	0	188	97.9	2.1	-	-	-
Elizabeth	9	54	75.9	5.5	-	16.7	1.9
	14	14	71.4	21.4	-	7.2	-
East	0	114	93.0	5.2	0.9	0.9	-
London	9	53	73.6	17.0	1.9	7.5	-
	14	16	75.0	25.0	-	-	-
Steynsburg	0	92	89.1	9.8	-	1.1	-
	9	29	82.8	6.9	-	10.3	-
	14	29	89.7	6.9	-	3.4	-
Cradock	0	103	92.2	7.8	-	-	-
	9	41	80.5	19.5	-	-	-
	14	12	66.7	33.3	-	-	-

* Normal = class 1 ovaries & <25% 9ODA, intermediate = all bees not falling into any other class, egg layer = class 3 ovaries & <25% 9ODA, false queen =class 1 or 2 ovaries & >45% 9ODA, surrogate queen = class 3 ovaries & >45% 9ODA.

No false queens were found in the colonies from Cradock (before or after queen loss). The number of false queens at the other localities had increased after 9 days without a queen and subsequently decreased by 14 days (Fig 3.3). The percentage of false queens in colonies from Port Elizabeth increased by 16.7% in the first 9 days and then decreased by 9.5%.

Those in East London colonies increased by 6.6% and then decreased by 7.5% and in Steynsburg bees they increased by 9.2% and then decreased by 6.9%. After 9 days of queenlessness Port Elizabeth had the greatest percentage of false queens, followed by Steynsburg and East London but by 14 days without a queen only Port Elizabeth and Steynsburg had any false queens and the colonies from Port Elizabeth had the greatest percentage (Table 3.4).

Only one surrogate queen was found throughout the experiments, and this came from a Port Elizabeth colony which had been queenless for 9 days (Table 3.4). The only egg layer was found 9 days after queen loss in a colony from East London. This represents an increase of 1% from the queenright state (Table 3.4).

3.4 DISCUSSION

As expected the number of bees with partially developed ovaries had increased at all localities after 9 days without a queen (Hepburn & Allsopp, 1994; Anderson, 1962). However, while the bees from Port Elizabeth, East London and Cradock continued to increase in this respect, over the entire 14 queenless days, those from Steynsburg did not and in fact the number of partially developed bees had returned to the queenright level after 14 days. One possible explanation for this could be unnoticed requeening but this is highly unlikely as the sample presented was obtained by pooling the results from many colonies originating from Steynsburg. The chance of all these requeening while none of the other localities colonies did, having undergone exactly the same queen control treatment is highly improbable. What is more likely is that it demonstrates two different developmental regimes. The thelytokous bees from Port Elizabeth, East London and Cradock (Hepburn *et al.*, 1998) show a continued increase in ovarial development after queen loss, even in brood right situations, while arrhenotokous bees from Steynsburg (Hepburn *et al.*, 1998) do not.

The trend of increasing ovarial development in the bees from the queenless colonies originating from Port Elizabeth, East London and Cradock match those reported in *A. m. capensis* (Anderson, 1962; Hepburn *et al.*, 1991; Allsopp, 1988) and *A. m. scutellata* (Allsopp, 1988; Hepburn & Allsopp, 1994) but the extent of development is much lower than that previously reported. After 2 weeks without a queen the percentage of bees with partially or fully developed ovaries from these 3 localities ranged between 25.0 and 28.6%. Hepburn and Allsopp (1994) reported 85% partial or full development in *A. m. scutellata* workers that had been queenless for the same amount of time while Allsopp (1988) reported that the percentage of queenless *A. m. scutellata* workers with active ovaries increased rapidly to a high of 40% after being made queenless and subsequently remained at this level. The percentages of *A. m. capensis* bees with partial or fully developed ovaries after 2 weeks without a queen are 32.6% according to Hepburn *et al.* (1991) and between 44-56% according to Anderson (1962) while Allsopp (1988) stated that the percentage of queenless workers with active ovaries for this subspecies increased rapidly to a high of 50% after being made queenless and then remained constant. The fact that in three of the 4 localities the number of bees in class 2 were still increasing after 14 days without a queen suggests that I would possibly have gained more valuable information had I let the experiment run longer. Comparisons of degrees of ovarial development with other studies are possibly limited to that of trends, as many external factors, for example seasonal variation can effect the extent of development.

It is surprising that so few of the bees, sampled from the queenless colonies had class 3 ovaries (mature sausage shaped eggs) after 9 and 14 days as it has been show that *A. m. capensis* workers can develop into laying workers within 4 - 7 days after queen loss (Anderson, 1962; Ruttner & Hesse, 1981). *A. m. scutellata* laying workers on the other hand develop in 10 days (Ruttner & Hesse, 1981). From the literature, however, we can see that the actual percentages of bees with mature eggs in natural native queenless colonies are surprisingly low, Hepburn & Allsopp (1994) reported less than 1% in *A. m. scutellata* and Hepburn *et al.* (1991) 2.6% in *A. m. capensis*, both after 2 weeks without a queen.

As for ovarial development, the decrease in the percentage of bees with normal reproductive status, as seen in those from Port Elizabeth, East London and Cradock, is expected after queen loss as is the increase in the percentage of intermediate workers. The bees from Steynsburg again, however, do not follow the expected trend. This despite the presence of false queens in some of these colonies on all sampling days prior and subsequent to queen loss.

After 14 days without a queen the percentage of reproductively normal bees (66.7-89.7%) is much higher than the reported 27% for *A. m. capensis* queenless workers (Hepburn, 1994) but comparable to the 70.3% reported for *A. m. scutellata* (Hepburn & Allsopp, 1994). The percentage of intermediate bees (6.9-25%) from the 4 localities after 14 days without a queen is lower than the reported values for *A. m. scutellata* and *A. m. capensis*, 28.8% and 57.4% respectively (Hepburn & Allsopp, 1994; Hepburn, 1994). The absence of egg layers after 14 days without a queen, in all the colonies examined, is surprising as 11.6% of the bees in queenless *A. m. capensis* colonies were reported to be egg layers by Hepburn (1994). Even queenless *A. m. scutellata* colonies were reported to have 0.9% egg layers after 14 days (Hepburn & Allsopp, 1994). False queens and surrogate queens have not been found in queenless *A. m. scutellata*, as despite having 9ODA as a component of their mandibular gland secretions it does not increase to levels appropriate to this status after queen loss (Hepburn & Allsopp, 1994). Hepburn (1994), however, found *A. m. capensis* to have small percentages of false queens and surrogate queens (3.4% and 0.6% respectively) after queen loss and while none of the bees from Cradock were either false or surrogate queens the other 3 localities had higher percentages of false queens than this. Port Elizabeth, the only locality were a surrogate queen was found is comparable to that found in *A. m. capensis* (Hepburn, 1994).

The observed increase in the percentage of 9ODA relative to the other compounds measured, after queen loss, is typical of *A. m. capensis* (Allsopp, 1988; Crewe, 1988; Hepburn, 1992) and *A. m. scutellata* (Hepburn & Allsopp, 1994) but rarely observed in other *A. mellifera* species (Plettner *et al.*, 1993). Despite having this increase in common the localities examined differed with regard to the changes in the various other compounds examined. These differences resulted in a distinction between the two ratios measured (a measure of how queen or worker-like the mandibular gland secretions are) in the bees from the different localities at various stages of queenlessness. Bees from East London while increasing in the amount of 9ODA present were the only ones not becoming significantly more queen like after queen loss and the bees from Port Elizabeth were the only ones to remain significantly more queen-like (with respect to both ratios) after 14 days.

While statistics show no significant difference in the means of the ratios 9ODA/10HDA and 9HDA/10HDAA after 14 days without a queen, in all 4 localities we can clearly see from the high mean and standard error values that the bees from Port Elizabeth are behaving somewhat differently to the bees from the other localities. This is supported by the fact that the colonies from Port Elizabeth, have the only surrogate queen encountered in the entire experiment, show the largest increase in the number of bees with partially or fully developed ovaries, after 14 days and have the highest percentage of false queens after 9 days without a queen.

The phenomenon of the development of queen like mandibular gland extracts dictates that the mean value is far less important than the individual potential of the bees within the colony as not all bees develop this queen like "smell" as a result of the balance between selection at the colony and individual level (Moritz & Hillesheim, 1985; Hillesheim *et al.*, 1989; Moritz, 1990). This combined with the fact that only one false or surrogate queen is needed to take over an entire *A. m. scutellata* colony emphasises the importance of individual development. From the maximum values for the 9ODA:10HDA ratios we can see that there is an individual in one of the colonies from Port Elizabeth that has 285 times more 9ODA than 10HDA, a value much higher than that reported in an *A. m. capensis* queen (Crewe, 1988; Table 2.3). The 3 most developed bees from Steynsburg, East London and Cradock have 9ODA:10HDA ratios that are higher (108, 72 & 24 respectively) than queens of *A. m. scutellata* but lower than *A. m. capensis* queens (Crewe, 1988; Table 2.3).

How we define a bee capable of dethroning a foreign queen is now of importance. If we chose to make the distinction by the presence of 9ODA:10HDA ratios higher than that of queens, certain bees from all localities are then capable of this, with regard to pure *A. m. scutellata* colonies. However, by restricting the definition to that of bees in which 9ODA predominates (in this case making up in excess of 45%) the localities potentially capable of usurping an A. *m. scutellata* colony is restricted to Port Elizabeth, East London and Steynsburg. It is clear that an appropriate definition is still to be refined, for many factors other than mandibular gland secretions are involved. This is demonstrated by the fact that bees without mandibular glands are still capable of becoming dominant over others (Saiovici, 1983).

Until the process is fully understood and a more complete definition is determined, what can be said from these results is that the bees from Port Elizabeth display ovarial and mandibular gland development that could give them an increased ability to assert themselves as reproductively dominant in foreign colonies. This is to be expected in light of the fact that they lie within the *A. m. capensis* morphocluster and exhibit thelytoky (Hepburn *et al.*, 1998). There are bees from both East London and Steynsburg, defined as morphometric *A. m. scutellata* (Hepburn *et al.*, 1998), that have the potential to acquire reproductive dominance in foreign colonies in terms of mandibular gland secretions. The probability of this occurring in the thelytokous bees from East London, is greater as more bees develop ovarially after queen loss. Cradock's bees, defined as morphometric hybrids that exhibit thelytoky (Hepburn *et al.*, 1998) unlike the other localities did not develop any surrogate or false queens (with 9ODA predominating) but by the ratio of 9ODA:10HDA are still capable of developing queen-like smells although to a lesser extent than bees from the other localities.

Ultimately this data shows that one must avoid assigning biological traits to bees, by inference due to them occurring within previously morphometrically and/or parthenogenetically defined areas. Clearly the 4 localities examined here respond in varying degrees (ovarially and pheromonally) subsequent to queen loss, not possibly predicted from previous studies of *A. m. capensis* and *A. m. scutellata* bees. This could partially be due to varying ratios of thelytokous to arrhenotokous workers between localities. Neumann et al. (2000a) has shown that even colonies from the same localities, as well as colonies from different localities, differ substantially in this regard.

CHAPTER 4

MANDIBULAR GLAND SECRETIONS AND OVARIAL DEVELOPMENT OF DRIFTED WORKERS

4.1 INTRODUCTION

Drifting in honeybees, the movement of individuals from 1 colony into another, is often observed in colonies that are close to each other and is very important due to the potential drifting bees have to spread various bee diseases. The majority of drifted bees are 1-2 weeks old and drift preferentially during their orientation flights (Free, 1958) presumably ending up in a foreign hive due to inexperience.

In South Africa, however, drifting poses an entirely different threat. Drifting is the mechanism by which *A. m. capensis* bees are thought to have gained access to foreign *A. m. scutellata* colonies, resulting in the usurpation of thousands of colonies in what has commonly become known as the '*capensis* problem" (Allsopp & Crewe, 1993; Allsopp, 1992; Hepburn & Allsopp, 1994).Usually the addition of workers to a colony represents a gain in fitness, however, newly incorporated workers can lay eggs, and in the case of thelytokous workers are able to rapidly develop ovarially (Hepburn & Allsopp, 1994) and produce queen-like mandibular gland secretions (Ruttner *et al.*, 1976; Hemmling *et al.*, 1979; Crewe, 1982). Thelytokous workers can thus potentially commandeer arrhenotokous colonies for the propagation of their own genes (Hepburn & Allsopp, 1994; Hepburn & Radloff, 1998).

Many have attributed this to the invasive or dispersive nature of *A. m. capensis* (Allsopp, 1992; Greeff, 1997). Onions (1912), Lundie (1954) and Johannsmeier (1983) all reported that the extent of drifting of *A. m. capensis* workers outweighed the possibility of it being due only to orientation errors.

With the obvious advantage of drifting to thelytokous bees, the possibility of raising your own offspring as opposed to that of your queen, and the biological traits that facilitate the ease with which *A. m. capensis* workers takeover *A. m. scutellata* colonies, the question of whether the extent of drifting is equal in the 2 subspecies once again arose. According to Neumann *et al.* (2000b) drifting (short-range) and dispersing (long-range drifting) represent different behaviours. *A. m. capensis* disperse more often (ie. significantly more frequently invasive) than either *A. m. scutellata* or thelytokous hybrids (Neumann *et al.*, 2000b).

Other than this most recent study by Neumann *et al.* (2000b), very little has been done on drifting and/or host finding mechanisms, the key feature in the takeover of *A. m. scutellata* colonies by *A. m. capensis*. With this in mind I analysed drifted and non drifted bees from various localities in order to answer the following questions:

- Do drifters differ from bees from their native colonies that did not drift, with respect to their mandibular gland secretions, ovarial development or reproductive status?
- Do drifters differ from bees from their host colonies that did not drift, with respect to their mandibular gland secretions, ovarial development or reproductive status?
- Do drifters from the same colony that drift to different host colonies differ with respect to their mandibular gland secretions, ovarial development or reproductive status?

4.2 MATERIALS AND METHODS

4.2.1 Marking and collection of bees

One week after collection of the colonies from the various localities (chapter 2), brood frames (with brood at the "purple eyed" stage) were removed from all remaining queenright and queenless colonies within the apiary. These were placed in incubators and the workers were left to emerge. The thoraxes of freshly emerged workers were marked with a colony specific colour in modelling paint. All marked bees were returned to their native colonies on the same day as painted. The queen state of the colonies were checked and controlled by removing any developing queen cells.

Ten days after reintroduction all colonies were screened for painted workers (either foreign or native) and samples of these bees were collected. As care was taken not to transport bees from one colony to another on our clothing it can be assumed that the foreign bees must have drifted into the host colonies of their own accord.

4.2.2 Mandibular gland secretions, ovarial development and reproductive status

The gas chromatographic and ovarial dissection processes are the same as those described in chapter 2 as are the classes of ovarial development and the grouping of reproductive status.

4.2.3 Data and statistical analyses

The number of painted bees collected were limited by both the number of drifters from each colony and the number of colonies remaining (those not having absconded) on termination of the experiment. Due to these constraints only selected results are presented here and these, by no means represent the extent or frequency of drifting by the bees. Details on this aspect of the experiment are presented in Neumann *et al.* (2000b).

Bees from colonies within one locality and with similar queen states were grouped together and the results of four types of drifted bees are presented (Fig. 4.1). Group A are bees that drifted from queenright colonies collected from Port Elizabeth, into other queenright Port Elizabeth colonies. Group B's bees drifted from queenright Port Elizabeth colonies into queenright colonies from Steynsburg. Group C consists of bees that drifted from queenless colonies from Port Elizabeth into queenright Port Elizabeth colonies. Group D's bees drifted from queenless Port Elizabeth colonies into queenright colonies from near East London.

The percentage composition of the 5 mandibular gland compounds measured were used to calculate the ratios of 9ODA:10HDA and 9HDA:10HDAA. Comparisons were drawn between drifted bees and bees from their host colonies of the same age that did not drift. Drifters from queenright colonies were also compared to non-drifters from native

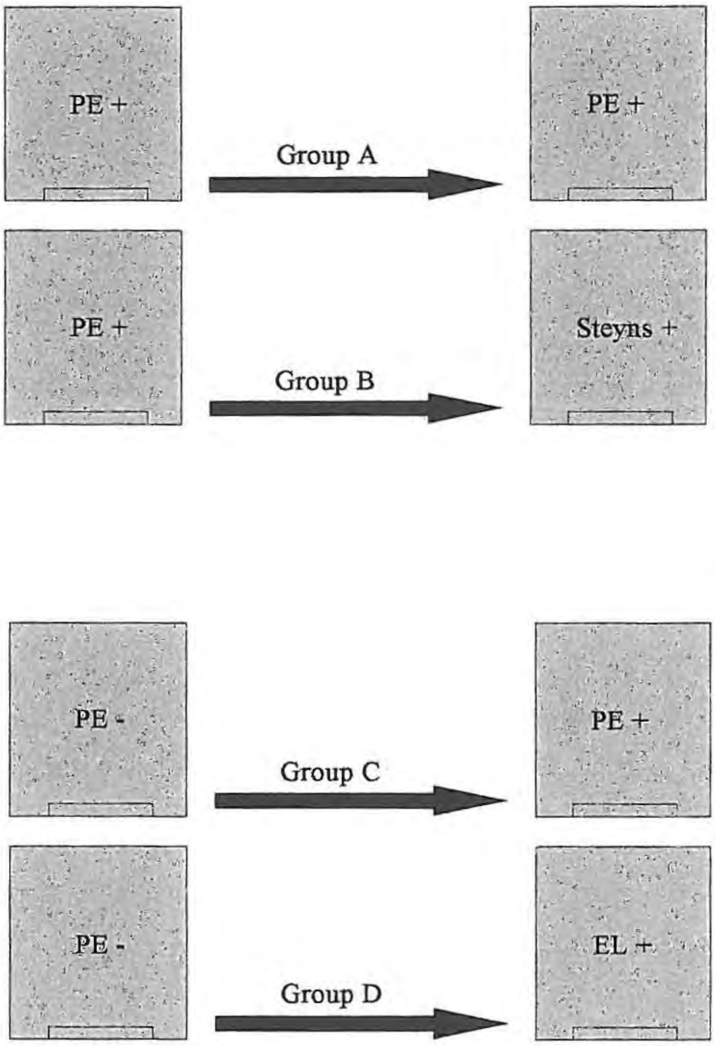

Fig. 4.1: The 4 groups of drifted bees, with their native colony on their left and host colony on their right. PE = Port Elizabeth, Steyns = Steynsburg and EL = East London. (+) = queenright and (-) = queenless.

colonies. The different groups of drifters were also compared. These comparisons were done by performing t-tests, on parametric data, and Mann-Whitney rank sum tests on non-parametric data. The percentage distribution of ovarial development and reproductive status were treated similarly.

4.3 RESULTS

4.3.1 Queenright drifters

4.3.1.1 Drifters versus non-drifters

In both groups of queenright drifters (group A & group B), there was no significant difference between their 9ODA:10HDA or 9HDA:10HDAA ratios and those of the bees from similar colonies that did not drift (Table 4.1, 9ODA:10HDA: T=130, P=0.4869; T=63, P=0.2123; 9HDA:10HDAA: T=162, P=0.4289; T=58, P=0.4808). In both cases, however, the drifters had higher percentages of bees with class 1 ovarial development and normal reproductive status than those that did not drift (Table 4.2).

4.3.1.2 Drifters versus hosts

Due to the fact that the results for bees from colonies from the same localities were combined Group A's native bees and host bees are as stated above. The drifted bees of group B did not differ significantly with respect to their ratio of 9ODA:10HDA or 9HDA:10HDAA to the bees from their host colony (Table 4.1, 9ODA:10HDA: t=1.53, P=0.1486; 9HDA:10HDAA: t=0.405, P=0.6917). The drifted bees, however, had higher percentages of class 1 ovarial development and normal reproductive status than their host colonies bees (Table 4.2).

4.3.1.3 Group A drifters versus group B drifters

There was no significant difference between the 2 groups of queenright drifters with respect to their 9ODA:10HDA or 9HDA:10HDAA ratios (Table 4.1& Fig. 4.2, 9ODA:10HDA:

T=96, P=0.1717; 9HDA:10HDAA: T=90, P=0.3337), or in regard to the number of bees with undeveloped ovaries and normal reproductive status (Table 4.2 & Fig. 4.2).

4.3.2 Queenless drifters

4.3.2.1 Drifters versus hosts

The queenless drifters from group C had significantly higher 9ODA:10HDA and 9HDA:10HDAA ratios than their queenright host colony's bees (Table 4.1, 9ODA:10HDA: T=83, P=0.0032; 9HDA:10HDAA: T=86, P=0.005). In addition to having a higher percentage of bees with class 1 ovarial development and normal reproductive status the bees from group C consisted of 11.1% false queens as opposed to their queenright hosts which had none (Table 4.2).

Similarly group D's drifters had higher 9ODA:10HDA and 9HDA:10HDAA ratios than their queenright host colony's bees (Table 4.1, 9ODA:10HDA: T=70, P=0.0054; 9HDA:10HDAA: T=63, P=0.0011), and a higher proportion of bees with class 1 ovarial development. They, however, had a lower percentage of bees with normal reproductive status than their hosts and a staggering 45.4% of false queens as opposed to their hosts which had none (Table 4.2).

4.3.2.2 Group C drifters versus group D drifters

There were no significant difference in the 9ODA:10HDA or 9HDA:10HDAA ratios of the 2 groups of queenless drifters (Table 4.1, 9ODA:10HDA: T=189, P=0.2909; 9HDA:10HDAA: T=191, P=0.2517). The mean values of these ratios are, however, quite distinct and despite not being significantly different the 9ODA:10HDA ratio of group D was 3 times that of group C's. This difference is reiterated in the ovarial development and reproductive status of these 2 groups. Group D was the only group to have bees with class 2 ovarial development and the highest, by far, proportion of false queens (Table 4.2 & Fig. 4.2).

Table 4.1: Percentage composition (mean ± SE) of 5 mandibular gland compounds[*] and 9ODA:10HDA and 9HDA:10HDAA ratios of these compounds, in drifted, native and host colony bees.

			Percentage composition (mean ± SE)					Ratio (mean ± SE)	
Type of bees	Group or locality	n	8HOA	9ODA	9HDA	10HDAA	10HDA	9ODA/10HDA	9HDA/10HDAA
Drifted	Group A	18	7.7 ± 1.0	2.8 ± 0.6	35.7 ± 4.8	36.8 ± 3.0	17.0 ± 3.5	0.2 ± 0.1	1.8 ± 0.7
	Group B	6	1.5 ± 0.2	2.4 ± 0.2	45.3 ± 8.8	26.6 ± 3.5	24.1 ± 5.9	0.1 ± 0.01	2.1 ± 0.6
	Group C	18	2.9 ± 0.5	15.7 ± 4.9	48.1 ± 3.9	16.9 ± 2.7	16.5 ± 2.8	5.1 ± 3.5	5.0 ± 1.0
	Group D	11	2.8 ± 1.0	31.7 ± 9.6	44.8 ± 6.5	10.9 ± 3.0	9.8 ± 2.5	17.3 ± 10.8	9.5 ± 3.2
Host / native[†]	Port Elizabeth +	10	4.0 ± 1.2	2.2 ± 0.8	30.1 ± 6.4	25.2 ± 4.0	38.4 ± 7.6	0.2 ± 0.1	1.7 ± 0.6
	Steynsburg +	10	4.4 ± 1.1	2.9 ± 0.7	30.7 ± 8.4	22.1 ± 5.3	39.8 ± 11.3	0.5 ± 0.2	2.7 ± 0.9
	East London +	10	4.4 ± 1.1	2.4 ± 0.5	34.8 ± 4.7	29.9 ± 3.3	28.5 ± 5.6	0.2 ± 0.1	1.6 ± 0.5

[*] 8HOA = 8-hydroxyoctanoic acid, 9ODA = (E)-9-oxo-2-decenoic acid, 9HDA = (E)-9-hydroxy-2-decenoic acid, 10HDAA = 10-hydroxydecanoic acid & 10HDA = (E)-10-hydroxy-2-decenoic acid.

[†] (+) = queenright.

Table 4.2: Ovarial development* and reproductive status† of drifted, native and host colony bees.

			Ovarial development			Overall reproductive status				
Type of bees	Group or locality	n	Class 1	Class 2	Class 3	Normal	Intermediate	Egg layer	False queen	Surrogate queen
Drifted	Group A	18	100	-	-	100	-	-	-	-
	Group B	6	100	-	-	100	-	-	-	-
	Group C	18	100	-	-	83.3	5.6	-	11.1	-
	Group D	11	72.7	27.3	-	36.4	18.2	-	45.4	-
Host / native	Port Elizabeth +	10	70	30	-	70	30	-	-	-
	Steynsburg +	10	80	20	-	80	20	-	-	-
	East London +	10	60	40	-	60	40	-	-	-

* Class 1 = inactive, class 2 = some degree of development & class 3 = mature sausage shaped eggs.

† Normal = class 1 ovaries & <25% 9ODA, intermediate = all bees not falling into any other class, egg layer = class 3 ovaries & <25% 9ODA, false queen =class 1 or 2 ovaries & >45% 9ODA, surrogate queen = class 3 ovaries & >45% 9ODA.

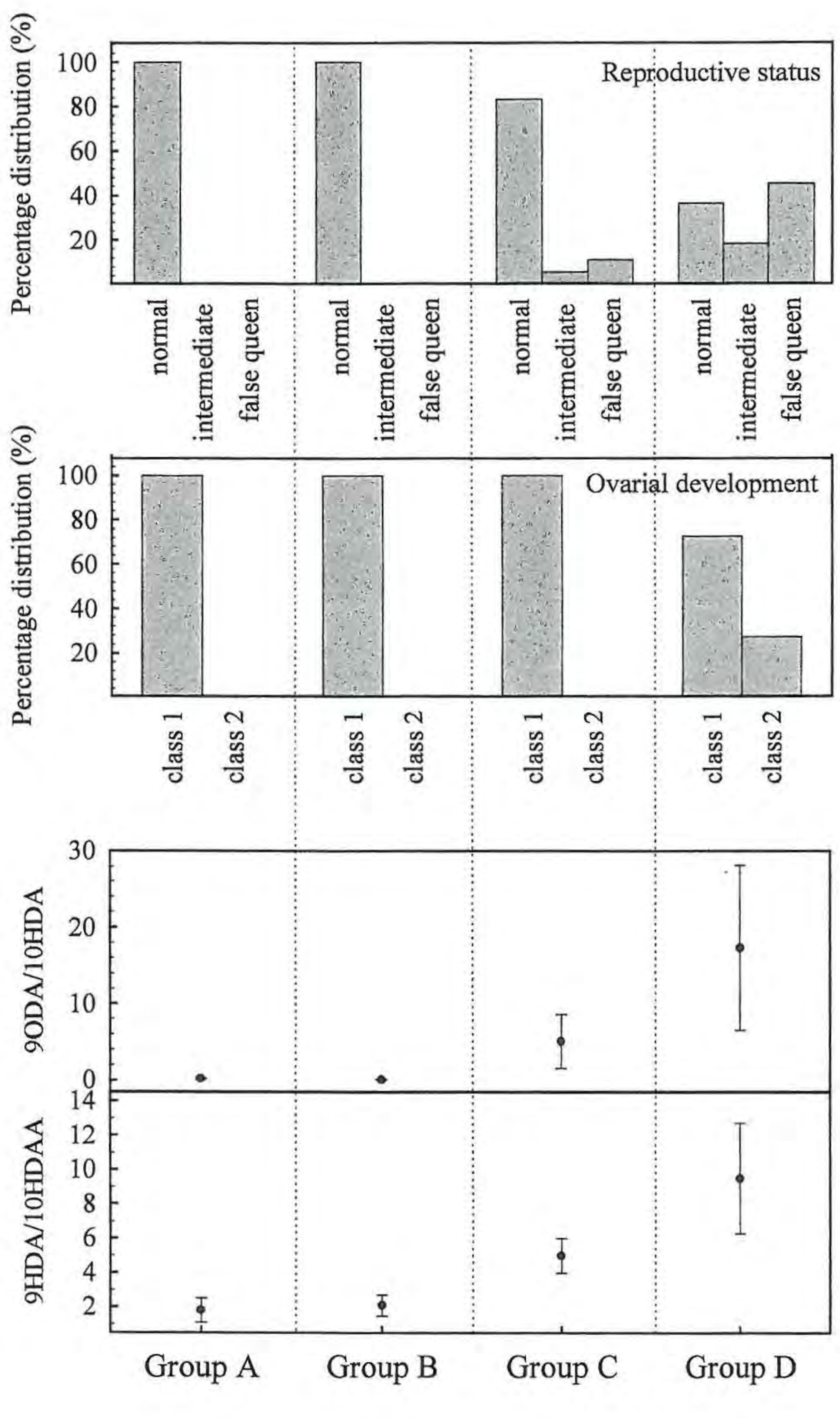

Fig. 4.2: Reproductive status, ovarial development and 9ODA:10HDA and 9HDA:10HDAA ratios (mean ± SE) of the 4 groups of drifted bees.

4.4 DISCUSSION

The results show that there was no more development (ovarial and mandibular gland) in the queenright drifters than in their queenright non-drifters or hosts of the same age. The 9ODA:10HDA ratios were much lower that those reported for the queens at the respective localities (Jones, 2000; Table 2.3). There are 2 possible explanations for this. Either the drifters had not been in the foreign hives long enough for development to take place or development of these drifters had been suppressed by the queens in the host colonies.

Unfortunately the experimental design did not allow any observations of when drifting occurred. All the drifters came from Port Elizabeth colonies, which are defined as thelytokous *A. m. capensis* by Hepburn *et al.* (1998). *A. m. capensis* laying workers are known to be present within 4 days of queen loss (Anderson, 1962; Fletcher, 1975, Hepburn et al., 1988) while elevated levels of 9ODA are observed after 2 days (Hemmling et al., 1979) and 9ODA predominates after 2 days (Crewe, 1987). Given that the majority of drifting takes place in the first week of a bee's life (Free, 1958) 10 days should be long enough to observe some degree of ovarial and mandibular gland development.

If one assumes then that time is not a factor and takes into account the latest classification of the bees used, it would seem unlikely that the queens in the hives from Steynsburg, arrhenotokous *A. m. scutellata* (Hepburn et al., 1998), would be able to prevent the ovarial and mandibular gland development of thelytokous *A. m. capensis* (Hepburn *et al.*, 1998) from Port Elizabeth. However, the ability of the bees from Steynsburg to themselves produce queen-like mandibular gland secretion and develop ovarially in the absence of a queen (chapter 3), together with the fact that the mandibular gland secretions of the queens from Port Elizabeth and Steynsburg are not significantly different (Jones, 2000) suggests that development of bees from Port Elizabeth might well be suppressed in colonies from Steynburg.

Once again this shows the conflicting results obtained by assigning biological traits to localities based on morphometric studies as opposed to direct experimentation. In addition, the fact that there was no difference in the ovary or mandibular gland development of the drifters from the same locality that flew into colonies from the 2 different localities emphasises this point. While the sample size is small, it seems from these results that queenright colonies from Steynsburg are resistant to these queenright drifting bees from Port Elizabeth.

The queenless drifted bees, however, differed from their respective queenright hosts. This may also be due to 2 reasons. Firstly, the bees drifting from the queenless colonies from Port Elizabeth could have developed partially due to their initial queenless environment (chapter 2). The extent of this pre-drifting development depends on how long they remained within their mother colonies, which unfortunately can not be determined. Secondly, their development could have occurred subsequent to drifting due to the host colonies not being able to suppress mandibular or ovarial development. These 2 possibilities are however not necessarily mutually exclusive.

If there was no suppression of the drifters in the foreign hive we would expect a difference in the development of the 2 groups of drifters, due to the host colonies being from different localities (assuming that the time of drifting was uniform). While there is no statistical difference between the development of these groups of drifters, due to large variation within the groups, the mean value of the 9ODA:10HDA ratio is 3 times more in the bees that drifted to a colony from the same locality than in the bees that drifted into a foreign colony. The bees that drifted to the foreign colony are also the only ones with class 2 ovaries in the entire experiment and have intermediate bees and false queens. It therefore seems that being queenless prior to drifting results in some degree of development, both ovarially and pheromonally, but in this case the Port Elizabeth bees that drifted into the queenright colonies from East London showed additional development to those that drifted into host colonies from the same locality. Hence it seems that the colonies from East London are not capable of preventing the development of queenless bees from Port Elizabeth. While queens from these two localities had similar 9ODA:10HDA ratios, it appears that being queenless prior to drifting allowed sufficient development to enable these drifters to subsequently develop false queens and 9ODA:10HDA ratios 3 times more than the East London queen value (Jones, 2000).

The presence of some degree of development (wherever it occurred) adds weight to my earlier assumption that 10 days is sufficient to observe some development. After 10 days the bees in the experiment were 12 days old and some had developed substantially. Prior to this it was, however, thought that ovarial and mandibular gland development only occurred in 15 day old *A. m. capensis* and 20 day old *A. m. scutellata* bees (Allsopp, 1988).

While this study can not prove that the drifters from queenless Port Elizabeth colonies actually took over the colonies from East London, it demonstrates that they are capable of gaining access to the colonies from East London and developing their ovaries and mandibular glands in the presence of their queens. This predrifting development may be an important preadaptation for taking over foreign colonies. By being partly developed prior to drifting (ovarially and pheromonally), these bees from queenless colonies have a head start, over the host bees as well as other queenright drifters, in obtaining reproductive dominance. If the host colony is unable to suppress the subsequent development of these drifters some of them may inturn drift to other colonies, and again have a head start thus further spreading their genes.

CHAPTER 5

DISCUSSION

The ability of *A. m. capensis* honeybees to develop queen-like mandibular gland secretions (Ruttner, 1976; Hemmling *et al.*, 1979; Crewe & Velthuis, 1980), rapidly develop their ovaries and lay female eggs, results in the usurpation of *A. m. scutellata* colonies (Allsopp, 1992; Allsopp & Crewe, 1993; Hepburn & Allsopp, 1994). While arrhenotokous bees do develop their ovaries upon queen loss in brood less situations, there are only a few insolated reports of these workers eliciting retinue behaviour, due to the development of queen-like mandibular gland secretions (Sakagami, 1958). Hence the ability to parasitise an unrelated colony and thereby produce your own offspring has previously been limited to morphometrically defined *A. m. capensis* honeybees, thought to be the only ones exhibiting thelytoky. It is now known, however, that the trait of thelytokous parthenogenesis has moved through the South African honeybee populations. Thelytoky now extends well past the limit of the *A. m. capensis* morphometric distribution (Hepburn et al., 1998) and into certain localities within the area of morphometric *A. m. scutellata* distribution.

The Eastern Cape is an interesting region with regard to honeybee interaction as it is an area where *A. m. capensis, A. m. scutellata* and their hybrid naturally occur. The 4 localities sampled in this study represent various morphologically defined groups and in addition display different reproductive mechanisms in workers. According to the latest classification of these bees (Hepburn *et al.*, 1998), Port Elizabeth, East London and Cradock's bees are morphometrically defined as *A. m. capensis, A. m. scutellata* and hybrids respectively, with the bees from all 3 localities exhibiting thelytoky to some degree. Steynsburg is the only locality included in this study that the bees have not been reported to exhibit thelytoky and they are bees morphometrically defined as *A. m. scutellata* (Hepburn *et al.* 1998).

While the mode of worker reproduction of the bees at these localities has been investigated, the mandibular gland profiles and ovarial development of these bees, under queenright and queenless conditions, has previously not been examined.

In this study the bees from queenright colonies from all 4 localities (chapter 2) were found to have 9HDA as apposed to 10HDA as the most abundant of the 5 mandibular gland compounds measured. This result while unexpected in light of the 'worker substance' views has previously been reported in *A. m. scutellata* by Hepburn and Allsopp (1994). All localities also had small percentages of 9ODA in their mandibular gland secretions. This abundance of 9HDA and the presence of 9ODA resulted in the ratios of 9ODA:10HDA and 9HDA:10HDAA being very high in respect to their queen status. Workers had consistently higher amounts of queen-like acids than worker-like acids. Despite this the percentage of bees with undeveloped ovaries was consistent with their queenright status and similar to *A. m. scutellata* (Hepburn & Allsopp, 1994; Jackson, 1982; Hastings, 1989) but higher than in *A. m. capensis* (Anderson, 1962; Hepburn *et al.*; 1991). The 9ODA:10HDA and 9HDA:10HDAA ratios for queenright bees from Port Elizabeth were significantly more worker-like than those from East London or Cradock, while the bees from Steynsburg were intermediate. The possibility that this enables the bees from East London and Cradock to gain a head start on mandibular gland development upon entering a foreign colony was therefore proposed. This however seems unlikely when one considers the development subsequent to queen loss.

Upon the loss of their queen (chapter 3) the bees from all localities had a significant increase in the percentage of 9ODA in their mandibular glands. The changes in the other 4 compounds, however, varied and this inturn resulted in the 9ODA:10HDA and 9HDA:10HDAA ratios changing to different degrees. The bees from East London were the only ones not to become significant more queen-like after queen loss. While the 2 ratios at the 4 localities were not significantly different after 14 days without a queen, the mean values of the 2 ratios, for the bees from Port Elizabeth were much higher than those from the other localities. Certain individuals from Port Elizabeth had 9ODA:10HDA ratios that were much higher than reported in *A. m. capensis* queens (Crewe, 1988). Port Elizabeth was also the only locality to display any surrogate queens and exhibited the largest increase in the number of bees with partially or fully developed ovaries. Certain individuals from Steynsburg, East London and Cradock had higher 9ODA:10HDA ratios than those reported in *A. m. scutellata* queens (Crewe, 1988). It therefore seems that the high queenright values of these 2 ratios, in bees from East London and Cradock have not enabled them to become more developed in less time, as was previously proposed. This initial characteristic does not seem to be an advantage with respect to the development of queen-like mandibular gland secretion upon queen loss but might serve another purpose.

If we suggest that a worker bee is capable of overthrowing a foreign queen due to it's 9ODA:10HDA and 9HDA:10HDAA ratios exceeding those of a foreign *A. m. scutellata* queen, all of these localities have bees potentially capable of usurpation. However, if we redefine this to include only the bees in which 9ODA predominates, Cradock no longer has any individuals capable of this. These are very basic definitions, and seriously underestimate the complexity of the process, but this discrepancy demonstrates the underlying problems when trying to solve the '*capensis* problem', especially since bees without mandibular glands have been shown to become dominant (Saiovici, 1983).

The traits displayed on queen removal are not surprising for the bees previously defined as exhibiting thelytoky (Hepburn *et al.*, 1998), regardless of their morphometric classification. As the ability to produce female brood spreads through the region, the percentages of workers capable of reproducing in this way will increase in colonies. This inturn increases the number of bees to which the traits of producing a queen-like mandibular gland secretions and rapidly developing their ovaries would be adaptive.

However, the observations for the bees from Steynsburg are puzzling. Steynsburg's bees differed from those of the other localities, in that they did not show a marked increase in ovarial development subsequent to queen loss, a trait consistent with brood right *A. m. scutellata* and other arrhenotokous colonies. However these bees also showed a significant increase in the percentage of 9ODA, resulting in certain individuals having higher 9ODA:10HDA and 9HDA:10HDAA ratios than *A. m. scutellata* queens (Crewe, 1988). The mean values of these ratios being higher than those of the bees from East London and Cradock. This might lead one to question the arrhenotokous status of this locality, as these traits are not consistent with a colony of entirely arrhenotokous workers. In addition to this the bees from Steynsburg were able to suppress the mandibular gland and ovarial development of bees from Port Elizabeth that drifted into their queenright colonies (chapter 4) and presumably avoid usurpation by these bees. This factor once again seems to contradict their *A. m. scutellata* status.

This discrepancy exposes the danger of assigning biological traits to bees from morphometrically defined localities. It is therefore no longer possible to assume that the bees from this Eastern Cape area behave in a certain way by mere inference. With the movement of various traits through the population at different rates, it is now essential that these traits be assigned to honeybees only by direct observation. This becomes very important when trying to solve the '*capensis* problem' as the present legislation in place for controlling the movement of hives is partially based on this dated morphometric approach.

As the bees from Steynsburg were able to suppress the mandibular gland and ovarial development of Port Elizabeth's drifted bees, it might be suggested that initial mandibular gland secretions in queenright situations being relatively more queen-like than expected, might play a role in invasion avoidance. However the bees from East London, that had a higher degree of queen-like mandibular gland development in a queenright situation than those from Steynsburg, were not able to suppress the mandibular gland or ovarial development of the bees from Port Elizabeth that drifted into their colonies, ruling out this suggestion.

Recently it has been suggested that a specialized social parasite, stemming from the initial invasion of the *A. m. scutellata* area, is responsible for the continuation of the '*capensis* problem' (Kryger & Van der Schyf, 2000). These clones reproduce unisexually, rendering these bees quite unadaptable, possibly over time culminating in their demise (Kryger & Van der Schyf, 2000). This does not, however, rule out the possibility of future invasion and subsequent selection of a new social parasite. Hence it is still important to continue researching this problem in an attempt to fully understand the process.

While the results of this study are limited to a small area, they highlight the need for more comprehensive studies. A more recent quantification of worker progeny sex ratios would clear up the ambiguity of how far thelytoky has spread. This together with further research to further our understanding of the complete set of traits that enable invaders to usurp foreign colonies, might provide some useful insight into the problem, possible allowing us to get one step closer to a solution.

REFERENCES

Allsopp MH (1988) Mandibular gland acids and laying workers in African honey bees. In: *Africanized Honey Bees and Bee Mites.* (Eds GR Needham, RE Page, M Delfinado-Baker & CE Bowman), 72-79, Ellis Horwood Ltd, Chichester.

Allsopp MH (1992) The *capensis* calamity. *South African Bee Journal* **64**: 52-55.

Allsopp MH (1993a) Description of *capensis* invasion of *scutellata* colonies. Unpublished.

Allsopp MH (1993b) Thelytokous parthenogenesis in *Apis mellifera capensis.* Proceedings of the Ninth Entomological Congress of the Entomological Society of Southern Africa, Johannesburg, South Africa, p 2.

Allsopp MH & Crewe RM (1993) The Cape honey bee as a Trojan horse rather than the hordes of Genghis Khan. *American Bee Journal* **133**: 121-123.

Anderson RH (1963) The laying worker in the Cape honeybee, *Apis mellifera capensis. Journal of Apicultural Research* **2**: 85-92.

Barbier J & Lederer E (1960) Structure chemique de la substance royale de la reine de l'abeille. *Comptes Rendus, L'Academie de Science, Paris, Série* D, **251**: 1131-1135.

Barker SA, Foster AB, Lamb DC & N Hodgson (1959a) Identification of 10-Hydroxy-Δ^2-decenoic acid in Royal Jelly. *Nature* **183**: 996-997.

Barker SA, Foster AB, Lamb DC & Jackman LM (1959b) Biological origin and configuration of 10-Hydroxy-Δ^2-decenoic acid. *Nature* **184**: 634.

Beekman M, Calis JNM & Boot WJ (2000) Parasitic honeybees get royal treatment. *Nature* **404**: 723.

Billen JPJ, Dumortier KTM & Velthuis HHW (1986) Plasticity of honeybee castes. *Naturwissenschaften* **73**: 332-333.

Blum MS & Fales HM (1988) Eclectic chemisociality of the honeybee: a wealth of behaviours, pheromones, and exocrine glands. *Journal of Chemical Ecology* **14**(11): 2099-2107.

Boch R & Shearer DA (1967) 2-heptanone and 10-hydroxy-trans-dec-2-enoic acid in the mandibular glands of worker honey bees of different ages. *Zeitschrift für vergleichende Physiologie* **54**: 1-11.

Butler CG (1956) Some further observations on the nature of 'queen substance' and its role in the organization of a honey-bee (*Apis mellifera*) community. *Proceedings of the Royal Entomological Society of London* **31**: 12-16.

Butler CG (1967) A sex attractant as an aphrodisiac in the honeybee (*A. mellifera* L.). *Proceedings of the Royal Entomological Society of London* **42A**: 71-76.

Butler CG, Callow RK & Chapman JR (1964) 9-Hydroxydec-trans-2-enoic acid, a pheromone stabilizing honeybee swarms. *Nature* **201**: 733.

Butler CG, Callow RK & Johnston NC (1961) The isolation and synthesis of queen substance, 9-oxodec-trans-2-enoic acid, a honeybee pheromone. *Proceedings of the Royal Society* **155B**: 417-432.

Buys B (1990) Features of basic reproduction in drones and queens of the Cape honeybee *Apis mellifera capensis*. In: *Bees and Beekeeping in Southern Africa* (Eds RH Anderson & B Buys), 106-109, Apimondia Symposium, Stellenbosch, South Africa.

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

Callow RK, Johnston NC & Simpson J (1959) 10-Hydroxy-2-decenoic acid in the honeybee (*Apis mellifera*). *Experentia* **15**: 421-422.

Cooke M (1992) Turnabout is fair play - Cape bee invades African bee territory. *American Bee Journal* **132**: 519-521.

Crewe RM (1976) Aggressiveness of honeybees and their pheromone production. *South African Journal of Science* **72**: 209-212.

Crewe RM (1981) Queens, false queens and *capensis*. *South African Bee Journal* **53**(6): 18-21.

Crewe RM (1982) Compositional variability: the key to the social signals produced be honeybee mandibular glands. In: *The Biology of Social Insects* (Eds MD Breed, CD Michner & HE Evans), 318-322, Westview Press, Boulder, USA.

Crewe RM (1984) Differences in behaviour and morphology between *capensis* and *adansonii*. *South African Bee Journal* **56**(2): 16-21.

Crewe RM (1987) Lability of the mandibular gland signal of three races of African honey bees. In: *Chemistry and Biology of Social Insects* (Eds J Eder & H Rembold), 433-434, Verlag J. Peperny, Munich, Germany.

Crewe RM (1988) Natural history of honey-bee mandibular gland secretions: development of analytical techniques and the emergence of complexity. In: *Africanized Honey Bees and Bee Mites* (Eds GR Needham, RE Page, M Delfinado-Baker & CE Bowman), 149-158, Ellis Horwood Limited, Chichester.

Crewe RM & Hastings H (1976) Production of pheromones by workers of *Apis mellifera adansonii*. *Journal of Apicultural Research* **15**(314): 149-154.

Crewe RM, Hepburn HR & Moritz RFA (1994) Morphometric analysis of 2 Southern African races of honeybee. *Apidologie* **25**: 61-70.

Crewe RM & Velthuis HHW (1980) False queens: a consequence of mandibular gland signals in worker honeybees. *Naturwissenschaften* **67**: 467-469.

Crewe RM, Wossler T & Allsopp MH (1990a) Workers in queens clothing: why *capensis* workers become pseudoqueens. In: *Bees and Beekeeping in Southern Africa* (Eds RH Anderson & B Buys), 83-89, Apimondia Symposium, Stellenbosch, South Africa.

Crewe RM, Wossler T & Allsopp MH (1990b) Intercaste or mosaic, the worker mandibular gland secretions of Cape honeybees (*Apis mellifera capensis*). In: *Social Insects and the Environment*, 11th International congress IUSSI, Bangalore, India (Eds GK Veeresh, B Mallik & CA Viraktamath), 415-416, Oxford and IBH Publishing Co. New Delhi, India.

Fletcher DJC (1975) First observations on Cape bees kept in Natal. *South African Bee Journal* **47**(3): 5-8.

Fletcher DJC (1978) The African bee, *Apis mellifera adansonii*, in Africa. *Annual Review of Entomology* **23**: 151-71.

Free JB (1958) The drifting of honey-bees. *Journal of Agricultural Science* **51**: 294-306.

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

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

Greeff JM (1996a) Thelytokous versus arrhenotokous worker reproduction in the Cape honeybee and other social Hymenoptera. *Hereditas* **124**: 99-103.

Greeff JM (1996b) Effects of thelytokous worker reproduction on kin-selection and conflict in the Cape honeybee, *Apis mellifera capensis*. *Philosophical. Transactions of the Royal Society, London* **B351**: 617-625.

Greeff JM (1997) The Cape honeybee and her way North: an evolutionary perspective. *South African Journal of Science* **93**: 306-308.

Guy RD (1976a) Whence the Cape bee? *South African Bee Journal* **48**(2): 7-8.

Guy RD (1976b) Whence the Cape bee?- Part 2. *South African Bee Journal* **48**(3): 9-11.

Hastings H (1989) *Two aspects of the biology of an African honeybee Apis mellifera scutellata (Hymenoptera, Apidae) laying workers and colony defence behaviour.* PhD thesis, University of Natal, Pietermaritzburg, South Africa.

Hemmling C, Koeniger N & Ruttner F (1979) Quantitative bestimmling der 9-ODA in lebenszykllis der Kapbiene (*Apis mellifera capensis* Escholtz). *Apidologie* **10**(3): 227-240.

Hepburn HR (1989) *Capensis* in the Eastern Cape. *South African Bee Journal* **61**(4): 87-89.

Hepburn HR (1990) The first report on the biology of the Cape honeybee. *South African Bee Journal* **62**(6): 128-131.

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

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

Hepburn HR & Allsopp MH (1994) Reproductive conflict between honeybees: usurpation of *Apis mellifera scutellata* colonies by *Apis mellifera capensis*. *South African Journal of Science* **90**: 247-249.

Hepburn HR & Crewe RM (1990) Defining the Cape honeybee: reproductive traits of queenless workers. *South African Journal of Science* **86**: 524-527.

Hepburn HR & Crewe RM (1991) Portrait of the Cape honeybee, *Apis mellifera capensis*. *Apidologie* **22**: 567-580.

Hepburn HR & Guye SG (1993). An annotated bibliography of the Cape honeybee, *Apis mellifera capensis* Escholtz (Hymenoptera: Apidae). *African Entomology* **1**(2): 235-252.

Hepburn HR & Jacot Guillarmod A (1991) The Cape honeybee and the fynbos biome. *South African Journal of Science* **87**: 70-73.

Hepburn HR, Jones GE & Kirby R (1994) Introgression between *Apis mellifera capensis* Escholtz and *Apis mellifera scutellata* Lepeletier: the sting pheromones. *Apidologie* **25**: 557-565.

Hepburn HR, Magnuson P, Herbert L & Whiffler LA (1991) The development of laying workers in field colonies of the Cape honey bee. *Journal of Apicultural Research* **30**(2): 107-112

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

Hepburn HR & Radloff SE (1998) *Honeybees of Africa.* Springer-Verlag, Berlin, Germany.

Hepburn HR, Radloff SE & Fuchs S (1998) Population structure and the interface between *Apis mellifera capensis* and *Apis mellifera scutellata. Apidologie* **29**: 333-346.

Hepburn HR, Reece SL, Neumann RFA, Moritz RFA & Radloff SE (1999) Absconding in honeybees (*Apis mellifera*) in relation to queen status and mode of worker reproduction. *Insectes Sociaux* **46**: 323-326.

Hillesheim E, Koeniger N & Moritz RFA (1989) Colony performance in honeybees (*Apis mellifera capensis* Esch.) depends on the proportion of subordinate and dominant workers. *Behavioral Ecology and Sociobiology* **24**: 291-296.

Jack RW (1916) Parthenogenesis amongst the workers of the Cape honey-bee: Mr G.W. Onions' experiments. *Transactions of the Entomological Society, London* **64**: 396-403.

Jackson ME (1982) *The effect of social interactions on the production of mandibular gland signals in the female African honeybees (Apis mellifera adansonii L.).* MSc thesis, University of the Witwatersrand, Johannesburg, South Africa.

Johannsmeier MF (1983) Experiences with the Cape bee in the Transvaal. *South African Bee Journal* **55**(6): 130-138.

Johannsmeier MF (1997) Overcoming the capensis problem. *South African Bee Journal* **69**(4): 63-70.

Jones GE (2000) *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.* PhD thesis, Rhodes University, Grahamstown, South Africa.

Kryger P & Van der Schyf A (2000) *The capensis honey bee problem: A genetic analysis using single locous DNA-fingerprinting.* Final report for the South African Bee-Farmers' Associations.

Lundie AE (1954) Laying worker bees produce worker bees. *South African Bee Journal* **29**(1): 10-11

Mackenson O (1943) The occurance of parthenogenic females in some strains of honeybees. *Journal of Economic Entomology* **36**(3): 465-467.

Moritz RFA (1990) Natural and artificial selection in the Cape honeybee *Apis mellifera capensis.* In: *Bees and Beekeeping in Southern Africa* (Eds RH Anderson & B Buys), 63-73, Apimondia Symposium, Stellenbosch, South Africa.

Mortiz RFA, Beye M & Hepburn HR (1998) Estimating the contribution of laying workers to population fitness in African honeybees (*Apis mellifera*) with molecular markers. *Insectes Sociaux* **45**: 277-287.

Moritz RFA, Cornuet JM, Kryger P, Garnery L & Hepburn HR (1994) Mitochondrial DNA variability in South African honeybees (*Apis mellifera* L.). *Apidologie* **25**: 169-178.

Moritz RFA, Crewe RM & Hepburn HR (2000) Attraction and repellence of workers by the honeybee queen (*Apis mellifera*). *Ethology*, in press.

Moritz RFA & Haberl M (1994) Lack of meiotic recombination in thelytokous parthenogenesis of laying workers of *Apis mellifera capensis* (the Cape honeybee). *Heredity* **73**: 93-102.

Moritz RFA & Hillesheim E (1985) Inheritance of dominance in honeybees (*Apis mellifera capensis* Escholtz). *Behavioral Ecology and Sociobiology* 17: 87-89.

Moritz RFA & Kauhausen D (1984) Hybridization between *Apis mellifera capensis* and adjacent races of *Apis mellifera*. *Apidologie* **15**: 211-221.

Moritz RFA, Kryger P & Allsopp MH (1996) Competition for royalty in bees. *Nature* **384**: 31.

Moritz RFA, Kryger P & Allsopp MW (1999) Lack of worker policing in the Cape honeybee (*Apis mellifera capensis*). *Behaviour*, in press.

Neumann P, Hepburn HR & Radloff SE (2000a) Modes of worker reproduction, reproductive dominance and brood cell construction in queenless honeybee (*Apis mellifera* L.) colonies. *Apidologie* **31**: 479-486.

Neumann P, Kryger P & Moritz RFA (1999) Genetic variability for polyandry in the honeybee. *Molecular Ecology*, submitted.

Neumann P, Radloff SE, Moritz RFA, Hepburn RH & Reece SL (2000b) Social parasitism by honeybee workers (*Apis mellifera capensis* Esch.): Host finding and resistance of hybrid host colonies. *Behavioral Ecology,* in press.

Onions GW (1912) South African "fertile-worker bees". *Union of South Africa Agricultural Journal* **1**: 720-728.

Onions GW (1914) South African "fertile" worker-bees. *Union of South Africa Agricultural Journal* 7: 44-46.

Plettner E, Slessor KN, Winston ML & Oliver JE (1996) Caste-selective pheromone biosynthesis in honeybees. *Science* **271**: 1851-1853.

Plettner E, Slessor KN, Winston ML, Robinson GE & Page RE (1993) Mandibular gland components and ovarian development as measures of caste differentiation in the honey bee (*Apis mellifera* L.) *Journal of Insect Physiology* **39**(3): 235-240.

Ratnieks FLW (1988) Reproductive harmony via mutual policing by workers in eusocial Hymenoptera. *American Naturalist* **132**: 217-236.

Ratnieks FLW & Visscher PK (1989) Worker policing in the honeybee. *Nature* **342**(Dec): 796-797.

Ruttner F (1977) The problem of the Cape Bee (*Apis mellifera capensis* Escholtz): Parthenogenesis - size of population - evolution. *Apidologie* **8**: 281-294.

Ruttner F (1988) *Biogeography and Taxonomy of Honeybees.* Springer-Verlag, Berlin, Germany.

Ruttner F & Hesse B (1981) Rassenspezifische unterschiede in ovarentloicklung und eiablage von weisellosen arbeiterinnen der honigbiene *Apis mellifera* L. *Apidologie.* **12**(2): 159-183.

Ruttner F, Koeniger N & Veith HJ (1976) Queen substance bei eierlegenden Arbeiterinnen der Honigbiene *Apis mellifica* L. *Naturwissenschaften* **63**: 434.

Saiovici M (1983) 9-Oxodecenoic acid and dominance in honeybees. *Journal of Apicultural Research* **22**(1): 27-32.

Sakagami SF (1958) The false-queen: fourth adjustive response in dequeening honeybee colonies. *Behaviour* **13**: 280-296.

Seeley TD (1995) *The Wisdom of the Hive: the social physiology of honey bee colonies.* Harvard University Press, Cambridge, Massachusetts, USA.

Steele GR, Villet MH, Radloff SE & Hepburn HR (1998) Clinal morphometric variation in wild honeybees (Hymenoptera: Apidae) in South Africa. *Diversity and Distribution* **4**: 17-25.

Tribe GD (1983) What is the Cape bee? *South African Bee Journal* **55**: 77-87.

Velthuis HHW (1970) Ovarian development in *Apis mellifera* worker bees. *Entomologia Experimentalis et Applicata* **13**: 377-394.

Velthuis HHW (1985) The honeybee queen and the social organization of her colony. *Fortschritte der Zoologie* **31**: 343-357.

Velthuis HHW (1990) Chemical signals and dominance communication in the honeybee *Apis mellifera* [Hymenoptera: Apidae]. *Entomologia Generalis* **15**(2): 083-090.

Velthuis HHW, Ruttner F & Crewe RM (1990) Differentiation in reproductive physiology and behaviour during the development of laying worker honey bees. In: *Social Insects. An evolutionary approach to castes and reproduction.* (Ed W Engels), 231-243, Springer-Verlag, Berlin, Germany.

Velthuis HHW & van der Kerk A (1988) Age, environment, and genes in relation to the mandibular gland secretion of pure and hybrid *Apis mellifera capensis* worker bees. In: *Africanized Honey Bees and Bee mites.* (Eds GR Needham, RE Page, M Delfinado-Baker & CE Bowman), 80-86, Ellis Horwood Ltd, Chichester.

Verma S & Ruttner F (1983) Cytological analysis of the thelytokous parthenogenesis in the Cape honeybee (*Apis mellifera capensis* Escholtz). *Apidologie* **14**(1): 41-57.

Weaver N & Law JH (1960) Heterogeneity of fatty acids from Royal Jelly. *Nature* **188**: 938-939.

Whiffler LA & Hepburn RH (1991) The queen in relation to wax secretion and comb building in honeybees. *Journal of Comparative Physiology* **A169**: 209-214.

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

Wossler TC (1993) Chemical characterization of the tergal gland secretions of the honeybee (*Apis mellifera*). Proceedings of the Ninth Entomological Congress of the Entomological Society of Southern Africa, Johannesburg, South Africa, 122.