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THE EFFECT OF SOCIAL INTERACTIONS ON THE PRODUCTION OF MANDIBULAR GLAND SIGNALS IN FEMALE AFRICAN HONEYBEES (*APIS MELLIFERA ADANSONII* L.)

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A Dissertation Submitted to the Faculty of Science,
University of the Witwatersrand, Johannesburg
for the Degree of Master of Science

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FORMAL DECLARATION

I, Michael Ernest Jackson, the undersigned, solemnly declare that the work submitted in this dissertation is my own unaided research, and that I did not receive assistance in any form other than that mentioned in my acknowledgments.

No part of the dissertation has been submitted in the past, or is being, nor is to be submitted for any degree in any University.

No part of this dissertation was obtained whilst I worked under the aegis of, or was employed by, any person or organisation other than the University of the Witwatersrand.

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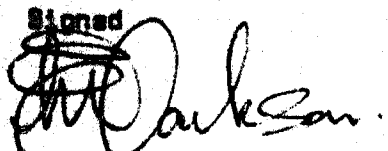

M. E. Jackson

ABSTRACT

The ability of queenless Apis mellifera adansonii workers to produce queen pheromones in their mandibular glands was examined gas chromatographically, and was compared with the degree of ovarial development that occurred in these individuals. Analyses were also made of the mandibular gland secretions of Apis mellifera adansonii queens and queenright workers.

The ovaries of workers were found to be extremely underdeveloped. Ovarioles were filamentous and often lacked a distinct germarium. Numbers per ovary varied from none to twelve (average 3.69) and in one sample from none to eight (average 2.88). Ovaries that lacked distinct ovarioles had only fine strands attached to the lateral oviducts to suggest their presence.

Ovary development was found to occur more readily in queenless bees that were less than three weeks old when the queen was removed than in workers that were older. While a number of individuals became functional laying workers in queenless Apis mellifera adansonii colonies, none evoked retinue behaviour and none suppressed ovary development in nestmates. Over 99 per cent of queenless bees were found not to secrete 9-keto decenoic acid. Two ten day olds that were secreting 9-keto decenoic acid had secretions that were qualitatively very similar to those of mated queens, however neither had mature eggs in their ovaries.

Levels of decenoic acid and 2-heptanone, which were both major components of the mandibular gland secretions of queenright workers, were found to diminish markedly with ovary development.

The secretions of mated queens of Apis mellifera adansonii were remarkably homogenous with regard to the ratio of 9-keto decenoic acid, 9-hydroxy decenoic acid and 10-hydroxy decenoic acid, although amounts varied substantially. The amounts of 9-keto decenoic acid in mated queen secretions varied between 116,6 micrograms and 390,3 micrograms (average 250,5 micrograms).

The secretions of a number of virgin queens of various ages were also analysed, and were found to be significantly different from those of mated queens.

To Gail, for her patience and understanding

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INTRODUCTION

Apis mellifera is probably the best known and most widely studied of all social insect species as it has been of particular interest to man since ancient times (Rembold, 1965). The species is thought to be represented in Africa by at least eleven geographical races of which two, A.m. capensis and A.m. scutellata are found in southern Africa. While the former is restricted to the south western Cape, the latter ranges throughout the south and east african savannah (Ruttner, 1977). As this classification is recent and incomplete, the name A.m. adansonii will replace A.m. scutellata throughout this text as the synonym at present identifies all bees south of the Sahara other than the Cape and Madagascan races (Anderson, 1977). The south african bee, A.m. adansonii, has evoked particular interest outside South Africa following its introduction into Brazil in 1956, where it has become renowned owing to its aggression and migrating tendencies in that country (Wiese, 1977) and because of the impending threat of it spreading to the United States through Central America (Weaver, 1977).

Two distinct female castes occur in honeybee colonies, a queen caste and a worker caste. In A. mellifera at least fifty morphologically distinct differences have been recorded between these castes, some of which are illustrated in Table 1.1. In addition to these morphological and anatomical features, the castes are also significantly different in their physiology and behaviour.

Within a colony, the queen caste usually consists of a single individual, the only fully developed female. She normally lays all the eggs and hence is the mother of the entire population. Unlike most other wasp and bee queens, she does not participate in any worker activity, but instead is highly specialised for reproduction commonly laying over a thousand eggs daily.

> Her mouthparts are reduced ; her eyes are smaller ... ; her antennae are short and bear fewer sensilla ; she has a conspicuously smaller brain ; she lacks pollen collecting hairs ; and her hypopharyngeal and wax glands, which in the worker are the principal sources of larval food and building material, are underdeveloped.
> (Wilson, 1971).

The queen is also conspicuously larger than the workers and in

Table 1.1. Some morphological and anatomical differences between the workers and queens of *Apis mellifera*

Feature	Worker	Queen
Body weight	80-100 mg	150-250 mg
Abdomen size	Smaller	Elongated
Hypopharyngeal glands	Large	Vestigal
Mandibular glands	Large	Very large
Nasanoff's glands	Present	Absent
Wax glands	Present	Absent
Dorsal abdominal gland	Absent	Present
Ovaries	Small	Enormous
Number of ovarioles	1-13[a]	150-350
Spermatheca	Small or Rudimentary[a]	Large
Sting axis	Straight	Curved
Sting barbs	Strong	Minute
Mandibular form	Slender	Robust
Mandibular tooth	Absent	Present
Corbicula	Present	Absent
Auricle or Pecten	Present	Absent
Length of Proboscis	Longer	Shorter

Modified from Michener (1974).

[a] *A.m. capensis* is an exception, having between 5-59 ovarioles and a spermatheca almost half the size of the queen (Anderson, 1963).

particular her abdomen, filled with active ovarioles, is distended to a noticeably greater degree.

In contrast the worker caste is comprised of many thousands of non-reproductive, smaller individuals which perform all the normal activities of the colony, including nest construction, nest cleaning, colony defense, nursing of brood and foraging. They have a comparatively short life time, living for little longer than a month, whereas the queen may live for a number of seasons (Winston and Otis, 1978).

It has been known for almost one hundred years that queens and workers are derived from the same type of egg and that they are therefore genetically identical (Perez, 1889). What remains largely unresolved though, is the mechanism by which genetically identical material produces two morphologically distinct forms, and secondly how such a mechanism could have evolved. It is hoped that the investigation undertaken in this study will broaden our knowledge of the effects of caste differentiation on exocrine secretions and improve our understanding of queen-worker social interactions in the species.

Some aspects of the mechanism of caste determination have been determined recently. It has been known for some time that it is a purely

physiological process implemented by differential treatment afforded to larvae by nurse workers particularly during their early development (Zander and Becker, 1925). Weaver (1957) timed the events of caste differentiation in the European honeybee and found that up to three days into larval development, larvae transferred from worker cells to queen cells were able to develop into distinct queens. After three days, worker characteristics began to appear and after only three and a half to four days, larvae developed into intermediate forms with distinct worker-like qualities. Woyke (1971) showed later that differentiation begins almost immediately when he found that queens had distinct differences in body weight and fertility depending upon the age of the brood from which they were reared. Queens reared from eggs averaged two hundred and nine milligrams and had over three hundred ovarioles per ovary, while those reared from four day old worker larvae averaged one hundred and nineteen milligrams and had nearly one hundred ovarioles per ovary fewer. The mean volume of the spermatheca of the latter queens was approximately half that of the former and contained only a quarter the number of spermatozoa.

It is interesting, then, that the development of both castes is synchronous during the first three days. The larvae of both moult three times within sixty hours and during this period both have similar weights. It is only during the fourth larval instar that differences begin to occur, these becoming more apparent during the final instar. During this period a marked regression in certain aspects of growth occurs in the worker caste. While at seventy two hours worker larvae have full sets of ovariole primordia identical to queen larvae of equivalent age, within twenty four hours only four to six remain while queen larvae have between one hundred and fifty and one hundred and eighty (de Wilde, 1976). In addition, fifth instar worker larvae pupate a day later than queen larvae and are almost half the weight (Wang, 1965). The pupal stage also extends over a longer period, stretching over nine days instead of four. Consequently the overall developmental periods in European bees are twenty one days for workers and only sixteen days for queens (Bertholf, 1925).

The developmental periods of the two castes of A.m. adansonii have been found to be somewhat shorter. While Smith (1958) found the developmental period of workers to be between nineteen and twenty days ; under more constant conditions Tribe and Fletcher (1977) showed that development can be completed in eighteen and a half days. Similarly the development

of queens was found to take between fourteen and fifteen days (Anderson, et al, 1973). Tribe and Fletcher (1977) found that the shorter development period of A.m. adansonii workers is due largely to a shorter larval stage which is apparently related to a smaller worker cell size (a characteristic of this race), this being expressed in the adults which are slightly smaller than those of European races. It is suggested that the faster rate of development together with smaller body size provide adaptive advantages in the African environment, particularly with respect to the utilization of the short and often unreliable honey flows from indigenous flora and to the rate at which colonies are able to build up to swarming strength during these flows (Tribe and Fletcher, 1977).

Returning to the changes that occur during the later stages of development of queen and worker larvae, a considerable amount of evidence has accumulated suggesting possible factors that influence the remarkable changes in growth rate that occur in the larvae. Histological data indicate that worker larvae ingest less food than queen larvae especially during their early development, although the quantity is obviously sufficient to ensure a growth rate similar to that of queen larvae (de Wilde, 1976). Their fat bodies contain little stores of glycogen and have large watery vacuoles, while those of queen larvae accumulate glycogen from hatching (Wirtz, 1973). In addition three day old queen larvae have six times as many mitochondria as worker larvae of the same age (Osanai and Rembold, 1968). The fact that there are significantly greater numbers of mitochondria in queen larvae supports earlier evidence that the rate of respiratory metabolism is higher in two or three day old queen larvae (Melampy and Willis, 1939), and indicates that while the weights of queen and worker larvae may be similar during early development, the rate of food intake must be substantially higher in queen larvae. This was demonstrated by Dietz and Lambremont (1970) who found that larvae which ingested approximately three hundred and eighty milligrams of royal jelly developed into queens, while those ingesting three hundred and eight milligrams developed into workers, a difference of only 19 per cent in the mean rate of ingestion.

In addition to differences in the rate of ingestion, worker larvae are known to receive food of a different quality. For instance, Rembold (1963) found that royal jelly and worker jelly, the foods fed to the larvae of the respective castes, differed in content of vitamins such as pantothenic acid, biopterin and neopterin, royal jelly always containing substantially greater levels of these biologically important substances even though worker

jelly contained greater amounts of most amino acids. Also, Shuel and Dixon (1959) found that the food of one to four day old queen larvae contained about 34 per cent (dry weight) reducing sugar while worker jelly fed to larvae less than thirty hours old contained only 12 per cent (dry weight). Finally there is evidence that queen larvae are fed proportionally more mandibular gland secretion than are worker larvae (Jung-Hoffman, 1967).

Weaver (1955) successfully reared adult queens *in vitro* on a diet consisting solely of fresh royal jelly. However he found that stored royal jelly lost its ability to promote queen characteristics and this led him to hypothesise that royal jelly contained specific substances that controlled or initiated differentiation and that an essential component was either highly labile or at least unavailable to larvae following storage. A number of researchers, including Weaver (1962), subsequently attempted to identify these compounds and Rembold *et al* (1974) claimed to have partially purified a queen bee determinator from royal jelly. Although the compound was not identified, tests showed that it was not a peptide, a sugar, a polyol or an aromatic compound.

While it is possible that additional chemical components of royal jelly such as that suggested by Rembold *et al* (1974) may yet be implicated as triggers for queen determination, Asencot and Lensky (1977) appear to have resolved the problem by showing that the apparent 'labileness' of royal jelly may be due to the evaporation of water and subsequent crystallisation of reducing sugars, for in this state the sugars appear to be unavailable to larvae. With the use of gas-liquid chromatography they determined that in stored royal jelly two thirds of the fructose and glucose was crystalline, and by supplementing the jelly with appropriate quantities of these sugars, they were able to rear queens, intermediates and workers in a ratio similar to that induced by fresh royal jelly. Earlier Shuel and Dixon (1959) showed that worker jelly contained approximately three times less sugar than royal jelly during early development, and Asencot and Lensky (1976) demonstrated that by adding glucose and fructose to worker jelly, the production of queens and intermediates was induced.

The exact process by which reducing sugars induce differentiation into queens is not understood, however it appears that in some way they have a phagostimulatory effect. This is supported by the fact that three to four day old larvae are known to enter a 'gorging' phase (Bertholf, 1925), for Shuel and Dixon (1959) found that worker jelly fed to these larvae contained up to 47 per cent (dry weight) reducing sugar, a fourfold increase

in sugar content of jelly fed to young worker larvae. In addition Goewie (1978) found that the chemoreceptors of larval mouthparts are particularly sensitive to sugars such as fructose and glucose, strengthening the suggestion that these chemicals may act as phagostimulants for larvae to ingest greater amounts of food.

For a number of years a substantial amount of evidence has accumulated indicating that caste differentiation is controlled by the endocrine system. A number of authors have suggested that a difference in hormone balance during early stages of larval development is a factor in caste determination (Haydak, 1943 ; Osanai and Rembold, 1968 ; Shuel and Dixon, 1960). It has only been during the last ten years, though, that juvenile hormone and the activity of the corpora allata have been associated with caste determination (Beetsma, 1979). Wirtz (1973) found that the corpora allata of worker larvae were considerably smaller than those of queen larvae and increased in both size and activity when worker larvae were transferred to queen cells. Copijn et al (1979) and Wirtz (1973) have also shown that three day old queen larvae have up to ten times more juvenile hormone in the haemolymph than worker larvae of the same age.

Ber-Lin Chai and Sheul (1970) found an increase in the number of ovarioles in workers they reared in vitro from four day old larvae into which they had implanted the corpora allata of three and four day old queen larvae. The effect is not induced by the corpora allata of worker larvae and therefore suggests a significant difference in the activity of the corpora allata of the two castes. A number of authors have also demonstrated that topical applications of juvenile hormones induce differentiation of queen characters in worker larvae (Asencot and Lensky, 1977 ; Goewie and Beetsma, 1976 ; Wirtz and Beetsma, 1972 ; Copijn et al, 1979). Wirtz and Beetsma (1972) showed that this was so even if three to five day old worker larvae receive normal amounts of worker jelly and grow up in worker cells, for they merely develop into worker-sized individuals with obvious queen features. Adults emerged in sixteen days instead of twenty to twenty one days and had between five and eighty ovarioles depending upon the age at which they were treated with juvenile hormone.

At present there appears to be some uncertainty concerning the precise role of juvenile hormone in differentiation. Wirtz (1973) and Wirtz and Beetsma (1972) found no evidence that the hormone has a phagostimulatory effect on larvae as suggested by Asencot and Lensky (1977). The precise link between the endocrine system and ingested reducing sugars is also not

understood (Beetsma, 1979 ; Asencot and Lensky, 1977). An important advance has recently been made by Hagenguth and Rembold (1979) who demonstrated by gas chromatography and isotope dilution that juvenile hormone 3 is the predominant hormone homolog in all three development stages of the honeybee, juvenile hormone 2 being absent and juvenile hormone 1 being present in only tract amounts.

In summary, according to our present knowledge female larvae possess the capacity for bipotent development (Weiss, 1978). Caste differentiation is the result of a dietary manipulation of larvae during the first three days of development. In presumptive worker larvae this appears to be due to reduced phagostimulation which in some way is related to the low level of juvenile hormone in the haemolymph. The low levels of reducing sugars in the diet, or the reduced rate of food intake by one to three day old worker larvae, somehow regulates corpora allata activity reducing the juvenile hormone titre by the third day to a level allowing for the determination of worker characters only. How this mechanism regulates genetic expression is unknown, as no-one has attempted to investigate this topic.

Far less understood is how a mechanism of this nature could have evolved. In examining the life histories of presocial and primitively eusocial bee and wasp species (see Michener, 1969 ; Michener, 1974 ; Spradbery, 1973 ; Wilson, 1971) it appears that the evolution of the sterile worker caste is the end result of a struggle for dominance between interacting females. In the eusocial halictine, allodapine and bombine societies, females are morphologically very similar, although in many species a gradation in size occurs. As far as is known all females are capable of being inseminated and many are in fact fertile. In most societies, however, it is usually only one female or at the most a few that actually function as egg layers. An uneasy oligarchy appears to exist, competition is rife and eating the eggs of rival layers is commonplace. That there exists a significant dominance hierarchy is evident especially among semisocial and primitively eusocial bees and wasps.

Michener and Brothers (1974) found that in the eusocial halictid bee, Lasioglossum zephyrum, colonies contain a single dominant female. What is interesting is that they were able to demonstrate that a dominance hierarchy appears to be in operation, for when they removed the queen, another bee soon adopted queenlike behaviour, and when she was removed a

third became the functional queen. They continued the process until just two bees remained, a queen and a worker. These observations suggest that the original queen inhibited ovary development and queenlike behaviour among nestmates, and it was determined that this control was achieved in part through the queen's high level of activity and frequent 'nudging' of others, the 'nudging' being particularly directed toward nestmates with larger ovaries.

Bombines also show a distinct social dominance hierarchy. Here the dominant females behave aggressively toward nestmates whenever they encroach upon their behavioural position as egg layers, and it is not uncommon for offending individuals to be mauled and even killed. Eggs laid by workers and rivals are immediately eaten by dominant females who replace them with their own eggs which are never eaten by subordinate individuals (Free and Butler, 1959).

In most primitive insect societies, therefore, queens establish and retain their behavioural position by physically dominating the other females of their colonies. Dominance based upon overt aggression is virtually absent in advanced eusocial societies such as spine bees, ants and termites. In ant and termite colonies, considered to be the most advanced eusocial insects, workers have never been observed to act aggressively towards nestmates, and there is no indication of a dominance hierarchy existing among them (Wilson, 1971). In the spine bees, the situation is not as refined. In the genus Melipona there is evidence of overt aggression among laying workers in queenless colonies and the social position of the queen may be influenced by aggression (Velthuis, 1976). Queenless workers of A. mellifera are also known to behave aggressively towards each other, in what might be considered a struggle for dominance, and in A.m. capensis large numbers of bees are actually killed (Anderson, 1963). In the presence of the Apis queen the quest for dominance is no longer an issue, for the queen is totally dominant and the workers totally dominated without any indication of overt aggression.

The question then is how can dominance by the queen be related to the mechanism of caste differentiation and the development of a totally sterile caste as found in A. mellifera? There is evidence in both halictids and bombines that their primitive caste systems are not entirely regulated by the behavioural and physiological consequences of the interactions of queens upon subordinate individuals. Knerer and Atwood (1966) found that in four North American halictine species, larvae reared in spring usually turned into small workers whereas those reared in summer were either larger

workers or queens. They found that in Evylaeus cinctipes spring reared larvae received almost half the amount of food, 33.9 milligrams compared to 61.7 milligrams in summer reared larvae. Similar observations have been made on a number of other halictine species strongly suggesting that differential feeding is a decisive factor influencing caste differentiation in this group of bees (Batra, 1966 ; Michener, 1969 ; Michener and Brothers 1974 ; Sakagami and Hayashira, 1960). Although not as well documented, a similar situation appears to exist in bombine colonies where generally undernourished larvae give rise to workers (Michener, 1969 ; Wilson, 1971 ; Free and Butler, 1959 ; Plowright and Jay, 1977).

However fortuitous differential feeding may be in these societies (it has been suggested that there may simply be too few workers or too little food to feed the brood sufficiently to induce queens) it suggests a possible early stage in the evolution of the more stringent process of differential feeding that occurs in Apis species. In bombines at least, there is some indication that the presence of the queen influences differential feeding, as the production of virgin queens is limited to the late summer. At present it is not known whether this is due simply to a reduced oviposition rate by the queen thereby increasing the worker : larva ratio (Cumber, 1949) or whether it is due to inhibitory substances or some other less obvious factor being lifted (Wilson, 1971). It is interesting that in queenless colonies of A. mellifera, where the absence of the queen ensures an increased worker : larva ratio, larvae are fed more frequently and develop into individuals having significantly more ovarioles in their ovaries than those reared under queenright conditions (Williams and Free 1975).

The suggestion that differential feeding occurs in a number of primitively eusocial bees is indeed interesting. It could be that a fortuitous situation of a similar nature existed in ancestral apine species resulting in the development of individuals that were smaller and had a lower reproductive potential, and that from this situation evolved the caste determining mechanism operative in Apis species. A fundamental difference between advanced eusocial species and semisocial and primitively eusocial species is that while females of the latter are morphologically very similar in most cases and workers are basically undernourished queens, in advanced eusocial species females are distinctly dimorphic and workers cannot be regarded as merely undernourished queens. Their dimorphism is so complete that it has affected not only the morphology, anatomy and physiology of the two castes but also their behaviour. Queens do not

attempt to forage and construct comb and do not possess the equipment to do so. They remain in the nest laying eggs and are fed by workers. Workers on the other hand have lost the ability to mate.

Several theories have been developed to attempt to explain the evolution of the worker caste. Alexander (1974) and Michener and Brothers (1974) have advocated a theory of control by the queen. They postulate that the worker caste arose by selection on queens resulting in their acquiring the ability to control the activities of their daughters, thus enhancing their own productivity. In their study of the halictid bee Lasioglossum zephyrum, Michener and Brothers (1974) found that the smaller females produced during summer were both reproductively inferior and environmentally disfavoured, and suggested that as such are subject to selective pressures at the individual level for remaining in the maternal nest. The relationship between the queen and her daughter workers is actually for their mutual good for the latter are more productive remaining with the queen than attempting to establish their own nests (which they are capable of doing) while at the same time their presence is beneficial to the queen.

An earlier theory (Lin and Michener, 1972) proposed that worker and queen castes arose by selection such that they evolved different social attributes simultaneously beneficial to each other. While agreeing that social attributes beneficial to both castes have arisen, Michener and Brothers (1974) suggest that such features could result from individual selection acting on the queen alone. Particularly, they suggest that the evolution of worker attributes that permit control by the queen could in fact have arisen by selection favouring queens that produce controllable workers. However while they emphasise the theory that queens have evolved to control workers, they do not exclude the possibility that workers subsequently evolved in some way to assist the queen.

The presence of altruistic traits in the worker caste, they maintain, is a consequence of advanced eusociality and cannot be used to explain its evolution as has been attempted by some authors (Hamilton, 1964 and 1972 ; West Eberhard, 1975 ; Wilson, 1971 and 1975). This conflicting theory, proposed by Hamilton (1964), is based upon the genetic asymmetry that exists in the Hymenoptera where males are haploid and females diploid, resulting in females sharing a greater proportion of their genes with their sisters than with their daughters. As every organism behaves so as to enhance the proportion of its own genes in the next generation and as the average fraction of genes shared by common descent among hymenopteran

females is 0.75 between sisters compared to 0.5 between mother and daughter, Hamilton (1964) proposed that workers sacrificed their reproductive potential in favour of caring for their younger sisters. Thus rather than advocating that sociality arose through selection upon dominant females to produce controllable nestmates, this theory proposed that workers evolved to assist their mothers produce more offspring, the reduced reproductivity of workers (which Hamilton regards as altruistic) being more than compensated in terms of fitness by the increased reproductivity of mothers.

The secretion of pheromones as continuous chemical signals by Apis queens toward workers, influencing both their physiology and behaviour, seems to support the view that dimorphism and particularly worker sterility are products of queen control and the struggle for dominance. Among certain species of the wasp subfamily Polistinae, and in all species of Vespinae, there is a complete dimorphism of queens and workers which appears to be entirely regulated by differential feeding as a result of the ratio of workers to larvae (Wilson, 1971). Queens are not known to produce pheromones and their influence on differential feeding, if any, is unknown. In bombines, where a similar process is in operation, there is some indication that queens exert an influence whether it be due to inhibitory substances or some other less obvious factor (Wilson, 1971). The presence of castes in these hymenopteran groups suggests that a simila· situation probably existed in the ancestors of Apis and that from this situation evolved chemical signals enforcing differential feeding and ultimately a totally sterile worker caste.

The evolution of chemical signals is thought to be one of the most significant milestones in the evolution of the honeybees. They enable the queens to retain control over the reproductive capacity of workers (apparent under queenless conditions when workers may become functional egg layers) by means other than overt aggression, thus allowing them to lay many more eggs. It is thought, therefore, that the advent of queen pheromones may be implicated in the evolution of colonies of huge proportions and of perennial existence. The signals seem to have established the behavioural position of the queen as egg layer within the Apis colony, so much so that workers are incapable of competing for the egg laying role as they are morphologically incapable of fertilisation. Ultimately, queen pheromones probably played a part in influencing subsequent evolution in the then dominant queen caste leading to

individuals that were, among other roles, specialist egg layers incapable of foraging or nursing. They evolved to become totally dependent on their daughter workers for their existence, yet their behaviourally dominant position assured them that very existence.

Undoubtedly the evolution of pheromones in general has been of enormous significance in the evolution of eusociality in insects, for while there are the expected visual, tactile and auditory signals common among all insect species, chemical signals have been associated with almost every communication system of eusocial species. Pheromones appear to be involved in the recognition of nestmates, in recruitment, alarm and assembly as well as in the regulation of social interactions between queens and workers (Wilson, 1971).

Apis mellifera has at least eleven major exocrine glands, the secretions of six of which are known to have pheromonal activity. Of these six, three are active in the worker caste only, the pheromones of which are associated with foraging and colony defence. As can be seen from Table 1.2. (compiled at the onset of this study) only the queen produces pheromones associated with dominance, and the only pheromone components that have been identified are found in the mandibular gland secretion.

Hess (1942) provided the first indication that ovary development in the worker caste was suppressed through direct contact between the queen and the workers. She postulated that ovary development occurred in the absence of the queen either through lack of an inhibitory substance which the queen produced and which workers obtained directly from her, or by the accumulation of food which workers would normally have fed to the queen. It was not until the next decade that Butler (1954a) suggested that the queen secreted a substance (which he termed 'queen substance') that enabled workers to recognise her presence in the hive. The following year, Voogd (1955) extracted a substance of fatty acid character from the queen that not only attracted attendant workers, but also inhibited their ovary development. Subsequently 'queen substance' was also found to inhibit queen rearing (Butler and Gibbons, 1958) and in the same year shown to be produced by the mandibular gland (Butler and Simpson, 1958).

'Queen substance' was finally isolated and identified as 9-keto-(E)-2-decenoic acid (hereafter designated 9KDA) by Callow and Johnston (1960) and Barbier and Lederer (1960), and was synthesised by Callow and Johnston (1960) and Barbier et al (1960). The acid itself

Table 1.2. Glandular source, chemical identity and function of some pheromones of Apis mellifera queens and workers

Glandular source	Queens	Workers
Mandibular gland	9-keto-(E)-2-decenoic acid Inhibition of queen rearing (Butler et al, 1962) Inhibition of ovary development in worker bees (Butler and Fairey, 1963) Sex pheromone (Gary, 1962 and 1963) Promotes foraging (Jaycox, 1970 ; Showers, 1967) Evokes retinue behaviour (Velthuis and van Es, 1964) Worker attractant during swarming (Morse and Boch, 1971) 9-hydroxy-(E)-2-decenoic acid Stabilises swarms (Butler et al, 1964) Functions with 9-keto in inhibition of queen rearing and ovary development in workers (Velthuis, 1976)	9-keto-(E)-2-decenoic acid Present in laying workers (Velthuis, 1976 ; Crewe and Velthuis, 1980 ; Ruttner et al, 1976) 2-heptanone Repels workers from foraging sites (Butler, 1966 ; Simpson, 1966) Aggressive responses in guard bees (Maschwitz, 1964) 10-hydroxy-(E)-2-decenoic acid Function unknown (Boch and Shearer, 1967)
Koschevnikov's gland	Pheromone unidentified Attracts workers in hive (Butler and Simpson, 1965)	-
Dorsal abdominal glands	Pheromone unidentified Arrestant in retinue behaviour (Velthuis, 1970a) Increases copulatory activity (Renner and Vierling, 1977)	-

Table 1.2. (continued)

Glandular source	Queens	Workers
Nassanoff's gland	-	Citral, Geraniol, Geranic acid, Nerolic acid Attraction leading to assembly (Butler, et al, 1970) Foraging (Boch and Shearer, 1962 ; (Butler and Calam, 1969) Swarming (Morse and Boch, 1971)
Sting Gland	-	Iso-pentyl acetate Alarm (Boch et al, 1962) Stops Nassanoff's scenting (Morse, 1972) Other chemicals Alarm (Blum et al, 1978 ; Koeniger et al, 1979)
Arnhart's gland	-	Pheromone unknown Followed by workers on foot (Chauvin, 1962 ; Butler et al, 1969)

Table 1.2. (continued)

Glandular source	Queens	Workers
Nassanoff's gland	-	Citral, Geraniol, Geranic acid, Nerolic acid Attraction leading to assembly (Butler, et al, 1970) Foraging (Boch and Shearer, 1962 ; (Butler and Calam, 1969) Swarming (Morse and Boch, 1971)
Sting Gland	-	Iso-pentyl acetate Alarm (Boch et al, 1962) Stops Nassanoff's scenting (Morse, 1972)
		Other chemicals Alarm (Blum et al, 1978 ; Koeniger et al, 1979)
Arnhart's gland	-	Pheromone unknown Followed by workers on foot (Chauvin, 1962 ; Butler et al, 1969)

was then shown to have a significant inhibitory influence on both queen rearing and ovary development in worker bees (Butler and Fairey, 1963 ; Butler and Paton, 1962 ; Butler et al, 1962) and was also found to be the primary sex pheromone (Gary, 1962 and 1963).

The possibility of additional substances being present in the mandibular gland pheromone led Callow et al (1964) to make a complete chemical study of the secretion. They determined by gas chromatography the presence of thirty two components, fourteen of which were identified by comparing relative retention times of natural and synthetic materials, and Butler and Fairey (1964), working with Callow were able to show that the secretion contained at least a second active chemical, 9-hydroxy-(E)-2-decenoic acid (hereafter designated 9HDA). They did not find that the substance reinforced the effects attributed to 9KDA, except that it may have acted as a secondary component attractive to drones. Blum et al (1971) subsequently showed that 9HDA was not attractive to drones, while Velthuis (1976) found that the chemical has some activity on worker physiology and behaviour in that it evokes a certain degree of retinue behaviour and decreases ovary development when presented to queenless workers. There is also some evidence that it suppresses emergency cell construction albeit to a lesser degree than 9KDA (Butler and Callow, 1968). Butler and Fairey (1964) and Butler et al (1964) found evidence that 9HDA was the only substance produced by the queen that functioned to stabilise swarm clusters. Later Butler and Simpson (1967) concluded that 9KDA and 9HDA were complementary in action during swarming, 9KDA acting to attract flying bees, 9HDA stimulating alighting and clustering, but Morse and Boch (1971) subsequently questioned this conclusion. They fou that alighting bees released Nasanoff's gland pheromones and proposed that these pheromones in concert with 9KDA were entirely responsible for attraction and stabilisation of the swarm cluster.

It remains then, that at present only two components of the queen mandibular gland secretion are known to have pheromonal properties, the role of one of which is questionable. 9KDA, the major component of the secretion appears to have a multiplicity of functions in the life of the honeybee colony. Outside the nest it not only functions as the primary sex pheromone during the mating flights of young virgin queens, but it also enables workers to locate the presence of their queen during the swarming process. Within the hive, 9KDA is largely responsible for the inhibition of queen rearing and the suppression of ovary development in worker bees. In addition, the special treatment afforded the queen

in the hive, including retinue behaviour, feeding and grooming is due at least in part to the influences of 9KDA (Gary, 1961a and 1974 ; Velthuis and van Es, 1964 ; Zmarlicki and Morse, 1964 ; Butler et al, 1973).

9KDA has never been found to be as effective as whole mandibular gland extracts. Velthuis (1976) demonstrated that 9HDA evoked similar responses from worker bees suggesting that while 9KDA is the major component of the mandibular gland secretion, the queen pheromone is at least a combination of both 9KDA and 9HDA, and is more likely to involve additional components as well. The fact that queens partially retain their dominant position without their mandibular glands caused some authors to investigate the possibility that other glands of the queen or other factors within the nest influence the physiology and behaviour of workers (Velthuis, 1970a ; Gary and Morse, 1962 ; Velthuis and van Es, 1964). Gary and Morse (1962) postulated that either 9KDA or other as yet unidentified inhibitory substances were being produced in glands other than the mandibular glands, or that other factors such as physical contact were involved.

Butler and Simpson (1965) found some evidence to indicate that a volatile substance secreted by Koschevnikov's gland in the sting chamber is attractive to workers, while Velthuis (1970a) proposed that dermal glands on the dorsal surface of the queen abdomen (beneath the second, third and fourth tergites) were responsible for enabling queens to maintain dominance without their mandibular glands. Vierling and Renner (1977) and Renner and Vierling (1977) have demonstrated that drones and young workers are strongly attracted to the abdominal gland secretion. They found that the secretion functioned as a close-up attractant increasing copulatory activity in drones and stabilising the worker court around the queen within the nest.

In addition to the possibility of other pheromone sources being suggested as influencing the dominant position held by the queen, there is evidence that the presence of worker brood has an inhibitory effect on ovary development in worker bees. Jay (1970 ; 1972) found that worker brood inhibited ovary development significantly more than did queens without brood, and suggested that a volatile scent produced by developing larvae was responsible. Similar results were obtained by Kropacova and Haslbachova (1970 ; 1971) when they examined ovary development in swarming honeybees. They found that workers showed significantly greater development both in the absence of larvae and in the absence of the queen, but the former had the greater influence.

It is apparent that the question of dominance cannot be completely answered in terms of the quantity of 9KDA alone. Other interacting factors are undoubtedly involved in creating an overall effect on the interactions between the two castes. Not yet mentioned are factors such as seasonal and circadian fluctuations in pheromone production by queens and workers observed by Pain and Roger (1970 ; 1978) and Pain et al (1972 ; 1974) and the marked seasonal variation in the trophallactic transfer of food among workers (Donault et al, 1975). Physiological changes of this nature strongly suggest that similar changes may occur in receptivity to dominance signals by worker bees, and also in the release of these signals by queens. These physiological factors may be responsible for the apparent fluctuations in the inhibitory effect of 9KDA observed by some authors. For instance, Jay (1975) found that the presence of worker comb appeared to stimulate ovary development in workers while the queen had little effect, but later Jay and Jay (1976) found the inhibitory effect of the queen to be greatly increased while comb had no effect. Kropacova and Haslbachova (1969) found evidence for both seasonal and intracolonial variations in ovary development in workers, including reduced development during honey flows and increased development at the onset of winter and immediately after swarming.

Undoubtedly the relationships existing between the queen and worker castes are complex, being influenced by a number of interrelated factors. The important role played by the mandibular gland secretion, and by 9KDA in particular, cannot be denied however. Velthuis (1972) concluded that the sensory perception of 9KDA on the body of the queen and on the bodies of workers that attended her played the major role in evoking queenright behaviour among worker bees, while Butler (1973) attributed the very 'spirit of the hive' to its influence and effect upon the physiology and behaviour of the worker caste. Since the mandibular gland secretion of the queen appears to be critical in determining social interactions between the female castes, the investigation of the secretions of these glands in both A.m. adansonii workers and queens will form the basis of this study.

The production of 9KDA by the mandibular glands of newly-emerged virgin queens of A.m. mellifera appears on average to be low until the fourth or fifth day immediately before their mating flights. Young queens between one and two days old were found to have less than ten micrograms (Velthuis, 1970 ; Butler and Paton, 1962) although Crewe and Velthuis (1980) have found their production to be much higher. That

the dominance of queens is influenced by the secretion of 9KDA is further evident in that young virgin queens are neither able to attract workers as effectively as mated laying queens (Gary, 1961a ; Szabo, 1974a and 1974b ; Yadava and Smith, 1971b) nor are their mandibular gland secretions able to inhibit ovary development effectively in workers (Jay, 1968) or suppress emergency queen cell construction (Butler and Paton, 1962). After about the fifth day, however, 9KDA production increases markedly. Butler and Paton (1962) estimated that the average production of five to ten day old virgins was about 132.5 micrograms, while Pain et al (1967) found that five to seven day olds produced between 28.5 and 356.0 micrograms with a mean of 108.2 micrograms. Pain et al (1972) and Pain et al (1974) found that twenty three day old virgins produced from two hundred to a thousand micrograms of 9KDA. These queens were confined in experimental cages with groups of only forty workers and Pain et al (1972) suggested that the large amounts of 9KDA were probably due to its accumulation because of the abnormally low level of distribution.

On average it has been found that mated laying queens normally have about two hundred micrograms of 9KDA (Callow et al, 1964 ; Shearer et al, 1970). Butler and Paton (1962) estimated that three to six week old mated queens average about one hundred and fifty micrograms, the amount dropping to less than one hundred in queens over eighteen months. Butler et al, (1973) who found between eight and five hundred and twenty micrograms of 9KDA in mated queens of various age, demonstrated that a positive correlation existed between the amount of 9KDA and the size of the court of workers attracted by the queen, the lower amounts attracting as few as four bees a minute while the higher amounts attracted up to sixty nine. Recently Crewe and Velthuis (1980) found an average of 71.2 micrograms of 9KDA in five mated A.m. mellifera queens, the chemical constituting 36.1 per cent of the total acid secretion of the mandibular glands.

The secretion of the worker mandibular glands has also been extensively studied. Shearer and Boch (1965) identified the presence of 2-heptanone, a volatile chemical which evoked alarm. However, as up to seventy times as much 2-heptanone is required to elicit the same responses as iso-pentyl acetate (an alarm pheromone from the sting gland) its significance as an alarm substance is equivocal (Boch et al, 1970). 2-heptanone has been shown to repel foragers (Butler, 1966 ; Simpson, 1966) and Simpson (1966) has suggested that as such the

chemical may be used to repel robbers from other nests. In workers secretion begins in the second week and gradually rises to about forty micrograms (Boch and Shearer, 1967 ; Crewe and Hastings, 1976). 2-heptanone is not secreted by queens.

Boch and Shearer (1967) have also followed the production of a second substance, 10-hydroxy-(E)-2-decenoic acid (hereafter 10HDA) with age, finding that while newly-emerged bees have only trace amounts, up to sixty micrograms may be found in bees after about three weeks. This chemical, although closely related in structure to 9KDA and 9HDA, and also secreted by the queen mandibular glands (Callow et al, 1964) has not been shown to have pheromonal activity. It was first identified in the mandibular glands of workers by Barker et al, (1959).

Until fairly recently it was thought that 9KDA was 'produced entirely by the queen's mandibular glands' (Wilson, 1971). This was accepted despite the fact that earlier Velthuis et al (1965) had shown that laying workers taken from queenless colonies and placed in experimental cages with fifty newly-emerged bees not only inhibited ovary development but also evoked retinue behaviour. Head extracts from these bees evoked the same reactions when presented on objects to small groups of bees and strongly suggested that laying workers were secreting 9KDA. Similar results were obtained by Jay and Nelson (1973) who found that functional laying workers effectively inhibited ovary development in worker bees. It is now known that the laying workers of A.m. capensis (Ruttner et al, 1976) and A.m. mellifera (Crewe and Velthuis, 1980) can produce significant amounts of 9KDA. The former effectively inhibit ovary development and attract courts of up to fifteen workers, a response comparable to that evoked by A.m. carnica queens (Ruttner et al, 1976).

Queenright workers of a number of A. mellifera races, on the other hand, are known not to produce 9KDA. These include A.m. ligustica (Pain et al, 1967), A.m. carnica (Ruttner et al, 1976) and A.m. mellifera (Crewe and Velthuis, 1980). At present the only known exception is A.m. capensis where queenright workers have been found to have very small amounts of 9KDA in their mandibular gland secretion (Crewe, unpublished data). No work has been done on the mandibular gland secretions of workers of other Apis species, and information of this nature would be most useful in the light of data recorded below.

It is interesting that the number of ovarioles in the ovaries of worker bees of A. mellifera races varies according to the amount of 9KDA in the mandibular gland secretion of the queens. A.m. mellifera workers

have on average about three to five ovarioles per ovary (Velthuis et al, 1971), A.m. ligustica workers between four and nine (Weaver, 1956 ; Levin and Haydak, 1951), and A.m. capensis workers have on average nearly twenty (Anderson, 1963). The size of the spermatheca also varies accordingly, that of A.m. mellifera measures approximately 0.11 x 0.08 millimetres (Hess, 1942), that of A.m. adansonii 0.13 x 0.17 millimetres (Anderson, 1963) while that of A.m. capensis measures 0.28 x 0.33 millimetres, nearly half the size of the spermatheca of the queen (Anderson, 1963).

The number of ovarioles in the ovaries of workers of the four Apis species also shows marked differences. With the exception of A.m. capensis, the races of A. mellifera average about four to six ovarioles per ovary with a range of between one and twenty four (Velthuis, 1970b; Chaud-Netto and Bueno, 1979). A. indica (=cerana) average nearly ten ranging from one to thirty (Velthuis, 1976). A. florea average nearly five ranging from one to nine (Velthuis, 1976) and A.m. dorsata twenty four ovarioles per ovary ranging between eleven and fifty three (Velthuis, 1976 ; Velthuis et al, 1971). The variations in the number of ovarioles in worker ovaries has little significance in itself, but Velthuis (1976) considers the difference in number between workers and their queens to be important and suggests that it may be indicative of the degree of difference between the castes. Using this as a criterion, the four species can be arranged in the following order : mellifera, florea, indica (=cerana) and dorsata as the queens of each species average one hundred and fifty, seventy, seventy and one hundred and thirty respectively (Velthuis, 1976). As it is generally considered that A. dorsata and A. florea are more primitive, the difference in the number of ovarioles in queens and workers may well be a good measure of the degree of differentiation between the castes.

Weaver (1955) estimated that in the A. mellifera colonies with which he was working, normal queens had between one hundred and fifteen to two hundred and thirty eight ovarioles per ovary (mean one hundred and sixty nine) while workers had only four. Queens reared in vitro from young undifferentiated larvae fed on fresh royal jelly, however, had one hundred and two to one hundred and sixty one ovarioles per ovary (mean one hundred and twenty four). Furthermore, larvae fed on various diets of stored royal jelly developed into intermediate individuals (whose ovariole count ranged from sixty six to one hundred and thirty five) and completely differentiated workers with only four

ovarioles.

Intermediates of this nature are almost entirely limited to artificial rearing and there is very little evidence to suggest they occur in natural colonies of A. mellifera except that Eckert (1934) did find an intermediate in a nucleus swarm which was no bigger than a worker. This individual had two hundred and thirty one ovarioles, substantially more than Weaver's estimates for average queens. Eckert (1934) also recorded substantially higher ovariole counts for queens, numbers varying from two hundred and sixty to three hundred and seventy three. These estimates compare favourably with those of Woyke (1971) who found that queens reared from eggs had between two hundred and ninety five and three hundred and forty ovarioles per ovary, the numbers steadily decreasing to only one hundred and seventy seven to two hundred and sixty three for queens reared from four day old larvae.

In A. mellifera caste differentiation appears to have evolved to a significantly higher level than in other Apis species. While the workers of the other species do not bear any morphological features indicating a degree of intermediacy, the fact that the ovariole ratio between queens and workers is substantially lower than in A. mellifera (with the exceptions of A.m. capensis and perhaps A.m. ligustica) and the fact that the laying workers function as 'false' queens, suggests that caste differentiation in these species is either of a different nature or of a lower degree.

While queens successfully maintain a dominant position among their daughters owing to the advantage gained by their differentiation, in their absence there is every indication that a struggle for dominance ensues among the remaining workers. If a new queen is not reared, certain individuals eventually become functional laying workers and may act as 'false' queens (Sakagami, 1958 ; Ruttner et al, 1976 ; Velthuis et al, 1971). That this has probably been the outcome of a struggle for dominance has been suggested by Anderson (1963) who found that in response to the loss of the queen in A.m. capensis colonies, widespread fighting among workers ensued until the emergence of functional laying workers, whereupon the fighting abated. Anderson (1968) commonly found that hive deaths rose ninefold to over five hundred daily for a period of about twelve days following loss of the queen. He (Anderson, 1963) suggested that this phenomenon may indicate that laying workers become dominant, stabilizing the colony in much the same way as the queen. The fights that occur in these queenless colonies are not the result of

interactions between laying workers, but are actually interactions between laying workers and other aggressive bees whose ovaries were found to be only partially developed (Velthuis, 1976). The reason for this is not clear. Velthuis (1976) concluded that the laying workers were at the same time dominant in a reproductive sense but submissive behaviourally. However in light of the work done by Yadava and Smith (1971a ; 1971b ; 1971c) and Gary (1961a ; 1961b) who found that workers behave aggressively toward young or strange queens that have a different degree of attraction from their own queen, the aggressiveness shown to laying workers may be due to a similar reaction, the laying workers being regarded as strange queens because of a change in their mandibular gland secretion.

It appears that further research on the mandibular gland secretions of queens and workers of other A. mellifera races (and indeed the other Apis species) is imperative for a complete explanation regarding the complex social interactions that occur between the castes. For this reason it was decided that an investigation of this nature on the south african honeybee, A.m. adansonii, would be significant in supplying some of the critical comparative data necessary. An important feature regarding this race is that while queenless colonies contain functional laying workers, these individuals are not known to attract a court of workers (Fletcher, pers. comm.). Furthermore fighting to the degree observed in queenless A.m. capensis colonies never occurs and increased death rates during the first few days of queenlessness are very rare (Fletcher, pers. comm).

These observations suggest that A.m. adansonii laying workers are unable to secrete sufficient amounts of 9KDA to elicit obvious queenright responses from nestmates during queenless periods. In order to test this hypothesis, it was decided to analyse the mandibular gland secretions of queens and workers, both queenright and queenless, to determine quantitative and qualitative differences of major components. In addition, it was decided to compare the mandibular gland secretions with the number of ovarioles and the degree of ovary development found in worker bees of both queenright and queenless colonies. It was thought that this information would indicate the social relationships or interactions occurring in A.m. adansonii colonies and in so doing increase our understanding regarding these interactions, dominance and caste differentiation in Apis.

MATERIALS AND METHODS

A small apiary consisting of a varying number of colonies (between five and ten) was established on the University of the Witwatersrand farm, Frankenwald, just north of Johannesburg in January, 1978. In addition, one and occassionally two observation colonies were kept in the Zoology Department from nucleus swarms split from parent colonies in the apiary. Almost all the biological material analysed during this study was obtained from either of these two sources. Apart from these sources, six queens were obtained from Zimbabwe, and a small queenless colony was provided by a local beekeeper.

The apiary colonies were housed in standard, ten-frame Langstroth hives, consisting of a brood box and none, one or two supers depending upon the strength of each colony. On one occasion when a large number of young bees was required for an experiment, a strong colony with a particularly active queen was supplied with a second brood box for a short period. The stronger colonies probably had on the order of sixty thousand workers during the summer while weaker ones had about thirty to forty thousand.

The observation colonies were established in structures consisting of three brood frames arranged vertically one above the other in a wooden framework with transparent perspex side walls. The perspex side wall sheets were perforated by a series of circular windows (diameter, five centimetres) normally closed by perspex squares held in position by fixings mounted onto the sheets. The perspex side walls enabled the bees to be observed without interference, while the windows allowed access to the colony for sampling purposes. The hives were normally enclosed by a warm woolen cover providing satisfactory insulation against excessive heat loss. Workers were able to forage via an entrance funnel passing through a hole cut into an adjacent window pane.

Because of the small size of the observation hives, colonies rapidly became overcrowded in spring and it was frequently necessary to remove excess bees to prevent swarming. Occasionally queens were also removed in order to control the population, their heads being retained for analytical purposes. The small size of the hives rapidly led to the reverse situation in winter, namely that numbers dropped to such a

degree that it was necessary for colonies to be strengthened by the addition of young bees hatched in incubators to avoid extinction. Also, as honey and pollen reserves were largely limited to a single comb, it was necessary to supply colonies with honey-sucrose water and pollen to prevent starvation, as winter conditions in Johannesburg usually restricted foraging to a few hours each day.

Biological Material

Queens

The head extracts of fifteen queens were analysed during the study, six of which were obtained from Mr M. Schmolke of Zimbabwe, the remaining nine coming from the Transvaal. The head extracts donated by Mr Schmolke were from queens that had been reared during his queen breeding programme. The programme is unique in that the queens are reared in queenright colonies in specially designed long hives in which the colony queens are restricted at one end of the structure while the programme is operated at the opposite end under an apparently induced queenless situation. Four of the queens supplied were newly-hatched virgins less than sixty hours old, while the remaining two were mated queens whose ages were unknown. Also unknown were the weights of the queens and the time of the year in which they were reared.

The Transvaal queens analysed during the study consisted of three virgin and six mated individuals. Significant details regarding each of the queens are as follows:-

Queen 6. A thirty six to forty eight hour old virgin queen weighing two hundred and twelve milligrams. This queen was one of a number donated by Mr Frank Steinhobel during his queen breeding programme at the Aloe honeyflow in August 1978. The unhatched queens were brought to the Frankenwald apiary in small 'hair-curler' cages in a nucleus hive containing bees that had been queenless. After two days it was found that all the queens had emerged, but that only two had survived. One was allowed to become the colony queen, while the other was killed for analytical purposes.

Queen 5. A twenty four to thirty six hour old virgin queen weighing two hundred and twenty milligrams, removed from one of the field hives in May 1979 while still in the queen cell. She eclosed eleven days after dequeening indicating that she had been a two to three day old larva when selected for queen rearing by the workers.

Queen 7. A forty day old virgin reared in a particularly strong field colony in November 1978. She eclosed fifteen days after dequeening indicating that she was reared as a queen throughout her larval development. Her queen cell was secured within a small gauze cage of approximately fifty cubic centimetres in volume and left in the brood chamber of the parent colony which at that time was queenless. What is remarkable is that she was not rejected by the workers as the colony was queenright throughout her confinement, the colony queen having been reared at the same time.

Queen 13. A fifteen week old mated, laying queen reared in an observation hive by queenless bees separated from one of the apiary hives in October 1978. She eclosed sixteen days after the hive was established indicating that she too was reared as a queen throughout larval development.

Queen 11. A three week old mated, laying queen weighing two hundred and fifty five milligrams when analysed. This queen was reared in an observation hive after natural swarming had occurred in mid October 1979.

Queen 10. A two week old mated queen weighing two hundred and sixty milligrams, reared in an observation hive in November 1979 following removal of Queen 11. She eclosed fourteen days after dequeening indicating that she was reared as a queen throughout larval development. When analysed she had just started to lay eggs.

Queen 12. A mated, laying queen weighing one hundred and thirty nine milligrams, removed from a moderately strong field colony in June, 1979. The presence of eggs and a queen in the hive only eight days later when frames were examined for queen cells, strongly suggesting the possibility that the queens had coexisted, a phenomenon apparently not as rare as once assumed (Pretorius, pers. comm.).

Queen 14. A mated, laying queen weighing two hundred and five milligrams, removed from a relatively weak field colony during May 1979.

Queen 15. A mated, laying queen weighing two hundred and six milligrams, removed from the same hive as Queen 14 in May 1979 only seven days after the latter was removed, suggesting that these queens also coexisted.

The procedure employed for the analysis of queen head extracts was the same as that used for analysis of worker head extracts and is described under laboratory methods elsewhere in this chapter.

Workers

Four separate analyses were made of unrelated groups of workers under

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