

THE UNIVERSITY OF MANITOBA

BEHAVIOURAL STUDIES OF QUEEN HONEY BEES
(APIS MELLIFERA L.) WITH WORKERS AND OTHER QUEENS

by

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ENTOMOLOGY

WINNIPEG, MANITOBA

May, 1976



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ABSTRACT

Observations of virgin queens, mated queens, and worker bees in one-frame observation hives were made to provide more information about the behaviour of queens and worker bees, and to investigate the problems which may be encountered in the establishment of multiple-queen colonies. The behaviour of queens and worker bees was observed under the following three conditions: (1) when there was one queen in a hive, (2) when there were two queens of the same kind in a hive, and (3) when there was one queen, with a set of queen cells containing queen brood of different ages, in a hive.

While moving among worker bees in the hive a virgin queen displayed both non-aggressive and aggressive behaviour while a mated queen displayed only non-aggressive behaviour. Worker bees displayed non-aggressive and aggressive behaviour towards both virgin and mated queens. Generally, older worker bees displayed various kinds of behaviour towards both kinds of queens more frequently than did younger worker bees. No regular sequence, in behavioural patterns of virgin queens, mated queens, and worker bees, was found. The number of times each type of behaviour was displayed, and the length of time spent by the virgin queens, the mated queens, or the worker bees in performing each type of

behaviour was also recorded.

The stings of virgin and mated queens were clipped in some experiments. When two virgin, or two mated queens, (with clipped, or unclipped, stings) met each other they usually fought by pawing, holding, biting, and attempting to sting each other, but only one queen was ever killed. The virgin queens appeared to be as aggressive as the mated queens. The worker bees, especially the older ones, reacted aggressively towards any pair of queens introduced to their hive and usually balled and killed one of them. The balling behaviour of worker bees, the cause(s) of balling, and the problems concerning the establishment of multiple-queen colonies are discussed.

Both virgin and mated queens attacked queen cells by making holes through the walls of the cells. They spent more time attacking the capped queen cells than they did the uncapped ones. Although virgin queens preferred attacking capped queen cells mated queens showed no preference. The behaviour of worker bees towards queen cells is also discussed.

Preliminary experiments were done to investigate why queens attack queen cells. The results suggest that the odour of the queen pupae and/or of other related materials (e.g. food, faeces,

cocoons, etc.) inside queen cells may stimulate queens to attack them.

The following are new observations made during the course of this study: (1) offering (food) behaviour displayed by virgin and mated queens towards worker bees, (2) holding, holding - biting, holding - biting - stinging behaviour displayed by virgin queens towards worker or drone bees, (3) "begging" (food) and receiving (food) behaviour displayed by worker bees towards virgin and mated queens, (4) the method queens used to kill each other, i. e. the "victorious" queen inserts her sting through the membrane around the base of the coxa of any leg of her rival queen, and (5) the difference in the preference of virgin queens and of mated queens in attacking different kinds of queen cells.

ACKNOWLEDGMENTS

I wish to express my sincere appreciation to Dr. S. C. Jay (Professor, Department of Entomology, University of Manitoba), for his generous encouragement and invaluable guidance during my graduate program. The helpful criticisms and suggestions of Dr. A. G. Robinson (Professor and Head, Department of Entomology, University of Manitoba), Dr. A. K. Storgaard (Professor, Department of Plant Science, University of Manitoba), and Dr. R. W. Shuel (Professor, Department of Environmental Biology, University of Guelph, Ontario) are also gratefully appreciated. I thank Mr. M. Bickis (Agriculture Canada Research Station, Winnipeg, Manitoba) for his help with the statistical analyses. I am also indebted to Dr. J. H. MacFarlane, Mr. R. A. Graham, Mr. J. Lintott, Miss J. Wright, and Miss L. Taylor for their invaluable assistance.

Special thanks are due to my wife, Phanna, for her understanding, patience, and continuous encouragement and to Professor and Mrs. A. G. Robinson, my Canadian parents.

Financial support for my Ph.D. program from the University of Manitoba Graduate Fellowship is gratefully appreciated.

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CHAPTER 1

INTRODUCTION

Under normal conditions a honey bee colony is a monogynous society consisting of one queen, several hundred drones, and 20,000-60,000 worker bees. A new queen is reared by worker bees when the old queen dies or becomes injured, or during the swarming period. The number of queen cells reared under the swarming impulse is generally higher than the number of queen cells reared under the emergency or supersedure impulses. Although more than one queen is usually produced in a colony, eventually only one queen survives. In cases of supersedure or swarming the mother and daughter queens might live and work together for a short period of time, particularly during inclement weather when the swarm cannot leave the hive.

It is well known that when virgin queens meet one another they fight vigorously. Many researchers (e. g. Huber, 1814; Park, 1952; Butler, 1954a, 1975; Gary, 1975) have reported that the "victorious" queen kills her rival queens either by fighting (if they have already emerged from the queen cells) or by chewing holes in the queen cells and stinging the emerging queens inside the cells.

1.1 The Problem

Only a few, rather brief, descriptions exist in the literature about how rival queens (virgin or mated) engage each other "in battle", how a queen kills her rival, or how queens treat queen cells containing queen brood of various ages. Such information would provide a better understanding of social behaviour fundamental to the survival of a honey bee colony. As well, it may provide a means of establishing multiple-queen colonies which would allow honey bee populations to be built up quickly for specific nectar flows and/or for the pollination of specific commercial crops.

1.2 Scope of Study

This study was done using observation hives containing worker bees, and concerned the behaviour of one newly emerged virgin queen, of two newly emerged virgin queens towards each other, and of one newly emerged virgin queen towards various kinds of queen cells. The above study was repeated using young mated queens. In addition, the behaviour of worker bees of known ages towards the queens and the queen cells in each of the above experiments was studied. Their behaviour and behavioural patterns were carefully observed, analyzed, described, and compared. Moreover, preliminary experiments were done to investigate why queen cells are

attacked by virgin or mated queens.

Because this study required many virgin queens, mated queens, and queen cells, I also investigated various methods of rearing queens and storing them.

CHAPTER 2

LITERATURE REVIEW

2.1 Behaviour of a Queen and Worker Bees in a Single-Queen

Colony

2.1.1 Behaviour of a Virgin Queen

After emerging from her cell, the virgin queen displays various types of behaviour. Butler (1954a) found that a queen feeds herself with honey contained in the combs or is fed by worker bees soon after she emerges from her cell. Solodkova (1958) observed that a queen moves quickly over the combs during the first two days after emerging from her cell but later she is slower and rests more frequently on the combs. Hammann (1958) reported that during the first few days of her life the queen always runs away from worker bees in the hive. However, if she is not disturbed, the queen usually wanders within the brood area of the colony (Butler, 1954a). When she is about three days old, if attacked by worker bees, the queen raises her body, stands on her third pair of legs, and strikes the attacking worker bees with her thorax and fore-legs (Hammann, 1958). At this age, the queen also opens and vibrates the tip of her

abdomen and occasionally dips it into some worker cells for short periods of time; Hammann (1958) suggested that this indicates that the queen is now sexually mature.

When a colony rears more than one queen at a time, "piping" sounds are usually heard from both the newly emerged virgin queens and the virgin queens which are about to emerge from their cells (Butler, 1954a, 1975). Snodgrass (1925) and Butler (1954a) suggested that the piping sounds are produced when a queen vibrates small sclerotized plates located at the wing bases without actually spreading the wings. Simpson (1964) concluded that the piping sound is radiated through the comb when the queen presses her thorax against it. Butler (1954a, 1975) wrote that virgin queens locate each other by their piping sounds. Huber (1814), Hammann (1958) and Butler (1975) found that the piping sounds of a queen also stop all activities of the worker bees around her.

2.1.2 Behaviour of a Mated Queen While Wandering in the Colony

The most important function of a mated queen is the laying of eggs. A mated queen usually starts laying eggs on the second to fourth day after her last successful mating flight (Park, 1952; Butler, 1954a). Gary (1975) stated that some mated queens

lay as early as 14 hours after mating. Butler (1954a) observed that, in searching for clean cells for laying eggs, the queen usually moves at random within the area on the comb which the worker bees have prepared for her. Park (1952), Butler (1975), and Gary (1975) stated that the queen examines each cell before laying in it.

Generally, the queen spends 9 - 12 seconds in laying an egg (i. e. pushing her abdomen into a cell, depositing one egg, and withdrawing the abdomen from the cell) (Gary, 1975). Butler (1954a) suggested that worker bees control the rate of egg laying of the queen by "expanding and contracting" the brood area in the colony according to the season. Chauvin (1956) reported that, during the active season, external conditions do not affect the rate of egg laying of the queen but that the quantity of food which worker bees feed the queen does.

2.1.3 Behaviour of Worker Bees Towards a Virgin and a Mated Queen

Generally, worker bees pay little or no attention to the newly emerged virgin queen (Butler, 1954a, 1975; Hammann, 1958; Solodkova, 1958). Hammann (1958) observed that from the second or third hour after the queen emerges from her cell the worker bees touch, lick, and feed the queen; they also display certain types of

behaviour towards the queen in order to stimulate her to fly. The types of behaviour described by Hammann (1958) are trembling movements, rocking movements, pulling movements, clinging movements, rolling the queen over and, from the third day of the queen's life, a piping movement; the trembling movement occurs much more frequently than do the other types of behaviour.

Hammann (1958) also found that the more the worker bees treat the queen in the above manner the earlier the queen takes her first exploratory flight.

The behaviour of worker bees towards an introduced virgin queen has also been studied in cages outside hives. Various types of behaviour, some of which were similar to those described by Hammann (1958), were observed; Yadava (1970) named and described the following four types of aggressive behaviour: threatening, seizing, pulling, and stinging. Velthuis (1972) classified six types of behaviour: avoidance, aggression, offering food, feeding, retinue behaviour, and negligence.

Several authors have studied the aggressive behaviour of worker bees of different ages towards queens. Hammann (1957, 1958) found that in an observation hive older worker bees display aggressive behaviour towards the virgin queen more frequently than

do younger worker bees. Szabo and Townsend (1974) observed that caged worker bees of different ages displayed aggressive behaviour towards an introduced virgin queen and found that worker bees 14 days old are the most aggressive.

Occasionally a newly introduced, or an established queen, is "balled" by worker bees (Huber, 1814; Allen, 1956). Walton and Smith (1969) defined the balling behaviour of worker bees as "the holding, abdomen inflecting, or stinging behaviour of a clustered group of workers". Yadava (1970) stated that balling occurs when many worker bees display one or more of the following aggressive behavioural patterns towards the queen: threatening, seizing, pulling, and stinging. Huber (1814) observed that if a mated queen is introduced into a colony that has been queenless for less than 24 hours she will be balled by the worker bees. Walton and Smith (1970) concluded that worker bees ball a newly introduced queen because they are stimulated by the queen pheromones produced from the queen's mandibular glands. The cause(s) of balling of an established mated queen in the colony (not disturbed by any means) is unknown (Allen, 1956). Huber (1814) reported that most of the queens which are balled eventually die. He postulated that the balled queens probably die from starvation and/or suffocation but

not from being stung by the balling worker bees. Minnick et al. (1967) reported that worker bees always ball and kill a virgin or a mated queen which is introduced into a queenright colony. Ribbands (1953) stated that when two queenright colonies are united the worker bees may ball both mated queens for some time; eventually they kill one queen and release the other. In establishing multiple-queen colonies, Lensky et al. (1970) found that worker bees ball and kill all but one of the introduced queens.

Solodkova (1958) reported that, from the second day of the virgin queen's life, 10 - 15 worker bees form a "court" (a circle) around the queen when she remains still on the comb; a court of worker bees is usually seen around a mated queen (Butler, 1954a). Taranov and Ivanova (1946), Butler (1954a), Butler et al. (1973), and Solodkova (1958) found that the bees forming a court do not follow the queen for long when she moves; the bees which are nearest to the queen form a new court each time the queen stops moving. Taranov and Ivanova (1946) reported that the court around a mated queen usually consists of 10 - 12 nurse bees (i.e. worker bees 3 - 13 days old). Allen (1957) found that the number of worker bees forming a court around a one-year old mated queen varies with the activity of the queen; the mean number in the summer

was six while the queen was moving over the comb, eight when laying, and ten when stationary. Allen (1960) also found that the ages of the bees forming a court varies from a few hours to 52 days.

Other than forming a circle around the queen, most of the attendant bees examine the queen with their antennae, with some licking her body (especially the abdomen) with their tongues, and some feeding her (Butler, 1954a, 1954b; Allen, 1955; Solodkova, 1958). Allen (1955) reported that attendant bees, between a few hours and 36 days old, examine the mated queen with their antennae. Allen (1955, 1960) concluded, from her observations, that attendant bees of any age lick their mated queen. Butler, (1954b) found that an attendant bee spent an average of 31.3 seconds examining the mated queen and 66.1 seconds licking her. Allen (1960) found that attendant bees, which feed a mated queen, are younger than those examining or licking her; most of them are two to four days old. Allen (1955) also found that the duration of feeding is not related to the age of the feeding bees. She recorded that the average duration of each feeding period was 47 ± 2.6 seconds. In addition to the above behaviour, some worker bees (most of which are 22 - 28 days old) "shake" the mated queen during the first few days after she starts laying eggs, and during swarm preparations.

(Allen, 1959). The description of shaking behaviour described by Allen (1959) is similar to that of trembling behaviour described by Hammann (1957, 1958).

2.2 Behaviour of Queens Towards Each Other

It is well known that when two queens (either virgin or mated queens) meet each other fighting between them usually occurs. Huber (1814) wrote that both queens run towards each other and fight, and sting each other. He reported that only one queen is usually killed in the fighting; if both queens have an equal opportunity to sting each other during the fighting (which could result in the death of both) they separate from each other immediately. He observed that the victorious queen kills her rival by first holding its wing base with her mandibles and then bending her abdomen in such a way that she can sting her rival. However, Lensky et al. (1970), in their studies about the behaviour of virgin queens towards each other without the presence of worker bees, found that both queens in one experiment died in combat. Butler (1954a) observed that a virgin queen kills her rival by inserting her sting through the soft membranes located between the segments of various parts of the body. Dines (1968) and Lensky et al. (1970) added that the sting goes through the membranes between the abdominal segments.

Huber (1814) reported that the worker bees take no part in the killing of the extra queen; when the queens separate from each other the worker bees cluster around each queen but disperse when the two queens renew their combat. Lensky et al. (1970), in their attempts to set up multiple-queen colonies, found that the worker bees always ball and kill the extra queens. Minnick et al. (1967) reported results similar to those of Lensky et al. (1970) when they introduced a virgin or a mated queen into a queenright observation hive.

The behaviour of two queens towards each other, in the absence of worker bees, has been studied by several researchers, e. g. Lensky et al. (1970), Waikakul (1973), and Szabo and Smith (1973). Waikakul (1973) reported that virgin queens display two types of behaviour towards each other: aggressive and non-aggressive behaviour. The aggressive behaviour displayed by one or both queens is similar to that reported by Huber (1814). The non-aggressive behaviour consists of examining, licking, begging food, and offering (food). Lensky et al. (1970) put two mated queens together in a cage and observed that the queens beg and offer food to each other. Szabo and Smith (1973) reported that, in addition to aggressive and non-aggressive behaviour, one or both virgin queens

display avoidance behaviour by discharging faeces during the first attack; some pairs which displayed this behaviour stopped fighting completely. Szabo and Smith (1973) also found that the aggression of virgin queens is not related to their previous fighting experience or their weights; usually the victorious queen is the one which attacks her rival first. Waikakul (1973) observed that the queen had more opportunity of winning the fight if she could hold the wing base of her rival with her mandibles and bend her abdomen to sting the venter of her rival's thorax around the bases of the coxae.

Under certain circumstances, queens live peacefully together in the same colony. Huber (1814) found that when he removed the antennae from a normal queen and a drone laying queen they lived together without fighting. Kovtun (1949) introduced many (4-8) one and one-half year old mated queens with clipped wings into a colony of emerging worker bees; the queens fought at first but in time they accepted each other and laid eggs in the hive. Kelsall (1940), Taranov (1951), and Ribbands (1953) reported that, after supersedure, an old queen and her daughter queen co-habit for some time. Mathis (1952) found that many virgin queens (Apis mellifera punica) in his two observation hives lived together without

serious fighting for over two weeks after emergence from their cells.

Anderson (1960) observed that when the sting tips of queens were amputated before their introduction into the same observation hive the queens tolerated each other, although they fought initially.

Skirkyavichyus (1965) reported that pairs of queens of the same age and condition (virgin or mated), introduced into a "box" of worker bees, always fight until one is killed, but those of different ages and conditions do not. Minnick et al. (1967) introduced virgin or mated queens into queenright observation hives and found that the established queens and the introduced virgin queens show no hostility towards each other.

Worker bees may react in one of two ways towards other queens in the same colony: acceptance or rejection. Only Huber (1814) and Kovtun (1949) reported that worker bees accept more than one queen present in each colony without showing any sign of hostility. The majority of researchers have found that worker bees consistently kill all but one queen present in the same colony (Smith, 1923; Anderson, 1960; Lensky and Darchen, 1963; Skirkyavichyus, 1965; Lensky et al., 1970).

The co-existence of queens in the absence of worker bees has been studied extensively. A report in *Bee World*

(Anonymous, 1972) mentioned that many virgin queens do not attack one another when they meet outside their hives in a mating cage.

Gary et al. (1967) observed that mated queens could adapt to each other if they are placed head to abdomen in a small cylindrical cage for 90 minutes. Lensky et al. (1970) and Riedel and Blum (1972) reported that pairs of mated queens fight less aggressively and for a relatively shorter period of time than do pairs of virgin queens.

Szabo and Smith (1973) observed that aggression between pairs of queens of different ages and conditions (virgin or mated) is far less than that between pairs of queens of the same age and condition.

Riedel and Blum (1972) found that pairs of virgin or mated queens do not fight with each other when their antennae have been amputated or masked with some volatile fluids such as citral and vanilla extract.

Several experiments have been carried out to investigate what factors stimulate queens to display aggressive behaviour towards each other. Velthuis (1967) reported that pairs of queens, with their mandibular glands removed, always fight with each other but those which have their mandibular glands removed and their abdominal tergites covered with nail polish do not. He suggested that secretions from the dermal glands associated with the tergites of the queen's abdomen

might act as releasers for the aggressive behaviour of other queens. Lensky et al. (1970) found that aggressive behaviour in a queen is stimulated by volatile substances produced from the sting glands of other queens.

2.3 Behaviour of Queens and Worker Bees Towards Queen Cells

Besides fighting with other adult queens, both virgin and mated queens display aggressive behaviour towards queen cells containing immature queens (Huber, 1814); he reported that when queens attack capped queen cells they usually bite holes through the walls of the cells near their bases and sting the emerging queens within the cells. He observed that the queens do not attempt to sting the pupae inside the cells. Butler (1954a, 1975) reported this same phenomenon and also observed that virgin queens seldom attack uncapped queen cells. Dines (1968) and Eckert and Shaw (1972) reported that a virgin queen attacks a queen cell by making a hole through the side of the cell and stinging the immature queen inside; however, they did not specify the developmental stage of the victim. Butler (1975) observed that a virgin queen may not attack a queen cell the first time that she locates it. Allen (1956) found that uncapped queen cells in a colony which is preparing to swarm are attacked by the mated queen of the colony; the queen tears the wall

of the cell apart but does not sting the larva inside.

Worker bees also display certain behavioural patterns towards queen cells. Huber (1814) observed that worker bees continue to destroy a damaged queen cell and its contents after a virgin (or mated) queen has finished attacking it. Huber (1814) also found that when queenless worker bees perceive the presence of a newly introduced mated queen they destroy all of the queen cells (uncapped) present in the colony. Butler (1975) stated that worker bees sometimes destroy certain queen cells which are not attacked by the virgin queen; he did not, however, specify the type of queen cells destroyed.

In addition to aggressive behaviour, worker bees display non-aggressive behaviour towards queen cells. Allen (1956) noticed that worker bees rebuild the uncapped queen cells bitten by a mated queen. Butler (1954a, 1975) reported that sometimes worker bees repair the damage done to capped queen cells by a virgin queen. It has also been reported that during the swarming period worker bees protect other queen cells from attacks by the first virgin queen to emerge by clustering around the cells (Butler, 1954a; Gary, 1975).

Waikakul (1973) studied the behaviour of virgin queens

towards queen cells in the absence of worker bees. He found that a queen shows no rigid pattern in attacking a set of queen cells containing queen brood of different ages. However, a queen attacks only the capped queen cells of each set. In addition, she also attacks empty capped queen cells previously containing queen pupae regardless of whether the cells are oriented vertically or horizontally.

CHAPTER 3

GENERAL METHODS

3.1 Rearing of Queens

Methods for rearing queens are basic to this study. The system used to rear queens was adapted from that described by Laidlaw and Eckert (1962) and from the systems used by queen breeders in the southern U.S.A. Queen rearing for this study was done from late June to late August in 1973, 1974, and 1975 on the University of Manitoba campus. Four breeder colonies* and twelve "queenright" (i. e. with a queen) cell building colonies** were used. All hives used for this study were of the ten-frame Langstroth type.

All colonies were headed by Starline hybrid queens obtained from Texas***. Each breeder colony consisted of one box (or chamber) containing bees, food, and a single-frame queen

*A breeder colony is a colony which provides a frame of young larvae for queen rearing.

**A cell building colony is a colony which constructs queen cells from the wax queen cell cups containing young larvae.

***Weaver Apiaries, Route 1, Navasota, Texas 77868, U.S.A.

excluder where a breeder queen was confined (Figure 1). An empty dark drawn comb was put into the single-frame queen excluder every fourth day so that young larvae of the same age would be available for grafting*. Cell building colonies consisted of two boxes (or chambers) with the queen confined to the bottom box by means of a queen excluder. The bottom box was filled with empty combs and frames of emerging and capped brood. Frames of grafted cells were put into the centre of the top box; it also contained frames of uncapped brood. The youngest brood was placed next to the grafted cells, while combs of honey and pollen were placed next to the two side walls. Queen cells were started and finished in the same cell building colony, as recommended by Roberts (1965). The cell building colonies were examined once a week, at which time combs of uncapped brood from the bottom box were exchanged for combs of emerging brood of the top one. Any queen cells which had been built on the combs by the bees were also removed at this time to prevent swarming and the destruction of any grafted queen cells by virgin queens which might emerge from them.

*Grafting is the process of transferring young larvae from worker cells into artificial wax queen cell cups by using a small curved needle called a "grafting" hook.

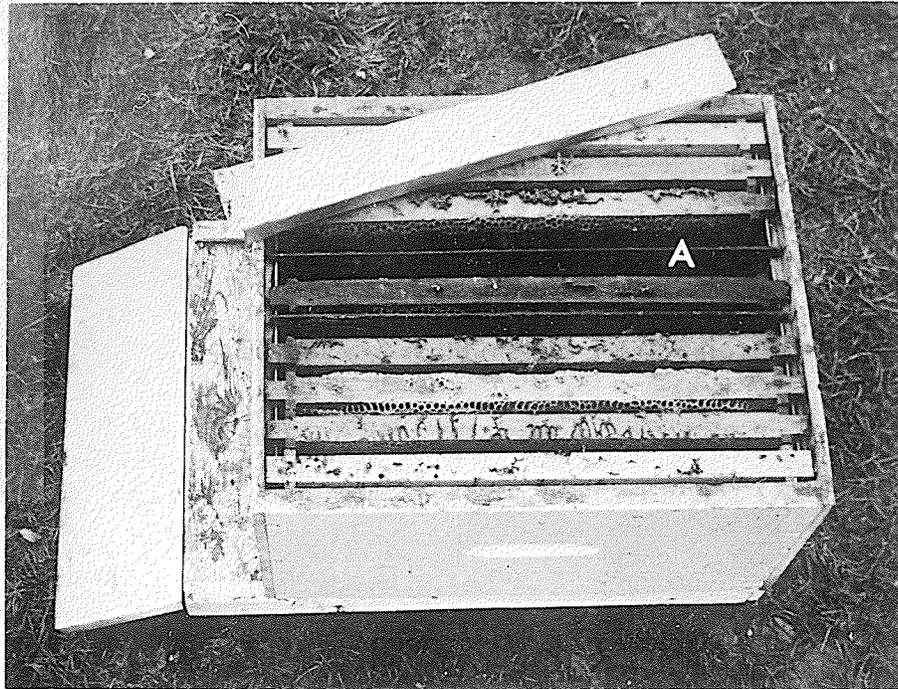


FIGURE 1: A BREEDER COLONY WITH A SINGLE-FRAME
QUEEN EXCLUDER.

A. Queen excluder.

Grafting was done every day during the first week of queen rearing. Later on it was done every day except Saturday and Sunday in the summer of 1973, every other day in the summer of 1974, and every fifth day in the summer of 1975. The equipment used for grafting is shown in Figure 2. Ten wax queen cell cups were attached, at one inch intervals, on each wooden bar. The cell cups were "primed" just before grafting with a royal jelly and distilled water mixture (1:1 by volume) using an eye dropper. According to Laidlaw and Eckert (1962), Vagt (1955), and Bilash (1963), good quality queens can be produced from 12-18 hour old larvae; therefore, young larvae, under 18 hours old, were used in all grafts. A comb of young larvae, from the breeder colony, was placed in an adjustable frame holder with a fluorescent light placed above it (Figure 2). A young larva was transferred from a worker cell onto the drop of royal jelly in each cell cup using a grafting needle. The bar of grafted cells was then placed in a bar-holding frame (see Figure 3) by hanging the cells downwards; one bar of grafted cells was given to each cell building colony.

On the eighth day after grafting the ripe cells were transferred from the cell building colonies into an incubator (at $30 \pm 2^{\circ}$ C and 70-80% R.H.). They were hung downwards within

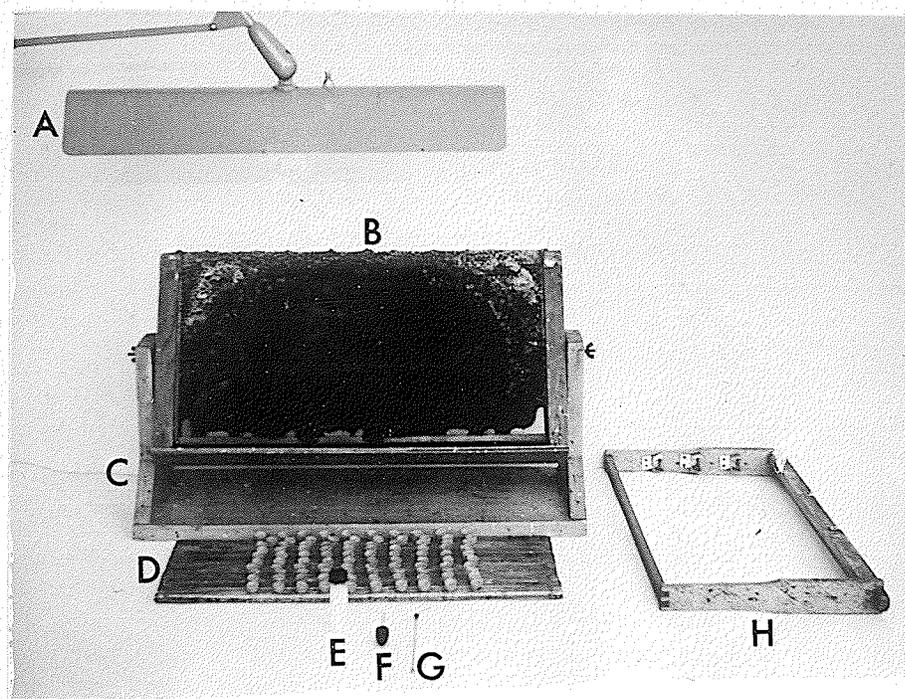


FIGURE 2. EQUIPMENT USED FOR GRAFTING

- A. Fluorescent light
- B. Comb of young larvae
- C. Adjustable frame holder
- D. Bars of wax cell cups
- E. Royal jelly
- F. Eye dropper
- G. Grafting needle
- H. Bar holding frame

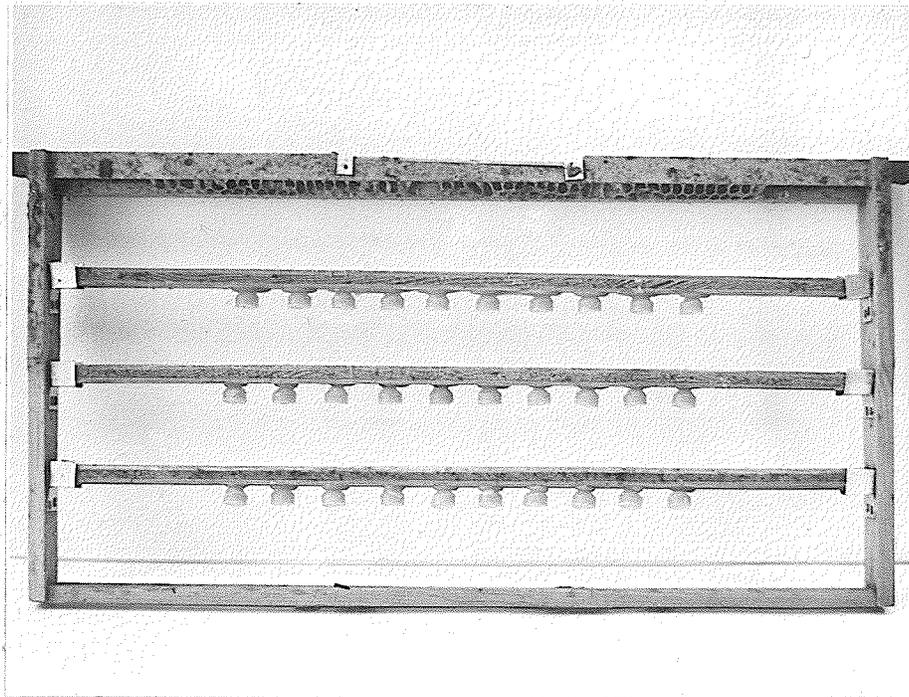


FIGURE 3. A BAR-HOLDING FRAME WITH GRAFTED CELLS.

glass vials and left there until emergence. The virgin queens usually emerged on the twelfth or thirteenth day after grafting. Both the queen cells and the adult queens reared by the above method were used throughout this study.

In order to obtain mated queens, a number of mating nuclei* were set up in the apiary. Each mating nucleus, which had a dimension of 13 cm x 49.5 cm x 24 cm, could accommodate three standard frames (Figure 4). One frame of brood in every stage of development (i.e. eggs, larvae, pupae), one frame of food (honey and pollen), and one frame of empty dark drawn comb were put into each nucleus; approximately 0.5 kg queenless bees were then added to the nucleus. The prepared nuclei were placed around the edge of the apiary so that the virgin queens from the nuclei would have ample opportunity to encounter drones from the apiary.

The nuclei were left queenless for three days. On the fourth day, after destroying any emergency queen cells that might have been constructed on the face of the combs, an adult virgin queen, two to three days old, and reared by the method previously mentioned,

*A mating nucleus is a miniature hive into which a "ripe" queen cell is introduced. The virgin queen emerges from her cell in the nucleus, flies out, mates in the air, and returns to the nucleus.



FIGURE 4. THREE-FRAME STANDARD LENGTH MATING NUCLEI.

was caged in a queen mailing cage. The cage was then introduced into the nucleus and left there for two days to allow the worker bees in the nucleus to become "familiar" with the queen before she was released. Normally, if the weather was favourable (i.e. temperature over 25° C with afternoon sunshine), the virgin queen would mate within 10 - 12 days after she was released from the cage. The presence of eggs and larvae in the worker cells indicated that the queen was mated. The mated queen was then caged and stored using the method described below. After removing the mated queen from the nucleus, it was left queenless for two days before a new virgin queen was introduced to it.

3.2 Storage of Queens

Both virgin and mated queens were stored using the method devised by Edwards and Poole (1971). However, instead of using their special storage box, an ordinary brood chamber box, with its bottom closed by a sheet of plywood, was used (Figure 5). Before placing queens on the box, three frames of emerging brood, which were covered with bees, (without a queen) were removed from a queenright colony, and put into the box; a piece of wire screen (3 - mm mesh) was then placed on top of the box (Figure 5). Queens, which were to be stored, were placed singly over the screen under

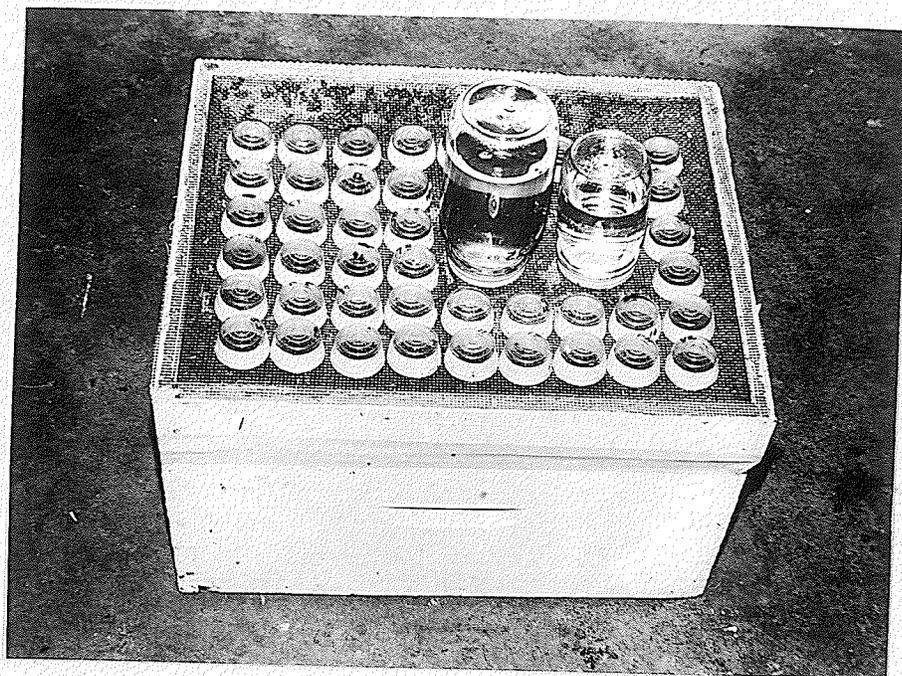


FIGURE 5. A QUEEN BEE STORAGE UNIT.

inverted clear plastic cups (top diameter 43 mm, bottom diameter 36 mm, height 25 mm). The side of each cup was covered over so that the stored queens could not see each other. A 909 ml (32 fluid ounces) glass jar with a perforated cap was inverted over the colony and used as a feeder. It was filled with a 50 percent sugar syrup solution with Fumidil B and Terramycin (2 gm of each per litre of the syrup) added to prevent Nosema disease and to reduce mortality in the confined worker bees. Water was given to the bees using the same method. Pollen was fed to the bees by pressing it through the screen top once a week. Both virgin and mated queens were stored on the same storage unit. The storage unit was kept in a dark incubator at $30 \pm 2^{\circ}$ C and 40 - 50 % R.H. One unit could accommodate about 75 queens. The bees, and the frames in the storage unit, were replaced every month with new frames of emerging brood covered with bees.

3.3 Selection of Experimental Queens and Queen Cells

Newly emerged virgin queens, one-day old virgin queens, and one-month old mated queens, were used in the various experiments. Queen cells, containing immature queens, were also used in some experiments. These were selected on the basis of their age using life tables (see Jay, 1963). The ages of the queen cells

selected were as follows:

- A. Uncapped queen cells containing young larvae selected from queen cells on the second day after grafting
- B. Uncapped queen cells containing old larvae selected from queen cells on the fourth day after grafting
- C. Capped queen cells containing young pupae selected from queen cells on the eighth or ninth day after grafting
- D. Capped queen cells containing old pupae selected from queen cells on the eleventh day after grafting

3.4 Observation Hives and the Experimental Shelter

Glass-walled observation hives, each of which held one Langstroth frame, were used to observe queen and worker bee behaviour (Figure 6). When the bees in the hive were not under observation, the glass walls on both sides of the hive were covered over by plywood sheets to keep the hive dark. If there was not enough food (i. e. honey and pollen) in the observation hive, the bees were fed with a mixture of honey and pollen (2:1 by volume) through the screened opening in the lid of the hive.

Each observation hive was prepared by putting a frame of brood in various stages of development (i. e. eggs, larvae, pupae) covered with 900 - 1000 worker bees (without a queen) into it. The

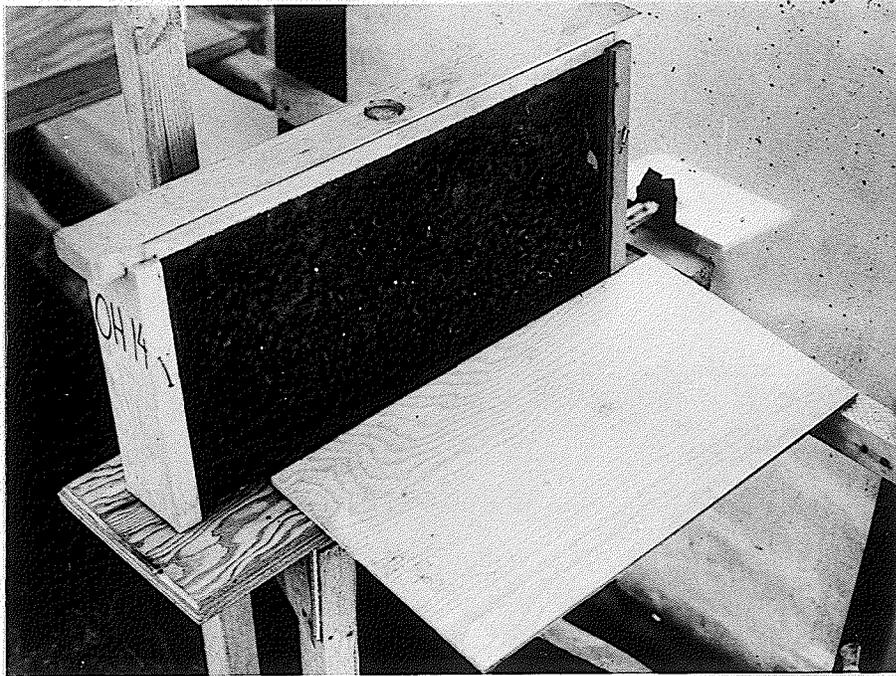


FIGURE 6. A GLASS-WALLED ONE-FRAME OBSERVATION HIVE.

number of adult bees on the frame was estimated from a series of photographs developed by Nelson and Jay (1972). According to Perepelova (1929), Milojevic and Filipovic-Moskovljevic (1958), and Jay (1970, 1972), ovary development of queenless worker bees should be inhibited by the presence of this frame of brood.

To reduce the extreme changes in temperature, which occurred over a 24 hour period, the observation hives were put into a shelter covered with polyethylene sheet (Figure 7). The shelter was equipped with a heater and thermostat so that the temperature inside was maintained between 28° and 32° C. During the day time both doors of the shelter were opened if the temperature inside was higher than 32° C. Each hive had a different coloured landing board at its entrance to prevent drifting. The shelter, which had a dimension of 1.8 m x 3.6 m x 1.8 m, could accommodate 16 observation hives.

3.5 Marking Queens and Worker Bees

Before introducing a queen into an observation hive, she was colour-marked with a small drop of aircraft model paint applied to the dorsal side of the thorax. If two queens were introduced into one observation hive at the same time, they were marked with different colours. After marking, the queens were held in storage

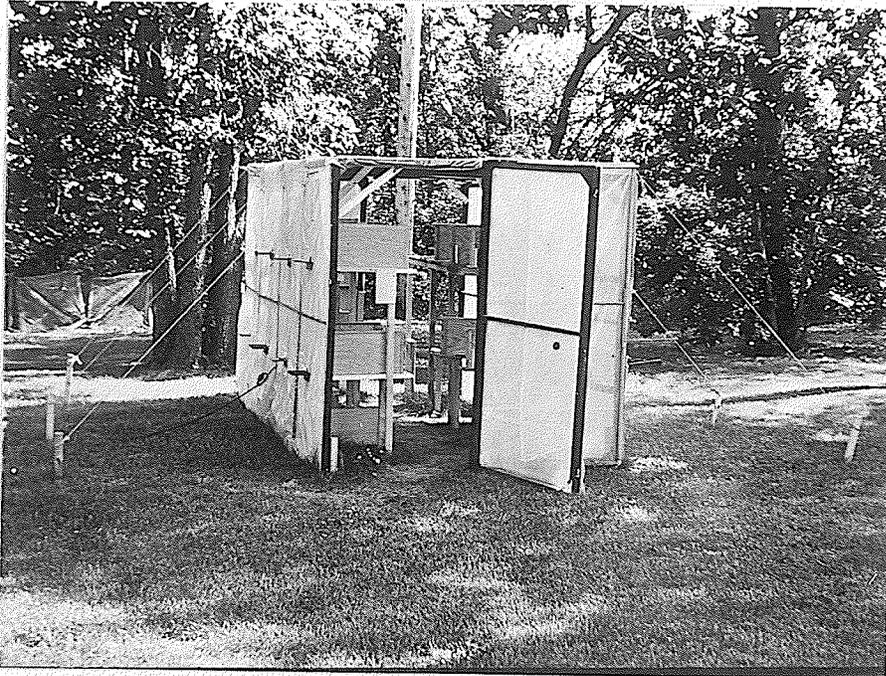


FIGURE 7. A SHELTER WITH A GROUP OF OBSERVATION
HIVES.

cages for a few minutes to allow the paint to dry and to eliminate any odour of the paint which might subsequently affect the behaviour of the worker bees in the observation hive towards them.

To obtain newly emerged worker bees for marking, frames of emerging worker bees were taken from hives and put into a box in an incubator (at $30 \pm 2^{\circ}$ C and 70-80% R.H.) over night. Each newly emerged worker bee was marked on the dorsal side of its thorax with a small drop of aircraft model paint. After marking, the bees were held in a plastic cage for about 30 minutes to allow the paint to dry before they were introduced into an observation hive. Carbon dioxide anaesthetic was never used while worker bees or queens were marked.

CHAPTER 4

BEHAVIOUR OF A VIRGIN OR A MATED QUEEN IN THE PRESENCE OF WORKER BEES

4.1 Introduction

After emerging from their cells, queens, workers, and drones behave in ways which ensure the survival of the colony. Understanding their behaviour is the key to successful management of a colony. In the following experiments an attempt was made to analyze the general behaviour of both virgin and mated queens and the behaviour of worker bees towards each.

4.2 Methods

4.2.1 Virgin Queen Experiment

A glass walled observation hive, containing one frame of brood in various stages of development (i. e. eggs, larvae, pupae)* covered with 900-1000 worker bees (without a queen) was used. The number of adult bees on the frame was estimated from

*According to Perepelova (1929), Milojevic and Filipovic-Moskovljevic (1958), and Jay (1970, 1972), ovary development of queenless worker bees should be inhibited by the presence of this frame of brood.

a series of photographs developed by Nelson and Jay (1972). One hundred and fifty newly emerged worker bees were marked and introduced into the hive each week for three weeks; the first group of marked bees was introduced on the same day the hive was set up. Different colours were used each week for marking the bees. Therefore, when the experiment began, the hive contained three different groups of marked worker bees of different ages; these were classified as group A (15 days old), B (8 days old), and C (1 day old). Emergency queen cells, constructed between the time when the first group of marked bees was introduced into the hive and the time when the last group of marked bees was introduced into the hive, were destroyed by hand.

On the day the last group of marked worker bees was introduced into the hive, a capped queen cell containing an emerging virgin queen was introduced into the hive. The queen cell was pressed gently onto the face of the comb (see Figure 8). The virgin queen usually emerged within 24 hours after the introduction of the cell. The behaviour of the emerged virgin queen, and the marked worker bees towards this queen, was observed for various 30 minute periods as follows: (a) after the emergence of the queen, (b) when the queen was three hours old, (c) when the queen was six hours old,

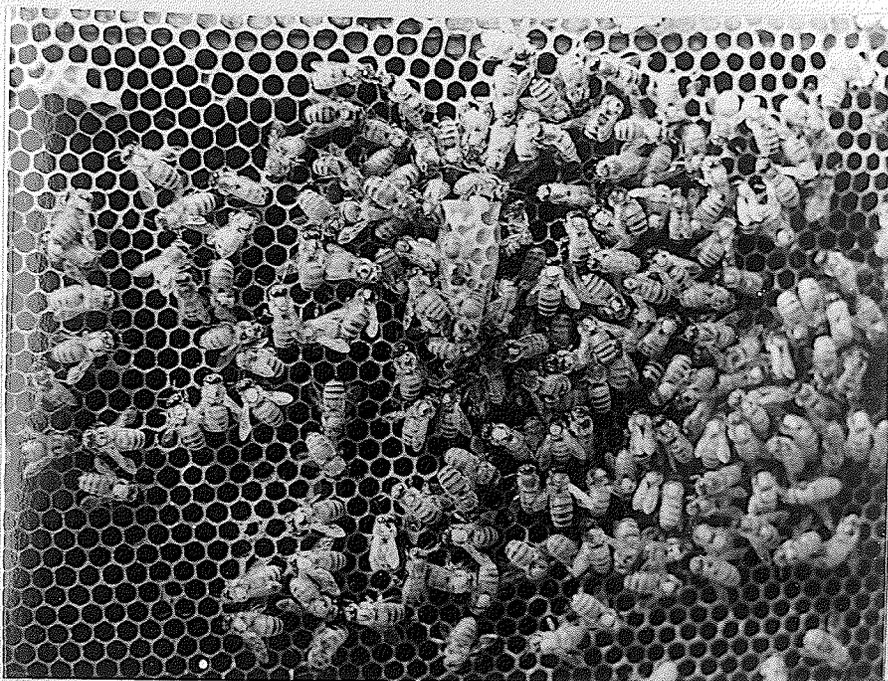


FIGURE 8. A CAPPED QUEEN CELL, CONTAINING AN EMERGING VIRGIN QUEEN, BEING INTRODUCED INTO A HIVE.

and (d) each afternoon thereafter, sometime between 1300-1400 hr until the queen was six days old. This experiment was replicated five times using five different observation hives and five different virgin queens. All replicates were done during July and August 1973.

4.2.2 Mated Queen Experiment

An experiment was done, similar in all respects to that of the virgin queen experiment described above, except that at the beginning of the fourth week before the experiment began (i.e. when the mated queen was released from her cage), an extra 150 newly emerged marked worker bees were added to the hive thus making four groups of marked bees (i.e. group A = 22 days old, B = 15 days old, C = 8 days old, D = 1 day old), rather than three, in this experiment. Emergency queen cells, constructed between the time when the first group of marked bees was introduced into the hive and the time when the last group of marked bees was introduced into the hive, were destroyed by hand.

Five days before the last group of marked bees was introduced into the hive, a young mated queen (aged 30 days) was introduced into the hive using a push-in cage (see Snelgrove, 1948) made of wire screen (Figure 9). The queen was confined in the cage with

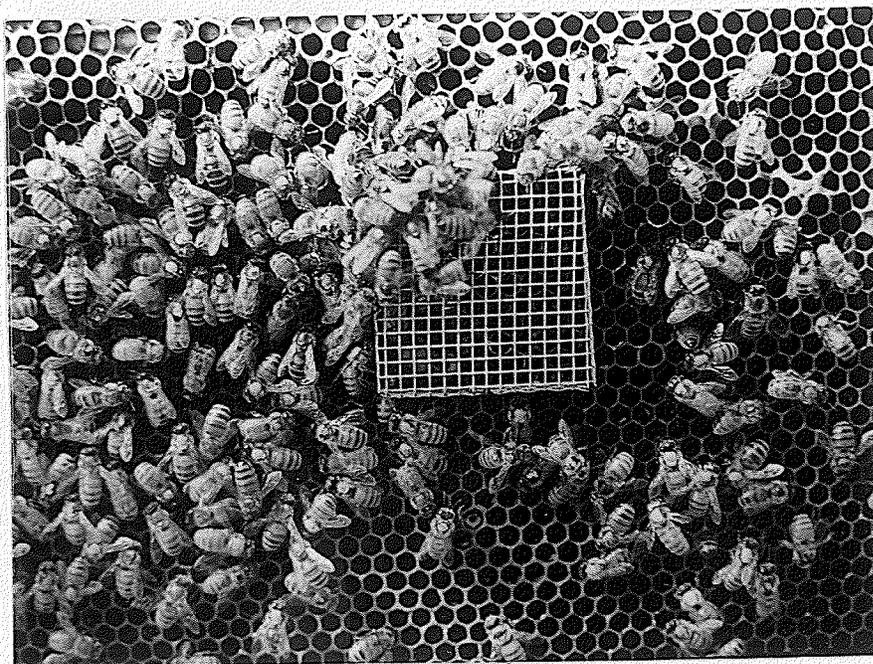


FIGURE 9. A MATED QUEEN IN A SCREENED PUSH-IN CAGE.

four to five worker bees for six days (to allow the worker bees in the hive to become "familiar" with her) before she was released. The behaviour of the queen, and the marked worker bees towards her, was observed for various 30 minute periods as follows: (a) immediately after the queen was released, and (b) each afternoon thereafter sometime between 1300-1400 hr for the next five days. This experiment was replicated five times between July and August 1974 using five different observation hives and five different mated queens.

4.3 Results and Discussion

4.3.1 Behaviour of Queens

The various types of behaviour of virgin and mated queens observed in the study, as each moved among the worker bees, are listed in Table I and described below:

4.3.1.1 Non-aggressive Behaviour

Grooming: Both virgin and mated queens groomed their antennae, heads, mouthparts, and abdomens by rubbing their abdomens, which swayed from side to side, with one or both of their hind legs and/or by cleaning their antennae, heads, and mouthparts with both of their fore legs. If worker bees had been licking the abdomen of a queen she would sometimes rub her abdomen with her hind legs. Butler

TABLE I. TYPES OF BEHAVIOUR*

<u>Virgin queens</u>	<u>Mated queens</u>
<u>Non-aggressive behaviour</u>	<u>Non-aggressive behaviour</u>
Grooming	Grooming
Examining cells	Examining cells
Placing abdomen into cells	Laying eggs
Feeding from cells	Feeding from cells
Begging food from worker bees	Begging food from worker bees
Receiving food from worker bees	Receiving food from worker bees
Offering food to worker bees	Offering food to worker bees
Holding	
<u>Aggressive behaviour</u>	<u>Aggressive behaviour</u>
Rocking	
Holding - biting	—
Holding - biting - stinging	

*Not all types of behaviour were performed by each queen.

(1975) reported this behaviour and postulated that while a queen is grooming herself she spreads queen substances* over her body.

Examining cells: Both virgin and mated queens examined cells by putting their heads into empty cells (mostly worker cells) while they were travelling over the combs. It is possible that either type of queen might look for food in the cells and that the mated queens might also be looking for places to lay their eggs. This type of behaviour was also reported by Park (1952) and Butler (1954a).

Laying eggs: Only mated queens displayed this type of behaviour. The queen stood over a cell, bent her abdomen, inserted it into the cell, and deposited an egg onto the bottom of the cell before withdrawing her abdomen. While selecting a cell, the queen did not move in concentric circles but walked randomly over the comb and frequently crossed and recrossed her path (see Butler, 1954a).

Park (1952), Butler (1975), and Gary (1975) observed that mated queens examine each cell before laying in it. It appeared to me, that

*Queen substance(s), originally named by Butler (1954a, 1954b), is one of the queen pheromones produced from the queen's mandibular glands (Butler and Simpson, 1958). It consists of at least two identified pheromones, 9 - oxodec - trans - 2 - enoic acid and 9 - hydroxydec - trans - 2 - enoic acid. Other unidentified pheromones are also produced from the other parts of the queen's body such as the abdomen (Velthuis, 1970).

some queens laid eggs in cells without first examining them.

Placing abdomen into cells: Only virgin queens displayed this behaviour, usually when they were about two days old. The queen usually inserted about half of her abdomen into a worker cell without first inspecting it; this looked like the laying behaviour of a mated queen. Hammann (1958) also reported seeing this type of behaviour among virgin queens.

Feeding from cells: Both virgin and mated queens put their heads into cells containing honey and sucked it from the cells. The queens were never seen feeding from pollen cells. Butler (1954a) also observed that virgin queens feed on honey from cells, but eat no pollen.

Begging food from worker bees: When approaching the worker bees which were in front of her, the queen extended her tongue to "beg" for food from them (see Butler, 1954a; Free, 1956). This behaviour was performed by both virgin and mated queens.

Receiving food from worker bees: The queen sucked liquid food from between the mandibles of the offering worker bees in front of her with her extended tongue. While sucking food, the antennae of the queen and the worker bee continuously stroked each other, similar to the stroking done by worker bees when feeding each other. Both virgin

and mated queens displayed this behaviour.

Butler (1954a, 1975) stated that whenever a queen "begs" for food, worker bees always feed her. I observed that the queen did not always receive food from the worker bees each time she "begged" for it.

Offering food to worker bees: The queen offered food to the worker bees by opening her mandibles and regurgitating a drop of liquid food onto the upper surface of the base of her tongue. This behaviour is also similar to that of a worker bee when she offers food to another worker bee. Sometimes the queen displayed this behaviour even when there were no begging worker bees in front of her. Both virgin and mated queens displayed this behaviour. It appears that this behavioural pattern has not been reported previously. Gary et al. (1967) found that mated queens did not offer food to other mated queens or worker bees when placed separately in a cage with a sheet of cellulose acetate having two holes between them. On the other hand, Lensky et al. (1970) reported that when a mated queen was paired with either a mated or a virgin queen each offered food to the other. Waikakul (1973), and Szabo and Smith (1973), also found that a virgin queen offered food to another virgin queen when they were caged together.

Holding: This behaviour was observed only among the virgin queens.

The queen held a worker bee or drone with her two front pairs of legs, or with all of her legs; sometimes when this happened the virgin queen would bite and/or sting the worker bee or drone. Nobody appears to have described this behaviour before.

4.3.1.2 Aggressive Behaviour

Only the virgin queens displayed aggressive behaviour when they met a worker bee or a drone.

Rocking: When meeting a worker bee, the queen stood on her two hind pairs of legs*, moved her body backwards and forwards, and tapped the worker bee with her thorax and fore legs. Hammann (1958) also reported seeing this type of behaviour.

Holding - Biting: The queen first held the worker bee with her legs and bit at its wings with her mandibles.

Holding - Biting - Stinging: The queen held and bit a worker bee as previously described; in addition she bent her abdomen in such a way as to attempt to sting the abdominal area of the worker bee. One virgin queen also displayed this behaviour towards a drone; however, in each case the queen was unable to injure her "victim".

It appears that the latter two descriptions of aggressive behaviour (i. e. the holding - biting and holding - biting - stinging behaviour of a virgin queen towards a worker bee, or drone) have

* This behaviour was similar to that of a guard bee at the entrance of its hive (see Butler, 1954a).

not been reported previously.

Many researchers have reported the "piping" sound* made by virgin queens. Hammann (1958) observed that virgin queens pipe in order to stop worker bees from chasing them. Butler (1954a, 1975) stated that virgin queens locate their rival queens by answering the piping sounds. However, I have never heard any virgin queens make "piping" sounds in my experiments. This is probably because each queen was not attacked by worker bees or because there were no rival queens within the hive to "challenge" her. Allen (1956) reported that a one-year old mated queen also piped when a colony was preparing to swarm, but I have never heard any of my mated queens pipe; this is probably because the experimental colonies were not preparing to swarm.

4.3.1.3 Behavioural Patterns

Virgin queen: After emerging from her cell, the virgin queen showed no regular sequence of behaviour. Generally, she wandered over both sides of the comb and displayed any of the behavioural patterns, outlined above, at any time during an observation period. The queen was usually chased by two or three worker bees whenever

*A virgin queen produces a "piping" sound by vibrating small plates (sclerites) located at her wing bases (Snodgrass, 1925).

she made a quick movement, especially during the first day of her life. A "court"* of worker bees, which was normally seen around the mated queen when she moved slowly or stayed still on the comb, was not seen around the virgin queen until the queen was about two days old; this is consistent with the findings of Solodkova (1958).

The behaviour of virgin queens for various 30 minute periods during the six days following their emergence, and the time spent by them to perform each behaviour, are shown in Table II and Table III respectively.

According to Table II, the number of times each type of behaviour was performed by the virgin queens, varied from day to day and did not increase with age. The queens groomed themselves more frequently than they performed the other types of behaviour. It is possible that the more the queens groom themselves the more queen substances are passed from the queens to worker bees via the queens' bodies (see Butler, 1975). The queens also examined cells quite often, especially during the first day of their lives; possibly the newly emerged queens were hungry and were

*A court is a circle of 8-12 worker bees around a queen. While forming a court, the worker bees also examine the queen's body with their antennae and/or lick her body with their tongues.

TABLE II. BEHAVIOUR OF VIRGIN QUEENS FOR VARIOUS 30 MINUTE PERIODS DURING THE SIX DAYS FOLLOWING THEIR EMERGENCE.

Types of behaviour	Number of times each type of behaviour was displayed by virgin queens for various 30 minute periods during the six days following their emergence									
	<0.5 hr.	3 hrs.	6 hrs.	1 day	2 days	3 days	4 days	5 days	6 days	Total
<u>Non-aggressive</u>										
Grooming	37*	18	20	15	16	19	23	24	13	185
Examining cells	21	3	23	22	8	9	2	7	2	97
Placing abdomen	0	0	0	0	2	0	0	1	0	3
Feeding from cells	2	1	3	1	1	0	3	3	6	20
Begging food	3	1	1	1	3	2	3	1	3	18
Receiving food	2	1	1	1	3	2	3	1	3	17
Offering food	9	1	7	0	2	1	1	0	2	23
Holding	0	1	0	0	0	0	1	0	0	2
<u>Aggressive</u>										
Rocking	0	0	0	1	0	1	3	3	2	10
Holding-biting	0	0	0	0	1	2	0	0	0	3
Holding-biting-stinging	0	1	0	1**	0	0	0	0	0	2

*Results of observations of five virgin queens for 30 minutes each.

**Behaviour of a virgin queen towards a drone.

TABLE III. TIME SPENT BY VIRGIN QUEENS IN PERFORMING VARIOUS TYPES OF BEHAVIOUR.

Types of behaviour	Times each behaviour was recorded	Mean time \pm S.E. and range (seconds)
<u>Non-aggressive</u>		
Grooming	116*	16.5 \pm 1.7 (2-94)
Examining cells	69	3.1 \pm 0.5 (1-24)
Placing abdomen	3	11.3 \pm 5.2 (2-20)
Feeding from cells	14	6.6 \pm 2.1 (2-26)
Begging food	12	4.0 \pm 0.6 (2-8)
Receiving food	11	23.4 \pm 7.3 (3-82)
Offering food	14	14.8 \pm 3.8 (2-43)
Holding	2	22.0 \pm 14.0 (8-36)
<u>Aggressive</u>		
Rocking	10	3.0 \pm 0.3 (2-5)
Holding-biting	3	10.0 \pm 1.0 (9-12)
Holding-biting-stinging	2	31.5 \pm 23.5 (8-55)

*Number of virgin queens observed = 5.

looking for food. Hammann (1958) observed that one virgin queen became aggressive towards the attacks of worker bees when she was about three days old. My results show that one of my virgin queens displayed aggressive behaviour (i. e. holding - biting - stinging behaviour) towards one worker bee when she was about three hours old and towards one drone when she was about one day old.

Table III shows that the mean period of time spent by a virgin queen in performing each behaviour varied from category to category. Although the longest period of time (mean 31 seconds) was recorded when a queen displayed holding - biting - stinging behaviour towards a worker bee and a drone, this figure was the average of only two observations. The other relatively long period of time (mean 23 seconds) was averaged from more observations, and was recorded when the queen received food from a worker bee. The shortest period of time (mean 3 seconds) was recorded when the queen examined a worker cell or performed rocking movements towards a worker bee. The range of time recorded from five different virgin queens for each behaviour showed that the queens spent the widest range of time (2 seconds to 1 minute and 34 seconds) grooming themselves and spent the narrowest range (2 to 5 seconds or 9 to 12 seconds) performing rocking or holding - biting behaviour towards worker bees.

Mated queen: Like a virgin queen, a mated queen wandered on both

sides of the comb and showed no regular sequence of behaviour after being released from the push-in cage. Normally, she was not chased by worker bees, as were the virgin queens, apparently because she moved slowly and quietly on the comb. While moving slowly or staying still on the comb, the queen usually had 8-12 worker bees forming a "court" around her. The behaviour of mated queens for various 30 minute periods during the six days following their release, and the time they spent in performing each behaviour are shown in Table IV and Table V respectively.

Table IV shows that the number of times each type of behaviour was performed by the mated queens, varied from day to day. It was found that the queens examined worker cells and displayed laying behaviour more frequently than they did the other types of behaviour; this is consistent with the fact that the main function of a mated queen is laying eggs.

It appears, from Table V, that the mean period of time spent by a mated queen in performing each behaviour varied considerably; the longest period (mean 28 seconds) was recorded when the queen groomed herself. The shortest period (mean 2 seconds) was recorded when the queen examined a worker cell. The range of time recorded for five different mated queens for each behaviour

TABLE IV. BEHAVIOUR OF MATED QUEENS FOR VARIOUS 30 MINUTE PERIODS DURING THE SIX DAYS FOLLOWING THEIR RELEASE.

Types of behaviour	Number of times each type of behaviour was displayed by mated queens for various 30 minute periods during the six days following their release						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Total
<u>Non-aggressive</u>							
Grooming	9*	10	10	3	5	7	44
Examining cells	7	65	87	57	95	106	417
Laying	2	43	54	40	81	50	270
Feeding from cells	0	1	0	0	0	1	2
Begging food	7	5	13	7	6	4	42
Receiving food	5	5	13	7	6	4	40
Offering food	1	0	0	2	1	0	4

*Results of observations of five mated queens for 30 minutes each.

TABLE V. TIME SPENT BY MATED QUEENS IN PERFORMING VARIOUS TYPES OF BEHAVIOUR.

Types of behaviour	Times each behaviour was recorded	Mean time \pm S.E. and range (seconds)
<u>Non-aggressive</u>		
Grooming	27*	28.3 \pm 7.4 (2-152)
Examining cells	414	1.7 \pm 0.1 (1-35)
Laying	202	19.3 \pm 1.0 (8-122)
Feeding from cells	2	22.5 \pm 10.5 (12-33)
Begging food	42	3.0 \pm 0.2 (2-5)
Receiving food	40	13.7 \pm 1.9 (2-53)
Offering food	4	7.5 \pm 2.9 (2-15)

*Number of mated queens observed = 5.

showed that the length of time spent in grooming was the most variable and the length of time spent in "begging" for food was the least variable. Gary (1975) observed that in general, a mated queen spends approximately 9 to 12 seconds laying an egg. My mated queens, however, spent a longer period of time than this (an average of 19 seconds and a range of 8 seconds to 2 minutes and 2 seconds); perhaps this is because my mated queens were young and had had little egg laying experience so that they spent more time per egg laid than did experienced queens.

4.3.2 Behaviour of Worker Bees

While observing the behaviour of a queen, the behaviour of the marked worker bees of known ages towards the queen was also observed. Their behaviour towards virgin or mated queens is shown in Table VI.

The marked worker bees displayed two behavioural patterns towards their queens arbitrarily categorized as non-aggressive and aggressive behaviour. Table VI shows that the worker bees performed more types of non-aggressive and aggressive behaviour towards virgin queens than they did towards mated queens. The behavioural patterns listed in Table VI are described below:

TABLE VI. WORKER BEES' BEHAVIOUR.

Towards virgin queensNon-aggressive behaviour

Trembling
 Licking
 Begging food from a
 queen
 Receiving food from
 a queen
 Offering food to a queen
 Holding - clinging

Aggressive behaviour

Rocking
 Holding - pulling
 Holding - biting
 Holding - stinging
 Holding - biting - stinging

Towards mated queensNon-aggressive behaviour

Licking
 Begging food from a
 queen
 Receiving food from a
 queen
 Offering food to a queen
 Holding - clinging

Aggressive behaviour

Rocking
 Holding - biting -
 stinging

4.3.2.1 Non-aggressive Behaviour

Trembling: A worker bee either held onto the comb (about 1 cm from the queen) or held onto the queen's body itself with its legs and moved its abdomen up and down quickly. This behaviour has been reported previously as "dorsal-ventral abdominal vibration" (DVAV), "trembling" movement, or "shaking", by Milum (1955), Hammann (1958), and Allen (1956, 1958, 1959) respectively. Hammann (1958) and Allen (1958, 1959) concluded from their experiments, that worker bees perform this behaviour towards their queen in order to stimulate her to fly. In my experiments, the worker bees displayed trembling behaviour only towards virgin queens. Hammann (1957) also confirmed this, but Allen (1959) reported that she saw the worker bees display this behaviour towards the mated queens during the few days after she first commenced laying, and during swarm preparations.

Licking: The worker bees, which surrounded the queen, extended their tongues and licked either her legs, thorax, or abdomen. They displayed this behaviour towards both the virgin and the mated queens. Licking behaviour of worker bees towards a queen has been reported by many researchers, e.g. Butler (1954a, 1954b), Allen (1955, 1957, 1960), and Hammann (1958). Butler (1954a, 1954b, 1956) stated that worker bees obtain queen substances from the queen in this way,

but Velthuis (1972) suggested that the substances pass from the queen's body to the worker bees when they come into direct contact with the queen.

Begging food from a queen: Sometimes a worker bee which was positioned in front of the queen extended its tongue to the mouth of the queen to "beg" for food. The worker bee "begged" for food from both virgin and mated queens. It appears that this behaviour has not been reported previously.

Receiving food from a queen: When a queen opened her mandibles and regurgitated so that a drop of liquid food appeared between them, the begging worker bee sucked up this food with its extended tongue. While receiving this food, antennal contact between the offering queen and the receiving worker bee was also observed. The worker bees received food from both virgin and mated queens. Again, this behaviour has not been reported previously.

Offering food to a queen: A worker bee regurgitated a drop of liquid food which appeared between its opened mandibles, and fed the queen; the offering worker bee did not move its tongue downwards and forwards as it did when feeding another worker bee (see Butler, 1954a). The worker bees displayed this behaviour towards both virgin and mated queens. Sometimes they offered food to the queen although she

had not begged from them. The worker bees also offered food towards the abdomen of the queen instead of her head. This agrees with the reports of Velthuis (1972).

Holding - Clinging: One or two worker bees held the queen's wings, then her abdomen or legs with their legs, and then clung to her for a period of time. Sometimes worker bees were dragged by the queen along the face of the comb while clinging to her. The worker bees displayed this behaviour towards both virgin and mated queens; this behaviour was reported by Hammann (1958) and Yadava (1970) towards virgin queens, but the same behaviour has not been reported previously towards mated queens.

4.3.2.2 Aggressive Behaviour

Rocking: When a worker bee came into contact with a queen, it stood on its two pairs of hind legs and rocked its body backwards and forwards. This looked as if the worker bee was "pecking" the queen. The worker bees displayed this behaviour towards both virgin and mated queens. Hammann (1958) reported this behaviour towards a virgin queen, but nobody has reported this behaviour of worker bees towards mated queens.

Holding - Pulling: One or two worker bees held the queen's wings and legs with their legs and mandibles and pulled her either sideways

or backwards. The worker bees only displayed this behaviour towards virgin queens. Hammann (1958) and Yadava (1970) also reported this behaviour of worker bees towards a virgin queen.

Holding - Biting: One or two worker bees held the queen by her legs, thorax, or abdomen with their legs and bit her wings with their mandibles. This behaviour was observed only in the virgin queen experiment. It was also observed by Hammann (1958) and Yadava (1970). Hammann (1958) included this behaviour under "pulling behaviour". However, I often observed that the worker bees held and bit the virgin queen without actually pulling her.

Holding - Biting - Stinging: One or two worker bees held and bit the queen as previously described. In addition, the worker bees bent their abdomens and extended their stings to either the dorsal or ventral side of the queen's abdomen. They also hung onto the back of the queen and kept on stinging her as she dragged them along. This behaviour was observed in both the virgin and the mated queen experiments. In none of my experiments did a virgin or a mated queen sustain injury from the stings of the worker bees. Yadava (1970) observed that caged worker bees displayed this behaviour towards an introduced virgin queen. Walton and Smith (1969) reported that worker bees also behaved this way towards virgin or mated

queens when they were "balling"* them. The balling behaviour of worker bees is outlined in Chapter 5.

4.3.2.3 Behavioural Patterns

Generally, the marked worker bees, of different ages, showed no regular sequence of behaviour towards a virgin or a mated queen. Allen (1960), who observed the behaviour of worker bees towards a one-year old mated queen, also reported this fact. However, I observed that most of the worker bees' aggressive behaviour was performed towards virgin and mated queens during the first 30 minute period after the virgin queens emerged from their cells, or after the mated queens were released from their cages. A few hours later, as the worker bees appeared to get used to the queen, they were less aggressive; for example they always used their antennae to examine the queen when she passed by, and often behaved in various ways (mostly in non-aggressive ways) towards the queen when they met her. It was observed that usually about 8-12 worker bees formed a "court" around the mated queen even on the first day

*Balling is the behaviour displayed by a group of worker bees towards intruders. A few worker bees hold, pull, bite, and try to sting the intruder while the other worker bees form a ball around it. The intruder may die due to being stung and/or possibly due to suffocation. The number of balling worker bees varies greatly.

after she was released from her cage; however, a "court" of worker bees was seldom seen around the virgin queen during the first day after her emergence. The worker bees, which constituted the court, did not usually follow the queen for long, and were continuously being replaced by other workers; this observation confirms the reports of Butler (1954a) and Butler et al. (1973). The court consisted of worker bees of various ages which were present in the area where the queen moved. They usually examined the queen's body with their antennae, licked the queen's abdomen with their extended tongues and/or offered food to her. Allen (1960) found that the ages of the worker bees forming a court around a one-year old mated queen varied considerably. The behaviour of worker bees of different ages towards virgin and mated queens during various 30 minute periods is shown in Table VII and Table VIII respectively.

In the virgin queen experiment (Table VII), worker bees licked the virgin queens more frequently than they displayed the other types of behaviour. Next to licking, the other three types of behaviour most frequently displayed by the worker bees towards the virgin queens were trembling, offering (food) and rocking. The results of Table VII also show that the oldest group of worker bees (15-21 days old) displayed each type of behaviour more frequently than did the

TABLE VII. BEHAVIOUR OF WORKER BEES OF DIFFERENT AGES TOWARDS VIRGIN QUEENS.

Types of behaviour	Total number of times each type of behaviour was displayed by workers of different ages			Total for all ages
	1-7*	8-14	15-21	
<u>Non-aggressive</u>				
Trembling	0**	0	61	61
Licking	9	5	98	112
Begging food	4	5	8	17
Receiving food	3	3	8	14
Offering food	0	2	26	28
Holding - clinging	1	0	11	12
<u>Aggressive</u>				
Rocking	4	0	18	22
Holding - pulling	1	0	5	6
Holding - biting	0	0	2	2
Holding - biting - stinging	0	0	6	6

*Age of worker bees in days.

**Results of nine 30 minute observations of five queens (i. e. total = 1350 minutes).

TABLE VIII. BEHAVIOUR OF WORKER BEES OF DIFFERENT AGES TOWARDS MATED QUEENS.

Types of behaviour	Total number of times each type of behaviour was displayed by workers of different ages				Total for all ages
	1-7*	8-14	15-21	22-28	
<u>Non-aggressive</u>					
Licking	40**	60	30	97	227
Begging food	2	1	0	4	7
Receiving food	1	1	0	3	5
Offering food	11	13	6	15	45
Holding-clinging	5	3	6	6	14
<u>Aggressive</u>					
Rocking	9	5	4	15	33
Holding - biting - stinging	0	0	2	0	2

*Age of worker bees in days.

**Results of six 30 minute observations of five queens (i. e. total = 900 minutes).

younger groups. Hammann (1957, 1958) also found that older worker bees displayed aggressive behaviour towards the virgin queen more often than did the younger worker bees.

In the mated queen experiment (Table VIII), worker bees licked the mated queens more frequently than they displayed the other types of behaviour. It also appears that most of the worker bees, which displayed this behaviour, were among the oldest group of worker bees (22-28 days old); Allen (1960) found that worker bees of all ages licked a one-year old mated queen but she did not report which age was the dominant one. The other two types of behaviour most frequently displayed by worker bees towards mated queens, which I observed, were the offering (food) and rocking behaviour. It is possible that the more the queen lays eggs the more frequently the worker bees feed her. The number of worker bees of each age offering food to the mated queens was close to one another (Table VIII); however, Allen (1960) found that most of the worker bees which fed a one-year old queen were two to four days old. The number of worker bees of each age which displayed rocking behaviour and other types of behaviour was constant.

The results of both the virgin and the mated queen experiments show that worker bees licked the virgin and the mated queens

more frequently than they displayed the other types of behaviour. Butler (1954a, 1954b, 1956) postulated that queen substances are passed from the queen to worker bees when the worker bees lick their queen. It is possible that the worker bees, in my experiments, lick their queens frequently because they are responding to the queen substances produced by their queens.

The period of time the marked worker bees (regardless of their ages) spent in performing various types of behaviour towards virgin and mated queens is shown in Table IX and Table X. In the virgin queen experiment (Table IX), the worker bees spent the longest period of time (mean 33 seconds) holding, biting and stinging virgin queens and spent the shortest period of time (mean 3 seconds) trembling or rocking. The widest range (7 seconds to 1 minute and 32 seconds) occurred when the marked bees displayed the holding - biting - stinging behaviour towards virgin queens. The narrowest range (2 to 7 seconds or 3 to 8 seconds) occurred when the marked bees displayed the begging (food), rocking, or holding - biting behaviour towards virgin queens.

In the mated queen experiment (Table X), the worker bees spent the longest period of time (mean 1 minute and 42 seconds) performing holding - clinging behaviour towards mated queens and

TABLE IX. TIME SPENT BY WORKER BEES IN PERFORMING VARIOUS TYPES OF BEHAVIOUR TOWARDS VIRGIN QUEENS.

Types of behaviour	Times each behaviour was recorded	Mean time \pm S. E. and range (seconds)
<u>Non-aggressive</u>		
Trembling	61*	3.2 \pm 0.2 (2-10)
Licking	47	16.8 \pm 2.6 (2-80)
Begging food	12	4.0 \pm 0.5 (2-7)
Receiving food	10	13.4 \pm 4.0 (3-38)
Offering food	14	18.9 \pm 6.2 (2-82)
Holding - clinging	9	15.0 \pm 6.3 (2-62)
<u>Aggressive</u>		
Rocking	15	3.0 \pm 0.3 (2-7)
Holding - pulling	6	7.2 \pm 1.1 (4-12)
Holding - biting	2	5.5 \pm 2.5 (3-8)
Holding - stinging	8	13.5 \pm 5.5 (4-52)
Holding - biting - stinging	4	33.5 \pm 19.8 (7-92)

*Number of virgin queens observed = 5.

TABLE X. TIME SPENT BY WORKER BEES IN PERFORMING VARIOUS TYPES OF BEHAVIOUR TOWARDS MATED QUEENS.

Types of behaviour	Times each behaviour was recorded	Mean time \pm S. E. and range (seconds)
<u>Non-aggressive</u>		
Licking	55*	40.3 \pm 9.6 (3-304)
Begging food	6	4.0 \pm 0.7 (2-6)
Receiving food	5	9.2 \pm 1.7 (4-15)
Offering food	44	12.8 \pm 1.8 (2-53)
Holding - clinging	10	102.4 \pm 30.7 (15-278)
<u>Aggressive</u>		
Rocking	27	6.4 \pm 2.0 (2-55)
Holding - biting - stinging	2	10.5 \pm 1.5 (9-12)

*Number of mated queens observed = 5.

spent the shortest period of time (mean 4 seconds) begging for food. The widest range (3 seconds to 5 minutes and 4 seconds) occurred when the marked bees licked mated queens. The narrowest range (9 to 12 seconds) occurred when the marked bees displayed the holding - biting - stinging behaviour towards mated queens.

Butler (1954b) recorded the length of time each worker bee spent licking the mated queen and found that the mean time was about one minute. The results of Table X show that the mean time spent by a worker bee in licking a mated queen is 40 seconds, which is close to that recorded by Butler (1954b). Allen (1955) observed worker bees feeding a one-year old mated queen and recorded that a worker bee spent an average of 47 ± 2.6 seconds in feeding a queen; the range of individual feedings varied between five and 156 seconds. In my experiments (Table X), the mean time for each feeding (13 seconds) was shorter, and the range of individual feedings (2 to 53 seconds) was narrower, than that recorded by Allen (1955).

The results of the experiments in this chapter show that while moving among worker bees in the hives, virgin queens displayed both non-aggressive and aggressive behaviour while young mated queens displayed only non-aggressive behaviour. The non-aggressive behaviour of virgin queens was similar to that of mated queens except

that laying behaviour was not displayed by virgin queens and holding behaviour was not performed by mated queens. Worker bees displayed both non-aggressive and aggressive behaviour towards virgin and mated queens. Generally, older worker bees displayed various types of behaviour towards the queens more frequently than did younger worker bees.

CHAPTER 5

BEHAVIOUR OF VIRGIN OR MATED QUEENS IN THE PRESENCE OF WORKER BEES

5.1 Introduction

The major goal of beekeeping is to produce a maximum bee population to coincide with the main nectar flows of a given area. A single-queen colony is not always capable of doing this. A colony with two or more queens (i. e. a multiple queen colony), laying eggs side by side, might be ideal for this purpose. Under normal conditions, however, a colony tolerates only one queen and eventually kills any extra queens that are present (see Lensky et al., 1970). To ascertain why more than one queen cannot live together within a hive I studied the behaviour of queens towards each other as well as the behaviour of worker bees towards these queens.

5.2 Experiments with Two Virgin or Two Mated Queens

5.2.1 Methods

5.2.1.1 Virgin Queen Experiment

A queenless observation hive, containing three different groups of marked worker bees of different ages (see the virgin queen

experiment in Chapter 4), was used. Emergency queen cells, constructed between the time when the first group of marked bees was introduced into the hive and the time when the last group of marked bees was introduced into the hive, were destroyed by hand.

On the day the last group of marked worker bees was introduced into the hive, two capped queen cells of the same age were introduced into the hive. They were pressed gently about 15 cm apart on the same side of the comb (Figure 10) about 24 hours before the virgin queens were due to emerge. The behaviour of the two newly emerged virgin queens towards each other and the behaviour of the marked worker bees towards both queens was observed continuously until one queen was killed. This experiment was replicated nine times using nine different observation hives. All replicates were done between July and August 1973.

5.2.1.2 Mated Queen Experiment

A queenless observation hive, containing four different groups of marked worker bees of different ages similar to that used for the mated queen experiment described in Chapter 4, was used. Emergency queen cells, constructed between the time when the first group of marked bees was introduced into the hive and the time when the last group of marked bees was introduced into the hive, were

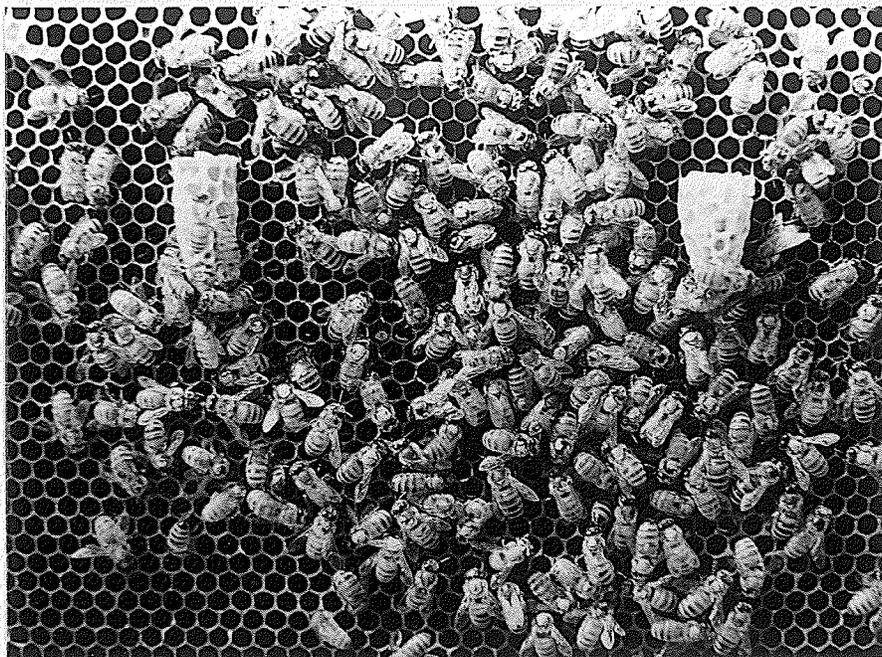


FIGURE 10. TWO CAPPED QUEEN CELLS, CONTAINING EMERGING VIRGIN QUEENS, BEING INTRODUCED INTO A HIVE.

destroyed by hand.

Five days before the last group of marked worker bees was introduced into the hive, two mated queens, about one month old, were introduced separately into the hive by using two wire screen push-in cages placed about 15 cm apart on the same side of the comb (Figure 11). They were confined in the cages with four to five worker bees for six days to allow the worker bees of the hive "to get used to them" before they were released. After their release, the behaviour of both queens towards each other and the behaviour of the marked worker bees towards both queens were observed continuously until one queen was killed. This experiment was replicated nine times using nine different observation hives. All replicates were done between July and August 1974.

Statistical analyses of both the virgin queen and the mated queen experiments were done by using the chi-square test (Steel and Torrie, 1960; Snedecor and Cochran, 1973).

5.2.2 Results and Discussion

5.2.2.1 Behaviour of Queens

The behaviour of the two virgin queens towards each other was similar to that of the two mated queens. The queens displayed only aggressive behaviour towards each other when they met. The

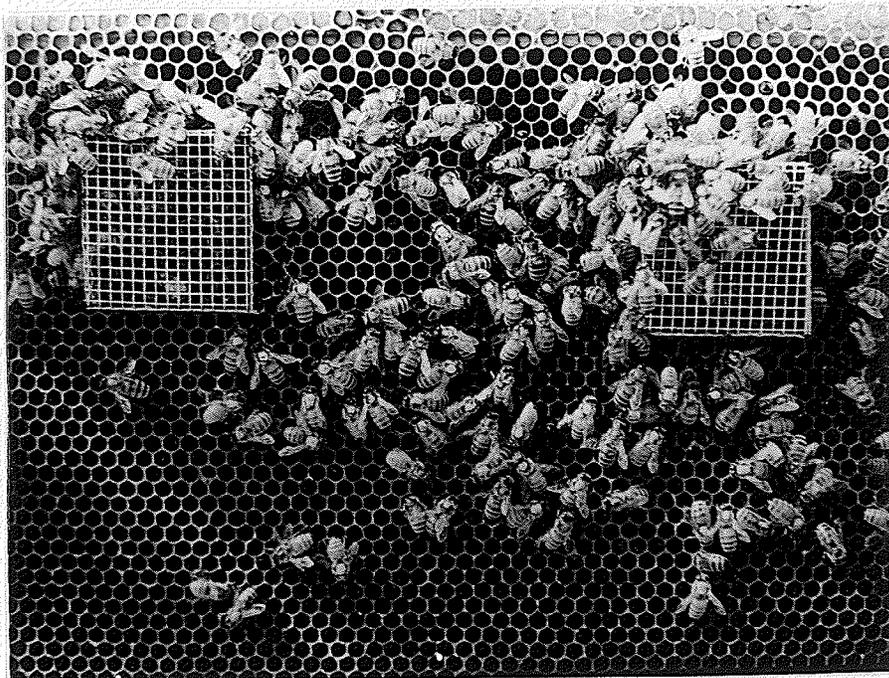


FIGURE 11. TWO MATED QUEENS IN SEPARATE PUSH-IN CAGES MADE OF WIRE SCREENING.

non-aggressive behaviour of the queens in the absence of worker bees, as reported by Waikakul (1973) and Szabo and Smith (1973), was not observed in this study.

The following aggressive behaviour was observed in both the virgin queen and the mated queen experiments:

Pawing: One queen stroked the other queen with her fore-legs when they met; on occasion each queen stroked the other simultaneously which usually led to holding, biting, and stinging. This behaviour usually occurred when both queens first met each other.

Holding - biting: One queen usually held the other queen by the legs, thorax, or abdomen with her legs and bit it (mostly on the wings or legs) with her mandibles. Usually, the queen was unable to injure her rival by biting. Sometimes the queens held and bit each other simultaneously.

Holding - biting - stinging: One queen held and bit the other queen as previously described; she then bent her abdomen and extended her sting towards the body of the other queen, mostly at the ventral regions of the thorax and abdomen. Sometimes both queens held, bit, and attempted to sting each other simultaneously. However, I observed that only one queen was ever killed in combat; this was consistent with the observation of Huber (1814).

The first behavioural pattern described above was reported by Waikakul (1973) and the last two were reported by Lensky et al. (1970), Waikakul (1973) and Szabo and Smith (1973) when they put two virgin queens together without worker bees present. Kovtun (1949) and Melnik (1951) reported aggressive behaviour of a mated queen towards other mated queens in one of his multiple-queen colonies, but Lensky et al. (1970) found that when mated queens are paired without worker bees present they usually do not fight with each other. Kovtun (1949) also reported that although the mated queens one and one-half years old in his multiple-queen colonies fought, they could not bend their abdomens, which were full of eggs, to sting and kill one another.

5.2.2.2 Behaviour of Worker Bees

Marked worker bees were observed to display both aggressive and non-aggressive behaviour towards the pair of virgin, or mated, queens. The types of behaviour displayed were similar to those displayed by worker bees in the one-queen experiments. Because I was mainly interested in how worker bees behave towards an extra queen, only aggressive behaviour (i. e. holding - biting - stinging) displayed by the marked worker bees of different ages was recorded. The data are shown in Table XI. The results show that

TABLE XI. THE OCCURRENCE OF AGGRESSIVE BEHAVIOUR BY WORKER BEES OF DIFFERENT AGES FROM THE TIME THE QUEENS WERE RELEASED UNTIL ONE QUEEN WAS KILLED.

Types of experiment	Occurrence of aggressive behaviour by marked bees of different ages (times)				Total
	1-7 days	8-14 days	15-21 days	22-28 days	
Virgin queen experiment ⁺	0	3	13	— ⁺⁺	16
Mated queen experiment ⁺	0	4	9	12	25

+ Recorded from nine replicates.

+ + This group of marked worker bees was not present in the virgin queen experiment.

in both the virgin queen and the mated queen experiments older marked worker bees usually displayed aggressive behaviour towards one or both of the introduced queens more frequently than did the younger ones. The results also show that the youngest group of marked worker bees (one to seven days old) did not display aggressive behaviour towards the queens. Szabo and Townsend (1974) obtained similar results when they introduced a virgin queen into a cage containing worker bees of known ages; they found that older worker bees (14-21 days old) usually attack the introduced queen and younger worker bees (seven days old) usually accept her.

It appears that any aggressive behaviour towards introduced queens by older worker bees usually attracts other worker bees which "ball" one, or both, of the queens. The term "balling" is generally used to describe how worker bees get rid of their intruders (see footnote in Chapter 4). The balling of a queen could be described as the aggressive behaviour displayed by a group of worker bees (mostly older bees) towards an introduced queen; first a few worker bees held, pulled, bit, and tried to sting the queen, then the other worker bees formed a ball around her (Figure 12). The number of the worker bees involved in the "balling" varied between 30 and 150 worker bees. Sometimes each queen was balled separately, although



FIGURE 12. A QUEEN BEING "BALLED", AS SEEN THROUGH THE GLASS WALL OF AN OBSERVATION HIVE.

in a few cases they were both balled while fighting (Figure 13); alternatively, one queen might be balled while the other queen ran around the ball trying to penetrate it (Figure 14). Usually, the worker bees killed one of the queens; however, in one of my mated queen experiments, in which both of the mated queens fought, they were balled, and eventually killed by the worker bees. The balled queen might be killed in the first or subsequent ballings by "suffocating" as suggested by Huber (1814) or by being stung by the balling worker bees. While a queen was being balled, some worker bees, which had chased and touched the queen, were subsequently balled and sometimes killed by their fellow worker bees; this kind of balling has also been reported by Butler (1954a).

Huber (1814) appears to have been the first observer to describe the balling behaviour of worker bees. Gary (1961), and Walton and Smith (1969) postulated that this behaviour of worker bees might be elicited by above normal concentration of the mandibular gland pheromone produced by the queen, by a trace factor in the pheromone that acts as a releaser for worker bee aggressive behaviour, and/or by an alarm pheromone produced by the attacking worker bees themselves. Yadava and Smith (1971a, 1971b) suggested that under certain stress conditions a queen might produce a "stress pheromone" from



FIGURE 13. TWO QUEENS BEING BALLED TOGETHER
WHILE FIGHTING.

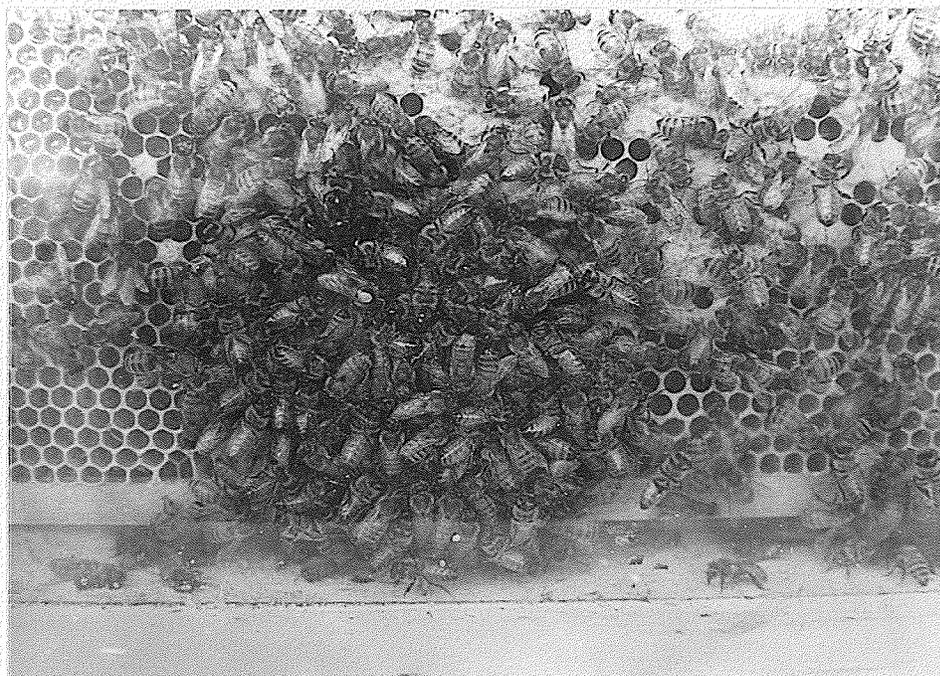


FIGURE 14. ONE QUEEN BEING BALLED WHILE THE OTHER QUEEN RUNS AROUND THE BALL.

her mandibular glands that evokes the balling behaviour of worker bees. From my observations, it seems that various kinds of pheromones, produced by both queens in large quantities when they meet each other or during their encounters, and/or an alarm pheromone that might be produced by the worker bees in response to the queens' pheromones, might be responsible for the initiation of worker bee balling behaviour.

5.2.2.3 Behavioural Patterns

Although each pair of virgin or mated queens acted differently from the others, any pair of virgin or mated queens displayed the same general behavioural patterns when they met each other. After they emerged from their cells, or were released from the cages, they did not fight each other immediately but wandered among worker bees. However, when they met each other, fighting between them usually occurred. The fighting might be initiated by one or both queens pawing the other, followed by holding, biting and stinging behaviour. Occasionally, if fighting did not occur when they first met each other, they pawed each other for two to three seconds and then separated. However, they always fought with each other in subsequent encounters. The fighting queens held legs, bit wings and/or legs and bent the abdomen to sting the other either on the

thorax or abdomen. The fighting pair usually dropped from the face of the comb onto the observation hive's floor. It appeared that one queen rarely injured the other during the first encounter. There was only one virgin queen experiment and one mated queen experiment in which one queen successfully stung and paralyzed her rival queen during the first encounter. In the other pairs, if one or both queens were not balled and killed by the worker bees, they fought with each other again, during which time one queen usually killed the other one. A queen would be able to paralyze or kill her rival queen if she could insert her sting through the membrane around the base of the coxa of any leg of her rival queen; this process has never been described previously. Butler (1954) reported that the sting is inserted between the segments of any part of the body and Dines (1968) and Lensky et al. (1970) stated that the sting might go through the membranes between the abdominal segments.

Worker bees displayed similar behavioural patterns towards pairs of virgin queens as well as pairs of mated queens. When fighting between queens occurred the worker bees clustered around them and sometimes tried to ball them while they were fighting. After both queens separated from each other the worker bees, mostly the older ones, chased and tried to ball and kill one, or occasionally,

both queens. In one of the mated queen experiments the worker bees killed both queens while balling them.

It is therefore apparent that when a pair of queens (either virgin or mated queens) are introduced into a queenless observation hive, they fight with each other and one of them is usually killed by the other or by the worker bees. The number of fighting pairs, the number of fighting pairs which fight when they first meet, and the number of fighting pairs in which one queen is killed by her rival queen or by the worker bees, are shown in Table XII, Table XIII, and Table XIV respectively.

Table XII shows that although a greater number of paired virgin queens fought this did not mean that the virgin queens were more aggressive than the mated queens. It appears that in all of the non-fighting pairs of mated queens one or both queens were balled by worker bees before they had a chance to meet each other. It is possible that if they had had an opportunity to meet they would have fought with each other.

The number of fighting pairs of virgin queens, which fought when they first met each other, was not significantly different from that of the mated queens ($0.250 < P < 0.500$) (Table XIII). The results from Table XIV show that the ability of the mated queens to

TABLE XII. NUMBER OF FIGHTING PAIRS OF QUEENS.

Kinds of queens	Total No. of pairs observed	No. of fighting pairs	No. of non-fighting pairs
Virgin	9	9	0
Mated	9	6	3 ⁺

+ One or both queens of all pairs were balled by worker bees before meeting each other.

TABLE XIII. NUMBER OF FIGHTING PAIRS WHICH FOUGHT WHEN THEY FIRST MET.

Kinds of queens	Total No. of the fighting pairs	No. of fighting pairs which fought when they first met
Virgin	9	8 ⁺
Mated	6	4 ⁺

+ Chi-square test between virgin and mated queens = 1.11 ($0.250 < P < 0.500$) non-significant.

TABLE XIV. NUMBER OF FIGHTING PAIRS IN WHICH ONE QUEEN WAS KILLED BY HER RIVAL QUEEN.

Kinds of queens	Total No. of fighting pairs	No. of fighting pairs in which one queen was killed by her rival	No. of fighting pairs in which one queen was killed by the workers
Virgin	9	6+	3
Mated	6	3+	3+ +

+ Chi-square test between virgin and mated queens = 0.42 ($0.500 < P < 0.750$) non-significant.

+ + Both mated queens were killed in one experiment.

kill other queens was not significantly different from that of the virgin queens ($0.500 < P < 0.750$). However, Kovtun (1949) reported that the mated queens one and one-half years old in his multiple-queen colonies were unable to kill one another. This suggests that the ability of mated queens to kill their rivals declines as their age progresses.

5.3 Experiments with Two Virgin or Two Mated Queens with Clipped Stings

5.3.1 Methods

One-frame observation hives, similar to those used in previous experiments, were used but no marked worker bees of known ages were added to them. The virgin and the mated queens used in the experiments were about one day and one month old respectively. Prior to the experiments, a quarter to a half of their stings were cut off with micro-scissors without using an anaesthetic. The thorax of the queen was held between the thumb and the index finger; her sting, which protruded while she was held, was then cut off. Two of these virgin (or mated) queens were then introduced separately into a hive using wire screen push-in cages similar to those used in the previous experiments. They were confined in the cages with four to five worker bees for six days to allow the worker bees of the hive

"to get used to them" before they were released. After their release, their behaviour towards each other, and the worker bees' behaviour towards them, were observed continuously until one queen was killed. Another observation hive, into which one virgin (or mated) queen with a clipped sting was introduced by means of a wire screen push-in cage was used as a control. Each experiment was replicated five times during August 1975.

5.3.2 Results and Discussion

It appears that the short sting of a queen does not affect her acceptance by worker bees. In each of the control hives into which one virgin or one mated queen with a clipped sting was introduced, the queen was well accepted by the worker bees. This indicates that if the worker bees in each of the two-queen experiments rejected either one or both queens they did so because they responded to other stimuli, not because the queen had her sting cut short.

In each of the two-queen experiments, both introduced queens were well accepted by the worker bees after they were released from their cages. When they met each other, fighting, initiated by one or both of them, usually occurred. The pawing, holding - biting, and holding - biting - stinging behaviour, similar to

that performed by a pair of virgin or mated queens with normal stings in the previous experiments, was also observed. Although they were deprived of their stings, the queens kept on fighting and stinging each other; however, they could not kill each other by stinging. Some pairs of queens without stings had their wings badly torn due to the long periods of fighting. The worker bees usually clustered around the fighting queens but took no part in the fighting. They tried, however, to chase and ball one or both queens when the queens separated from each other. In some of the virgin queen and the mated queen experiments the worker bees balled both queens while they were fighting. However, most of the queens tried to escape from the worker bees. Three virgin queens, from two different observation hives, fought back by grabbing the chasing bees and stinging them; however, they were unable to injure the bees. The balling behaviour of the worker bees was the same as that observed in the previous experiments. The number of fighting pairs, and the number of the fighting pairs which fought, when they first met each other are shown in Table XV.

Table XV shows that each pair of virgin and mated queens without stings always fought with each other when they were in the same hive. It also appears that the number of pairs of virgin queens

TABLE XV. NUMBER OF FIGHTING PAIRS OF QUEENS WITH THEIR STINGS CUT SHORT.

Kinds of queen	Total No. of pairs observed	No. of pairs which fought	No. of pairs which fought when they first met
Virgin	5	5	4+
Mated	5	5	5+

+ Chi-square test between virgin and mated queens = 1.11 ($0.250 < P < 0.500$) non-significant.

which fought when they first encountered each other was not significantly different from that of the pairs of mated queens ($0.250 < P < 0.500$).

These results suggest that virgin and mated queens deprived of stings have the same degree of aggressiveness when they meet the same kind of queens in the presence of worker bees.

In all experiments one queen was usually balled and killed by the worker bees; there was one exception in which one of the virgin queens was driven out of the hive by the worker bees. In two virgin queen experiments the queens ceased fighting but the worker bees interfered and killed one of the queens. In the other experiments, one queen was usually killed by the worker bees before she had an opportunity to co-habit with the other queen. Anderson (1960) and Lensky et al. (1970), who put two mated queens deprived of their stings into a one-frame observation hive and a standard Langstroth hive respectively, obtained the same results.

The results from the experiments with two ordinary queens, and two queens deprived of their stings, raise two important problems relating to the use of multiple-queen colonies, (1) the killing of one queen by the other queen and (2) the killing of one, or both, queens by the worker bees. The first problem might be overcome by cutting the queens' stings short before introducing them into

a hive. The second problem seems to be more difficult to overcome. Smith (1923), Lensky and Darchen (1963), and Lensky et al. (1970) emphasized the importance of the behaviour of the worker bees in initiating and maintaining multiple-queen colonies. To overcome the worker bee problem, further studies which might involve the masking of the odours of the queens so that they do not attract the worker bees, and the use of newly emerged worker bees when the multiple-queen colonies are initiated, should be undertaken.

CHAPTER 6

BEHAVIOUR OF A VIRGIN OR A MATED QUEEN AND WORKER BEES TOWARDS QUEEN CELLS CONTAINING QUEEN BROOD OF DIFFERENT AGES

6.1 Introduction

Besides attacking other adult queens, a virgin queen usually attacks any queen cells present in her colony (Huber, 1814; Butler, 1954a, 1975). If a queen's rivals can be eliminated before they emerge from their cells, there is less chance of a queen being killed in the ensuing "battles". Little information is available on how the queen attacks queen cells, and in what sequence the various cells are attacked. Huber (1814) and Butler (1954a) reported that usually both virgin and mated queens attack capped queen cells. Allen (1956), however, found that a mated queen also attacks uncapped queen cells. The following experiments were conducted to investigate the behaviour of a virgin queen, or a mated queen, and worker bees towards queen cells as well as the preference of virgin and mated queens in attacking queen cells containing queen brood of different ages.

6.2 Methods

6.2.1 Virgin Queen Experiment

A queenless observation hive was prepared in the same way as that used for the virgin queen experiment described in Chapter 4, except that no marked worker bees of known ages were added to it.

On the third day after the hive was set up, and after all natural emergency queen cells were destroyed by hand, a set of four queen cells, one containing a young larva, one an old larva, one a young pupa, and one an old pupa, was introduced at random into each hive. The cells were pressed gently onto the same side of the comb about six cm from one another (Figure 15). Another queen cell, containing a queen which would emerge in the next few hours was also placed in the hive on the same side of the comb. After the virgin queen emerged from her cell, her behaviour towards the four queen cells containing queen brood was observed continuously for 24 hours. After 24 hours this set of queen cells was removed from the hive and a new set was presented to the same virgin queen. This experiment was replicated nine times using nine different observation hives and nine different virgin queens. The number of sets of queen cells which were presented to each virgin queen is shown in Table XX. All replicates were done in August 1973.

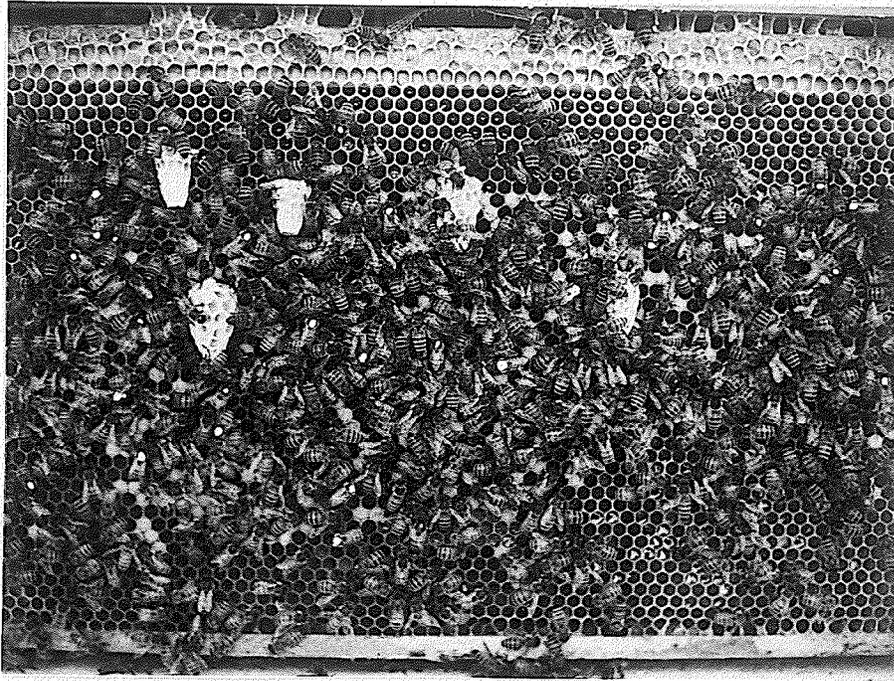


FIGURE 15. A SET OF FOUR QUEEN CELLS EACH CONTAINING A LARVA OR PUPA, AND A QUEEN CELL CONTAINING AN EMERGING QUEEN, BEING INTRODUCED INTO A HIVE.

6.2.2 Mated Queen Experiment

A queenless observation hive was prepared in the same way as that used for the mated queen experiment described in Chapter 4, except that no marked worker bees of known ages were added to it.

On the third day after the hive was set up, and after all natural emergency queen cells were destroyed by hand, one mated queen about one month old was introduced into each hive by using a wire screen push-in cage. The queen was confined with four to five worker bees for six days to allow the worker bees in the hive "to get used to her" before she was released. On the day the queen was released, a set of four queen cells containing queen brood of various ages (see above) was introduced into the hive in the same manner as in the virgin queen experiment (Figure 16). After the queen was released from the cage, her behaviour towards the four queen cells, containing queen brood, was observed continuously for 24 hours. After 24 hours this set of queen cells was removed from the hive and a new set was presented to the same mated queen. This experiment was replicated five times using five different observation hives and five different mated queens. Four sets of these queen cells were presented to each mated queen. All replicates were done in



FIGURE 16. A SET OF FOUR QUEEN CELLS EACH CONTAINING A LARVA OR PUPA, AND A MATED QUEEN BEING INTRODUCED INTO A HIVE.

August 1974.

Statistical analyses of both the virgin queen and the mated queen experiments were done by using the t-test, chi-square test (Steel and Torrie, 1960; Snedecor and Cochran, 1973), or a multiple comparison analysis based on Friedman Rank Sums (Hollander and Wolfe, 1973).

6.3 Results and Discussion

6.3.1 Behavioural Patterns

After a virgin queen emerged from her cell (or a mated queen was released from her cage) she did not rush to attack the queen cells immediately but spent some time wandering on the surface of the comb. The length of time spent by the virgin (and the mated) queens from the time they emerged from their cells (or were released from their cages) until they started attacking the first queen cells is shown in Table XVI. It appears that the virgin queens spent significantly more time ($P < 0.005$) wandering over the combs than did the mated queens before they started attacking the first queen cell of each set.

When the virgin (or mated) queen first located a queen cell, she did not always attack it; this agrees with the reports of Butler (1975). Sometimes she examined the cell for 5-10 seconds

TABLE XVI. LENGTH OF TIME SPENT BY QUEENS WANDERING ON THE COMB BEFORE STARTING TO ATTACK THE FIRST QUEEN CELL OF EACH SET.

Kind of queens	Total number of queens observed	Total number of cell sets observed	Mean length of time before the first queen cell was attacked ($\bar{x} \pm$ S. E.)(hours : minutes)
Virgin	9	40	5:14 \pm 1:01 **
Mated	5	20	0:21 \pm 0:06

**t-test between virgin and mated queens = 3.38 (P<0.005).

and then left without displaying any signs of aggression towards the cell; later she returned to the cell and attacked it.

The virgin and the mated queens displayed similar behavioural patterns in attacking the various kinds of queen cells. When attacking an uncapped queen cell (either containing a young or an old larva), the queen usually held the cell with her legs, oriented her head upwards, and bit the cell wall with her mandibles. Before biting the wall of the uncapped cell (or sometimes while doing so), the queen would put her head through the opening of the cell to examine the larva inside. This behaviour by a mated queen was reported by Allen (1956), but no author has previously reported this behaviour by a virgin queen. It is possible that the queen might have also bitten the larva inside the cell while examining it but this was difficult to determine.

When attacking a capped queen cell (containing either a young or an old pupa), the queen usually held the queen cell with her legs, oriented her head upwards, and bit through the thin part of the cell's wall with her mandibles (Figure 17). However, Huber (1814) and Butler (1954a) observed that when attacking capped queen cells virgin queens usually start by biting holes close to the tips of the cells, but later bite holes nearer the bases. The queen bit the cell



FIGURE 17. A QUEEN BITING THROUGH THE WALL OF A CAPPED QUEEN CELL.

until she was able to make a small hole about two mm in diameter in the cell and then went on biting and enlarging the hole in every direction. While enlarging the holes, most queens also put their heads through the holes to examine the pupae inside the cells (Figure 18). It is possible that the queens might have also bitten the pupae when examining them but again, this was difficult to determine. Waikakul (1973) observed the behaviour of virgin queens towards queen cells in the absence of worker bees and saw the queens bite the pupae, or emerging queens, inside the queen cells. It appears that this biting behaviour of both virgin and mated queens has not been reported previously.

Some queens did not bite the cells continuously but left the cells, wandered in the vicinity of the cells for a half to one minute, and then came back to bite the cells again. Some of the mated queens also laid one or two eggs when they temporarily left the cells. After biting and leaving a cell, the queen would usually come back sometime during the next 10-15 minute period to examine the hole she had made previously. I observed that regardless of how large a hole was made, the queen never pulled the larva or the pupa out of its cell. The time spent, by virgin, and mated queens, in attacking different kinds of queen cells is shown in Table XVII and Table XVIII



FIGURE 18. A QUEEN EXAMINING A PUPA INSIDE A
CAPPED QUEEN CELL.

respectively.

It appears, from Table XVII, that the virgin queens spent significantly more time attacking the capped queen cells containing old pupae than they did the uncapped queen cells containing old larvae ($P < 0.01$). The time spent by the virgin queens in attacking the capped queen cells containing old pupae was not significantly different from the time they spent attacking the capped queen cells containing young pupae. Table XVII also shows that there was no significant difference between the time spent by the virgin queens in attacking the capped queen cells containing young pupae and the uncapped queen cells containing old larvae. Because only one uncapped queen cell, containing a young larva, was attacked by a virgin queen, no statistical analysis was done (see Table XVII). The results shown in Table XVII, however, suggest that the virgin queens appeared to spend less time attacking the uncapped queen cells containing young larvae than the other kinds of queen cells.

Table XVIII shows that the mated queens spent significantly more time attacking the capped queen cells containing young or old pupae than they did the uncapped queen cells containing young or old larvae ($P < 0.01$). The time spent by the mated queens in attacking the uncapped queen cells, containing young larvae, was not significantly

TABLE XVII. TIME SPENT BY VIRGIN QUEENS IN ATTACKING
 QUEEN CELLS CONTAINING BROOD OF DIFFERENT AGES.

Kinds of queen cells ¹	No. of queen cells observed	Mean time spent by virgin queens in attacking queen cells ($\bar{x} \pm$ S. E.) (mins:secs) ²
A	1	07:00
B	7	11:26 \pm 02:04, a
C	16	26:45 \pm 03:43, a, b
D	19	34:22 \pm 04:06, b

¹ A = uncapped queen cells containing young larvae.

B = uncapped queen cells containing old larvae.

C = capped queen cells containing young pupae.

D = capped queen cells containing old pupae.

² Figures within a column followed by the same letter are not significantly different at the 1% level by t-test.

TABLE XVIII. TIME SPENT BY MATED QUEENS IN ATTACKING
QUEEN CELLS CONTAINING BROOD OF DIFFERENT AGES.

Kinds of queen cells ¹	No. of queen cells observed	Mean time spent by mated queens in attacking queen cells ($\bar{x} \pm$ S.E.) (mins:secs) ²
A	11	14:11 \pm 04:10, a
B	11	11:27 \pm 01:41, a
C	10	46:30 \pm 05:34, b
D	12	48:45 \pm 06:23, b

¹ A = uncapped queen cells containing young larvae.

B = uncapped queen cells containing old larvae.

C = capped queen cells containing young pupae.

D = capped queen cells containing old pupae.

² Figures within a column followed by the same letter are not significantly different at the 1% level by t-test.

different from the time they spent in attacking the uncapped queen cells containing old larvae. There was also no significant difference between the time spent by mated queens in attacking capped queen cells containing young and old pupae (see Table XVIII).

It appears that the mated queens spent significantly more time in attacking the queen cells containing young pupae than did the virgin queens ($P < 0.01$) (Table XIX). However, the time spent by the mated queens in attacking the queen cells containing old larvae and old pupae was not significantly different from that spent by the virgin queens. It is not possible to statistically compare the time spent by the virgin queens with that spent by the mated queens in attacking the queen cells containing young larvae because only one of the queen cells containing young larvae was attacked by a virgin queen. The mated queens, however, appeared to spend more time attacking the queen cells containing young larvae than did the virgin queens (Table XIX).

In all experiments neither the virgin nor the mated queens were observed to sting the larvae or the pupae inside the queen cells. Huber (1814) and Butler (1954a, 1975) reported that virgin queens sting only the emerging queens inside the queen cells. It seems that the factor(s) that evokes stinging behaviour of queens is

TABLE XIX. TIME SPENT BY VIRGIN AND MATED QUEENS IN ATTACKING EACH KIND OF QUEEN CELLS.

Kinds of queen cells ¹	Mean time spent by virgin queens in attacking queen cells ($\bar{x} \pm S.E.$)(mins:secs)	Mean time spent by mated queens in attacking queen cells ($\bar{x} \pm S.E.$)(mins:secs)
A	7:00	14:11 \pm 04:10
B	11:26 \pm 02:04	11:27 \pm 01:41 ^{ns}
C	26:45 \pm 03:43	46:30 \pm 05:34**
D	34:22 \pm 04:06	48:45 \pm 06:23 ^{ns}

¹ A = uncapped queen cells containing young larvae.

B = uncapped queen cells containing old larvae.

C = capped queen cells containing young pupae.

D = capped queen cells containing old pupae.

**Significantly different at the 1% level by t-test.

^{ns}Not significantly different at the 5% level by t-test.

not yet known.

The preference of virgin queens in attacking different kinds of queen cells, analyzed by a multiple comparison based on Friedman Rank Sums (Hollander and Wolfe, 1973), is different from that of mated queens; the virgin queens preferred to attack the capped queen cells containing young or old pupae (C or D type) before they attacked the uncapped queen cells containing young or old larvae (A or B type) ($P < 0.01$) (see Table XX). In 1973 I obtained the same results when I placed a virgin queen in a cage with queen cells containing queen brood of different ages (see Waikakul, 1973). In the present study I also observed that the experimental virgin queens seldom attacked the uncapped queen cells; this agrees with the reports of Butler (1954a, 1975). The mated queens, unlike the virgins, showed no preference for attacking a particular kind of queen cell (Table XXI).

The total numbers of queen cells containing young larvae, old larvae, young pupae, and old pupae respectively, attacked by virgin and mated queens were also compared (see Tables XXII, XXIII, XXIV, and XXV). The mated queens attacked significantly more queen cells containing young larvae (Table XXII) or old larvae (Table XXIII) than did the virgin queens ($P < 0.005$); no significant

TABLE XX. PREFERENCE OF VIRGIN QUEENS IN ATTACKING QUEEN CELLS CONTAINING QUEEN BROOD OF DIFFERENT AGES.

Queen No.	No. of sets of queen cells	Preference of virgin queens in attacking queen cells ¹															
		A				B				C				D			
		1st	2nd	3rd	4th	1st	2nd	3rd	4th	1st	2nd	3rd	4th	1st	2nd	3rd	4th
1	6	0*	0	0	0	0	0	2	0	3	3	0	0	3	3	0	0
2	6	0	0	0	0	0	0	0	0	2	1	0	0	4	2	0	0
3	6	0	0	0	0	0	0	1	0	4	2	0	0	2	3	0	0
4	5	0	0	1	0	0	1	0	1	3	2	0	0	2	2	1	0
5	5	0	0	0	0	0	1	0	0	1	3	1	0	4	1	0	0
6	5	0	0	0	0	0	1	1	0	1	3	1	0	4	0	0	0
7	4	0	0	0	0	1	1	0	0	0	1	1	0	1	0	1	0
8	4	0	0	0	0	0	0	1	0	2	0	0	0	0	1	0	0
9	3	0	0	0	0	0	1	0	0	1	0	1	0	2	0	0	0
Total	44	0	0	1	0	1	5	5	1	17	15	4	0	22	12	2	0

¹A = uncapped queen cells containing young larvae.

B = uncapped queen cells containing old larvae.

C = capped queen cells containing young pupae.

D = capped queen cells containing old pupae.

* Number of queen cells attacked by a queen.

TABLE XXI. PREFERENCE OF MATED QUEENS IN ATTACKING QUEEN CELLS CONTAINING QUEEN BROOD OF DIFFERENT AGES.

Queen No.	No. of sets of queen cells	Preference of mated queens in attacking queen cells ¹															
		A				B				C				D			
		1st	2nd	3rd	4th	1st	2nd	3rd	4th	1st	2nd	3rd	4th	1st	2nd	3rd	4th
1	4	1*	1	1	0	1	0	0	2	2	1	0	0	0	2	1	0
2	4	0	1	0	0	1	0	2	0	1	1	2	0	2	2	0	0
3	4	3	0	0	1	0	4	0	0	0	0	2	2	1	0	2	1
4	4	2	2	0	0	1	1	2	0	1	1	0	2	0	0	2	2
5	4	0	1	0	1	2	0	1	0	1	0	3	0	1	3	0	0
Total	20	6	5	1	2	5	5	5	2	5	3	7	4	4	7	5	3

¹A = uncapped queen cells containing young larvae.

B = uncapped queen cells containing old larvae.

C = capped queen cells containing young pupae.

D = capped queen cells containing old pupae.

* Number of queen cells attacked by a queen.

TABLE XXII. NUMBER OF QUEEN CELLS CONTAINING YOUNG LARVAE WHICH WERE ATTACKED BY VIRGIN AND MATED QUEENS.

Kinds of queens	Total number of queen cells given to queens	Total number of queen cells attacked	Total number of queen cells not attacked
Virgin ¹	44	1 ⁺	43
Mated ²	20	14 ⁺	6

¹Observed from nine different virgin queens.

²Observed from five different mated queens.

⁺Chi-square test between virgin and mated queens = 35.15 ($P < 0.005$) highly significant.

TABLE XXIII. NUMBER OF QUEEN CELLS CONTAINING OLD LARVAE WHICH WERE ATTACKED BY VIRGIN AND MATED QUEENS.

Kinds of queens	Total number of queen cells given to queens	Total number of queen cells attacked	Total number of queen cells not attacked
Virgin ¹	44	12 ⁺	32
Mated ²	20	17 ⁺	3

¹Observed from nine different virgin queens.

²Observed from five different mated queens.

⁺Chi-square test between virgin and mated queens = 18.49 ($P < 0.005$) highly significant.

TABLE XXIV. NUMBER OF QUEEN CELLS CONTAINING YOUNG PUPAE WHICH WERE ATTACKED BY VIRGIN AND MATED QUEENS.

Kinds of queens	Total number of queen cells given to queens	Total number of queen cells attacked	Total number of queen cells not attacked
Virgin ¹	44	36 ⁺	8
Mated ²	20	19 ⁺	1

¹Observed from nine different virgin queens.

²Observed from five different mated queens.

⁺Chi-square test between virgin and mated queens = 1.98 (0.100 < P < 0.250) non-significant.

TABLE XXV. NUMBER OF QUEEN CELLS CONTAINING OLD PUPAE WHICH WERE ATTACKED BY VIRGIN AND MATED QUEENS.

Kinds of queens	Total number of queen cells given to queens	Total number of queen cells attacked	Total number of queen cells not attacked
Virgin ¹	44	36 ⁺	8
Mated ²	20	19 ⁺	1

¹Observed from nine different virgin queens.

²Observed from five different mated queens.

⁺Chi-square test between virgin and mated queens = 1.98 (0.100 < P < 0.250) non-significant.

difference was found between the numbers of queen cells containing young pupae (Table XXIV) or old pupae (Table XXV) that were attacked by the virgin or the mated queens.

I observed that a virgin or a mated queen must first attack capped queen cells before worker bees would destroy them. However, Butler (1975) reported that worker bees also attack queen cells even though the cells have not been attacked first by a virgin queen. I observed that approximately six to eight worker bees, some of which were continuously being replaced, clustered around each cell without showing any aggressive behaviour towards it. After the queen finished biting the cell, the clustering worker bees carried on the destruction by enlarging the hole first started by the queen, by sucking liquid from the pupa inside the cell, by pulling the pupa out of the cell, and by disposing of it outside the hive. It appeared that when the queen was biting the cell some of the worker bees also assisted her. However, if the queen made only a very small hole (i.e. less than 1 mm in diameter) in the wall of either a capped or uncapped queen cell, the worker bees usually did not enlarge the hole but repaired it; this verifies the report of Butler (1954a, 1975).

When an uncapped queen cell was not attacked by the queen, the worker bees either destroyed it or fed the larva inside it

throughout the experimental period. The worker bees destroyed an uncapped queen cell in the same manner as they destroyed a capped queen cell as described above.

The results indicate that the virgin and the mated queens exhibit similar behavioural patterns in attacking queen cells. However, the virgin queens usually preferred to attack capped queen cells while few attacks were directed towards the uncapped ones. The mated queens displayed no preference in attacking queen cells as the uncapped queen cells were attacked as frequently as the capped ones.

CHAPTER 7

PRELIMINARY EXPERIMENTS TO INVESTIGATE WHY QUEENS ATTACK QUEEN CELLS

7.1 Introduction

Usually, a virgin (or mated) queen attacks queen cells, particularly the capped ones, that appear in her hive (Huber, 1814; Butler, 1954a, 1975). It is possible that certain factors, e. g. odour, orientation, and texture of a queen cell may assist the queen in recognizing the cell and stimulating her to attack it; however, little is known about this subject. Therefore, the following experiments were done to ascertain the role of odour and queen cell orientation in attracting queens to queen cells.

7.2 Methods

One-frame observation hives were used in the experiments (see Chapter 6). In the virgin queen experiments a mature queen cell, containing an emerging queen, was introduced into each hive by pressing it gently onto the face of the comb. In the mated queen experiments, a mated queen aged 30 days, was

introduced into each hive by means of a wire screen push-in cage (see Chapter 6).

The following four experiments, each with a different virgin queen, were done on the second day after the queens had emerged from their cells; These experiments were repeated, using four different mated queens, on the second day after they were released from their cages.

A. One capped queen cell, containing an old pupa, was placed vertically in a hive which contained either a virgin or a mated queen. This experiment was used as the "control".

B. One empty capped queen cell, previously containing an old pupa, was placed vertically in a hive which contained either a virgin or a mated queen. The pupa was removed from the cell by making a "T" shaped incision through the wall of the cell. The incision was then carefully sealed over by using a heated metal wire placed directly on the incision to melt the wax and to seal the incision.

C. One capped queen cell, containing an old pupa, was placed horizontally (Figure 19) in a hive which contained either a virgin or a mated queen.

D. One empty capped queen cell, previously containing an old pupa, was placed horizontally in a hive which contained either a

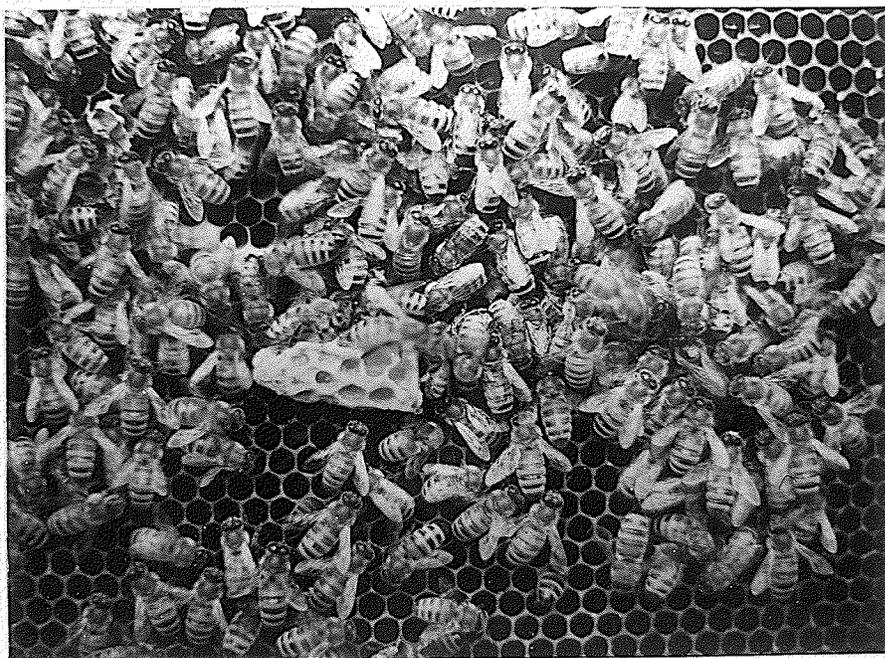


FIGURE 19. A HORIZONTALLY PLACED QUEEN CELL
CONTAINING AN OLD PUPA.

virgin or a mated queen (as in Experiment C). The pupa was removed from the cell in the same manner as was done in Experiment B.

Each experiment was replicated five times by using five different virgin or mated queens and five different queen cells. After the introduction of a queen cell, the behaviour of the queen and the worker bees in the hive was observed continuously for 24 hours. If the queen cell was attacked before the end of the 24 hour period, the observation was terminated at that point. All experiments were conducted in August 1975 as I was completing my main study.

7.3 Results and Discussion

The number of queen cells destroyed, in various experiments, is shown in Table XXVI and XXVII. It appears from these tables that queen cells containing old pupae were attacked more frequently by both virgin and mated queens than were empty cells, regardless of their orientation.

The behaviour of both kinds of queens in attacking the queen cells in various experiments in this chapter was similar to

TABLE XXVI. NUMBER OF QUEEN CELLS, IN VARIOUS EXPERIMENTS,
WHICH WERE ATTACKED BY VIRGIN QUEENS.

No. of experiment ¹	No. of replicates	No. of queen cells which were attacked by queens	No. of queen cells which were not attacked by queens	No. of queen cells which were attacked by worker bees
A	5	5	0	0
B	5	3	2	0
C	5	5	0	0
D	5	1	4	2

¹A = one capped, vertically oriented queen cell containing an old pupa was presented to each queen (control).

B = one empty, vertically oriented queen cell previously containing an old pupa was presented to each queen.

C = one capped, horizontally oriented queen cell containing an old pupa was presented to each queen.

D = one empty, horizontally oriented queen cell previously containing an old pupa was presented to each queen.

TABLE XXVII. NUMBER OF QUEEN CELLS, IN VARIOUS EXPERIMENTS,
WHICH WERE ATTACKED BY MATED QUEENS.

Type of experiment ¹	No. of replicates	No. of queen cells which were attacked by queens	No. of queen cells which were not attacked by queens	No. of queen cells which were attacked by worker bees
A	5	4	1	0
B	5	3	2	0
C	5	5	0	0
D	5	1	4	1

- ¹A = one capped, vertically oriented queen cell containing an old pupa was presented to each queen (control).
- B = one empty, vertically oriented queen cell previously containing an old pupa was presented to each queen.
- C = one capped, horizontally oriented queen cell containing an old pupa was presented to each queen.
- D = one empty, horizontally oriented queen cell previously containing an old pupa was presented to each queen.

that of the queens in Chapter 6. It also appears that worker bees would not destroy a queen cell unless a queen first attacked it (see Experiment D for one exception). In Experiment D, the worker bees destroyed two (of four) empty horizontally placed queen cells even though they had not been previously attacked by virgin queens (Table XXVI). The experiment was repeated using mated queens; one (of four) queen cell was destroyed by the worker bees (Table XXVII). The worker bees destroyed the cells by tearing the walls of the cells with their mandibles.

The results of the experiments described in this chapter indicate that orientation is not the factor which stimulates queens to attack queen cells. Further studies are required to identify the stimulating factor(s), which may be the odour of the pupae and/or of other materials related to the larvae (e.g. food, faeces, cocoons, etc.) inside queen cells. For example, an experiment could be set up by introducing an empty artificial queen cell into an observation hive which contains either a virgin or a mated queen. Another experiment could be done by introducing an artificial queen cell containing a queen pupa into an observation hive which contains either a virgin or a mated queen; the bottom side of the artificial cell should be made of wire screen in order to allow the odour of the pupa to escape from the cell.

I was unable to complete these experiments myself because the necessary biological materials were not available in the fall of 1975.

CHAPTER 8

GENERAL SUMMARY AND CONCLUSIONS

The purpose of the research done in this thesis was to provide more basic information about the behaviour of queens and worker bees with the hope that such information might be useful for the successful establishment of multiple-queen colonies. Experiments were conducted using virgin queens, mated queens, and worker bees in one-frame observation hives when (1) there was one queen in a hive, (2) there were two queens of the same kind in a hive, and (3) there was one queen, and a set of queen cells containing queen brood of different ages, in a hive.

The virgin queens showed two main types of behaviour while wandering in the hives; these were arbitrarily classified as non-aggressive and aggressive behaviour. Non-aggressive behaviour consisted of grooming, examining cells, placing the abdomen into cells, feeding from cells, begging food from worker bees, receiving food from worker bees, offering food to worker bees, and holding other bees. Aggressive behaviour consisted of rocking, holding-biting, and holding-biting-stinging. The mated queens showed only non-aggressive behaviour. The types of non-aggressive behaviour displayed by the mated queens were similar to those of the virgin queens. However, the mated queens did not hold other bees as did

the virgin queens; they did, however, lay eggs, which was not done by the virgin queens.

There was no regular sequence of behavioural patterns shown by virgin or mated queens. The number of times each type of behaviour was performed by the virgin and the mated queens varied from queen to queen and from day to day. A virgin queen groomed herself more frequently than she performed the other types of behaviour. She spent a relatively long period of time receiving food from a worker bee and spent a relatively short period of time examining a worker cell or performing rocking movements towards a worker bee. A mated queen examined worker cells and displayed laying behaviour more frequently than she did the other types of behaviour. She spent a relatively long period of time grooming herself and spent a relatively short period of time examining a worker cell.

Marked worker bees performed non-aggressive and aggressive behaviour towards both the virgin and the mated queens. Their non-aggressive behaviour towards the virgin queens consisted of trembling, licking, begging food from the queens, receiving food from the queens, offering food to the queens, and holding - clinging. The non-aggressive behaviour of marked worker bees towards the mated queens was similar to that shown towards the virgin queens

except that the worker bees did not perform the trembling behaviour towards the mated queens. The worker bees displayed more types of aggressive behaviour towards the virgin queens than they did towards the mated queens. They performed rocking, holding - pulling, holding - biting, holding - stinging, and holding - biting - stinging behaviour towards the virgin queens and performed rocking and holding - biting - stinging behaviour towards the mated queens.

Generally, the marked worker bees showed no regular sequence of behaviour towards virgin or mated queens. They licked both kinds of queens more frequently than they displayed the other types of behaviour. In the virgin queen experiment, the worker bees spent a relatively long period of time displaying holding - biting - stinging behaviour, and spent a relatively short period of time displaying trembling or rocking behaviour, towards virgin queens. In the mated queen experiment, the worker bees spent a relatively long period of time performing holding - clinging behaviour towards mated queens and spent a relatively short period of time "begging" for food from the queens. It was also observed that older worker bees usually displayed various types of behaviour towards the virgin and the mated queens more frequently than did younger worker bees.

Worker bees offered food more often to mated queens than to virgin queens and these mated queens obtained food more often than did virgin queens. This is probably related to the fact that mated queens require more food for ovary development and egg laying. However, virgin queens offered food more often to worker bees than did mated queens and workers obtained food more often from virgin queens than they did from mated queens. It appeared that the virgin queens often displayed this type of behaviour in an attempt to decrease the aggressiveness of the worker bees towards them.

Eighteen experiments were done using pairs of virgin or mated queens in one-frame observation hives to observe their behaviour towards one another as well as the behaviour of the worker bees in the hives towards them. Nine pairs of newly emerged virgin queens and nine pairs of one-month old mated queens were used. In addition, the behaviour of pairs of virgin or mated queens, whose stings had been clipped, and the behaviour of worker bees towards them, were ascertained.

The behaviour of pairs of virgin queens towards each other was similar to that of pairs of mated queens towards each other. There were no behavioural differences between queens, whether or not the queens had their stings clipped. When two queens met each other, they usually fought by pawing, holding, biting, and stinging

each other. For each pair that fought, only one queen was stung to death. A queen was able to paralyze or kill her rival queen if she could insert her sting through the membrane around the base of the coxa of any leg of her rival queen. Usually, one queen could not successfully sting the other during the first encounter. It appeared that the virgin and the mated queens showed the same degree of aggressiveness when they met the same kind of queens.

Generally, the older worker bees clustered around the fighting queens and tried to ball one or both queens while they were fighting and after they separated from each other. In some experiments one or both queens were balled by worker bees before they had a chance to meet each other. It appeared that when one queen could not kill her rival in the fighting (especially in the experiments where the stings of both queens had been clipped), the worker bees usually balled one or both queens until one queen died. In one experiment both mated queens were balled and killed by the worker bees. When attacked by worker bees, some virgin queens fought back by grabbing and attempting to sting some of the bees.

The behaviour of virgin queens, mated queens, and worker bees towards sets of queen cells containing queen brood of different ages was studied. Newly emerged virgin queens, one-month old mated queens, and queen cells of four different ages (i. e. containing

a young larva, an old larva, a young pupa, or an old pupa) were used in the experiments.

Both virgin and mated queens behaved similarly when attacking the various kinds of queen cells. The queens did not always attack queen cells the first time that they located them. They attacked queen cells by making holes through the thin parts of the cell walls with their mandibles. It is not certain if they bit the larvae or pupae inside the cells. However, no queen ever pulled a larva or pupa out of its cell. The virgin queens spent more time wandering over the combs than did the mated queens before they started attacking the queen cells presented to them. Both the virgin and the mated queens spent more time attacking the capped queen cells than they did the uncapped ones. The virgin queens preferred attacking the capped queen cells rather than attacking the uncapped ones. Unlike the virgin queens, the mated queens displayed no preference as to the kind of queen cells they attacked.

Worker bees displayed no aggressive behaviour towards capped queen cells if the cells were not first attacked by the queens. However, after the queens finished biting the cells, the worker bees usually continued to destroy the cells and the pupae inside them. On the other hand, worker bees destroyed uncapped queen cells and the larvae inside them even though the cells had not been bitten by the

queens; sometimes they even fed the larvae throughout the experimental period.

Preliminary experiments were conducted to investigate why queens attack queen cells. Capped queen cells, with or without old pupae, were presented to virgin and mated queens in either a vertical or horizontal position. It appeared that both the virgin and the mated queens attacked the queen cells containing old pupae more frequently than they did empty queen cells which previously contained old pupae regardless of the orientation of the cells. It is possible that the queens attack the queen cells because they are attracted to the odour of the pupae and/or of other materials related to the larvae (e.g. food, faeces, cocoons, etc.) inside the cells. Worker bees did not usually destroy the queen cell unless the cell was attacked first by a queen (see also Chapter 6).

The results of the experiments in this thesis have shown various types of queen and worker bee behaviour which have not been reported previously. The new observations are as follows: (1) offering (food) behaviour displayed by virgin and mated queens towards worker bees, (2) holding, holding-biting, and holding-biting-stinging behaviour displayed by virgin queens towards worker and drone bees, (3) "begging" (food) and receiving (food) behaviour displayed by worker

bees towards virgin and mated queens, (4) the method queens used to kill each other, i. e. the "victorious" queen inserts her sting through the membrane around the base of the coxa of any leg of her rival queen, and (5) the difference in the preference of virgin queens and of mated queens in attacking different kinds of queen cells.

These observations should provide a better understanding of the social behaviour of queens and worker bees. They also indicate that two important problems may be encountered in the establishment of multiple-queen colonies: (1) the killing of one queen by the other queen and (2) the killing of one, or both, queens by the worker bees. The results of Chapter 5 have suggested some methods that might lead to the solution to these problems. These methods include the masking of the odours of queens, the use of queens with clipped stings, and the use of newly emerged worker bees when the multiple-queen colonies are initiated. However, further experiments are required to verify these suggestions.

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