FACTORS AFFECTING THE PRODUCTION OF HONEY BEE QUEENS (*Apis mellifera* L.) IN MANITOBA

A Thesis
Submitted to the Faculty
of
Graduate Studies
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by
Donald Peter Dixon

In Partial Fulfillment of the
Requirements for the Degree

of

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Plaint of the Queen Bee

Workers, oh sisters striving near,
Envy not me whom ye serve here
Queen but in name. A slave I fill
The endless cradles of your will.
Proudly I soared up to the sun
And with my lover bold was one.
But for that rapturous moment I
Burdens must bear and prisoner, die.
You may fare forth to nectar sip
On many a soft and bloomy lip,
May rest on many a golden heart.
Causing its flow'ry dreams to start.
Workers, oh sisters striving near,
Envy not me whom ye serve here.
Queen am I none; I strive to fill
The endless cradles of your will.
No more the blue, where you take flight,
Only my dreams and endless night.

- Romanie Van de Poele

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ABSTRACT

Each year an increasing number of beekeepers are overwintering their honey bee colonies for economic reasons. Thus, there is a great need for a reliable supply of queen honey bees for these colonies. In an attempt to solve some of the problems associated with queen availability, the following four aspects of queen rearing and queen supply were examined; (1) spring queen rearing in the laboratory using caged worker bees, (2) a comparison of spring reared queens from British Columbia with spring reared queens from the United States, (3) queen rearing during the summer in Manitoba, and (4) studies in the orientation of queens during orientation and mating flights.

Caged worker bees that had been confined to hives throughout the winter did not accept grafted larvae or nourish accepted larvae as successfully as did caged overwintered worker bees that had taken recent "cleansing" flights.

When brood and surplus honey production of colonies headed by spring reared queens from British Columbia were compared with those of queens imported from the United States the former colonies produced significantly more sealed brood and surplus honey than did the latter ones.

Summer conditions in Manitoba proved to be adequate for rearing high quality queens during the two seasons encompassed in this study. The highest level of acceptance

of grafted larvae by honey bee colonies was found during July and early August.

The introduction of virgin queens to queenless colonies was most successful when the queen was first caged in the colony for 24 hours and then upon release was sprayed, along with its surrounding workers with a sugar syrup, vanilla mix.

Under some test conditions, queens were found to make orientation errors and enter the wrong colony. This usually resulted in the queen being attacked or expelled from the colony. Various orientation cues (i.e. landmarks) appeared to reduce orientation errors of the queens to a marked degree.

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INTRODUCTION

Commercial beekeeping in Western Canada has grown in close association with the package bee industry of the southern United States. The common practice among many Canadian commercial beekeepers, and especially beekeepers in the Prairie Provinces, has been to start their colonies in the spring from package bees imported from the southern United States, kill the bees and harvest the honey crop in late summer, and then place the equipment in storage for the winter. The following spring, colonies are again initiated with imported package bees. However, in the last 3-5 years several factors have influenced the beekeeping industry of Central Canada and promoted interest in methods that could contribute to a more self-sufficient approach to Canadian beekeeping.

The number of honey bee colonies has increased dramatically throughout North America since about 1972. This has promoted an increase in the price of package bees imported from the United States and has also enhanced the possibility that present sources of supply, during unfavourable weather conditions, may not be able to meet all of Canadian beekeepers spring package bee requirements. Since 1972 inflation has also reduced beekeepers' profits, and honey prices and markets have remained unstable and unpredictable. Coincident with these problems there still remains a general concern that the Brazilian or "Africanized" bee of South America may eventually invade the queen breeding yards of the southern

United States making them unsuitable as a source of bees for Canada.

In an attempt to avoid some of these problems Canadian beekeepers have shown a renewed interest in both indoor and outdoor overwintering of honey bee colonies. Indeed, many beekeepers are already overwintering large numbers of colonies with varying degrees of success.

Those beekeepers that do successfully overwinter colonies are faced with the problem of finding a reliable source of quality queen bees either for spring and fall requeening, or for making increase from strong colonies. Traditionally, caged mated queens have originated from the same source as package bees, namely the bee breeders of the southern United States.

The importance of developing methods that would allow Canada to move towards self-sufficiency in production of queens has become more recognized as increasing numbers of beekeepers show interest in overwintering.

Based on our present knowledge of the honey bee, there appear to be four basic approaches to this problem of queen honey bee supply for Canada;

1. Queen Storage.

This method would involve rearing and mating large numbers of queens during the summer and then placing them in some form of overwinter storage for introduction to successfully overwintered colonies in the spring. Several methods have been developed for storing queens (see; Avetisyan and Vasiliadi,

1967; Cook, 1969; Edwards and Poole, 1971; Gary, 1966; Griffin, 1963; Harp, 1969; Nelson and Roberts, 1967; Poole et al, 1973; Szabo, 1975; Walsh, 1967; Smarlicki and Morse, 1963.). Most of these methods have been reviewed by Reid (1975).

Szabo (1977) has attempted to overwinter large numbers of caged queens in queenless colonies in northern Alberta but has had discouraging results, probably due to the length of storage time required and the severity of the winter weather. An attempt was made to store a small number of queens during the winter of 1976-77 at the University of Manitoba overwintering building but the results were also discouraging.

2. Laboratory Rearing of Queens.

This method involves the rearing of large numbers of queens in the laboratory, late in the winter, for distribution to beekeepers early in spring. This approach is dealt with in Chapter III.

3. Spring Queen Rearing in Canada's Warm Climatic Regions.

If large queen breeding yards could be established in the early spring in areas like south western Ontario or the Okanagan Valley of British Columbia, many beekeepers could be supplied with spring queens. This possibility is dealt with in Chapter IV.

4. Summer Queen Rearing in the Field.

Large numbers of queens have already been reared during the summer in many parts of Canada for introduction to colonies

in the late summer and early fall. This method of queen supply is dealt with in Chapter V.

During the course of the summer of 1977 while queens were being reared in the University of Manitoba apiary, a preliminary study was also made of queen drift during orientation and mating flights. This study is described in Chapter VI.

CHAPTER I

REVIEW OF LITERATURE

The Queen Honey Bee

A normal honey bee colony is usually composed of one queen, several hundred drones and several thousand workers. The queen's presence in a colony is fundamental to both the survival of the colony as a monolithic structure and to the colony's perpetuation and growth. The queen, normally the only egg layer, is the source of all individual members in the honey bee colony and under ideal conditions is capable of laying in excess of 2000 eggs per day (Moeller, 1958). Because of her central role as the source of future generations within the colony, the queen not only influences the number of individuals being produced, but also is the source (through the eggs she produces and the sperms stored in her spermatheca) of all genetically controlled traits of those individuals.

The queen also exerts considerable influence on the adult members of the colony. The presence of pheromones or "queen substances" produced by the queen's mandibular glands, or from her abdomen are transmitted throughout the colony and help maintain colony cohesion and organization, inhibit queen rearing by the workers and also prevent development of the workers' ovaries (Butler, 1975).

The queen is undoubtedly the most important single bee in the honey bee colony and any colony that does not have a queen or the material necessary to rear a queen will eventually dwindle and die.

Sex Determination

Of the three castes in the honey bee colony, the queen and workers are female and the drones are male. Sex determination in the honey bee normally follows the haplodiploidy pattern, which is characteristic of the Hymenoptera. In the haplodiploidy model the males (drones) develop by parthenogenisis from unfertilized eggs and are therefore haploid and the females (queens and workers) develop from fertilized eggs and are therefore diploid (Kerr, 1974).

Research in the last 30-40 years however, has shown that, although sex determination in the honey bee generally follows the haplodiploidy model, there are some important exceptions. Mackensen (1951) showed that in matings of closely related individuals there was a higher than normal mortality of brood. He postulated, for the honey bee, a series of alleles that allowed viability when they occurred in the haploid and heterozygous condition but were lethal in the homozygous diploid condition. Kerr (1974), in a review article on this subject, stated that sex is determined by a multiple sex allele (known as the "x" allele) which results in females when found in the heterozygous condition and males when found in the homozygous or hemizygous condition.

Woyke, found that diploid drone eggs are in fact viable and will hatch (1962b) but that the larvae are eaten by worker bees (1963a). Woyke was also able to raise diploid drones to maturity in the incubator isolated from workers (1963b). The occurrence of diploid drones resulting from

"x" allele homozygosity can usually be prevented in a breeding program by disallowing extensive inbreeding.

Another interesting anomaly in honey bee sex determination is the occurrence of females that have developed from unfertilized eggs. Laying workers (i.e. workers that have functionally developed ovaries, usually because of queenless conditions) of the Cape honey bee (Apis mellifera capensis Esch.) can produce unfertilized eggs which will develop into females. In an A. mellifera capensis queenless colony, laying workers are capable of maintaining the worker population of the colony for several generations by laying unfertilized diploid eggs, and eventually even rearing a queen from these eggs, thus restoring the colony to a normal queenright condition (Anderson, 1963). The mechanism that allows this formation of diploid unfertilized eggs in the honey bee is not well understood. Tucker (1958) has suggested that these eggs develop from an improper spindle formation during meiosis I that results in the two pronuclei instead of one pronucleus and three polar nuclei. The two pronuclei then unite to form a zygote.

Development of abnormal traits in the honey bee can also result from multiple sperm penetration of the egg. It is believed (Rothenbuhler, 1958a, cited by Rothenbuhler et al, 1968) that gynandromorphic honey bees (i.e. individual bees that show both male and female characteristics) develop from a zygote and one or more accessory sperms. The zygote results from the syngamy of a single egg and a single sperm

and develops into female tissue, the accessory sperm or sperms develop into male tissue. Laidlaw and Tucker (1964) have also shown that two accessory sperms in the cytoplasm of an egg can unite and give rise to female tissue.

Caste Determination

Since both the queen and worker honey bees are female and normally develop from fertilized eggs, differences between these two castes cannot be attributed to any genetical differences. Lukoschus (1955, 1956 - cited by Wilson, 1974) cited 56 morphological differences by which a queen can be recognized. There are also many obvious physiological and behavioural differences between the queen and worker honey bee.

Eggs transferred from worker cells to queen cells develop into queens while eggs from queen cells transferred to worker cells become workers. Weaver (1957a) found that up to three days into larval development a larva transferred from a worker cell to a queen cell would develop into a queen. Larvae transferred after three days tended to result in adults that showed more worker-like and intermediate (intercaste) characteristics.

Weaver (1957b) has proposed that both large amounts of food and food of a special quality are required for the differentiation of queens. Conceivably the different types of cells (either worker or queen) act as the releaser promoting the nurse bees to feed the larvae in the respective cells differently. It has been known for some time that larvae in queen cells are fed differently than their counter-

parts in worker cells. Planta (1888 - cited by Butler, 1975) observed that larvae in queen cells appear to be fed throughout their development on glandular secretions whereas larvae in worker cells receive food more diluted with honey beginning after about the third day of larval life.

It is also well known that larvae in worker and queen cells receive different quantities of food during their larval development. Haydak (1943) pointed out that from the time of hatching until about the third day of larval development all female larvae (i.e. both those in worker and queen cells) normally receive a surplus of food but that after the third day only the larvae in queen cells receive a continuous surplus of food and those in worker cells are apparently undernourished. Weaver (1966) believed that starvation may reinforce other caste determining mechanisms but that it was not a cause.

In the last 30 years there has been extensive research into the biochemistry of "royal jelly" (the food fed to the larvae in queen cells) in an attempt to determine what compounds are essential for queen determination. The results of this work have shown that royal jelly is a very complex mixture of many compounds but nobody has been able to point, with confidence, to any one compound or combinations of compounds that are responsible for queen determination. The work done on the chemistry of royal jelly has been reviewed by; Townsend and Shuel (1962); Rembold (1965); Weaver (1966); Weaver *et al* (1968); Painter (1969); Liu (1973); and Wilson (1974).

Recent research into caste determination has led to some significant advances. Wirtz (1973 - cited by Goewie, 1976) has shown that caste determination in the honey bee involves the action of juvenile hormone (JH) which is more abundant in queen larvae than worker larvae. Wirtz and Beetsma (1972 - cited by Goewie and Beetsma, 1976) have shown that queen differentiation can be induced by a single topical application of JH-I to three or to three-and-a-half day old worker larvae when reared in worker cells and Goewie and Beetsma (1976) have been able to obtain the same results with repeated topical applications of JH-III to three or to three-and-a-half day old worker larvae in worker cells. The mechanism by which the activity of the corpus allatum (the endocrine gland responsible for JH production) is regulated is still unknown. The possibility that nutrients in the larval food influence endocrine activity still remains. Wirtz (1973 - cited by Goewie, 1976) has suggested that some differences in larval food may be detected by chemoreceptors in the mouthparts of developing larvae and that this sensory stimulation may influence endocrine activity. This hypothesis has received some support from Goewie (1976) who has shown, with the use of scanning electron microscopy, that oral chemoreceptors are present in young (less than 72 hours old) larvae of both castes and that structural differentiation of these sensilla in the queen and worker larvae occurs during the fourth and fifth instar. Research now in progress will probably go far to finally determining the exact mechanism of honey bee caste determination.

Natural Queen Rearing

Honey bee colonies will rear queens naturally under three basic situations; 1. "emergency" conditions, which result from the sudden loss of a queen, 2. supercedure of the old queen which is still heading the colony, and 3. swarming, or the division of the colony into two or more colonies, each of which requires their own queen (Butler, 1975).

In an emergency situation, as when the original queen dies, the adult workers will construct emergency queen cells from normal worker cells containing young female larvae or eggs. The workers will float the existing larva out to the mouth of the cell by secreting large amounts of food into the cell. The bees then construct a large "peanut shaped" queen cell that typically hangs downward from the face of the comb. The larvae which are selected for emergency queen rearing are usually less than two days old, but can sometimes be as much as three days old (Butler, 1975).

The supercedure impulse usually develops when the queen grows old, is injured, diseased or is unable to sustain a normal rate of egg laying. Under any of these circumstances the workers will usually construct two or three queen cells into which the failing queen may deposit fertilized eggs (Laidlaw and Eckert, 1974).

To supply queens to satisfy the swarming impulse, workers build relatively large numbers of queen cell cups on the bottom or sides of combs. If the colony is going to swarm,

the queen will lay eggs in several of the prepared queen cell cups. The number of cells started usually depends on the strength of the colony and the intensity of the swarming impulse (Laidlaw and Eckert, 1974).

Artificial Queen Rearing

Beekeepers first began manipulating colonies of honey bees to produce needed queens about two centuries ago. Since then the knowledge of queen development and the refinement of artificial queen rearing techniques has increased to the extent that a very major industry in the southern United States has grown up around the commercial production of package bees and caged mated queens. The basis of queen production in the past, and continuing with modern techniques, is the common genetic origin of both workers and queens.

Schirach (1787 - cited by Laidlaw and Eckert, 1974) proved that queen bees could be reared from larvae in worker cells. He found that if he confined a small number of worker bees in a box with a piece of comb of worker cells containing eggs and larvae that after a few days the bees would have modified several of the worker cells into queen cells.

Francis Huber (1814 - cited by Laidlaw and Eckert, 1974) reaffirmed that queens could be reared from larvae taken from worker cells. In his experiments he replaced larvae in queen cells with young (48 hours old) larvae taken from worker cells. When normal queens resulted, he not only proved that queens could be reared from young worker larvae

but he was also probably the first researcher to transfer larvae from worker cells to queen cells. This technique of transferring young worker larvae to queen cells or artificial queen cell cups later became known as "grafting" and is now used almost exclusively by commercial queen breeders.

Quinby (1853 - cited by Pellett, 1918) used a queen rearing technique similar to that of Schirach. He described how small colonies containing about one quart of bees could rear queens if they were given small pieces of comb containing two to three day old worker larvae. Quinby also recommended the use of swarm cells, cut from colonies preparing to swarm, as a source of queens for other queenless colonies.

It was Larch (1876 - cited by Laidlaw and Eckert, 1974) who first coined the term "grafting" to describe the technique of substituting worker larvae for those found in naturally built queen cells. Boyd (1878 - cited by Laidlaw and Eckert, 1974) suggested cutting out and saving naturally built queen cell cups into which newly hatched larvae could be transferred and then placed in queenless colonies. Boyd also stated that the A.I. Root Company was preparing to market artificially built queen cells into which larvae could be grafted and then transferred to a strong colony for rearing.

Townsend (1880 - cited by Laidlaw and Eckert, 1974) developed a new technique of queen rearing by cutting worker comb containing young larvae or eggs into one cell wide strips and fastening them, with cells pointing downward, to the underside of combs. The combs were then intro-

duced to queenless colonies where many of the inverted worker cells would be converted to queen cells.

Henry Alley (1883 - cited by Pellet, 1918) developed several important advances in queen rearing. The queens that he wanted to use as a source of eggs and larvae for further queen rearing were confined to small "breeder colonies" from which he would take worker comb containing the desired eggs and young larvae. He also introduced the idea of a "swarming box" which would serve as the "cell building colony". The "swarming box" was prepared by removing the queen from a strong colony, brushing the worker bees into a holding box which was then kept queenless in a cool dark place for at least ten hours. At night the bees were then returned to their original location and given the material from which they were to rear queens. This was an important advancement since it recognized the need to make special preparations to the "cell building colonies" before successful queen rearing could take place.

Doolittle (1888 - cited by Pellet, 1918) outlined a step by step queen rearing method that, with some modifications, has become the method used almost universally by commercial queen breeders. Doolittle used artificial queen cups attached to a wooden bar which was fastened to a regular sized frame below a piece of brood comb. At the time of grafting he placed a small drop of previously collected royal jelly ("primer") into each of the cell cups and then transferred a worker larva, less than 36 hours old, into each of the "primed" cell cups. After experimenting with different

types of cell building colonies he decided that the best method was to have the queen cells built in the second storey of a strong colony in which the queen was confined to the lower chamber by a queen excluder.

Dr. C.C. Miller (1912 - cited by Laidlaw and Eckert, 1974) developed a system in which strips of beeswax foundation, hung from the top bar of a frame, were placed in a breeder colony. After several days the outer margins of the comb, which contained eggs were cut away and the remaining young larvae and comb placed in a cell builder colony to be reared as queens.

Jay-Smith (1949 - cited by Laidlaw and Eckert, 1974) adopted the "Alley system" for large scale queen rearing. He confined the breeder queen to small areas of new comb, thus forcing her to fill the comb with eggs. The comb was then cut into strips, attached to bars and then introduced to queenless cell builder colonies.

Today, beekeepers involved in queen rearing often use various techniques from many different sources. For example the two colony system of queen rearing has become popular in many areas. This involves placing the grafted larvae first in a queenless "cell starter colony", where the building of the queen cells is begun. Later the queen cells are placed in "cell finisher colonies" which are strong queenright colonies with the queen confined to the lower storey. The queen cells are left in the finisher colonies until they are capped (York, 1975).

Queen cells are usually removed from the cell building colonies ten days after grafting. At this time each cell can be introduced to a queenless mating colony or placed separately in containers in an incubator until emergence of the adults (Laidlaw and Eckert, 1974).

Queen Mating

Francis Huber (1814 - cited by Laidlaw and Eckert, 1974) showed that if a newly emerged (virgin) queen was confined to a hive until it started laying, then only drones were produced. If, however, the virgin queen was allowed to leave the hive at will, when she began laying, she was capable of producing both workers and drones. This was perhaps the first experiment demonstrating that queens mate outside the hive.

Risga (1931) observed that most virgin queens left the colony only once but many went on two flights and a small number left the colony three times before the commencement of egg laying. Taber (1954) and Roberts (1944 - cited by Harbo, 1971) found that mating flights usually take place when the queen is about one week old, with each successful flight averaging about 14 minutes. Taber (1954) was able to show that most queens mate more than once (i.e. with more than one drone) during each mating flight, and Woyke (1962a) showed later that the queen usually mates with seven to ten drones on each successful mating flight.

Gary (1962) discovered that the "queen substance"

(9-oxodec-trans-2-enoic acid), discovered earlier by C.G. Butler, was in fact a sex attractant that was used by the queen, during mating flights, to attract drones. Gary was able to show that drones could be attracted to a tethered queen, the extract of a queen or to queen substance elevated 30-80 feet into the air. Gary and Murston (1971) were even able to entice drones to mate with wooden models of queens that had been treated with queen pheromones and elevated 5-15 meters.

The "queen substance" produced by the queens' mandibular glands may not be the only sex attractant pheromone in operation during honey bee mating. Butler (1971) believed that there may be other unidentified pheromones that stimulate the drones, when close to the queen, to sieze and copulate with her. These pheromones would have their maximum effect close to the queen whereas the "queen substance" would act as a general attractant over a greater distance.

Gary (1963) was able to induce drones to mate with tethered queens that had had their sting chamber dissected open and was thereby able to observe many matings and give detailed accounts of the sequence of events that occur during mating. Gary found that, while in flight, the drone approaches the queen from behind, grasps onto the dorsal sides of the queen's abdomen and then everts his copulatory apparatus into the sting chamber of the queen. Following completion of successful copulation, part of the drone's penis is detached and remains in the queen's vagina forming a plug. This plug, known as the "mating sign", is obvious

and if it is seen on a queen returning to the hive, it is a good indication that she has been successfully mated. The mating sign also acts to prevent the semen from leaving the queen before it can be stored in the queen's spermatheca. According to Harbo (1971) a queen which has been properly mated accumulates five to eight million spermatozoa in her spermatheca.

Zmarlicki and Morse (1963b) found that some areas had a higher concentration of drones than others and they termed these areas "drone congregation areas". Strong (1970) was able to map three drone congregation areas near Ithaca, New York, and was also able to show that these areas persist from year to year. It is not known whether the queen seeks out drone congregation areas during a mating flight or whether the drones will leave the congregation area in pursuit of a queen.

The optimum time for virgin queens to leave the hive on mating flights is about the first two weeks of life. Zmarlicki and Morse (1963a) found that if virgin queens were confined to a colony for over a month, most of them would not go on mating flights when given the opportunity and therefore were capable of laying only drone eggs.

Although Kulincevic (1967) found evidence that a queen went on a mating flight after she had started laying eggs, this is considered exceptional (Woyke, 1962).

Usually once a queen has started laying she will not go on any further mating flights. Therefore continued egg

fertilization is dependent on the quantity of spermatozoa that was acquired and stored in the spermatheca during the first few weeks of life.

CHAPTER II

GENERAL METHODS AND MATERIALS

A. Queen Rearing

In this study queen rearing was done under two different conditions; using caged nurse bees maintained in an incubator in the laboratory, and using the more traditional methods in the field as described by Laidlaw and Eckert (1974).

1. Laboratory Rearing of Queens

a. The Cage.

The cage used for queen rearing in the laboratory measured 10 cm. x 8 cm. x 12 cm. (see Figures 1 and 2). The top, bottom, front, and back of the cage were constructed of plexiglass and the two sides were covered with removable metal screens (with apertures about 6 mm. square). Two round holes, 2.5 cm. in diameter, were made in the top of the cage to hold glass feeding tubes. Two oval holes, approximately 4 cm. x 2 cm., were made in the upper part of the front piece of plexiglass and special corks were made to fit these holes. One of these corks was fitted with an aluminum bar which had 3 plastic queen cups attached to it. Two round holes, 2.5 cm. in diameter, were drilled directly beneath the oval holes and fitted with plastic feeding trays, one on each side. On the opposite side of the bottom round holes, four small holes (.3 cm. in diameter for each), two on each side, were drilled and used to fasten the two feeding

Figure 1. Queen rearing cage

a) glass tube feeders
b) plastic food trays (partially removed)
c) plastic queen cell cups attached to an aluminum strip


a
b
c

Figure 2. Queen rearing cage containing bees.

trays to the back of the cage.

b. Food

Two semi-tubular plastic food trays (9 cm. in length, 2.2 cm. in diameter) and one glass tube feeder (Figure 1) were used for feeding the bees during the period of confinement. The plastic feeding trays were inserted into tubes made of wire screen through which the bees could feed.

Ground pollen and honey (1:3 by volume) was put into one food tray, and pure honey was put into the other food tray daily. The tube feeder was filled with a honey and water solution (1:1 by volume).

c. Nurse Bees

Nurse bees, for all of the laboratory queen rearing experiments were obtained from normal queenright overwintered colonies. The bees were shaken from frames containing unsealed larvae, to increase the chances of obtaining large numbers of bees with active "food" glands.

One hundred and seventy-five ml. of worker bees (about 400 bees) were added to each cage (see Figure 2) except for the first preliminary experiment on March 14, 1977 when 250 ml. (about 650-700 bees) were added.

The caged bees were fed and kept queenless in the incubator for 48 hours (except for the March 14, 1977 experiment which were kept for 24 hours) before grafting (see Chapter I) was done.

d. Grafting

Following the grafting methods developed by Lai (1969) and Liu (1973), three plastic queen rearing cups (diameter; 10 mm. ± 1 mm., depth 9 mm. ± 2 mm.) were attached with melted beeswax to a 6 cm. x 1.3 cm. strip of aluminum connected to a cork (Figure 1). The aluminum strip, with attached queen cups, was placed in the cage at the time the bees were introduced to the cage so that the bees could clean the cups before grafting was done.

Brood frames, containing newly hatched female larvae, were removed from overwintered colonies, brought into the laboratory, and placed in "grafting stands" (see Figure 5). Larvae, less than 48 hours old, were removed from the brood combs using a grafting hook and transferred into the queen rearing cups which had been previously "primed" with a drop of distilled water. The queen cups containing these larvae were then placed directly into the cages containing the bees and the cages returned to the incubator.

e. Incubation

The cages containing nurse bees and grafted larvae were kept in incubators at 30°C ± 2°C with 40-60% relative humidity.

When queen cells were sealed by the bees they were removed from the cages and placed separately in glass vials (2 cm. in diameter and 5 cm. in height). These vials were incubated at 30°C ± 2°C until emergence of the queens. Adult queens were either preserved for later anatomical examination

or shipped to Vernon, British Columbia by air for mating.

2. Queen Rearing in the Field

The method used to rear queens in the field was adopted from the Doolittle or "grafting" method as described by Laidlaw and Eckert (1974). Queen rearing was done on the University of Manitoba campus from mid-July to mid-August in 1976 and from late May to early September in 1977. In the summer of 1976 two "breeder"[1] colonies and five queenright "cell builder"[2] colonies were used. In 1977 five breeder colonies and six queenright cell builder colonies were used.

All breeder colonies were headed by queens imported from California. In 1976 the breeder queens were allowed to head normal colonies and had free access to all parts of the hive. In 1977 the breeder queens were restricted to a single frame (comb) within a single-frame queen excluder in a standard 10-frame Langstroth type box. Every three or four days an empty brood comb (i.e. a dark comb) was placed with the queen in the single-frame excluder in order to obtain larvae of known age for grafting.

The cell builder colonies consisted of two standard Langstroth boxes each containing nine frames with the queen confined to the lower box by means of a queen excluder (Figure 3). In 1976 the cell builder colonies were made in July

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1. See Chapter I

2. ibid.

Figure 3. Cell builder colony showing metal queen excluder between the first and second storey.

from colonies maintained at the University of Manitoba apiary and in 1977 they were started in May from four pound (1.8 kg.) packages of bees imported from California.

Once a week the cell builder colonies were examined and rebuilt as follows: frames containing unsealed larvae and/or pollen were removed from the lower box and placed in the upper box and frames with sealed brood were placed in the lower box. During poor weather or periods of low nectar and/or pollen availability, frames containing honey and pollen were added to the upper storey. During periods of heavy nectar flow, frames that became filled with honey were removed and replaced with either empty drawn comb or beeswax foundation. All frames were inspected for swarm cells which, if present, were removed, to prevent virgin queens emerging into the second storey and destroying the grafted queen cells.

Grafting was normally done on Monday, Wednesday and Friday of each week, except at the beginning of the season when grafting was done every day until the cell builder colonies became more accustomed to accepting the grafted larvae. The frames, used to hold the grafted larvae, consisted of three removable bars each having ten queen cell cups about 2.5 cm. apart attached with beeswax. Just before grafting, the cell cups were "primed", using an "eye dropper", with a small drop of royal jelly diluted with distilled water, 1:1 by volume. A comb containing young larvae was removed from one of the breeder colonies and brought to a small "grafting hut" where the temperature was maintained at approximately 30°C.

Figure 4. "Grafting hut" at the University of Manitoba apiary used for grafting during 1977.

(see Figure 4). As recommended by Vagt (1955), Bilush (1963), and Laidlaw and Eckert (1974), one young female larva less than 12 hours old, was transferred from the brood comb to each of the queen cell cups. The brood comb was placed in an adjustable frame holder illuminated with a fluorescent lamp and each young larva was transferred from a worker cell with a grafting needle and floated on the drop of "primer" in the cell cup (see Figure 5). Three bars containing the grafted cells were placed on a bar holding frame with the cells hanging downward and the frame was then introduced to the upper box of the cell builder. The frames containing grafted larvae were always placed beside at least one frame containing unsealed larvae to increase the chance that nurse bees would find the grafted larvae and begin feeding them. Acceptance of the grafted larvae by the cell builder colony was determined on the second day after grafting.

On the tenth day after grafting the finished cells were cut from the grafting bar and placed in individual glass vials in the incubator (at 30° $\pm$ 2°C) until emergence.

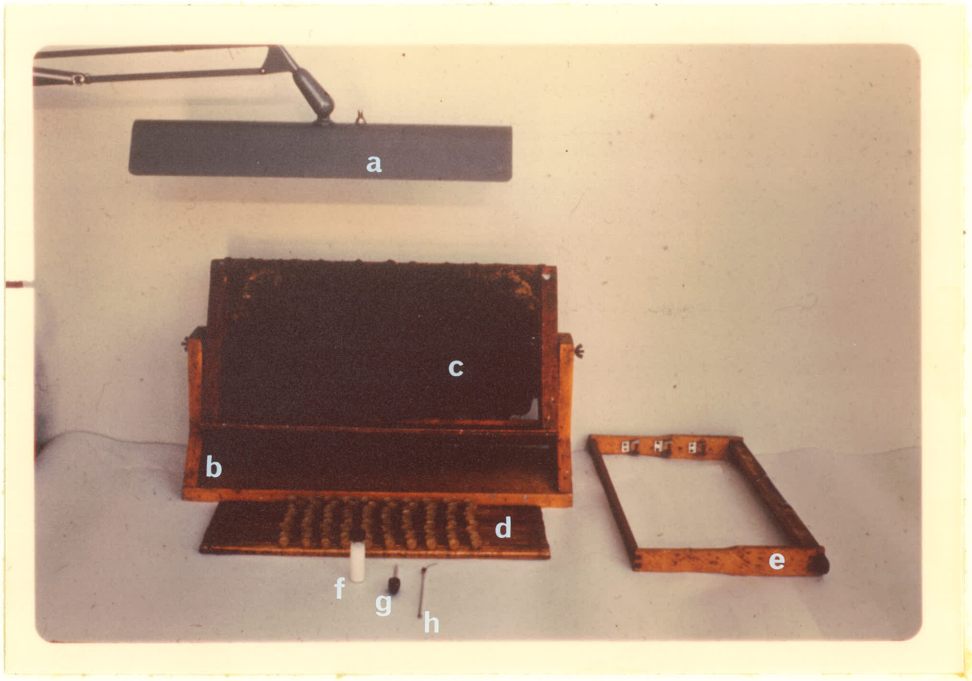
After emergence some queens were weighed and preserved with Weaver's fixative (see Weaver and Thomas, 1956) for later dissection, some were introduced immediately into mating colonies, while others were stored in petri-dishes with honey and water for later introduction into mating colonies.

B. Anatomical Measurements

The external and internal morphological characteristics

Figure 5. Equipment used for grafting

a) fluorescent lamp
b) frame stand
c) brood frame containing worker larvae
d) grafting bars each with ten beeswax queen cell cups
e) bar holding frame
f) "royal jelly"
g) "eye dropper"
h) grafting needle


a
c
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h

of adult queens were measured using a binocular microscope having an eye piece fitted with a linear microscale. The following measurements were taken.

1) The Basitarsus

The inner surface of the first segment of the tarsus (basitarsus) of the right hind leg was examined to determine if it was worker or queen-like in structure (see Snodgrass, 1956). The basitarsus was also measured as shown in Figure 6.

a) Length: Measurement was made from the outer tip of the auricle, along the outer portion of the basitarsus, to its most distal point.

b) Width: This was a perpendicular measurement from one margin across to the other, at the widest part of the basitarsus.

2) The Spermatheca

The diameter of the spermatheca, including it's tracheal covering was measured at its widest part.

3) The Ovarioles

The right ovary was removed and the number of ovarioles it contained was counted.

4) The Mandibles

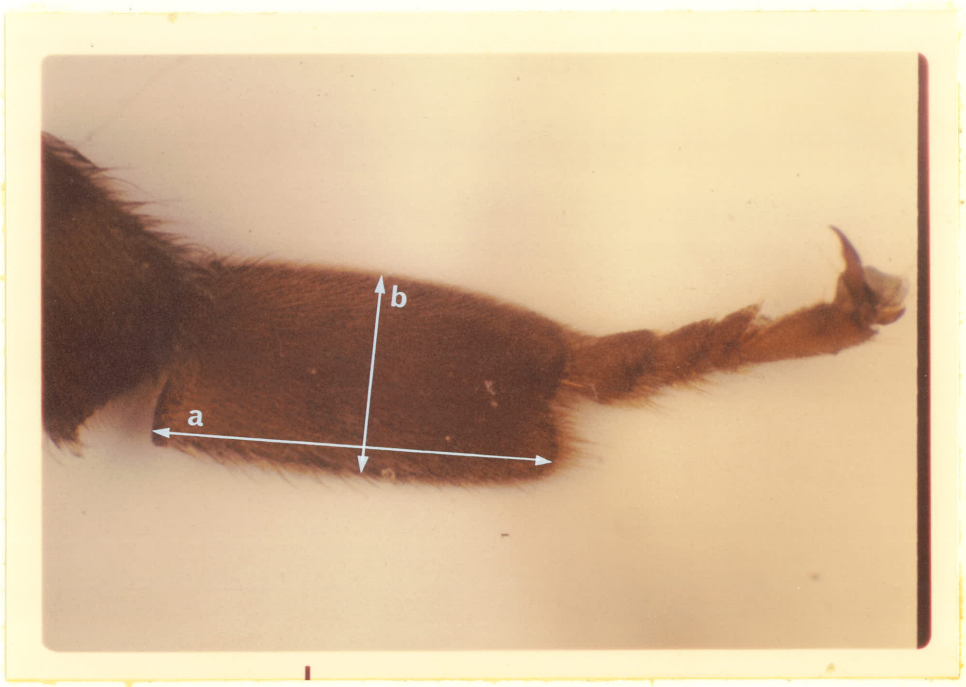
The mandibles were examined to determine if they were worker or queen-like in structure.

C. Measurement of Pupa

As a method of comparing fecundity in queens reared in British Columbia, California, and Manitoba brood production

Figure 6. Basitarsus measurements

a) length
b) width


b
a

of some queens was measured by determining the area of "sealed" brood at various time intervals.

Sealed brood area was first measured at 19 days after queen introduction to the colony and every 12 days thereafter until the readings were terminated. Areas of sealed brood were determined by placing a grid marked off in one inch squares (2.54 cm.) over the area to be counted and then counting the number of squares containing pupae (see Figure 7).

D. Surplus Honey Production

In the comparison of British Columbia reared queens and California reared queens (see Chapter IV), the amount of surplus honey (i.e. all honey stored above the bottom two hive storeys) was determined per hive. This estimate of colony productivity was determined by weighing all honey supers before and after the honey was extracted and then attributing the weight difference to honey production.

E. Other Measurements

1) Queen Weight

Queens that were destined for later anatomical examination were first weighed with an analytical balance and then either frozen or preserved with Weaver's fixative. Only queens that had not expelled their meconium[1] were used.

2) Nectar Flow

During the summers of 1976 and 1977 the nectar flow was monitored at the University of Manitoba apiary by placing colonies on platform scales and recording the weight changes throughout the season.

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1. Intestinal waste that is discharged after the adult emerges.

Figure 7. Grid used to estimate sealed brood area

3) Barometric Pressure

Air pressure was recorded during the summer of 1977 with a recording barometer.

4) Acceptance of Introduced Virgin Queens

The number of virgin queens accepted out of the total number introduced to mating colonies was recorded for the various introduction methods.

F. Queen Drift and Orientation

1) Mating Colonies (Nucleii)

Virgin queens were introduced into two types of small mating hives; a four frame mating nucleus which held four standard Langstroth sized frames, and a "split box nucleus" which was a standard Langstroth ten frame box divided in half with a central partition, i.e. four frames could be placed on each side of the partition (see Figure 8). During all tests only one side of the split box nucleus was used.

2) Daily Weight Changes of Hived Virgin Queens

During the late part of the summer in 1977 several queens were weighed every day in the morning, beginning at emergence and continuing until several days after the commencement of egg laying. The first weight was taken in the laboratory using an analytical balance and subsequent weights were taken in the field using a precision torsion balance. Queens were removed from the mating colonies and placed in small screen cages (see Figure 9) for transport to the torsion balance where they were transferred to a pre-balanced screen

Figure 8. Mating colonies

A. Four frame nucleus colony

B "Split box" nucleus colony

A

B

Figure 9. Marked queen being placed in screen cage for transport to torsion balance.

cage in which they were weighed. After the queens were weighed they were returned to their original colonies.

3) Orientation tests

To ascertain the ability of queens, in pre-egg laying condition, to find their way back to their hive, queens were removed from their hive, placed in a small enclosed box (measuring 2 cm. x 6 cm. x 8.5 cm.), moved various distances and directions from the hive and then released.

a) Queen marking

Each queen in a given pattern of hives was given an individual identifying mark on it's thorax. This was done with paint and consisted of either a single colour or a combination of colours.

b) Weather measurements

During days when queens were being tested, the following weather measurements were taken; wind speed and direction at 2M above ground, temperature and barometric pressure.

c) Queen drift

When queens were removed from the mating colonies, records were kept of those that had drifted from their original hives and were accepted by other colonies.

CHAPTER III

SPRING QUEEN REARING IN THE LABORATORY

A. Introduction

Many beekeepers, with successful overwintering operations, replace low quality queens, and divide strong colonies so as to increase their total number of colonies early in the spring. Both of these practices require a reliable source of young mated queens.

Traditionally, the major source of spring queens for Canadian beekeepers has been from the bee breeders of the southern United States. Because the time that these queens are required (i.e. April and May) is usually too early for outdoor queen rearing in Manitoba (see Chapter V) it was decided to test the feasibility of rearing queens in the laboratory, late in the winter, as an alternative to spring importation of queens. Queens thus reared, could be either allowed to mate naturally in the out-of-doors during years with warm spring weather, instrumentally inseminated in the laboratory, or shipped to areas of Canada where weather conditions would normally permit natural matings outside.

Artificial queen rearing in the laboratory has been performed successfully for many years. The methods employed

often involve placing young female larvae into small containers such as petri-dishes which are held in incubators. These larvae are usually fed by hand with food previously collected from queen cells in the colony or are fed with artificial diets (Rhein, 1933; Haydak, 1943; Weaver, 1955, 1957; Mitsui, et al, 1964; Jay, 1964, 1965a, 1965b; Dietz, 1969; Nobusawa, 1970). Most of the methods developed by previous researchers have been designed largely for studies of nutrition and caste determination. These methods often require too much labour and skill to be practical for rearing large numbers of queens for commercial beekeeping.

A method of queen rearing in the laboratory using relatively small numbers of caged workers was developed by Lai (1969), and further studied by Liu (1973) at the University of Manitoba. Lai was able to rear morphologically normal queens, using ten day old worker bees, in cages containing 25, 50, 100, 200 or 400 nurse bees. Liu used the same method to study the importance of the mandibular gland substance of nurse bees during queen rearing. This technique of laboratory queen rearing seemed to offer promise as a practical alternative to traditional queen rearing practices during periods of unfavourable climatic conditions. Labor and space requirements are minimal and since nurse bees are employed to do the feeding, larval nutrition is not directly dependent on the researcher.

Lai's and Liu's studies were done using young bees during the summer when the bees had ample opportunity to collect fresh pollen, nectar, and water before being caged

and placed in the laboratory.

The purpose of the present study was to determine if this same method could be used to rear normal queens in the late winter and early spring using bees of mixed ages taken from overwintered colonies.

B. Methods and Materials

1. General Design

In all of the cage rearing trials worker bees were taken from normal queenright colonies that had been overwintered in a heated building at about 5°C (40°F) and maintained in total darkness.

Worker bees were collected by removing frames, containing unsealed larvae, from normal colonies and shaking the adhering bees into a collecting funnel. The bees were then collected in a graduated beaker and poured into their respective cages (see Chapter II). Approximately 175 ml. of worker bees (about 400 bees) were added to each cage, except for the first preliminary experiment on March 14, 1977 when 250 ml. (about 650-700 bees) were added. Bees were shaken from frames containing unsealed larvae so as to increase the chance of obtaining nurse bees (i.e. bees with their "food" glands developed).

The study was divided into three parts:

Preliminary Experiments - Bees for this trial were removed from hives which were still inside the wintering facility. This was done on a small scale to determine if the workers were capable of accepting and rearing grafted

larvae, and to determine the optimum numbers of bees required for each cage.

Trial 1 - Worker bees were removed from colonies that had been brought outside but, because of cold weather, the bees had not had the opportunity to leave the hive to deficate and forage.

Trial 2 - Worker bees were taken from the colonies at a later date after they had had ample opportunity to leave the hive thereby permitting defication and the collection of fresh pollen and water.

2. Incubation and Grafting Procedures (see also Chapter II).

Thirty-one replicates (cages) were used in each of Trials 1 and 2. After the worker bees had been removed from the colonies and placed in the cages, the cages, without queens, were placed in incubators for 48 hours.

After incubation the bars containing the three queen cups were removed from the cages and young female larvae, less than 24 hours old, were grafted into the cups. The queen cups containing the larvae were returned to their original cages and then the cages were returned to the incubators. After 24 hours the acceptance of the grafted larvae was checked with a microscope. In each cage only the larva which appeared to have the most royal jelly around it was retained; all other accepted larvae were removed.

In the cages that had not accepted larvae from the first graft, a second graft was performed and these cages were then examined after 24 hours to determine the acceptance and to remove any extra larvae if more than one had been accepted.

Each day the larvae were examined and if any had died this was recorded and the cage removed. Water and food were replenished daily as required. After a queen cell had been sealed by the attendant workers, it was removed from the cage and placed in a glass vial in the incubator. Following emergence, queens were weighed and then either frozen for later dissection or shipped to Vernon, British Columbia for mating.

3. Measurements (also see Chapter II).

The following adult measurements were taken; weight, width of basitarsus, length of basitarsus, basitarsal indices, number of ovarioles in the right ovary, the diameter of the spermatheca and the shape of the mandibles.

The development time of each queen was also recorded from the time of grafting to the time the cells were sealed from sealed to emergence, and the total development time.

4. British Columbia Mating Test.

On May 5, 1977 eleven queens that had emerged from the Trial two (T_2) cages were placed in individual queen cages which were then suspended in a screened shipping package containing about .45 kg. (1 lb.) of worker bees. This package was then shipped "air express" to the British Columbia Provincial Apiarist who introduced the queens to mating nuclei in the Okanagan Valley.

Due to poor weather conditions only one queen mated and started egg laying. This queen was returned to Winnipeg on June 13, 1977 and introduced to a queenless colony containing about three frames of sealed brood and adult bees.

For comparison, two young mated queens, imported from the United States were introduced to queenless colonies of similar strength at the same location and at the same time.

Two sealed brood readings (see Chapter II) were taken on all three colonies after which the queens were preserved in Weaver's fixative (Weaver and Thomas, 1956) on July 22, 1977 for later dissection. This was done because, by accident, the experimental queen was removed from her colony.

C. Results and Discussion

1. Preliminary Experiments

In the first preliminary experiment (March 14, 1977), five cages with 250 ml. of bees (about 650-700 bees) were placed in an incubator. After 24 hours the first graft was done; acceptance, after 24 hours, was found to be zero. The cages were returned to the incubator for 48 hours and then a second graft was done. After 24 hours one cage had accepted two larvae which appeared to be well supplied with royal jelly and one cage had accepted one larva which appeared to be poorly fed. The remaining three cages had not accepted any larvae. In this first experiment the mortality of the attendant bees was very high and they seemed to be under a high level of stress as many of the bees had moisture collecting on their bodies and the cages had large amounts of feces in them. In an attempt to solve these problems, fewer bees per cage were used in the next trials.

In the second preliminary experiment (April 1, 1977) three cages, each containing 175 ml. of bees (about 400 bees), were used. After 72 hours three larvae were grafted

into each cage. After 24 hours the bees from one of the cages had escaped and one larva had been accepted in each of the remaining two cages. In these cages the bees were quiet, mortality was much lower and the cages did not accumulate as much fecal matter as in the previous test using 250 ml. of bees per cage. It was therefore decided to use 175 ml. of attendant bees in future tests.

2. Trials One and Two

a) Rearing Results

The initial acceptance of the first graft in Trial one (T_1G_1), was very low (see figure 10). Of 93 grafted larvae, only six (6.45%) were still alive after 24 hours. In the second graft of Trial one (T_1G_2), acceptance was significantly greater ($P < 0.05$) than the first graft with 23 (30.7%) of the 75 grafted larvae being accepted after 24 hours. Acceptance of the first graft of Trial two (T_2G_1), was significantly greater ($P< 0.05$) than the first graft of T_1 with 35 (37.6%) of 93 larvae being accepted after 24 hours. Acceptance remained relatively high on the second graft of Trial two (T_2G_2) with 12 (36.4%) of 33 being accepted.

The ability of the nurse bees to nourish the developing larvae was measured by examining the cages daily and recording the mortality (see Figure 11 and Table 1). Since each cage was allowed to keep only one larva it was decided to indicate survival by the number of cages that had a viable larva. Therefore at time zero (i.e. at the time the graft was done) survival was 100%. After one day there were six cages (19.4%)

Figure 10. Percent acceptance of grafted larvae, in Trials 1 and 2, 24 hours after grafting.

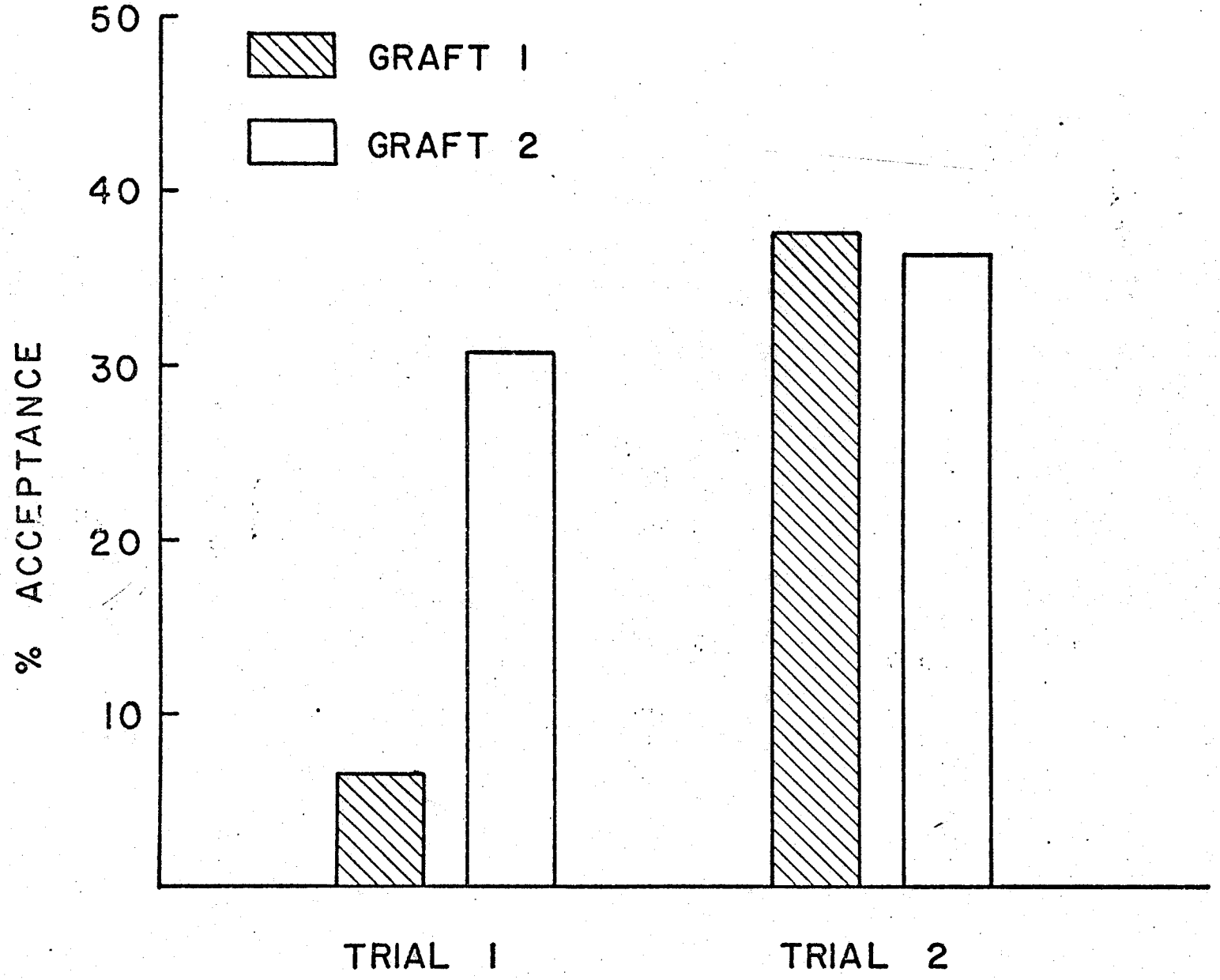
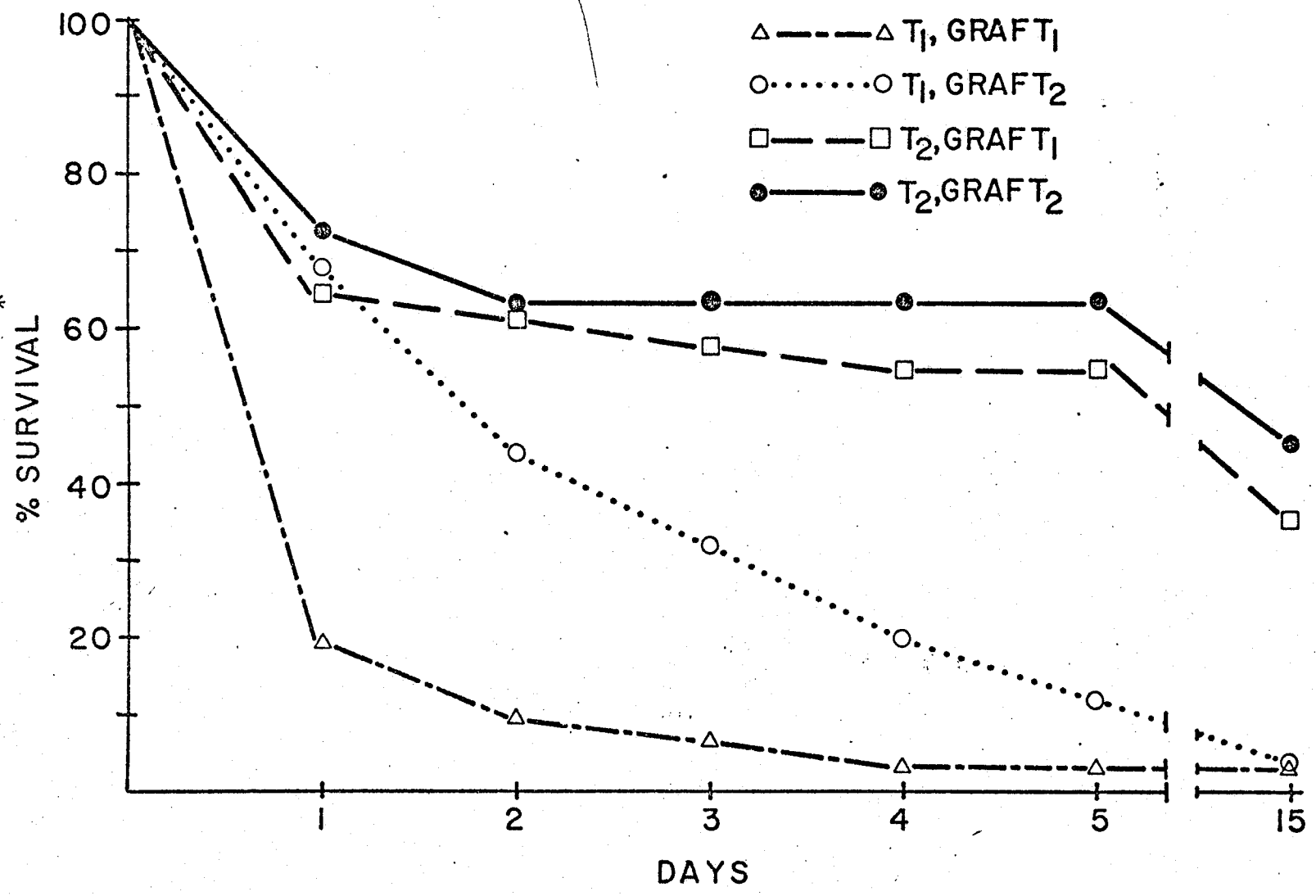

50
40
30
20
10
% ACCEPTANCE
GRAFT 1
GRAFT 2
TRIAL 1
TRIAL 2

Figure 11. Percent survival of cage reared queens, during the total period of development. For statistical interpretation see Table 2.



* % CAGES WITH AT LEAST ONE LARVA SURVIVING

Table 1. Rearing Results of Spring Queen Rearing With Caged Bees in the Laboratory (1977)

Trial	Date of graft	No. of ages	Total no. of larvae grafted	Acceptance		Survival by cage (days) 1		2		3		4		5		Adult		Days from grafting to capping		Days from capping to emergence		Total development time (days)	
				No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	$\bar{x}$	Range	$\bar{x}$	Range	$\bar{x}$	Range
T_1G_1	Apr.9	31	93	6	6.45	6	19.4	3	9.7	2	6.5	1	3.2	1	3.2	1	3.2	5	--	8	--	13	--
T_1G_2	Apr.10	25	75	23	30.7	17	68	11	44	8	38	5	20	3	12	1	4	5	4-6	9	--	13	--
T_2G_1	Apr.21	31	93	35	37.6	20	65	19	61	18	58	17	55	17	55	11	36	5.18	5-6	9.18	9-10	13.4	13-14
T_2G_2	Apr.22	11	33	12	36.4	8	73	7	64	7	64	7	64	7	64	5	46	5.71	5-8	9.4	9-11	13.8	13-15

Table 2. Comparison of Survival of Caged Reared Queens in Trials One and Two at Various Time Intervals ($P < 0.05$)

Time Interval (Days)	Contrast
0-1	$T_1G_1 < T_1G_2$
	$T_1G_1 < T_2G_1$
	$T_2G_1 \not< T_2G_2$
	$T_1G_2 \not< T_2G_2$
1-2	$T_1G_1 \not< T_1G_2$
	$T_2G_1 \not< T_1G_1$
	$T_2G_1 \not< T_2G_2$
	$T_2G_2 \not< T_2G_2$
2-5	$T_1G_1 < T_1G_2$
	$T_2G_2 < T_1G_2$
	$T_2G_1 \not< T_2G_2$
	$T_1G_1 = T_2G_1$
Emergence	$T_1G_1 < T_2G_1$
	$T_1G_2 < T_2G_2$
	$T_1G_1 \not< T_1G_2$
	$T_2G_1 \not< T_2G_2$

from T_1G_1 that had at least one larva surviving, 17 cages (68%) from T_1G_2, 20 cages (65%) from T_2G_1 and eight cages (73%) from T_2G_2.

According to Jung-Hoffman (1966 - cited by Haydak, 1970) nurse bees feeding queen larvae deposit two types of secretions; watery-clear and milky-opaque. The watery-clear substance is secreted by nurses averaging 17±2 days of age and the milky-opaque by those 12±2 days of age. The ratio of these two different substances is dependent on the age of the nurses, with the older nurses providing less of the white component. The nutritional demand that the queen larvae place on the nurses increases with time and the nurse bees have to make more feedings per day as each larva increases in age.

In experiments where nurse bees are forced to continuously rear brood, the ability of these nurses to produce larval food may be substantially prolonged past the normal age when nursing activities usually occur. However, the older nurse bees are not as efficient at feeding larvae as both the quantity and quality of food produced tends to decrease with the age of the nurse bees. Queens, produced by older bees, also tend to exhibit intercaste characteristics (Haydak, 1963; Haydak *et al.*, 1964; Weiss, 1972).

Because brood rearing is much reduced during the winter (Harris, unpublished data) and workers tend to live much longer during the winter than the summer (Anderson, 1931) there is a very high proportion of old bees in a colony, in the spring.

Because, in the present experiment, the workers were taken from overwintered colonies early in the spring, it would be safe to assume that a large proportion of the bees taken were quite old.

When comparing the ability of nurse bees in T_1 and T_2 to rear queens, the ability of the bees in T_1G_1 to accept the transferred larvae is much less than with all other grafts and the inability of the bees in T_1G_2 to nourish the accepted larvae is apparent.

Even though the initial acceptance in T_1G_2 was not significantly less ($P < 0.05$) than T_2G_2 mortality of the larvae in T_1G_2 during development was much higher than in any of the other grafts. A linear regression on each of the lines in Figure 11 between days 2 and 5 show that the only line with a slope significantly less than zero ($P < 0.05$) is the line representing T_1G_2. The mortality of T_1G_1 was relatively low but this was probably due to the low level of initial acceptance when only six of 93 larvae were accepted.

The mortality was so high throughout the development of the T_1G_2 larvae that at emergence there was only one viable adult. Too, only one adult emerged from T_1G_1. In T_2 However, there was little difference in the percent survival at emergence between G_1 and G_2 (T_2G_1 was not significantly less ($P < 0.05$) than T_2G_2) and the percent survival at emergence in T_2G_2 was significantly greater ($P < 0.05$) than T_1G_2 (see Table 2).

Development time was, on the average, slightly longer than normal in both of the trials with some individuals taking a very long time to develop. Normal development for a queen, from egg deposition to emergence as an adult, is about 16 days (Butler, 1975). The tendency for development time to be prolonged was also found by Lai (1969) and Liu (1973) in their queen rearing trials in the laboratory, but no explanation was given. The author observed that the royal jelly being supplied by the nurses in T_1 was often low in quantity and in many cases, clear and watery in appearance, whereas, the royal jelly found in the queen cells in T_2 was usually abundant and was quite thick and milky-white in appearance. This seems to indicate that the bees in T_1 were less able than the bees in T_2 to supply the glandular secreted portion of the royal jelly. There were also some obvious differences in the general condition of the nurse bees in T_1 and T_2. In T_1 the cages showed greater amounts of accumulated fecal matter and a higher level of mortality among the nurses than in the T_2 cages. This was probably the result of the T_2 bees being the only ones able to take "cleansing flights" before being caged. The nurse bees in T_2 also were much more able to produce wax and showed a higher level of "hoarding" behaviour (i.e. they constructed large pieces of comb and stored sugar syrup in the wax cells; see Figure 12).

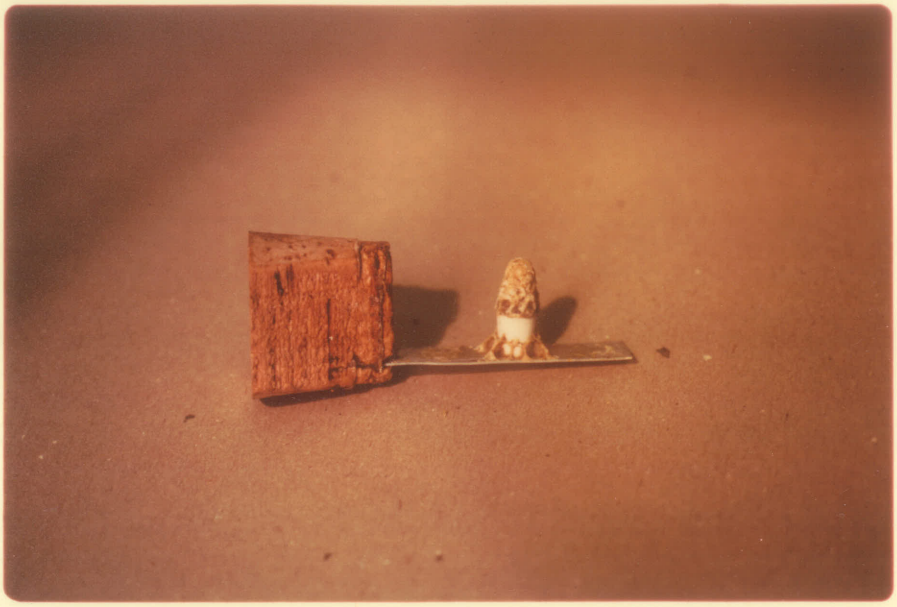
Lai (1969) found that in grafts done into cages containing seven day old nurse bees the acceptance was higher

Figure 12. Comparison of comb construction around queen cells by workers in Trials 1 and 2.

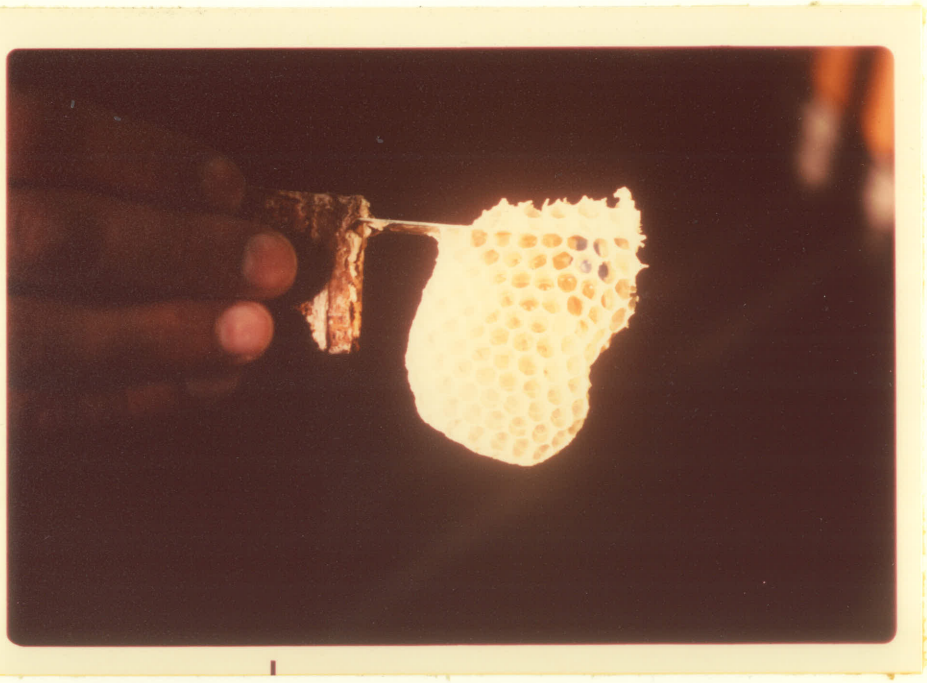
A. Finished cell from Trial 1 showing little new wax construction around the queen cell.

B. Finished cell from Trial 2 completely covered with new comb construction. Notice the presence of sugar syrup in some of the wax cells.

A



B



in the cages containing 200 and 400 bees than in the cages with 50 or 100 bees. Too, ten day old nurse bees had the highest larval acceptance, especially in cages with 200 and 400 nurse bees. The larger groups of bees tended to produce larger queens and with a shorter development time than did the cages containing smaller numbers of nurse bees.

Liu (1973), using ten day old nurse bees, found that acceptance was usually two or three of three grafted larvae, which would be about twice as much as the highest acceptance observed in the present experiment. Liu found that in cages with 30 nurse bees, initial acceptance of grafted larvae was high and the larvae were well supplied with royal jelly but as the larvae grew, the amount of food they received was less than that of larvae of the same age reared by larger numbers of workers. Of 23 replicates in 1970, only two pupae and six adults were reared by the groups of 30 bees, and in 1971 no pupae or adults were reared from five replicates. Queens reared by groups of 30 nurse bees showed lower pupal weights, some intercaste characteristics, lower ovariole numbers, lower spermathecal diameters and longer development times.

The results obtained by Liu (1973), using groups of 30 summer nurse bees ten days old, compare closely to the results of the present experiments using groups of about 400 bees from overwintered colonies.

There are several variables that could be responsible, either individually or cumulatively, for the differences

in behaviour and queen rearing ability within the experiments conducted in the summer using young bees (see Liu, 1973) and T_1 and T_2 of the present experiments. It is possible that the proportion of young bees may have been slightly higher in T_2 than T_1. It is however, unlikely that the difference would be great because the T_2 bees were taken only 11 days after the T_1 bees and at the time the T_1 bees were obtained there did not appear to be large amounts of brood in any of the hives. Having the opportunity to defecate probably relieved the T_2 bees of a large amount of physiological stress which might put them in a better condition for brood rearing. The opportunity to collect water and fresh pollen under good spring weather conditions probably stimulated the T_2 bees to rear brood.

b. Morphological Characteristics

The morphological characteristics of both adult queens that emerged from T_1G_1 and some randomly selected queens that emerged from T_2G_1 and T_2G_2 are listed in Table 3. Unfortunately during storage the internal structures of the bees deteriorated and therefore measurements could not be taken of spermatheca diameter or ovariole number.

The basitarsal index of the adult queens was either lower than, or at the low end of the range for, normal queens described by Weaver (1957b). Weight, at emergence, was also below normal for queens reared in colonies; (see Chapter V) the mandibles of all stored queens appeared to show intercaste

Table 3. Measurements of Some Spring Laboratory Reared Queens from Trials One and Two (1977)

Trial	Graft	Bee no.	Days from grafting to sealing of cells	Days from capping to emergence	Total development time (day)	Wt. at emergence (mg.)	Mandibles W	Mandibles I	Mandibles Q	Basitarsus L (mm.)	Basitarsus W (mm.)	Basitarsus L/W	Basitarsus W	Basitarsus I	Basitarsus Q
1	1	30	5	8	13	-		x		1.64	.88	1.86		x	
1	2	23	4	8	12			x		1.96	1.08	1.82		x	
2	1	3	4	8	12	168		x		2.52	1.24	2.03		x	
2	2	14	4	10	14	95.6		x		2.16	1.04	2.08		x	
2	2	23	4	8	12	194		x		2.6	1.44	1.81		x	
2	2	30	5	8	13	173		x		2.48	1.36	1.82		x	
Mean standard error			4.33± .211	8.33± .334	12.67± .334	157.7± 21.44				2.23± .184	1.17± 0.86	1.90± 0.49			
Queen											1.9-2.3				
Worker (Weaver, 1957b)											1.6-1.9				

characteristics. Although Laidlaw and Eckert (1974) believe that the only reliable method for determining the quality of a queen is to judge her and her offsprings' performance in a colony, Szabo (1973) showed that it is possible to ascertain, to some degree, the queen's egg laying potential through anatomical measurements. Based on measurements taken, the queens examined from T_1 and T_2 generally showed a low level of queen differentiation.

c. British Columbia Mating Test.

Throughout the period that the queen that was reared in Manitoba and mated in British Columbia was in the colony it appeared to perform well compared to the queens from the United States. Egg laying (as measured by sealed brood readings) was slightly lower than the two American queens, but the brood pattern was uniform and there was no attempt at supercedure by the workers.

On close examination of the Manitoba queen, the mandibles were found to be queen-like, there were 140 ovarioles in the right ovary, the pattern of hairs on the basitarsus was queen-like; however, the basitarsus index (length = 2.56mm., width = 1.36mm., L/W = 1.88) did not fall into the range of normal queens described by Weaver (1957b).

D. Conclusion

More research is required to determine the limiting factors that prevented the nurse bees in this experiment from rearing as many well differentiated queens as was done in previous experiments with ten day old bees reared in the summer.

If age is the primary determining factor for a bee to feed larvae then colonies destined to supply nurse bees for spring queen rearing will have to be stimulated to produce large amounts of worker brood in February and March. This can be accomplsihed by feeding pollen, a balanced pollen supplement, or a substitute as well as sugar syrup or honey.

It is reasonable to assume that the older bees which have been restricted to the hive throughout the winter, and therefore have not had the opportunity to take "cleansing flights" will be under a considerable amount of physiological stress by March and April. Because of this stress and the age of the bees they could not be expected to be very effective as nurse bees. It would be of interest to determine, in future experiments, if allowing these bees to take "cleansing flights" in an indoor cage or flight room would promote a more effective level of nursing activity. The low level of brood rearing usually found in an over-wintering colony, and therefore the decreased need for nurse bees, may cause many of the older bees to lose their food-producing capabilities as winter progresses.

The low level of acceptance and the prevalence of intercaste characteristics, in the present experiment, are probably a function of a single factor, namely the inability of the nurses to produce adequate amounts of high quality royal jelly.

The technique of using caged bees in the incubator

to rear queens early in the spring still has potential as a viable method. However, considerable refinement of the technique is required before it will be useful as a dependable source of high quality queens early in the spring.

CHAPTER IV

A COMPARISON OF CALIFORNIA SPRING REARED QUEENS VERSUS BRITISH COLUMBIA SPRING REARED QUEENS

A. Introduction

Canadian beekeepers who overwinter their honey bee colonies and find themselves in need of young mated queens in the spring have traditionally turned to the bee breeders of the southern United States for these queens. It has generally been assumed by most beekeepers that the Canadian spring is not warm enough to permit the production of mated queens for introduction to colonies by late April or early May. For most parts of Canada this is undoubtedly true; cold weather on the prairies is common well into April and May in some years and wet weather is common during the spring on both the west and east coasts of Canada.

There are however, small regions of Canada that may hold promise as areas for rearing and mating queens early enough for spring introduction to colonies. For example, the Point Pelee and Pelee Island region of south-western Ontario is on a similar latitude (42° - 43° N) to that of Northern California and usually has an early spring. The southern Okanagan Valley of British Columbia also has a relatively early spring. With this in mind, the Provincial Apiarist of British Columbia, Mr. John Corner, was asked in the fall of 1976 if he could supply Manitoba with young mated

queens in mid-April of the following spring. He agreed to a pilot project in which he would attempt to supply about 80 queens. It was decided to distribute most of these queens to commercial beekeepers in Manitoba for their evaluation and to keep a small number for a more rigorous comparison to queens that arrived with package bees from the United States.

The British Columbia queens were derived from Homer Park, Styles and New Zealand stock. All of the British Columbia queens were reared and mated at the British Columbia Department of Agriculture Research Station located at Vernon, British Columbia.

B. Methods and Materials

Due to inclement weather in the mating yard, the first group of queens was not sent from British Columbia until May 2, 1977 and the second group on May 17, 1977. Altogether a total of 79 queens were received with none dying during transit. Twenty of the queens were kept by the author and the remaining 59 were distributed to five commercial beekeepers at various locations in Manitoba. The beekeepers were asked to compare the productivity and activity of these queens (and their colonies) to the other colonies that they had in their apiaries.

On May 14, 1977, fifteen two pound packages, originating from the United States, were purchased from the Manitoba Co-operative Honey Producers Ltd. in Winnipeg. In seven of the packages the queen was removed and replaced with a British Columbia queen. The remaining eight packages were

allowed to keep their original queens. All 15 queens, to be hived with the package worker bees, were marked on the thorax with a dot of paint to ensure identification throughout the summer. The 15 packages were then hived on May 14 and the queens were slow released on May 17. On May 18, 1977, when the 15 colonies were checked for acceptance, all the queens were found to be present and laying normally.

The packages were hived in brood chambers containing similar amounts of honey and pollen. Each colony also received the equivalent of about one frame of sealed brood (pre-pupa or early pupa stage) to strengthen the colony. All colonies were established in the same location about 2.5 km. north of Winnipeg. The 15 colonies were established in two groups and were arranged with their entrances facing various directions (offset entrance pattern) to reduce the drifting of worker bees between colonies (Jay, 1966a).

The colonies received second brood chambers and honey supers as required to insure that no crowding occurred.

1. Sealed Brood Readings

It was decided to measure the fecundity of the queens by taking retular measurements of the amount of sealed brood present. According to Butler (1975) and Garofalo (1977) the total development time from egg to emergence as adult of the worker honey bee is about 21 days and the time required from prepupa (i.e. - the time the cell is sealed) to adult emergence is about 12 days. To avoid duplicating measurements of sealed brood, the first

measurement was taken 19 days after the queen was released and every 12 days thereafter, except for the last reading which was delayed until 14 days after the preceding reading because of inclement weather.

The technique of estimating the amount of sealed brood present was based on a method originally developed by Nolan (1925, 1932). Nolan's technique involved removing the frames containing sealed brood from the colony, shaking the adhering bees off the frame, placing the frame in front of a wire grid, with one inch squares, and then photographing the frame. The resulting photograph showed the grid superimposed over the frame and area of sealed brood. Using the grid outline as a reference, an estimate of the area of sealed brood could then be obtained and the photograph kept as a permanent record.

A calibrated grid placed over the comb containing sealed brood and used for an immediate visual estimate was used by Jeffree (1958); Moeller (1958, 1961); Pankiw (1969); Nelson (1970) and Smirl (1970). This was the technique used in the present study (See Chapter II). Frames containing sealed brood were removed from the colony, the adhering bees were shaken from the frame back into the hive and a grid marked off in one inch squares was laid over the face of the brood comb. An estimate of sealed brood area was obtained by counting the number of squares containing sealed brood. Grid squares only partially filled with sealed brood were added to similar squares until the equivalent of a full square inch of sealed brood was obtained. The procedure

was carried out as quickly as possible to prevent chilling of the brood and to insure minimal disruption of colony activities.

Readings were recorded in square inches and later converted to square decimeters.

2. Surplus Honey Production

Since all of the colonies used in this experiment were going to be overwintered, only the honey stored above the second brood chamber ("surplus honey") was removed. Any honey stored in the brood chambers was left for winter feed. The amount of surplus honey was determined by weighing all honey supers before and after the honey was extracted and then attributing the weight difference to surplus honey production.

3. Other Observations

Records were also kept of any supercedures, abnormal brood patterns, and diseases present.

C. Results and Discussion

1. Commercial Beekeepers' Evaluations

No data were obtained from one beekeeper. All of the other beekeepers who co-operated in evaluating the British Columbia queens gave favourable reports on the performance of the queens. All believe that the British Columbia queens performed at least as well as the other queens in their apiaries. All reported that the British Columbia queens produced good brood patterns and that honey production was at least as good as colonies under similar

conditions. Several supercedures were reported by the beekeepers and this may have been due to the queen being confined for a long time in the mating nucleii in British Columbia because of poor weather conditions. Most of the beekeepers said they would use the queens in their commercial operation should they become available in the future.

2. Comparison of British Columbia Queens With Package Bee Queens

The data collected for sealed brood and surplus honey production are shown on Figures 13 and 14, and Table 4.

British Columbia queen BC6 was superceded and package queen P5 became a drone laying queen before the readings were completed so data from these two colonies are not included in any of the Figures or Table 4.

The mean amount of sealed brood present was significantly greater in the British Columbia colonies than in the package colonies at each reading with the following probabilities; June 4 ($P < 0.0005$); June 16 ($P < 0.0005$); June 28 ($P < 0.025$) and July 12 ($P < 0.025$) (see Figure 13).

The colonies headed by British Columbia queens also showed a significantly higher ($P < 0.005$) mean surplus honey production than the colonies headed by the package queens (see Figure 14). The mean surplus honey production for the British Columbia colonies was almost twice as much as the mean for the package colonies with no overlapping individual

Figure 13. Comparison of sealed brood readings between British Columbia (B.C.) queens and Package (P.) queens. Vertical lines represent standard errors.

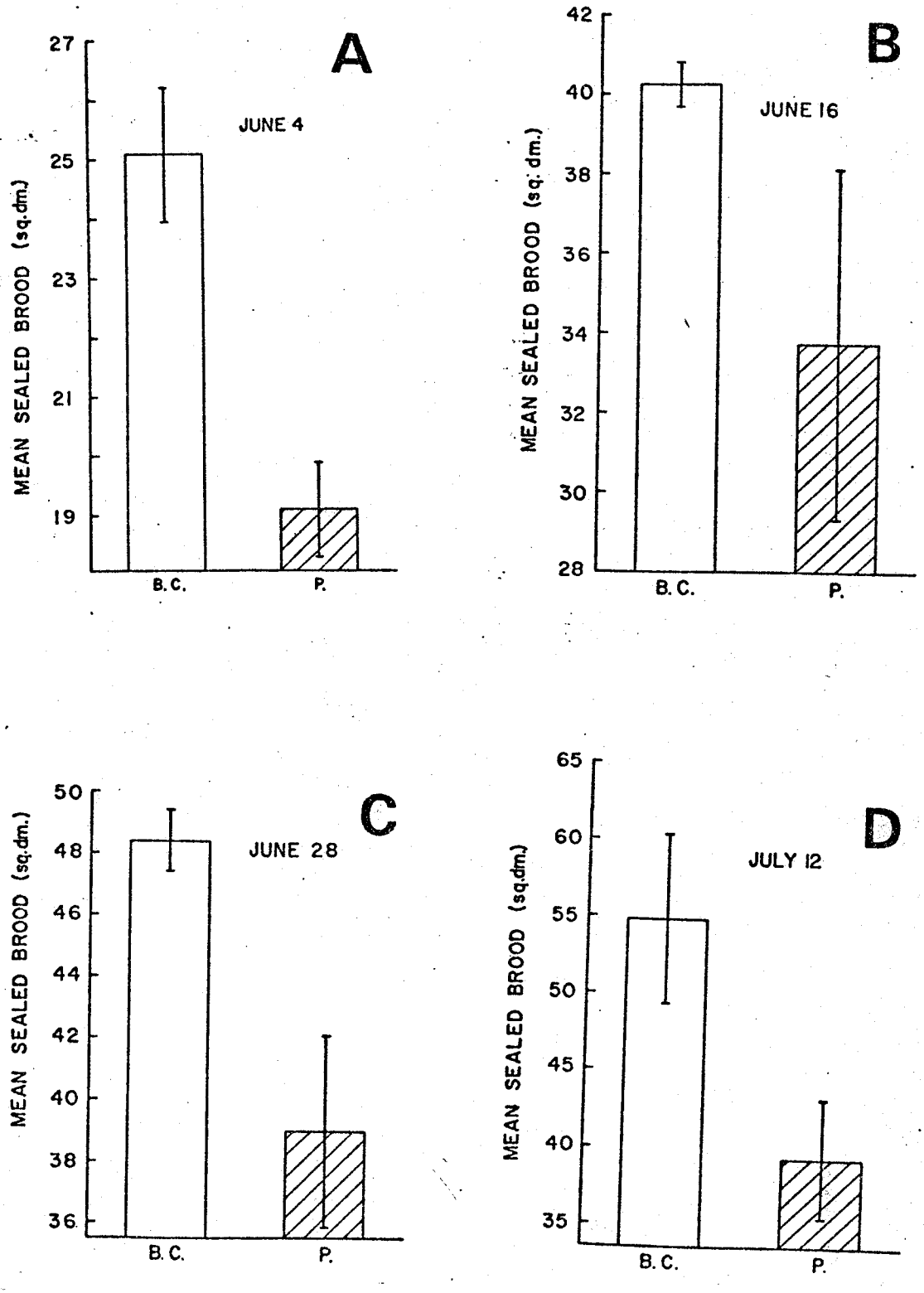

A
JUNE 4
MEAN SEALED BROOD (sq.dm.)
27
25
23
21
19
B.C.
P.
B
JUNE 16
MEAN SEALED BROOD (sq.dm.)
42
40
38
36
34
32
30
28
B.C.
P.
C
JUNE 28
MEAN SEALED BROOD (sq.dm.)
50
48
46
44
42
40
38
36
B.C.
P.
D
JULY 12
MEAN SEALED BROOD (sq.dm.)
65
60
55
50
45
40
35
B.C.
P.

Figure 14. Comparison of surplus honey production between British Columbia (B.C.) queens and package (P.) queens. Vertical lines represent standard error.

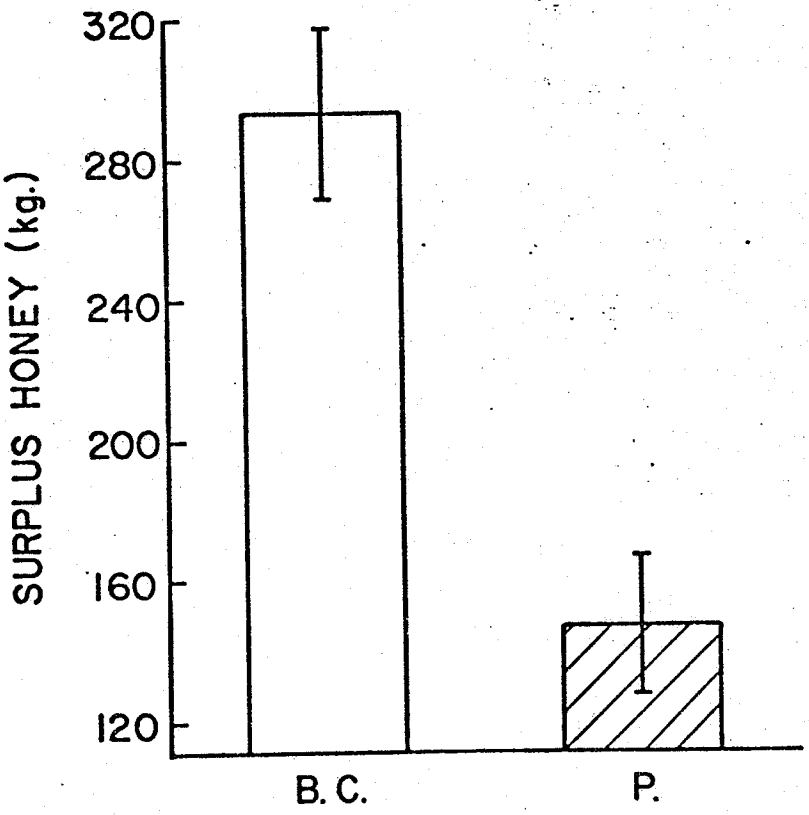

320
280
240
200
160
120
SURPLUS HONEY (kg.)
B. C.
P.

Table 4. Comparison of Sealed Brood and Surplus Honey Production Between British Columbia and United States Reared Queens (1977)

Hive no.	Sealed brood readings (dm^2). June 4	June 16	June 28	July 12	Surplus honey production (kg.)
BC 1	29.36	38.45	46.58	36.58	226.7
BC 2	24.52	41.68	52.00	63.94	222.2
BC 3	23.29	40.65	46.00	63.48	310.0
BC 4	27.29	38.77	46.90	38.45	323.3
BC 5	24.39	40.65	48.26	64.97	373.3
BC 7	21.81	41.42	50.90	62.00	307.8
Mean Standard Error	25.10± 1.123	40.26± .5484	48.45± 1.007	54.90± 5.516	293.9± 24.00
P 1	18.52	33.10	42.65	37.82	210.0
P 2	21.42	37.87	43.32	29.36	188.9
P 3	21.36	41.29	52.71	54.39	180.0
P 4	17.16	29.16	27.10	29.42	84.44
P 6	19.74	30.77	32.00	54.45	145.6
P 7	19.29	33.54	37.42	34.32	74.44
P 8	16.32	30.33	38.45	35.87	147.8
Mean Standard Error	19.10± .7936	33.97± 4.396	38.97± 3.103	39.23± 4.065	147.3± 19.56

values (see Table 4).

The striking differences in brood rearing and honey production between the British Columbia and package queens and the colonies they headed was unexpected and the reasons for such a significant divergence of performance are unknown.

Sealed brood readings are at best only a "rough" indication of the egg laying ability and potential of the queen. There are many factors that can contribute to the number of eggs the queen will deposit and the survival of larvae in the colony after hatching. Some factors that influence the amount of sealed brood present include; the viability of the eggs, the number of adults capable of fulfilling nurse activities, the availability of food (pollen and nectar) and the ability of the adults to get this food to the brood, the size of the colony and their ability to maintain proper brood nest conditions (temperature and humidity), available space for brood rearing and the presence of brood and adult diseases. Woyke (1977) has shown that through "brood cannibalism" the adult workers of a colony will, to a limited extent, regulate the number of larvae present. The number of larvae eaten is dependent on the season. The presence of a large sealed brood area would, however, depend directly on the presence of a healthy and active queen.

Honey production is dependent on the egg laying capacity of the queen (Moeller, 1958) and behavioural characteristics of the worker bees (Kulincevic and Rothenbuhler, 1973). The fecundity of a queen will be affected by her inheritance,

her environment during development and the conditions of the colony that she occupies. Behavioural characteristics of the workers will depend largely on the genetic background of the queen and the drones with which she has mated.

The results of the present experiment demonstrate the importance of the queen in determining how productive a colony of honey bees will be. It is not known why there was such a great difference between the performance of the two groups of queens studied. Because it was the first year of this pilot project and because the British Columbia Apiarist knew we would be watching and testing his queens carefully, he probably took extra care during the queen rearing and in the selection of queens supplied for this study. Also the author did not know the actual identity of the shipper of the queens that arrived with the package bees or how conscious of quality control he was in his queen rearing operation.

D. Conclusions

Good quality queen honey bees can be reared and mated in British Columbia early enough for spring introduction to colonies in Manitoba. Further research should be encouraged to determine;

a) what geographical areas of Canada would allow early spring rearing and mating of queens for distribution to other parts of Canada and

b) how many queens could be expected from these areas.

Spring rearing of queens in the moderate climatic regions of Canada may prove to be an important contribution to a more self-sufficient approach to queen supply in Canada.

CHAPTER V

SUMMER QUEEN REARING IN MANITOBA

A. Introduction

Queen rearing and mating during the summer in Manitoba has been carried out successfully for a number of years. Harcus (1967) was the first graduate student, at the University of Manitoba, to rear large numbers of queens during the summer. Since then several graduate students at the University of Manitoba have maintained an active program of queen rearing during the summer (see; Lai, 1969; Liu, 1973; Waikakul, 1973, 1976; Graham,(unpublished data)and a small number of Manitoba commercial beekeepers have been rearing queens during the summer for several years.

Because this method of queen supply has already achieved a high level of efficiency and success in Manitoba, it offers the most promise as a viable alternate source of queens for Canadian prairie beekeepers. Queens reared during the summer could be used either for late summer introduction to colonies to be overwintered or put into storage during the winter for introduction to colonies in the spring. Harris (unpublished data) found that colonies requeened in the late summer (late July - early August) with Manitoba reared queens produced an average of about 45 kg. (100 lb.) more honey the next year than overwintered colonies that had not been similarily requeened. This was probably due to their higher egg laying rate in the fall and winter

which increased the number of bees surviving overwinter and allowed for a more rapid spring population increase.

The techniques used for queen rearing during the summer are relatively simple and because weather conditions during July and August are usually conducive to queen rearing and mating, productivity is usually high. Unlike the other sources of Canadian queens discussed in this thesis - summer queen rearing is a tested and dependable method which could be relied on even during summers with adverse weather conditions.

The purpose of the present queen rearing studies were (a) to supply other researchers with mated queens for colonies to be overwintered, (b) to test various queen rearing and mating techniques during the summer months and (c) to supply queens for queen orientation studies (see Chapter VI).

B. Methods and Materials (also see Chapter II)

1. Queen Rearing

The techniques used for queen rearing in this study were based on the Doolittle or "grafting" method (see Chapters I and II) described by Laidlaw and Eckert (1974).

a. Queen Cell Cups

Two types of artificial queen cell cups were used during the summer of 1977; (a) beeswax queen cell cups with an open end diameter of about 11 mm. $\pm$ 2 mm., an inside depth of about 10 mm. $\pm$ 2mm., with the inside

of the cell rounded at the bottom; (b) plastic queen cell cups with an open end diameter of about 10 mm.± 1 mm., an inside depth of about 9 mm.± 2 mm., and the inside of the cell tapering gradually to a more flattened base with a diameter of about 3 mm.

The frames used to hold the grafted larvae, consisted of three removable bars each having 10 queen cell cups about 2.5 cm. apart attached with beeswax. These frames containing fresh queen cell cups were placed in the cell builder colonies over-night, to allow the bees to clean the queen cell cups, before receiving the grafted larvae. Just before grafting, the cell cups were "primed" with a small drop of royal jelly diluted with distilled water. 1:1 by volume using an "eye dropper" (see Figure 15).

To determine if either of the two queen cell cups would be preferred for rearing queens by the adult bees in the cell builder colonies a test was initiated in 1977 to determine if there was any difference in the rate of acceptance of grafted larvae based on queen cell cup type.

For this test the bars of the grafting frame were constructed in the usual way except that both types of queen cell cups were used in equal numbers on each bar. The queen cups were arranged in an alternating wax, plastic, pattern (see Figure 15). Each frame therefore held 15 beeswax queen cell cups and 15 plastic queen cell cups. About 48 hours after grafting, the queen cell cups were checked for acceptance of the grafted larvae and records

Figure 15. Queen cell cups being primed with royal jelly, water mix before grafting. Notice the alternating arrangement of wax and plastic cells on the cell cup bar.

were kept based on the queen cell cup type. This test was done on 12 grafting days using all six cell builder colonies during the summer of 1977.

b. Acceptance of Grafted Larvae

Records were kept of the number of surviving grafted larvae about 48 hours after grafting for each cell builder colony.

c. Emergence of finished cells

Ten days after the queen cell cups had received their grafted larvae, the finished cells were removed from the cell builder colonies (see Figure 16). Each individual queen cell was cut from the grafting bar and placed in a glass vial which was held in an incubator (at 30° $\pm$ 2°C) until adult emergence. At various intervals throughout the summer of 1977, records were kept of the number of adults emerging from the finished cells.

2. Acceptance of Introduced Virgin Queens

Records were kept of the acceptance of virgin queens introduced to queenless mating nucleus colonies under various conditions. These records were based on: date of introduction, type of nucleus colony (four frame colony or split box colony), length of time the colony had been queenless, location of mating colony and type of colony pattern it was found in, and method of queen introduction.

3. Anatomical Measurements

At various times during the summer of 1977 sample queens were randomly selected from the groups of emerging queens for anatomical examination at a later time.

Figure 16. Finished queen cells being removed from the cell building colony.

Queens that were to be used for anatomical examination were first weighed on an analytical balance and then either frozen or injected in the thorax with Weaver's fixative (Weaver and Thomas, 1956) and placed in labelled vials filled with Weaver's fixative. Only queens that had not expelled their meconium were used.

a. Basitarsus

The inner surface of the first segment of the tarsus (basitarsus) of the right hind leg was examined to determine if it was worker or queen-like in structure (see Snodgrass, 1956). The length and width of the basitarsus was also recorded (see Chapter II).

b. The Spermatheca

The diameter of the spermatheca was measured at it's widest part including the trachea.

c. The Ovarioles

The right ovary was removed and the number of ovarioles it contained was counted.

d. The Mandibles

The mandibles were examined to determine if they were worker or queen-like in structure.

4. Other Measurements

a. Honey Flow

The duration and intensity of the honey flow was monitored at the University of Manitoba apiary using two scale colonies.

b. Barometric Pressure

Barometric pressure was recorded at the University of Manitoba apiary.

C. Results and Discussion

1. Acceptance of Grafted Larvae

The main purpose of the queen rearing program during the summer of 1976 was to test and become familiar with the queen rearing techniques to be used the following year. Queen rearing was done from July 25 until August 16 in 1976 (see Table 5). During this period, the overall acceptance of grafted larvae was generally quite low with only three of 12 grafting days having an overall acceptance exceeding 50 per cent and only one exceeding 60 per cent. This relatively low level of acceptance may have been the result of starting the queen rearing program late in the summer.

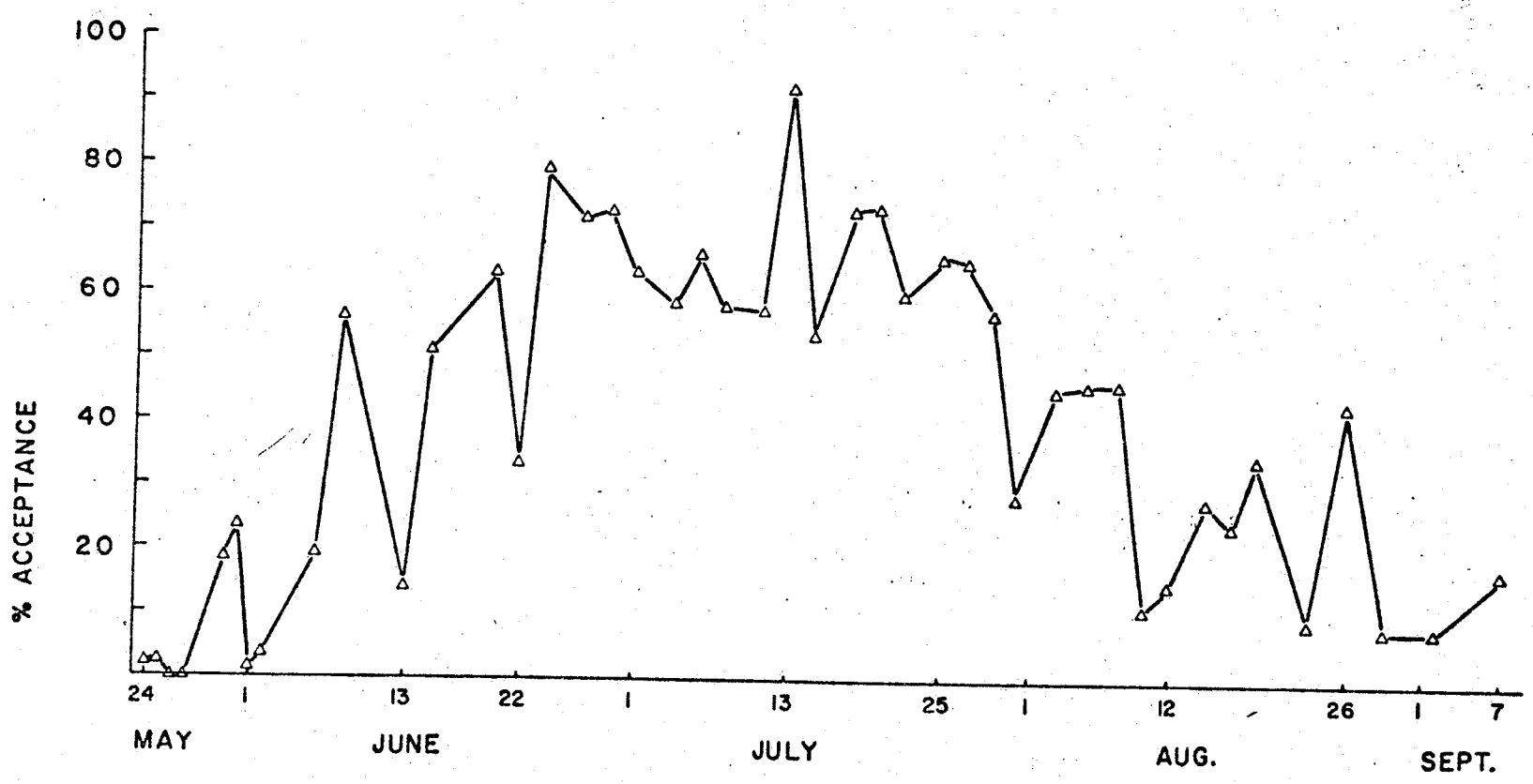
Acceptance of grafted larvae in 1977 (see Figure 17) showed a gradual increase from the time grafting began (May 24) and maintained a relatively high level of acceptance beginning in late June and continuing until late July. Acceptance during August and early September was erratic but generally lower than during July. The highest level of acceptance was recorded on July 13, 1977 when two of the six cell building colonies had 100 per cent acceptance and all except one of the cell building colonies had an acceptance exceeding 90 per cent. Interestingly, this particular graft was performed on a rainy, cool day (Mean temperature 17.5°C) when the bees were not flying.

Why worker bees, in queenright cell builder colonies either reject or accept larvae introduced in artificial queen cups is not well understood. Laidlaw and Ekcert (1974) have suggested that when a queen is restricted to

Table 5. Acceptance of Grafted Larvae, after 48 Hours by Five Colonies (Summer, 1976).

	Acceptance by each cell building colony										
	1		2		3		4		5		Mean
Day	No.	%	No.	%	No.	%	No.	%	No.	%	%
July 23	24	80	6	20	1	3.3	16	53.3	0	0	31.3
July 22	17	56.7	3	10	0	0	0	0	0	0	13.3
July 27	13	43.3	14	46.7	3	10	22	73.3	22	73.3	49.3
July 28	22	73.3	12	40	12	40	21	70	18	60	56.7
July 29	15	50	9	30	1	3.3	23	76.7	6	20	36
July 30	8	26.7	13	43.4	0	0	19	63.3	8	26.7	32
Aug. 4	9	30	19	63.3	0	0	21	70	14	46.7	42
Aug. 6	12	40	12	40	0	0	18	60	23	76.7	43.3
Aug. 10	18	60	25	83.3	21	70	23	76.7	9	30	64
Aug. 11	18	60	25	83.3	9	30	21	70	8	26.7	54
Aug. 12	14	46.7	12	40	6	20	9	30	7	23.3	32
Aug. 16	0	0	3	10	0	0	17	56.7	2	6.7	14.7

Figure 17. Mean per cent acceptance of grafted larvae during the summer of 1977.


% ACCEPTANCE
100
80
60
40
20
24
1
13
22
1
13
25
1
12
26
1
7
MAY
JUNE
JULY
AUG.
SEPT.

the bottom storey of a two storey colony and young unsealed brood are continually brought up to the second storey (i.e. the young brood and queen are separated) that this approximates a queenless or "emergency" condition in the second storey and the nurses there are stimulated to rear queens. If this was the primary motivation for queen rearing in the second storey I think one would expect to find, at least occasionally, emergency queen cells constructed on the face of combs containing unsealed brood. At no time during the two seasons of queen rearing described in this study, was one typical emergency queen cell found on the face of any of the brood combs in the cell builder colonies. Emergency conditions may be better approximated when the cell builder consists of three storeys where the queen is being excluded to the bottom storey where the second storey containing food, and the third storey contains the frames of young larvae and frames of grafted larvae. Under these conditions the queen is far removed from the area of queen rearing and conceivably her pheromones would have less of an influence on the nurse bees in the third storey.

When using a two storey queenright cell builder colony, I think the primary factor being "exploited" by the beekeeper to promote queen rearing, is the natural swarming impulse. Usually when the bees are restricted to only two boxes throughout the year there is a high level of congestion and this is one important prerequisite for swarming (Stephen, 1975). Colonies, with a relatively small population, do not

make good cell builders (Laidlaw and Eckert, 1974) and this is probably one reason why acceptance was so low during May in the 1977 queen rearing program. Since the bees had been started from four pound (8.89 kg.) packages in April they had not had time to attain a high population by late May. Furthermore, when the artificial queen cups, with grafted larvae, were introduced to the cell building colonies, they were introduced in a way which closely approximated natural swarm cells. Unlike typical emergency queen cells, and some supercedure cells, which are found on the face of brood comb, the artificial queen cups are constructed on the underside of bars with the open end facing downward, similar to natural swarm cells. The author also observed that it was not necessary for the queen to be excluded to the lower box for grafting to be successful and even whenthe queen had free access to the second storey, the grafted larvae could be accepted and the artificial queen cells finished by the workers. This also tends to discount the theory that the second box must simulate "emergency" conditions before queen rearing will take place.

There is little statistical information, to be found in the literature, on acceptance of grafted larvae by cell building colonies or on artificial queen rearing in general. Free and Spencer-Booth (1961) have found in Britain, that there is no correlation between time of year and the proportion of larvae accepted and reared; however, their cell building colonies were started from overwintered colonies. Because their colonies were overwintered colonies,

the worker population would have been relatively high all year long, whereas, colonies started from packages as done in my study, begin with a very small population. They also found, in the first year of study, but not in the second, that better results were obtained by first introducing grafted larvae to prepared starter colonies (see Chapter I) and then introducing the accepted cells to cell building colonies rather than by grafting larvae directly into cell building colonies. Queenless and queenright colonies completed about the same proportion of cells introduced directly to them in one year, but queenless colonies completed a greater proportion during one month of another year. Generally there was no difference in acceptance between grafted larvae that received larval food in the cell cup and those that received no food (Free and Spencer-Booth, 1961).

Krol (1974) found a difference in the rate of acceptance of grafted larvae based on the strain of bees used in the cell building colonies. The highest level of acceptance was achieved with hybrids and the lowest levels with local strains of honey bees.

Several researchers (Myser, 1952: Woyke, 1962, 1963, 1977; Alber, 1965, and Fukuda and Sakagami, 1968) have observed worker bees eating eggs and larvae; this is probably an important mechanism by which the nurse bees control the number of eggs and larvae present in the colony at any time.

Alber (1965) found that more larvae were accepted in the lower rows on the grafting frame and that the food

introduced with the larvae began to dry up within ten minutes. Some larvae received fresh food from the nurse bees within ten minutes whereas others were not fed for 1 1/2 hours or more. Too, some of the grafted larvae were eaten by the nurse bees and some were completely neglected.

Woyke (1977) found that the youngest brood were the most likely to be eaten and that the highest level of this type of cannibalism occurred during spring and fall and the lowest level during summer. He also found that by simply altering the position of brood in the colony that brood cannibalism could be elicited in the adult workers. Woyke (1977) hypothesized that the apparent seasonal variation in the rate of cannibalism may be a function of pollen availability. Low levels of protein coming into the colony may cause the workers to use the existing larvae as a protein source, thus increasing the amount of protein available and decreasing the need for it. This type of brood cannibalism is common in many Wasp genera during periods of low protein availability (Wilson, p280, 1974).

The rate of acceptance of grafted larvae during the 1977 summer appears to relate to the nectar flow (see Table 7) that was recorded in the same area. The strongest part of the nectar flow began about the last week of June and continued until about the first week of August. A consistently high level of acceptance of grafted larvae began about the last week of June and continued until about the last week of July (see Figure 17).

Table 6 . Acceptance of Larvae, After 48 Hours in Wax or Plastic Cells by Six Colonies (Summer, 1977)

Number of larvae accepted in wax or plastic cells												Mean % acceptance		
Colony 1		Colony 2		Colony 3		Colony 4		Colony 5		Colony 6				
Wax	Plastic	Wax	Plastic	Wax	Plastic	Wax	Plastic	Wax	Plastic	Wax	Plastic	Wax	Plastic	Total
0	--*	1	--	1	--	2	--	0	--	0	--	2.2	--	2.2
0	--	0	--	0	--	0	--	5	--	0	--	2.2	--	2.2
0	--	0	--	0	--	0	--	0	--	0	--	0.0	--	0.0
0	--	0	--	0	--	0	--	0	--	0	--	0.0	--	0.0
2	--	14	--	12	--	1	--	1	--	4	--	18.9	--	18.9
0	--	0	--	1	--	21	--	0	--	21	--	23.9	--	23.9
0	--	2	--	0	--	0	--	0	--	1	--	1.7	--	1.7
0	--	6	--	1	--	0	--	0	--	0	--	3.9	--	3.9
1	--	4	--	19	--	10	--	0	--	1	--	19.4	--	19.4
26	--	17	--	25	--	14	--	5	--	15	--	56.7	--	56.7
5	--	10	--	4	--	1	--	6	--	0	--	14.4	--	14.4
25	--	16	--	21	--	21	--	7	--	2	--	51.1	--	51.1
14	--	20	--	20	--	20	--	23	--	18	--	63.9	--	63.9
13	--	12	--	9	--	2	--	16	--	9	--	33.9	--	33.9
5	14**	13	14	12	13	9	12	12	15	11	14	68.9	91.1	80.0
13	13	10	14	14	4	3	1	13	10	13	8	72.2	55.6	64.4
6	14	8	14	13	10	10	9	13	8	13	14	70.0	76.7	73.3
4	14	7	11	8	5	7	11	9	7	5	12	44.4	66.7	61.1
11	12	8	12	6	5	12	15	11	10	5	6	57.8	66.7	63.3
10	15	5	10	2	3	10	15	10	13	12	15	54.4	78.9	66.7
19	--	19	--	12	--	15	--	16	--	24	--	58.3	--	58.3
7	--	20	--	23	--	18	--	16	--	20	--	57.8	--	57.8
6	14	14	15	15	13	15	15	14	15	15	15	71.1	80.	92.2
3	11	12	15	1	4	12	15	7	14	0	3	38.9	68.9	53.8
14	10	8	12	9	11	14	15	12	14	6	7	65.3	76.7	73.3
21	--	23	--	25	--	25	--	27	--	12	--	73.9	--	73.9
24	--	12	--	21	--	26	--	25	--	9	--	65.0	--	65.0
18	--	22	--	20	--	22	--	21	--	16	--	66.1	--	66.1
17	--	21	--	23	--	23	--	25	--	9	--	65.5	--	65.5
20	--	22	--	13	--	16	--	20	--	12	--	57.2	--	57.2
5	--	16	--	15	--	4	--	6	--	5	--	28.3	--	28.3
16	--	25	--	10	--	11	--	8	--	12	--	45.6	--	45.6
17	--	22	--	11	--	13	--	16	--	4	--	46.1	--	46.1
12	14	8	13	11	3	3	2	6	6	4	2	48.9	44.4	46.7
1	5	1	1	0	1	4	7	0	0	0	0	6.7	13.6	11.1
0	--	2	--	10	--	3	--	10	--	2	--	15.0	--	15.0
14	--	7	--	6	--	9	--	5	--	10	--	28.3	--	28.3
10	--	17	--	0	--	6	--	2	--	9	--	24.4	--	24.4
4	10	10	12	0	3	2	5	2	11	1	3	21.1	48.9	35.0
3	--	1	--	2	--	4	--	4	--	3	--	9.4	--	9.4
19	--	15	--	7	--	14	--	19	--	5	--	43.9	--	43.9
1	--	4	--	4	--	4	--	2	--	0	--	8.3	--	8.3
0	--	1	--	7	--	0	--	2	--	3	--	7.2	--	7.2
11	--	21	--	1	--	2	--	7	--	7	--	27.2	--	27.2

indicates that plastic queen cell cups were not used in an experiment; therefore arvae were transferred to the wax queen cell cups.

r in this column indicates that 15 wax and 15 plastic queen cell cups each received

Nectar and/or pollen availability may be the necessary stimulus required to maintain a high level of acceptance of grafted larvae by the cell building colonies. Mitchener (1955) showed over a ten year period, that in Manitoba the nectar flow began about mid-June, increased sharply near the end of June, reached a peak in July and gradually decreased until it was virtually over in all locations by the end of August. Based on these findings, it would seem that the best time to depend on queen rearing in Manitoba would be July. The warm, dry weather of late July and August would also be excellent for queen orientation and mating flights.

In the past most commercial beekeepers in Manitoba have not shown very much interest in rearing their own queens. This has probably been due to several reasons; 1) until recently there has not been a great need for queens because most beekeepers were not overwintering their colonies and would therefore obtain their queens with packages in the spring; 2) up until about 1973 queens could be purchased from breeders in the United States very inexpensively especially in the fall when the suppliers were trying to sell all of their queens before winter; 3) many beekeepers felt intimidated by the amount of work they thought would be required to raise queens at a time when they were already very busy with other beekeeping responsibilities.

However, since 1973 many beekeepers have begun overwintering their colonies (this trend will probably continue, with more beekeepers overwintering more colonies); as well, the cost of queens from the United States has risen sharply. The work that is required to rear queens is probably less than what most beekeepers would expect. For example, in the present study, on any given grafting day during the summer of 1977, a total of 180 larvae were transferred to queen cell cups and introduced to the six cell building colonies (i.e. 30 larvae per colony). This required about 1 1/2 - 2 hours work, which could be done at any time of the day. If the acceptance of the grafted larvae was 60% (not an unreasonable expectation during July) this would represent a production of 108 virgin queens - a very good return for one morning's work. A beekeeper rearing his own queens would also have greater control over stock selection and quality control, and would therefore be able to develop and maintain strains of bees that he preferred for his particular type of operation.

For the commercial beekeeper who has a large overwintering program it would probably be profitable to employ someone for four to six weeks during the summer to work half days on queen rearing. For the smaller beekeeper perhaps some form of queen rearing co-operative could be formed, with other beekeepers, to help reduce costs and the time that each individual would have to devote to the project. This type of co-operative is not without precedent; there is presently a co-operative of six commercial beekeepers

with a total of about 40,000 colonies operating in northern Saskatchewan who rear virtually all of their queens.

Queen rearing in Manitoba has been shown to be viable at the University of Manitoba and the author believes it is one area of beekeeping that has been largely overlooked as a method of increasing both efficiency and profit for the Western Canadian beekeeper.

2. Queen Cell Cup Test

The results of the tests in which the rate of acceptance of larvae in plastic versus beeswax queen cell cups were compared, are found in Table 6. With the exception of cell building colony number three, all of the cell building colonies showed an overall higher level of acceptance using plastic cell cups rather than beeswax cell cups. During the 12 test grafts, the overall acceptance of larvae grafted into wax cell cups was 56.2% whereas the acceptance of larvae in plastic cell cups was 64.1%. Acceptance was greater in the plastic cups with a high level of significance ($P < 0.0003$). The reasons for the higher level of acceptance with plastic cups is unknown; perhaps the plastic is less likely to carry "foreign" odors than the beeswax and is therefore more acceptable to the workers. Bognoczky (1967) was able to rear queens in hexagonally shaped plastic queen cell cups but he did not compare the rate of acceptance with that of beeswax cell cups. One advantage of using plastic cell cups, other than the apparent higher level of acceptance associated

Table 7. Nectar Flow for 1977 Recorded by Two Scale Colonies at the University of Manitoba

Five Day Period	Average Change Per Day (kg.) Colony 1	Colony 2
May 13-18	.89	1.8
19-23	0.0	.89
24-28	0.0	.44
29-June2	.44	-.89
3-7	0.0	0.0
8-12	-1.8	-2.7
13-17	0.0	-.89
18-22	.44	2.2
23-27	2.2	4.4
28-July 1	2.2	0.0
2-6	8.2	3.6
7-11	6.0	4.9
12-16	10.4	1.3
17-21	6.9	4.9
22-26	12.	12.
27-31	4.9	1.8
Aug. 1-5	5.8	5.8
6-10	0.0	1.8
11-15	-3.1	0.0
16-20	-.44	1.8
21-25	-1.8	-.89
26-30	-2.2	-2.2
31-September 4	-.89	-3.1
5-9	-3.6	-1.3

with them, is that they can be re-used. Wax, dried royal jelly etc. can be removed easily from the plastic with hot water.

3. Emergence of Queens from Finished Cells

With increased numbers of beekeepers overwintering their colonies there has been an increase in experimentation with different methods of requeening colonies. One of the methods that is presently causing interest in Canada is the "cell method" of requeening. For the purpose of requeening colonies this method involves placing a finished queen cell in a colony above the brood chambers. Usually the cell is wedged between two frames in the third or fourth super. When the virgin queen emerges from the cell, it is hoped that she will kill the existing mated queen, become mated and take over the duties as the new queen in the colony. This is a very appealing method of requeening colonies since it entails so little labour by the beekeepers. There is, however, a considerable amount of controversy regarding the effectiveness of this technique in replacing old queens and several researchers in Canada are presently evaluating the effectiveness of this method.

One of the factors that contributes to the success or failure of the "cell method" of requeening is the level of pupal mortality occurring in the queen cells to be introduced. The viability of a pupa is difficult to determine by superficial examination of the cell and a more detailed examination of each cell would be impractical

for large numbers of cells. To obtain some indication of pupal mortality during the 1977 summer queen rearing program records were kept of the proportions of "finished" cells that gave rise to viable adults.

Of the queens reared at the University of Manitoba between June 8 and August 5 (1977), pupal mortality was recorded for 11 grafts. The mean percent emergence of adults from finished queen cells was 83.2 $\pm$ 3.22% (see Table 8).

Mortality of pupae is dependent on several factors; the prevalence of brood disease, rough handling by the beekeeper, and exposure to high or low temperature conditions, to mention only a few. Beekeepers intending to use this method of requeening should leave the queen cells in the cell building colonies for ten days after grafting to ensure that the pupae can develop in the proper temperature and humidity. After removal of the cells care should be taken not to jar the pupae inside their cocoons; too, the cells should be transferred quickly to their recipient colonies or else placed in an incubator to prevent excessive chilling. Chilling the pupa in the late stages of development may result in wing deformities (Laidlaw and Eckert, 1974). The relatively high level of pupal mortality, observed in this experiment, may not be representative of all queen rearing operations since different techniques and rearing conditions will affect mortality. Anyone interest in using the cell method of requeening should first monitor the level of pupal mortality in their queen rearing operation to ensure that a high mortality does not go unnoticed.

Table 8. Emergence of Adult Queens from Finished Cells Taken from Six Cell Building Colonies (1977)

Date	No. of queen cells finished	No. of adults emerged	Emergence of Adults from finished cells (%)
June 8	97	91	94
June 15	79	74	94
June 20	106	84	79
June 27	107	97	91
July 1	95	67	71
July 4	82	71	87
July 6	102	95	93
July 11	98	64	65
July 13	94	85	90
Aug. 3	82	64	78
Aug. 5	72	51	71
Mean			83.2±
Standard Error			3.22

4. Introduction of Virgin Queens to Mating Colonies

Records of virgin queen introduction and acceptance in small mating nuclei during 1977 are found in Table 12. The purpose of these experiments was to find a rapid and simple method of queen introduction to mating colonies that would also ensure a relatively high level of acceptance of the virgin queens by the recipient colonies. During the summer the author attempted to evolve an acceptable introduction method by testing different techniques as the season progressed. Therefore, different techniques were often being tested under different environmental conditions and as a result, detailed statistical analysis of the data would not be possible.

a. Length of Time Recipient Colonies are Queenless

Virgin queens were introduced to colonies that had been queenless for varying lengths of time. The following results were obtained:

Table 9. Acceptance of Introduced Virgin Queens Relative to the Length of Time Recipient Colonies were Queenless

No. of Days Colony Queenless	No. of Replicates (i.e. Colonies)	Mean Acceptance of Introduced Queens (%)
3	57	73.4 ± 11.4*
4	120	87.4 ± 4.6
5	33	68.4 ± 13.6
6	20	93.3 ± 6.7

*Standard Error

It is well known (Johansson and Johansson, 1971) that virgin queens are more difficult to introduce to queenless

colonies than mated ones. The length of time the recipient colony has been queenless is also an important factor in determining how readily the colony will accept an introduced queen. Free and Spencer-Booth (1961) found that a lower percentage of mated laying queens was produced from cells introduced within two days of dequeening than from cells introduced after the queen had been removed for three days or more. The time of year when introduction takes place is also important. Langstroth (1890 - cited by Szabo (1974) found that queens were most readily accepted by queenless colonies during a nectar flow and that during periods of dearth it is advisable to feed a colony into which a queen is to be introduced.

In the present experiments the acceptance of introduced queens was quite high in all cases; this was probably because the minimum queenless period for any colony was three days. Colonies that were queenless for six days showed the highest level of acceptance with a mean percent acceptance of 93.3$\pm$ 6.7. It would probably be an inefficient use of equipment to leave mating colonies queenless for six days before each queen introduction, but beekeepers should leave their colonies queenless for at least two to three days to ensure an acceptable level of success in the queen introductions.

b. Acceptance Based on Introduction Method

As the summer (1977) progressed, various methods of virgin queen introduction to mating colonies were tested. The following results were obtained;

Table 10. Acceptance of Introduced Virgin Queens Relative to Introduction Method

Method	No. of Replicates (Colonies)	Mean Acceptance of Introduced Queens (%)	
A) Caged queen in colony 24 hours followed by direct release	14	35.5±	21.5*
B) Caged queen in colony 24 hours followed by direct release with sugar syrup spray	21	71.0±	15.
C) Caged queen in colony 24 hours followed by direct release with sugar syrup and vanilla spray	66	96.6±	2.8
D) Direct queen release with sugar syrup and vanilla spray	124	78.3±	6.2

*Standard Error

It is difficult to draw any firm conclusions from these data because there were so many other variables operating at the time these tests were done (see Table 12). By mid-summer we decided to use the direct queen release with sugar syrup and vanilla spray method because it usually gave an acceptable level of introduction and because it was more expedient than the other methods used. The best method was to have the queen caged in a colony for 24 hours and then release her directly into the colony with a spray of sugar syrup and vanilla. Although this method was very successful (Mean Percent acceptance = 96.7± 2.8) it

did require an extra trip to each colony which was very time consuming. The sugar and vanilla spray which was sprayed onto the queen being introduced and the surrounding workers is probably an effective way of "masking" any foreign "odors" on the queen that may cause the workers to reject her. By the time the sugar and vanilla have been cleaned off the queen and bees, most queens will have been accepted. Covering the queen in some form of "masking" substance is a well known introduction technique that has been used by beekeepers for many years (see Johansson and Johansson, 1971).

c. Acceptance Based on Hive Type and Hive Arrangements

Various hive types and arrangements of hives were used for queen orientation studies (see Chapter VI) during the summer of 1977. Records of successful virgin queen introductions were kept, based on hive types and hive arrangements to determine if the position and location of nearby colonies could affect the acceptance of a queen being introduced to a queenless colony (see Table 11).

During virgin queen introductions, in the summer of 1976, it seemed that in some mating colony arrangements, the virgins were more readily accepted than in other arrangements. Colonies found beside trees in the forest seemed to accept introduced queens more readily that did those in straight lines found in the open fields. It was also observed that there were often more dead worker bees found at the entrances of colonies in pattenrs that had low levels of introduced queen acceptance.

Table 11. Acceptance of Introduced Virgin Queens Relative to Hive Type and Hive Arrangement

Hive type and pattern*	No. of replicates (colonies)	Mean Acceptance of introduced queens (%)
F) Four frame nuc.[x] in straight line of 5, facing south, 1 m. apart.	40	65.8± 14.*
G) Four frame nuc. in row of 5 pairs, facing south; pairs 1.5 m. apart. individuals of pair 1 m. apart.	24	74.3± 12.
I) Four frame nuc, in row of 5 with offset entrances	24	100. ± 0.0
J) Split box nuc. each located beside a tree, in forest.	23	84.5± 16.
K) Split box nuc. facing south, in random arrangement	13	95.5± 4.5
L) Four frame nuc, in row of 5, 1 m. apart, facing south center colony only with coloured entrance	97	83.0± 8.1

* "H" is not included because there were only eight replicates (see Table 12). *S.E.

[x]Nuc. - nucleus

Large number of dead bees at the entrances of colonies may result from fighting, brought about by the drifting of worker bees to neighbouring colonies. If this is so, then perhaps those colonies, with few orientation cues, may not accept queens as readily as colonies with orientation cues because of the high level of worker drifting. The high levels of drifting may cause the colonies to become more "guard conscious" or defensive and therefore less likely to accept foreign queens introduced to their colonies.

Although the data collected in 1978 are inconclusive, is does tend to support the idea that colonies with distinct orientation cues will accept queens more readily than colonies without orientation cues (see Table 11). The lowest levels of queen acceptance were found in the colony arrangements with the fewest orientation cues (i.e. colonies in straight lines all facing one direction). By adding color to the centre colony of a pattern of five hives an improvement in queen acceptance was obtained (see Table 11). The highest (100%) level of queen acceptance was found in patterns where the colony entrances were facing different directions (see I, Table 12). Split box mating colonies placed in the forest beside trees or randomly in the field also had relatively high levels of queen acceptance.

When establishing bee yards with many mating colonies, it is important that the colonies be establihsed in such

Table 12. Results of Virgin Queen Introductions Under Various Conditions (Summary 1977)

Exp. no.	Date of introduction	Length of time colonies queenless (days)	No. of queens introduced*	Type of introduction A**	B	C	D	E	Hive type and pattern F	G	H	I	J	K	L	Replicates of hive pattern	Location of replicates Shade	Forest	Open field	No. of queens accepted	% Acceptance
1	June 14	3	7	X					X							2	X			4	57
1	June 14	3	7	X					X							2			X	1	14
2	June 22	3	8		X					X						1			X	4	50
2	June 22	3	8		X						X					2			X	5	63
2	June 22	3	5		X							X				2			X	5	100
3	June 28	3	10			X							X			-		X		10	100
3	June 28	3	3			X								X		-			X	3	100
4	July 10	4	31			X									X	7			X	30	97
4	July 10	3	9			X						X				2			X	9	100
5	July 11	4	6			X				X						1			X	5	83
5	July 11	4	7			X			X							2			X	7	100
6	July 19	5	9				X		X							2			X	4	44
6	July 19	5	13				X					X				-		X		9	69
7	July 25	4	10				X			X						1			X	9	90
7	July 25	4	36				X								X	8			X	25	69
7	July 25	5	11				X							X		-			X	10	91
8	July 27	6	10				X					X				2			X	10	100
8	July 27	6	5				X		X							1			X	4	80
8	July 27	6	5					X	X							1			X	5	100
9	Aug. 8	4	30				X								X	6			X	25	83

* This value does not include caged queens that died before they were released into the colony.

** A Caged queen in colony 24 hrs. followed by direct release
B Caged queen in colony 24 hrs. followed by direct release with sugar syrup spray
C Caged queen in colony 24 hours. followed by direct release with sugar syrup and vanilla spray
D Direct queen release with sugar syrup and vanilla spray
E Direct queen release with smoke only

Hive type and pattern
F Four frame nucleus colonies in straight line of 5, 1 metre apart
G Four frame nucleus colonies in row of 5 pairs, 1.5 metres apart, individuals of pair 1 metre apart
H Four frame nucleus colonies in straight line of 5, 1 metre apart, all entrances a different colour
I Four frame nucleus colonies in rows of 5 with offset entrances
J Split box nucleus colonies each located beside a tree, in forest
K Split box nucleus colonies random arrangement
L Four frame nucleus colonies in rows of 5, 1 metre apart, centre colony only with coloured entrance

Table 13. Anatomical Measurements of Selected Queens Reared During the Summer (1977)

Date of graft	Bee no.	Wt. at emergence (mg.)	Mandibles W	Mandibles I	Mandibles Q	Right basitarsus (mm.) L	Right basitarsus (mm.) W	Right basitarsus (mm.) L/W	Right basitarsus (mm.) W I Q	Diam. of spermatheca	No. of ovarioles in right ovary
June 5	1	205									
	2	211									
	3	258									
	5	259			x	2.56	1.32	1.94	x		
	6	222									
	7	266									
	8	240			x	2.56	1.32	1.94	x		
	9	242									
	10	236									
	11	233									
	12	243			x	2.64	1.28	2.06	x		
	13	213			x	2.6	1.28	2.03	x		
	14	200			x	2.76	1.32	2.09	x		
	15	228			x	2.6	1.28	2.03	x		
	16	181									
Mean ± Standard error		229. ± 6.18				2.62 ± .031	1.3 ± 0.009	2.02 ± .026			
Queen								1.9-2.3		1.0-1.3	129-197
Worker (Weaver, 1957)								1.6-1.9			1-8

Table 13. Cont'd

Date of graft	Bee no.	Wt.at emergence (mg.)	Mandibles W	Mandibles I	Mandibles Q	Right basitarsus (mm.) L	Right basitarsus (mm.) W	Right basitarsus (mm.) L/W	Right basitarsus (mm.) W I	Right basitarsus (mm.) Q	Diam. of spermatheca	No. of ovarioles in right ovary
June 20	1	194			x	2.6	1.2	2.17		x		
	2	192			x	2.52	1.24	2.03		x		
	3	231										
	4	220			x	2.48	1.28	1.94		x		
	5	216			x	2.52	1.16	2.17		x		
	6	193										
	7	193										
	8	224										
	9	208										
	10	223			x	2.56	1.2	2.13		x		
	11	208										
	12	211			x	2.64	1.2	2.2		x		
	13	241										
	14	227			x	2.68	1.24	2.16		x		
Mean ± Standard error		213± 4.22				2.57± .027	1.22± .015	2.11± .036				
Queen								1.9-2.3			1.0-1.3	129-197
Worker (Weaver, 1957)								1.6-1.9				1-8

Table 13. Cont'd

Date of graft	Bee no.	Wt. at emergence (mg.)	Mandibles W	Mandibles I	Mandibles Q	Right basitarsus (mm.) L	Right basitarsus (mm.) W	Right basitarsus (mm.) L/W	Right basitarsus (mm.) W	Right basitarsus (mm.) I	Right basitarsus (mm.) Q	Diam. of spermatheca	No. of ovarioles in right ovary
July 4	1	204											
	2	172											
	3	189											
	4	178			x	2.64	1.40	1.89			x	1.2	162
	5	194											
	6	203											
	7	175			x	2.76	1.28	2.16			x	1.2	152
	8	222											
	9	211			x	2.68	1.28	2.09			x	1.28	152
	10	219			x	2.68	1.36	1.97			x	1.08	140
	11	222			x	2.48	1.28	1.94			x	1.12	159
	12	236											
	13	220											
	14	223											
	15	213											
Mean ± Standard error		205 ± 5.11				2.65± .046	1.32± .025	2.01± 0.050				1.18± .035	153± 3.80
Queen								1.9-2.3				1.0-1.3	129-197
Worker (Weaver, 1957)								1.6-1.9					1-8

Table 13. Cont'd

Date of graft	Bee no.	Wt. at emergence (mg.)	Mandibles W	Mandibles I	Mandibles Q	Right basitarsus (mm.) L	Right basitarsus (mm.) W	Right basitarsus (mm.) L/W	Right basitarsus (mm.) W I Q	Diam. of spermatheca	No. of ovarioles in right ovary
July 15	1	227			x	2.60	1.32	1.97	x	1.12	166
	2	237			x	2.68	1.36	1.97	x	1.32	154
	3	237			x	2.40	1.28	1.88	x	1.2	124
	4	207			x	2.72	1.32	2.06	x	1.28	144
	5	246									
	6	234									
	7	240									
	8	234									
	9	229			x	2.56	1.32	1.94	x	1.0	136
	10	198			x	2.56	1.32	1.94	x	1.28	149
	11	231									
	12	205									
	13	189									
Mean ± Standard error		224 ± 5.01				2.59± .046	1.32± .010	1.96± .025		1.2± 0.050	146± 5.94
Queen								1.9-2.3		1.0-1.3	129-197
Worker (Weaver, 1957)								1.6-1.9			1-8

Table 13. Cont'd

Date of graft	Bee no.	Wt. at emergence (mg.)	Mandibles W	Mandibles I	Mandibles Q	Right basitarsus (mm.) L	Right basitarsus (mm.) W	Right basitarsus (mm.) L/W	Right basitarsus (mm.) W I Q	Diam. of spermatheca	No. of ovarioles in right ovary
Aug. 12	1	222			x	2.76	1.36	2.03	x	1.16	135
	2	175			x	2.64	1.32	2.0	x	1.16	146
	3	188									
	4	187			x	2.72	1.36	2.0	x	1.2	158
	5	216			x	2.6	1.32	1.97	x	1.2	152
	6	178			x	2.44	1.28	1.91	x	.92	158
	7	202			x	2.68	1.56	1.72	x	1.2	125
	8	257			x	2.72	1.32	2.06	x	1.36	128
	9	265			x	2.64	1.32	2.0	x	1.52	142
Mean ±Standard error		210 ± 11.0				2.65± .035	1.36± .031	1.96± .038		1.22± .061	143± 4.54
Queen								1.9-2.3		1.0-1.3	129-197
Worker (Weaver, 1957)								1.6-1.9			1-8

Table 13. Cont'd

Date of graft	Bee no.	Wt. at emergence (mg.)	Mandibles W	Mandibles I	Mandibles Q	Right basitarsus (mm.) L	Right basitarsus (mm.) W	Right basitarsus (mm.) L/W	Right basitarsus (mm.) W	Right basitarsus (mm.) I	Right basitarsus (mm.) Q	Diam. of spermatheca	No. of ovarioles in right ovary
Aug. 17	1	197											
	2	233			x	2.68	1.4	1.91			x	1.16	161
	3	205			x	2.6	1.44	1.81			x	1.2	134
	4	248			x	2.68	1.52	1.76			x	1.2	128
	5	213											
	6	221											
	7	219			x	2.64	1.28	2.06			x	1.24	150
	8	245			x	2.68	1.24	2.16			x	1.16	148
	9	281			x	2.6	1.28	2.03			x	1.12	139
	10	242			x	2.76	1.24	2.22			x	1.2	120
	11	232											
Mean± Standard error		231± 7.08				2.66± .021	1.34± .042	2.00± .066				1.18± .015	140± 5.31
Queen								1.9-2.3				1.0-1.3	129-197
Worker (Weaver, 1957)								1.6-1.9					1-8

Table 13. Cont'd

Date of graft	Bee no.	Wt. at emergence (mg.)	Mandibles W	Mandibles I	Mandibles Q	Right basitarsus (mm.) L	Right basitarsus (mm.) W	Right basitarsus (mm.) L/W	Right basitarsus (mm.) W	Right basitarsus (mm.) I	Right basitarsus (mm.) Q	Diam. of spermatheca	No. of ovarioles in right ovary
Aug. 26	1	193			x	2.68	1.28	2.09			x	1.2	164
	2	216			x	2.76	1.28	2.16			x	1.36	149
	3	194											
	4	198			x	2.72	1.36	2.0			x	1.16	171
	5	202			x	2.64	1.32	2.0			x	1.2	172
	6	212											
	7	257			x	2.68	1.4	1.91			x	1.16	159
	8	227			x	2.72	1.16	2.35			x	1.2	143
	9	232			x	2.8	1.32	2.12			x	1.2	159
	10	252											
	11	209			x	2.76	1.36	2.03			x	1.16	153
	12	169			x	2.68	1.32	2.03			x	1.24	150
	13	226			x	2.76	1.36	2.03			x	1.2	155
Mean ± Standard error		214 ± 6.81				2.72± .016	1.32± .021	2.07± .037				1.21± .019	158± 2.99
Queen								1.9-2.3				1.0-1.3	129-197
Worker (Weaver, 1957)								1.6-1.9					1-8

Table 13. Cont'd

Date of graft	Bee no.	Wt. at emergence (mg.)	Mandibles W	Mandibles I	Mandibles Q	Right basitarsus (mm.) L	Right basitarsus (mm.) W	Right basitarsus (mm.) L/W	Right basitarsus (mm.) W I	Right basitarsus (mm.) Q	Diam. of spermatheca	No. of ovarioles in right ovary
Sept. 7	1	197			x	2.56	1.36	1.88		x	1.28	147
	2	175			x	2.68	1.4	1.91		x	1.32	120
	3	174			x	2.72	1.32	2.06		x	1.28	117
	4	192										
	5	227										
	6	186										
	7	187										
Mean ±Standard error		191± 6.76				2.65± .048	1.36± .023	1.95± .055			1.29± .013	128± 9.54
Queen								1.9-2.3			1.1-1.3	129-197
Worker (Weaver, 1957)								1.6-1.9				1-8

a way as to give the bees access to as many orientation cues as possible. This reduces the amount of worker drifting between colonies and will also help the queen find her way back to the proper colony during orientation and mating flights (see Chapter VI).

d. Morphological Characteristics

The morphological characteristics of the sample of adult queens reared during the summer (1977) are shown in Table 13.

For queens obtained from the June 5 and June 20 grafts, spermatheca diameter and ovariole numbers were not obtained because these queens deteriorated during storage. With the exception of ovariole numbers, for queens sampled from the September 7, 1977 graft, all other characteristics from the other grafts fell well within the categories for normal queens described by Weaver (1957). The reason that some queens from the September 7 graft showed low numbers of ovarioles is not known, but may have been due to cool temperatures or inadequate feeding during development.

D. Conclusions

It is possible to rear large numbers of high quality queen honey bees during the summer in Manitoba. The best time to rear queens during the summer appears to be during the peak of the nectar flow period, which usually occurs in July. Queen rearing started in July would have the benefit of the warm, sunny days of late July and early

August which would be ideal for queen mating. At this time, queen rearing during the summer appears to be the most dependable method by which to obtain mated queens independent of the United States sources.

CHAPTER VI

DRIFTING OF QUEEN HONEY BEES DURING ORIENTATION AND MATING FLIGHTS

A. Introduction

It has been known for many years that the queen honey bee receives her lifetime supply of spermatozoa during several aerial mating flights which occur in the first few weeks of adult life. (see: Risga, 1931; Taber, 1954; Woyke, 1962; Zmarlicki and Morse, 1963a; Gary, 1962, 1963). To accomplish this with large numbers of queens, commercial queen breeders have had to establish "mating yards" consisting of hundreds of small mating colonies. Each mating colony usually consists of a few small frames of wax comb and several hundred bees. A finished queen cell or newly emerged virgin queen is introduced to each mating colony and if, after about two weeks, eggs are found in the worker cells then this is taken as a reasonable sign that the queen has been properly mated; she is then removed from the colony for shipment and replaced by another queen cell or adult virgin queen.

Although there has been a considerable amount of research into the mechanism of sex attraction and copulation during mating flights, little is known about how the queen orients to the home hive so that she can successfully return after her mating flight. Knowledge of queen orientation would have important practical applications to both the design of mating colonies and the layout of the mating yard.

It is well known that both worker and drone honey bees will make orientation errors and "drift" into a colony other than their own. Generally, drifting occurs when a number of colonies are located close together in similar type hives (Free, 1958).

King (1932) found that drifting of workers between colonies was often over 30 percent of the total number of bees. When colonies are arranged in rows, with a few landmarks or orientation cues, the bees tend to drift from the centre hives to the hives at the ends of the rows (Free, 1958; Jay, 1965c). Several methods have been shown to reduce drifting (see Free, 1958; Free and Spencer-Booth 1961; Free, 1961: Jay, 1966a, 1966b, 1968, 1969a, 1969b, 1971a; Levin, 1966). Most of the methods used in reducing worker drift involve supplying the bees with orientation cues that will help them to distinguish their parent colony from other nearby colonies; such cues as colours and other markings above the entrance, physical landmarks (trees, fences, rocks, etc.) located close to the hive, facing nearby hives in different directions and placing the hives in irregular patterns, all help to reduce worker drift.

Although Butler (1939) found virtually no drifting of drones during one season of study, most researchers believe that drones drift to, and are accepted by other colonies even more readily than workers. Free (1961) stated that drones drift two or three time as much as workers; Witherall (1965) found that after one week, 11.43 percent

of the drones in one hive had drifted to two neighboring hives and after two weeks 12.25 percent of the drones had drifted. Free (1961) also found that more drones from queenright colonies drift to queenless than to queenright colonies.

Very little is known about drifting of queen honey bees during orientation and mating flights and there are only a few references to queen drifting in the literature. Gontarski (1952) believed that up to 25 percent of queens returning from mating flights try to enter the wrong mating nucleus. He also suggested that when a queen is turned away by the guard bees at one colony, she may seek out the most receptive foreign nucleus to enter. In a series of experiments performed by Mathis (1960) with mating colonies placed in pairs on five window ledges (i.e. two colonies per ledge) it was found that queens often entered colonies other than their own. Mathis found that the queens usually drifted to hives on the same side of the pair as their parent hive; Jay (1966b) found similar results with worker bees. Mathis also found that the queens tended to drift towards the east; Jay (1971b) found that workers tend to drift more towards the west.

The purpose of the present research was to determine how prevalent queen drift is under different conditions and to determine what happens to queens when they enter colonies other than their own.

In an attempt to gain a better understanding of the anatomical changes that take place in queens before and after mating, weight readings of some queens were taken daily from the time

of emergence until several days after egg laying began.

B. Methods and Materials

1. Daily Weight Changes of Hived Virgin Queens (see also Chapter II)

To determine the changes in the weight of adult queens between emergence and the begining of egg laying, several queens were weighed each day beginning less than one hour after emergence and continuing each day for several weeks. The queens that were to be used in this experiment were first given an identifying paint mark on the thorax, weighed with an analytical balance in the laboratory and then introduced directly to queenless mating colonies in the University of Manitoba apiary. Each morning the queens were removed from the mating colonies and placed in small screen cages (see Figure 9, Chapter II) for transport to the "grafting hut" (see Figure 4, Chapter II) where they were weighed indivually. Once inside the grafting hut, each queen was transferred to a pre-balanced screen cage and then weighed on a precision torsion balance. After the queens had been weighed they were returned to their original colonies.

The first trial of this experiment began July 25, 1977 and 12 queens were introduced to mating colonies; the second trial began August 9, 1977, also with 12 queens. In the July 25 trial weights were taken for 15 days and in the August 9 trial, weights were taken for 24 days.

2. Queen Release Tests

The method used to test the queens' ability to return to her colony was based on a technique used by Gary (1971). After the queens had been in the colony for about eight days, depending on preceding weather conditions, they were removed from the colony, placed in a small enclosed box (measuring 2 cm. x 6 cm. x 8.5 cm.), moved various distances and directions from the hive and then released. One person made the release and one person observed the hives in order to capture the queen after it landed. The hive entrances were screened to prevent the queen entering the hive before being captured. The person at the hives also recorded the time which elapsed between release of the queen and its' return to a colony.

3. Queen Drift

In any given pattern of mating colonies each queen was given an identifying mark of paint on the thorax. Whenever the colonies were inspected, either to remove queens for release tests, or to remove laying queens, records were kept of any queens that had drifted from their original colony to nearby colonies.

c. Results and Discussion

1. Daily Weight Changes of Hived Virgin Queens

During the course of the summer many queens were observed each day from the time they emerged as adults until they began egg laying. During these observations, it became apparent that the queens underwent a large reduction

in size after about the second or third day after emergence and then began to increase in size just before the beginning of egg laying. Newly emerged queens usually could not fly and were typically very slow moving. By about the fifth or sixth day after emergence the queens that were in colonies had a much shorter abdomen than the newly emerged queens, and were very active. At this time the queens could be found running rapidly on the comb and would readily fly if disturbed or removed from the colony. Five or six day old queens were often difficult to find in the colony because they were not much larger than the workers.

By the time the queens began laying eggs they had again reached a very large size because of their distended abdomens. Most laying queens were also incapable of flight.

After doing several dissections of queens at various stages of development it appeared that the large size at emergence was due primarily to accumulated reserves of fat tissue in the abdomen. This fat tissue is apparently metabolized without being replaced during the first week of life, hence the reduction in size of the abdomen. After the queen has been properly mated, her ovaries begin to develop and as egg production begins, these get very large; this results in the characteristically enlarged abdomen of a laying queen. Harbo (1971), found that a decrease in hemolymph volume accounted for about 30 percent of a queen's weight loss between emergence and the time she reached her lowest weight. The amount of food and waste stored in the queen's gut may also decrease as the queen

loses weight. A comparison of queens at the three basic stages of development discussed here is illustrated in Figure 18.

Because most of the queens observed seemed to undergo these changes, it was decided to try and quantify each stage by taking a daily weight reading for several queens. If the pattern of weight changes was found to be consistent, then taking daily weights of young queens could be an effective way of anticipating, a few days in advance, when a queen was going to begin egg laying.

The problem of trying to determine if a queen has been on orientation or mating flights, is that unless a direct observation has been made of a queen leaving the hive then one cannot be sure that she has actually left until she begins laying fertilized eggs. By the time the queen begins laying it is too late to perform orientation tests because, normally, laying queens cannot fly well, if at all. If it were possible to anticipate egg laying, by detecting the beginning of a gradual increase in queen weight, then it might be an effective tool for determining if a queen had been on mating flights and therefore was oriented to the home colony. This technique would eliminate the need for direct observation of queen mating flights and would therefore free the researcher to work with a much larger number of queens at one time.

Figure 18. Three queens at various stages of development.

a) newly emerged adult; weight 220 mg.
b) eight day old virgin; weight 155 mg.
c) mated and laying queen; weight 306 mg.

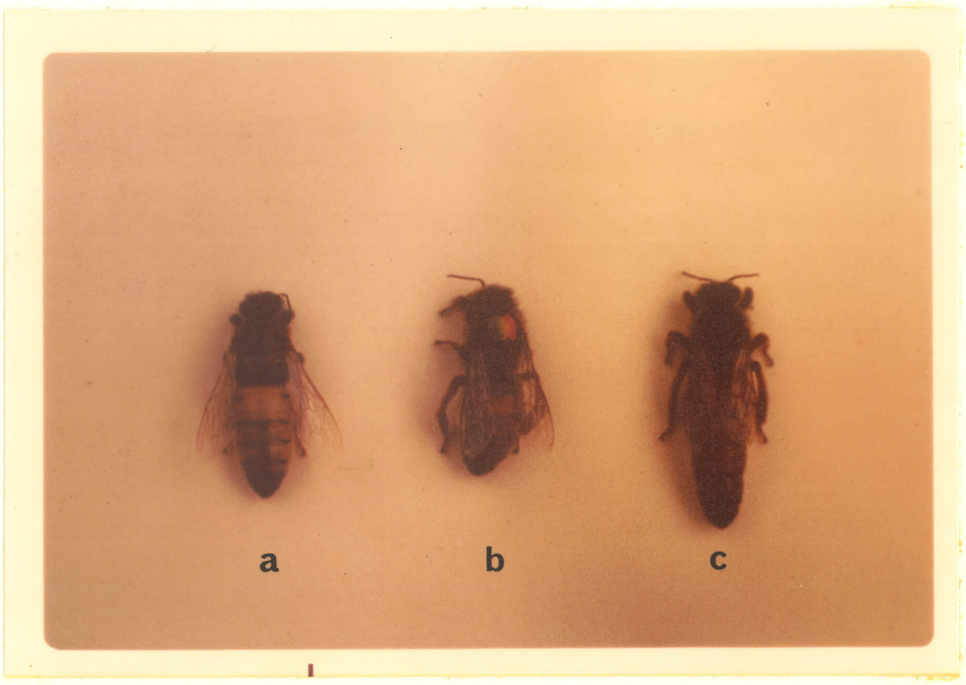

a
b
c

Figure 19. Changes in queen weight during early development for queens introduced July 25, 1977. "L" represents the beginning of egg laying.

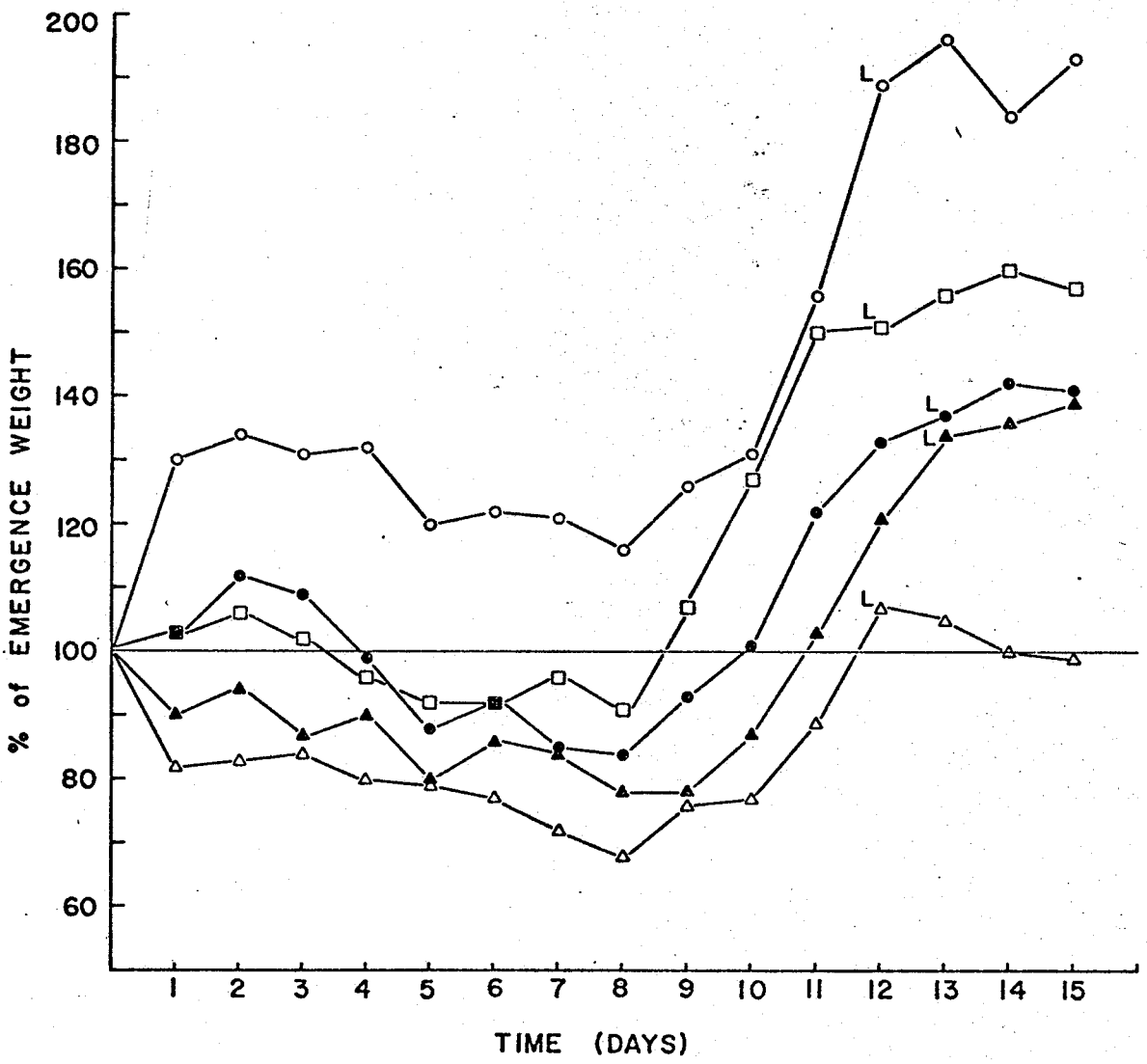

200
180
160
140
120
100
80
60
% of EMERGENCE WEIGHT
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
TIME (DAYS)
L
L
L
L
L

Table 14. Changes in Queen Weight During Early Development for Queens Introduced July 25, 1977

Queen no.	Emergence		Daily weight and % of emergence weight													
			1		2		3		4		5		6		7	
	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%
1	218	100	195	90	205	94	190	87	197	90	174	80	185	85	183	84
3	194	100	199	103	218	112	211	109	191	99	170	88	178	92	164	85
8	140	100	182	130	188	134	184	131	185	132	168	120	171	122	170	121
9	185	100	191	103	193	104	188	102	177	96	170	92	171	92	175	96
11	257	100	210	82	213	83	215	84	206	80	202	79	199	77	185	72

(cont'd)

Table 14. (cont'd)

Queen no.	8		9		10		11		12		13		14		15	
	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%
1	171	78	170	78	189	87	224	103	263	121	293[L]	134	297	136	302	139
3	162	84	180	93	196	101	237	122	257	133	266[L]	137	276	142	272	141
8	163	116	176	126	184	131	219	156	265[L]	189	272	196	257	184	270	193
9	168	91	197	107	235	127	277	150	280[L]	151	289	156	296	160	290	157
11	174	68	196	76	197	77	229	89	275[L]	107	269	105	256	100	255	99

"L" represents the beginning of egg laying.

In the first trial (July 25), of the 12 queens that were introduced to mating colonies; two were not accepted after 24 hours; five were missing during the course of the experiment and five were mated and began laying eggs within 13 days after emergence. The changes in weight that occurred between emergence and 15 days for the five mated queens are recorded in Table 14 and illustrated in Figure 19.

All of the queens, from the July 25 trial, showed a great increase in weight just before egg laying started. The average number of days (± standard error) at which the lowest weight was attained was 8.2 ± .2 days. The mean lowest weight attained was 167.4± 2.33 mg. and the mean weight at the outset of egg laying was 257.8± 5.3 mg.; a substantial increase over the mean of the lowest weight. The mean number of days which elapsed until laying began was 12.4± .24.

One queen did not show a drop below it's emergence weight. This queen (queen no. 8) was the smallest queen in the group, weighing only 140 mg. at emergence (see Table 14); this may have been the reason it gained weight after emergence rather than losing it. It may have been slightly under-nourished during pupation and therefore did not emerge with the same quantity of stored nutrients as the other queens.

This weight reduction is probably an adaptation to allow easy and efficient flight and most mating flights occur close to the time when the lowest weight is attained. After

the queens had mated they gained weight rapidly over three to four days until egg laying began (see Figures 19 and 20A).

According to Harbo (1971) the characteristic loss in weight of a virgin queen is a natural rhythm that occurs independent of the environment the queen is found in.

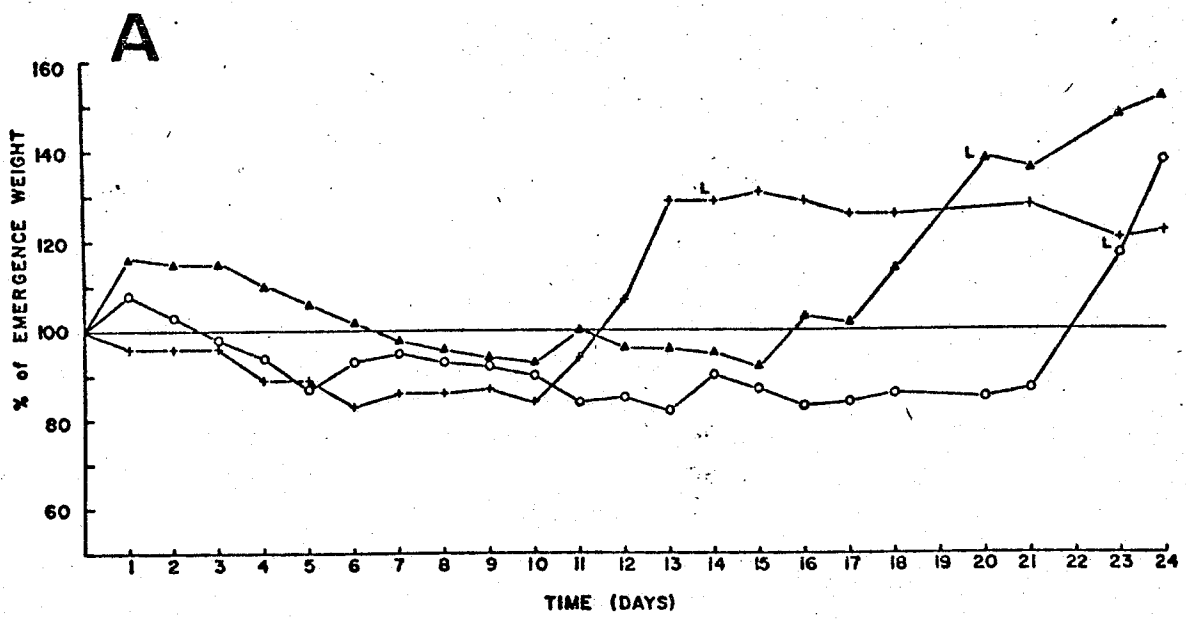
In the second trial (August 9), of the 12 queens that were introduced to mating colonies three were laying by 24 days after emergence, five were still in the colonies but not laying, and four were missing during the course of the experiment. Of the five queens that had not started laying by the twenty-fourth day (when the weight readings were stopped) two began laying about 10 days later (i.e. about 34 days after emergence). (The results from this trial are found in Table 15 and Figure 20).

The results from the August 9 trial are similar to those of the July 25 trial except that most of the queens did not begin laying within the experimental period and those that did were delayed (see Figure 20A). The fact that most of the August 9 queens took much longer than the July 25 queens to begin egg laying was primarily due to adverse weather conditions that prevented successful mating. It is interesting to note that queens will apparently retain their weight at a constantly low level for long periods of time until they can go on successful mating flights (see Figure 20B).

Figure 20. Changes in queen weight during early development for queens introduced August 9, 1977.

A. Queens that began egg laying within 24 days. "L" represents the beginning of egg laying.

B. Queens that did not begin egg laying within 24 days.


A
% of EMERGENCE WEIGHT
160
140
120
100
80
60
L
L
L
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
TIME (DAYS)

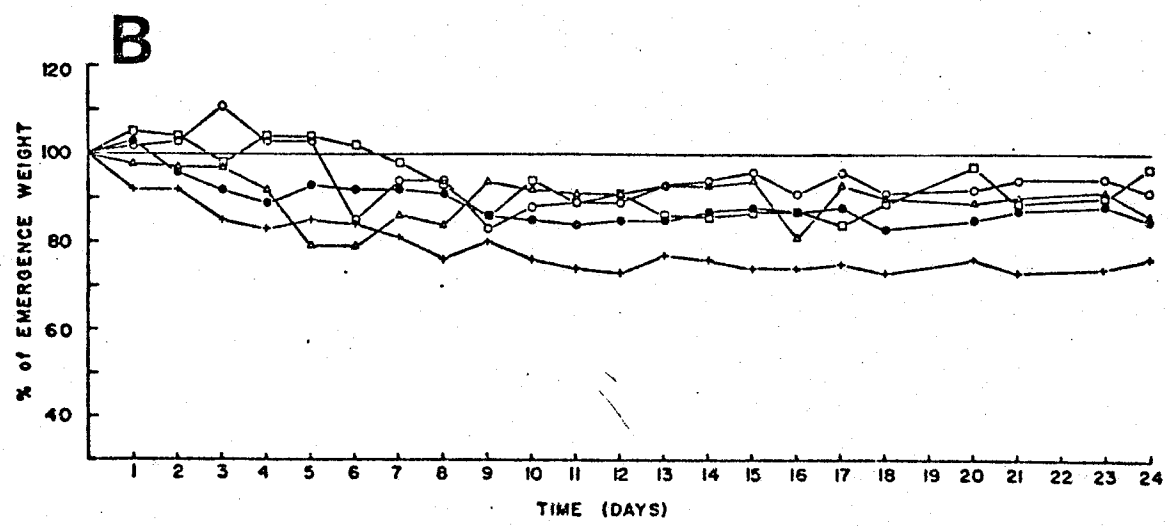

B
% of EMERGENCE WEIGHT
120
100
80
60
40
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
TIME (DAYS)

Table 15. Changes in Queen Weight During Early Development for Queens Introduced August 9, 1977

Queen no.	Emergence		Daily weight and % of emergence weight													
			1		2		3		4		5		6		7	
	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%
1	216	100	207	96	207	96	207	96	193	89	193	89	180	83	185	86
3	190	100	206	108	195	103	186	98	179	94	165	87	176	93	181	95
10	178	100	206	116	204	115	204	115	195	110	188	106	182	102	174	98
2	201	100	206	103	193	96	185	92	178	89	187	93	184	92	184	92
4	196	100	192	98	186	97	186	97	180	92	154	79	154	79	168	86
9	189	100	193	102	194	103	209	111	194	103	194	103	160	85	178	94
11	198	100	207	105	206	104	194	98	206	104	206	104	202	102	193	98
12	207	100	190	92	190	92	176	85	171	83	176	85	173	84	168	81

(cont'd)

Table 15. (cont'd)

Queen no.	Daily weight and % of emergence weight															
	8		9		10		11		12		13		14		15	
	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%
1	185	86	188	87	181	84	202	94	230	107	278	129	279[L]	129	282	131
3	176	93	174	92	170	90	160	84	161	85	156	82	171	90	165	87
10	171	96	167	94	166	93	178	100	170	96	170	96	169	95	163	92
2	182	91	172	86	170	85	169	84	171	85	170	85	175	87	176	88
4	164	84	184	94	181	92	178	91	179	91	183	93	182	93	185	94
9	178	94	156	83	166	88	169	89	169	89	175	93	177	94	182	96
11	185	93	171	86	186	94	177	89	181	91	171	86	170	86	172	87
12	157	76	166	80	158	76	153	74	152	73	160	77	157	76	153	74

(cont'd)

Table 15. (cont'd)

Queen no.	Daily weight and % of emergence weight													
	16		17		18		20*		21		23		24	
	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%
1	278	129	273	126	271	126	-	-	276	128	261	121	264	122
3	158	83	160	84	163	86	162	85	166	87	222[L]	117	262	138
10	184	103	182	102	203	114	246[L]	138	242	136	264	148	270	152
2	174	87	177	88	167	83	171	85	174	87	177	88	171	85
4	181	92	183	93	177	90	175	89	177	90	178	91	169	86
9	172	91	181	96	171	91	173	92	178	94	178	94	173	92
11	172	87	167	84	176	89	191	97	177	89	179	90	192	97
12	153	74	155	75	150	73	157	76	151	73	153	74	158	76

"L" represents the beginning of egg laying.

* Weights were not taken on days 19 and 22 because of inclement weather.

2. Queen Release Tests

a. General Observations

The queens normally flew without hesitation when they were released from the transportation box. If their ovaries were beginning to produce eggs, flight was difficult to sustain and they would often drop to the ground after a few seconds. Those queens that could fly well seemed to repeat a behaviour similar to that observed by Gary (1971), i.e. they usually flew in circles of increasing diameter and height around the releaser until they could no longer be observed. Sometimes I saw them flying directly towards the home pattern of colonies and they would often pass over the colonies and fly out of sight.

On three separate occasions, with three different queens, a more unusual type of behaviour was observed when the queens were released. These queens seemed to be attracted to the releaser and were "reluctant" to leave. They hovered around the releaser, landed on the releaser's head and clothing and one even tried to re-enter the transport box. One of these queens took repeated short flights of increasing diatance from the releaser in several directions up to a maximum of about 60 meters. After each short flight she would return to the releaser, hover nearby for a few seconds, and then leave on another short flight. The apparent desire of these queens to remain close to the releaser and to keep the releaser in sight leads one to speculate that perhaps these queens had never been outside

their colonies before and that they were orienting to the releaser as their closest point of reference on their first flight. A similar type of hovering behaviour is often exhibited by queens that fly when package bees are being hived. These queens often hover nearby and can sometimes be captured in mid-flight.

Released queens were often observed being pursued by drones both during flight and even a few times while they were walking on the ground. Only once was a drone seen to actually mount a queen. When this queen returned to her colony she did not show a mating sign (see Chapter I). Taber (1954) has shown that a queen can return to a hive without a mating sign yet still have spermatozoa in her spermatheca indicating that she must have been mated.

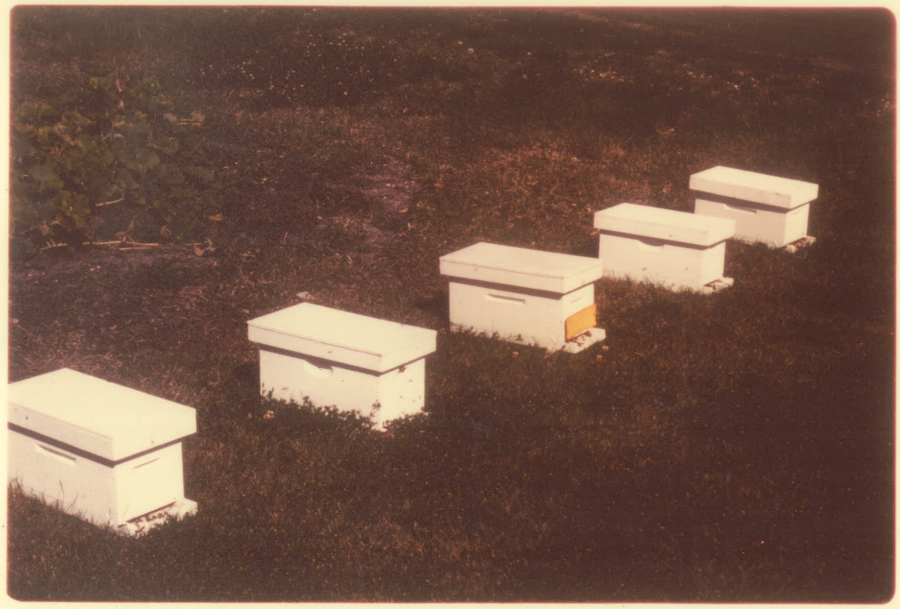
Queens that tried to enter colonies other than their own were usually attacked immediately by the workers. I observed that some queens that returned to the pattern of colonies containing their home colony were sometimes attacked in mid-flight by workers as they hovered in the vicinity of other colonies. This could be a significant source of queen loss in mating yards that have a large number of closely placed colonies. Queens, returning from a flight, sometimes landed on the ground near the colonies; these queens were sometimes attacked and sometimes fed by workers but were usually just ignored.

Figure 21. Two arrangements of mating colonies.

A. Four frame nucleus colonies in row of five with center colony colored.

B. Four frame nucleus colonies in row of five pairs.

A



B

Table 16. Experiment 1. Return of Queens Removed From Pattern of Five, Four Frame Mating Colonies, One Meter Apart Facing South With All Hive Entrances A Different Colour. All Queens Released 100 Meters South of Home Hive.

Date	Queen	Original position*	Colour**	Treatment	Return flight duration (min.)	Return to Position 1	2	3	4	5	Colour O	G	W	Y	B	Comments
July 4	C16	1	O	-	4.0	x					x					Correct position and colour
July 4	C16	1	O	switch G. & O.	1.03	x						x				Correct position, wrong col.
July 4	C19	4	Y	-	2.5			x							x	Correct position and colour
July 4	C19	4	Y	switch Y. & G.	11.58			x				x				Correct position, wrong col.
July 4	C5A	1	O	-	.87	x					x					Correct position and colour
July 4	C5A	1	O	switch O. & G.	1.02	x						x				Correct position, wrong col.
July 4	C5A	1	O	same as above	.92		x				x					Wrong position, correct col.
July 4	C5A	1	O	G-W-O-Y-B	1.58		x						x			Wrong position, wrong colour
July 4	C5A	1	O	return to orig. pattern	.98	x					x					Correct position and colour
July 4	C4A	2	G	-	4.57			x							x	Wrong position, wrong colour
July 4	C4A	2	G	-	1.22			x							x	Wrong position, wrong colour
July 4	C4A	2	G	O-G-W-B-Y	3.0			x							x	Wrong pos. & col., seems oriented to position 4.
July 5	C1A	5	B	-	2.75		x					x				Wrong position and colour
July 5	C1A	5	B	-	4.47				x						x	Correct position and colour
July 11	C3A	3	W	-	2.20				x						x	Wrong position and colour
				Mean ± standard	2.85 ± .71											

** Original pattern of entrance colours from west to east was; O(Orange), G (Green), W (White), Y (Yellow), B (Blue).

* Colonies were numbered 1-5 from west to east.

On two occasions during the summer (1977) a queen was seen to leave her colony voluntarily. The first queen left at 4:30 P.M. on July 25 and returned after eight minutes; the second queen observed, left in the afternoon (exact time unknown) on August 19 and returned after one minute and seventeen seconds. In both cases no mating sign was observed in the returning queens.

b. Experiment 1.

The queens were introduced to patterns of five mating colonies (four frame nucleus colonies); the colonies were one meter apart facing south, with all hive entrances of each pattern having a different colour. The results from this experiment are recorded in Table 16. After eight days some queens were removed from the colonies and released 100 meters south of the home colony. If a queen returned successfully to it's original colony, then the sequence of colours was changed and the queen released again to determine if it preferred to orient to position or colour. Six different queens were used giving a total of 15 releasals.

The mean return flight duration ($\pm$ standard error) was 2.85 $\pm$ .71 minutes. The first two queens tested seemed to be oriented primarily to the position of the hive in the pattern; when the original colour sequence was altered they chose their original position rather

than their original colour. The third queen was "confused" after the original colour sequence had been changed; first she went to her original position, then she chose her original colour over position and then she chose a hive unrelated to both the colour or position of her home hive. However, after the original colour pattern was re-established she returned to her original hive. The fourth queen tested (C4A) seemed to be oriented to hive number four, even though she was from hive number two. The last two queens tested did not seem well oriented to their home hive.

c. Experiment 2.

The queens were introduced to patterns of five, four frame nucleus colonies, one meter apart, facing south with only the centre hive coloured (see Figure 21A). The results of the releasals of these queens are recorded in Table 17. There were a total of 21 queens tested with altogether 35 releasals.

The mean (± standard error) return flight duration was 4.22± 1.08 minutes. Of the mistakes that were made, six were towards the east and seven towards the west.

d. Experiment 3.

The queens were introduced to various patterns of colonies and then the queens were released from different directions and distances from the home colony. The results of this experiment are found in Table 18.

One of the queens (CYA5) returned to the home hive correctly after each releasal; queen CYA1 returned correctly each time except once when it landed on the ground instead

Table 17. Experiment 2. Return of Queens Removed From Pattern of Five, Four Frame Mating Colonies, One Meter Apart Facing South With Center Hive Only Coloured. All Queens Released 100 Meters South of Home Hive.

Date	Queen	Time in hive (days)	Original* location	Return flight duration (min.)	Return to (position)	Comments
July 18	CGA5	8	5	4.05	3	incorrect
July 18	CGA5	8	5	1.9	5	correct
July 18	CGA4	8	4	1.28	5	incorrect
July 18	CGA4	8	4	DNR		
July 19	CYA4	8	4	>36.0	4	correct
July 19	CYA5	7	5	.68	5	correct
July 19	CYA5	7	5	1.28	5	correct
July 19	CGB4	8	4	3.8	4	correct
July 19	CYB2	8	2	1.73	4	incorrect
July 19	CYB2	8	2	.88	3	incorrect
July 19	CYB2	8	2	.73	2	correct
July 19	CYB2	8	2	.72	2	correct
July 19	CGA5	9	5	>9.0	5	correct
July 25	HB1	8	1	DNR		
July 25	HB3	8	3	6.17	3	correct
July 25	HB3	8	3	2.25	2	incorrect
July 25	HB3	8	3	DNR		

(cont'd)

Table 17. (cont'd)

Date	Queen	Time in hive (days)	Original* location	Return flight duration (min.)	Return to (position)	Comments
July 26	HB2	8	2	DNR		
Aug. 1	CGA2	8	2	7.7	1	incorrect
Aug. 1	CGA3	8	3	11.17	4	incorrect
Aug. 1	CBA3	8	3	DNR		
Aug. 2	CGA1	7	1	1.13	1	correct
Aug. 2	CGA1	7	1	4.03	1	correct
Aug. 2	COB3	7	3	DNR		
Aug. 2	COB4	7	4	2.52	4	correct
Aug. 2	COD3	7	3	2.7	2	incorrect
Aug. 2	COD3	7	3	DNR		
Aug. 3	COB4	8	4	DNR		
Aug. 3	COC3	7	3	DNR		
Aug. 16	CGA3	8	3	DNR		
Aug. 19	CYA3	11	3	22.72	2	incorrect
Aug. 19	CYA3	11	3	3.07	2	incorrect
Aug. 19	COB3	11	3	12.93	4	incorrect

(cont'd)

Table 17. (cont'd)

Date	Queen	Time in hive (days)	Original* location	Return flight duration (min.)	Return to (position)	Comments
Aug. 19	COC3	10	3	1.3	2	incorrect
Aug. 19	COC3	10	3	2.3	4	incorrect
			Mean± Standard Error**	4.22± 1.08		

* Colonies were numbered 1-5 from west to east.
** Does not include values from CYA4 and CGA5 on July 19.

of the entrance; one queen did not return on the second releasal and the remaining two queens tested were able to find the correct hive pattern after each releasal but made several errors in selecting the specific colony to enter.

3. Queen Drift

a. Inter-pattern drift

Each queen that was introduced to each discreet pattern of mating colonies had an identifying paint mark on the thorax. There was, however, duplication of the colour code used between patterns of colonies and therefore at any one time there may have been several queens in the mating yard with the same colour code on their thorax. During the course of the summer (1977) there were three observed instances of queens entering and being accepted to colonies in patterns other than their own. Because of this duplication of the colour code used it was impossible to say with certainty which colony patterns these stray queens originated from.

The first example of inter-pattern drift was discovered on August 5, 1977 when two queens were found to have drifted into a pattern of five, four frame colonies with the centre hive entrance painted green. On August 12, 1977 another queen was found to have drifted into a pattern of five, four frame colonies with entrances facing different directions ("offset entrance pattern"). In both of these instances the nearest pattern from which a queen could drift was about 50 meters and the farthest pattern about 250 meters.

b. Intra-pattern drift

Examples of the queens that drifted and were accepted by other colonies in patterns of five, four frame colonies with the centre hive coloured are illustrated in Figure 22. Of the 11 queens that drifted, seven were towards the west and four towards the east. Most of the queens drifted to colonies immediately adjacent to their original colonies with only two queens drifting to a colony not adjacent to it's own. It should be noted for Figure 22 that in two cases (5 and 6 and 10 and 11) two queens in the same pattern drifted, and in both cases one queen replaced another that had drifted to another colony.

One queen drift was found in a pattern of five, four frame colonies with no colour on the entrances. This drift is illustrated in Figure 23.

The queens that drifted to, and were accepted by, colonies in the patterns of five pairs of four frame colonies (see Figure 21B) are recorded in Figure 24. Two of the queens drifted to adjacent colonies and two drifted farther than their adjacent colonies. Most (three of four) of the queens drifted towards the east (see Mathis, 1969). However, only one of these queens drifted to a hive on the same side of the pair as it's parent hive (see Mathis, 1960).

Table 18. Experiment 3. Return of Queens Released Various Distances and Directions From Different Hive Patterns

Date	Queen	Time in hive (days)	Original location*	Hive pattern	Direction of release	Distance of release (M.)	Return flight duration (min.)	Return to (position)	Comments
July 4	S15	8	5	row of 5: offset entr.	S	200	5.4	5	correct
July 4	S15	8	5	"	W	200	4.73	5	correct
July 4	S15	8	5	"	N	200	3.68	5	correct
July 4	S15	8	5	"	E	200	2.58	4	incorrect
July 4	S12	8	2	"	S	200	4.35	2	correct
July 4	S12	8	2	"	W	200	DNR		
						Mean ± standard error	4.15 ± .48		
July 20	CYA5	9	5	row of 5: centre hive coloured	S	100	2.55	5	correct
July 20	CYA5	9	5	"	S	100	3.58	5	correct
July 20	CYA5	9	5	"	W	100	.72	5	correct
July 20	CYA5	9	5	"	E	100	.85	5	correct
July 20	CYA5	9	5	"	N	100	.92	5	correct
						Mean ± standard error	1.72 ± .57		
Aug. 19	CYA1	11	1	"	S	100	.53	1	correct
Aug. 19	CTA1	11	1	"	W	100	1.75	on ground between 1 & 2	-
Aug. 19	CYA1	11	1	"	N	100	.92	1	correct
Aug. 19	CYA1	11	1	"	E	100	.77	1	correct
						Mean ± standard error	.99 ± .26		
July 20	P4	6	4	row of 5 pairs	S	100	15.07	5	incorrect
July 20	P4	6	4	"	S	100	7.0	4	correct
July 20	P4	6	4	"	W	100	1.15	6	incorrect
July 20	P4	6	4	"	E	100	2.6	8	incorrect
July 20	P4	6	4	"	W	100	.78	6	incorrect
						Mean ± standard error	5.32 ± 2.68		

*Colonies were numbered from west to east.

Figure 22. Drifting of queens in patterns of five, four frame colonies with the center hive coloured.

Y = yellow
G = green
O = orange

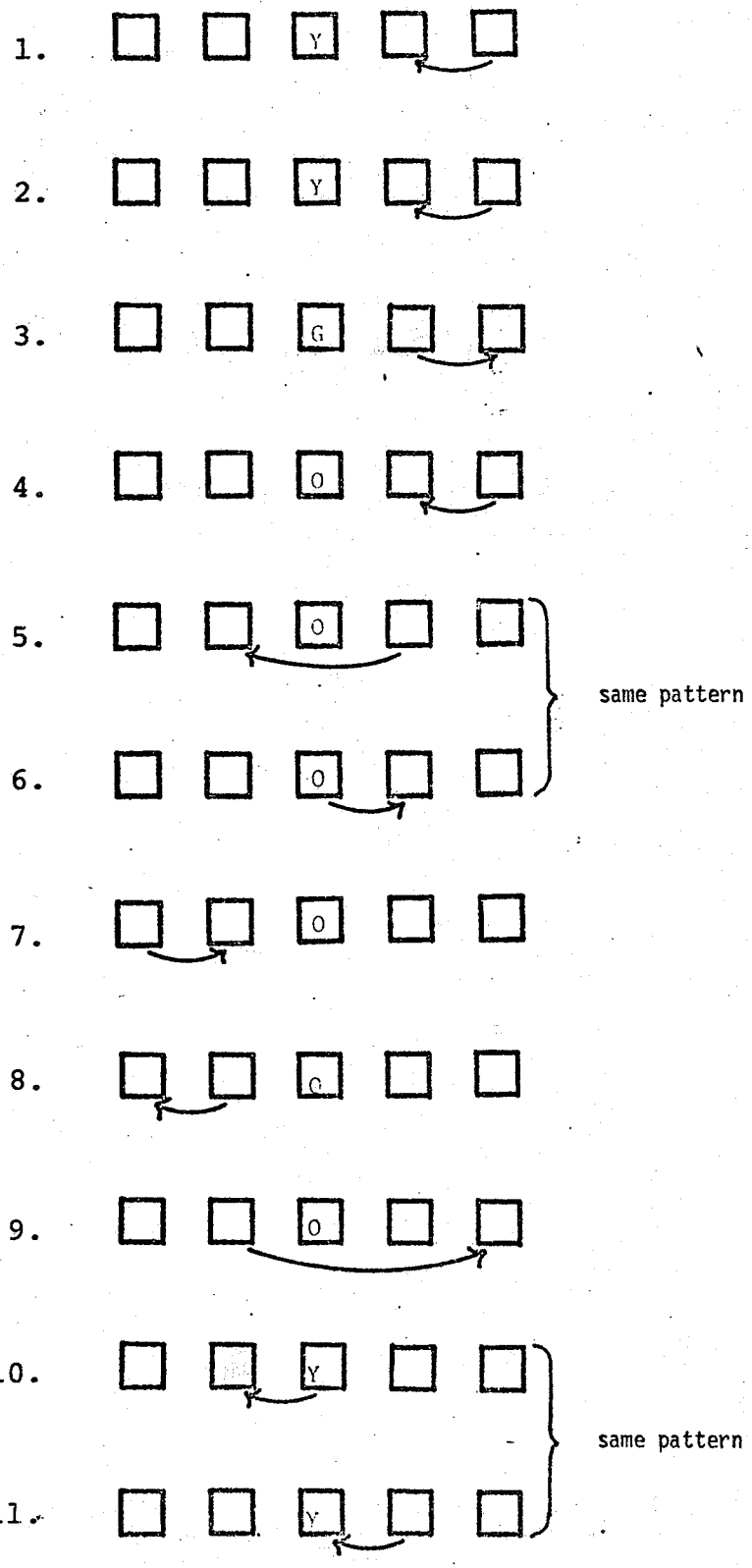

1.
Y
2.
Y
3.
G
4.
O
5.
O
same pattern
6.
O
7.
O
8.
O
9.
O
10.
Y
same pattern
11.
Y

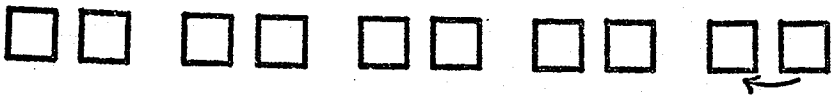
Figure 23. Drift of a queen in a pattern of five, four frame colonies with no colour on the entrance

Figure 24. Drifting of queens in patterns of five pairs of four frame colonies.

1. 

2. 

3. 

4. 

D. Conclusions

Most queens will undergo a substantial reduction in size and weight between the time they emerge as adults and the time they begin taking mating flights. Shortly after successful mating has occurred, the ovaries will gradually become functional and the size and weight of the queen will increase. These changes in the queen can be quantified by monitoring queen weight and therefore the various stages of adult queen development can be approximately determined. When the queen's weight reaches the lowest level, the queen is ready for taking mating flights and when her weight begins to increase this probably indicates that she has been mated and that she will begin laying within two to four days. Queens can apparently retain their weight at a relatively low level during periods of inclement weather, until conditions improve so that they can leave on mating flights.

Queens will make orientation mistakes and enter the wrong colony. When this happens the queen is often attacked by the workers and killed or repelled from the colony, however, sometimes the colony will accept a foreign queen. Queens on orientation flights or returning from mating flights may be attacked in mid-flight by workers from nearby hives. To avoid these problems in a mating yard, colonies should be supplied with orientation cues and should be well spaced.

Further research is required to determine if queen drift follows similar or different patterns to that of worker drift.

GENERAL SUMMARY

The purpose of this study was to develop methods that would help the Canadian beekeeping industry move towards greater self-sufficiency in the production and supply of queen honey bees.

Chapter III

Using a technique developed by Lai (1969) spring queens were reared in the laboratory using cages containing approximately 400 worker bees per cage.

In Trial 1 the worker bees used were taken from over-wintered colonies that had been brought outside but had not had the opportunity to take cleansing flights.

In Trial 2 the worker bees used were taken from over-wintered colonies at a later date after they had had ample opportunity to take cleansing flights and collect fresh pollen and water.

In each trial 31 cages were used and each cage received a total of three female larvae during each grafting. The first graft was performed on all cages and the second only on those cages that had not accepted any larvae from the first graft.

The worker bees in Trial 1 could not rear queens as well as those used in Trial 2. In the first graft of Trial 1 only 6 of 93 grafted larvae were accepted whereas in the first graft of Trial 2, 35 of 93 grafted larvae were accepted. Acceptance of larvae in the second graft was not significantly different between the two trials with

30.7% of the larvae being accepted in Trial 1 as compared to 36.4% in Trial 2. Mortality of accepted larvae was higher in Trial 1 than in Trial 2. In the first and second grafts of Trial 1, only 3.2% and 4% respectively of the accepted larvae survived to become adults, whereas in Trial 2, 36% from the first graft and 46% from the second survived to become adults.

Chapter IV

A total of 79 spring reared British Columbia queens were shipped to Manitoba in May, 1977. Twenty of these queens were kept by the author and 59 were distributed to five commercial beekeepers. Seven of the British Columbia queens were compared to 8 queens arriving with package bees from the United States. Sealed brood measurements were used to compare fecundity of the two types of queens and surplus honey weight was used to compare productivity of colonies headed by the different queens.

All of the commercial beekeepers who received B.C. queens reported that these queens performed at least as well as the other queens in their apiaries.

In the comparison of B.C. queens with the U.S. queens the colonies headed by the B.C. queens produced significantly more sealed brood at each reading. The B.C. colonies also produced significantly more surplus honey than the colonies headed by U.S. queens. This research indicates that high quality queen honey bees can be reared and mated in British Columbia early enough for spring introduction to colonies in Manitoba.

Chapter V

Using queenright cell building colonies, queen rearing was carried out successfully during the summer of 1976 and 1977. During one complete season (1977) of queen rearing the highest level of acceptance of grafted larvae was found during July.

In a test comparing the acceptance of grafted larvae based on queen cell cup type, acceptance of grafted larvae in plastic cell cups was found to be significantly greater than acceptance of grafted larvae in beeswax cell cups.

Numerous methods of introducing virgin queens to colonies were tested and the most successful method was to leave the queen caged in the colony for 24 hours and then upon release, spray her and the surrounding workers with a sugar syrup vanilla mix.

At this time, queen rearing during the summer appears to be the most dependable method by which to obtain mated queens independent of the U.S.

Chapter VI

To gain better understanding of the anatomical changes that take place in queens before and after mating, weight readings of some queens were taken daily from the time of emergence until several days after egg laying began. Typically, newly emerged queens were relatively large and would not fly; after a few days their weight usually dropped markedly and they became capable of flight; a few days before egg laying commenced they began gaining weight and gradually lost the ability to fly easily.

To determine how prevalent queen drift is under different conditions and to determine what happens to queens when they enter colonies other than their own, queens were released at various directions and distances from their home hive and their behaviour observed. Also, individual queens were given identifying marks to determine if they drifted during orientation or mating flights.

Under some test conditions queens were found to make orientation errors and tried to enter the wrong colony. This usually resulted in the queen being attacked or expelled by the workers of that colony. However, sometimes a foreign queen would be accepted by a colony. To avoid orientation problems in a mating yard, colonies should be supplied with orientation cues and should be well spaced. Further research is required to determine if queen drift follows similar or different patterns to that of worker drift.

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