

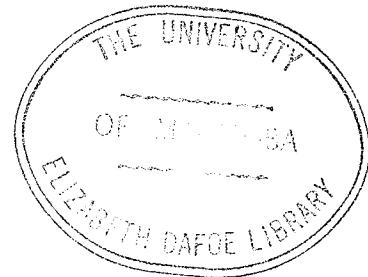
REARING QUEENS IN THE LABORATORY
WITH SMALL GROUPS OF WORKER HONEY BEES

A Thesis

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by

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ABSTRACT

Various factors which affect the rearing of queen honey bees in the laboratory were examined in an attempt to provide a technique which could be used for studying caste determination, behaviour, and the development of the various glands of nurse bees; the technique might also provide queens economically and at any time of the season for use by the commercial beekeeping industry.

Measurements, to determine the "caste" of the bees reared in the laboratory, were done during both the pupal and adult stage. The effect of the following factors on the number and "quality" of the queens accepted by the nurse bees and reared by them were examined : number of nurse bees, age of nurse bees, method of grafting, use of egg versus larval grafts, number of larvae per cage, type of cage, foods, temperature, and humidity.

It was found that groups of 50, 100, 200, and 400 nurse bees, 10 days old, could accept larvae and rear them to adult queens. Queens could be reared by 25 and 50 bees from larvae which had first been accepted by 200 or 400 bees. The effect of the other factors, and their significance in rearing queens, are discussed in the thesis.

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CHAPTER I
INTRODUCTION

Certain areas involving basic research with honey bees have been hampered because a simple efficient method for rearing queen bees in the laboratory, away from the complexities of the natural environment, has not been available. Such a technique would provide a means of rearing queen bees under controlled conditions for studying caste determination, behaviour, and the development of the various glands of the nurse bees.

Little or no data are available about the minimum number of nurse bees or the age of such bees that are required to rear adult queens in cages in the laboratory. The practicability of using common hive methods for "grafting" larvae (i.e. "direct" versus "indirect" method) and/or the use of honey bee eggs in queen rearing cups or larvae in wax comb, and the numbers of larvae, for rearing queens in the laboratory has not been investigated. Finally, little is known about the type of cage, food, arrangement of queen rearing cups, temperature, humidity, and general procedures required for success in rearing queens in the laboratory.

This thesis represents an attempt to study the various factors outlined above in order to rear queen honey bees in the laboratory. Apart from its use in fundamental research, such a technique might provide queens economically and at any time of the season for use by the commercial beekeeping industry. The use of such queens, however, is contingent on

a method of mating these queens under controlled conditions.

CHAPTER II
LITERATURE REVIEW

Both queen and worker honey bees are females and developed from fertilized eggs (Dzierzon, 1845). In the natural condition, the worker bees build queen cells from normal worker cells containing worker larvae to rear emergency queens when the original queen of the colony is old, injured, diseased, or the colony is so crowded that it divides. On the other hand, a large number of queens can be reared by transferring young female larvae from the worker cells into artificial queen cells (i.e. grafting) in colonies which are in a queenless condition (Laidlaw and Eckert, 1950). Worker-like, intercaste, and queen-like pupae and adult honeybees have been reared on both natural and artificial diets in incubators by many researchers (Rhein, 1933; Haydak, 1943; Weaver, 1955, 1958; Smith, 1959; Jay, 1964, 1965(a), 1965(b)). However, the rearing of honeybee queens by small groups of nurse bees in the laboratory has not been reported to date.

The hypopharyngeal glands (food glands) of nurse bees begin to fill when they are 4 days old (Haydak, 1957) and reach a peak of secretory activity when they are 6-11 days old (Wetzig, 1964); they degenerate when the bees are 15 days old (Haydak, 1957; Wetzig, 1964). Laidlaw and Eckert (1950) suggested that the hypopharyngeal glands of nurse bees are more active when the bees are between 5-16 days old; however, nurse bees 89 to 105 days old appear to be capable of rearing normal queens (Haydak, Petal and Dietz, 1964).

The differentiation of queen and worker honeybees begins on the first larval day; as the larval age at the time of transfer is increased, a higher percentage of workers and intermediate forms are obtained (Weaver, 1957; Rembold, 1967). Queens can be reared from 3 day old larvae taken from worker cells, but these queens are not perfect. Simpson (1961), for example, found that the younger the larvae are when transferred, the greater the number of ovarioles which they develop. Woyke (1967) showed that the adult queen bees, reared from eggs or from larvae 1, 2, 3, or 4 days old had an average ovariole count of 319, 305, 291, 374 and 233, and an average spermathecal volume of 1.23, 1.15, 1.0, 0.89 and 0.62 mm³ respectively; the average weights of the queens produced showed a decrease as the age of the larvae increased. Weaver (1957) reared normal queens from larvae that were 1 or 2 days old, anatomically poor queens from larvae that were 3 days old, and slightly queen-like adults from larvae that were 4 days old. Thus, very young larvae should be used for queen rearing experiments (see also Laidlaw & Eckert, 1950; Weaver, 1957; Vuillaume, 1957; Smith, 1959; Jay, 1964; Mitsui, et al, 1964; Rembold, 1967; Marcus, 1967).

A queenless condition is usually necessary for the nurse bees to accept grafted larvae although the presence of the queen does not always inhibit the building of queen cells (e.g. Vuillaume, 1957). High correlation coefficients between the volume of the queen cell and (a) the weight of the queen emerging from it, and (b) the number of her ovarioles, was found by Avetisyan (1967); Levicheva (1964) also showed a positive correlation between these factors, and suggested that large queen cells should

be used for rearing queens. Vuillaume (1956), found that worker bees preferred cells with rounded rather than flat bases, and cylindrical rather than hexagonal shaped cells. Inoue and Inoue (1963), noted that the acceptance of grafted larvae in plastic queen cups was higher than that in wax cups.

The temperature in the brood chamber, found by various authors, ranges from 32-35° C. It was suggested that the temperatures during the transferring of larvae from worker cells to queen cups should be kept at least in the range of 27-32° C., and a moderately high humidity should be maintained (Grout, 1966). The nurse bees are able to regulate the temperature and humidity of the hive.

For a complete literature review about the rearing of honeybee queens under both natural and artificial conditions, and the method of measuring pupal and adult bees see Weaver (1957), Smith (1959) and Marcus (1967).

CHAPTER III
GENERAL METHOD

A. Types of Cages

A plastic cage (Figure 1), 10 cm. x 8 cm. x 12 cm., was designed and built to confine and feed groups of nurse bees. Two of its sides were covered with removable metal screens (8 mesh). Two round holes, 2.5 cm. in diameter, were made in the top of the cage for either corks or water tubes. Two oval holes, 4 cm. x 2 cm., were drilled on the upper part of the back of the cage and special corks were made to fit into these holes. Two round holes, 2.5 cm. in diameter, were drilled directly beneath the oval holes and fitted with corks. This type of cage was used in most of the experiments and is defined as the "standard cage" in this thesis. The other types of cages were as follows:

1. Cage 2 : 7 cm. x 5 cm. x 10 cm. plastic cage
2. Cage 3 : 10 cm. x 7 cm. x 10 cm. plastic cage
3. Cage 4 : 10 cm. x 8 cm. x 11 cm. wooden cage

B. Food

A plastic dish, 3.5 cm. in diameter and 2.5 cm. in height was kept half filled with honey and placed on the floor of the cage. The honey was covered with a screen through which the bees could feed without drowning. Two or three strips of screen were fastened to the inside edge of the dishes to assist the nurse bees when landing.

Ground pollen and honey were mixed (1:1 by volume) into a paste and placed in dishes, similar to those described above, or in pieces of pollen comb, 3.5 cm. x 3.5 cm.; these were placed on the bottom of the cages. A removable semi-tubular container, filled with a ground pollen and honey mixture (1:4 by volume), was used in all cages but the "standard" ones. Sterilized glass tube feeders (Figure 1) were filled with tap water and placed on the top of the cages during all tests.

C. Nurse Bees

Large numbers of newly emerged yellow (Italian) worker bees were introduced into colonies of dark bees (Caucasian), or vice versa, in order to obtain bees of known ages at a later date. One day before a "grafting"* was done for an experiment, bees of a certain age (e.g. 4, 6, or 9 days old) were removed from the hives with tweezers and placed in groups of 25, 50, 100, 200, or 400 bees in cages (one person can collect and cage 2,000 bees in a 3 hour period). The caged bees were supplied with food and kept queenless in an incubator for 24 hours.

D. Grafting

Depending on the experiment 3, 4, or 5 plastic queen rearing cups, 1 cm. in diameter and 1 cm. in height, were attached with melted beeswax to a 6 cm. x 1.3 cm. strip of metal connected to a cork (Figure 2). Before an experiment was begun, a metal strip with queen rearing cups

* the transference of female larvae from worker comb to artificial queen rearing cups.

fastened to it, was installed inside the cages so that the bees could clean them up during the first day when the bees were caged. This appeared to assist in larval acceptance by the bees. Brood frames, containing newly hatched larvae (some were of a selected genetic line, depending on the experiment) were brought into the laboratory and placed in "grafting stands" (see Marcus 1967). Female larvae, less than 12 hours old, were removed from a brood comb with a grafting hook and transferred into queen rearing cups which had been cleaned by the caged bees and which contained a small drop of distilled water or a 1:1 mixture of royal jelly and distilled water. These larvae were placed directly into the cages containing the bees; in this thesis this method is defined as the "direct" grafting method and coded as "B" in the experiments in the thesis. The other grafting method, defined in this thesis as "indirect" grafting, was done as follows: Young larvae were grafted into frames containing queen rearing cups, were accepted by a queenless colony in the field, and then after 24 hours were transferred into cages containing the nurse bees. This "indirect" grafting method was coded as "A" in the experiments in this thesis.

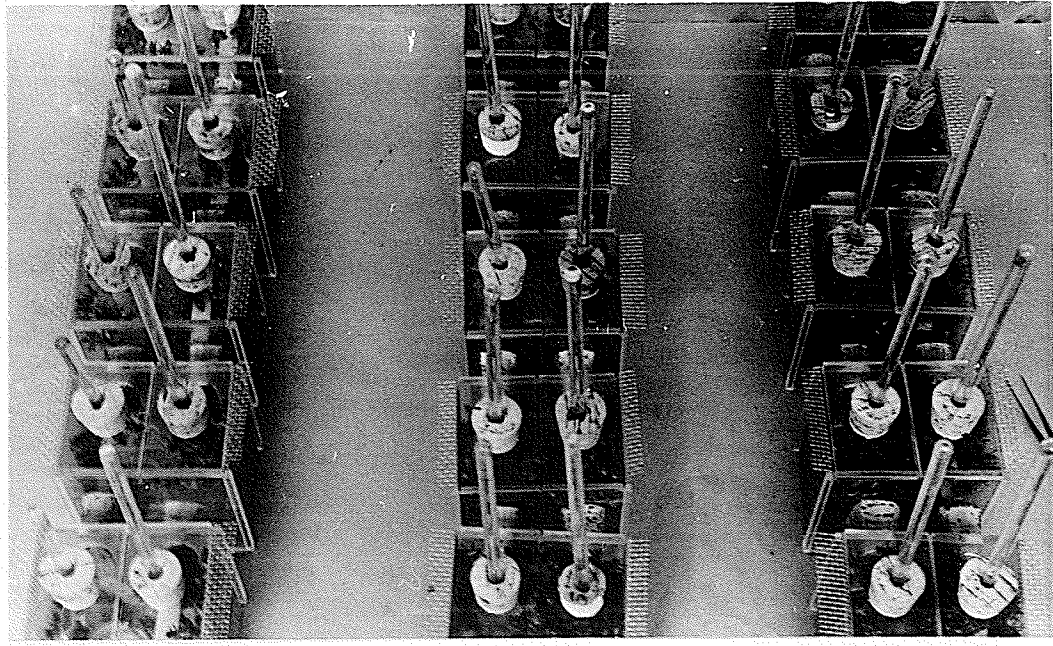
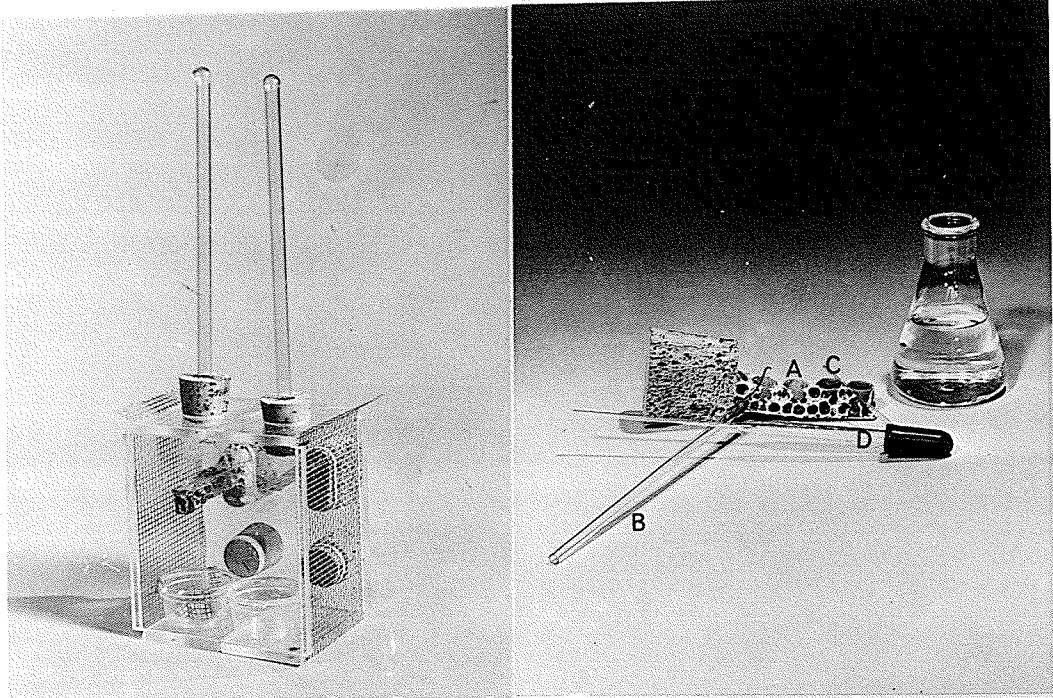
E. Incubator

The cages containing the nurse bees and grafted larvae were kept in an incubator at 30° C. or 35° C. with 40-60% relative humidity and good ventilation. The Bee Flight Room (Jay, 1964), had the same conditions as the incubator except that the temperature ranged from 30-35° C., and was therefore used in most of the experiments (Figure 3) because of its large size.

Figure 1
"Standard" plastic cage containing water tubes, feeders, and metal strip with queen rearing cups attached to it.

Figure 2
Equipment for rearing queens. A. Metal strip with queen rearing cups. B. Grafting hook. C. Glass rod for transferring eggs. D. Eye dropper and water for "priming" the queen cells.

Figure 3
Groups of "standard" cages containing queen cells and worker bees.



F. Procedures Used During the Rearing Period

Larval acceptance was checked 24 hours after the larvae were either grafted or transferred into the cages containing bees. Usually only the larvae with the largest amount of royal jelly inside the queen cups after acceptance were allowed to be reared to adults in each cage. The mortality of the nurse bees as well as that of the larvae were recorded each day. Abundant amounts of food was always supplied. The time of capping of the queen cells was also recorded. Five days after the cell was capped (i.e. 3 days after pupation), the pupa was ready to be measured (i.e. the pupa had dark pink purple compound eyes and ocelli, dark brown mandibles, light yellow abdomen, legs, and antennae, and a light brown head and thorax, as described by Jay, 1962. This matured pupa could not be injured easily. After a capped queen cell was carefully removed from a cage a "T"-shaped cut was made in its wall with a dissecting knife (see Figure 4). The pupa was taken out, measured (for details, see Chapter IV), and returned to its cell in the same position it had occupied formerly. The cells were placed in separate glass vials (Figure 5) and held in the incubator until emergence; the time of emergence was then recorded. The newly emerged queen was injected with F. A. A. solution with a fine medical syringe and then preserved in F. A. A. solution in a small bottle for further investigation and measurement.

The code numbers used in this thesis are usually written as : Experiment, Grafting method (Temperature)-Group (Treatment) [Age of nurse bees]- Replicate (Larva); e.g., the experiment number is written in Arabic numerals, "A" and "B" indicates "indirect" and "direct" grafting respec-

tively, the temperature is indicated in centigrade degrees, the treatment was shown when the groups of certain ages of nurse bees* were kept in different types of cages or used for different purposes. For example, 4A-200(b)(13)-3(b) is a bee number that was the "b" bee reared by replicate 3 of the 2nd treatment (cage) of 200 nurse bees, 13 days old, in the fourth experiment by the "indirect" grafting method.

* the term "5 + 10" means 5 and 10 day old bees in equal numbers.

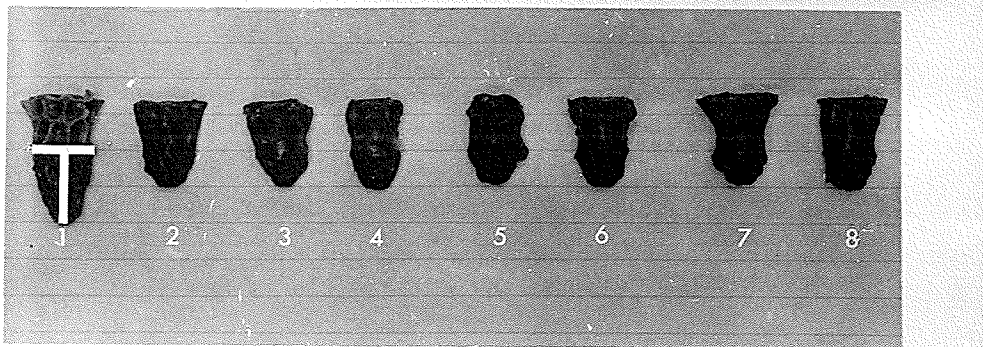


Figure 4 Cells containing developing queens reared by nurse bees in cages.
Cell #1 - hive control with "T"-shaped cut for removing pupa, cells #2-6 - reared by 100 bees, cells #7 and 8 - reared by 50 bees.



Figure 5 Emergence bottles used to confine queens and prevent them from fighting.

CHAPTER IV
MEASUREMENTS

The external and internal morphological characteristics of the adult bees obtained in this study were measured with a binocular microscope having an eye piece fitted with a linear microscale. The measurements used are listed below.

A. Pupal Measurements

1. Length of tongue:

This was measured from the base of the mandibles to the tip of the tongue. (Figure 6)

2. Length of pupa:

The sum of the length from the frons on the head to the small anterior indentation of the second true abdominal segment (a), and the length from the small anterior indentation of the second true abdominal segment to the tip of the abdomen (exclusive of the sting shaft). (Figure 6)

3. Weight of pupa:

This was measured to the nearest milligram.

B. Adult Measurements

1. The head: anterior view

a). Width: this was measured across the widest part of the head from one lateral edge (parietal area of the head capsule) to the other. (Figure 7)

b). Length: this was measured from the vertex of the head to the dis-

tal edge of the labrum. (Figure 7)

2. The basitarsus:

The first segment of the tarsus of the right hind leg was measured as shown in Figure 7. The inner surface of the basitarsus was also examined to ascertain if it was worker- or queen-like in structure (Snodgrass, 1956).

a). Length: this was measured 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 which was approximately one half to three fifths of the total length from the proximal end. (Figure 7)

3. The spermatheca:

The diameter of the spermatheca was measured at its widest part, after the trichea and the spermathecal gland had been removed.

4. The ovarioles:

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

C. Other Measurements

Other records kept included larval acceptance, the queen rearing ability of the nurse bees (see Table I - IV), the time of cell capping, the length of the cell, and the total developmental time.

1. Larval acceptance:

The number of larvae accepted out of the total number of larvae

grafted in each cage was recorded.

2. The queen rearing ability of the nurse bees:

The time of death, or the number of adults produced from the grafted larvae, was recorded to show the queen rearing ability of the nurse bees.

3. The time of cell capping:

This was measured from the time when the larvae were grafted directly into the cages for the "direct" grafting method, and from the time when the larvae were grafted into the queen colony for the "indirect" grafting method, to the time when the cell was capped, for both methods.

4. The length of the cell:

This was measured from the rim of the plastic cup to the tip of the wax cell, which the nurse bees had built.

5. The total developmental time:

This was measured from the time when the egg was laid and hatched (average figure used was 3 days) to the time of emergence of the adult; this total period was subdivided (see Tables X - XVI). The estimated total developmental time has an error of ± 6 hours.

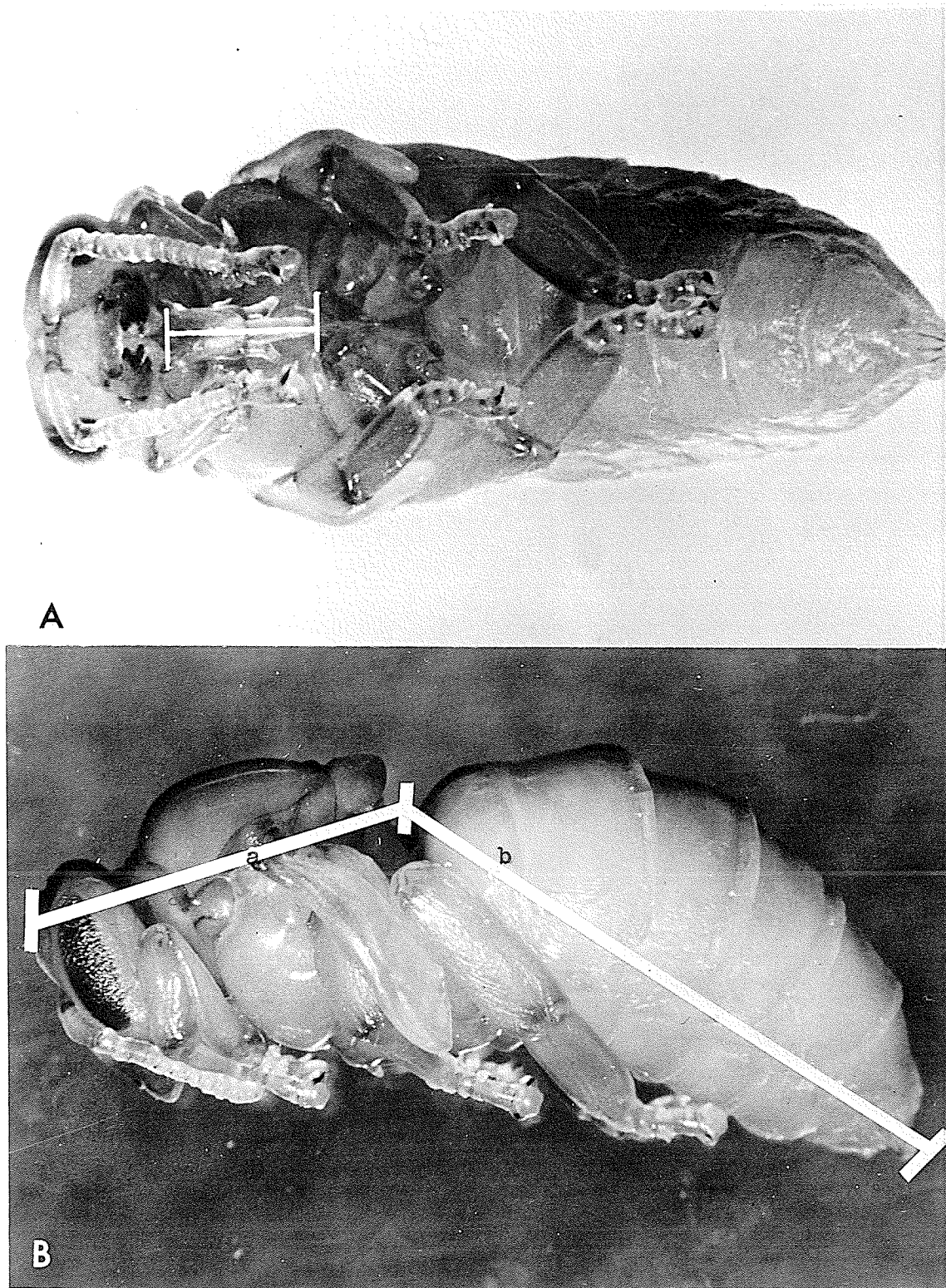
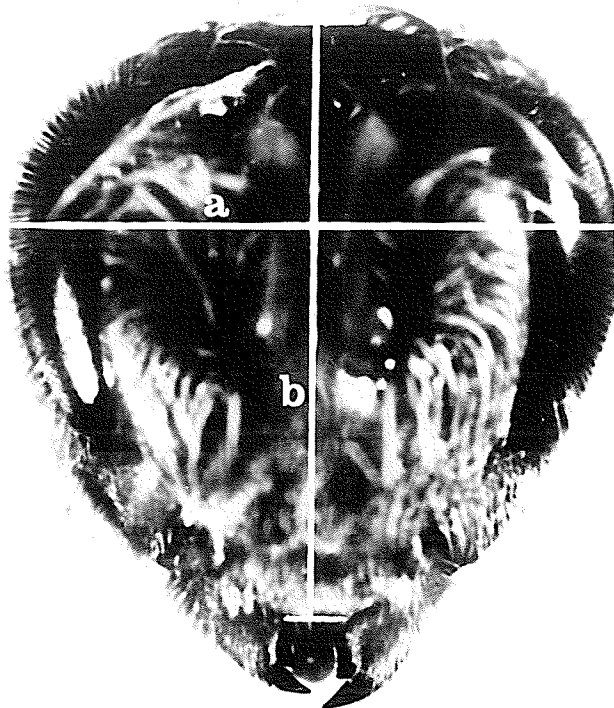
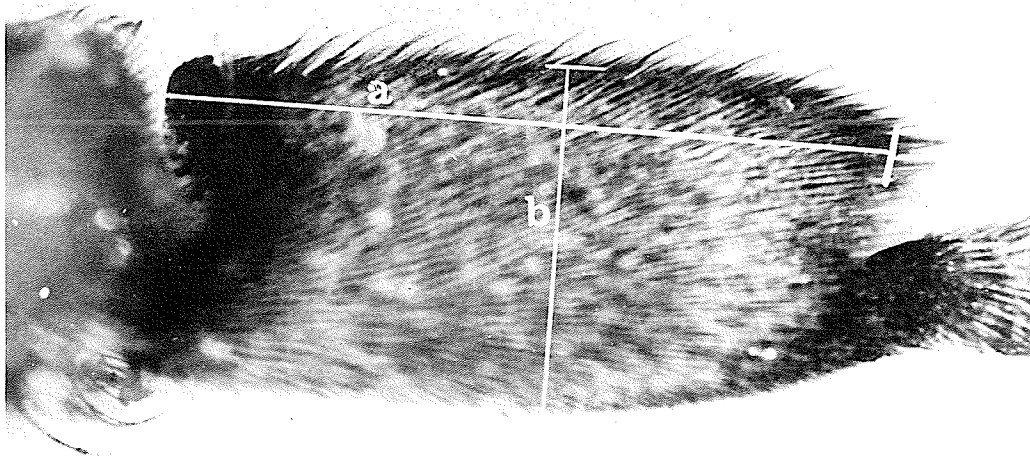


Figure 6
A. Length of tongue.
B. Total length of pupa (a+b).



1



2

Figure 7 Adult Measurements

1. Head

a. width

b. length

2. Basitarsus

a. length

b. width

CHAPTER V

LARVAL ACCEPTANCE BY SMALL GROUPS OF QUEENLESS WORKER BEES OF VARIOUS AGES KEPT IN CAGES

A. Larval Acceptance by Groups of Queenless Seven Day Old Bees (1967)

Four preliminary experiments were done in 1967, comparing larval acceptance by 50, 100, 200, and 400 bees in different types of cages at temperatures of 30° C. and 35° C. Larvae were grafted either directly or indirectly and their acceptance was checked one day after the larvae were either transferred from the colonies to the cages or were grafted directly into to cages, depending on the experiment. Each test had three replicates with 3 larvae grafted in each replicate (i.e. nine larvae were grafted in each test). The results are shown in Table I. No larvae were accepted in Experiments 3B and 4A; the reason(s) for this is unknown. There was no difference between the number of larvae accepted at the two temperatures in Experiment 2A. Because the hive temperature varies between 30-35° C. in the brood chambers of a honey bee colony and because of the results of the experiments indicated above, Experiments 3 and 4 were conducted in incubators at 35° C. In any given test no difference was found between the number of larvae accepted in the different types of cages. However the number of nurse bees does appear to affect larval acceptance. Although larvae, which had been grafted indirectly, appeared to be better accepted by the caged bees nevertheless sufficient numbers of larvae were accepted by the nurse bees when grafted directly to make the method feasible. In addition direct grafting requires less equipment,

TABLE I

LARVAL ACCEPTANCE BY GROUPS OF QUEENLESS
SEVEN DAY OLD BEES IN CAGES (1967)

Experiment	Temperature (°C.)	No. of bees per cage	No. of larvae accepted ^x			Total acceptance	Notes	
			Replicates					
			1	2	3			
1A ^z	30°	100	1	2	-	3	Cage ^y 2	
		200	1	2	-	3		
2A	30°	50	1	1	1	3	Cage 2	
		100	1	2	2	5		
	35°	50	2	1	0	3	Cage 2	
		100	1	3	2	6		
3 A	35°	50	3	2	2	7	Cage 2	
		100	2	3	3	8		
		200(a)	3	3	3	9		
		200(b)	3	3	3	9		Cage 3
		400	3	3	3	9		
	B	35°	50	0	0	0	0	Cage 2
100			0	0	0	0		
200(a)			0	0	0	0		
200(b)			0	0	0	0	Cage 3	
400			0	0	0	0		
4 A	35°	50	0	0	0	0	Cage 2	
		100	0	0	0	0		
		200(a)	0	0	0	0		
		200(b)	0	0	0	0		Cage 3
		400(a)	0	0	0	0		
		200(c)	0	0	0	0		Cage 4
		400(b)	0	0	0	0		
	B	35°	50	0	0	0	0	Cage 2
			100	1	1	0	2	
			200(a)	2	0	1	3	
200(b)			1	2	1	4	Cage 3	
400(a)			1	2	2	5		
200(c)	1	1	1	3	Cage 4			
400(b)	2	1	0	3				

x 3 larvae grafted / cage x 3 replicates (i.e. 9 larvae)

y Cage 1 - "standard" cage (1968); Cage 2 - small plastic cage;

Cage 3 - large plastic cage; Cage 4 - large wooden cage (see Chapter III).

z A - Indirect grafting; B - Direct grafting.

is simpler, and is less time consuming.

B. Larval Acceptance by Groups of Queenless Bees of Various Ages (1967)

A second series of preliminary experiments were done in 1967 as follows: Experiments, in which no larvae were accepted (i.e. 3B and 4A) or which had low numbers of larvae accepted (i.e. 4B) were regrafted several times. Thus by the time the nurse bees accepted the grafted larvae, they varied in age in the different cages. Three tests using nurse bees of mixed ages were done in Experiments 1A, 4A and 4B. The results are shown in Table II. The number of grafts and the age of the nurse bees when larvae were first accepted in each test are shown in Table II.

As stated earlier the reason(s) that the nurse bees did not accept the grafted larvae in Experiments 3B and 4B was unknown. Apparently it is possible for groups of nurse bees of mixed ages to accept grafted larvae on the first graft. However nurse bees apparently are able to accept grafted larvae, when they are as old as 18 days, if during this time they have been in a queenless condition.

C. Larval Acceptance by Groups of Queenless Bees Which are Five and/or Ten Days Old (1968)

Laidlaw (1950) suggested that nurse bees should be between 5-15 days old if they are to rear high quality queens. It is well-known that the major food producing glands of nurse bees (the hypopharyngeal glands) become less active, to a varying extent, when the nurse bees are 15 days or older (Wetzig, 1964). Therefore in 1968 several experiments were done

TABLE II

LARVAL ACCEPTANCE BY GROUPS OF QUEENLESS
BEES OF VARIOUS AGES IN CAGES (1967)^a

Experiment		No. of bees per cage	Age (days) of bees when larvae grafted	No. of larvae accepted ^b			Total acceptance	Notes		
				Replicates				Types of cages ^c	Grafts	
				1	2	3				
1	A ^e	200(b)	mixed	1	-	-	1	Cage 2	1st-G ^d	
3	B	50	12	0	0	1	1	Cage 2	3rd-G	
		100	12	2	2	1	5	"	"	
		200(a)	12	2	1	0	3	"	"	
		200(b)	12	0	0	0	0	Cage 3	3rd-G	
		400	12	1	1	0	2	"	"	
4	A	50	18	2	1	1	4	Cage 2	6th-G	
		100	13	2	2	2	6	"	5th-G	
		200(a)	13	-	-	2	2	"	"	
			18	2	1	-	3	"	6th-G	
		200(b)	13	-	-	2	2	Cage 3	5th-G	
			18	2	2	-	4	"	6th-G	
		400(a)	13	-	1	-	1	"	5th-G	
			18	0	-	1	1	"	6th-G	
		200(c)	13	1	-	2	3	Cage 4	5th-G	
			18	-	1	-	1	"	6th-G	
		400(b)	13	-	2	-	2	"	5th-G	
			18	2	-	1	3	"	6th-G	
		200(d)	mixed	1	-	-	1	Cage 2	1st-G	
		B	50	13	1	1	-	2	Cage 2	2nd-G
				18	-	-	1	1	"	3rd-G
100	18			1	1	-	2	"	2nd-G	
200(a)	13			-	1	-	1	"	2nd-G	
200(b)	13			1	-	-	1	Cage 3	2nd-G	
400(a)	18			0	0	2	2	"	"	
200(c)	13			1	1	1	3	Cage 4	2nd-G	
400(b)	13			-	-	1	1	"	"	
	14			1	-	-	1	"	"	
200(d)	mixed			1	-	-	1	Cage 2	1st-G	

a All cages were kept at 35° C. except 1A.

b 3 larvae grafted / cage x 3 replicates (i.e. 9 larvae)

c Cage 2 - small plastic cage; Cage 3 - large plastic cage;
Cage 4 - large wooden cage.

d G - Graft.

e A - Indirect grafting; B - Direct grafting.

in which the acceptance of larvae and the rearing ability of 5 and 10 day old nurse bees were compared.

Nurse bees, in 3 age groups, were used. These bees, 5 days old, 5+10 days old in equal numbers, and 10 days old were collected in groups of 50, 100, 200, and 400 respectively in each experiment (see Table III). Each test consisted of five replicates; three experiments were done using the "standard" cages in all tests. Five larvae were grafted directly on a drop of distilled water placed inside the plastic queen cup. The caged bees were kept in the Bee Flight Room at 30-35^o C. and 40-60% relative humidity with good ventilation. The results are shown in Table III.

Within any group of tests using the same number of bees in each cage but with nurse bees of different ages (i.e. 5, 5+10, or 10 days old), there was little difference between the numbers of larvae accepted by the nurse bees of the various age groups. Two exceptions did occur in Experiment 7B using 50 or 100 nurse bees; however this was probably because low numbers of bees were used, many of which were very young (i.e. they were not of nursing age). Within any age category of nurse bees acceptance usually increased with increase in the number of nurse bees. However, low numbers of larvae were usually accepted in all tests where 50 nurse bees, 5 or 5+10 days old, were used or where 100 nurse bees, 5 days old, were used. When large numbers of bees, 5 days old or younger, were used as nurse bees or when large numbers of them were used in combination with 10 day old ones acceptance was improved (see Experiment 7B). In all tests where 10 day old bees were used alone as nurse bees or in combination with 5 day old bees acceptance was high - especially when 200 or 400 of them

TABLE III

LARVAL ACCEPTANCE BY GROUPS OF QUEENLESS
FIVE AND/OR TEN DAY OLD BEES IN CAGES (1968)^v

Experiment	No. of bees per cage ^w	Age(days) of bees when lar- vae grafted	No. of larvae accepted ^y					Total acceptance
			Replicates					
			1	2	3	4	5	
5 B	50	5	1	1	1	1	1	5
		5+10 ^x	1	1	1	2	1	6
		10	1	1	2	4	2	10
	100	5	2	1	2	2	5	12
		5+10	2	3	3	2	3	13
		10	5	3	3	3	4	18
	200	5	3	3	4	4	3	17
		5+10	5	5	4	4	5	23
		10	5	4	5	5	3	22
	400	5	4	3	5	4	5	21
		5+10	5	5	4	5	5	24
		10	4	4	3	3	5	19
6 B	50	5	1	2	3	1	1	8
		5+10	3	0	2	3	3	11
		10	2	3	2	2	3	12
	100	5	3	1	3	1	4	12
		5+10	4	3	3	3	1	14
		10	5	4	3	4	2	18
	200	5	4	3	3	3	5	18
		5+10	5	5	5	5	5	25
		10	5	2	5	5	5	22
	400	5	5	3	4	3	4	19
		5+10	5	5	4	4	5	23
		10	5	5	5	5	3	23
7 B	50	≤5 ^z	0	1	0	0	1	2
		≤5+10	2	0	2	2	3	9
		10	2	1	1	3	2	9
	100	≤5	4	1	2	3	0	10
		≤5+10	4	4	2	5	5	20
		10	5	3	5	2	2	17
	200	≤5	3	3	4	3	4	17
		≤5+10	4	4	5	4	5	22
		10	5	4	5	3	5	22
	400	≤5	4	5	3	4	4	20
		≤5+10	4	3	5	5	4	21
		10	4	4	5	4	5	22

^v Temperature:- 30°-35° C. ^w "standard" cage

^x Equal numbers of 5 and 10 day old bees.

^y 5 larvae grafted / cage x 5 replicates (i.e. 25 larvae)

^z Bees were less than 5 or 5 days old.

were used. It was also observed that the youngest nurse bees in most tests, and the lowest numbers of nurse bees in most tests, had the least amount of royal jelly in the queen cups which contained larvae.

D. Larval Acceptance by Groups of Queenless Ten Day Old Bees (1968)

To ascertain the relative ability of various numbers of 10 day old nurse bees to accept different numbers of grafted larvae the following experiments were done.

Two experiments were done in which 10 day old nurse bees were caged in groups of 200 and 400 bees (see Table IV). The groups of nurse bees which received 4 larval grafts were replicated 5 times and the nurse bees receiving 8 grafts were replicated 7 times in order to supply extra accepted larvae for additional experiments (see Chapter VI).

The results are shown in Table IV. The two experiments were combined for statistical analysis. When 4 larvae were grafted there was no significant difference between the numbers accepted by the groups of 200 and 400 nurse bees but more larvae were accepted by the 400 nurse bees than the 200 ones when 8 larvae were grafted ($P < 0.01$). More larvae were accepted by the groups of 200 and 400 bees when 4 larvae were grafted than when 8 larvae were grafted ($P < 0.01$ in both cases).

TABLE IV

LARVAL ACCEPTANCE BY GROUPS OF QUEENLESS
TEN DAY OLD BEES IN CAGES (1968) ^a

Experiment	No. of bees per cage ^b	No. of larvae grafted per cage	No. of larvae accepted							Total acceptance
			Replicates							
			1	2	3	4	5	6	7	
8 B ^c	200(a)	4	3	4	4	4	3	-	-	18
	400(a)	4	3	4	3	3	2	-	-	15
	200(b)	8	4	4	4	5	5	4	3	29
	400(b)	8	7	7	6	7	3	8	6	44
9 B	200(a)	4	4	3	3	3	3	-	-	16
	400(a)	4	4	4	1	4	4	-	-	17
	200(b)	8	4	3	4	4	5	5	6	31
	400(b)	8	6	7	5	4	5	5	6	38

a Temperature: 30°-35° C.

b "standard" cage.

c Direct grafting.

CHAPTER VI

QUEEN REARING ABILITY OF NURSE BEES

The ability of different numbers of nurse bees of various ages to rear accepted larvae to adults was tested. The time of death of any of the larvae or pupae was recorded as well as the number of adults produced. The mean length of the wax cell (from the outer edge of the plastic cup to the tip of the wax cell), the time from the grafting of the larvae to the capping of the cells, and the total developmental time were also recorded. The experiments outlined in this chapter are a continuation of the acceptance experiments of the previous chapter.

A. The Queen Rearing Ability of Groups of Queenless Seven Day Old Bees Kept in Cages (1967)

Either all of the accepted larvae in each test were allowed to be nursed or all but one were removed (see Figure 8 and Table V). The results are shown in Table V and VI. Table V shows the rearing ability of the seven day old bees which were allowed to nurse all of the larvae which they accepted. Little can be said about Experiments 1A and 2A because of the high mortality which occurred. Table VI shows the rearing ability of the bees when all but one accepted larvae were removed from each cage (except in 4B-400(a)-3 where two larvae were left to be reared). By removing all but one of the accepted larvae and/or by using higher numbers of nurse bees in any given cage, it appears that the chances of obtaining adults are slightly increased.

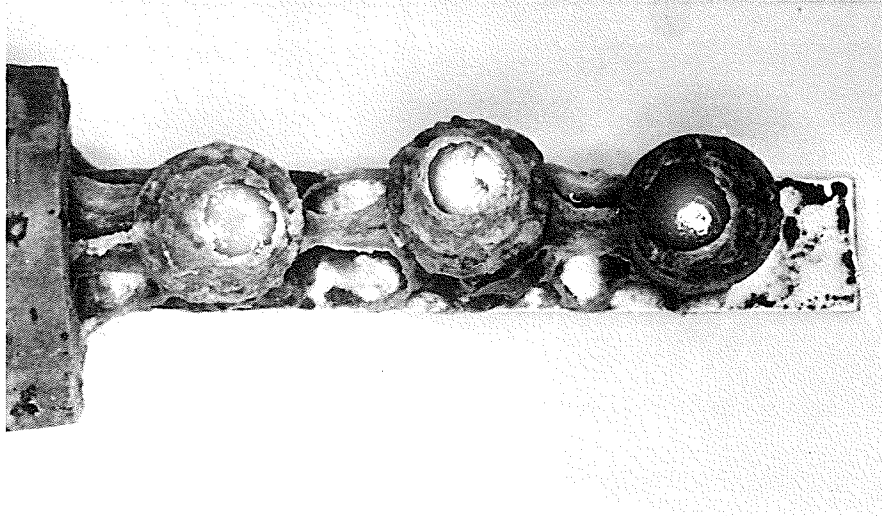


Figure 8 Underside view of a queen cell containing a queen larva (see central cell).

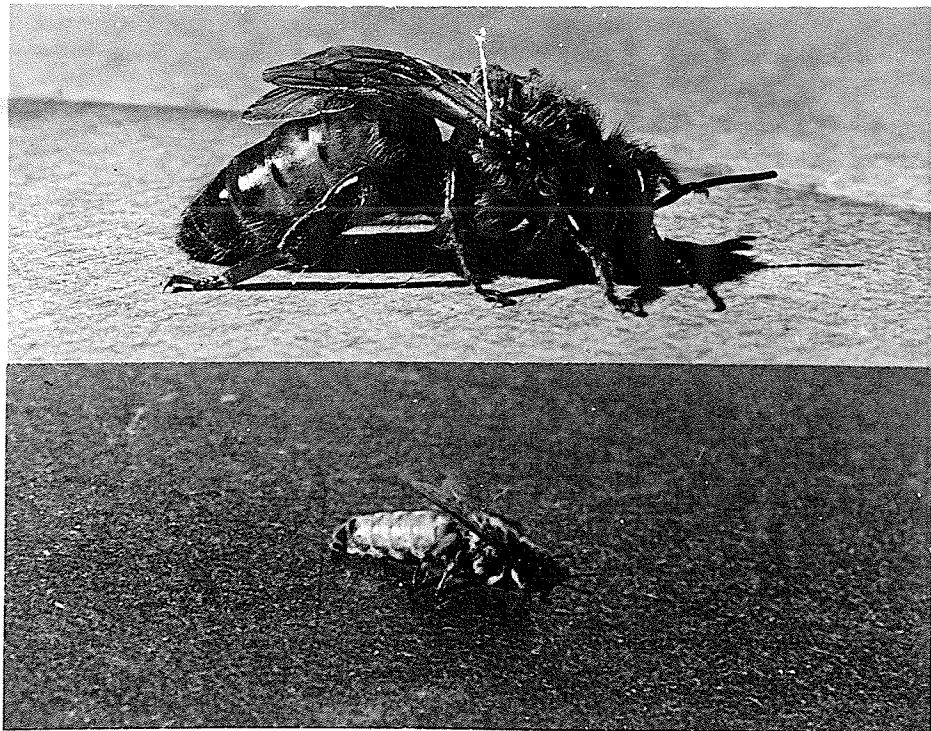


Figure 9 Virgin queens reared by 200 nurse bees in cages.

TABLE V

THE QUEEN REARING ABILITY OF GROUPS OF QUEENLESS SEVEN DAY OLD BEES KEPT IN CAGES (1967)

Experi- ment	Tempera- ture (°C.)	# of N. B. per cage	Re- pli- cate #	# L. ^e nursed	Time of death				Emer- gence Adult reared	Mean length (mm.) of cell	Mean time (days) from G. to C. ^b	Total develop- mental time (days) ^c
					unsealed # L.	Sealed ^a						
					# Late L. &/or Prep.	# Late P.	# A.					
1A	30°	100	1	1	1	-	-	-	-	-	-	
			2	2	1	-	-	-	1	11	5	17
	200	1	1	1	-	-	-	-	-	-	-	
		2	2	2	-	-	-	-	-	-	-	
2A	30°	50	1	1	1	-	-	-	-	-	-	
			2	1	1	-	-	-	-	-	-	
			3	1	1	-	-	-	-	-	-	
	100	1	1	1	-	-	-	-	-	-	-	
		2	2	1	1	-	-	-	8	4	-	
		3	2	-	-	-	-	2	6.5	6	20	
	35°	50	1	2	-	2	-	-	-	8	3	-
			2	1	-	1	-	-	-	7	4	-
		100	1	1	1	-	-	-	-	-	-	-
			2	3	-	2	-	1	-	8.3	4.3	-
			3	2	-	1	1	-	7.5	4	-	

- a # Late L. &/or Prep. - Bees died at late larval or prepupal stage.
 # Late P. - Bees died at late pupal stage. # A. - Bees died after last molt.
 b Time from grafting of larvae to capping of cell.
 c $\pm 1/4$ day (± 6 hrs.) from egg stage to emergence.
 d Nurse bees.
 e L. - Larva(e)

TABLE VI

THE QUEEN REARING ABILITY OF GROUPS OF QUEENLESS SEVEN DAY OLD BEES KEPT IN CAGES AT 35° C. (1967)

Experiment	# of N.B. ^a per cage	Re- pli- cate #	Accep- tance ^b	Time of death				Emer- gence Adult reared	Mean length (mm) of cell	Mean time (days) from G. to C. ^g	Total develop- mental time (days) ^f
				Unsealed # L. ^c	Sealed ^d						
					# Late L. &/or Prep.	# Late P.	# A.				
3 A. g	50	1	3	-	1	-	-	-	8	4	-
		2	2	-	-	1	-	-	8	4	-
		3	2	-	1	-	-	-	9	4	-
100	100	1	2	1	-	-	-	-	-	-	-
		2	3	-	-	-	-	1	11	4	17
		3	3	-	-	1	-	-	11	4	-
200(a)	200(a)	1	3	-	-	1	-	-	10	4	-
		2	3	1	-	-	-	-	-	-	-
		3	3	-	-	-	-	1	12	4	17
200(b)	200(b)	1	3	1	-	-	-	-	-	-	-
		2	3	1	-	-	-	-	-	-	-
		3	3	1	-	-	-	-	-	-	-
400	400	1	3	1	-	-	-	-	-	-	-
		2	3	1	-	-	-	-	-	-	-
		3	3	1	-	-	-	-	-	-	-

a Nurse bees.

b All but one of the accepted larvae were removed from each replicate.

c Larva(e)

d # Late L. &/or Prep. - Bees died at late larval or prepupal stage.

Late P. - Bees died at late pupal stage. # A. - Bees died after last molt.

e Time from grafting of larvae to capping of cell.

f $\pm 1/4$ day (± 6 hrs.) from egg stage to emergence.

g - Indirect grafting.

Continued ...

TABLE VI (Continued)

Experiment	# of N.B. ^a per cage	Re- pli- cate #	Accep- tance ^b	Time of death				Emer- gence Adult reared	Mean length (mm.) of cell	Mean time (days) from G. to C.	Total develop- mental time (days) ^f
				Unsealed # L. ^c	Sealed ^d						
					# Late L. &/or Prep.	# Late P.	# A.				
4 B ^g	100	1	1	-	1	-	-	-	5	4	-
		2	1	-	1	-	-	-	8	5	-
200(a)		1	2	1	-	-	-	-	-	-	-
		3	1	-	-	-	-	1	8	6	19
200(b)		1	1	1	-	-	-	-	-	-	-
		2	2	-	-	-	-	1	8	5	17
		3	1	1	-	-	-	-	-	-	-
400(a)		1	1	-	-	1	-	-	11	4	-
		2	2	-	-	-	-	1	8	4	-
		3	2	-	-	-	-	2	9	4	16
200(c)		1	1	1	-	-	-	-	-	-	-
		2	1	1	-	-	-	-	-	-	-
		3	1	-	-	-	-	1	9	5	16
400(b)		1	2	-	1	-	-	-	13	4	-
		2	1	-	-	-	-	1	15	6	18

a Nurse bees.

b All but one of the accepted larvae were removed from each replicate except 4B-400(a)-3 where two larvae were left.

c Larva(e)

d # Late L. &/or Prep. - Bees died at the late larval or prepupal stage.

Late P. - Bees died at late pupal stage. # A. - Bees died after last molt.

e Time from grafting of larvae to capping of cell.

f $\pm 1/4$ day (± 6 hrs.) from egg stage to emergence.

g Indirect grafting.

B. The Queen Rearing Ability of Groups of Queenless Bees of Various Ages in Cages (1967)

Different numbers of nurse bees (50, 100, 200, and 400) of different ages were put into cages. Only one accepted larva per cage was allowed to be nursed except in test 4A-200(b)-3 where two larvae were left. The grafted larvae were either second graft, third graft, fifth graft, or sixth graft in the tests. Only those in the tests of mixed ages in 1A, 4A, and 4B were first grafts (see Table II). The results are shown in Table VII.

A few more adults were reared by the larger numbers of bees than by the smaller ones. Caged bees were able to rear queen larvae successfully until they were 22 days old (e.g. see test 4A-200(a)-1 and 2). The mixed age groups were able to rear queen larvae. The results show that the small groups, (i.e. 50 bees), do not rear grafted larvae as well as do the larger numbers. On the whole the sealed wax cells capped by 50 bees were shorter (e.g. 4B-50's) than when capped by the groups of 200 or 400 bees. Little can be said about the other data because of the low numbers of adults reared.

C. The Queen Rearing Ability of Five and/or Ten Day Old Queenless Bees in Cages (1968)

The acceptance of grafted larvae by the nurse bees was higher in 1968 due to the experience of the author. The queen rearing ability of different sized groups of bees of different ages was therefore inves-

tigated. Cages with 50, 100, 200, and 400 bees 5, 5+10, and 10 days old were tested (see Table VIII). Each test had five replicates with 5 larvae grafted per replicate. After acceptance all but one of the accepted larvae were removed from each replicate. Those which had no larvae accepted (e.g. 7B-50{5+10}-2 and 7B-100{≤5}-5) were supplied with accepted larvae from the other replicates of the same group size and age within the same experiment. Nevertheless, the total acceptance of test 7B-50{≤5}'s was lower than 5 and therefore only two larvae were nursed. Thus, all tests but 7B-50{≤5} had five accepted larvae to be nursed. Three experiments were conducted in the standard cages kept in the Bee Flight Room at 30-35° C. and 40-60% relative humidity. The results are shown in Table VIII.

The results indicate that groups of 50 and 100 bees, 5 days old, and ≤5 days old, and groups of 50 bees, 5+10 days old, do not generally rear larvae successfully, although 3 emerged or unemerged adults were reared in tests 5B-50{5}, 5B-50{5+10} and 6B-50{5+10}. The larger groups of the same age usually have a greater chance of rearing accepted larvae successfully. The rearing ability of the nurse bees of mixed ages (i.e. 5+10) probably depends mostly on the presence of 10-day-old bees in the test. Generally speaking, it appears that the larger groups of nurse bees produce larger queen cells than those reared by the smaller groups, and the developmental time of the queens reared in these is shorter than that of the queens reared by the smaller groups.

TABLE VII

THE QUEEN REARING ABILITY OF GROUPS OF QUEENLESS BEES OF VARIOUS AGES IN CAGES ^a (1967)

Experi- ment	# of N.B. ^b per cage	Re- pli- cate #	Accep- tance	Age of N. B. when larvae grafted	Time of death				Emer- gence Adult reared	Mean length (mm.) of cell	Mean time (days) from G. to C. ^f	Total develop- mental time (days) ^g
					Unsealed		Sealed ^e					
					# L. ^d	# Late &/or Prep.	# Late P.	# A.				
1A ^h	200(b)	1	1	mixed	-	-	-	-	1	13	5	18
3B	50	3	1	12	1	-	-	-	-	-	-	-
	100	1	2	12	-	-	1	-	-	5	5	-
		2	2	12	-	-	-	-	1	6	5	18
		3	1	12	1	-	-	-	-	-	-	-
	200(a)	1	2	12	-	-	-	-	1	9	5	16
		2	1	12	-	-	-	-	1	8	5	17
	400	1	1	12	-	-	-	-	1	13	5	15
2		1	12	1	-	-	-	-	-	-	-	
4A	50	1	2	18	-	1	-	-	-	6	4	-
		2	1	18	-	-	-	1	-	6	4	-
		3	1	18	1	-	-	-	-	-	-	-
	100	1	2	13	-	-	-	1	-	6	4	-
		2	2	13	-	-	-	-	1	6	4	-
		3	2	13	-	1	-	-	-	6	4	-
	200(a)	1	2	18	-	-	-	-	1	8	4	17
		2	1	18	-	-	-	-	1	7	4	15
		3	2	13	-	1	-	-	-	8	4	-
	200(b)	1	2	18	-	-	-	1	-	7	4	-
		2	2	18	-	-	-	-	1	7	4	-
3		2	13	-	-	-	1	1	7.5	4	17	

Continued ...

a Temperature - 35° C.; only 1 accepted larva was allowed to be reared except 4A-200(b)-3 where 2 larvae were left.

b Nurse bees.

d- Larva(e)

e # Late L. - Bees died at late larval or prepupal stage.

Late P. - Bees died at late pupal stage. # A. - Bees died after last molt.

f Time from grafting of larvae to capping of cell.

g $\pm 1/4$ day (± 6 hrs.) from egg stage to emergence.

h A - Indirect grafting. B - Direct grafting.

TABLE VII (Continued)

Experi- ment	# of N.B. b per cage	Re- pli- cate #	Accep- tance	Age of N. B. when larvae grafted	Time of death				Emer- gence Adult reared	Mean length (mm.) of cell	Mean time (days) from G. to C. f.	Total develop- mental time (days) g
					Unsealed		Sealed e					
					# L. d	# Late L. &/or Prep.	# Late P.	# A.				
4A h	400(a)	2	1	18	-	1	-	-	-	7	4	-
		3	1	13	-	-	-	1	-	8	4	-
	200(c)	1	1	13	-	-	-	-	1	5	4	19
		2	1	18	-	1	-	-	-	5	4	-
		3	2	13	-	-	-	1	-	7	4	-
	400(b)	1	2	18	-	-	-	-	1	6	4	-
		2	2	13	-	1	-	-	-	6	4	-
		3	1	18	-	1	-	-	-	6	4	-
	200(d)	1	1	mixed	-	-	-	-	1	11	6	14
	4B	50	1	1	13	-	1	-	-	-	2	5
2			1	13	-	1	-	-	-	2	5	-
3			1	18	-	1	-	-	-	1	6	-
100		1	1	18	-	1	-	-	-	5	6	-
		2	1	18	-	1	-	-	-	5	6	-
200(a)		2	1	13	-	1	-	-	-	8	5	-
200(b)		1	1	13	-	1	-	-	-	8	5	-
400(a)		3	2	18	-	1	-	-	-	11	6	-
200(c)		1	1	13	-	-	-	-	1	8	6	-
		2	1	13	1	-	-	-	-	-	-	-
		3	1	13	1	-	-	-	-	-	-	-
400(b)		1	1	14	-	-	-	-	1	10	5	16
		3	1	13	-	-	-	-	1	12	6	16
200(d)	1	1	mixed	-	-	-	-	1	13	5	16	

TABLE VIII

THE QUEEN REARING ABILITY OF GROUPS OF 5 AND/OR 10 DAY OLD QUEENLESS BEES IN CAGES ^a (1968)

Experiment	# of N.B. ^b per cage	Age of N.B. when larvae grafted	Time of death				Emer- gence Adult reared	Mean length of cell (mm.)	Mean time from G. ^e to C. ^e (days)	Mean developmental Time (days) ^f	
			Unsealed # L. ^d	Sealed ^c		#					
			# Late L. &/or P.	L. Prep.	Late P.	A.					
5 B ^g	50	5	4	-	-	1	-	8	5	-	
		5+10	4	-	-	-	1	7	5	18.5	
		10	4	-	-	1	-	7	4	-	
	100	5	2	-	-	2	-	1	10	6	18.5
		5+10	-	1	-	-	2	2	8.4	4.6	16.5
		10	-	-	-	1	-	4	8.4	5	17.75
	200	5	-	1	-	-	1	3	11.4	5.4	17.8
		5+10	-	-	-	-	2	-	12	5.8	17.2
		10	-	-	-	-	-	5	13	4.6	16.1
	400	5	-	-	-	-	1	4	13.4	4.4	17.5
		5+10	-	-	-	-	2	1	14.8	5	16.5
		10	-	1	-	-	-	4	15	4.8	16.5
6 B	50	5	5	-	-	-	-	-	-	-	
		5+10	4	-	-	1	-	6	5	-	
		10	1	1	2	-	1	5.75	4.5	17.5	
	100	5	4	-	-	1	-	-	7	4	-
		5+10	2	-	-	2	-	1	7.3	4.67	17.5
		10	1	1	2	-	1	7.75	4.25	16.5	
	200	5	2	-	-	2	-	1	10.67	5.3	16.5
		5+10	2	-	-	-	1	2	10	4.67	16.5
		10	-	1	-	-	2	2	10.6	3.8	16
	400	5	4	-	-	-	-	1	14	5	16.5
		5+10	-	-	-	1	-	4	13.4	4.6	16.5
		10	-	-	-	1	1	3	16.2	4.4	16.8

Continued ...

TABLE VIII^a (Continued)

Experiment	# of N.B. ^b per cage	Age of N. B. when larvae grafted	Time of death				Emergence of Adult reared	Mean length (mm.) of cell	Mean time (days) from G. to C. ^e	Mean developmental time (days) ^f	
			Unsealed # L. ^d	Sealed ^c							
			# L. ^d	# Late L. &/or Prep.	# Late P.	# A.					
7 B ^g	50	≤ 5 ^h	2	-	-	-	-	-	-		
		≤ 5+10	5	-	-	-	-	-	-		
		10	4	-	-	-	1	9	7	18.5	
	100	≤ 5	5	-	-	-	-	-	-	-	
		≤ 5+10	1	-	-	2	-	2	9.25	5.5	19
		10	-	-	-	1	-	4	9.8	5	16.75
	200	≤ 5	1	-	-	2	-	2	11.75	6.25	17
		≤ 5+10	-	-	-	1	2	2	10.6	4.8	15.5
		10	-	-	-	-	-	5	10	4.8	15.9
400	≤ 5	-	-	2	3	-	-	12.6	5.4	-	
	≤ 5+10	-	-	1	-	1	3	13.2	4.8	15.8	
	10	-	-	-	-	-	5	12	4	15.5	

a Temperature: 30-35° C.; 5 accepted larvae nursed in 5 cages in each test, except 7B-50(5), where only 2 accepted larvae were nursed.

b Nurse bees.

d- Larva(e)

c # Late L. &/or Prep. - Bees died at late larval or prepupal stage.

Late P. - Bees died at late pupal stage.

A. - Bees died after last molt.

e Time from grafting of larvae to capping of cell.

f ±1/4 day (±6hrs.) from egg stage to emergence.

g B - Direct grafting.

h Bees were less than 5 or 5 days old.

D. The Queen Rearing Ability of Groups of Ten Day Old Queenless Bees with High (8) and Low (1) Numbers of Larvae in Cages (1968)

Because the 10 day old bees were considered to be the best age for rearing queen bees in cages (see Chapter V), groups of 200 and 400 bees were used to test this ability in rearing high numbers (8) and low numbers (1) of accepted larvae per cage. Two experiments were conducted in "standard" cages kept in the Bee Flight Room at 30-35° C. and 40-60% relative humidity. Each test had five replicates. Those tests having fewer than 8 accepted larvae received larvae from additional cages of bees which contained bees similar in numbers and ages. The results are shown in Table IX.

Experiments 9B-400(a), 9B-200(b), and 9B-400(b) did not rear larvae successfully. Nevertheless, the groups of 200 and 400 bees reared queen bees successfully when there was only one larva present per cage. Generally speaking, the larger groups of bees (i.e. 400) reared queens in longer cells and took less time to rear them from the time of grafting to capping. Little difference was noticed however in the total developmental time between the two size groups.

TABLE IX

THE QUEEN REARING ABILITY OF GROUPS OF 10 DAY OLD QUEENLESS BEES REARING HIGH (8) AND LOW (1) NUMBERS OF LARVAE IN CAGES ^a (1968)

Experiment	# of N. B. ^b per cage	Total # larvae nursed in 5 cages	Time of death				Emergence Adult reared	Mean length of cell (mm.)	Mean time from G. to C. ^e (days)	Mean developmental time (days) ^f
			Unsealed		Sealed ^c					
			# L. ^d	# Late L. &/or Prep.	# Late P.	# A.				
8 B ^g	200(a)	5	-	-	-	-	5	11.4	4	14.5
	400(a)	5	-	-	-	-	5	14	4	15.3
	200(b)	40 ^x	11	-	15	2	12	8.07	4.13	15.08
	400(b)	40	8	-	7	6	19	9.53	4.2	15.18
9 B	200(a)	5	-	-	-	-	5	13.2	4.4	15.9
	400(a)	5	1	-	3	-	1	12.8	4	16.5
	200(b)	40 ^y	23	2	9	1	5	8.76	4.76	16.3
	400(b)	40 ^z	30	1	7	-	2	10.3	4	16.5

a Temperature: 30-35° C.

b Nurse bees.

c # Late L. &/or Prep. - Bees died at late larval or prepupal stage.

Late P. - Bees died at late pupal stage.

A. - Bees died after last molt.

d Larva(e).

e Time from grafting of larvae to capping of cell.

f $\pm 1/4$ day (± 6 hrs.) from egg stage to emergence. g - Direct grafting.

x 11 out of 40 larvae were accepted by 8B-200(a) & transferred to 8B-200(b).

y 9 out of 40 larvae were accepted by 9B-200(a) & transferred to 9B-200(b).

z 3 out of 40 larvae were accepted by 9B-400(a) & transferred to 9B-400(b).

CHAPTER VII
MORPHOLOGICAL COMPARISON OF ADULTS REARED BY GROUPS
OF QUEENLESS BEES OF VARIOUS AGES

This chapter is a continuation of the results obtained from Chapter V and VI. In order to compare the ability of different numbers of nurse bees of various ages in cages to rear adults (either unemerged or emerged), it was necessary to use several measurements to define the particular caste of the adults. The bees that died at the late pupal stage or at the last molt, were also measured when possible. All of the measurements were based on the methods outlined in Chapter IV. In addition to these, the mandibles were examined and depending on their shape were divided into three categories; worker (i.e. spoon shaped), intercaste (i.e. the edge of the mandible is not smooth but has a small notch), and queen mandible (i.e. with sharp edge and deep notch).

"Control" queens were reared by queenless colonies in the summers of 1967 and 1968; the results are shown in Tables XII and XV. "Control" queens (Table XII), grafted in July, 1967, appeared to be of a better "quality" than those grafted in August, 1968. This was particularly noticeable when diameters of spermathecae and numbers of ovarioles were compared; these are considered to be two very important caste characteristics in honey bees. Many factors affect the "quality" of queens produced under natural conditions-- e.g. weather factors, time of season when larvae are grafted, strength and genetic background of the colony, age distribution of bees, stores in the hive, nectar and pollen flows, number of grafts

put into the colony, experience of the queen rearer, etc. Thus it is difficult to use field controls for comparisons with laboratory experiments. However, field controls were reared to give some indication of the "quality" of the queens reared in these experiments.

Both numbers of ovarioles and spermathecae of the "control" queens reared for these experiments fall within the range of normal queens reared by Weaver (1957) (see Table XV). However the weights of pupae in the above experiments were lower than those reported by Weaver (1957), Jay (1964), and Marcus (1967). The lower pupal weights obtained in this study probably resulted from their being reared at a different time of the season; as well Weaver (1957) measured his bees immediately following pupation rather than on the fourth day as was done in this study (see Chapter III).

A. Morphological Comparison of Queens Reared by Groups of Seven Day Old Queenless Bees (1967)

Either one or two adults were reared successfully by groups of 100, 200 (Figure 9), and 400 queenless bees which were 7 days old when they received grafted larvae. Four experiments were conducted at 30° and 35° C. and the preserved bees were measured (see Chapter IV). The results are shown in Table X. No pupal measurements were done. All of the adult measurements showed that the bees belonged to the queen caste except that the basitarsal indices of 2A(30)-100-3(a), 3A-200-3(a), and 4B-200(a)-3(a) were low (see Weaver, 1957, and Table XII); all of the bees except

4B-400(b)-2(a), had a lower ovariole count than those recorded by Weaver (1957) and those shown in Table XII.

The relationship between the number of ovarioles and the egg laying ability of a queen has not been investigated to date. The spread between the number of ovarioles of queens (116-217 for left ovary, 129-197 for right, found by Weaver, 1957; 160-180 for each found by Snodgrass, 1956) and workers (1-9 for left, 1-8 for right, found by Weaver, 1957; 2-12 for each found by Meier (1916) described by Snodgrass, 1956) is very large. No author has recorded an ovariole count lower than 110 (see Mitsui et al, 1964) for either ovary for hive-reared queens; the lowest count recorded among the controls (Table XII and XV) was 133. The possible factors which account for this variation were outlined earlier in this chapter. Many of the bees (Table X) had ovariole counts lower than 110 but were often queen-like in most other characteristics. The numbers of ovarioles were reduced when more than one adult was reared by a single group of nurse bees (e.g. 2A(30)-100(a), (b) and 4B-400(a)-3(a), (b)). The mandibles were those of queens. The length of the queen cells was usually shorter than those of the controls. Total developmental time of the bees was longer than is usual (i.e. 16 days).

B. Morphological Comparison of Queens Reared by Groups of Queenless Bees of Various Ages (1967)

One adult (see Table XI) was reared in each cage containing 50, 100, 200, and 400 nurse bees except that the nurse bees in cage 4A-200 (b){13} reared two adults (3(a) and 3(b)). Most of the bees showed

TABLE X

A MORPHOLOGICAL COMPARISON OF QUEENS REARED BY GROUPS OF SEVEN DAY OLD QUEENLESS BEES IN CAGES (1967)

Experiment	Temperature (°C)	# of N.B. per cage	Replicate #	Adult queen #	Length (mm.) of cell	Time (days) from G. to C. ^x	Total developmental time (days) ^y	Adult Measurements									# of ovarioles ^z	
								Head			Mandibles			Basitarsus				Dia ^v (mm.) of spermatheca
								Width (mm.)	Length (mm.)	Index W/L	W	I	Q	Length (mm.)	Width (mm.)	Index L/W		
1A	30°	100	2	a	11	5	17	3.79	3.64	1.04		X	2.24	1.12	1.97	1.12	102	
2A	30°	100	3 ^v	a	7	5	20	3.88	3.61	1.07		X	2.32	1.24	1.87	1.06	60	
				b	6	5	20	3.82	3.64	1.05		X	2.32	1.20	1.93	1.06	90	
3A	35°	100	2	a	11	3	17	3.85	3.67	1.05		X	2.24	1.12	2.00	1.00	90	
		200	3	a	12	3	17	3.82	3.67	1.04		X	2.32	1.24	1.87	1.06	77	
4B	35°	200(a)	3	a	8	6	19	3.91	3.64	1.07		X	2.32	1.24	1.87	1.12	116	
		200(b)	2	a	8	5	17	3.85	3.73	1.03		X	2.52	1.24	2.03	1.12	84	
		400(a)	2	a	8	4	16	3.79	3.67	1.03		X	2.48	1.12	2.21	1.20	110	
			3 ^v	a	9	4	16	3.94	3.76	1.05		X	2.36	1.16	2.03	1.20	96	
		b		11	5	17	3.76	3.67	1.02		X	2.36	1.20	1.96	1.12	90		
		200(c)	3	a	9	5	16	3.85	3.70	1.04		X	2.48	1.20	2.06	1.16	140	
400(b)	2	a	15	6	18	3.94	3.79	1.04		X	2.48	1.24	2.00	1.16	140			

x Time from grafting of larvae to capping of cell.

y $\pm 1/4$ day (± 6 hrs.) from egg stage to emergence.

z The right ovary of the queen was counted.

v Two adults were reared in the same cage at the same time.

queen characteristics. However, some deviations follow: The basitarsal indices and the diameters of spermathecae of 4A-200(b)[18]-1(a) and 2(a) were smaller than that of the control (Table XII) and that found by Weaver (1957). Worker combs were found on the basitarsi of both bees; the mandibles were worker-like and intercaste in bees 2(a) and 1(a) respectively. Bee 4B-50[18]-2(a) had 55 ovarioles, intercaste mandibles, a small spermathecal diameter (1.00 mm.) and a short wax cell (6 mm.). Bee 4A-200(c)[13]-1(a) had intercaste mandibles, a short cell (5 mm.), and a long developmental time (both ovaries and spermatheca were decayed). Bee 4A-200(b)[13]-3(a) and (b) had low numbers of ovarioles, small spermathecae, short cells, high basitarsal indices, but only (b) had intercaste mandibles. Most of the wax cells were shorter than the "control" queens (Table XII).

C. Morphological Comparison of Queens Reared by Groups of Queenless Five and/or Ten Day Old Bees (1968)

These data are a continuation of those presented in Table VIII and are shown in Table XIII. The bees reared by 50 or 100 nurse bees of the three age groups usually showed characteristics of workers or intercastes including very short wax cells; 6B-50[10]-2 had worker mandibles, a low pupal weight, short tongue and wax cell; eight bees of 5B and 6B had intercaste mandibles with low numbers of ovarioles, low pupal weights, short wax cells, but with high basitarsal indices and short tongues. Although 5B-100[5+10]-3, 5B-100[10]-2 and -4 had lower basitarsal indices

TABLE XI

A MORPHOLOGICAL COMPARISON OF QUEENS REARED BY GROUPS OF QUEENLESS BEES OF VARIOUS AGES AT 35° C. (1967)

Experiment	# of N. B. per cage	Age of N.B.	Replicate #	Adult queen#	Length (mm.) of cell	Time (days) from G. to C. ^x	Total development time (days) ^y	Adult Measurements										Notes	
								Head			Mandibles			Basitarsus			Dia ¹ (mm.) of spermatheca		# of ovarioles ^z
								Width	Length	Index				Length	Width	Index			
								(mm.)	(mm.)	W/L	W	I	Q	(mm.)	(mm.)	L/W			
1A	200(b)	mixed	1	a	13	5	18	3.88	3.76	1.03		X	2.52	1.20	2.10	1.16	122	at 30°C.	
3B	100	12	2	a	6	5	18	3.82	3.58	1.07		X	2.32	1.16	2.00	1.12	105		
	200	12	1	a	9	5	16	3.88	3.67	1.06		X	2.36	1.16	2.03	1.12	140		
		12	2	a	8	5	17	3.85	3.73	1.03		X	2.36	1.16	2.03	1.16	150		
	400	12	1	a	13	5	15	3.88	3.64	1.07		X	2.36	1.24	1.90	1.24	158		
4A	50	18	2	a	6	3	-	3.67	3.58	1.03		X	2.28	1.16	1.96	1.00	55	"a."	
	200(a)	18	1	a	8	3	17	3.70	3.61	1.02		X	2.16	1.12	1.92	1.06	111		
			2	a	7	3	15	3.85	3.64	1.06		X	2.28	1.12	2.03	1.12	125		
	200(b)	13	3	a	7	3	17	3.82	3.64	1.05		X	2.36	1.16	2.03	1.06	100		
				b	8	3	-	3.82	3.70	1.03		X	2.28	1.12	2.03	1.02	95	"a."	
		18	1	a	7	3	-	3.70	3.61	1.02		X	2.20	1.20	1.83	1.02	97	"a."	
	2			a	7	3	-	3.67	3.58	1.03	X		2.21	1.12	1.89	1.00	70	"a."	
	400(a)	18	3	a	8	3	-	3.82	3.64	1.05		X	2.32	1.20	1.93	1.08	150	"a."	
	200(c)	13	1	a	5	3	19	3.82	3.82	1.00		X	2.28	1.12	2.03	-----	---		
	400(b)	18	1	a	6	3	-	3.85	3.73	1.03		X	2.40	1.16	2.06	1.12	100	"a."	
200(d)	mixed	1	a	11	5	14	3.94	3.79	1.04		X	2.44	1.20	2.03	1.16	114			
4B	400(b)	13	3	a	12	4	16	3.82	3.70	1.03		X	2.44	1.20	2.03	1.12	130		
		14	1	a	10	5	16	3.82	3.67	1.04		X	2.36	1.16	2.03	1.16	135		
	200(d)	mixed	1	a	13	4	16	3.88	3.73	1.04		X	2.36	1.16	2.03	1.16	133		

"a." - not emerged adult.

x - Time from grafting of larvae to capping of cell.

y - $\pm 1/4$ day (± 6 hrs.) from egg stage to emergence.

z - The right ovary of the queen was counted.

TABLE XII

MORPHOLOGICAL RECORDS OF QUEENS REARED BY A QUEEN-REARING COLONY
(CONTROL) ^a (1967)

Adult queen #	Length (mm.) of cell	Adult Measurements									# of ovarioles ^z	
		Head			Mandibles			Basitarsus				Dia [†] (mm) of spermatheca
		Width	Length	Index	W	I	Q	Length	Width	Index		
		(mm.)	(mm.)	W/L				(mm.)	(mm.)	L/W		
1	16	3.91	3.82	1.02			X	2.60	1.20	2.17	1.16	161
2	16	3.85	3.82	1.01			X	2.52	1.16	2.17	1.20	207
3	17	3.88	3.82	1.02			X	2.52	1.24	2.03	1.24	189
4	15	3.88	3.82	1.02			X	2.52	1.24	2.03	1.20	147
5	14	3.85	3.82	1.01			X	2.52	1.24	2.03	1.20	148
6	14	3.91	3.85	1.02			X	2.52	1.20	2.10	1.20	166
7	17	3.94	3.85	1.02			X	2.56	1.20	2.13	1.24	161
8	15	3.94	3.79	1.04			X	2.56	1.24	2.06	1.24	168
9	18	3.94	3.88	1.02			X	2.56	1.20	2.13	1.24	152
10	16	4.00	3.85	1.04			X	2.60	1.24	2.09	1.28	185
11	17	3.91	3.82	1.02			X	2.48	1.20	2.06	1.16	171
12	18	3.97	3.85	1.03			X	2.48	1.20	2.06	1.20	187
13	17	3.88	3.82	1.02			X	2.44	1.24	1.97	1.16	209
Mean	16.15	3.91	3.83	1.02				2.53	1.22	2.07	1.21	173
S.D.	.04	.02						.04	.02		.04	

^a The larvae were grafted on July 18th, 1967; 19 queens emerged on July 29th. The time from egg stage to emergence ranged 14-15 days.

^z The right ovary of the queen was counted.

S.D. Standard deviation.

and short wax cells the number of ovarioles were high and the mandibles were queen-like; bee 5b-100{5+10}-3 had a long tongue, a high pupal weight, and a short developmental period. Bee 5B-100{10}-2 had a short tongue, a low pupal weight and a long developmental period. The rest of the bees showed queen-like characteristics except for 7B-400{5+10}-2 which had a low number of ovarioles, small spermatheca, a low weight, and a long developmental period. All four groups of 10 day old nurse bees were able to rear queens, especially the groups of 200 and 400 bees. Most of the late pupae had worker or intercaste mandibles, low numbers of ovarioles, and small spermathecae.

D. Morphological Comparison of Queens Reared by Groups of Ten Day Old Queenless Bees with High (8) and Low (1) Numbers of Larvae in Cages (1968)

As Table IX indicated, the adults (either emerged or unemerged) and late pupae (last molt) were reared by 200 and 400 nurse bees. Only one larva was reared in each cage in the other group of tests. The results are shown in Table XIV. Four pupae, 8B-200(b)-1(c), -4(a), 9B-200(b)-5(c), and -400(b)-4(a), had worker mandibles; two of them had high basitarsal indices, but low pupal weights and shorter wax cells (the ovaries and spermathecae were decayed). Four bees, 8B-400(b)-2(b), -3(f), 9B-200(b)-1(a) and (c), had intercaste mandibles, low pupal weights, and high basitarsal indices; bee 9B-200(b)-1(a) had a high number of ovarioles large spermatheca and a developmental period of 17 days. Two hundred and 400

nurse bees were able to rear queens whenever they had only one grafted larva to be reared or when fewer than 8 larvae were present. Nevertheless, the bees reared by the groups with 8 larvae present had shorter cell lengths, worker-like or intercaste mandibles as described above, and lower numbers of ovarioles (e.g. 8B-200(b)-2(a) and 2(c), -400(b)-1(d), 4(e), and 5(e)) than those reared by the groups with one larva present (except 9B-200(a)-1(a) which had 98 ovarioles). The other characteristics (i.e. developmental time, pupal weights, tongue length, diameters of spermathecae) of the bees reared by the two groups differed little.

TABLE XIII

A MORPHOLOGICAL COMPARISON OF QUEENS REARED BY GROUPS OF QUEENLESS FIVE AND/OR TEN DAY OLD BEES IN CAGES (1968)

Experiment	# of N. B. per cage	Age of N. B.	Replicate #	Length (mm.) of cell	Time (days) x Total developmental time (days) from G. to C.	Pupal Measurements						Adult Measurements										Notes	
						Body			Tongue	Head		Mandibles			Basitarsus		Dia. (mm.) of spermatheca	# of ovarioles					
						Length (mm.)	Weight (mgs.)	Length (mm.)	Width (mm.)	Length (mm.)	Index W/L	W	I	Q	Length (mm.)	Index L/W							
																	a	b	Total	(mgs.)	(mm.)		(mm.)
5B	50	5	3	8	5	-	5.81	6.76	12.57	159	2.54	3.79	3.52	1.08	X	2.36	1.20	1.96	----	---	"a."		
			5+10	4	7	6	18.5	5.97	7.86	13.83	166	2.54	3.82	3.58	1.07		X	2.20	1.12	1.96	1.04	76	
			10	1	7	5	-	5.66	6.60	12.26	136	2.38	3.85	3.61	1.07	X	2.28	1.12	2.03	.94	80	"p."	
100	5	5+10	1	10	6	18	6.28	6.60	12.88	150	2.50	3.85	3.64	1.06		X	2.36	1.12	2.10	1.08	74		
			5	9	5	-	6.76	7.86	14.62	208	2.50	3.82	3.73	1.02		X	2.44	1.20	2.03	----	---	"p."	
			3	9	5	16	6.60	8.17	14.77	216	2.66	3.94	3.79	1.04		X	2.32	1.24	1.87	1.16	100		
			4	10	5	16	6.76	7.86	14.62	221	2.66	3.82	3.73	1.02		X	2.44	1.24	1.97	1.12	123		
			5	10	5	-	7.07	9.43	16.50	245	2.42	3.64	3.67	.99	X	2.44	1.28	1.91	1.12	---	"p."		
			10	1	6	4	-	6.60	7.20	13.80	107	2.54	----	----	----	X	2.24	1.10	2.03	.98	80	"p."	
			2	8	5	19	5.66	6.44	12.10	133	2.42	3.79	3.58	1.06		X	2.24	1.20	1.86	1.04	103		
			3	9	5	17	6.44	8.01	14.45	210	2.50	3.88	3.73	1.04		X	2.48	1.24	2.00	1.12	117		
			4	10	6	17	6.28	7.86	14.14	186	2.58	3.91	3.76	1.04		X	2.20	1.16	1.89	1.08	119		
			5	9	5	16	7.07	8.33	15.40	222	2.62	3.88	3.76	1.03		X	2.56	1.24	2.06	1.20	109		
200	5	5+10	2	8	4	-	7.15	8.80	15.95	195	2.54	3.91	3.73	1.05		X	2.36	1.20	1.96	----	---	"a."	
			3	13	5	16	7.38	8.48	15.86	239	2.66	3.94	3.79	1.04		X	2.48	1.20	2.06	1.24	145		
			4	12	6	17	7.07	8.33	15.40	236	2.70	3.97	3.79	1.05		X	2.52	1.20	2.10	1.12	155		
			5	12	5	18	6.60	8.01	14.61	198	2.54	3.79	3.67	1.03		X	2.44	1.24	1.97	1.08	117		
			1	12	5	17	7.07	8.01	15.08	236	2.62	3.97	3.85	1.03		X	2.48	1.28	1.94	1.20	112		
			2	14	5	17	6.76	8.33	15.09	230	2.62	3.91	3.76	1.04		X	2.52	1.24	2.03	1.12	139		
			3	13	4	-	7.15	8.25	15.40	185	2.54	3.94	3.67	1.07		X	2.36	1.20	1.96	----	---	"a."	
			4	11	5	-	6.28	8.17	14.45	193	2.54	3.85	3.64	1.06		X	2.44	1.24	1.97	1.06	---	"a."	
			5	10	5	16	6.76	7.38	14.14	191	2.58	3.88	3.76	1.03		X	2.36	1.24	1.90	1.12	118		

continued ...

TABLE XIII (continued)

Experiment	# of N. B. per cage	Age of N. B.	Replicate #	Length (mm.) of cell	Time (days) x from G. to C.	Total develop- mental time (days)	Pupal Measurements					Adult Measurements										Notes	
							Body			Tongue	Head		Mandibles			Basitarsus		Dia [†]	#				
							a	b	Total	Length (mgs.)	Length (mm.)	Width (mm.)	Length (mm.)	Index W/L	W	I	Q	Length (mm.)	Width (mm.)	Index of L/W	of sperma- theca		of ovario- les ^y
5B	200	10	1	12	5	16	7.07	8.64	15.71	231	2.58	3.88	3.73	1.04		X	2.44	1.24	1.97	1.20	131		
			2	14	4	15	7.15	8.25	15.40	192	2.58	3.88	3.67	1.03		X	2.40	1.16	2.06	1.16	124		
			3	14	5	16	7.07	9.43	16.50	243	2.62	3.97	3.79	1.05		X	2.48	1.24	2.00	1.24	179		
			4	10	4	15	7.80	8.40	16.20	193	2.58	3.85	3.70	1.04		X	2.44	1.20	2.03	1.08	148		
			5	15	5	16	7.38	9.27	16.65	243	2.58	3.91	3.73	1.05		X	2.52	1.24	2.03	1.16	149		
	400	5		1	15	5	17	7.07	8.17	15.24	232	2.66	3.88	3.73	1.04		X	2.48	1.28	1.94	1.16	113	
				2	11	4	-	7.07	8.33	15.40	224	2.50	3.88	3.61	1.07		X	2.52	1.24	2.03	1.08	---	"a."
				3	11	4	16	7.07	8.64	15.71	234	2.50	3.91	3.64	1.07		X	2.44	1.16	2.10	1.20	146	
				4	14	4	15	6.76	8.17	14.93	296	2.62	3.85	3.76	1.02		X	2.48	1.20	2.06	1.12	152	
				5	16	5	17	7.07	8.64	15.71	243	2.54	3.91	3.79	1.03		X	2.48	1.24	2.00	1.12	120	
		10	5+10	1	16	5	-	7.07	8.33	15.40	239	2.62	3.94	3.73	1.06		X	2.48	1.20	2.06	1.06	---	"a."
				3	15	5	16	7.07	8.80	15.87	250	2.68	3.94	3.70	1.06		X	2.56	1.24	2.06	1.20	72	
				4	14	5	16	7.07	8.95	16.02	238	2.66	3.94	3.73	1.06		X	2.40	1.20	2.00	1.20	111	
				5	12	5	-	6.60	8.01	14.61	196	2.62	3.91	3.73	1.05		X	2.40	1.20	2.00	---	---	"p."
				1	15	5	16	7.07	8.80	15.87	249	2.66	3.91	3.79	1.03		X	2.48	1.20	2.06	1.20	148	
				2	13	5	16	7.07	8.64	15.71	236	2.66	3.91	3.79	1.03		X	2.48	1.24	2.00	1.16	136	
				4	15	5	16	7.07	8.33	15.40	237	2.70	3.97	3.76	1.06		X	2.48	1.20	2.06	1.16	138	
				5	15	5	16	6.76	8.64	15.40	221	2.66	3.88	3.73	1.04		X	2.44	1.20	2.03	1.24	144	
				6B	50	5+10	1	6	5	-	5.97	6.60	12.57	135	2.46	3.73	3.61	1.03		X	2.32	1.16	2.00
10	1	7	5				17	5.97	8.80	14.77	161	2.50	3.79	3.67	1.03		X	2.24	1.12	2.00	1.06	92	
2	5	4	-				5.18	5.50	10.68	100	2.66	---	---	---	X		2.20	1.10	2.00	---	---	"p."	
100	5	5+10	4		7	5	-	5.66	5.81	11.47	114	2.22	---	---	---	-	-	---	---	---	---	---	"p."
			1		7	4	-	5.81	6.28	12.09	121	2.26	3.76	3.61	1.04		X	2.12	1.10	1.92	---	---	"p."
			1		8	5	17	6.28	8.64	14.92	185	2.54	3.85	3.70	1.04		X	2.32	1.16	2.00	1.12	121	
			4		6	4	-	5.50	5.03	10.53	95	2.22	---	---	---	X		2.16	1.06	2.03	---	---	"p."

continued ...

TABLE XIII (continued)

Experiment	# of N. B. per cage	Age of N. B.	Replicate #	Length (mm.) of cell	Time (days) from G. to C. x	Total develop- mental time (days)	Pupal Measurements					Adult Measurements										Notes		
							Body			Tongue	Head			Mandibles			Basitarsus		Dia ^o	#				
							Length (mm.)	Weight (mgs.)	Length (mm.)	Length (mm.)	Width (mm.)	Index	W	I	Q	Length (mm.)	Width (mm.)	Index of sperma-	of ovari-					
							a	b	Total	Length (mm.)	Length (mm.)	W/L				(mm.)	(mm.)	L/W	theca	oles ^y				
6B	100	10	1	7	4	-	6.28	7.07	13.35	131	2.46	3.76	3.64	1.03		X	2.20	1.06	2.07	----	---	"p."		
			4	9	4	-	5.50	7.54	13.04	135	2.42	3.73	3.58	1.04		X	2.24	1.16	1.93	----	---	"p."		
			5	8	5	16	6.28	8.64	14.92	150	2.42	3.88	3.70	1.05			X	2.40	1.20	2.00	1.16	148		
	200	5	10	1	12	6	16	6.60	8.95	15.55	197	2.42	3.88	3.73	1.04			X	2.36	1.20	1.96	1.16	154	
				5	11	5	-	5.81	6.60	12.41	145	2.50	-----	-----	-----	-	-	-	-----	-----	-----	-----	-----	"p."
		5+10	2	10	5	16	7.07	8.64	15.71	203	2.58	3.94	3.79	1.04			X	2.36	1.16	2.03	1.12	143		
			4	12	5	16	6.44	9.11	15.55	210	2.54	3.97	3.79	1.05			X	2.44	1.24	1.97	1.12	151		
			5	8	4	-	5.97	7.07	13.04	145	2.46	3.73	3.61	1.03			X	2.28	1.20	1.90	-----	---	"a."	
		10	1	12	5	16	7.38	9.74	17.12	208	2.50	3.97	3.79	1.05			X	2.48	1.24	2.00	1.20	118		
			3	9	3	-	6.28	7.38	13.66	151	2.42	3.82	3.67	1.04			X	2.12	1.10	1.92	-----	---	"a."	
			4	10	3	-	6.28	7.07	13.35	157	2.54	3.79	3.64	1.04			X	2.24	1.12	2.00	-----	---	"a."	
	400	5	10	5	14	5	16	7.07	9.11	16.18	228	2.58	3.88	3.76	1.03			X	2.48	1.20	2.06	1.16	114	
				1	12	4	-	5.97	7.54	13.51	177	2.34	-----	-----	-----	-	-	-	-----	-----	-----	-----	---	"p."
		5+10	2	14	4	16	6.44	9.11	15.55	214	2.58	3.94	3.73	1.06			X	2.48	1.24	2.00	1.20	148		
			3	12	5	16	7.07	9.11	16.18	230	2.54	3.97	3.79	1.05			X	2.44	1.16	2.10	1.12	140		
4			14	5	16	6.28	10.21	16.49	241	2.54	3.97	3.82	1.04			X	2.44	1.28	1.91	1.20	151			
5			15	5	16	6.28	10.05	16.33	240	2.54	4.00	3.82	1.05			X	2.52	1.20	2.10	1.12	141			
10			2	14	5	16	6.76	10.21	16.97	225	2.50	3.94	3.79	1.04			X	2.40	1.16	2.06	1.16	122		
			3	16	4	16	7.07	9.11	16.18	227	2.54	3.94	3.79	1.04			X	2.52	1.16	2.11	1.24	143		
			4	18	4	17	7.54	9.43	16.97	255	2.62	4.00	3.82	1.05			X	2.48	1.24	2.00	1.24	150		
5			17	5	-	7.07	10.37	17.44	216	2.54	3.97	3.79	1.05			X	2.48	1.24	2.00	-----	---	"a."		
7B	50	10	5	9	7	18	5.66	6.60	12.26	136	2.38	3.97	3.70	1.07			X	2.40	1.20	2.00	1.08	137		
	100	5+10	1	10	6	-	6.76	9.58	16.34	234	2.54	3.94	3.79	1.04			X	2.36	1.24	1.90	-----	---	"p."	
			4	10	5	17	6.76	8.48	15.24	211	2.62	3.91	3.70	1.06			X	2.44	1.20	2.03	1.12	75		
			5	10	6	20	6.76	8.64	15.40	210	2.58	3.88	3.67	1.06			X	2.24	1.16	1.93	1.08	82		
	10	1	12	6	17	7.07	9.90	16.97	234	2.54	3.88	3.70	1.05			X	2.32	1.20	1.93	1.16	104			
2		11	5	16	6.76	8.17	14.93	221	2.50	3.85	3.73	1.03			X	2.40	1.24	1.93	1.16	150				

continued ...

TABLE XIII (continued)

Experiment	# of N. B. per cage	Age of N. B.	Replicate # Length (mm.) of cell		Time (days) from G. to C. x	Total developmental time (days)	Pupal Measurements					Adult Measurements								# of ovarioles ^y	Notes													
							Body			Tongue	Head		Mandibles			Basitarsus		Dia ^o																
							Length (mm.)		Weight	Length	Width	Index	W	I	Q	Length	Index	(mm.)																
							a	b	Total	(mgs.)	(mm.)	Length	W/L	(mm.)	(mm.)	L/W	of spermatheca																	
7B	100	10	3	7	4	16	6.28	8.80	14.08	203	2.50	3.82	3.73	1.02																				
			4	7	5	-	6.28	7.38	13.66	203	2.42	3.88	3.73	1.04																"a."				
			5	12	5	16	7.07	9.58	16.65	238	2.50	3.85	3.70	1.04																				
	200	≤5	2	12	7	-	6.28	7.86	14.14	203	2.58	-	-	-																"a."				
			3	12	7	17	6.60	10.53	16.13	234	2.50	3.85	3.73	1.03																				
			5	10	6	16	6.60	10.21	16.81	234	2.50	3.88	3.70	1.05																				
		≤5+10	1	13	6	-	7.07	8.17	15.24	208	2.54	-	-	-																	"p."			
			2	11	5	15	6.60	10.37	16.97	242	2.50	3.88	3.76	1.03																				
			3	8	4	-	6.28	7.07	13.35	160	2.54	3.82	3.64	1.06																		"a."		
			4	11	4	15	6.60	8.17	14.77	188	2.54	3.85	3.73	1.03																				
			5	10	5	-	6.28	7.86	14.14	162	2.46	3.88	3.73	1.04																		"a."		
		10	1	9	4	15	6.91	8.64	15.55	208	2.54	3.94	3.73	1.06																				
			2	11	5	15	6.91	9.74	16.65	207	2.54	3.94	3.73	1.06																				
			3	9	5	15	7.07	9.43	16.50	215	2.54	4.00	3.79	1.06																				
			4	9	5	16	6.60	8.95	15.55	217	2.58	3.97	3.76	1.06																				
			5	12	5	16	6.60	9.11	15.71	231	2.62	3.91	3.73	1.05																				
	400	≤5+10	1	15	4	14	6.60	9.74	16.34	225	2.42	3.97	3.82	1.04																				
			2	11	6	17	6.60	9.43	16.03	195	2.50	3.88	3.73	1.04																				
			3	12	5	15	6.76	9.74	16.50	204	2.50	3.94	3.73	1.06																				
			4	14	5	-	6.60	9.58	16.18	239	2.46	3.97	3.79	1.05																				"a."
		10	1	13	5	15	6.76	9.74	16.50	227	2.46	3.94	3.79	1.04																				
			2	12	5	15	6.76	9.27	16.03	232	2.58	3.88	3.76	1.03																				
			3	13	5	15	7.07	9.58	16.65	250	2.58	3.94	3.79	1.04																				
			4	12	5	15	7.07	8.64	15.71	235	2.58	3.91	3.76	1.04																				
			5	10	5	15	7.07	9.74	16.81	226	2.46	3.91	3.79	1.03																				

x Time from grafting of larvae to capping of cell.

y The right ovary of the queen was counted.

"a." Unemerged adult.

"p." Late pupa.

TABLE XIV

MORPHOLOGICAL COMPARISON OF QUEENS REARED BY GROUPS OF TEN DAY OLD QUEENLESS BEES
WITH HIGH (8) AND LOW (1) NUMBERS OF LARVAE IN CAGES (1968)

Experiment	# of N. B. per cage	Replicate #	Adult queen # Length (mm.) of cell	Time (days) from G. to C. x	Total developmental time (days)	Pupal Measurements						Adult Measurements											
						Body			Tongue	Head		Mandibles			Basitarsus		Dia ^r (mm.)	# of ovarioles ^y	Notes				
						Length (mm.)	Weight (mgs.)	Length (mm.)	Width (mm.)	Length (mm.)	Index W/L	W	I	Q	Length (mm.)	Width (mm.)				Index L/W			
																	a	b	Total				
8B	200(a)	1	a	13	4	14	6.60	9.58	16.18	195	2.50	4.00	3.76	1.06		X	2.48	1.24	2.00	1.12	146		
		2	a	13	4	14	6.91	9.43	16.34	189	2.50	3.97	3.76	1.06		X	2.44	1.20	2.03	1.16	162		
		3	a	10	4	14	6.44	9.90	16.34	202	2.54	3.88	3.70	1.05		X	2.40	1.20	2.00	1.12	144		
		4	a	11	4	14	6.60	9.74	16.34	205	2.50	3.94	3.73	1.06		X	2.44	1.20	2.03	1.16	151		
		5	a	10	4	14	6.60	9.74	16.34	210	2.50	3.82	3.67	1.04		X	2.32	1.16	2.00	1.12	116		
400(a)	1	a	14	4	14	6.76	8.80	15.56	215	2.54	3.97	3.79	1.05		X	2.48	1.24	2.00	1.16	178			
		2	a	14	4	15	6.60	8.33	14.93	175	2.50	3.94	3.70	1.06		X	2.36	1.20	1.96	1.12	144		
		3	a	14	4	15	7.07	9.43	16.50	265	2.54	3.97	3.79	1.05		X	2.44	1.20	2.03	1.16	154		
		4	a	13	4	14	6.91	9.43	16.34	222	2.50	3.94	3.70	1.06		X	2.44	1.20	2.03	1.16	142		
		5	a	15	4	16	6.76	10.37	17.13	304	2.54	3.97	3.79	1.05		X	2.52	1.24	2.03	1.24	141		
200(b)	1	a	9	4	15	7.07	8.95	16.02	210	2.50	3.85	3.67	1.05		X	2.40	1.20	2.00	1.08	172			
		b	11	4	15	6.60	9.43	16.03	212	2.50	3.91	3.67	1.07		X	2.36	1.20	1.96	1.16	110			
		c	7	4	-	5.97	7.23	13.20	120	2.34	3.73	3.61	1.03	X		2.32	1.16	2.00	-----	----	"p."		
		d	12	5	-	6.28	7.23	13.51	210	2.46	3.85	3.61	1.06		X	2.12	1.16	1.82	-----	----	"p."		
		e	10	4	14	6.44	9.27	15.71	195	2.50	3.85	3.64	1.06		X	2.28	1.12	2.03	1.12	140			
	2	a	8	4	14	6.44	7.54	12.98	210	2.38	3.88	3.70	1.05		X	2.36	1.16	2.03	1.16	76			
		b	7	4	15	6.28	9.43	15.71	240	2.50	3.85	3.70	1.04		X	2.40	1.20	2.00	1.20	135			
		c	7	4	15	6.44	7.54	13.98	200	2.34	3.82	3.67	1.04		X	2.28	1.16	1.96	1.12	65			
	3	a	10	4	15	7.07	10.21	17.28	260	2.50	3.94	3.79	1.04		X	2.52	1.24	2.03	1.16	166			
		b	9	4	14	6.28	9.43	15.71	227	2.46	3.94	3.73	1.06		X	2.40	1.20	2.00	1.16	114			
	4	a	8	4	15	6.60	9.74	16.34	220	2.50	3.88	3.67	1.06		X	2.36	1.20	1.96	1.12	115			
		b	7	4	-	5.97	7.07	13.04	185	2.34	3.76	3.61	1.04	X		-----	-----	-----	-----	----	"p."		
																							"a."

continued

TABLE XIV (continued)

Experiment	# of N. B. per cage	Replicate #	Adult queen #	Length (mm.) of cell	Time (days) from G. to C. X	Total developmental time (days)	Pupal Measurements					Adult Measurements											
							Body			Tongue	Head		Mandibles			Basitarsus		Dia ^r	#				
							Length (mm.)		Weight	Length	Width	Length	W	I	Q	Length	Width	Index of	sperma-	of			
							a	b	Total	(mm.)	(mm.)	(mm.)	W/L			(mm.)	(mm.)	L/W	theca	ovari-oles ^y	Notes		
8B	200(b)	5	a	8	4	14	6.28	8.64	14.92	200	2.42	3.88	3.67	1.96		X	2.48	1.24	2.00	1.08	119		
			b	10	5	15	7.07	8.17	15.24	220	2.46	3.85	3.67	1.05		X	2.44	1.24	1.97	1.12	134		
			c	8	4	-	6.28	7.07	13.35	192	2.26	3.82	3.64	1.05		X	2.32	1.16	2.00	-----	----	----	"a."
400(b)	1	a	9	4	14	6.60	9.43	16.03	205	2.46	3.82	3.64	1.05		X	2.36	1.16	2.03	1.12	166			
		b	9	4	15	6.60	9.11	15.71	188	2.50	3.88	3.67	1.06		X	2.28	1.12	2.03	1.12	129			
		c	10	4	15	7.07	9.74	16.81	234	2.50	3.85	3.73	1.03		X	2.44	1.24	1.97	1.16	151			
	2	d	10	5	15	6.44	8.33	14.77	227	2.50	3.88	3.70	1.03		X	2.40	1.20	2.00	1.20	88			
		e	14	5	-	6.28	8.01	14.29	204	2.34	-----	-----	-----	-	-	-	-----	-----	-----	-----	----	"p."	
		f	13	5	16	6.60	7.86	14.46	210	2.58	3.85	3.64	1.06		X	2.32	1.16	2.00	1.16	172			
	3	a	10	4	15	6.44	8.80	15.24	220	2.50	3.88	3.67	1.06		X	2.24	1.12	2.00	1.08	116			
		b	8	4	-	6.28	9.43	15.71	190	2.46	3.82	3.61	1.06		X	2.44	1.20	2.03	-----	----	----	"a."	
		c	10	4	14	6.60	9.90	16.50	240	2.50	3.97	3.79	1.05		X	2.48	1.24	2.00	1.16	168			
	4	d	9	4	-	6.13	7.54	13.67	180	2.34	3.79	3.58	1.06		X	2.20	1.16	1.89	-----	----	----	"a."	
		e	9	4	14	6.28	9.43	15.71	200	2.42	3.88	3.67	1.06		X	2.40	1.20	2.00	1.16	148			
		f	10	4	14	6.44	9.74	16.18	205	2.46	4.00	3.82	1.05		X	2.48	1.20	2.06	1.16	163			
	5	a	6	4	15	7.07	8.80	15.87	225	2.42	3.85	3.64	1.96		X	2.40	1.20	2.00	1.12	199			
		b	11	4	-	6.44	8.17	14.61	197	2.42	3.82	3.64	1.05		X	2.32	1.16	2.00	-----	----	----	"p."	
		c	6	4	-	6.28	8.64	14.92	200	2.34	3.76	3.61	1.04		X	2.40	1.08	2.21	-----	----	----	"a."	
5	d	12	5	15	7.07	9.43	16.50	282	2.42	3.97	3.79	1.05		X	2.52	1.24	2.03	1.16	174				
	e	10	4	-	6.28	7.54	13.82	213	2.34	3.82	3.64	1.05		X	2.36	1.20	1.93	-----	----	----	"a."		
	f	8	5	15	6.60	9.74	16.34	230	2.46	3.97	3.70	1.07		X	2.36	1.16	2.03	1.12	124				
5	a	12	4	15	6.60	8.80	15.40	254	2.34	3.88	3.67	1.06		X	2.44	1.24	1.97	1.16	89				
	b	10	4	14	6.44	9.43	15.87	210	2.46	3.94	3.76	1.05		X	2.40	1.24	1.93	1.16	116				
	c	10	4	-	6.28	8.17	14.45	212	2.34	3.82	3.64	1.05		X	2.36	1.08	2.19	-----	----	----	"a."		
5	a	11	4	14	6.76	9.58	16.34	210	2.50	3.91	3.70	1.06		X	2.40	1.20	2.00	1.12	139				
	b	10	4	14	6.28	9.43	15.71	190	2.50	3.73	3.64	1.02		X	2.28	1.16	1.96	1.12	100				
			c	10	4	-	6.44	8.33	14.77	210	2.46	3.73	3.61	1.03		X	2.36	1.16	2.03	-----	----	----	"a."

continued ...

TABLE XIV (continued)

Experiment	# of N. B. per cage	Replicate #	Adult queen # of Length (mm.) of cell	Time (days) from G. to C. x	Total developmental time (days)	Pupal Measurements						Adult Measurements										
						Body			Tongue	Head		Mandibles			Basitarsus		Dia ^o (mm.)	# of ovarioles ^y	Notes			
						Length (mm.)	Weight (mgs.)	Length (mm.)	Width (mm.)	Index	W	I	Q	Length (mm.)	Width (mm.)	Index of L/W	of spermatheca					
																		a		b	Total	
8B	400(b)	5	d	9	5	15	6.60	7.38	13.98	208	2.46	3.85	3.64	1.06		X	2.32	1.16	2.00	1.06	136	
			e	9	4	14	6.60	9.74	16.34	210	2.50	3.91	3.70	1.06		X	2.24	1.12	2.00	1.08	115	
			f	9	5	16	6.76	8.17	14.93	243	2.54	3.91	3.70	1.06		X	2.44	1.24	1.97	1.16	134	
9B	200(a)	1	a	12	4	16	6.44	8.64	15.08	160	2.50	3.88	3.70	1.05		X	2.32	1.24	1.87	1.12	98	
			a	12	5	16	7.07	8.33	15.40	208	2.50	3.94	3.76	1.05		X	2.40	1.24	1.93	1.20	127	
			a	14	5	15	6.76	9.74	16.50	224	2.46	3.97	3.79	1.05		X	2.44	1.20	2.03	1.16	131	
			a	15	4	15	6.91	8.95	15.86	210	2.48	3.91	3.73	1.05		X	2.48	1.20	2.06	1.20	121	
			a	13	4	15	7.07	8.80	15.87	212	2.50	3.91	3.76	1.04		X	2.40	1.24	1.97	1.20	119	
400(a)	1	4	a	17	4	-	-----	-----	-----	-----	-----	3.94	3.73	1.06		X	2.44	1.24	1.97	-----	-----	"p."
			a	14	4	16	6.76	8.17	14.93	180	2.34	3.85	3.64	1.06		X	2.40	1.20	2.00	1.20	129	
200(b)	1	4	a	10	4	15	6.60	9.11	15.71	169	2.34	3.91	3.67	1.07		X	2.32	1.20	1.93	1.12	118	
			b	10	4	15	6.91	9.58	16.49	195	2.46	3.94	3.73	1.06		X	2.44	1.24	1.97	1.16	131	
			c	9	4	-	6.60	8.80	15.40	160	2.46	3.70	3.58	1.03		X	2.24	1.12	2.00	-----	-----	"p."
			d	9	5	17	6.13	6.76	12.89	153	2.50	3.82	3.64	1.05		X	2.36	1.20	1.96	1.12	134	
			a	8	4	16	6.13	6.76	12.89	150	2.70	3.82	3.61	1.06		X	2.32	1.28	1.81	1.12	138	
			a	8	4	-	6.28	7.70	13.98	140	2.38	3.82	3.64	1.05		X	2.32	1.16	2.00	-----	-----	"a."
			a	10	4	16	6.76	7.38	14.14	172	2.50	3.88	3.64	1.07		X	2.32	1.24	1.87	1.12	162	
			a	10	4	-	6.28	7.07	13.35	168	2.46	3.67	3.46	1.06		X	2.28	1.16	1.96	-----	-----	"p."
			b	9	5	-	6.28	7.38	13.66	138	-----	3.70	3.55	1.04		X	2.28	1.20	1.90	-----	-----	"p."
			c	7	4	-	4.87	4.87	9.74	100	2.14	3.88	3.70	1.05		X	-----	-----	-----	-----	-----	"p."
400(b)	1	3	a	13	5	17	6.60	7.38	13.98	175	2.42	3.88	3.70	1.05		X	2.36	1.28	1.84	1.16	146	
			a	12	4	-	6.76	9.90	16.66	205	2.14	3.79	3.64	1.04		X	2.08	1.06	1.95	-----	-----	"p."
			b	10	4	-	6.28	7.07	13.35	136	2.42	3.85	3.64	1.06		X	2.36	1.24	1.90	-----	-----	"p."
			c	8	4	15	6.28	9.27	15.55	156	2.42	3.82	3.64	1.05		X	2.32	1.20	1.93	1.16	127	
			a	10	4	-	6.28	8.64	14.92	153	2.42	3.73	3.55	1.05		X	2.24	1.16	1.93	-----	-----	"p."

x Time from grafting of larvae to capping of cell.
y The right ovary of the queen was counted.

"a." Unemerged adult.
"P." Late pupa.

TABLE XV

MORPHOLOGICAL RECORDS OF QUEENS REARED BY A QUEEN-REARING COLONY (CONTROL) ^x (1968)

Adult queen of #	Length (mm.) of cell	Pupal Measurements					Adult Measurements							Dia. of spermatheca (mm.)	Number of ovarioles ^y	
		Body			Tongue	Head			Mandibles			Basitarsus				
		Length (mm.)	Weight (mgs.)	Length (mm.)	Width (mm.)	Length (mm.)	Index W/L	W	I	Q	Length (mm.)	Width (mm.)	Index L/W			
1	12	7.07	9.43	16.50	225	2.56	4.00	3.79	1.06		X	2.44	1.28	1.91	1.16	185
2	13	7.07	9.74	16.81	241	2.54	4.03	3.73	1.08		X	2.52	1.20	2.10	1.16	142
3	15	7.07	10.53	17.60	240	2.50	3.88	3.73	1.04		X	2.52	1.28	1.97	1.20	205
4	15	7.23	9.90	17.13	264	2.58	4.06	3.82	1.06		X	2.52	1.20	2.10	1.24	208
5	14	6.76	10.05	16.81	225	2.54	3.88	3.76	1.03		X	2.48	1.32	1.88	1.12	180
6	13	6.91	8.48	15.39	201	2.54	3.97	3.67	1.08		X	2.32	1.16	2.00	1.12	179
7	12	6.76	9.90	16.66	218	2.42	3.94	3.76	1.05		X	2.44	1.20	2.03	1.24	223
8	12	7.07	10.53	17.60	230	2.46	3.97	3.70	1.07		X	2.40	1.20	2.00	1.20	167
9	12	6.60	9.74	16.34	227	2.50	3.88	3.73	1.04		X	2.48	1.24	1.97	1.20	176
10	11	6.76	9.74	16.50	221	2.46	3.94	3.70	1.06		X	2.48	1.24	1.97	1.20	164
11	12	6.76	9.58	16.34	220	2.50	3.85	3.73	1.03		X	2.48	1.24	1.97	1.20	151
12	13	7.07	10.21	17.28	230	2.50	3.85	3.73	1.03		X	2.48	1.28	1.91	1.24	170
13	12	6.76	10.53	17.29	225	2.42	3.91	3.73	1.05		X	2.48	1.20	2.06	1.16	164
14	14	7.07	10.05	17.12	231	2.50	3.94	3.67	1.07		X	2.52	1.32	1.91	1.24	152
15	15	7.54	10.05	17.59	245	2.46	3.88	3.70	1.05		X	2.52	1.24	2.03	1.20	170
16	15	7.07	10.37	17.44	248	2.50	4.09	3.79	1.08		X	2.52	1.32	1.91	1.20	170
17	15	7.07	10.37	17.44	243	2.50	3.94	3.82	1.03		X	2.56	1.24	2.06	1.20	133
18	15	7.07	9.90	16.97	234	2.52	3.94	3.79	1.04		X	2.48	1.24	2.00	1.24	152
19	14	6.91	9.74	16.75	232	2.50	4.03	3.79	1.06		X	2.20	1.16	1.89	1.24	141
20	15	7.07	10.68	17.75	260	2.42	3.97	3.76	1.06		X	2.56	1.28	1.97	1.20	166
21	13	7.07	11.14	18.21	255	2.50	3.97	3.85	1.03		X	2.60	1.36	1.91	1.20	138
22	11	6.76	9.90	16.66	235	2.50	3.91	3.70	1.06		X	2.48	1.24	2.00	1.16	135
23	14	7.07	10.84	17.91	250	2.50	3.94	3.79	1.04		X	2.60	1.28	2.03	1.28	171
Mean	13.35	6.98	10.06	17.04	234.8	2.50	3.95	3.75				2.48	1.25		1.20	167
S.D.		.20	.30			.04	.06	.05				.09	.05		.04	
Range of Normal Queens (Weaver, 1957)												2.4-2.8	1.9-2.3		1.0-1.3	129-197
Range of Normal Workers (Weaver, 1957)												2.0-2.4	1.6-1.9			1-8

^x Larvae were grafted on July 27th, 1968.

S.D. Standard deviation.

^y The right ovary of the queen was counted.

CHAPTER VIII

ADDITIONAL EXPERIMENTS AND OBSERVATIONS

A. The Acceptance of Larvae by Large Groups and Their Subsequent Rearing by Small Groups of Queenless Nurse Bees

Although larval acceptance by small groups (e.g. 50, and 100) of nurse bees was low, experiments were done to show their rearing ability. Female larvae, accepted by groups of 200 and 400 nurse bees, 10 days old, were transferred into cages containing 25, 50, and 100 nurse bees which had been in a queenless condition for at least one day. Some of the transferred larvae were reared successfully to pupae and adults as follows (see Table XVI):

(1). Three tests, each test with 5 replicates, were conducted at 30-35° C. with 40-60% relative humidity. Larvae accepted by 200 nurse bees were transferred into 15 cages containing 50 and 100, 5 day old bees, and 50, 10 day old bees. One adult was reared successfully in each test. One of the bees was classified as intercaste having shallow notches on the mandibles and 79 ovarioles; the other two were within the queen-category having queen-mandibles and 113 and 114 ovarioles respectively. The other characteristics of these three adults were similar.

(2). Larvae accepted by 400 nurse bees were transferred into two and three cages containing 50 and 100, 5 day old, and ten cages containing 25 and 50, 10 day old, nurse bees.

(a). One pupa and one adult were reared by 50, 5 day old nurse bees. The adult had queen mandibles and 120 ovarioles.

(b). One pupa and one adult were reared by 100, 5 day old nurse bees.

The adult had queen mandibles and 145 ovarioles.

(c). Two adults and one pupa were reared by 25, 10 day old nurse bees. The two adults had queen mandibles and 118 and 169 ovarioles respectively. The pupa had intercaste mandibles, worker basitarsi on the third legs, and malformed ovaries.

(d). One adult and two pupae were reared by 50, 10 day old nurse bees; each had queen mandibles and the adult had 120 ovarioles.

All of the above had pupal weights ranging from 141 to 212 mgs.; the emerged bees had a total developmental time ranging from 16 to 18 days, and their spermathecae ranging from 1.06 to 1.16 mm.

The results of these experiments raise many questions about caste determination in honey bees. The effect of the age and number of nurse bees as well as the effect of transferring larvae from large to small groups of nurse bees on caste determination requires further investigation - particularly as these involve the nutrition of the larvae. This is particularly important because the adults reared in these tests with 50 nurse bees (which had received larvae accepted by large numbers of nurse bees) had higher ovariole counts and larger spermathecae than the adults reared by 50 nurse bees which had accepted the larvae themselves (see Table XIII). Furthermore, queens with high ovariole counts and large spermathecae were reared by 25 nurse bees in these tests.

B. The Grafting of Eggs into Cages of Bees

(1). Grafting Eggs into Plastic Queen Rearing Cups

Two experiments were done in which eggs were removed from comb

TABLE XVI

MEASUREMENTS OF QUEENS REARED BY SMALL GROUPS OF NURSE BEES (1968)

Experiment	# of N. B. per cage	Age of N. B.	Replicate #	Length (mm.) of cell	Time (days) from G. to C. ^x	Total developmental time (days)	Pupal Measurements					Adult Measurements											
							Body			Tongue	Head		Mandibles			Basitarsus		Dia ^o of spermatheca	# ^y of ovarioles	Notes			
							Length (mm.)		Weight	Length	Width	Index	W	I	Q	Length	Width				Index		
							a	b	Total	(mgs.)	(mm.)	(mm.)	W/L			(mm.)	(mm.)	L/W					
10	50	5	1 ^z	7	5	16.5	6.60	8.64	15.24	165	2.46	3.82	3.67	1.04		X	2.24	1.12	2.00	1.12	113		
		5	2	6	5	-	6.28	8.80	15.08	150	2.46	-----	-----	-----	-	-	-	-----	-----	-----	-----	-----	"p."
		5	3	7	5	17.5	6.28	9.11	15.39	170	2.50	3.94	3.79	1.04		X	2.28	1.24	1.84	1.12	120		
100	5	5	1 ^z	8	4	16.5	6.76	8.01	14.77	183	2.54	3.85	3.73	1.03		X	2.28	1.12	2.03	1.06	114		
		5	2	6	4	-	6.13	7.54	13.67	149	2.54	-----	-----	-----	-	-	-	-----	-----	-----	-----	-----	"p."
		5	3	10	6	16.5	6.76	9.43	16.19	194	2.42	3.88	3.70	1.05		X	2.16	1.08	2.00	1.12	145		
25	10	10	1	10	4	16.5	6.76	8.01	14.77	168	2.50	3.88	3.67	1.06		X	2.20	1.12	1.96	1.08	118		
		10	2	10	4	16	6.60	8.17	14.77	170	2.42	3.94	3.73	1.06		X	2.32	1.20	1.93	1.12	169		
		10	3	10	4	-	5.97	7.07	13.04	141	2.42	3.73	3.58	1.04		X	2.24	1.08	2.07	-----	-----	"p."	
50	10	10	1	11	5	-	6.60	8.17	14.77	170	2.42	3.88	3.70	1.05		X	2.40	1.24	1.93	-----	-----	"a."	
		10	2	11	5	17	6.60	9.58	16.18	212	2.50	3.97	3.79	1.05		X	2.36	1.24	1.90	1.16	120		
		10	3	11	5	-	6.60	9.74	16.34	197	2.50	3.91	3.70	1.05		X	2.40	1.20	2.00	-----	-----	"a."	
		10	4 ^z	11	5	18	6.60	6.91	13.51	146	2.34	3.85	3.67	1.05		X	2.00	1.12	1.78	1.08	79		

x Time from grafting of larvae to capping of cell.

y The right ovary of the queen was counted.

z Larvae were first accepted by 200 nurse bees; all others accepted by 400 nurse bees.

"p." Late pupa.

"a." Unemerged adult.

with glass rods (Figure 2) and placed in dry plastic rearing cups. Two hundred and 400 nurse bees, 10 days old, were kept in "standard" cages each with 5 plastic cups. Each test had 4 replicates; several eggs were grafted into each plastic cup but only one accepted larva was allowed to be reared in each cage. Only one pupa and one unemerged adult were reared by the 200 and 400 nurse bees respectively in Experiment XI. However Experiment XII was more successful; four adults were reared by 200 nurse bees; they had queen mandibles with 187, 136, 135, and 160 ovarioles respectively. The diameters of the spermathecae were 1.20, 1.24, 1.20, and 1.24 mm., and the pupal weights were 217, 245, 208, and 228 mgs. respectively. One pupa and two adults were reared by the 400 nurse bees; the pupa had intercaste mandibles and a pupal weight of 173 mgs.; the ovaries and spermatheca were malformed. The two adults had queen mandibles, pupal weights of 230 and 234 mgs., 160 and 154 ovarioles, and spermathecae 1.16 and 1.20 mm. in diameter respectively. All of the adults reared were queen-like in all other respects.

(2). Rearing Eggs in Pieces of Comb

A piece of comb, 2 cm. x 5 cm., containing eggs was cut from a brood comb and eggs were removed from alternate cells to leave space for the building of queen cells. These pieces of comb were then attached to the metal bar and inserted into the cages containing either 200 or 400 ten day old bees which had been in queenless condition for at least one day.

Only one adult was reared by one of the 8 replicates of 200 nurse bees; it had queen mandibles, 134 ovarioles, a spermathecal diameter of

1.20 mm., and a pupal weight of 190 mgs. Three pupae and one adult were reared by 4 of the 6 replicates of 400 nurse bees; the pupae had queen mandibles, pupal weights of 220, 150, and 215 mgs., and malformed ovaries and spermathecae. The adult had queen mandibles, 146 ovarioles, a spermathecal diameter of 1.16 mm., and a pupal weight of 205 mgs. In general, this experiment was not as successful as the one in which eggs were grafted into queen cups.

Another method, in which brood cell bases containing eggs were transferred into cages with 200 and 400, 10 day old nurse bees, was done but without success.

C. Ovarial Development of Nurse Bees

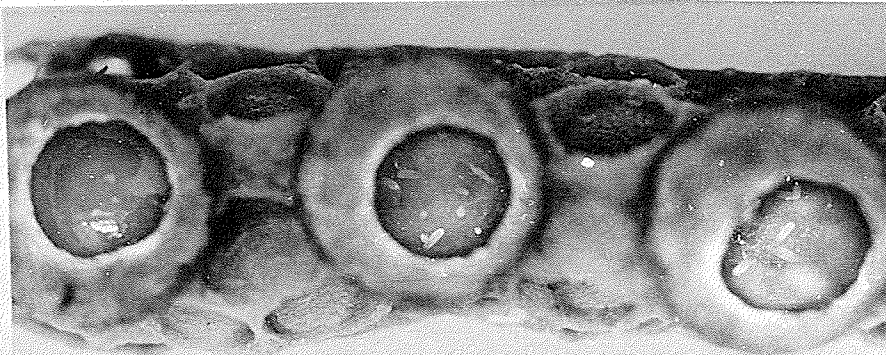
It was found that nurse bees, from 12 to 24 days old, which had been in a cage for at least 3 days laid eggs either in the empty plastic cups or in the wax cells that they built around the plastic cups (Figure 10). Three eggs hatched in 3B-50-1, one of the larvae from them lived for one day; two eggs hatched in one wax cell in 4A-200(d)-1 but the larvae from them lived for only one day. The larvae were supplied with a milky food by the nurse bees. Up to 40 eggs have been found in a plastic cup. Nurse bees in all size groups (50, 100, 200, and 400 bees) laid eggs in the tests.

D. Wax Production

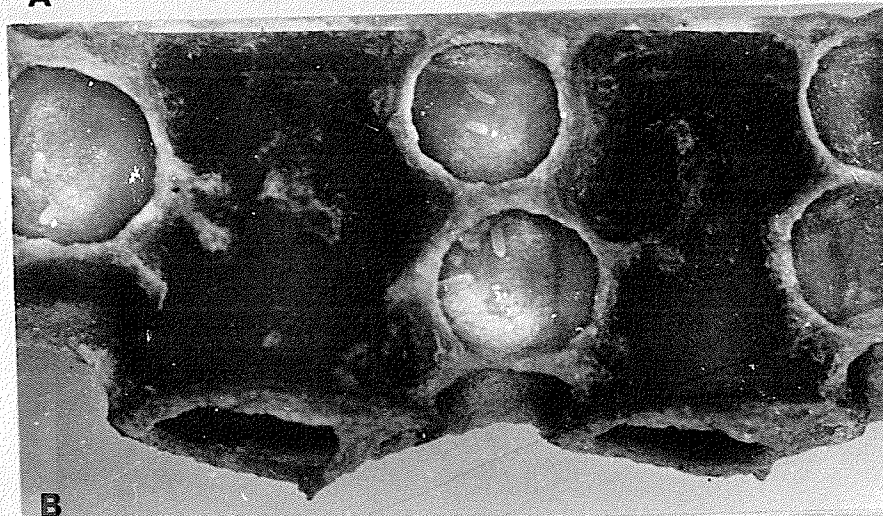
During the rearing period, the nurse bees in cages also produced

Figure 10 Eggs and Larva Produced by Worker Bees in Cages.

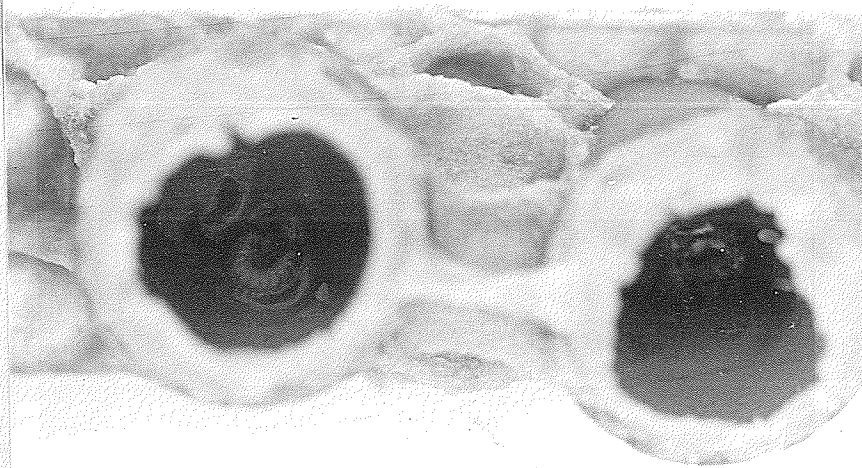
- A. Eggs laid within queen rearing cups.
- B. Eggs laid in wax cells on side of queen cup.
- C. Larva within queen cup.



A



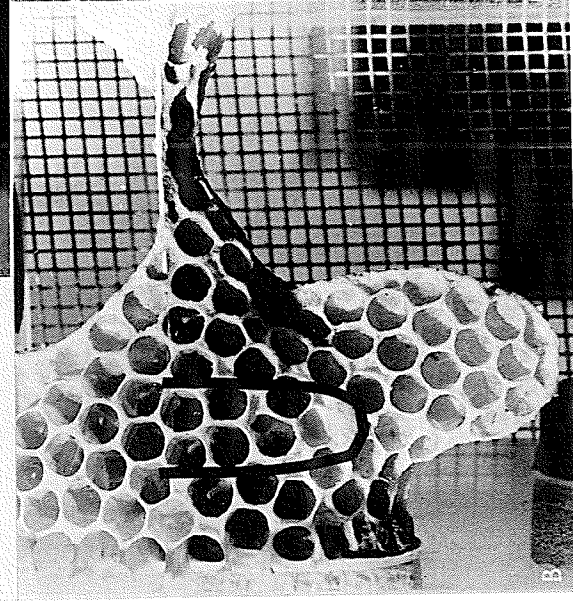
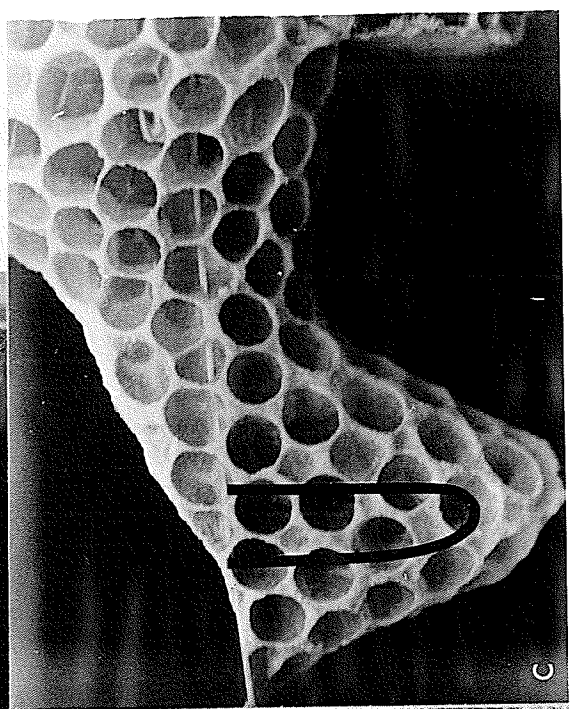
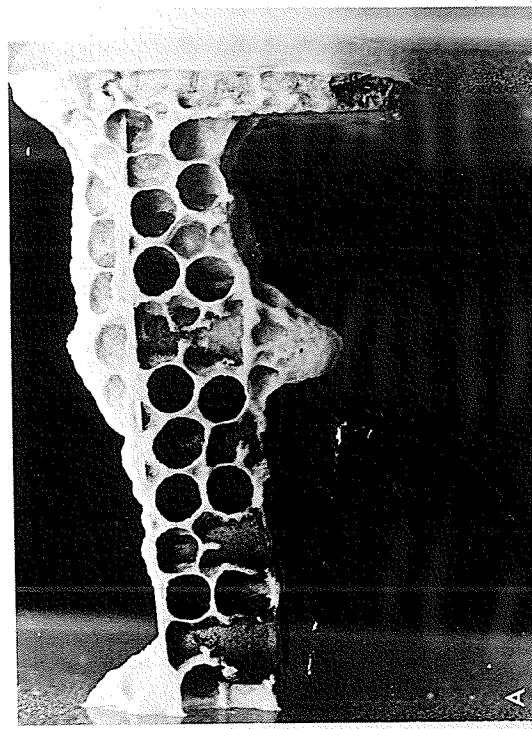
B



C

wax and built cells around the plastic cups. After the grafted larvae had been sealed in their cells, the nurse bees produced wax cells around the sides of the capped queen cells (Figure 11); the bees frequently filled these cells with honey collected from the food dishes. The nurse bees secreted fresh wax (white in color) and used it to build wax cells. The larger the number of nurse bees the more wax that was produced.

Figure 11 Wax Construction by Worker Bees in Cages.
A. 200, 5 day old bees.
B. 400, 10 day old bees.
C. 400, 5+10 day old bees.
The black lines indicate position of queen cells.



GENERAL SUMMARY

The experiments described in this thesis were done in the summers of 1967 and 1968. Groups of 50, 100, 200, and 400 nurse bees, 5, 7, 10, and of mixed ages, were kept in cages for rearing queen bees.

CHAPTER III

A "standard" plastic cage, 10 cm. x 8 cm. x 12 cm., was designed and built to confine and feed groups of nurse bees. One day before "grafting" (the transferring of female larvae from worker comb to artificial queen rearing cups) was done for an experiment, bees of a certain age (e.g. 4, 6, or 9 days old) were collected and placed in groups of 25, 50, 100, 200, or 400 bees in cages. These caged nurse bees were fed with honey, a mixture of ground pollen and honey (1:1 or 1:4 by volume), and water. Holding the bees in a queenless condition for 24 hours appeared to be necessary to ensure good acceptance of the larvae.

Newly hatched female larvae (some were of a selected genetic line, depending on the experiment), less than 12 hours old, were removed from a brood comb with a grafting hook and transferred into queen rearing cups (e.g. 3, 4, or 5 plastic queen rearing cups per cage, depending on the experiment) which had been cleaned by the bees and which contained a small drop of distilled water or a 1:1 mixture of royal jelly and distilled water. Both "direct" and "indirect" methods of grafting were used. The cages, containing the nurse bees and grafted larvae were kept in an in-

cubator at 30° C. or 35° C. with 40-60% relative humidity or in the Bee Flight Room which had the same conditions as the incubator except that the temperature ranged from 30-35° C.

Larval acceptance was checked 24 hours after the larvae were grafted directly or indirectly. The time of capping of the queen cells was also recorded. Five days after the cell was capped (i.e. 3 days after pupation), the capped queen cells were carefully removed from the cages and the pupae in them removed, measured, and returned to their cells in the same position they had occupied formerly. All newly emerged queens were injected with F. A. A. solution and then preserved in it for further measurements.

CHAPTER IV

The external and internal morphological characteristics of the adult bees obtained in this study were measured with a binocular microscope having an eye piece fitted with a linear microscale. Pupal measurements included the length of tongue and the length and weight of the pupa. The adult measurements used were the width and length of head, the length and width of the basitarsus, the diameter of the spermatheca, and the number of ovarioles of the right ovary. Other measurements included larval acceptance, the queen rearing "ability" of the nurse bees, the time of cell capping, and the total developmental time of the reared adult bees.

CHAPTER V

Groups of 50, 100, 200, and 400 nurse bees, of various ages from 5-18 days old, accepted larvae which were grafted either "directly" or "indirectly" depending on the experiment. The cages containing nurse bees and grafted larvae were kept at temperatures of 30°, 35°, or 30-35° C. (i.e. brood nest temperature); nurse bees accepted grafted larvae at each of these temperatures. Although larvae, which had been grafted "indirectly", appeared to be better accepted by the caged bees nevertheless sufficient numbers of larvae were accepted by the nurse bees when grafted "directly" to make the method feasible. It is possible for groups of queenless nurse bees of mixed ages to accept grafted larvae, as well as for bees to do so when they are as old as 18 days. In general, the 10 day old nurse bees had the highest larval acceptance for all groups especially when they were in groups of 200 and 400 bees. When low numbers (4) of larvae were grafted there was no significant difference between the numbers accepted by the groups of 200 and 400 nurse bees, 10 day old, but more larvae were accepted by the 400 nurse bees than the 200 ones when high numbers (8) of larvae were grafted ($P < 0.01$).

CHAPTER VI

The experiments outlined in this chapter are a continuation of the acceptance experiments of the previous chapter. Caged bees were able to rear queen larvae successfully until they were 22 days old. The groups of 50 and 100 bees, 5 days old, and ≤ 5 days old, and groups of 50 bees,

5+10 days old, seldom reared larvae successfully. The larger groups of the same age usually had a greater chance of rearing accepted larvae successfully; they also reared queens in longer cells and took less time to rear them from the time of grafting to capping. The groups of 200 and 400 bees, 10 days old, reared queens successfully when there was only one larva present per cage.

CHAPTER VII

The results outlined in this chapter are a continuation of those obtained from chapters V and VI. Several measurements were done to define the particular caste of the adults reared by the different numbers of nurse bees of various ages in cages. Many factors affect the "quality" of queens produced under natural conditions - e.g. weather factors, time of season when larvae are grafted, strength and genetic background of the colony, age of distribution of bees, stores in the hive, nectar and pollen flows, number of grafts put into the hive, experience of the queen rearer, etc. Thus it is difficult to use field controls for comparisons with laboratory experiments; therefore the comparisons of adults (or pupae) reared by different ages in combination with different numbers of nurse bees were done. However, field controls were reared to give some indication of the "quality" of the queens reared in these experiments. Most of the adults reared by the groups of different ages had queen-like mandibles, tongues, basitarsal indices, developmental times, spermathecae, and high numbers of ovarioles; a few intercaste and worker-like bees were

also produced. It was found that the number of ovarioles was reduced when more than one adult was reared by a single group of nurse bees. All groups of 10 day old nurse bees were able to rear queens, especially the groups of 200 and 400 bees; these two groups were able to rear queens whenever they had only one grafted larva, and when 8 or fewer larvae were present they were also able to rear a high number of adults.

CHAPTER VIII

Adult queens were reared from larvae, accepted by 200 and 400 nurse bees, 10 days old, and transferred to 25, 50, and 100 nurse bees, 10 days old. The adults reared in these tests with 50 nurse bees (which had received larvae accepted by 200 or 400 nurse bees) had higher ovariole counts and larger spermathecae than the adults reared by 50 nurse bees which had accepted the larvae themselves. Furthermore, queens with high ovariole counts and large spermathecae were reared by 25 nurse bees in these tests.

Queen-like adults were reared by 200 and 400 nurse bees, 10 days old, by grafting eggs into queen cups or by transferring pieces of comb containing eggs into the cages.

Caged nurse bees, from 12 to 24 days old, were found to lay eggs either in empty plastic cups or in wax cells which they had built around the plastic cups. Five eggs hatched and 3 of them lived for one day. Up to 40 eggs were found in one plastic cup. The wax glands of the caged nurse bees were well developed; the larger the number of nurse bees the

more wax they produced.

It is concluded that small groups (25, 50, 100, 200, and 400) of queenless nurse bees, 5, 7, 10, and of mixed ages are able to rear queen-like adults, especially the groups of 200 and 400, 10 day old ones. Too, small groups of nurse bees, confined in cages are able to lay eggs and produce wax. This technique might provide queens economically throughout a given season for use by the commercial beekeeping industry as well as provide a tool for studying caste determination, behaviour, and the development of the various glands of nurse bees.

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