THE UNIVERSITY OF MANITOBA

BIOLOGICAL FACTORS AFFECTING THE LABORATORY REARING OF QUEEN HONEY BEES

by

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ENTOMOLOGY

WINNIPEG, MANITOBA

MAY, 1973

ABSTRACT

Queen-like adult bees were reared in the laboratory by groups of 75, 100, 200 and 400, caged worker bees as a means of studying caste determination under controlled conditions. As few as 30 nurse bees could rear queen-like adult bees; however, some of these adult bees had intercaste characteristics.

When some of the laboratory reared adult bees were tested under field conditions they were able to mate and produce good brood patterns. Thus, it appears that adult bees, reared in the laboratory, not only have queen-like characteristics but also can function normally in hives. This technique therefore has commercial possibilities.

Larvae were exposed for different feeding periods to groups of 75 nurse bees without mandibular glands to ascertain if these glands secrete certain nutrients or a special substance(s) essential to queen differentiation. After a specific time larvae were fed by normal nurse bees for the balance of their feeding period. Although most of the resulting pupae and adults were queen-like some had intercaste characteristics. Generally, the longer the larvae were fed by bees without mandibular glands the smaller were the pupae produced from these larvae. Adult bees, reared by groups of 75 nurse bees, without their mandibular glands, were small but queen-like in appearance in all experiments. Adults

reared by groups of 75 nurse bees (without their mandibular glands) for 45 hours before being fed by normal bees, had lower ovariole counts and had smaller spermathecae than those reared in other experiments. Whether a substance or substances from the mandibular glands is essential for queen determination is still not certain; it is possible that queen-like adults were obtained because the larvae were not fed long enough by nurse bees, without their mandibular glands, to pass the supposed "critical" period of 72 hours for caste determination. Too, following the feeding of these larvae, by the bees without mandibular glands, they may have obtained from the normal bees some of the determining substance(s) and/or important nutrients necessary to develop into queen. The technique for removing mandibular glands should provide the bases for studying the chemical control of behavior in bees using the glandular material.

Caged worker bees, which had been queenless, usually had developed ovaries even when their mandibular glands were removed and/or they were feeding larvae. It also appears that some worker bees, with developed ovaries ("laying workers"), inhibit the ovary development of other worker bees.

ACKNOWLEDGMENTS

I wish to express my sincere appreciation to my advisor, Dr. S. C. Jay (Professor, Department of Entomology, University of Manitoba), for his continuous encouragement, guidance and helpful criticism during the investigation and the preparation of this thesis. My appreciation is also extended to Dr. A. J. Thorsteinson (Professor and Head, Department of Entomology, University of Manitoba), Dr. C. M. Wong (Associate Professor, Department of Chemistry, University of Manitoba), and to Dr. R. W. Shuel (Professor, Department of Environmental Biology, University of Guelph, Ontario). I am also indebted to Dr. U. Soehngen, Mr. D. Nelson and Mr. Y. Waikakul, for invaluable assistance received at various times throughout the project, and to student technicians, Miss V. Wright, Miss J. Lintott, Mrs. W. Laszlo, Mr. J. Lintott and Mr. D. Wright for help both with the field work and the laboratory work.

I also wish to thank my parents, Mr. & Mrs. F. Liu, for their encouragement throughout these years.

This investigation was supported by funds from the National Research Council of Canada.

INTRODUCTION

Female dimorphism of honey bees is thought to be determined trophogenically rather than genetically. The differentiation between the queen caste and the worker caste begins early in the larval stage, and becomes progressively more fixed. The first three days of the larval stage are bipotent, that is, the larva has the potential of developing into either a worker or a queen depending on the type of diet it receives. The qualitative differences between larval foods is considered to be the main factor in determining caste in honey bees. However, chemical analyses of the foods of young larvae of the two female castes show certain similarities. Therefore, the presence of an unstable differentiating component in royal jelly and/or of a different "balance" in certain essential nutrients could be the mechanism which controls the differentiation.

Recent research has indicated that the mandibular glands of nurse bees contain the determining substance(s). It seems that if queens could be reared using nurse bees, with their mandibular glands removed, it might show if this hypothesis is tenable.

In this study, therefore, an attempt was made first, to rear queens in the laboratory using small numbers of caged bees, and second to develop a technique for removing the mandibular glands from the nurse bees.

If a rearing technique, could be developed, it would have commercial queen rearing possibilities, while a technique for removing the mandibular glands from worker bees would have possibilities for studying the chemical control of behavior in bees using the glandular material.

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CHAPTER I

REVIEW OF LITERATURE

CASTE DIFFERENTIATION IN HONEY BEES

In the honey bee society, there are three distinct castes; queens, workers and drones. They are reared in three different types of cells, i.e., queen, worker and drone cells. The drones are male and are produced from unfertilized (haploid) eggs (Dzieron, 1845); the other two castes are female (Butler, 1634; Swammerdam, 1732; Huber, 1814), and arise from fertilized, diploid eggs (Sanderson and Hall, 1948, 1951; Ruttner and Mackensen, 1952; Hachinohe and Onishi, 1952; Rothenbuhler and Kulincevic, 1968).

Queen bees and worker bees, although genetically identical, differ in morphological and anatomical characteristics, as well as in their behavior in the colony. Although the fact that queen and worker bees arise from the same type of fertilized eggs has been recognised by naturalists, and shown by experiments, for some time (Schirach, 1771; Huber, 1814), the actual mechanism which determines how the female larvae differentiate into two different castes still remains largely unknown. Biologists have approached this problem in many ways, biologically, physiologically and chemically. However, the central question still remains

unanswered, and generates considerable interest on the part of biologists.

To date it has been well established that female caste determination is trophogenic in origin, i.e., determined by food factors (Brian, 1959). Very young female larvae are bipotent, or are not yet caste determined, and can develop either into a queen or a worker depending on the type of food they are fed.

I. BIOLOGICAL FACTORS

A. Age of the Larvae

In nature, queen bees can be produced under three conditions in a colony: swarming, supersedure or through loss of the queen. When the queen is lost from the colony worker bees select young worker larvae and build emergency queen cells. In this way, a new queen can be produced to continue the life of the colony.

Huber (1814) demonstrated that queens are produced from very young larvae rather than from larvae which are three or four days old. In one test, he removed larvae from queen cells and replaced them with others which were known to be only 48 hours old. Two queens emerged from five of the queen cells; in this experiment, Huber not only demonstrated that the bees could produce queens from young worker larvae but in doing he used a method of transferring larvae from worker cells to queen cells which is now used almost exclusively in rearing queens commercially.

When larvae, varying in age from one-half day to three days, are transferred from worker cells to queen cells, certain morphological characters in the resulting imagos are intermediate between queens and workers, depending on the age of the larvae which are transferred (Klein, 1904; Becker, 1925; Zander, 1925, cited by Ribbands, 1953; Vagt, 1955; Weaver, 1955, 1956, 1957). Other reciprocal transfer experiments, carried out during the bipotent period, illustrate the influence of larval age on the effectiveness of a given diet. Weaver (1957) found that when one year old larvae were transferred to queen cells for 24 hours and then returned to worker cells, they developed into normal workers, whereas two or three day old larvae similarly treated exhibited some queen characteristics. One day old larvae, transferred for two days and returned to worker cells, also developed some queen-like features. However, intermediate forms are quite common among laboratory reared bees (Jay, 1959, 1963, 1964; Smith, 1959).

Evidently, differentiation begins on the first day and is progressive. As the larval age, at the time of transfer, is increased, a higher percentage of workers and intermediate forms are obtained (Shuel and Dixon, 1960; Towsend and Shuel, 1956).

B. Physiological Aspects of Differentiation

From the queen rearing experiments and the reciprocal transfer experiments, it appears that caste determination

begins early in larval life. Confirmation of the early initiation of caste determination has come from various anatomical, metabolic, and biochemical studies.

1. Growth Rate

Bertholf (1925, cited by Smith, 1959) stated that up to the fourth molt (about 3 1/2 days old) both queen and worker larvae grow at approximately the same rate. Stabe's data indicate that for the first two days both queen and worker larvae grow at approximately the same rate. Worker larvae then grow more rapidly than do queen larvae, until at the age of 84 hours both are approximately equal in weight. From this point on, the queen larvae grow much more rapidly. According to his growth measurements, the critical stage of caste differentiation would lie between 72 and 85 hours in what would be the fourth instar (Townsend and Shuel, 1956). Wang (1965) found that after about 90 hours the growth rate of queen larvae increased, and by 102 hours they were 50% heavier than workers. These data agree with those of Stabe's for the period after 48 hours, but the data were contrary to that of Stabe for young larvae. It was found that the mean weight of worker larvae was greater than that of queens for every age group between 6 and 90 hours. Dixon and Shuel (1963) also found that laboratory reared larvae grew faster on worker jelly than on royal jelly from the first day. In incubator queen rearing, it was always noted that the larvae, which grew most rapidly, also reached a greater

maximum weight. Slower growing larvae were never among the largest (Smith, 1959).

Bishop (1961) found that the weight of queen larvae increased exponentially by a factor of five each 24 hours during the first four days after hatching; the weight of worker larvae increased similarly, but after two days, their increase was slower, and workers matured later than did queens. Worker larvae were heavier than queen larvae for the first four days (Wang, 1965). However, there is a great variability in the weight of larvae of the same age in both castes (Stabe, 1930; Nelson and Sturtevant, 1924; Smith, 1959).

Determination of a larva towards a queen or worker form is essentially complete by the time the larva has attained a weight of 20 to 46 mg (Von Rhein, 1933; cited by Dixon and Shuel, 1960).

2. Morphological and Anatomical Aspects

The development of the ovaries is similar in both queen and worker larvae for the first day. By the end of the second day, the queen ovary is considerably larger than the worker one (Zander et al, 1916; cited by Dixon and Shuel, 1960). However, Wang and Shuel (1965), using more precise methods, found no difference in ovary size or ovariole number between queens and workers before 90 hours. Although a qualitative difference appears on the second day, a certain degree of change of the reproductive system is still possible

(Ribbands, 1953; Weaver, 1957). The reduction in number of ovarioles in the worker during the prepupal stage and the retrogression of the spermatheca in the pupal stadium (Zander et al, 1916) are also evidence of an early dichotomy in queen and worker development.

Other anatomical differences between queen and worker, such as size of ovaries (Von Rhein, 1933; Waver, 1955, 1957), number of ovarioles (Von Rhein, 1933; Smith, 1959; Weaver, 1955, 1957), size and shape of spermatheca (Von Rhein, 1933; Weaver, 1957; Vagt, 1955), size and shape of the abdomen (Weaver, 1955, 1957), characteristics of the meta-thoracic legs, the sting and mandibles (Von Rhein, 1933; Smith, 1959; Weaver, 1955, 1957), tongue length (Weaver, 1955, 1957; Smith, 1959), size of the mandibular glands (Von Rhein, 1933; Weaver, 1955, 1957), and duration of the development period (Weaver, 1955), are considered as criteria of female dimorphism.

3. Biochemical and Metabolic Aspects

The cytological studies of Mickey and Melampy (1941) show that nuclear fragmentation in fat cells begins during the third larval day in the queen and a day later in the worker. Chemical analyses of larval tissue by Melampy et al (1940) have revealed marked differences between castes in 3 to 4-day old larvae. The tissue of queen larvae contains a higher percentage of lipids and a lower percentage of nitro-gen and reducing substances. During the next 2 or 3 days

there is a substantial percentage increase in reducing substances in the queen but little change in nitrogen or lipids. In the worker, there is a marked reduction in nitrogen accompanied by a moderate increase in lipids and a large percentage increase in reducing substance.

An investigation of the changes in haemolymph protein in developing larvae of both castes shows that throughout the larval stage the queen caste has fewer haemolymph protein fractions than has the worker caste. Protein concentration is highest at 48 hours and lowest at 72 hours. The queen larvae show lower total haemolymph protein around 72 hours (Liu and Dixon, 1965). Before 72 hours, tissue protein is higher in workers (Shuel, 1963). It is concluded that haemolymph protein concentration reflects developmental differences both with age within a caste and between castes. The differences may be caused by the diet and/or hormonal regulation (Liu an Dixon, 1965). This suggests that haemolymph protein can be used as an indicator of caste differentiation in the early larval stages.

There is a qualitative difference in amino acids in the haemolymph of female larvae. In both castes, the total amino acid concentration increases at first and then, around the third day in the worker and the 4th day in the queen, it undergoes a steady decrease with age. The amino acid concentration is roughly the inverse of protein concentration in the haemolymph. Since there is a striking difference in concentration of aspartic acid with time, both within castes

and between castes, it is suggested that this acid might be a criterion of dimorphism on a molecular level (Lue and Dixon, 1967).

Using ^{14}C labelled sugar and aspartic-^{14}C acid, fourteen amino acids were defined as essential amino acids for larva development. Comparative radioautographic studies showed that worker larvae haemolymph contained more proteins and amino acids synthesized from sugars than did that of queen larvae (Lue and Dixon, 1967).

Haemolymph esterase patterns differ qualitatively and quantitatively with age both within a caste and between castes. Esterase activity is significantly higher in workers until they are 72 hours old and thereafter show a general decline. In contrast, esterase activity in queens remains significantly lower for about 72 hours and thereafter increases (Tripathi and Dixon, 1968). The correlation between esterase activity and development has been described for various insects. Qualitative and quantitative differences in haemolymph esterases have been found, not only between species, but also during development within a species. Clements (1967), for example, showed that esterase patterns of gut and haemolymph varied during different stages of the life cycle of Pieris brassicae L. Similar changes in esterase patterns have also been found in Hyalophora cecropia and Samia cynthia (Laufer, 1960, 1961); Musca domestica L. (Menzel et al. 1963; Van Asperen Mazijk, 1965); Bombyx mori L. (Eguchi et al., 1965; Eguchi and Sugimoto, 1965); Periplaneta america-

na L. (Cook and Forgash, 1965), and Oncopeltus fasciatus (Dallas) (Salkeld, 1965).

Differences in respiratory activity between castes are also manifested early in larval life. Two to three day old queen larvae have a much higher rate of gas exchange than do worker larvae when measured with the direct Warburg method (Melampy and Willis, 1939). Cartesian diver measurements of gas exchange in larvae during the first 24 hours, have revealed a much higher net carbon dioxide evolution on a substrate of royal jelly than on a diet supplied to worker larvae during the first three days (Shuel and Dixon, 1959). Rembold and Hanser (1964) found that groups of laboratory reared larvae exhibiting comparatively high average rates of oxygen uptake contained more individuals that developed into queens than did groups with comparatively low rates of oxygen uptake. Shuel and Dixon (1968) also studied the differences between queen and worker larval oxygen consumption. The larvae were fed on worker jelly or royal jelly. They found that the dichotomy between the two castes, with respect to oxygen consumption, appeared at about 50 hours. It was characterized by a progressive decrease in the rate of uptake by worker larvae and a progessive increase in queen larvae. During this period, oxygen uptake was sensitive to the influence of diet. The fact that the divergence in rates of oxygen uptake between larvae from queen and worker cells is a two-way divergence from a common level suggested that the worker represented a distinctive form of development from a

common prototype, and that it should not be regarded merely as a "failed queen." Oxygen consumption varied rapidly with season in queen larvae, but not in worker larvae. It has been pointed out that if the rate of oxygen consumption is independent of diet, it could be used as an indicator of caste differentiation (Weaver, 1966). However, Shuel and Dixon (1968) concluded that although oxygen consumption is higher in queen larvae, caution is necessary in adopting absolute rates as indicators of caste, because of dietary and seasonal influences.

The oxygen activity of mitochondrial enzymes in the queen is higher than in the worker, especially in the larval stage. Both castes are deficient in cytochrome C in the early developmental stages, and worker larvae contain less cytochrome oxidase than do queen larvae (Osani and Rembold, 1968).

Evidently, metabolic differences are established very early in the larval period and are a reflection of nutritional differences.

II. NUTRITIONAL FACTORS

A. Quantitative Differences in Larval Food Supply

In nature, queen larvae are reared in queen cells, which are larger in size and supplied with so much food that a surplus is usually left uneaten when the larvae pupate. Worker larvae are reared in much smaller sized worker cells, and receive an excess of food for only the first three days;

later they are supplied with a smaller quantity of food (Nelson and Sturtevant, 1924). Haydak (1943) supposed that differentiation between queens and workers was not due to a change in the composition of the food, but rather due to the amount of essential nutrients consumed by the queen and worker larvae, respectively. He cited the conclusions of other researches in support of his hypothesis, and pointed out that from the time of hatching until about the third day of life all female larvae are supplied with a greater amount of food than they can eat, and that queen larvae continue to be fed in this surplus manner; worker larvae however, are fed at intervals and are undernourished. He suggested that only richly nourished queen larvae acquire fully developed ovaries, and that the ovaries secrete enough hormones to produce the secondary sex characters of queens. Because the worker larvae are undernourished, the ovaries are not well developed and therefore do not secrete hormones in sufficient amounts to cause the larvae to develop into queens. In his experiments he was able to rear normal queens and workers as well as intercaste pupae to support his hypothesis; his results have been verified through other laboratory rearing and starvation experiments (Jay, 1959, 1963, 1964; Weaver, 1957; Smith, 1959). However, most authors agree that starvation is not the only mechanism which determines the queen.

B. Qualitative Differences in Food Supply

1. Evidence from Rearing Experiments

From the results of laboratory rearing experiments, Von Rhein (1933) proposed that queen differentiation takes place in two steps. First, the bipotent larva is predetermined by feeding on royal jelly of young larvae and then the change to royal jelly of older larvae brings about the final step in queen differentiation. Therefore, queen larvae receive a different kind of food at the beginning of the differentiation than they do later in their development. He believed that queen larvae receive a specific secretion directly from the nurse bees, instead of having it deposited in the cells. Other researches have not been able to verify this (Weaver, 1958; Smith, 1959; Jay, 1959, 1960).

Biological differences in larval food, from different sources have been shown in queen rearing experiments in the laboratory (Smith, 1959; Weaver, 1955, 1957). Smith's incubator-reared larvae, although they grew well on young worker jelly, always failed to pupate and no adults were reared on this food. However, the failure to pupate could be corrected by adding sugar to worker jelly (Shuel and Dixon, 1968). Similar results have been obtained with drones (Takeuchi et al., 1972). Feeding tests using royal jelly taken from cells containing young larvae resulted in such poor larval development that they usually died before completing their development. Queens were often reared on the royal jelly taken from cells containing older larvae

without being given the "predetermining" feeding of royal jelly of young larvae which Von Rhein believed was necessary.

Based on the results of feeding larvae on fresh royal jelly vs stored royal jelly, it has been proposed that some substance(s) in royal jelly initiates or controls, the differentiation of queens, and that some part of it is either unstable or is not available to larvae when it has been stored (Weaver, 1955, 1958). The qualitative differences between larval food have been demonstrated through rearing experiments and chemical analyses. With respect to the significance in caste differentiation, it is suggested that some substance(s) in royal jelly, which initiates or controls the differentiation of queens, could be an essential nutrient or queen-differentiating factor, or a substance functioning as both (Jay, 1964).

Evidently, nutritional factors play an important role in caste differentiation. As a consequence, the comparative analyses of larval food and the determining whether an assumed differentiating substance(s) is present are indispensable to the understanding of the mechanism of caste determination.

2. Chemical Comparison of Larval Foods

Many chemical analyses have been done relating to the chemical composition of royal jelly and worker jelly (see reviews by Ribbands, 1953; Johansson, 1955, 1958; Rembold, 1960, 1964, 1965; Shuel and Dixon, 1959). It is generally

accepted that larval foods fed to young larvae in worker and queen cells and to older larvae in queen cells is similar in composition. The modified worker jelly (Haydak, 1690; Shuel and Dixon, 1959), which is fed to older worker larvae, differs from the other food because it contains pollen and honey (Ribbands, 1953).

Comparative analyses of larval foods fed to queens and workers of different ages has been done by Elser (1929), Haydak (1943), and Shuel and Dixon (1959). Shuel and Dixon (1959) found a distinct difference in the protein and carbohydrate content of foods fed to worker larvae and queen larvae of 0-30 hours old. Worker jelly is higher in protein but lower in carbohydrate than royal jelly. The quantity of carbohydrate and protein in royal jelly from queen cells with larvae 0-30 hours compared to those with larvae 72-96 hours old remains constant. However, honey is added to cells containing worker larvae, 72-96 hours old, and therefore the quantity of protein is reduced and the quantity of carbohydrate becomes substantially greater. Lipid content is slightly lower in worker jelly than in royal jelly (Shuel and Dixon, 1959; Butenandt and Rembold, 1957). Lipid content of royal jelly varies little with the age of the queen larvae, while in worker jelly, it decreases with the age of the worker larvae. There is a decrease in water content of worker jelly taken from cells containing older larvae (Haydak, 1943, 1957; Smith, 1959; Dietz and Haydak, 1967). In royal jelly this change is reversed; the moisture content increases with the

age of the larvae. This gradual change in the moisture content of larval food may be directly related to the different growth patterns of these larvae, and according to Dietz and Haydak (1967) may be instrumental in initiating caste differentiation.

Electrophoretic studies of larval foods show that there are no qualitative differences in the proteins of the food of young queen and worker larvae, or of the food of the older larvae. In worker jelly, the weakening of the electrophoretic bands after the third day may be due to the dilution of protein by the addition of honey (Habowsky and Shuel, 1959; Patel, Haydak and Gochnauer, 1960). However, qualitative electrophoretic studies strongly suggest that female dimorphism is not attributable to qualitative variation in the protein fraction of the diets (Habowsky and Shuel, 1959). Quantitative analyses of protein show that total protein content remains constant in royal jelly from cells with larvae of any age, while in worker jelly, there is a marked drop around the third day (Habowsky, 1958). It is suggested that the quantitative variation in proteins might contribute both to differences in form and differences in growth rate of the larvae (Shuel, 1959; Rembold, 1964).

Rembold (1960), using UV absorption, found no qualitative differences between royal jelly and worker jelly amino acid content, but found quantitative differences in phenylalanine and glycine in the larval food.

Mitsui and Sagawa (1966) did quantitative and quali-

tative analyses of nucleotides of worker and royal jelly from cells containing larvae of different ages; differences were found to be quantitative rather than qualitative. The total amount of nucleotides was highest in the royal jelly from queen cells containing larvae 48 hours old, and lowest in worker jelly and royal jelly collected from cells containing 72 hours old worker larvae or queen larvae respectively.

Chemical analyses also show that there are quantitative differences in the vitamin content of royal jelly and worker jelly (Rembold and Lingens, 1959). Royal jelly is rich in pantothenic acid, biotin, folic acid and biopterin. However, in spite of quantitative differences in these biological active substances, none of them is thought to be the caste-determining substance (Rembold, 1964). An active dialyzable fraction has been extracted from royal jelly, which is necessary for the growth of the differentiation regulating mechanism (Weaver, 1956, 1962). The presence of the differentiating component in royal jelly has also been demonstrated by means of rearing experiments (Hanser and Rembold, 1964). Rembold found that the determining principle is located in the lower molecular, water soluble components of royal jelly, and can be enriched into a chromatographic "fraction F." The absence of this fraction results in a total loss of the determining effect in larval rearing experiments. If "fraction F" is added at a concentration of about twice that found in the native state a highly active larval food is obtained which causes the majority of the adults reared on it to develop into

intermediates or queens. It can also be concentrated from the heads of nurse bees. Whether it consists of a mixture of chemically very similar compounds or of a single substance, has not yet been ascertained. Rembold (1969) proposed that caste formation is a consequence of hormonal deficiency in a certain stage of imaginal development. The function of the determining substance, which may be identical with the deficient hormone, is to repair this deficiency.

III. HORMONAL ASPECTS AND NUTRITIONAL BALANCE

The role of hormone balance in metamorphosis has been thoroughly reviewed by Wigglesworth (1954). He regards metamorphosis as a special case of polymorphism. Data to date indicate that the general concept of hormone balance in relation to metamorphosis is applicable to the honey bee (Fyg, 1956, 1959; Schaller, 1951, 1952; Lukoschus, 1955, 1955, 1956, 1956, cited by Shuel and Dixon, 1960).

In the honey bee, metabolic, biochemical, chemical, physiological and histological evidence suggest that the endocrine system mediates between diet and form, in the initiation of caste differences during the first 3 or 3 1/2 days of larval life, and in the completion of these differences in the post-determination period (Shuel and Dixon, 1960). Within the first two days of larval life, distinct histological differences between the corpora allata of the two castes have been shown (Canetti et al., 1964). During the period of caste determination, the corpora allata appears to play an

intermediary role, and is the only endocrine gland in which conspicuous caste differences are visible. Differences in other endocrine organs are apparent at 96 hours after hatching (Ritcey, 1969; Ritcey and Dixon, 1969). Caste differences in size and appearance are indicative of greater activity in the corpora allata of the queen larvae. In addition to their role in maintaining juvenile characters throughout insect larval moults, the corpora allata produce a secretion identical with the juvenile hormone and is involved in lipid and protein metabolism during ovary development (Chai and Shuel, 1970). Juvenile hormone activity has been found in farnesol and its methyl derivatives (Wigglesworth, 1963). The development of ovaries is closely related to the activity of the corpora allata. The retrogression of the reproductive system of the worker honey bee normally begins about the fifth day of larval life, after caste has been determined. Implantation of corpora allata from queen larvae, 3 or 4 days old, into a worker larva 4 days old reduces the degree of retrogressive development; implantation of corpora allata from 3 day old worker larvae did not give this effect. These data suggest that the corpora allata of queens are involved in the post-determination development of her reproductive system, and that the corpora allata of 72 hours workers are less active in promoting reproductive development (Chai and Shuel, 1970). This is also consistent with the histological evidence of Canetti et al (1964). Corpora allata of 48 hour old worker larvae are capable of

activating the accessory glands of Periplaneta female. Corpora allata from queen larvae of the same age are repressive in action. By 72 hours corpora allata of worker larvae have reduced activity but queen larvae of the same age have allata of the activating type. Thus, corpora allata show a duality in function during caste development (Dixon and Moser, 1972). The experimental effects on ovariole numbers are consistent with the hypothesis of endocrinal control over retrogressive changes in the reproductive system of the worker honeybee (Chai and Shuel, 1970).

Rapid development of the prothoracic glands in the queen around 4 days has been observed (Lukoschus, 1952). A marked reduction in the size of the neurosecretory cells of queen larvae has also been observed and has been interpreted as indicating a massive release of neurosecretion around 96 hours (Ritcey, 1969). However, its significance in differentiation is not clear.

IV. ORIGIN OF LARVAL FOODS

A. Food Sources

In 1888, Von Planta stated that larval foods of the honey bee were glandular in origin and Koehler (1922) confirmed that worker larvae, as well as queen larvae, are fed such secretions. The secretion of larval foods mainly involves the hypopharyngeal glands, mandibular glands and salivary glands (Kratky, 1931; Ribbands, 1953; Snodgrass, 1956; Simpson, 1960, 1961).

1. Hypopharyngeal gland

The contribution of the hypopharyngeal glands in larval food has been confirmed by chemical studies (Schiemeny, 1883; Langer, 1912, cited by Ribbands, 1953). Judging from the pH, it is known that the clear substance in royal jelly is from this gland and from the crop. The milky-white secretion is a mixture of secretions of the mandibular and the hypopharyngeal glands (Jung-Hoffmann, 1966). Comparative paper chromatography and electrophoretic studies also support this conclusion (Jung-Hoffmann, 1966). The protein component in larval food is secreted from the hypopharyngeal glands of the nurse bees (Kratky, 1931; cited by Ribbands, 1953; Patel et al., 1960; Simpson, 1961; Jung-Hoffmann, 1966; Rembold, 1964). The comparative biochemical analyses of larval food and glandular extractions show that this gland also contributes a high amount of lipid material (Patel, Haydak and Lovell, 1961; Rembold, 1964). The gland is high in free fatty acids and weak acids (Rembold, 1964). Hypopharyngeal glands also contribute 10-hydroxy-Δ^2-decenoic acid, which is the main fatty acid found in worker and royal jelly (Rembold and Hanser, 1960). The heterocyclic component, characteristic of queen and worker foods, is only found in the hypopharyngeal glands. It has been suggested that the basic food product of the hypopharyngeal glands is worker brood food (Rembold, 1964).

2. Mandibular glands

Simpson (1961) suggested that the mandibular glands produce the fat in the larval food (1961). It has been shown that the mandibular glands contain 10-hydroxy-Δ^2-decenoic acid (Barker et al., 1959; cited by Shuel, 1963). This fatty acid has been found also in the hypopharyngeal gland (Rembold and Hanser, 1960). These glands contribute more pantothenic acid and biopterin than do the hypopharyngeal glands. When environment changes occur, such as during queen rearing activity, the amount of these vitamins increases in the mandibular glands, but remains at the same level in the other glands (Rembold and Hanser, 1964). On the basis of comparative analytical and histoautoradiographic studies, Rembold and Hanser (1964) have suggested that, during queen rearing, the activated mandibular glands of the nurse bees secrete certain substances into this basic brood food and further that the mandibular glands synthesize the determining substance(s) (Hanser and Rembold, 1964).

3. Salivary Glands

The salivary glands include the postcerebral and thoracic glands. They are possible sources of dietary components (Towsend and Shuel, 1962). Both glands contain low amounts of biopterin and pantothenic acid (Hanser and Rembold, 1964). The quantity of biopterin and pantothenic acid in the thoracic gland changes slightly with changes in the environment; however, no such change occurs in the postcerebral gland.

B. Age of Nurse Bees

The hypothesis that either nurse bees are able to change the compositon of the hypopharyngeal gland secretions at will, or that different age classes of nurse bees provide qualitatively different foods to larvae of different ages and castes is improbable (Shuel, 1956). Evidence to date show that a nurse bee can feed larvae of any age. Nurse bees, even 89 or 105 days old, appear capable of rearing queens (Haydak, Patel and Dietz, 1964). Hypopharyngeal glands begins to fill when worker bees are 4 days old, and start to degenerate when they are 15 days old (Haydak, 1957). Laidlaw and Eckert (1950) also stated that these glands are active when the adults are 5-16 days old. Electrophoretic studies of different glands and larval foods show that fully active hypopharyngeal glands are the source of young brood food protein (Patel, Haydak and Gochnauer, 1960). Nurse bees, one day old, have their mandibular glands full of a milky secretion. Bees, 69 days old, and the majority of winter bees (even when they are 90 days old), also have their mandibular glands full of a milky secretion (Ribbands, 1953). However, in worker bees, the mandibular gland is also involved in the secretion of pheromones; whether the worker bee is involved in larval food secretion or in pheromone secretion depends on the physiological activity of the worker bees rather than chronological factors (Simpson, 1966; Simpson et al., 1968; Boch and Shearer, 1967). There is histological evidence suggesting that the corpora allata may

regulate the function of the hypopharyngeal glands (Laere, 1965). Kratky (1931) stated that the salivary glands did not vary with the age or occupation of the worker bees. However, other evidence shows that, the secretory activity of the salivary glands varies with temperature, the presence of larvae, and flight behavior. The abundance of oil in the postcerebral glands (head glands) is correlated with an increase in the age of the worker bees, a change to foraging duties, or a retrogression of the hypopharyngeal glands; however, the thoracic gland shows little change with age or occupation (Simpson, 1960).

OVARIAN DEVELOPMENT OF WORKER BEES

It has been known for a long time that if the queen is removed from a colony of honey bees, the ovaries of some of the workers will develop from their normal rudimentary condition and the appearance of laying workers will occur. Butler (1954), De Grott and Voogd (1954) and Pain (1954) independently proposed that a substance produced by the queen is involved in the inhibition of ovary development in worker honey bees. Later, the significance of the queen's mandibular gland in this inhibition was revealed (Butler and Simpson, 1958). The pheromone 9-Oxo-decenoic acid (Barbier and Lederer, 1960; Barbier, Lederer and Nomura, 1960; Butler, Callow and Johnston, 1962) and 9-hydroxy-decenoic acid (Butler, Callow and Chapman, 1964; Brown and Felauer, 1961) were isolated from the queen's mandibular glands, and synthesized

artificially. The 9-oxo-trans-2-decenoic acid (usually called queen substance), only partially inhibits ovary development in worker honey bees (Butler and Fairey, 1963) but has a strong inhibitory effect on queen cell construction (Morse, Gary and Johansson, 1962; Pain, 1961; Butler, 1961). The 9-hydroxy-trans-2-decenoic acid contains an "inhibitory scent." However, queens without mandibular glands can still inhibit ovary development (Gary, 1961; Morse, Gary and Johansson, 1962; Velthuis and Van Es, 1964). This suggests a second source of inhibitory material which appears to come from the abdomen of the queen (Velthuis, 1967). There is evidence suggesting that other queen pheromones are produced in glands on the abdomen, most probably in the glands described by Renner and Bauman in 1964 (Velthuis, 1970).

In queenless colonies, the ovaries of many worker bees develop but only a few laying workers appear (Perepelova, 1926, 1928; Dreischer, 1956; Sakagami, 1959). Evidence suggest that laying workers, or workers with the most highly developed ovaries, have an inhibitory effect on ovarian development in other bees (Sakagami, 1959; Velthuis et al., 1965; Jay, 1967; Velthuis, 1970). Jay (1967, 1970, 1972) also showed that worker larvae or pupae can inhibit the ovarian development of worker bees in queenless hives.

CHAPTER II

GENERAL METHODS

MATERIAL

I. TYPES OF CAGES

Two types of plastic cages were used to confine and feed groups of nurse bees: a larger sized cage (Type A) and a smaller sized one (Type B). Type A cages were used to confine bees of more than 100 bees, while Type B cages were used for groups of bees of less than 100 bees.

A. Type A Cage

Type A cage measured 10 cm. x 8 cm. x 12 cm. (see Figure 1). Two of its sides were covered with removable metal screens (with apertures about 6 mm. square). Two round holes, 2.5 cm. in diameter, were made in the top of the cage, one for a water tube and one for a sugar solution tube. 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. A piece of waxed aluminium bar, with 3 plastic queen cups attached to it, was fitted to one of the special corks. Before placing bees into a cage, the cork, with queen cups attached, was fitted into the oval hole. Two round holes, 2.5 cm. in diameter, were drilled

directly beneath the oval holes and fitted with feeding trays, one on each side. On the opposite side of the bottom round holes, which were used for the feeding trays, four small round holes (0.3 cm. in diameter for each), two on each side, were drilled and used to fasten the two feeding trays to the cage itself.

B. Type B Cage

Type B cage measured 7.5 cm. x 5 cm. x 10 cm. (see Figure 2). Two of its open sides were covered with removable plastic wire screens with apertures about 1 mm. square. A round hole, 2.5 cm. in diameter, was made on the top of the cage to hold a water tube. Another two round holes (both 2.5 cm. in diameter) were drilled on the top and bottom parts of the back piece of the cage. The upper hole was used to hold queen cups by means of a piece of aluminum bar fitted to a cork. The bottom one was used to hold the feeding tray.

II. FOOD

Two semi-tubular plastic food trays (9 cm. in length, 2.2 cm. in diameter) were used for feeding the bees in the larger cages. One tray was filled with honey and the other one with a honey and pollen mixture. Only one tray was used with the smaller cages, and it was divided into two compartments with beeswax. One compartment contained honey and the other one a honey and pollen mixture. This feeding tray was inserted into a tube made of wire screen and through which

the bees could feed (see Figure 1 and 2).

Ground pollen and honey (1:3 by volume), and pure honey were put into the food trays daily. Two sterilized glass tube feeders (Figure 1), one filled with 60% sugar solution, the other one filled with water, were placed on top of the cages during all tests. Only water tubes were supplied to the bees in the small cages.

III. NURSE BEES

Frames, containing capped brood (of a yellow strain) just about to emerge, were incubated for one day at 30-35°C. On the following day, large numbers of newly emerged worker bees were introduced into colonies of dark bees (Caucasian), in order to obtain bees of a known age at a later date. One day before a "grafting" was done, or the mandibular glands were removed from the nurse bees (i.e. when the bees were 9 days old), the bees were removed from the hives and placed in cages in groups of 30, 75, 100, 200, or 400. The caged bees were supplied with food and kept queenless in an incubator for 24 hours, if they were to be used for queen rearing, or for one or two hours if they were to have their mandibular glands removed.

IV. GRAFTING

Plastic queen rearing cups (1 cm. in diameter) were attached with melted beeswax to a 6 cm. x 1.3 cm. strip of aluminum connected to a cork (see Figure 3). Two queen cups

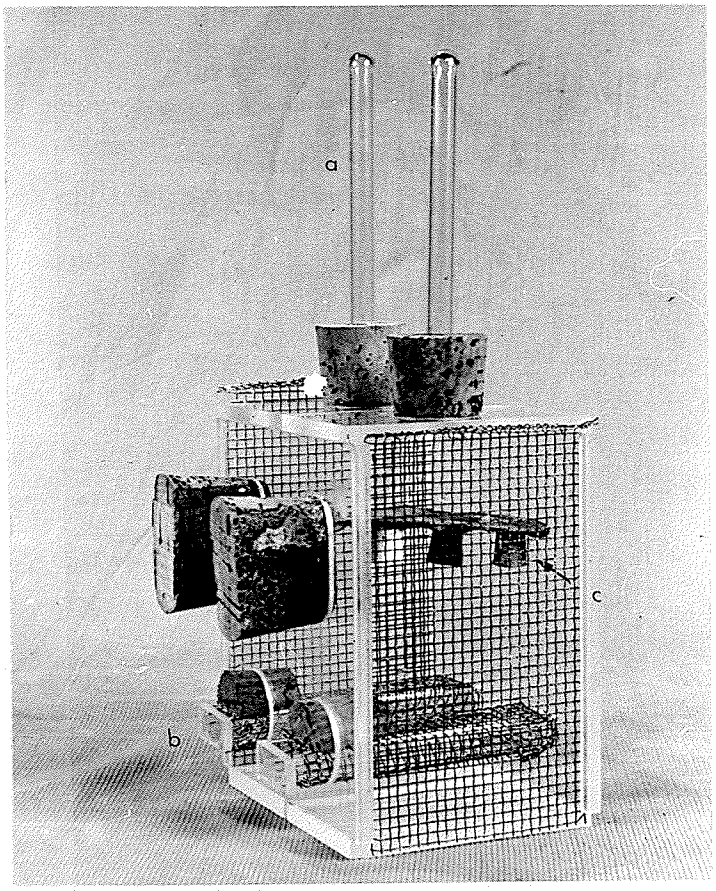


Figure 1. Type A cage.

Water tubes (a) and two removable feeders (b) were used for supplying food. Plastic queen-cell cups (c) were used for rearing queens.

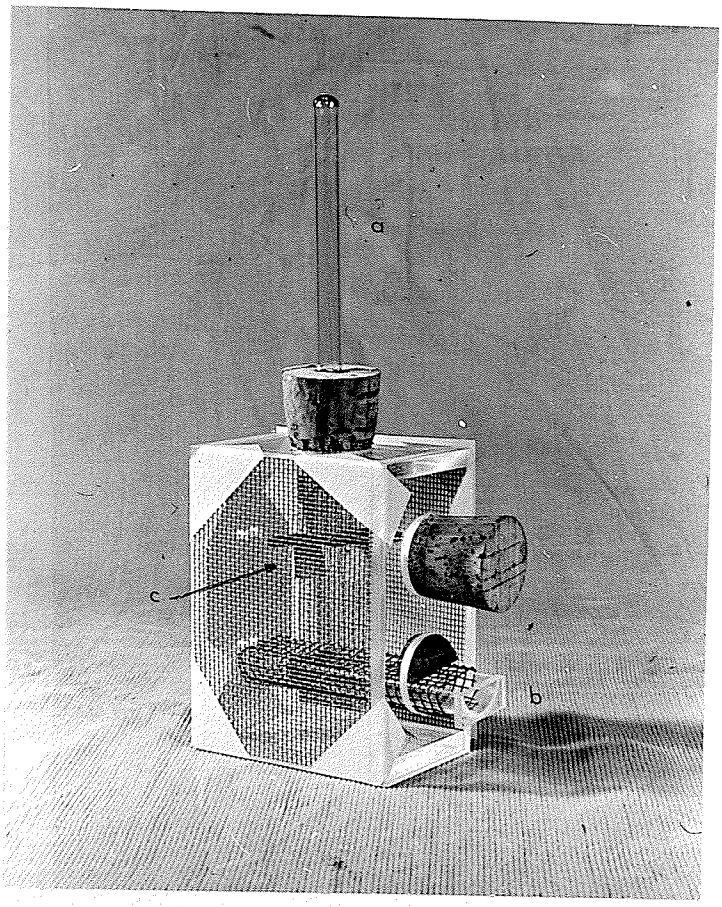


Figure 2. Type B cage.
Water tube (a), feeder (b), and
plastic queen-cell cups (c).

were used in the small cages and three queen cups were used in the big ones.

Before an experiment was begun, a metal strip, with queen rearing cups fastened to it, was installed inside the cages so that the bees could clean them; this appeared to assist in acceptance of the larvae by the bees. Young larvae, less than 12 hours old, were used in the rearing experiments, while larvae less than 6 hours old, were used in the dissection experiments.

Brood frames, containing newly hatched female larvae, were brought into the laboratory and placed in "grafting stands" (see Harcus, 1967). Larvae were removed from brood combs using a grafting hook and tranferred into queen rearing cups which had been cleaned previously by the caged bees, and into which had been placed a small drop of distilled water. These larvae were then placed directly into the cages containing the bees.

V. INCUBATION

The cages containing the worker bees and grafted larvae were kept in incubators at 30-35°C, with 40-60% relative humidity.

When any queen cells were capped by the bees they were removed from the cages and placed separately in glass vials (2 cm. in diameter and 5 cm. in height). These vials, containing queen cells, were then incubated at 35°C until the queens emerged (see Figure 4 and 5).

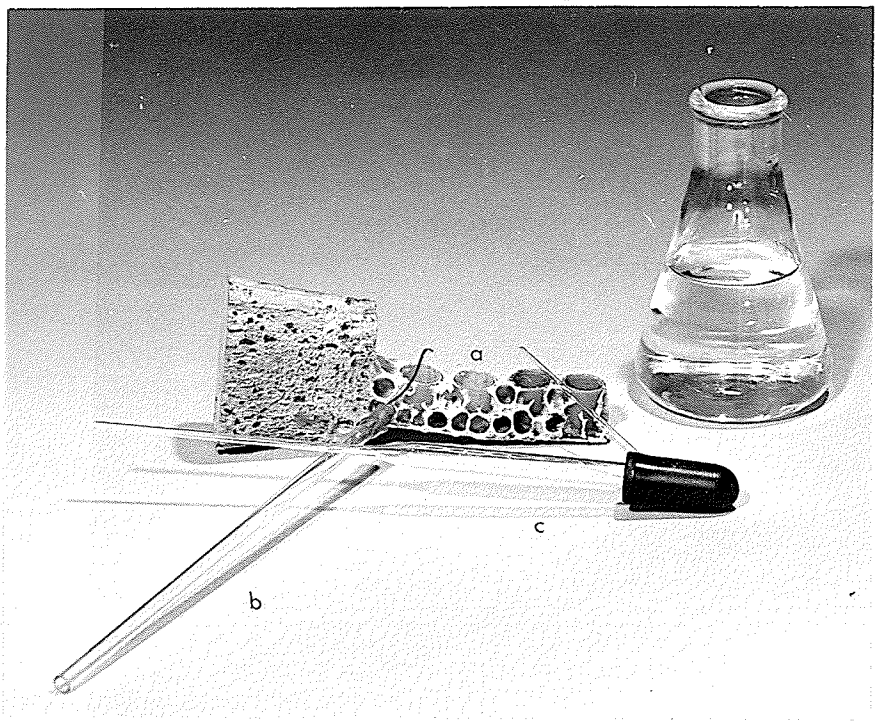


Figure 3. Plastic queen-cell cups and grafting tools. Queen cups (a) attached to a strip of aluminum, grafting hook (b) and eye dropper (c).

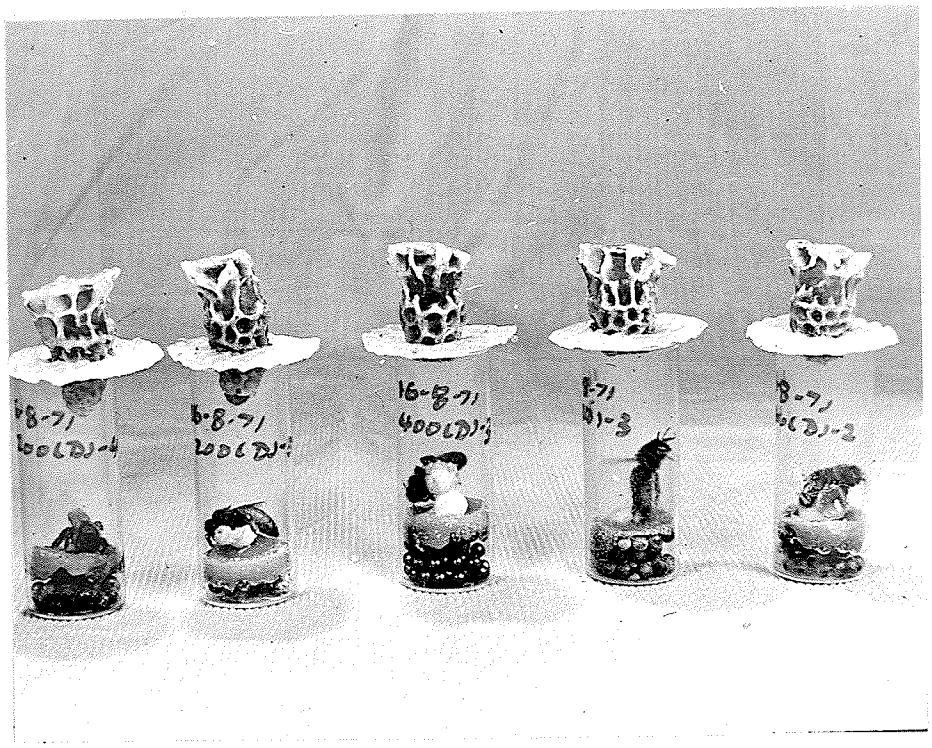


Figure 4. Caged worker bees in an incubator.

Figure 5. Vials into which queens emerged from their cells.

VI. DISSECTION TOOLS AND APPARATUS

A. Dissection Tools

Electrolytically sharpened No. 5 Dumont Tweezers were used for grasping and removing the mandibular glands. Irex microscalpels were used in the dissections. A hook was made from an insect pin and a wooden swab stick. The hook, which was also sharpened electrolytically, was used in removing the mandibular glands from the nurse bees. All of the dissecting tools were sterilized in an autoclave before all operations. During dissections, cotton-wool balls, soaked in 70% ethanol, were used for cleaning all dissection tools.

B. Dissection Apparatus

The dissecting apparatus consisted of two parts, the dish-holding stand and the dissecting dish itself. The dissecting dish was also made of two parts consisting of a plastic petri-dish (9 cm. in diameter), and a polystyrene foam base (see Chapter V). Both the petri-dish and the polystyrene base contained five holes (each 0.7 cm. in diameter on the petri-dish and 0.9 cm. in diameter on the polystyrene foam base). These holes, overlapped the gas-hole on the dish-holding stand by rotating the plate, and were used for mounting and holding bees for dissection. The dish-holding stand, consisted of a stand on a rotating table. The table, holding the dissecting dish, could be adjusted to

any desirable angle for dissection. The table was also drilled with one hole, which was connected with the gas tube. The flow of carbon dioxide (i.e. anaesthetic) through the gas tube could be regulated (for more detail, equipment, etc., see Chapter 6).

REARING PROCEDURES

I. LABORATORY QUEEN REARING PROCEDURES

In the queen rearing and mating experiments, the rearing procedure was the same. Worker honey bees were collected and grouped in cages, in different numbers, on the ninth day after emergence. They were kept caged in a queenless condition for 24 hours. On the following day, two or three young worker larvae were grafted into each cage. On the next day, the acceptance of the larvae was checked. Only one of the larvae was kept for the bees to rear as queens. Two or three days after the queen cells were capped, they were removed from the cages, and put into vials until emergence. When the pupae were 5 or 6 days old, a "T" shaped cut was made in the wall of the queen cell with a microscalpel (see Figure 6). The pupa was carefully removed from the cell and measured. After the measurement was made, the pupa was returned to its original position until it emerged. After emergence, the adults were injected, in the thorax, with a preservative (see Weaver, 1955), and stored in the same solution in vials for measurements of adult characters at a

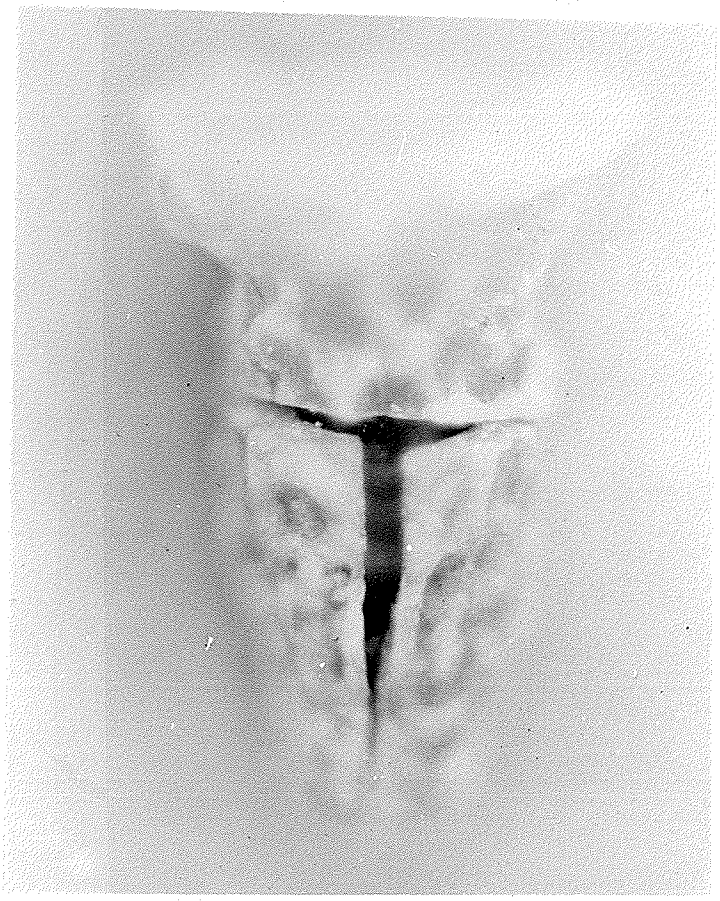


Figure 6. "T"-shaped cut made in the wall of a queen cell.

later date (for Rearing Procedure, see Figure 2A in Chapter V).

II. QUEEN REARING PROCEDURES USED IN THE DISSECTION EXPERIMENTS

Thirty, or seventy-five, worker bees were caged when they were 9 days old and incubated for two to three hours before their mandibular glands were removed. It took two hours to remove the glands of 75 bees. After an operation, the bees were incubated for 24-28 hours ("recovery period"). Two larvae, which had been accepted by 400 bees for six hours, were then introduced into each cage containing bees, whose mandibular glands had been removed previously, After 24 hours one larva was selected and kept with the treated worker bees for various periods of time, depending on the experiment. Where a larva was to be left with the treated bees only for a certain period, it was removed from its original cell with grafting tools, and put into a queen cell containing royal jelly, suited to its age (the larva originally in that cell was first removed). The cell containing the larva was then placed in a cage with 400 normal worker bees. After capping, and after the larva had stopped feeding, it was removed from its cell, and put into small waxed pupation cell until emergence (see Figure 7). These pupation cells, which were covered with a plastic film punctured with holes, were then put into petri dishes for incubation (see Figure 7). Pupal and adult measurements were done as in the

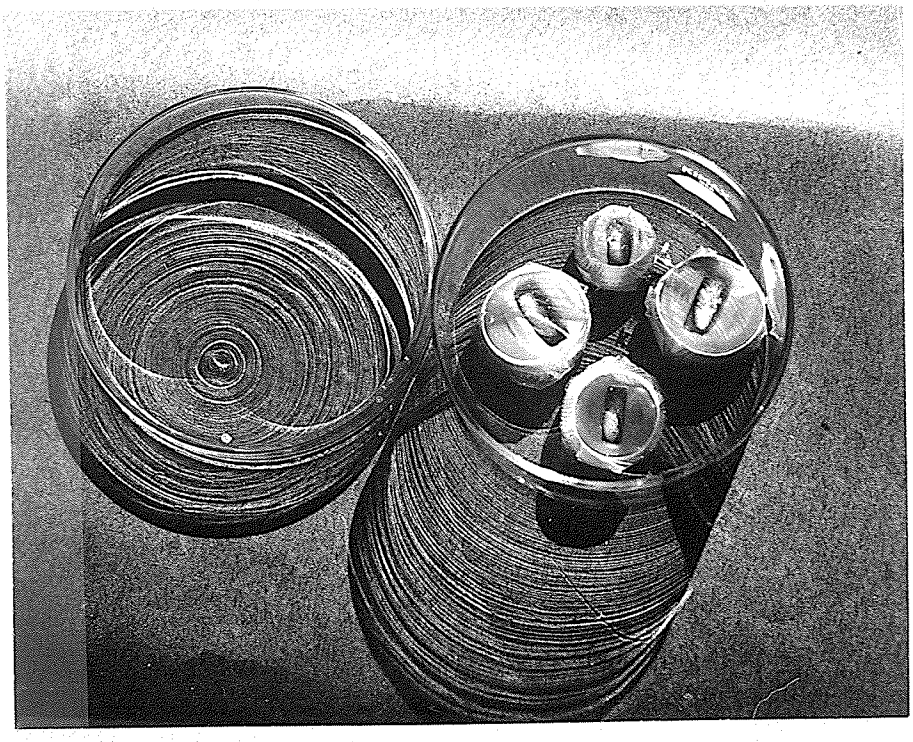


Figure 7. Wax pupation cells used in experiments (1972).

queen rearing experiments (for Rearing Procedure, see Figure 2C in Chapter V).

III. DISSECTION PROCEDURE

Worker bees were anaesthetized with carbon dioxide and mounted on the dissecting dish. A triangular-shaped incision was made between the compound eye and the mandible of a bee. The cuticular flap thus made was raised to expose the gland. With tweezers and a hook, the gland was removed intact. Then the flap was lowered back into its original position for sealing. The dissection dish was rotated and the angle of the holding stand was also adjusted to expose the other side of the gena for gland removal. The dissection process was carried out under a binocular microscope with the magnification of 80 X. The above apparatus and dissection procedures are shown in Figures 7, 8 and 9 in Chapter V.

IV. MATING TESTS

Four-frame Langstroth mating nuclei were set up three or four days before the day that a mating experiment was to be done. The mating nuclei consisted of one frame of food, one frame of mixed uncapped and capped brood along with worker bees, and an empty frame. The queen introduction was either by direct release or by caging the queen, depending on the experiment. In the case of the direct release method, vanilla and sugar solution was sprayed on

the bees and frames of the nuclei when introducing the queen. Queen acceptance was checked on the day following the introduction. After acceptance, mating (i.e. egg laying) was checked every four days. Brood patterns and brood counts were done twice for each nucleus (i.e. at 12 day intervals) during the experiment. Brood counts included eggs, larvae and capped and uncapped brood.

MEASUREMENTS

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 8A).

2. Length of Pupa

The sum of the length from the front of 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) (b) (Figure 8B).

3. Weight of Pupa

This was measured to the nearest milligram on the fifth or sixth day of pupation.

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 9A).

(b) Length: this was measured from the vertex of the head to the distal edge of the labrum (Figure 9A).

2. The Basitarsus

The first segment of the tarsus of the right hind leg was measured as shown in Figure 9B. 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 9B).

3. The Spermatheca

The diameter of the spermatheca was measured at its widest part, after the tracheae and the spermathecal gland

had been removed (Figure 10).

4. The Ovarioles

The right ovary was removed and the number of ovarioles it contained was counted. A staining technique (Weaver, 1956) was used for accuracy in counting.

C. Other Measurements

Other records kept included larval acceptance, the queen rearing ability of the nurse bees, the time of cell capping, 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 number of pupae and adults obtained 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 into the cages to the time when the cells were capped.

4. The Total Development Time

This was measured from the time the larvae were grafted to the time of emergence of the adult.

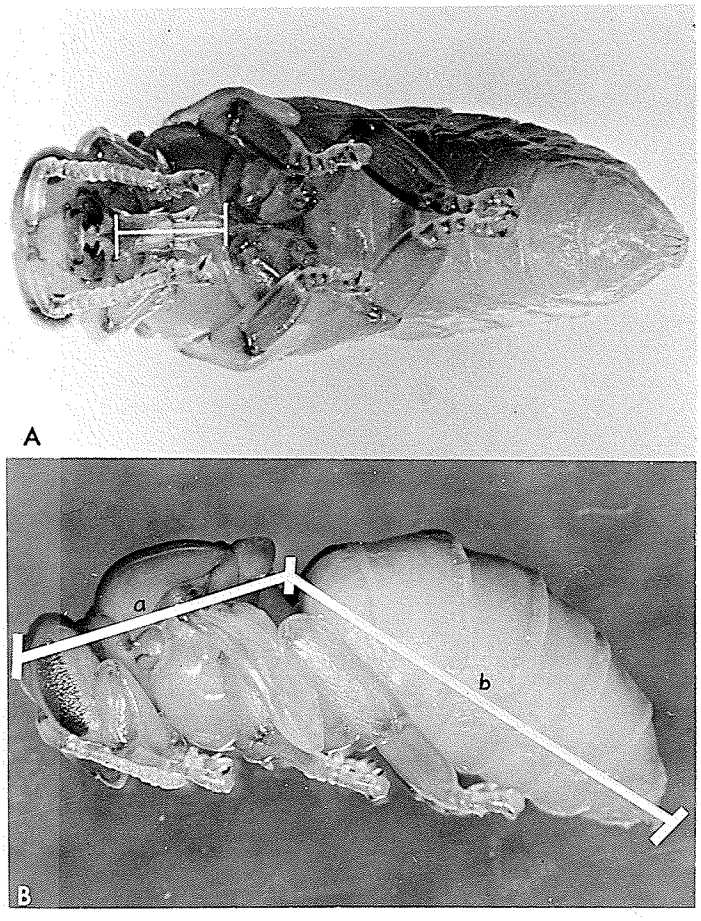


Figure 8. Pupal measurements. (A) tongue length (B) body length; (a) and (b).

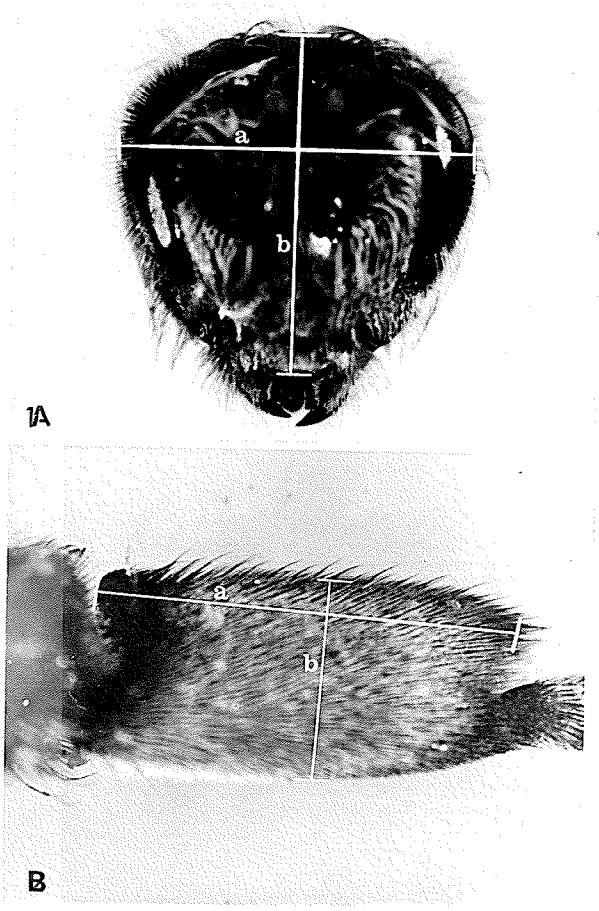


Figure 9. Adult measurements. A. Head; width (a) and length (b). B. Basitarsus; length (a) and width (b).

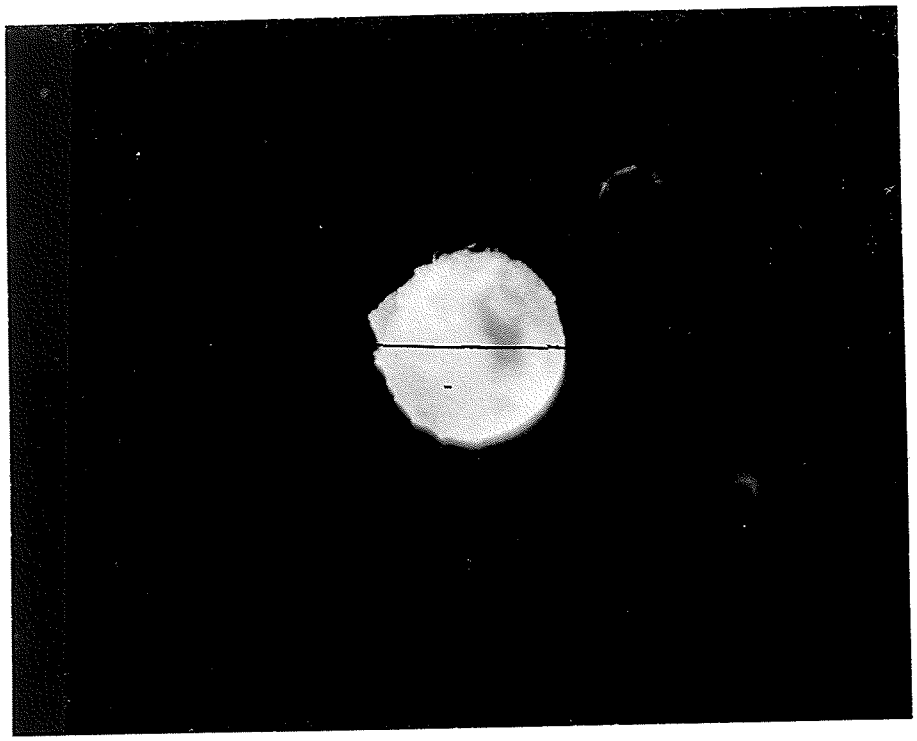


Figure 10. Diameter of spermatheca used in adult measurements.

COMMON ABBREVIATIONS AND DEFINITIONS USED IN THE THESIS

The definition of abbreviated terms used in this thesis are outlined in this section.

I. Terms Relating to Nurse Bees

A. Types of Treatments Received by a Group of Nurse Bees

(C): a group of caged nurse bees each of which was left intact (i.e. not dissected) as controls.

(S): a group of caged nurse bees on each of which was performed a "sham" operation (i.e. incisions were made in both genae but no glands were removed).

(S)(L): a group of caged nurse bees on each of which was performed a "sham" operation on the left gena only.

(Md): a group of caged nurse bees each of which had both mandibular glands removed.

(Md)(L): a group of caged nurse bees each of which had only the left mandibular gland removed.

B. Abbreviations Used to Denote A Particular Group of Nurse Bees

EXPERIMENT NO.	NO. OF NURSE BEES / CAGE	TREATMENT OF (NURSE BEES)
___		

EXAMPLES:

Code	Definition
I-30(Md)	Experiment I in which a group of 30 nurse bees had their mandibular glands removed.
III-75(S)(L)	Experiment III in which a group of 75 nurse bees each of which had a "sham" operation performed on their left gena.

C. Special Terms Used Mainly in Chapter V.

Usually three distinct time periods were followed in rearing larvae in the laboratory:

(1) Acceptance Period: the period of time (6 hours in all tests) during which a larva was accepted and fed by a group of 400 "normal" nurse bees.

(2) Test Period: varying periods of time (following the Acceptance Period) when a larva was fed, by a second group of bees which had received some special treatment (e.g. they may have no mandibular glands).

(3) Feeding Period: the period of time, following the Test Period, during which a larva was fed by a group of 400 "normal" nurse bees until its cell is capped.

D. Abbreviations used for the Test Periods are as follows:

EXPERIMENT NO. — [NO. OF NURSE BEES/CAGE (TREATMENT OF NURSE BEES), TEST PERIOD: HOURS]

EXAMPLE:

Code	Definition
II-[75(Md), TP:45]	Experiment II in which a group of 75 nurse bees had their mandibular glands removed and fed a larva for 45 hours during the Test Period.

II. Terms Relating to Larvae, Pupae or Adults

A. When denoting a particular larva (L), pupa (P) or adult (A) reared by a specific group of nurse bees, the number of the replicate for the larva in that experiment is added after the code of the treatment as follows:

EXPERIMENT NO. ___ NO. OF NURSE BEES / CAGE (TREATMENT OF NURSE BEES) ___

NO. OF REPLICATE STAGE

EXAMPLES:

Code	Definition
I-75(Md)-1 L	Experiment I in which larva number 1 was reared by 75 nurse bees each of which had had their mandibular glands removed.
III-75(S)(L)-2 P	Experiment III in which pupa number 2 was reared by 75 nurse bees each of which had had a "sham" operation performed on its left gena.

B. When denoting a particular larva (L), pupa (P) or adult (A) in a dissection experiment in Chapter V, the particular number of the replicate is added to the end of the code:

EXPERIMENT NO. — { NO. OF NURSE BEES / CAGE (TREATMENT OF) NURSE BEES — } NO. OF REPLICATE, STAGE

TEST PERIOD: HOUR ___ NO. OF REPLICATE,

EXAMPLE:

Code	Definition
III-{75(Md)-2, TP:42}-1 A	Experiment III in which adult number 1 in replicate number 2 was fed during Test Period for 42 hours by 75 nurse bees each of which had no mandibular glands.

CHAPTER III

LABORATORY QUEEN REARING BY SMALL NUMBERS OF CAGED WORKER HONEY BEES

I. INTRODUCTION

The queen honey bee is an indispensable part of a normal honey bee colony, because she assumes the major reproductive role, and therefore is essential for the "welfare" and development of the colony. The relationship between the queen's reproductive capacity and honey production in the colony is extremely important to the beekeeping industry itself. Besides this, the queen honey bee also controls queen cell production (Butler, 1954, 1961; Pain, 1954, 1961; Morse and Gary, 1962), inhibits the ovarian development of worker bees (Butler, 1961; Butler and Fairey, 1963; Velthuis, 1964), by means of her "queen substance" and other glandular secretions (Butler, 1961; Butler and Callow, 1968; Pain1961). Thus queen rearing is of great importance both in beekeeping and in the study of bee biology.

Artificial methods of queen production have been reviewed by many authors (e.g. Laidlow and Eckert, 1950; Volosevich, 1954; Harcus, 1967). However, queen rearing can be arbitrarily classified into two categories; (1) natural methods and, (2) artificial methods. In the former, queen

rearing involves the rearing of queens without transferring individual eggs or larvae to other cells or by cutting up, and transferring, the comb containing them. Rearing queens under natural conditions makes use of the swarming, supersedure or queenless behavior of honey bees, in which queens are produced in small numbers. The production of queens, by artificial methods, involves the rearing of queens by the manipulation of individual eggs or larvae. The swarming, supersedure, or queenless behavior of honey bees may be utilized, but grafting and/or the cutting up of the comb is always involved (Harcus, 1967).

Artificial queen rearing, involving the grafting of female larvae into artificial cells, is the major method used in commercial queen bee production. By this method, queen honey bees, of good quality, can be produced in large numbers. However, artificial queen rearing has also been done in the laboratory. This also involves the grafting of young larvae, but rather than rearing them in the colony, they are reared in petri-dishes or other containers in an incubator. They are fed with food, collected by hand from queen cells in the colony or they are fed with an artificial diet (Rhein, 1933; Haydak, 1943; Weaver, 1955, 1957; Jay, 1959, 1964, 1965, 1965; Rembold, 1964). Rearing queens in the incubator is largely used for academic studies at the present time. However, because to date it involves much skill and labor, this method of queen rearing cannot be applied to beekeeping unless the technique can be simplified

and improved.

A method of queen rearing in the laboratory by small numbers of caged worker bees was developed by Lai (Lai, 1969). She was successful in rearing morphologically normal queens using 10-day old worker bees; the larvae were reared by 25, 50, 100, 200 or 400 nurse bees. The rearing procedure is simple, and appears to be practical. This method of queen rearing can be used year round under controlled conditions. It could be valuable in regions where weather is a limiting factor in queen production. This queen rearing procedure also provides a reliable method for studying female caste differentiation.

The rearing of queens, in this thesis, was done during 1970, 1971 and 1972; however, there were modifications in food supply, and feeding methods. These changes and the morphological aspects of the queens reared are discussed below.

II. METHOD

A. General Design of Tests

Ten day old worker bees were used in the 1970 and 1971 experiments. In 1970, the queen rearing experiments were done from June to August. In each experiment, groups of 30, 100, 200 or 400 worker bees were collected from their incubating colonies (see Chapter II) and caged. The 30 bee groups were put into the small cages while the 100, 200 and

400 groups were put into the large cages. Five replicates of each group were used in each experiment. The rearing experiments were done seven times between June and August.

In 1971, 75, 100, 200 and 400 bees were caged and incubated in the laboratory. In each group, there were five replicates and the test was repeated six times between June and August. A similar number of control queens were reared in a colony in the apiary. In each test, 10 larvae were grafted, and the test was done three times.

B. Incubation and Rearing Procedure (also see Chapter II)

After collecting the 10-day old worker bees from the colonies, they were incubated in cages in the incubators without queens for 24 hours. After incubation, the queen-cups were removed from the cages. Young female larvae, less than 12 hours old, were grafted into these queen cups; with three larvae grafted into each large cage, and two larvae grafted into each small cage (see Grafting Methods, Chapter II). The queen cups, containing grafted larvae, were then put back into their original cages for acceptance and feeding. These cages containing bees and larvae were then incubated for 24 hours. On the next day, the acceptance of the larvae of the bees was checked under a microscope.

One larva which appeared to be the largest and which had the most royal jelly around it was selected and kept in each cage. The larvae were then incubated with the worker bees until the queen cups were capped by the workers. Water

and food were supplied daily to worker bees, and any dead bees or fecal matter were removed. Three days after capping, the queen cups were removed from each cage, and hung in an emergence vial. On the fifth or sixth day after the pupa was formed, a "T"-shaped cut was made in the wall of the wax cell, and the pupa was removed for measurement. Following this it was returned to its vial, and incubated until it emerged. The newly emerged queen bees were injected and stored in a preservative solution for future adult measurements.

C. Measurements (for details, see Chapter II)

1. Pupal measurements: these included tongue length, length of body, and pupal weight.

2. Adult measurements: these included width of head, length of head, head index, width of basitarsus, length of basitarsus, basitarsal indices, number of ovarioles in the right ovary, the diameter of the spermatheca, and the shape of the mandibles.

3. Development time: time was measured from the time of grafting to when the cells were capped, from capping to emergence, and the total development time.

III. RESULTS AND DISCUSSION

A. Queen Rearing by Groups of Thirty Bees

1. Rearing Results

Larval acceptance was high, with usually two out of

two larvae being accepted. The extension of the wax cells was done by the worker bees with fresh beeswax; later, wax from the coating on the aluminum bars was used. Generally, the sealed queen cells were much smaller than the queen cells built by groups containing a larger number of bees. The amount of food supplied to the young larvae on the first two or three days was similar to that of the young larvae reared by the groups containing a larger number of bees. However, as the larvae grew, the amount of food they received was less than that of larvae of the same age reared by larger numbers of workers. It was also observed that there was not much food left in the queen cells after the adults emerged from them. It appears that although 30 nurse bees can supply enough food for the larvae up to 3 days, after that, the supply cannot keep up with the consumption of the growing larvae simply because there are not enough nurse bees.

Of 23 replicates in 1970, only 2 pupae and 6 adults were reared by the groups of 30 bees; no pupae or adults were reared in 1971 from 5 replicates (see Table 1). The morphological measurements are shown in Table 1.

2. Morphological Characteristics

All pupae were small and had lower weights (range, 154-181 mg.), compared to the pupal weights of pupae reared by the other groups with larger numbers of nurse bees. However, the pupal weights of adult bees reared by 25 and 50 nurse bees were also low (see Lai, 1969). Two of the adult

Table 1

The Rearing Results and the Morphological Measurements of Bees Reared by 30(C) Bees in the Laboratory (1970)

Bee No.	Days from grafting to capping	Days from capping to emergence	Total Dev. time (day)	Pupal measurements: Tongue length (mm.)	Body length (mm.) a	b	total	Weight (mg.)	Adult measurements: Head (mm.) W	L	w/L	Mandibles W	I	Q	Basitarsus L	W	L/W	Dia. Spermatheca (mm.)	No. of ovarioles
1	5	-	-	2.30	5.28	6.10	11.38	157	-			-			-			-	-
2	5	-	-	2.30	4.65	6.10	10.75	181	-			-			-			-	-
3	5	10	15	2.40	4.65	5.10	9.75	165	3.60	3.40	1.06			x	2.20	1.20	1.83	0.90	96
4	6	10	16	2.40	4.30	5.10	9.40	168	3.50	3.20	1.09		x		2.00	1.10	1.82	0.90	84
5	5	12	17	2.30	4.20	5.20	9.40	157	3.70	3.50	1.03			x	2.30	1.30	1.77	1.10	113
6	5	10	15	2.10	4.30	5.20	9.50	155	3.90	3.50	1.11		x		2.20	1.20	1.83	1.00	83
7	6	9	15	2.10	4.30	5.30	9.60	155	3.60	3.00	1.20			x	2.30	1.20	1.92	0.90	96
8	6	10	16	2.20	4.30	5.30	9.60	154	3.70	3.40	1.09			x	2.00	1.20	2.00	1.00	103
Mean ± standard error		10.83± 0.75	16.33± 0.61	2.26± 0.04	4.50± 0.13	5.43± 0.15	9.87± 0.27	161.5± 3.32	3.67± 0.06	3.35± 0.05	1.10± 0.02				2.17± 0.06	1.20± 0.03	1.86± 0.03	0.97± 0.03	95.82± 4.66
Queen																	1.9-2.3	1.0-1.3	129-197
Worker (Weaver, 1957)																	1.6-1.9		1- 8

bees (Bee No. 4 and No. 6) had slightly notched mandibles and were classified as intercastes (Table 1). The ovariole counts were lower than that of the adults reared by larger numbers of nurse bees (Table 1). The diameter of the spermathecae were also low. A longer time was required for the bees to develop than was observed in the other groups. In general, however, the adult bees, reared by cages of 30 nurse bees, were queen-like in external appearance. Although queen-like honey bees can be reared by as few as 30 nurse bees, it would be better to use larger numbers of bees in this type of queen rearing.

B. Queen Rearing by the Groups of 75, 100, 200 and 400 Bees

1. Rearing Results

The acceptance of the larvae by the caged worker bees in all groups was high. In most of the cases, 2 or 3 larvae out of 3 were accepted per cage. The acceptance of grafted larvae by 200 or 400 nurse bees was a little better than that of 100 nurse bees. Most of the three grafted larvae were accepted in each cage in the 200 and 400 bee groups; therefore, there was a good chance that a healthy normal larva could be selected for rearing to the adult stage on the following day. Lai (1969) also obtained high acceptance of the grafted larvae in each cage.

A large quantity of royal jelly was observed in the queen cells 24 hours after grafting. Extension of queen

cells with fresh beeswax (about 0.5 cm. in length) was also observed.

The number of pupae and adults, reared by the different groups of bees in 1970 and 1971, are given in Table 2. High numbers of adults were obtained in both 1970 and 1971; 24, 30 and 30 adults were reared by 100, 200 and 400 bees respectively in 1970; 22, 20, 25 and 25 adults were reared by 75, 200, 400 groups and control colonies respectively in 1971 (Table 2). Failure in rearing was sometimes due to experimental errors, i.e., the accidental dropping of a larva during feeding, etc. Some of the larvae died in the late larval stage for unknown reasons. Lai (1969) also obtained high numbers of adults using 100, 200 or 400 nurse bees in cages.

No differences were found in developmental time, from grafting to capping, between the different groups within years (see Table 2). As for the time from capping to emergence, a difference was found only between the 75 groups and the control groups ($P<0.05$) in 1971. Total developmental time of bees reared by the 400 groups was significantly shorter than that of the other groups both in 1970 and 1971 ($P<0.05$ in both cases). No differences were found between the others in 1970 or 1971, nor were there any differences between years, within different groups.

The total development time in this experiment was found to be longer than that found by other research workers (e.g. Huber, 1814; Langstroth, 1890; Zander et al., 1916;

Table 2

The Development Time of the Bees Reared in the Laboratory in 1970 and 1971

	No. Nurse Bees per cage	No. Replicate	No. pupa reared	No. adult reared	Days from Grafting to capping	Days from capping to emergence	Total development time (day)
(I)	1970						
	100	35	1	24	4.67 ± 0.15	10.38 ± 0.17	14.88 ± 0.22
	200	35	-	30	5.23 ± 0.11	9.30 ± 0.43	14.83 ± 0.25
	400	35	-	30	4.70 ± 0.11	9.39 ± 0.11	14.10 ± 0.15
(II)	1971						
	75	30	-	22	6.32 ± 0.14	10.55 ± 0.23	15.86 ± 0.24
	200	30	-	20	4.57 ± 0.11	10.70 ± 0.19	15.15 ± 0.18
	400	30	-	25	4.32 ± 0.10	9.88 ± 0.17	14.12 ± 0.12

Bertholf, 1925; Leuenberger, 1929; Herrod-Hempsall, 1930; Wedmore, 1932; Rhein, 1933). However, they used whole colonies to rear their queens rather than bees in cages. The developmental time of the queens reared by Lai (1969) also took a longer period of time, (i.e., about 17-18 days); her times were close to those observed in these experiments.

2. Morphological Characteristics

The morphological characteristics of the adult bees, reared by the different groups of nurse bees in 1970 and 1971 are shown in Table 3.

In 1970, the pupal weight, body length and basitarsal index of the adult bees reared by 100 and 200 nurse bees (Table 3) were lower than the range of normal queens reared in colonies (Weaver, 1957). Other characteristics fell within the normal queen category (Weaver, 1957). The mean value of the ovariole counts of adult bees, which were reared by the different groups of nurse bees, was also below the range of normal queens (Weaver, 1957). Significant differences were found in pupal tongue length, pupal weight and basitarsal index between adults, reared by different numbers of nurse bees (Table 3). They were higher in the adults reared by 400 bees than in the adults reared by other groups. However, the adult bees, reared by different numbers of nurse bees, were queen-like with respect to morphological and anatomical characteristics.

In 1971, basitarsal indices, number of ovarioles and

diameter of spermathecae of the adult bees, reared by different numbers of nurse bees, fell within the queen category (Weaver, 1957) (Table 3). Tongue length, body length and weight of the pupae also fell within the queen category. The pupal tongue length was significantly higher in the adults reared by 400 nurse bees than the adults reared by 75 or 200 nurse bees ($P < 0.01$ in each case) (Table 3). No differences were found in the characteristics of pupal body length, pupal weight or head index of the adult bees reared by different groups (see Table 3). The basitarsal indices, ovariole counts, as well as diameter of spermathecae of adults reared by 75, 200 and 400 bees were significantly lower than that of the adults reared by the control colonies ($P < 0.05$ in each case). Nevertheless, the adults reared by 75, 200 or 400 nurse bees were queen-like and possessed high numbers of ovarioles and large spermathecae. Only the adults reared by 200 nurse bees had ovariole counts lower than the range of normal queens, that of the other adults fell within the queen category (see Weaver, 1957).

The morphological characteristics of the adults reared in 1970 and 1971, by different groups of nurse bees, were combined in Table 4. The pupal tongue length of the adults reared by groups of 75 nurse bees was significantly longer than the ones reared by groups of 100, or 400 nurse bees ($P < 0.01$). However, they all fell within the queen category (Weaver, 1957). No difference was found in the pupal length of adults reared by different groups of bees

Table 3

The Morphological Characteristics of the Bees Reared by Different Groups of Nurse Bees in the Laboratory in 1970 and 1971

Number of nurse bees per cage	No. Pupae measured	No. adults measured	Pupal measurements: Tongue length (mm.)	Pupal measurements: Body length (mm.) a	b	total	Pupal measurements: Weight (mg.)	Adult measurements: Head (mm.) W	L	w/L	Mandibles W	I	Q	Basitarsus L	W	L/W	Dia. Spermatheca (mm.)	No. of ovarioles
(I) 1970																		
100	25	25**	2.24± *0.03	6.30± 0.05	7.76± 0.10	14.12± 0.11	171.52± 2.80	3.83± 0.04	3.45± 0.03	1.11± 0.01	1	24		2.31± 0.03	1.18± 0.01	1.09± 0.05	1.09± 0.01	124.25± 3.86
200	30	30	2.39± 0.02	6.43± 0.07	8.32± 0.02	14.70± 0.21	178.83± 2.52	3.73± 0.04	3.45± 0.05	1.09± 0.06		30		2.25± 0.03	1.22± 0.01	1.85± 0.03	1.09± 0.01	124.07± 3.34
400	30	30	2.25± 0.03	6.60± 0.08	8.67± 0.09	15.32± 0.16	205.30± 6.02	3.71± 0.03	3.45± 0.04	1.06± 0.00		30		2.43± 0.01	1.21± 0.01	2.01± 0.01	1.11± 0.06	121.57± 4.34
F-Test			P< 0.01			P< 0.01	P< 0.01		P> 0.01						P< 0.05		P> 0.01	P> 0.01
(II) 1971																		
75	22	22	2.42± 0.04			14.98± 0.12	209.55± 5.76		1.15± 0.01		1	22			2.01± 0.03	1.12± 0.02		130.68± 3.21
200	20	20	2.44± 0.07	6.23± 0.09	9.48± 0.32	15.21± 1.69	210.95± 7.16	3.97± 0.04	3.56± 0.03	1.11± 0.01		20		2.45± 0.02	1.24± 0.01	1.96± 0.02	1.10± 0.01	124.30± 3.01
400	25	25	2.50± 0.04	6.27± 0.06	9.31± 0.08	15.58± 0.11	211.04± 6.55	3.90± 0.03	3.50± 0.03	1.13± 0.01		25		2.36± 0.03	1.24± 0.02	1.91± 0.02	1.11± 0.00	131.16± 2.68
Control	-	-						4.10± 0.02	3.54± 0.03	1.14± 0.01		25		2.56± 0.01	1.26± 0.02	2.04± 0.02	1.15± 0.01	143.96± 6.50
F-Test			P< 0.01			P>0.05	P>0.01		P>0.05						P<0.05		P< 0.05	P <0.05

*Standard error.
**One is pupa.

(see Table 4). Pupal weights of adults, reared by 100 nurse bees, were significantly lower than the weights of those reared by other groups of bees; no difference was found between adults reared by 75, 200 or 400 nurse bees (see Table 4).

During the rearing process, I often observed that 75 nurse bees kept in a cage, paid more attention to the larvae than did groups of 100 bees, kept in the larger cages. It appears that small cages should be used for rearing adults when fewer than 100 bees are put in a cage.

The head and basitarsal indices were also found to be significantly larger in the adults reared either by 75 nurse bees in cages or control colonies in the field than those reared by other groups of nurse bees ($P<0.01$, see Table 4); there were no significant differences in these characteristics in the adults reared by 100, 200 or 400 groups of bees. Ovariole counts did not differ between the adults reared by the different groups of nurse bees or by the control colonies (see Table 4). The diameter of the spermathecae was found to be significantly higher in the adults reared by the control colonies than in the adults reared by the other groups of nurse bees ($P< 0.05$). Ovariole counts of the adults, reared by 100, 200 or 400 nurse bees, were lower than those in the queen category (Weaver, 1957); the other adult measurements of these adults all fell within the queen category (Weaver, 1957). Generally speaking, adults reared by groups of 400, 200 or 75 bees more closely

Table 4

A Comparison of the Morphological Characteristics of Bees Reared by Different Numbers of Nurse Bees in the Laboratory in 1970 and 1971

No. nurse bees per cage	No. pupae measured	No. adults measured	Pupal Measurements			Adult Measurements			
			Tongue length (mm.)	Total body length (mm.)	Weight (mg.)	Head Index (W/L)	Basitarsal index (L/W)	No. ovarioles	Dia. Spermatheca (mm.)
75	22	22	2.42± 0.04	14.98± 0.12	209.55± 5.76	1.15± 0.01	2.01± 0.03	130.68± 3.21	1.12± 0.02
100	25	25	2.24± 0.03	14.12± 0.11	171.52± 2.90	1.11± 0.01	1.89± 0.05	124.25± 3.86	1.09± 0.01
200	50	50	2.41± 0.03	15.71± 0.70	191.88± 3.90	1.10± 0.01	1.89± 0.02	124.16± 2.32	1.09± 0.01
400	55	55	2.37± 0.03	15.44± 0.10	207.91± 4.41	1.09± 0.01	1.96± 0.01	125.93± 2.73	1.11± 0.01
Control	-	25				1.14± 0.01	2.04± 0.02	143.96± 6.50	1.15± 0.01
Queen									
Worker (Weaver, 1957)									
F-Test			$P<0.01$	$P>0.01$	$P<0.01$	$P<0.01$	$P<0.01$	$P>0.05$	$P<0.05$

resembled normal queens their morphological and anatomical measurements than did adults reared by 100 bees.

Frequently in this study the adults, reared by small numbers of bees, had queen-like characteristics; however, some of them overlapped with worker caste or intercaste characters when Weavers' (1957) data are used. It is also very possible that this type of variation occurs in nature. It is important also to note that the ranges, which occur in normal queens with regard to each of the characteristics, are still not known in full. One queen may be long and slender or she may be short or stubby, or she may be of average or larger proportions. The number of ovarioles in the ovaries of queens do not vary in direct proportion to her external size, and no correlation has been found between the number of ovarioles and the amount of brood produced by a queen (Eckert, 1934). Therefore, the performance of a queen and of her colony is probably the best criterion for judging her quality, rather than her general appearance (Laidlaw and Eckert, 1962). The mating and brood production tests discussed in Chapter IV, show that these adults can perform normally in nature. Therefore, this method of queen rearing appears to be of economic importance to the beekeeping industry and could be done as a year round basis.

CHAPTER IV

MATING TESTS OF QUEEN HONEY BEES REARED IN THE LABORATORY BY SMALL NUMBERS OF CAGED WORKER BEES

I. INTRODUCTION

Smith (1959) reared two virgin queens in the laboratory on fresh royal jelly, collected from queen cells containing three day old larvae, and introduced them to mating nuclei. Both of the queens were accepted by the bees in the nuclei, but one queen was lost before it was mated. The other queen started laying eggs thirteen days after the introduction, and a week later normal capped brood was present in the nucleus. Later the queen, along with four frames of her brood, were transferred to a standard hive. This colony failed to survive the winter and the queen was lost. Smith suggested that although laboratory reared queens were not fully queenlike individuals, they were still capable of mating and functioning normally. Dietz (1969) reared four adult queens from egg to adult in the laboratory and artificially inseminated them before introducing them to mating nuclei or colonies. Two of the queens, which were accepted by bees in nuclei, laid eggs fourteen and fifteen days after their introduction. One of the two queens, introduced later to a colony, was killed while the other queen was accepted

and found laying eggs thirteen days later. However, one of the two queens in the nuclei ceased laying eggs several weeks later and was lost for an unknown reason. The queen, accepted by the colony, was compared to a normal queen; her hind legs, mandibles, number of ovarioles and size of spermatheca were not fully queen-like, although the spermatheca did reveal the presence of sperm. Dietz also stated that the brood patterns of the different queens appeared normal and that only a few drones were reared. The brood patterns differed little from those of a normal colony of similar size and normal worker progeny were produced by all of the queens.

Each of the above experiments dealt with small numbers of incubator-reared adults, in the absence of worker bees, and showed the feasibility of mating and brood production by these individuals. However, few data are given about the acceptance rate of the virgin queens by the bees in the nuclei, the probability of natural mating, actual brood production within a certain period, or the anatomical and the morphological characteristics of these queens. These data are important for assessing the functional qualities of incubator-reared queens.

It is necessary to establish some reliable parameters as criteria for determining a functional queen, drone layer, or intermediate queen. It would also be desirable to know to what extent morphologically and anatomically defined queens, reared in the laboratory, can behave as a normal, functioning

queens in a colony. Too, no research has been done to show the results of introducing queens, reared by small groups of caged worker bees to mating nuclei.

The object of this preliminary experiment was to determine if laboratory reared queens are accepted by bees in mating nuclei and whether they can be mated and produce brood.

II. METHOD

In an attempt to test the mating ability of queen honey bees, which are reared by small groups of caged worker bees in the laboratory, preliminary studies were done from July to September 1971 using bees of a yellow strain. Four frame Langstroth nuclei were set up four or five days before the test, so that the bees could build emergency queen cells. At the time when queen cells, or virgin queens were introduced, all queen cells built previously in the hives were destroyed.

In July 1971, groups of 75, 200 or 400 bees reared five queen cells each; this was repeated three times. Each queen cell was introduced into a nucleus by attaching it to a comb surface. Because all fifteen queen cells were destroyed when checked the following day, this method of introducing queen cells was not used again.

A second method was tested: newly emerged virgin queens were put separately into petri-dishes with honey as food and were then incubated for 1-2 days before being introduced to the nuclei. Before introducing a virgin queen into

a nucleus, all three frames were sprayed with a mixture of vanilla and sugar solution. The virgin queen was also sprayed with the mixture and then released directly onto the frame. This method of virgin queen introduction was used in years 1971 and 1972, with little modification.

In 1971, fifteen virgin queens emerged on 18-19th August and were incubated for one to two days. On August 20, all the queens were introduced into mating nuclei. Their acceptance was checked on the following day, and thereafter the presence of those that were accepted, along with their egg laying, was checked every four days. The dates when the first group of eggs were laid by the mated queens were recorded. Later observations included brood patterns, queen checks, etc. In the beginning of October, the queens were removed from the nuclei, and preserved (see Weaver, 1956, for preservation method).

In 1972, this technique was modified as follows: a virgin queen was put into a queen cage and sprayed lightly with the vanilla mixture. Then the queen cage was inserted between two frames. On the following day, the virgin queen was released slowly onto the frames, and the behavior of the worker bees toward the virgin queen was observed. When no antagonistic behavior was observed, the virgin queen was left with the bees. The acceptance of the virgin queen was checked the next day after the introduction, and every four days thereafter. The date when the first eggs were laid by a mated queen was recorded. Brood counts were done at

twelve and twenty four days after introduction, for each nucleus in 1972. In order to obtain five mated queens for each group in this experiment it was necessary to make two or three queen introductions into each group.

Totally, 7, 8 and 8 virgin queens were introduced from the 400, 200 and 75 groups respectively, as well as 6 virgin queens for the control groups. The introduction of virgin queens was carried out from July 1 to July 17.

III. RESULTS

The results of the preliminary mating test done on August 15, 1971 showed that some of the honey bee queens reared in the laboratory by small groups of worker bees could mate with drones and became fertile (Table 5). Three of the five queens, reared by 400 (c) bees were accepted, and two were killed by the worker bees. Two of the accepted queens were found laying eggs twelve and thirteen days after introduction. The other virgin queen remained unmated and was killed sometime during the third week after introduction. Of the four accepted queens reared by 200(c) bees, two were found laying eggs on the 17th and 18th day. One of the mated queens (Queen No. 3, Table 6) was a drone layer. The queens reared by 75 worker bees, had the lowest acceptance rate, but both accepted queens were mated on the 18th day after introduction. Five of the queens produced good patterns of worker brood (see Figure 3).

The adult measurements of these six mated queens are

Table 5

Acceptance and Matings of Five Virgin Queens in Nuclei (1971)

Rearing Test	Total No. of queens introduced	Total No. of queens accepted	Total No. of queens mated
400 (c)	5	3	2
200 (c)	5	4	2
75 (c)	5	2	2

Table 6

Measurements of the Laboratory Reared Queens which were Introduced to Nuclei in 1971

Queen No.	Rearing Test	Time from introduction to laying (days)	Adult Measurements							
			Head (mm.)			Basitarsus (mm.)			Dia.spermatheca (mm.)	No. ovarioles in the right ovary
			Width	Length	W/L index	Width	Length	L/W index		
1	400(C)-1	13	3.99	3.88	1.03	1.35	2.84	2.10	1.38	148
2	400(C)-2	12	3.13	3.92	1.05	1.29	2.65	2.05	1.44	135
3	200(C)-3*	18	4.13	3.55	1.16	1.23	2.65	2.15	1.28	113
4	200(C)-5	17	4.06	3.48	1.17	1.23	2.45	1.99	1.11	161
5	75(C)-7	18	3.99	3.88	1.03	1.35	2.84	2.10	1.18	148
6	75(C)-1	18	4.19	3.81	1.10	1.23	2.27	2.25	1.44	137
	(mean ± standard error)				1.09± .03			2.11± .04	1.31± .06	130.33± 6.66

* Drone layer.

given in Table 2. The head index, basitarsal index diameter of the spermathecae and numbers of ovarioles in the right ovary fell within the normal queen category (Weaver, 1957), except for queen No. 3 (a "drone layer"), which had a lower number of ovarioles.

The mating test of 1972 was designed to give five mated queens for each group of workers and also five control queens reared by queen rearing colonies. The results are shown in Table 7. One of the five mated queens, reared by 400 bees, was a drone layer. Two of the five were drone layers in each of the 200 (c) and 75 (c) groups.

In 1972, the frequency distribution of the time interval between acceptance and the laying of the queens indicates that most of the queens were laying eggs around the 16th day after they were introduced to the nuclei, which was greater than the time needed in 1971 (see Figure 1).

The anatomical and morphological measurements of the adult queens, introduced to the nuclei, in 1972 are given in Table 8. The basitarsal index, diameter of spermatheca and number of ovarioles in the right ovary of all queens reared by 200 or 400 bees fell within the queen category (Weaver, 1957). However, queens No. 4, 7 and 10 were "drone layers." Queens No. 11, 12 and 20 had a basitarsal index within the worker category (Weaver, 1957), while queen No. 11 and 12 were normal in other aspects and produced worker brood. Queen No. 14 was a "drone layer" whose spermathecal diameter and number of ovarioles fell within the queen category.

Table 7

Acceptance, Mating and Fertility of the Laboratory Reared Queens and the Colony Reared Control Queens (1972)

Rearing Test	Total No. of queens introduced	Total No. of queens accepted	Total No. of queens mated	No. of queens producing worker brood	No. of drone layers
400 (C)	7	5	5	4	1
200 (C)	8	5	5	3	2
75 (C)	8	6*	5	3	2
Control	6	5	5	4	1

* One of the queens was accepted by the nucleus but remained unmated and was later killed by the colony.

Table 8

Measurements of the Laboratory Reared Queens and the Control Queens which were Introduced to Nuclei in 1972

Queen No.	Rearing Test	Time from introduction to laying (days)	Adult Measurements							
			Head (mm.)			Basitarsus (mm.)			Dia.spermatheca (mm.)	No. ovarioles in the right ovary
			Width	Length	W/L index	Width	Length	L/W index		
1	400(C)-1	16	4.19	3.55	1.08	1.16	2.65	2.28	1.39	139
2	400(C)-2	16	4.39	3.74	1.17	1.29	2.58	2.00	1.44	145
3	400(C)-7	18	3.94	3.48	1.13	1.29	2.52	1.95	1.34	151
4	400(C)-9	16	4.06	3.62	1.12	1.29	2.52	1.95	1.39	132*
5	400(C)-5	16	3.87	3.55	1.09	1.23	2.52	2.05	1.39	172
	(mean ± standard error)				1.14± 0.02			2.05± 0.09	1.39± 0.02	147.80± 6.82
6	200(C)-1	11	4.00	3.68	1.09	1.36	2.58	1.90	1.34	197
7	200(C)-3	13	3.87	3.48	1.11	1.20	2.40	2.00	1.36	140*
8	200(C)-4	14	4.19	3.61	1.16	1.29	2.52	1.95	1.41	168
9	200(C)-5	16	3.94	3.48	1.13	1.23	2.58	2.10	1.36	154
10	200(C)-9	16	3.87	3.61	1.07	1.29	2.45	1.90	1.26	152*
	(mean ± standard error)				1.11± 0.02			1.90± 0.08	1.38± 0.07	162.20± 9.77
11	75(C)-1	16	4.13	3.48	1.19	1.29	2.32	1.80	1.39	159
12	75(C)-2	13	4.13	3.48	1.19	1.29	2.39	1.80	1.31	163
13	75(C)-4	19	4.13	3.55	1.16	1.23	2.58	2.10	1.56	146
14	75(C)-6	17	4.00	3.48	1.13	1.16	2.32	2.00	1.39	141*
15	75(C)-8	19	3.94	3.48	1.13	1.23	2.39	1.94	1.31	127*
	(mean ± standard error)				1.16± 0.01			1.94± 0.05	1.39± 0.05	147.20± 6.47

Table 8 (continued)

Queen No.	Rearing Test	Time from introduction to laying (days)	Adult Measurements							
			Head (mm.)			Basitarsus (mm.)			Dia.spermatheca (mm.)	No.ovarioles in the right ovary
			Width	Length	W/L index	Width	Length	L/W index		
16	Control-1	16	4.19	3.81	1.10	1.29	2.58	2.00	1.44	148
17	Control-2	16	3.87	3.35	1.09	1.16	2.58	2.22	1.36	163
18	Control-5	16	4.06	3.61	1.12	1.29	2.58	2.00	1.41	164
19	Control-8	12	4.13	3.48	1.19	1.23	2.52	2.05	1.44	170
20	Control-9	16	4.19	3.61	1.16	1.29	2.32	1.80	1.31	133*
	(mean ± standard error)				1.13± 0.02			2.01± 0.07	1.39± 0.03	155.60± 6.71

* Drone layers.

Brood measurements are given in Figure 2. Queen No. 6 which had the highest number of ovarioles was accidently injured during a queen check and died before the second reading was taken. The histogram of the brood readings show a general picture of the brood producing ability of the queens, and also shows that these queens had a high potential for brood production. There was no significant difference in total brood and sealed brood production by queens reared by different numbers of bees. The drone layers had a significantly lower number of ovarioles ($P<0.05$) than the worker-brood producing queens. However, the mean number of ovarioles, mean diameters of spermathecae were still within the queen category (Weaver, 1957).

IV. DISCUSSION

Generally speaking, it was more difficult to have virgin queens accepted by worker bees in colonies than mated tested queens, either with the direct release method or the cage method (Snelgrove, 1940; Ribbands, 1953; Butler and Simpson, 1956; Free and Spencer-Booth, 1961). This was found to be true in my queen mating tests both in 1971 and in 1972. It seemed that if the queen was accepted, she would than be able to mate successfully and rear brood. However, the data of Table 5 (1971) showed a decrease in the number of mated queens. Loss of queens was probably due either to the bad weather in August 1971, or the low acceptance rate of queens which is usual late in the season (see Free and

Spencer-Booth, 1961).

The occurrence of drone layers was found not only in the treated groups but also in the control groups, but in lower numbers. The number of the queens reared by 400 bees was the same as for control queens.

Among the drone layers, only queen No. 3 (1971) and queen No. 17 (1972) had fewer ovarioles than the normal queens. All of the other drone layers had a higher number of ovarioles. The mean value of the number of ovarioles of all drone layers fell within the normal queen range. Therefore, it was difficult to explain if the number of ovarioles affected the occurrence of drone layers or not. However, the data show that the number of ovarioles in drone layers was significantly lower than that of the queens producing worker brood ($P<0.005$), but with the mean number of ovarioles within the queen range. All of the drone layers and queens, which produced worker brood, compared well to normal queens with regard to spermathecal diameter (Weaver, 1957). Although the spermathecae were significantly smaller in the drone layers than in the worker layers ($P<0.05$), it was obvious, from microscopical examinations, that they actually contain sperm.

Queen No. 20 had a small basitarsal index; this was also true of queen No. 11 and 12, which both produced worker brood. Anatomical and morphological observations of the drone layers did not show any malformation of the reproductive system, e.g., ovaries remaining in the primitive stage,

disconnection of the oviduct from the ovaries (Fyg, 1951, 1964), or blockage of oviducts (Köhler, 1956). Therefore, the occurrence of drone layers, in the present experiment, could be caused by other factors, for instance, poor weather during nuptial flights, insufficient numbers of sperm received from the drones during mating, (Jay, 1967) or some unknown disease (Fyg, 1964).

The brood production histogram of all the introduced queens suggest that laboratory reared queens have high potential as brood producers. Even the queens reared by only 75 worker bees, did equally well in brood production, compared to control queens and queens reared by high numbers of bees. Therefore, normal, functioning queens can be reared by as few as 75 worker bees and small numbers of 10-day old young worker bees are able to supply enough food during the queen rearing process (Lai, 1969).

This experiment has an economic aspect in that the possibility of producing queens in the laboratory seems feasible. It is possible to rear normal queens with small numbers of workers bees under controlled laboratory conditions during any season of the year. However, it is too early to make any definite conclusions about the quality of the laboratory reared queens. The above data indicate that the queens reared in the laboratory can function as normal colony mothers, but more research is needed in this area. I suggest that the performance of a queen and of her colony is probably the best criterion for judging her quality (see

Laidlow and Eckert, 1962). If a queen is prolific enough to maintain a strong colony, and if the colony has a majority of desirable characteristics then the queen is a good one regardless of her size and appearance.

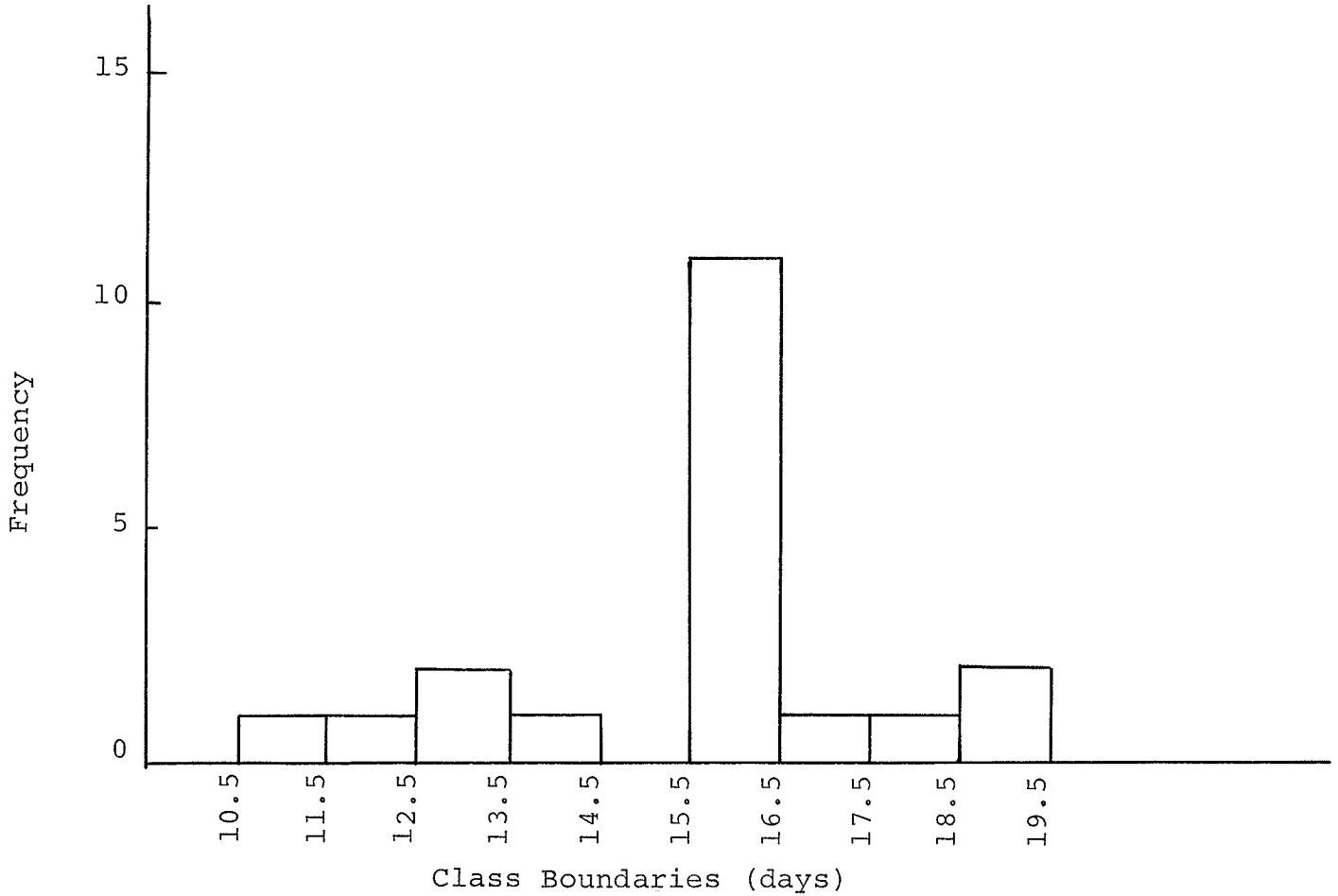


Figure 1. Frequency distribution of the time interval between the time the queens were accepted by the nuclei and the first eggs were observed.

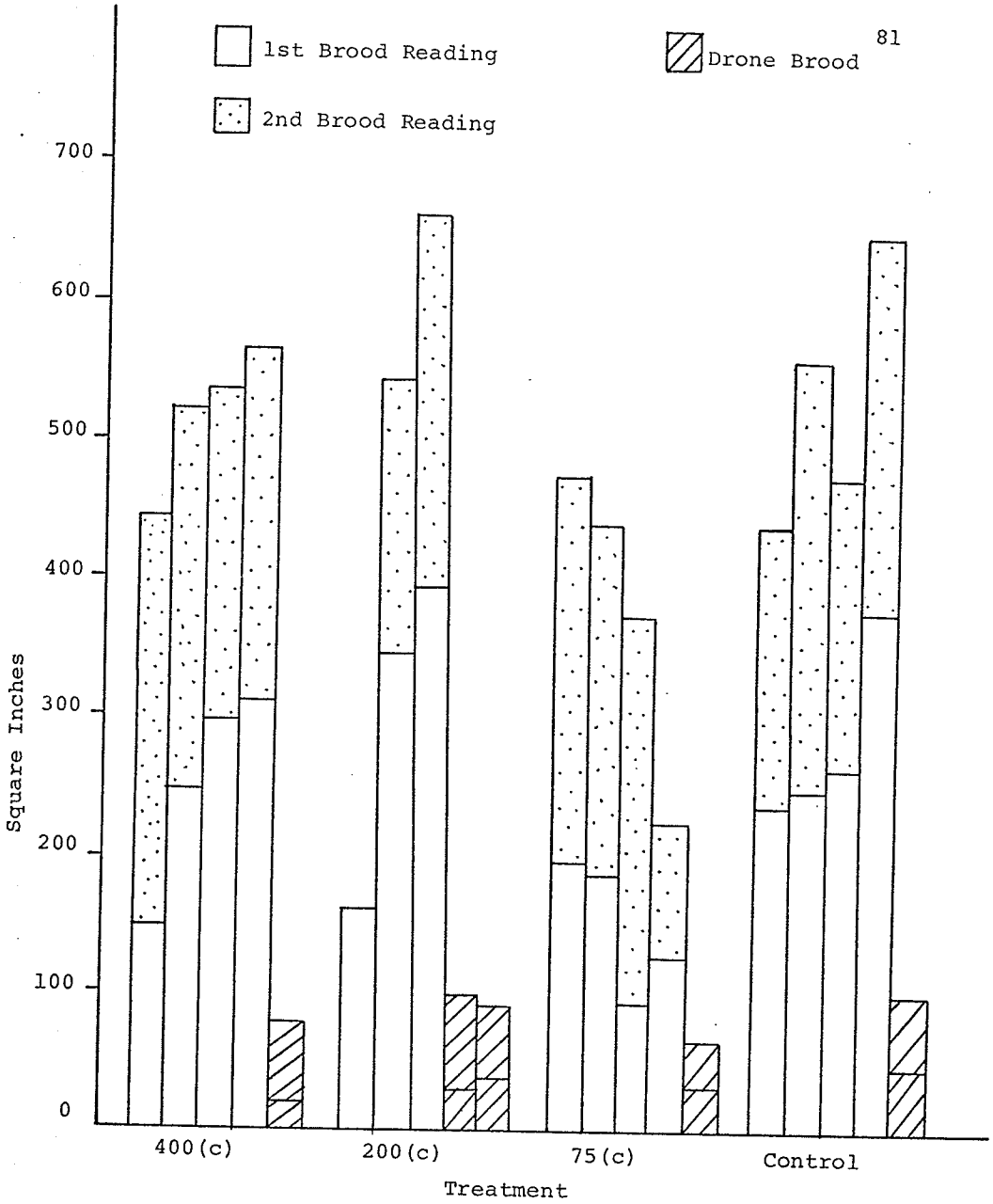


Figure 2. The total brood measurements of the twenty nuclei which were produced by the queens reared in the laboratory and by the control colony in July and August in 1972.

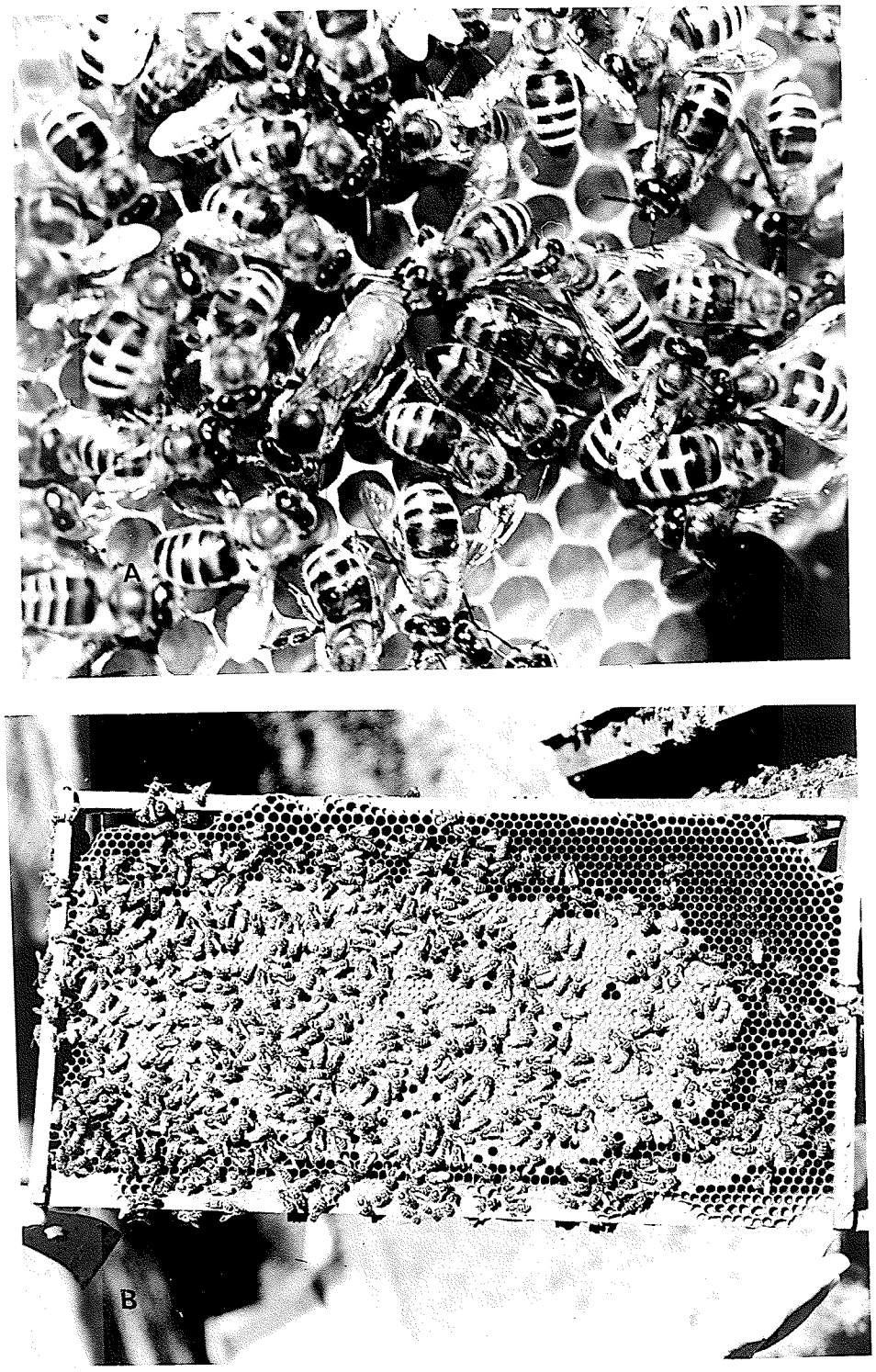


Figure 3. Queen No. 5 reared in the laboratory by seventy-five bees in 1971. (A) showing the queen, (B) the brood produced by her.

CHAPTER V

LARVAL REARING BY NURSE BEES LACKING THEIR MANDIBULAR GLANDS

I. INTRODUCTION

Physiological and histological differences found between worker larvae and queen larvae indicate that the differentiation of female honey bees begins early in their larval life. Studies of growth rates of female larvae show that the critical stage of caste differentiation in the larval stage lies between 72 hours and 85 hours in what is the fourth instar (Townsend and Shuel, 1956). Between 6 and 90 hours, worker larvae are heavier than queen larvae, but after 90 hours, the growth rate of queen larvae increases; consequently, queen larvae are heavier than worker larvae at 102 hours (Wang, 1965). Chemical analyses of larval tissue show marked differences between castes in 3 to 4-day old larvae (Melampy et al, 1940). Total haemolymph protein (Liu and Dixon, 1965), tissue protein concentration (Shuel, 1963) and the activity of haemolymph esterase (Tripathi and Dixon, 1968) differ, at approximately 72 hours in the larval stage, between castes. Differences in respiratory activity, between castes, are also manifested early in larval life. The dichotomy between the two castes, with respect to oxygen

consumption, appears at 50 hours (Shuel and Dixon, 1968). However, distinct histological differences between the corpora allata of the two castes have been shown within the first two days of larval life (Canetti et al., 1964). The activity of corpora allata also differs at that time (Ritcey and Dixon, 1969). Therefore, it has been well established that female dimorphism of the honey bee begins during the first day of larval life (Shuel and Dixon, 1959, 1963) and becomes progressively "fixed" (Weaver, 1957).

An attempt was made in this section to determine the significance of the mandibular glands in the caste differentiation of female honey bees. An adaptation of the queen rearing method using small numbers of nurse bees developed by Lai (1969), and an adaptation of the mandibular gland removal technique developed for queens by Gary (1961), were used in this study. Briefly, the mandibular glands were removed from small numbers of worker bees and these bees then attempted to rear female larvae (grafted into artificial queen cups) to adults. Of particular interest was whether the adults thus reared were worker-like or queen-like in their morphological and anatomical characters.

II. EXPERIMENTS IN 1970

A. Methods and Materials

In a preliminary experiment, methods, developed by Gary (1961), were adapted for removing mandibular glands from worker bees. Using this method, the mandibular glands

of no more than 15 bees per hour could be removed. It was therefore necessary to modify the method and apparatus in order to increase the speed of dissection.

1. Dissection Apparatus and Tools

Several Pyrex glass petri-dishes (10 cm. in diameter) were filled with beeswax and 5 holes (1 cm. in diameter) were drilled through the wax in each dish. The holes served as holders for mounting the worker bees which were to be dissected. In this way, 5 bees could be held for dissection at the same time in order to speed up the operation.

"V"-notched paper collars (see Gary, 1961) were used to hold the bees. Number 5 Dumont tweezers were filed to pin-point sharpness and Irex microscalpels, which use razor blades, were used for the dissection.

2. Dissection Procedure

Bees were anaesthetized with CO_2 gas before being mounted. A paper collar was placed around the neck of a bee after which, it was put into one of the wax holes. The paper collar was pinned to the wax to hold the bee firmly.

A triangular shaped incision was made in one gena (see Figure 1; Gary, 1961) with the scalpel, using a binocular dissection microscope with a magnification power of 80X. The cuticular flap thus made was raised, and using the tweezers the duct of the mandibular gland was seized and the intact gland removed. After the gland was removed, the flap was put back into its original position. The dissection

dish was revolved 180° to display the other gena of the same bee, and a similar operation was performed to remove the opposite mandibular gland. After dissection, the bees were placed in small cages (Type B cages), 30 to a cage (denoted as 30(Md) groups later in the text). The same dissection tools and methods were used in performing a "sham" operation as was done in the actual removal of the mandibular glands. In the "sham" operation, cuticular incisions were made on both genae of a nurse bee without actually removing the glands. After a "sham" operation was performed on 30 bees, they were placed together in a small cage and were denoted as one 30(S) group.

It took 2-2 1/2 hours to finish removing the glands from one group of 30 bees, and 1 1/2 hours to finish the "sham" operations for 30 bees.

B. Experimental Design and Rearing Procedures

Each experiment consisted of a control group (C), a group with their glands removed (Md), and a group upon which was performed a "sham" operation (S). In Experiments I to Experiments III, there were 5 replicates of each of the above treatments (see Table 2). The whole test was conducted between mid June and the beginning of September, 1970.

After the treatments (dissections), the worker bees were caged and incubated for 24 hours for recovery. After this recovery period, two larvae, less than 12 hours old, were grafted into each cage for feeding and rearing.

Acceptance of larvae and selection of larvae were done 24 hours after grafting (see Chapter II), at which time the mortality of the nurse bees was recorded. In the control groups (C), grafting and selection of larvae were carried out as described in Chapter III.

C. Results and Discussion

1. Mortality of the Nurse Bees

High mortality occurred among the nurse bees of the 30 (Md) and 30 (S) groups (Tables 9 and 17). Mortality was significantly higher in the 30(Md) and 30(S) groups than in the 30(C) groups ($P < 0.01$). Mortality in the 30(S) groups was significantly lower than in that of 30(Md) groups ($P < 0.05$).

The high mortality in the 30(Md) and 30(S) groups was probably caused by the type of dissection tools, and dissection techniques used during the operation. It was observed that, in the 30(Md) groups, the tweezers were not fine enough to remove the gland intact by grasping its duct, and thus the gland frequently ruptured during the dissection; bleeding from the incision was also observed. The mortality was especially high on the second day after an operation. Some dead nurse bees were examined under the microscope, and it was observed that the incision had failed to heal. Some of the living (Md) bees were also examined under a microscope, while they were rearing larvae, and their incisions appeared to be healed. If the incision was opened, a cavity was observed where the original mandibular gland had been; other

Table 9

Rearing Results of Groups of Thirty Nurse Bees (1970)

Treatment of nurse bees	No. of Replicates	Total of larvae introduced	Total of pupae reared	Total of adults reared	Total of late larvae reared	Total* development time (day)	Mortality of* nurse bees (%) (7 days)
(Md)	23	46	0	0	3	-	56.19
(S)	23	46	2	1	11	17	26.34
(C)	23	46	2	6	6	15	6.06

* mean value

tissues around the former site of the gland appeared to be normal. Of particular importance was that the hypopharyngeal gland appeared normal and functional.

The dissection procedure itself was also too time consuming. It seemed important, from the above work, that the dissection tools, equipment and general method had to be improved to decrease the mortality of the nurse bees as well as to increase the dissection speed to meet the purposes of this experiment.

2. Observations and Rearing Results

The rearing results of the 30(Md) and 30(S) were poor probably because of the high mortality of the nurse bees. The acceptance of the grafted larvae by the 30(Md) and 30(S) groups was also low (Table 10). In the 30(Md) groups, few of the larvae were accepted, and around the accepted larvae, there was only a small amount of yellowish watery secretion. The amount of this type of food was about 1/2-1/3 of the amount of royal jelly found in the cells of the 30(C) groups. This showed that the 30(Md) bees, even without their mandibular glands, could still secrete food for the larvae after a "recover" period, though the quantity (and appearance) of the food was much lower (and different). The larvae in the 30(Md) groups, examined at 24 hours after grafting, were also quite small compared to the larvae reared by the 30(C) groups or 30(S) groups. The 30(Md) nurse bees usually ceased feeding the larvae around 48 hours after grafting and

as a consequence, the larvae soon starved to death and were removed from the queen cells by the nurse bees. Occasionally, a few nurse bees continued to add a watery-yellowish secretion to the cells, and in these cells larvae (I-30(Md)-3L, I-30(Md)-5L, V-30(Md)-2L, see Table 10) survived to about 48 to 60 hours after grafting. These cells were then sealed by the nurse bees (Table 10). Any larvae that were grafted a second or third time into those 30(Md) cages, which had failed to accept larvae the first time, were also not accepted.

However, in some cages, where larvae had died, some food was placed around them up to 48 hours after grafting. No signs of injury could be seen on these dead larvae under the microscope. In this case, another young larva was grafted into each queen cell thus replacing the dead one. However, when examined at 24 hours after the second grafting, the queen cells were usually found to be empty; no yellowish watery larval food had been added to the queen cells by the nurse bees and consequently the larvae died and were removed from the cells. A third grafting gave the same result.

The ability of the 30(Md) groups to cap queen cells was tested as follows: on the fourth and fifth day after dissection, a queen cell containing a larva from a 30(C) group and which was about to be capped, was introduced into a 30(Md) cage. It was observed that in the II-30(Md)-2, III-30(Md)-2, III-30(Md)-4, and IV-30(Md)-1 groups (Table 10) that the queen cells were capped 2-3 days after their intro-

duction.

In the 30(S) groups, the acceptance of larvae was lower than that of the 30(C) groups (see Table 10). The appearance of the larval food, secreted by groups 30(S) bees, was similar to that of royal jelly. The amount of food in the queen cells of the 30(S) groups was lower than that in the cells of the 30(C) groups. Only a small quantity of food was fed to the growing larvae 2-3 days after grafting; the 30(S) larvae grew much more slowly than those in the 30(C) groups, and were smaller. However, most of the cells reared by the 30(S) groups were capped (see Table 10). The majority of the larvae, reared by the 30(S) groups, died in the late larval stage after their cells had been capped. However, only one adult and two pupae (see Table 10) were reared by the 30(S) groups. The poor rearing success was probably due to the decrease in the quantity of larval food fed to the larvae. The high mortality of the nurse bees in the 30(S) groups (Table 9) likely caused this decrease in the amount of food fed to the larvae.

In the 30(C) groups, the acceptance of the larvae was high (except in II-30(c)-3, in which none were accepted). The amount of royal jelly in each 30(C) group was much less than that secreted by 100, 200, or 400 bees (see Chapter III). Two pupae and six adults were obtained from these groups.

Thirty nurse bees were able to rear the grafted larvae to the adult stage but the amount of larval food secreted by them was much less than that secreted by larger

numbers of bees (see Chapter III). Because of the high mortality among the nurse bees in the 30(S) and 30(Md) groups, the quantity of larval food was decreased when compared with that of the 30(C) groups. Therefore, it appears that the number of nurse bees should be increased in order to obtain more food for the larvae.

3. Morphological characteristics of the pupae and adults reared by 30(S) and 30(C) groups are shown in Table 11.

Pupae, reared by the 30(S) groups, were small and had low pupal weights (see Table 11). Adult measurements were done on these pupae; one had intercaste mandibles, while the other one had queen-like mandibles.

The pupal measurements of the adults reared by the 30(C) groups (Table 11) showed that they were generally small with a body length of 9.87 ± 0.27 mm., a weight of 155.00 ± 4.5 mg., and a short tongue. Four adults possessed queen-like mandibles, and two (III-30(C)-5A, V-30(C)-4A) (see Table 11) had intercaste mandibles. The ovariole numbers were also low with a mean of 95.83.

Because of the low numbers of pupae and adults reared by the 30(S) and 30(C) groups, the low quantity of larval food observed, and the frequent occurrence of intercastes, etc., it seems that the number of worker bees must be increased in order to feed the larvae a sufficient amount of food for proper development.

Table 10

Results of Larval Acceptances of all Experiments (1970)

Treatment of nurse bees	Experiment No.	Replicate No.	No. larvae grafted	No. larvae accepted	Capping ability	Days from grafting to capping	Days from capping to emergence	Time of death
(Md)	I	30(Md)-1	2	1	-	-	-	L
		-2	2	0	-	-	-	L
		-3	2	1	+	6	-	L
		-4	2	0	-	-	-	L
		-5	2	0	+	7	-	L
	II	30(Md)-1	2	0	-	-	-	L
		-2	2	1	+	-	-	L
		-3	2	0	-	-	-	L
		-4	2	0	-	-	-	L
		-5	2	0	-	-	-	L
	III	30(Md)-1	2	0	-	-	-	L
		-2	2	1	+	-	-	L
		-3	2	0	-	-	-	L
		-4	2	1	+	-	-	L
		-5	2	0	-	-	-	L
	IV	30(Md)-1	2	0	-	-	-	L
		-2	2	1	-	-	-	L
		-3	2	1	-	-	-	L
		-4	2	0	-	-	-	L
	V	30(Md)-1	2	0	-	-	-	L
		-2	2	1	+	7	-	L
		-3	2	1	-	-	-	L
		-4	2	0	-	-	-	L

Table 10 (continued)

Treatment of nurse bees	Experiment No.	Replicate No.	No. larvae grafted	No. larvae accepted	Capping ability	Days from grafting to capping	Days from capping to emergence	Time of death
(S)	I	30(S)-1	2	0	-	-	-	L
		-2	2	1	+	6	-	L
		-3	2	1	+	6	-	L
		-4	2	1	+	6	-	L
		-5	2	1	+	6	-	L
	II	30(S)-1	2	0	-	-	-	L
		-2	2	0	-	-	-	L
		-3	2	1	+	5	-	L
		-4	2	1	+	7	-	L
		-5	2	1	-	-	-	L
	III	30(S)-1	2	1	+	7	-	L
		-2	2	1	-	-	-	L
		-3	2	1	-	-	-	L
		-4	2	1	+	5	-	L
		-5	2	1	+	6	11	A
	IV	30(S)-1	2	1	+	6	-	P
		-2	2	1	+	6	-	P
		-3	2	1	+	-	-	L
		-4	2	0	-	-	-	L
	V	30(S)-1	2	1	+	6	-	L
		-2	2	1	+	6	-	L
		-3	2	1	+	6	-	L
		-4	2	1	-	-	-	L

Table 10 (continued)

Treatment of nurse bees	Experiment No.	Replicate No.	No. larvae grafted	No. larvae accepted	Capping ability	Days from grafting to capping	Days from capping to emergence	Time of death
(C)	I	30(C)-1	2	1	-	-	-	L
		-2	2	2	-	-	-	L
		-3	2	1	-	-	-	L
		-4	2	1	+	8	-	L
		-5	2	1	-	-	-	L
	II	30(C)-1	2	1	+	6	-	P
		-2	2	2	+	6	-	L
		-3	2	1	-	-	-	L
		-4	2	2	+	7	-	P
		-5	2	1	+	6	-	L
	III	30(C)-1	2	2	-	-	-	L
		-2	2	2	-	-	-	L
		-3	2	0	-	-	-	L
		-4	2	1	+	5	13	A
		-5	2	1	+	6	9	A
	IV	30(C)-1	2	1	+	6	9	A
		-2	2	2	+	6	9	A
		-3	2	1	+	7	9	A
		-4	2	1	+	6	-	L
	V	30(C)-1	2	1	-	-	-	L
		-2	2	1	+	5	-	L
		-3	2	1	+	5	-	L
		-4	2	2	+	7	10	A

Table 11

Morphological Charachteristics of Bees Reared by the 30(C) and 30(S) Groups (1970)

Code of Individual Bees	Pupal measurements					Adult measurements										
	Tongue length (mm.)	Body length (mm.) a	b	Total	Weight (mg.)	Head (mm.) W	L	w/L	Mandibles W	I	Q	Basitarsus L	W	L/W	Dia. Spermatheca (mm.)	No. of ovarioles
IV -30(S)-1P	2.00	4.10	5.00	9.10	142	3.70	3.20	1.51		x		2.00	1.20	1.67	1.00	75
IV -30(S)-3P	2.00	4.20	5.10	9.30	150	3.50	3.00	1.50			x	2.00	1.10	1.82	0.90	82
III-30(S)-5A	2.10	4.30	6.10	10.40	169	3.50	3.20	1.09			x	2.30	1.20	1.92	0.90	94
II -30(C)-1P	2.30	5.28	6.10	11.38	157	-	-	-		-		-	-	-	-	-
II -30(C)-4P	2.30	4.65	6.10	10.75	131	-	-	-		-		-	-	-	-	-
III-30(C)-4A	2.00	4.30	5.10	9.40	165	3.60	3.40	1.06			x	2.20	1.20	1.83	0.90	96
III-30(C)-5A	2.00	4.20	5.10	9.40	168	3.50	3.20	1.09		x		2.00	1.10	1.82	0.90	84
IV -30(C)-1A	2.00	4.30	5.20	9.50	157	3.70	3.60	1.03			x	2.30	1.30	1.77	1.10	113
IV -30(C)-2A	2.10	4.30	5.20	9.50	155	3.90	3.50	1.11		x		2.20	1.20	1.83	1.00	83
IV -30(C)-3A	2.10	4.30	5.30	9.60	155	3.60	3.00	1.20			x	2.30	1.20	1.92	0.90	96
V -30(C)-4A	2.20	4.30	5.10	9.40	154	3.70	3.40	1.09			x	2.00	1.00	2.00	1.00	106

III. EXPERIMENTS IN 1971

A. Methods and Materials

The technique used in 1970 was too time consuming to assist in meeting the objectives of the experiment. Also, the points of the tweezers were too large to grasp the ducts of the glands properly. Therefore, injuries at the site of the incisions frequently occurred causing high mortality among the dissected worker bees. In order to increase the speed of dissection and to decrease the mortality, further improvements were made in 1971. The improvements included the modification of the dissecting apparatus and a means of obtaining very sharp dissecting tools.

1. Sharpening Method

Sand paper was used to file the tweezers initially, followed by an electrolytic method. The electrolysis unit consisted of an electrolytic solution, a carbon cathode and a metal anode. The electrolytic solution was made by mixing one part of concentrated hydrochloric acid and one part of 3 M KCl solution. The supernatant was poured from the precipitate, and covered with a thin layer of xylene to prevent fumes from rising from the solution. The tweezers were attached to the anode with a clip and dipped into the solution. Three volts were passed through the solution.

When sharpening the tweezers, only their tips were dipped into the solution for 1-2 seconds. The depth of the

tips in the solution and the electrolysis time determine the shape and the sharpness of the points. No. 5 Dumont tweezers could be sharpened to very fine points for grasping the ducts of the mandibular glands.

2. Dissecting Apparatus and Tools

The dissecting apparatus consisted of two major parts: the dissecting dish and the dish-holding stand. The individual parts of the dissection apparatus and the assembled apparatus are shown in Figures 3, 4, and 5.

(a) Dissecting dishes:

Plastic petri-dishes (9 cm. in diameter) had 5 holes (0.7 cm. in diamter) drilled on their undersides. A piece of polystyrene foam material, with five holes (0.9 cm. in diameter) drilled in it, was placed on each petri-dish so that the holes overlapped those of the dish, thus permitting the flow of carbon dioxide gas over the insects during the operation (see Chapter II).

(b) The Dish-holding Stand:

The dish-holding stand was made of plywood and was designed to serve as a holder for the dissecting dish and gas tube (see Figure 4). The angle of the dish-holding stand (12.5 cm. x 8.5 cm. x 5.5 cm.) was adjustable for ease of dissection. A hole in the edge of the stand was connected by a series of tubes to a carbon dioxide tank which was equipped with a regulator. While anaesthetizing bees, the gauge on the carbon dioxide tank was set at the lowest level

to allow a small amount of CO_2 gas to pass from the tank; this flow was further reduced by a small valve near the holding stand. The dissecting apparatus was fitted to the stand of a binocular microscope (see Figure 6) during dissection. The magnification used in dissection was 80X.

The dissecting tools are the same as described in Chapter II.

3. Gland Removal Procedure

The procedure for mandibular gland removal can be separated into two major parts: mounting, and dissection.

(a) Mounting (see Figure 6):

A worker bee was first anaesthetized with CO_2 gas; then picked up with forceps, after which, a paper collar (Gary, 1961) was placed around its neck. The bee was placed in a hole leaving only the head outside with the collar pinned to the polystyrene base. In this way, the bee was inside the hole with its head exposed through the collar. When all five holes were filled with bees, the dissecting dish was placed on the dish-holding stand, which was then placed beneath the microscope. The two valves attached to the CO_2 gas lines allowed CO_2 gas to flow over the bee during the dissection process.

(b) Dissection (see Figures 7, 8 and 9):

Two incisions were made between the area of the compound eye and the mandibular articulation (see Diagram 1, and Gary, 1961) to form a triangular shaped flap which could be lifted

at its apex with forceps (see Figure 7). The left hand was used to lift the cuticular flap to expose the gland, while the tweezers, held in the right hand, were used to grasp the duct. Sometimes, the hook had to be used to pull out the large part of the gland, after which the tweezers were used to grasp the duct and pull out the gland intact (see Figures 8, 9). After pulling out the gland, the flap was put back into its original place, and the incisions left to heal themselves. The whole process was carried out as quickly as possible to eliminate bleeding. The dissecting dish was rotated to expose the opposite gena; the angle of the dish-holding stand was then regulated to a proper position, and the same operation was repeated. The other bees on the same dish were dissected in the same way; five bees could be dissected within 5-6 minutes. With help in mounting bees, only one and half to two hours were required to remove the glands from all the bees in a cage (i.e., 75 bees). Mounting the bees so that all of the heads were arranged clockwise or anticlockwise (depending on which hand is preferred in handling the tweezers) was easier and saved time during the dissection.

The nurse bees, after the removal of their mandibular glands, were coded as 75(Md) (or 30(Md), depending on the numbers of nurse bees). If only an incision was made without removing a gland (i.e., "sham" operation), it was coded as 75(S) (or 30(S)). If only the left gena received a "sham" operation, the bees were coded as 75(S)(L). The in-

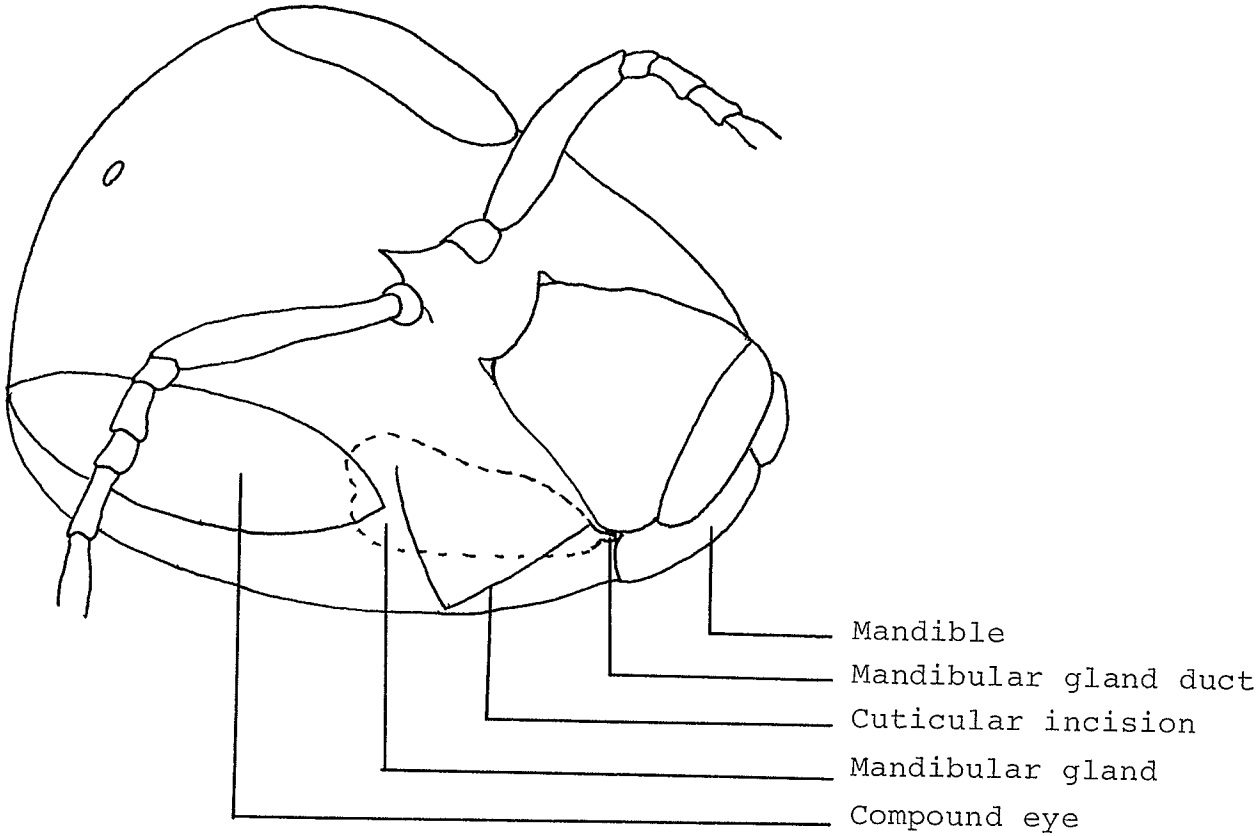


Figure 1. The head of a worker bee showing the position used for removing the mandibular gland.

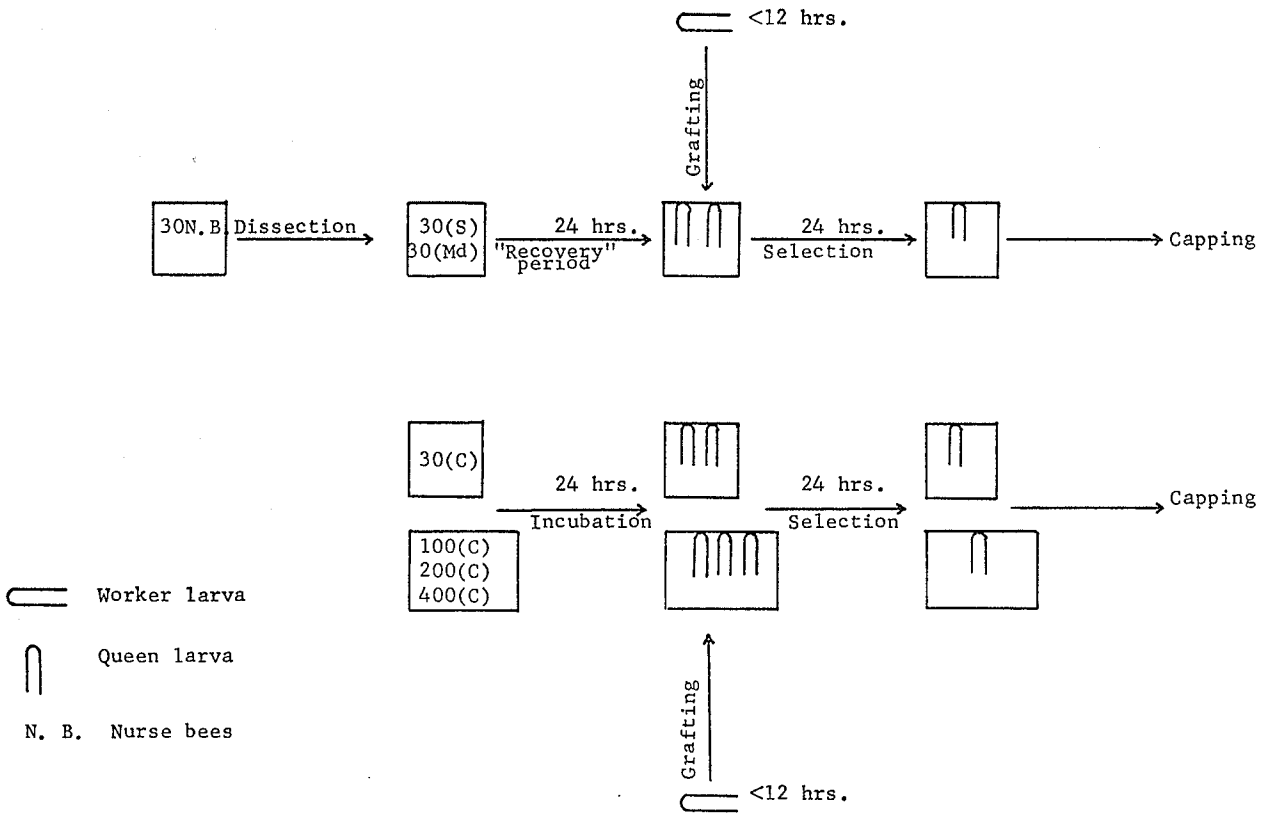


Figure 2A. Rearing procedures used in the rearing experiments and the mandibular gland removal experiments of 1970 and 1971.

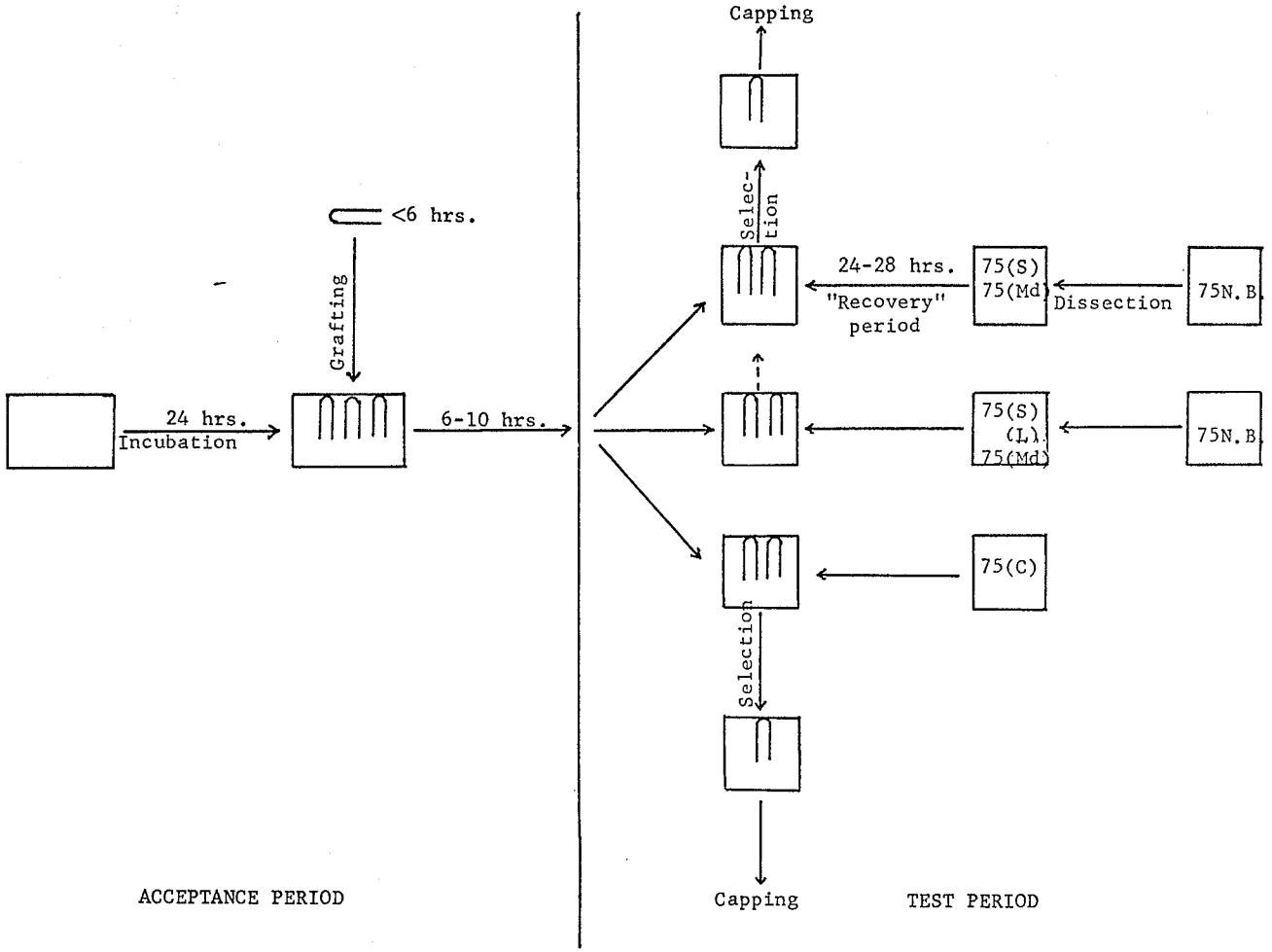


Figure 2B. Rearing procedures used in the mandibular gland removal experiments of 1971.

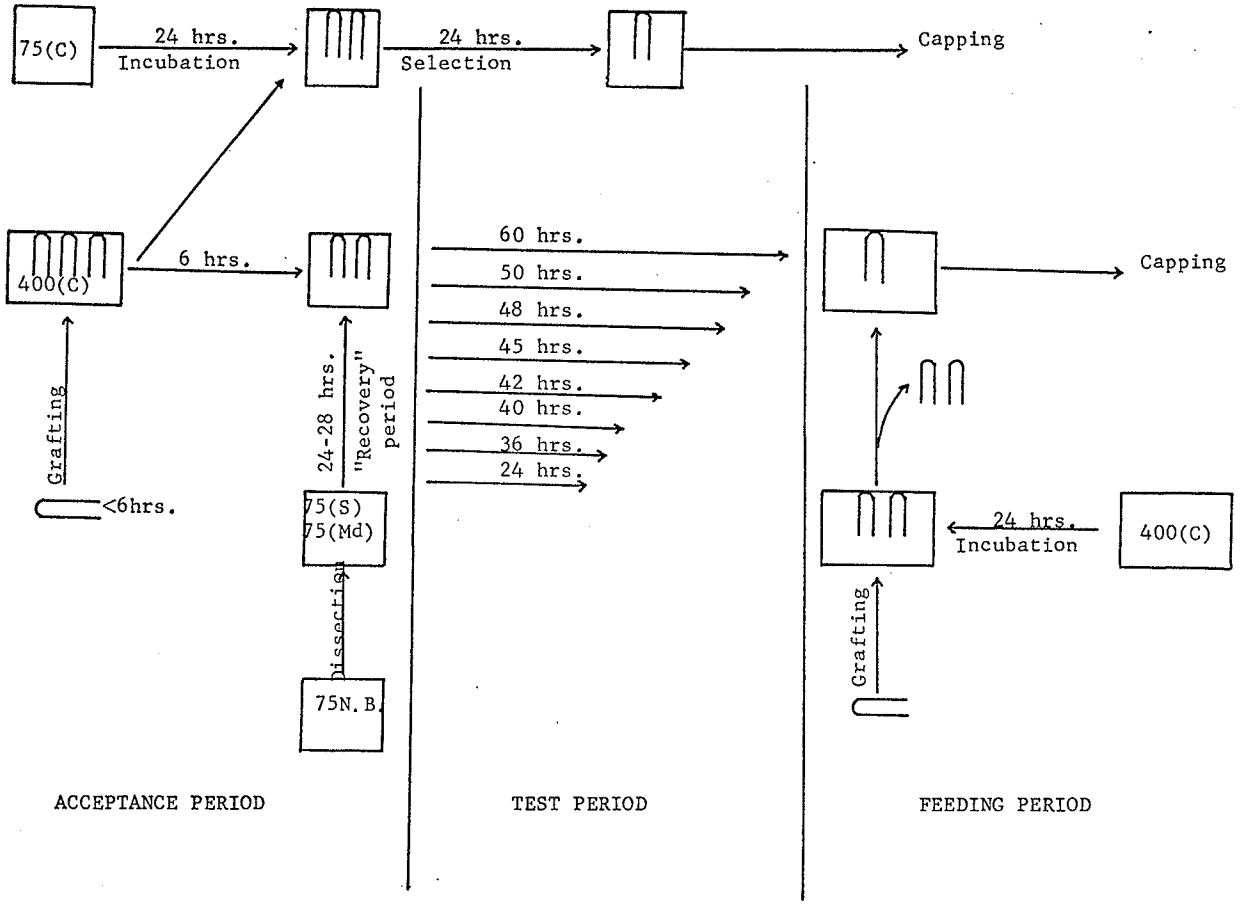


Figure 2C. Rearing procedures used in the mandibular gland removal experiments of 1972.

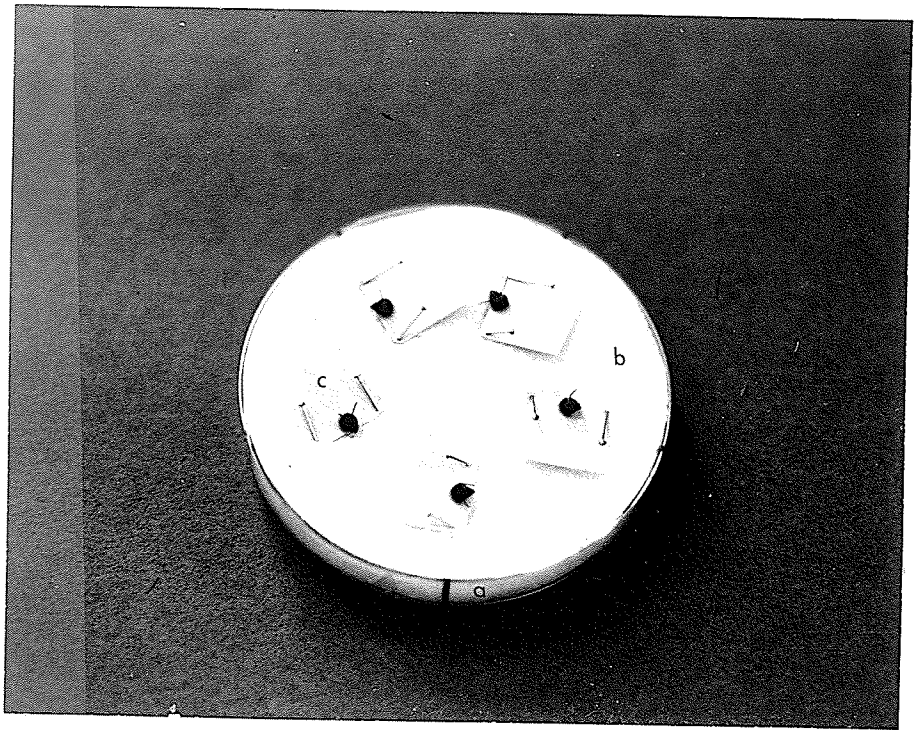


Figure 3. Dissection dish used in 1971 and 1972. (a) Plastic petri-dish, (b) polystyrene foam base, (c) paper collar.

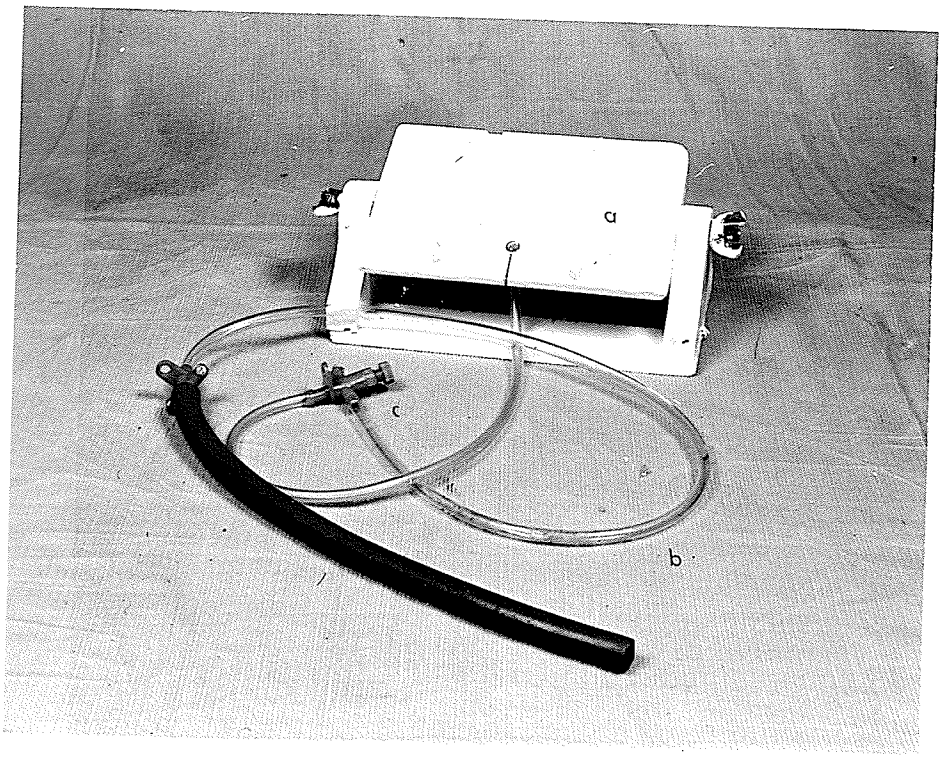
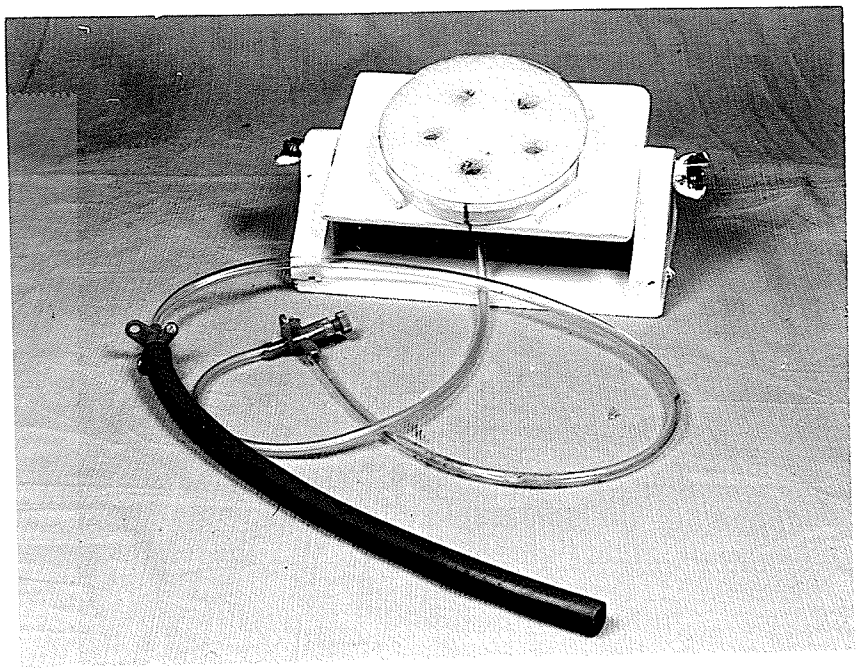


Figure 4. Dissecting apparatus used in 1971 and 1972. (a) The dish-holding stand, (b) gas tube, (c) valve for regulating the flow of CO_2 gas.



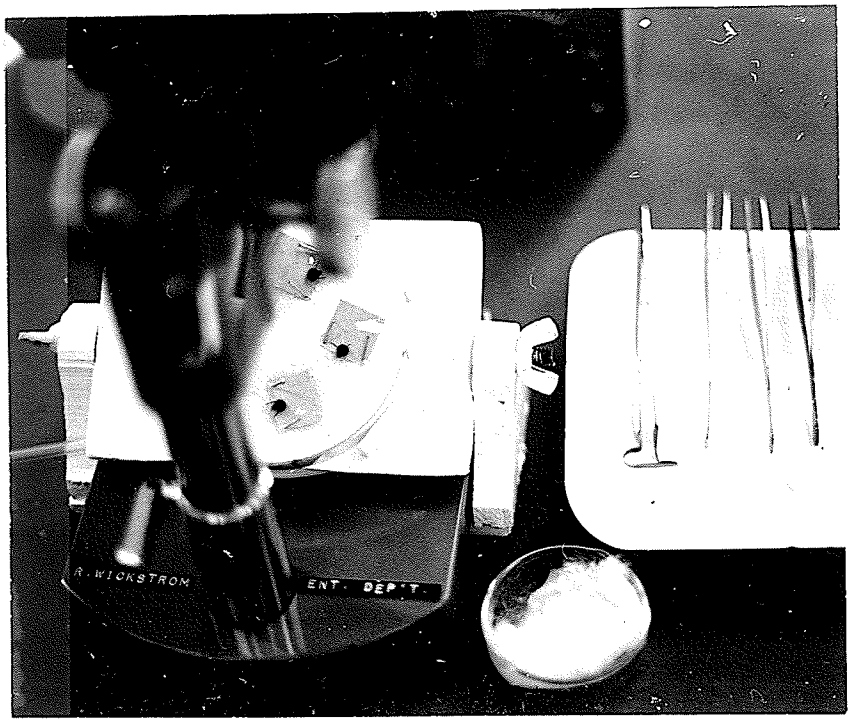


Figure 5. The dissecting apparatus; dish-holding stand with a dissecting-dish sitting on it.

Figure 6. Dissecting equipment and tools used in 1971 and 1972.

Figure 7. The incision position on the gena of a nurse bee. A cuticular, triangular-shaped flap (a) was made between the compound eye (b) and the articulation of the mandible (c).

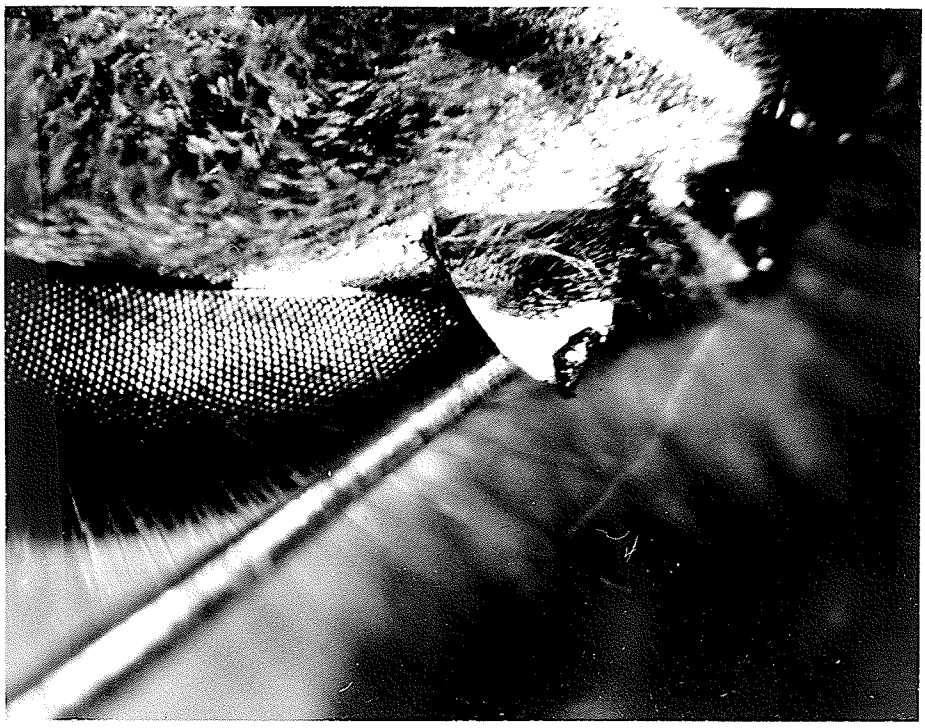


Figure 8. The removal of the mandibular gland. A hook was used for removing the mandibular gland intact.

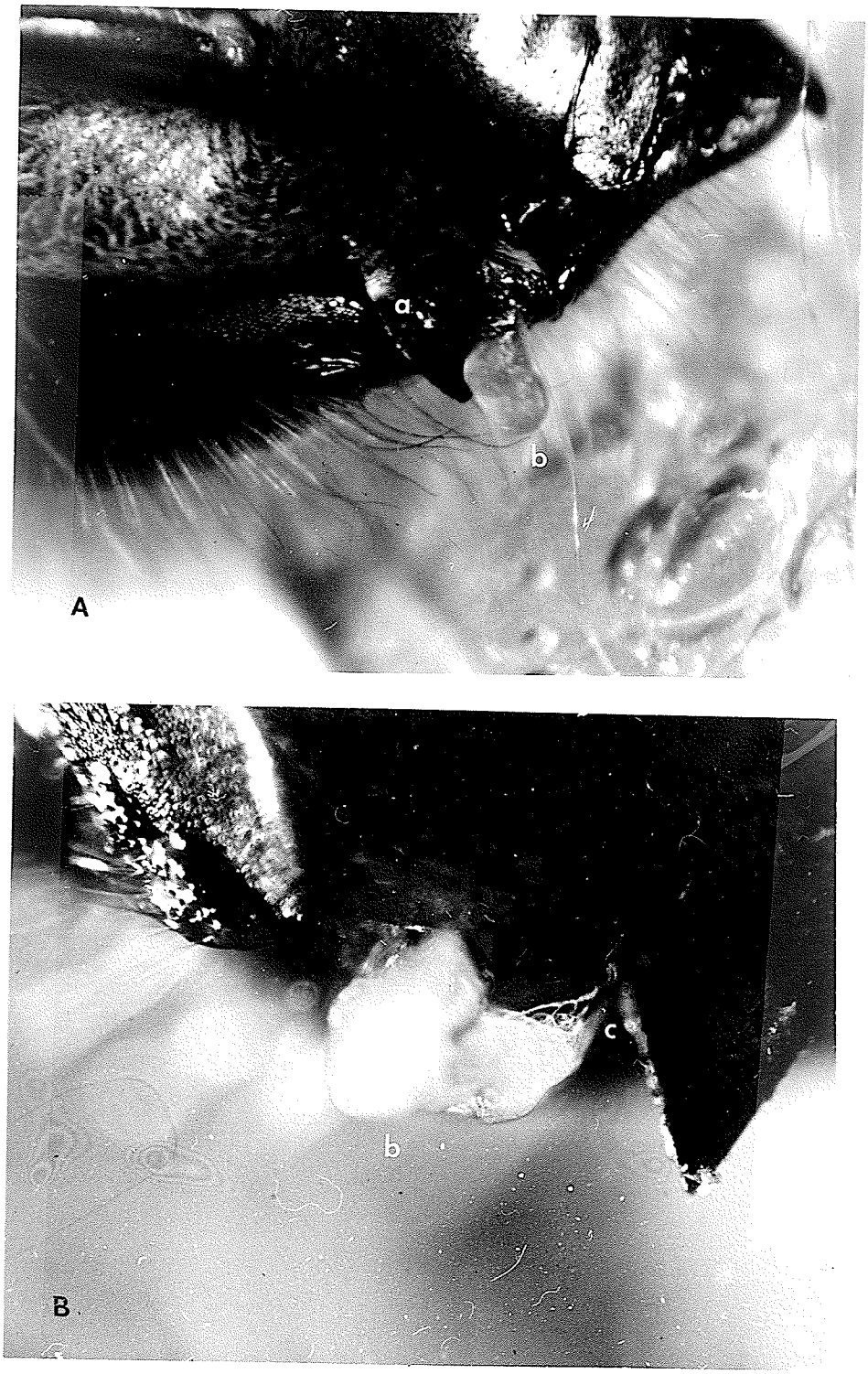


Figure 9. A view of the mandibular gland. A. Flap (a) with part of the gland protruding on the right gena. B. Showing the intact gland (b) and the duct of the gland (c).

tact 75 bees caged as one group (i.e., without either a "sham" operation or a gland removal), were coded as 75(C) (see Chapter II for abbreviations).

B. Experimental Design and Rearing Procedures

The experiment in 1971 consisted of two parts, 1. carbon dioxide tests and 2. the rearing experiments.

1. Carbon Dioxide Experiment

To test the effect of CO_2 (used in the dissections) on the mortality of nurse bees, an experiment was conducted as follows:

Carbon dioxide was used to anaesthetize nurse bees in the 30(Md) and 30(S) groups during the dissection process, but in the 30(Md)(X)* and 30(S)(X) groups, carbon dioxide was not used during the dissections, nor were the 30(C) groups treated (see Table 12). In each group, there were four replicates.

Rearing procedures were the same as described in 1970, except that each of the two larvae which were introduced into each group had previously been accepted and been fed by groups of 400 nurse bees for 6-10 hours. The acceptance of larvae in each cage was checked at twelve hour intervals. The mortality of nurse bees was checked every 24 hours. The rearing procedures are illustrated in Figure 2B.

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*Nurse bees not anaesthetized with carbon dioxide during dissection.

2. Rearing Experiments Using Groups of Seventy-Five Nurse Bees

In Experiments I to VI, the nurse bees received one of three treatments: 75(Md), 75(S) or 75(C). In each experiment, there were two replicates of the 75(Md) and 75(C) groups, and one replicate of the 75(S) group. In Experiments VII to XI, the nurse bees received one of three treatments: 75(Md)(L), 75(S)(L) or 75(C). In each experiment, there were two replicates of each treatment.

In the rearing procedure, each group was incubated 25-28 hours after its operation. Two larvae (less than 12 hours old), which had been accepted and fed by groups of 400 nurse bees for 6-10 hours, were introduced into each cage. Twelve hours later, larval acceptance, and selection of larvae were done as described in the preceding section.

C. Results and Discussion

1. Mortality of Nurse Bees

The results of the CO_2 test on the mortality of nurse bees is shown in Table 18. The mortality in 30(Md) groups was significantly lower than that of the 75(Md)(X) groups ($P<0.05$). No significant difference was found between the 75(S) and 75(S)(X) groups. However, the mortality was significantly higher in the 75(Md), 75(Md)(X) and 75(S)(X) groups as compared with the 75(C) groups. The mortality of the 75(Md), 75(S), 75(Md)(L), 75(S)(L) and 75(C) groups is also shown in Table 18. Both the 75(Md) and the 75(S) groups had

higher mortalities than did the 75(C) groups ($P<0.05$). It appears therefore that carbon dioxide can be used as an anaesthetic in the dissections.

Mortality of the nurse bees was significantly reduced by using the modified dissection method developed in 1971 ($P<0.05$) (Table 17). With the newly developed dissection apparatus and the electrolytically sharpened tools, the speed of dissection also increased. The gland removal operation, for each group of 75 nurse bees, could be finished within 2 hours.

2. Observations and Rearing Results

(A) Groups of Thirty Nurse Bees

Larval acceptances in the 30(Md), 30(S), 30(Md)(X) and 30(S)(X) groups was low (Table 9) when checked 12 hours after introduction. In the 30(Md) or 30(Md)(X) groups, only a small quantity of watery-yellowish secretion was supplied to the accepted larvae by the nurse bees (see 1970 results).

Because the 30(Md)-1, 30(Md)-2, 30(Md)-4, 30(S)-3 groups did not accept the larvae that were grafted first, a second, third and fourth larval grafting was done using the same nurse bees. None of the third or fourth introductions were accepted. No food was supplied to the larvae, although they were healthy and normal when grafted. One pupa was reared by the 30(S)-1 group from the first larva; it had a tongue length of 1.58 mm., was 14.62 mm. long and weighed 176 mg.

Table 12

Rearing Results of Groups of Thirty Nurse Bees (1971)

Treatment of nurse bees	No. of replicates	Total of larvae introduced	Total of pupae reared	Total of adults reared	Age of larvae when introd. (hr.)	Time of N.B.* when larvae introd. after dissection (hr.)	Mortality of nurse bees (%) (7 days)
(Md)	4	8	0	0	6.31**	36.57	11.26
					12.41***	46.73	
					17.61****	67.13	
(S)	4	8	1	0	6.76	37.68	10.17
					12.89	49.88	
					17.88	79.00	
(C)	4	8	0	0	-	-	7.83

*Nurse Bees
**Second introduction
***Third introduction
****Fourth introduction

In this experiment, the quantity of larval food in the cells was similar to that observed in the 1970 tests and the mortality of the nurse bees was decreased in the (Md) and (S) groups. This test also indicates that the number of nurse bees should be increased in order to supply more food to the grafted larvae.

(B) Groups of Seventy-Five Nurse Bees

Larval acceptance in the 75(S) and 75(S)(L) groups was as good as that in the 75(C) groups. Usually two out of two larvae were accepted in each cage. The larval acceptance was lower in the 75(Md) and 75(Md)(L) groups.

Those larvae which were accepted by the 75(Md) or 75(Md)(L) groups were fed with a watery-yellowish secretion by the nurse bees. The quantity of secretion was more than that in the 30(Md) groups studied in 1970 and 1971. However, the amount of secretion was only about one half the amount of royal jelly found in the 75(C) groups. Slightly more watery-yellowish secretion was found in the 75(Md)(L) groups than in the 75(Md) groups. The accepted larvae grew more slowly and were smaller in the 75(Md) and the 75(Md)(L) groups than in the 75(S), 75(S)(L) or 75(C) groups during the 36 hours after introduction. Although these larvae were never supplied with as much food as the 75(C) groups they were still surrounded by a small amount of food for 36 hours by the nurse bees without mandibular glands. The nurse bees of the 75(Md) and 75(Md)(L) groups appeared to stop secreting

larval food around 48-60 hours and larvae in those cages soon died from starvation. The larvae, which were introduced next, were also largely ignored by the nurse bees, although occasionally a nurse bee deposited a small quantity of yellowish secretion in their cells. The larvae died, and were soon removed by the nurse bees from the queen cells.

In the 75(S) and 75(S)(L) groups, the larvae also failed to reach the pupal stage and all died in the late larval or prepupal stages after capping. It appears that lack of sufficient food to complete development within the capped cells was at least partly responsible for the death of the larvae. Only one pupa (III-75(S)-1P) and three adults (I-75(S)-1A, IV-75(S)-2A, VI-75(S)-2A) were reared by the 75(S) groups. There were three pupae (VII-75(S)(L)-2P, XI-75(S)(L)-1P, XI-75(S)(L)-1P, XI-75(S)(L)-2P) and three adults (VII-75(S)(L)-1A, IX-75(S)(L)-1A, IX-75(S)(L)-2A) reared by the 75(S)(L) groups out of 10 larvae. In the 75(C) groups, twenty two adults were reared from 22 larvae (Table 15).

Because the 75(Md) groups stopped feeding the larvae around the 48-60 hour period, most of the larvae in these cages died about that time. Some larvae survived longer, and in a few cases, survived to the pupal stage (IV-75(Md)-1P, VII-75(Md)(L)-2P, VIII-75(Md)(L)-1P, X-75(Md)(L)-1P). It is possible that the under-nutrition of the larvae was due to the lack of an essential nutrient in the secretion of the nurse bees without mandibular glands. However, the

Table 13

Rearing Results of Groups of Seventy-Five Nurse Bees (1971)

Treatment of nurse bees	No. Replicates	Total of Pupae reared	Total of adults reared	Age of larvae when introd. (hr.)	Time of N.B.* when larvae introd. after dissection (hr.)	Mortality of nurse bees (%) (7 days)	Total Development time (day)
75(Md)	12	1	0	6.79**	25.18	15.33	-
				26.97***	49.15		
				89.33***	92.57		
75(Md)(L)	10	3	0	8.93*	30.89	11.87	-
75(S)	6	1	3	9.99	26.15	13.33	17
75(S)(L)	10	3	3	8.88	27.64	8.00	17
75(C)	12	-	12	8.23	-	10.11	16
75(C)	10	-	10	9.34	-	8.40	16

* Nurse Bees
**Second introduction
***Third introduction
****Fourth introduction

question as to whether failure to rear larvae to adults is due to quantitative starvation or due to the lack of certain caste determining substances and/or nutrients in the diet as yet remains unanswered.

3. Morphological Characteristics

The morphological characteristics of the adults and pupae reared by different groups of bees are given in Table 7.

The only pupa reared by the 75(Md) groups (IV-75(Md)-2P), had a tongue length of 2.54 mm., a body length of 9.35 mm. and body weight of 142 mg. This pupa died in the early pupal stage. The reproductive system looked normal, but the ovarioles were slightly decayed, and an accurate count could not be made. Three pupae were reared by the 75(Md) (L) groups, had short tongues and low weights (Table 15). These pupae were small and died in the early pupal stage.

The adults and pupae reared by the 75(S) and 75(S) (L) groups possessed queen-like and intercaste characteristics (see Table 15). Two of the pupae reared by the 75(S) groups had intercaste mandibles. The pupae, reared by the 75(S) groups, were small (see Table 15) and their tongues were short. The basitarsal indices of the adults fell within the queen category (see Weaver, 1957). The number of ovarioles was lower than that of normal queens (Weaver, 1957). The pupae and adults reared by the 75(S)(L) groups were

Table 14

Morphological Characteristics of Pupae and Adults Reared

By Seventy-Five Nurse Bees (1971)

Treatment of nurse bees	No. of pupae measured	No. of adults measured	Pupal measurements					Adult measurements										
			Tongue length (mm.)	Body length (mm.) a	b	Total	Weight (mg.)	Head (mm.) W	L	w/L	Mandibles W	I	Q	Basitarsus L	W	L/W	Dia. Spermatheca (mm.)	No. of ovarioles
75(Md)	1	0	2.54			9.35	142		-			-			-		-	-
75(Md)	3	0	2.52± 0.11			14.02± 0.37	142.67± 9.21		-			-			-		-	-
75(S)	4	*4	2.46± 0.07			12.85± 0.18	146.75± 5.73	3.48± 1.87	2.905± 0.31	1.34± 0.14	2		2	2.18± 0.15	1.05± 0.06	2.08± 0.07	0.95± 0.11	113± 4.93
75(S)(L)	6	3	2.86± 0.16			14.97± 0.31	181.0± 16.31	3.97± 0.04	3.45± 0.10	1.15± 0.02	2		5	2.43± 0.04	1.13± 0.03	2.17± 0.03	1.05± 0.02	107± 12.57
75(C)	12	12	2.69± 0.108			14.99± 0.204	199.75± 9.75	4.05± 0.04	3.49± 0.04	1.16± 0.01			12	2.57± 0.05	1.28± 0.03	2.02± 0.04	1.14± 0.04	129.83± 4.07
75(C)	10	10	2.68±			14.93±	221.3±	3.98±	3.55±	1.14±			10	2.46±	1.23±	2.00±	1.11±	131.7± 5.32

* = one is a pupa.

queen-like (see Table 15), and the ovariole counts were lower than that of normal queens (Weaver, 1957).

The adults reared by the 75(C) groups were queen-like. Generally speaking, pupae and adults reared by the 75(S) and the 75(S)(L) groups were smaller than the ones reared by the 75(C) groups and some had intercaste characteristics. However, the low amount of food left in the cells after capping could be partly responsible for this.

IV. EXPERIMENTS IN 1972

A. Methods and Materials

The dissection equipment, methods and techniques were the same as those used in 1971.

B. Experimental Design and Rearing Procedures

The experiments in 1972 were designed to elucidate nutritional effects on the development of larvae during and after the "critical" period (see Chapter I) in their caste differentiation.

The rearing procedure consisted of three successive periods (see Chapter II), (1) Acceptance Period, (2) Test Period, and (3) Feeding Period. The Acceptance Period and Test Period were similar to those in 1971, except that the larvae were fed for 6 hours during the Acceptance Period. However, the Feeding Period was introduced in an effort to avoid the starvation, and death of the larvae, following the

"critical" period (the first 72 hours in larval stage). An attempt was made, in these experiments, to prolong the Test Period as long as possible so as to pass the "critical" period of larval differentiation. The rearing observations and results of 1971, showed that 75(Md) bees had a tendency to decrease or stop the secretion of larval food about 2-3 days after dissection. The first Test Period of 60 hours [TP:60] was used for the 12 hour old larvae (aged 6 hours when they were grafted, plus 6 hours in the Acceptance Period). Later Test Periods were shortened to 50, 48, 45, 42, 36 and 24 hours, because of rearing problems in the [TP:60].

The rearing procedures are shown in Figure 2C. During the Test Period, larvae were fed by bees of the 75(Md) or 75 (S) groups and were examined every 6 hours for acceptance and growth. Each larva, which survived a particular Test Period, was grafted into a queen cell which, until this transfer, had contained a larva, similar in age to the test larva; the test larva was then fed by a group of 400 nurse bees. During this feeding, the larva in each group of 400 nurse bees was examined for acceptance and growth every 6 hours until the cell was capped. The queen cell, containing the larva, was then removed from the cage and hung in a vial in an incubator until feeding was completed. Later it was put into a wax cell for pupation (see Chapter II). Pupal and adult measurements were done at the proper time.

In the [TP:60], [TP:50], [TP:48] and [TP:24] test, there were two replicates in both the 75(Md) and the 75(C)

groups, and one replicate in the 75(S) group. In each [TP: 45] or [TP:42] test, there were two replicates in the 75(Md) and 75(C) groups, and one replicate in the 75(S) group (i.e., there were 4 larvae in two replicates and 2 larvae in one replicate). These two experiments were replicated four times. In the [TP:40] and [TP:36] tests, there were two replicates of the 75(Md), and 75(C) groups and one replicate of the 75(S) group. These experiments were repeated twice.

C. Results and Discussion

1. [TP:60], [TP:50] and [TP:48] Experiments

In the [TP:60] experiment, all four larvae died between the 42-48 hour check during the Test Period (Table 15). The larvae were small and similar to those in the 1971 tests. There was a tendency for the 75(Md) groups to decrease or stop the amount of secretion about this time. One adult, reared by the 75(S) groups and 4 adults, reared by the 75(C) groups, were obtained in this experiment.

In the [TP:50] experiment, one larva, I-[75(Md)-1, TP:50]-2L survived for 50 hours during the Test Period. It died after 6 hours in the Feeding Period, even though a large quantity of royal jelly had been given to it. This larva was 3.9 mm long and weighed 2.25 mg. According to Wang's data (1965) its weight was close to that of a queen or worker larva 48 hours old. Larva I-[75(Md)-2, TP:50]-1L died during the 50 hour Test Period. It only weighed 1.87 mg. and was 3.6 mm. long. Its weight was close to a larva

Table 15

Summary of Rearing Results for 1972

Test Period (hr.)	Treatments of Nurse Bees								
	(Md)			(S)			(C)		
	Total of larvae grafted	No. of pupae reared	No. of adults reared	Total of larvae grafted	No. of pupae reared	No. of adults reared	Total of larvae grafted	No. of pupae reared	No. of adults reared
60	4	0	0	4	0	1	4	0	4
50	4	0	0	4	0	2	4	0	4
48	8	2	0	-	-	-	4	2	2
45	16	2	4	8	2	6	16	3	13
42	16	1	7	8	-	8	16	1	16
40	8	1	7	4	1	3	8	0	8
36	8	0	7	4	0	4	8	0	8
24	4	0	4	4	1	3	4	0	4

42 hours old (see Wang, 1965). Larva I-[75(Md)-1, TP:50]-1L was not accepted at the beginning of the Test Period. Larva I-[75(Md)-2, TP:50]-2L died before 50 hours and was removed from the cell by the 75(Md) bees. Two adults were reared by the 75(S) groups, while in the 75(C) groups, four adults were obtained in the [TP:50] experiment (Table 16).

In the [TP:48] experiments, larvae I-[75(Md)-1, TP: 48]-1L and I-[75(Md)-2, TP:48]-1L, both survived for 48 hours, but died after 6 hours in the Feeding Period. Their body weights were 0.79 and 0.91 mg. respectively. These are close to larvae 30-36 hours old (Wang, 1965). Their body lengths were 2.8 mm., and 3.0 mm. respectively. The other two larvae were not accepted at the beginning of the Test Period. Two pupae and two adults were reared by the 75(C) groups in this experiment.

Some larvae, although they survived the Test Period, were small and did not complete growth in the Feeding Period. Probably, these larvae, were undernourished and could not survive even when supplied with normal fresh royal jelly in large quantities. The Test Period was later shortened to 45, 42, 40, 36 and 24 hours.

2. [TP:45], [TP:42], [TP:40], [TP:36] and [TP:24] Experiments

(1) Rearing Results:

The numbers of pupae and adults obtained in the test are shown in Table 16. Higher numbers of pupae and adults were obtained when the Test Period was decreased.

The acceptance of larvae in the 75(Md), 75(S) or 75(C) groups were high. However, most of the larvae in the 75(Md) groups died during the Test Period or during the Feeding Period in the [TP:45] and [TP:42] experiments. In the [TP:40], [TP:36] and [TP:24] experiments the larvae usually survived both the Test Period and the Feeding Period, and therefore higher numbers of adults were obtained (Table 16). Four and seven adults were reared from 16 larvae in the [75(Md), TP: 45] and the [75(Md), TP:42] experiments respectively. Seven adults were reared out of 8 larvae in both the [75(Md), TP: 40] experiment, and the [75(Md), TP:36] experiment. Four adults were reared out of four larvae in the [75(Md), TP:24] experiment. High numbers of adults and pupae were also reared by the 75(S) or 75(C) groups (Table 16).

These larvae were smaller than those reared by the 75(S) bees during the same Test Period. In the [75(Md), TP: 45] experiments, trace amounts of a watery-yellowish secretion were observed in the cells after the larvae were removed to the cells used during the Feeding Period. They appeared to be normal but were small.

(2) Morphological Characteristics:

The characteristics of the adults and pupae reared in the different experiments are summarized in Table 16; the results of the factorial analysis of variance are summarized in Table 17.

There was a significant difference in tongue length between the different Test Periods and within treatments of

the nurse bees (75(Md), 75(S) and 75(C) groups) (Table 17) ($P<0.05$). Tongue lengths of pupae reared in the [TP:45] experiments were significantly shorter than those reared in the [TP:40], [TP:36] and [TP:24] experiments. Body lengths of the pupae reared by the 75(Md) groups was significantly lower than that of pupae in the 75(S) or 75(C) groups ($P<0.05$); no differences were found between pupae in the 75 (S) and 75(C) groups. Significant differences were found between Test Periods and within treatments for pupal weights (Table 18) ($P<0.01$). Weights of pupae reared by the 75(Md), in different Test Periods, were significantly lower than those reared by the 75(S) or 75(C) groups ($P<0.05$). Weights of pupae reared in the [TP:45] experiments were lower than those reared in the [TP:24] or [TP:36] experiments. Generally, pupae reared by the 75(Md) groups were small regardless of the Test Period used. Pupae, reared in the [TP:45] experiments were also smaller in size and lower in weight than those reared in the shorter Test Period (Table 16). However, those larvae reared by the 75(Md) groups were small but queen-like in appearance in all of the Test Period experiments.

Head indices of adults reared in the [TP:45] and [TP: 42] tests were significantly larger than those reared in the other Test Periods (Table 18). No significant differences were found in the head index, within treatments, regardless of Test Period. Adult, III-[75(Md)-1, TP:45]-2A, and III-[75(Md)-2, TP:45]-3A, I-[75(Md)-2, TP:42]-3A and I-[75(Md)-2, TP:40]-3A had intercaste mandibles. No significant differen-

ce was found in basitarsal indices between adults reared in the various Test Periods and within treatments (Table 17). Except for the adults reared in [75(Md), TP:36] none had a basitarsal index lower than those in the queen range (Weaver, 1957); adults, reared in other experiments, all had basitarsal indices within the queen range (Weaver, 1957); significant differences however were found between the bees in the various Test Periods ($P<0.05$) (Table 17). Adults reared in the [TP:42] experiments had significantly higher ovariole numbers than those reared in other experiments ($P<0.05$). Adults, reared in the [75(Md), TP:45] and [75(Md), TP:42] experiments, had lower ovariole counts than those in the queen range (Weaver, 1957) (Table 17). Adults, reared by [75(Md), TP:45] groups had only 96.00 ± 7.50 ovarioles in their right ovaries, which is still higher than the worker range (Weaver, 1957). There were significant differences in the diameters of spermathecae within treatments ($P<0.05$). Adults, reared in the [75(Md), TP:45] groups, had smaller spermathecae than those reared in other experiments (Table 16).

Adults reared in these experiments were queen-like in appearance, and generally had queen-like morphological characteristics (Weaver, 1957). The rearing experiments, along with various physiological and biochemical studies (see Chapter I), suggest that caste differentiation in honey bees is progressive, and is fixed about the third day in the larval stage. Many physiological differences between queen

larvae and worker larvae are found around 72 hours and 84 hours (see Chapter I). Therefore, the fact that the adults, which were reared in experiments with different Test Periods, were queen-like might be due to the fact that the Test Periods are not long enough to exceed the "critical" period in caste differentiation (presumably around 72 hours of larval age), thus the larvae may obtain some substance(s), during the Feeding Period which are not produced by the bees without mandibular glands during the Test Period. Since a certain degree of change in the reproductive system is possible in the later larval stage (Ribbands, 1953; Weaver, 1957), the larvae could develop their reproductive systems to a certain degree in the Feeding Period while ingesting normal royal jelly. Therefore, adults with high ovariole counts and of normal sized spermathecae might be obtained in this experiment. The other possible reason is that, in the absence of mandibular glands, other glands might be able to synthesize the determining substance or the nutrients which are thought to be only produced by the mandibular glands. Therefore these larvae, although they had been fed less food during a certain period of their larval life, differentiated into queen-like or intercastes, in the presence of these substances presumably produced by other glands.

Actually, the adults and pupae which showed queen-like characters were 57-59 hours old when they were removed from the nurse bees without mandibular glands and trans-

Table 16

Morphological characteristics of bees reared for various Test Periods by Groups of Nurse Bees which have Received Various Treatments (1972)

Test Period (Hr.)	Treatment of nurse bees	No. of pupae measured	No. of adults measured	Pupal measurements: Tongue length (mm.)	Body length (mm.) a	b	total	Weight (mg.)	Adult measurements: Head (mm.) W	L	W/L	Mandibles W	I	Q	Basitarsus L	W	L/W	Dia. Spermatheca (mm.)	No. of ovarioles
	(Md)	6	6	*2.21± **0.04	6.48±0.07	8.00±0.07	14.47±0.12	187.2±5.28	3.32±0.09	3.91±0.03	1.18±0.04		2	4	2.21±0.11	1.15±0.04	1.92±0.05	0.99±0.01	96.00±7.50
45	(S)	8	8	2.32±0.04	6.43±0.11	8.79±0.19	15.16±0.24	196.5±8.81	3.56±0.08	4.05±0.04	1.14±0.02			8	2.45±0.05	1.27±0.02	1.92±0.03	1.07±0.02	127.25±5.38
	(C)	16	13	2.44±0.05	6.64±0.12	8.77±0.13	15.41±0.20	219.75±5.52	3.57±0.08	3.94±0.04	1.24±0.02			8	2.39±0.14	1.23±0.03	1.95±0.06	1.11±0.02	132.13±8.87
	(Md)	8	7	2.47±0.04	6.42±0.04	8.50±0.22	14.86±0.29	188.13±5.44	3.38±0.04	3.31±0.04	1.16±0.02			6	2.33±0.05	1.21±0.03	1.96±0.06	1.07±0.02	113.29±6.41
42	(S)	8	8	2.30±0.04	6.61±0.12	8.35±0.31	14.96±0.41	189.00±7.32	3.69±0.04	4.19±0.04	1.14±0.01			8	2.57±0.03	1.27±0.02	2.02±0.02	1.09±0.01	141.25±7.75
	(C)	16	16	2.40±0.03	6.63±0.12	8.63±0.13	15.26±0.22	206.00±6.28	3.59±0.09	3.98±0.06	1.11±0.01			8	2.31±0.06	1.23±0.04	1.90±0.06	1.08±0.02	142.25±8.32
	(Md)	8	7	2.48±0.05	6.43±0.03	8.52±0.05	14.95±0.06	198.63±4.53	3.58±0.10	4.01±0.08	1.12±0.02			6	2.27±0.11	1.20±0.03	1.90±0.07	1.04±0.02	120.71±7.30
40	(S)	4	4	2.28±0.02	6.64±0.05	8.27±0.14	14.91±0.18	194.25±7.12	3.47±0.13	4.16±0.04	1.20±0.03			4	2.30±0.09	1.26±0.02	1.98±0.04	1.08±0.01	134.00±4.40
	(C)	8	8	2.51±0.04	7.04±0.06	8.91±0.10	15.95±0.14	205.50±4.18	3.61±0.06	3.90±0.05	1.08±0.01			8	2.45±0.04	1.27±0.03	1.94±0.04	1.05±0.02	127.25±11.09

Table 16 (continued)

Test Period (Hr.)	Treatment of nurse bees	No. of pupae measured	No. of adults measured	Pupal measurements					Adult measurements										
				Tongue length (mm.)	Body length (mm.) a	b	total	Weight (mg.)	Head (mm.) W	L	W/L	Mandibles W	I	Q	Basitarsus L	W	L/W	Dia. Spermatheca (mm.)	No. of ovarioles
	(Md)	7	7	2.45± 0.04	6.45± 0.03	8.79± 0.09	15.24± 0.10	201.29± 4.19	3.56± 0.04	3.98± 0.06	1.11± 0.02			7	2.34± 0.09	1.26± 0.02	1.84± 0.05	1.05± 0.02	127.14± 3.96
36	(S)	4	4	2.42± 0.04	6.78± 0.09	8.38± 0.07	15.15± 0.16	200.00± 8.09	3.54± 0.09	4.02± 0.03	1.14± 0.02			4	2.44± 0.08	1.26± 0.02	1.93± 0.07	1.11± 0.03	134.50± 11.14
	(C)	8	8	2.52± 0.06	6.84± 0.10	8.78± 0.12	15.62± 0.21	206.68± 5.43	3.57± 0.05	3.99± 0.05	1.12± 0.02			8	2.41± 0.06	1.24± 0.03	1.96± 0.05	1.10± 0.01	121.50± 4.95
	(Md)	4	4	2.36± 0.03	6.72± 0.11	8.93± 0.10	15.67± 0.16	208.75± 5.27	3.74± 0.10	3.97± 0.09	1.05± 0.02			4	2.39± 0.04	1.23± 0.03	1.99± 0.02	1.04± 0.03	146.25± 4.68
24	(S)	4	4	2.44± 0.03	6.86± 0.07	8.53± 0.05	15.39± 0.09	207.75± 4.66	3.65± 0.13	3.97± 0.10	1.09± 0.00			4	2.32± 0.15	1.80± 0.04	1.96± 0.11	1.02± 0.03	126.00± 13.92
	(C)	4	4	2.42± 0.08	6.70± 0.17	8.80± 0.10	15.49± 0.23	209.25± 5.62	3.60± 0.06	3.89± 0.05	1.05± 0.02			4	2.29± 0.06	1.26± 0.06	1.96± 0.04	1.08± 0.02	135.25± 5.38

Table 17

Summary of Analysis of Variance

	Pupal Measurements			Adult Measurements			
	Tongue length	Total body length	Pupal Weight of Pupa	Adult head index	Basitarsal index	No. of ovarioles	Dia. of Spermatheca
Time (5)	S ($P<0.005$)	NS($P>0.05$)	S($P<0.05$)	S ($P<0.05$)	NS($P>0.05$)	S ($P<0.05$)	NS($P>0.05$)
Treatment (3)	S ($P<0.05$)	S($P<0.01$)	S($P<0.01$)	NS($P>0.05$)	NS($P>0.05$)	NS($P>0.01$)	S ($P<0.005$)
Time x Treatment	NS($P>0.05$)	NS($P>0.05$)	S($P<0.01$)	S ($P<0.01$)	NS($P>0.05$)	NS($P>0.05$)	NS($P>0.05$)

Table 18

The Mortality of Bees Used in Various Experiments
(1970, 1971 and 1972)

Year	30(10)(Md) %	30(10)(S) %	30(10)(C) %	30(Md)(X) %	30(S)(X) %
1970	56.19(23)*	26.34(23)	6.06(23)		
1971	11.26(4)	10.17(4)	7.83(4)	18.33(4)	13.33(4)
	75(10)(Md) %	75(10)(S) %	75(10)(C) %	75(Md)(L) %	75(S)(L) %
1971	15.33(12)	13.15(6)	9.78(12)		
1971			8.40(10)	11.87(10)	8.00(10)
1972	7.31(30)	5.26(18)	3.06(30)		

* = No. of replicates.

ferred to normal bees. It appears that the quantity of food fed to these larvae by these nurse bees during the Test Period was insufficient to feed the older larger larvae. It therefore seems important now that a larger group of nurse bees without mandibular glands (e.g., 400-500) be used in an attempt to extent the Test Period to as close to 72 hours (the "critical" period) as possible. Only then can we be reasonably sure that quantity of food is not masking qualitative considerations.

CHAPTER VI

OVARY DEVELOPMENT OF GROUPS OF WORKER BEES HELD IN CAGES

I. INTRODUCTION

The ovary development of worker bees, in hives or in cages has been extensively studied by many research workers (Hess, 1929; Butler, 1957, 1961; De Groot and Voogd, 1954; Jay, 1968, 1970, 1972; Velthuis, Verheijin and Gottenbos, 1965; Velthuis, 1970). It has been established that pheromones of adult queens can inhibit the ovary development of worker honey bees. Therefore, when worker bees are left in a queenless condition, their ovaries develop. However, worker larvae, or pupae, also play a role in the inhibition of ovary development of worker bees (Jay, 1968, 1970).

Most of the above research has been done with nuclei or hives. The investigation of ovary development of caged worker bees in the laboratory has been done by Butler (1957), Butler and Fairy (1963), De Groot and Voogd (1954), Voogd (1955, 1956), Velthuis, Verheijen and Gottenbos (1965), Velthuis (1970), and of worker bees in the observation hives by Sakagami (1958). It was found that when small numbers of caged worker bees are incubated in a queenless condition, their ovaries develop after 14-21 days (Velthuis, 1970).

II. METHOD

Experiments were done to determine seasonal and source effects on the ovary development of worker bees used in this study as well as the effect of removing the mandibular glands of workers on their ovary development.

A. Experiment I.

Ten day old worker bees of a yellow strain were collected on July 19 and August 9, 1972, from two colonies. Groups of ten-day old worker bees, 75 to a cage, were incubated in the laboratory as described in Chapter III. 75(Md) groups and 75(C) groups, collected on these two dates, were kept and used for this experiment. If, in these groups, the larvae survived through the test period, the queen cells containing young larvae were removed from the cage, and an empty queen cup was put in the place of the original one. If the larvae failed to survive through the test period, the dead larvae were removed, but the queen cells were left with worker bees. The queen cups in the cages served as a laying place for the worker bees. The worker bees were incubated about four more days in their cages with the empty queen cells and the wax cells which they had built by themselves. The occurrence of laying workers in the cages was observed and recorded after the dissection experiment. When the worker bees were 17 days old, they were stored in a refrigerator for ovary examinations at a later date.

B. Experiment II.

Ten day old bees of a yellow strain were collected on July 26 (a), August 3 (b) and August 24 (c, d, e), 1972, as shown in Table 20. In addition to 75(Md) and 75(C) treatments, other 75(G) groups were included, each with two queen cells containing one larva each (less than 12 hours old). The larva from one of the cells was removed 24 hours later while the larva in the other cell was reared until its cell was capped. In the 75(C)(M) and 75(G)(M) groups, the treatment procedure was essentially the same as that of the 75(C) and 75(G) groups except that a piece of worker comb was attached to the aluminum bar. The bees of the various groups were stored, for dissection, when they were seventeen days old.

Twenty-five worker bees, from each cage, were dissected; their ovaries were classified as 0, undeveloped (score = 1); I, slightly developed (swelling and (or) constriction of ovarioles, score = 2); or II, well developed (ova usually present in various stages, score = 3). The few ovaries that were between categories and difficult to classify were assigned alternately to the next higher category or the next lower one (Jay, 1972).

III. RESULTS

The results are summarized in Tables 19, 20 and 21.

Development of the workers' ovaries occurred in these experiments as indicated by the ovary development in-

dex (Table 19). There was no significant difference in worker ovary development between the two treatments in which the mandibular glands of nurse bees were removed on different dates (75(Md)), nor in the two control groups (75(C)). Too, there was no significant difference between the two different treatments (75(Md) vs 75(C)) (Table 19). The data suggest that samples of bees collected at different times or from different colonies and kept queenless in cages, do not differ in their ovary development.

In Table 20, no significant difference was found among all the treatments. The worker bees generally had developed ovaries at seventeen days regardless of treatment, (i.e., after the removal of both mandibular glands and the rearing of female larvae in the queen cups for 42-45 hours (75(Md) groups), after rearing female larvae in queen cups for 42-45 hours (75(C) groups), or when 75(C)(M), 75(G)(M) groups were supplied with a piece of worker comb. Laying workers occurred often among caged worker bees during the experiments as shown in Table 21. The eggs could be found in the queen cups or in the wax cells on the 5th-8th day after caging (Figure 1). Usually, up to ten eggs could be found in a single queen cell cup, and wax cells often contained more than one egg.

IV. DISCUSSION

The ovary development of worker honey bees in queenright colonies is inhibited by pheromones produced in the

heads of queens (Voogd, 1955; Verheijen Voogd, 1959) and in particular the mandibular glands (Butler et al., 1962; Butler and Fairy, 1963), as well as by glands in the abdomen of the queen (Velthuis, 1967). After queens have been mated, they produce enough pheromone to inhibit the ovary development of worker bees of a colony. However, queen pheromones, or the queen alone, are unable to fully inhibit the ovary development of worker bees when sealed or unsealed worker brood is absent (Perepelova, 1929; Müssbichler, 1952; Jay, 1968, 1970). Nor can queen larvae or pupae, in small numbers, inhibit the ovary development or workers in queenless colonies (Jay, 1968, 1970). Small groups of caged queenless worker bees, in the above studies, showed a high degree of ovary development (see also Butler, 1957; Butler and Fairy, 1963; De Groot and Voogd, 1954; Voogd, 1955, 1956; Velthuis, Verheijen and Gottenbos, 1965). The data of the present experiment show that even caged queenless worker bees without their mandibular glands, which have been feeding queen larvae for a certain period, develop their ovaries. Thus the feeding of queen larvae does not appear to affect the ovary development of the nurse bees nor do queen larvae appear to inhibit their ovary development. The introduction of worker comb seemed to provide laying space rather than to stimulate ovary development. Although many workers had developed ovaries, only a few were involved in egg laying as indicated by the ovary development index (Tables 20 and 21). Their ovary development index was lower than that of the

Table 19

Ovary Development of Seventeen Day Old Caged Worker Bees Collected from Different Hives on Different Dates (1972)

Experiment	Collection date	Treatment	Development of ovaries after 17 days												Ovary development index **
			Rep. I			Rep. II			Rep. III			Rep. IV			
			1	2	3	1	2	3	1	2	3	1	2	3	
I.	July 19	75(Md)	11	9	5	7	4	14	7	5	13	4	3	18	55.250 ± 4.151
	July 19	75(C)	9	3	13	8	6	11	7	4	14	14	5	16	51.500 ± 3.279
II.	August 9	75(Md)	8	7	10	8	11	6	5	7	13	15	8	2	45.500 ± 3.175
	August 9	75(C)	3	12	10	10	8	7	13	8	5	16	9	0	45.500 ± 3.735

** Not significant at $\alpha = 0.05$ with t-test.

Table 20

Effect of Various Treatments on the Ovary Development of Seventeen Day Old Caged Worker Honey Bees

Treatment of Nurse Bees	Collection Date	Development of ovaries after 17 days												Ovary development index
		Rep. I			Rep. II			Rep. III			Rep. IV			
		1	2	3	1	2	3	1	2	3	1	2	3	
75 (Md)	July 26	11	8	6	7	8	10	7	11	7	10	10	5	50.750 ± 2.175
75 (C)	August 3	8	12	5	16	6	3	12	6	7	10	6	9	44.500 ± 2.630
75 (G)	August 24	7	8	10	9	9	7	10	12	3	10	11	4	46.750 ± 2.056
75 (C)(m)	August 24	6	13	6	8	11	6	9	11	5	13	8	4	46.250 ± 1.931
75 (G)(m)	August 24	12	9	4	7	11	7	5	15	5	9	10	6	47.250 ± 1.887

Table 21

The Occurrence of Laying Workers in Experiments III. and IV. (1971)

Experiment	Collection date	Treatment	Replicate No.	Testing time in dissection experiment (hr.)	No. days eggs found after caging	Position of eggs found	
						Queen cup	Wax cells
III.	July 19	75(Md)	1	45	-	-	-
			2	45	-	-	-
			3	42	7	+	-
			4	42	-	-	-
		75(C)	1	45	6	+	-
			2	45	-	-	-
			3	42	5	+	+
			4	42	7	+	-
	August 9	75(Md)	1	45	8	+	-
			2	45	7	+	-
			3	40	-	-	-
			4	40	-	-	-
		75(C)	1	45	5	+	+
			2	45	7	+	-
			3	40	6	+	+
			4	40	-	-	-
IV.	July 26	75(Md)	1	45	-	-	-
			2	45	7	+	-
			3	48	8	+	-
			4	48	-	-	-
	July 26	75(C)	1	45	7	+	-
			2	45	-	-	-
	August 3		3	48	-	-	-
			4	48	8	+	+

Table 21 (continued)

Experiment	Collection date	Treatment	Replicate No.	Testing time in dissection experiment (hr.)	No. days eggs found after caging	Position of eggs found	
						Queen cup	Wax cells
IV.	July 26	75(G)	1		6	+	+
			2		6	+	+
	August 3		3		-	-	-
			4		7	+	+
	August 24	75(C)(m)	1	45	8	+	+
			2	45	7	+	+
			3	42	-	-	-
			4	42	7	+	+
		75(G)(m)	1		7	+	+
			2		6	+	-
			3		-	-	-
			4		5	+	-

75(Md) groups and the 75(G) groups (see Table 20). However, the ovaries of some bees did not develop even though the bees had been kept in the same condition as the others (see Table 19 and 20). This observation coincides with that of Perepelova (1926, 1926), Dreischer (1956) and Sakagami (1958). It appears that some bees, with developed ovaries ("laying workers") can inhibit the ovary development of some of the other bees (see Sakagami, 1958; Velthuis et al., 1965, Jay, 1968, 1970 and 1972).

The occurrence of laying workers was observed often during the rearing period. When provided with plastic queen cell cups or wax cells built by the worker bees, 15-17 day old bees could lay eggs, even after their mandibular glands had been removed, or when they were involved in feeding larvae (see Table 21). In the queen rearing experiments, those cages in which the grafted larvae died two or three days after grafting, laying workers were usually 13 to 14 days old. Similar observations were also made by Lai (1969). Therefore, when supplied with space to lay, many laying workers can be found between the ages of 13 to 14 days. The eggs laid by laying workers can hatch, but this was not observed very often. The worker bees sometimes fed the larvae which hatched with milky food; however, they only survived for one or two days because the worker bees stopped feeding the larvae for some reason. This was also observed by Lai (1969).

The wax cells built around the queen cup consisted

of various sized cells, and were used not only for the storing of eggs but also for storing honey which the worker bees collected from the food trays (see Figure 2).

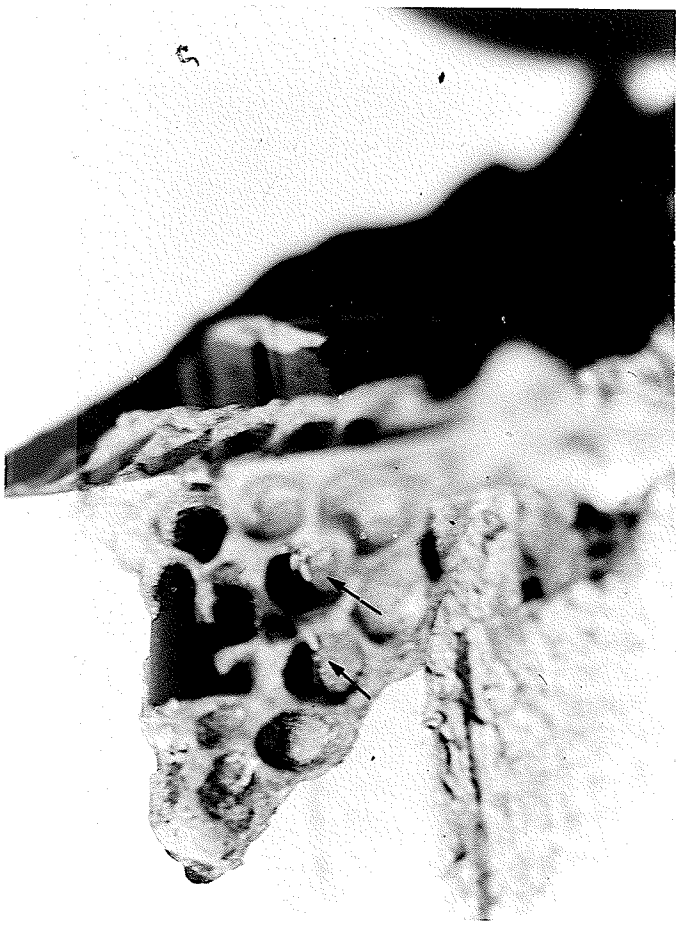

Figure 1. Eggs in the wax cells laid by laying workers 5-8 days after caging.

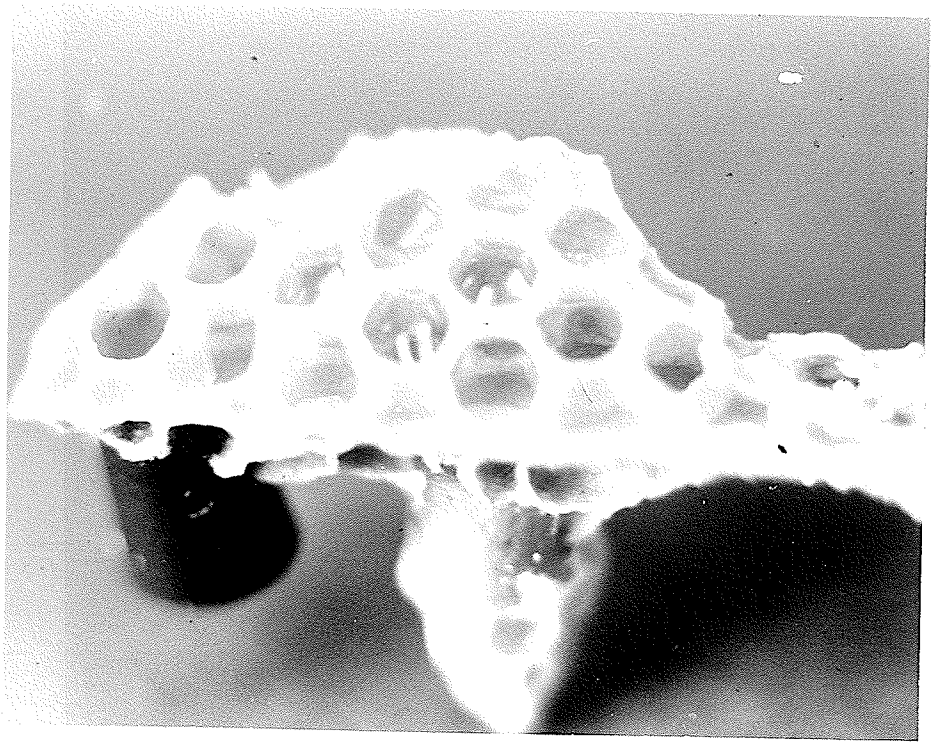

Figure 2. Wax cells built by the caged bees were sometimes used by the bees for storing honey collected from the food trays.

CHAPTER VII

SUMMARY

Throughout the summers of 1970, 1971 and 1972, experiments were done in an attempt to rear queen bees using small numbers of caged nurse bees. The mating ability and brood production of such queens was also tested in the field. This method of queen rearing, combined with a technique for removal of the mandibular glands of nurse bees, was used to study caste determination. The cage technique has commercial queen rearing possibilities while the technique for obtaining large numbers of mandibular glands of worker bees has possibilities for studying the chemical control of behavior in bees.

Groups of 30, 75, 100, 200 and 400, 10 day old nurse bees were caged and used for the queen rearing trials in the laboratory. Groups of 30 nurse bees reared only six adult bees and two pupae. Twenty two, 25, 50 and 55 queen-like adult bees were reared by groups of 75, 100, 200 and 400 nurse bees respectively.

Variation in several morphological characters in relation to the size of the nursing population was evaluated statistically. Significant differences were found in the adult head indices, basitarsal indices and diameter of the spermathecae of control queens and the adults reared by

different numbers of nurse bees ($P<0.01$, $P<0.01$ and $P<0.05$, respectively). Pupal tongue length and pupal weight differed significantly between bees reared by different numbers of nurse bees ($P<0.05$). Groups of 30 nurse bees could also rear queen-like adult bees, although they were smaller in size. Intercaste characteristics occurred in adult bees reared by groups of 30, 75 and 100 nurse bees. Because there was a higher frequency of these characteristics in the adults reared by the groups of 30 nurse bees, it is suggested that larger numbers of nurse bees be used in this type of queen rearing.

During the summers of 1971 and 1972, fifteen adult bees, reared by groups of 75, 200 and 400 nurse bees (5 from each group) in the laboratory, were introduced into mating nuclei. In 1971, of the 15 adults which were introduced, six were mated and produced good brood patterns. The mated bees had queen-like characteristics, except queen No. 3 which had an ovariole count below that of the normal queen range, and was a "drone layer."

In 1972, 7, 8, and 8 adult bees, reared by 75, 200 and 400 nurse bees respectively, as well as 6 adult queens reared by control colonies, were introduced into mating nuclei. Five adult bees of each group were mated and produced brood. Brood measurements of these adult bees showed that they had a high potential for brood production and that there was no significant difference in total brood and sealed

brood production by queens reared by different numbers of nurse bees. All of the mated queens compared well to normal queens with regard to spermathecal diameter, and ovariole counts, although the drone layers had significantly smaller spermathecae and lower numbers of ovarioles ($P<0.05$ in each case). These data show that adult bees, reared by small numbers of caged worker bees in the laboratory, not only possess queen-like morphological characteristics, but also can function normally in colonies.

In an attempt to rear queens, using nurse bees without their mandibular glands, a dissection apparatus was designed to assist in removing the mandibular glands from large numbers of nurse bees within a short period of time and with little mortality among the dissected bees. It was observed that these nurse bees, after the removal of the mandibular glands, could secrete a watery-yellowish material which they fed to the larvae. However, the amount of this secretion was only 1/3-1/2 of the amount of royal jelly observed in the control cages of equal numbers of nurse bees. No pupa or adult was obtained from groups of 30(Md)*bees in 1970 or 1971. Because of the limited quantity of larval food secreted by these nurse bees, most of the larvae died of starvation about 2-3 days after being introduced into the cages of nurse bees. Therefore, the number of nurse bees per group was increased to 75. The amount of food and the size of the larvae increased as the number of nurse bees was increased. However, the nurse bees had a tendency to stop

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* 30 bees without mandibular glands.

secreting larval food about 48-60 hours after their mandibular glands were removed and therefore most of the larvae died during this period. Only one pupae was obtained from the groups of 75(Md) bees in 1971.

In an attempt to reduce larval mortality due to starvation, larvae which had been reared by 75(Md) bees for certain time periods ("Test Periods") (i.e., 60, 50, 48, 45, 42, 36 and 24 hours), were then grafted into queen cells containing normal royal jelly; following this, they were fed by groups of 400, normal nurse bees until their cells were capped ("Feeding Period"). No success was obtained in Test Periods of 60, 50 and 48 hours. Some of these larvae survived the Test Period, only to die in the Feeding Period. Four, 7, 7, 7 and 4 adults were reared in [75(Md),TP:45]*, [75(Md),TP:42], [75(Md),TP:40], [75(Md),TP:36] and [75(Md), TP:24] experiments respectively. Two, one and one pupae were obtained from the [75(Md),TP:45], [75(Md),TP:42] and [75(Md),TP:40] experiments respectively. Higher numbers of pupae and adults were reared by 75(S)** and 75(C)*** groups in different Test Periods. Generally, pupae reared by the 75(Md) groups were small regardless of the length of the Test Period. The pupae, reared in the [TP:45] experiments were smaller in size and lower in weight than those reared in the shorter Test Periods. However, the adult bees,

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* Test Period equals 45 hours.

** Bees receiving a "sham" operation, see text.

*** Control bees.

reared by the 75(Md) groups, were queen-like in appearance regardless of the Test Period. Adult bees, reared in the [75(Md),TP:45] groups had lower ovariole counts, and smaller spermathecae than those reared in the other experiments ($P<0.05$ in each case). However, they were all queen-like in appearance.

Studies of ovarian development of caged nurse bees (in the absence of queens), which had received different treatments were conducted in 1972. The caged worker bees usually had developed ovaries at 17 days whether or not their mandibular glands were removed and/or they were rearing female larvae. Eggs laid by the laying workers could be found in the empty queen-cups or in the wax cells on the 5th-8th day after caging. The ovary development indices of the nurse bees within the different treatments suggested that some worker bees, with developed ovaries ("laying workers") could inhibit the ovary development of other worker bees. The data of the present experiments also suggest that ovary development in worker nurse bees is not affected by the feeding of queen larvae or by the presence of queen larvae.

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APPENDIX A

APPENDIX A

Table 1. Measurements of Bees Reared by 75(C)* Groups (1971)

Experiment No.	Replicate No.	Time of death	Days from grafting to capping	Days from capping to emergence	Total development time (days)	Pupal measurements					Adult measurements									Dia. of spermatheca (mm.)	No. of ovarioles
						Tongue length (mm.)	Body length (mm.) a	b	total	Weight (mg.)	Head (mm.) W	L	w/L	Mandibles W	I	Q	Basitarsus L	W	L/W		
I	75(C)-1	A**	5	10	15	2.63	6.30	8.40	14.70	205	4.19	3.61	1.16			x	2.71	1.42	1.91	1.34	151
	-2	A	5	10	15	2.31	6.41	8.50	14.91	129	4.06	3.48	1.17			x	2.19	1.09	2.01	0.88	128
	-3	A	6	11	16	2.73	6.41	9.98	16.39	225	4.13	3.61	1.14			x	2.71	1.23	2.21	1.24	125
	-4	A	6	11	17	3.15	5.78	8.40	14.18	158	3.87	3.42	1.13			x	4.51	1.23	2.00	1.03	113
	-5	A	6	12	18	3.15	5.76	9.98	15.74	200	3.87	3.16	1.22			x	2.45	1.23	2.00	1.08	122
II	75(C)-1	A	5	12	17	3.15	6.30	8.50	14.80	152	3.87	3.55	1.09			x	2.45	1.29	1.90	1.08	130
	-2	A	5	10	15	2.63	6.30	8.40	14.70	220	4.06	3.55	1.15			x	2.52	1.36	1.86	1.08	125
	-4	A	5	13	18	3.26	6.30	9.77	16.07	224	4.19	3.68	1.14			x	2.65	1.36	1.95	1.24	139
III	75(C)-1	A	4	10	14	2.94	6.09	8.51	14.60	217	4.13	3.42	1.21			x	2.71	1.42	1.91	1.08	127
	-3	A	5	10	15	2.10	6.15	8.51	14.12	221	3.94	3.35	1.17			x	2.58	1.23	2.10	1.08	112
	-5	A	6	10	16	2.63	6.41	8.40	14.81	226	4.19	3.48	1.20			x	2.71	1.29	2.10	1.21	154
IV	75(C)-2	A	6	10	16	2.31	6.30	8.61	14.91	220	4.06	3.61	1.13			x	2.77	1.23	2.26	1.24	142
	-3	A	4	12	16	2.63	6.30	8.40	14.70	210	3.87	3.49	1.11			x	2.57	1.16	2.16	1.13	163
	-4	A	5	12	17	3.05	6.41	8.72	15.12	220	4.00	3.68	1.09			x	2.32	1.25	1.85	1.13	127
	-5	A	5	10	15	2.94	6.40	8.50	14.90	227	3.94	3.22	1.22			x	2.47	1.16	2.13	1.08	141
V	75(C)-1	A	5	9	14	3.05	6.30	8.93	15.53	230	3.94	3.29	1.20			x	2.47	1.16	2.13	1.07	113
	-4	A	5	10	15	3.05	6.41	8.51	14.91	221	4.00	3.62	1.10			x	2.51	1.25	2.01	1.11	152
	-5	A	6	10	16	2.63	5.67	9.35	15.02	221	4.06	3.68	1.10			x	2.39	1.25	1.91	1.13	135
VI	75(C)-1	A	5	11	16	2.10	6.09	8.51	14.60	217	4.12	3.68	1.12			x	2.58	1.25	2.06	1.21	124
	-3	A	6	10	16	2.63	6.62	8.51	15.12	221	4.00	3.54	1.13			x	2.47	1.25	1.98	1.07	121
	-4	A	6	10	16	2.31	6.41	8.40	14.81	226	4.31	3.68	1.17			x	2.51	1.29	1.95	1.13	132
	-5	A	6	9	15	2.42	6.30	8.61	14.91	220	4.12	3.62	1.14			x	2.32	1.25	1.86	1.07	109

*Control Group.
**Adult stage.

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Table 2. Measurements of Bees Reared by 100(C) Groups (1970).

Experiment No.	Replicate No.	Time of death	Days from grafting to capping	Days from capping to emergence	Total development time (days)	Pupal measurements: Tongue length (mm.)	Body length (mm.): a	b	total	Weight (mg.)	Adult measurements: Head (mm.): W	L	w/L	Mandibles: W	I	Q	Basitarsus: L	W	L/W	Dia. of spermatheca (mm.)	No. of ovarioles
I	100(C)-1	A	6	10	16	2.40	6.50	8.00	14.50	149	3.80	3.60	1.06			x	2.40	1.30	1.85	1.15	106
	-5	A	5	11	16	2.05	6.40	8.15	14.55	145	3.80	3.70	1.03			x	2.50	1.20	2.01	1.00	113
II	100(C)-3	A	4	12	16	2.35	6.18	7.20	13.38	167	3.80	3.70	1.03			x	2.00	1.10	1.82	1.10	105
	-5	A	6	12	18	2.20	6.19	7.20	13.39	158	3.80	3.70	1.03			x	2.30	1.20	1.92	1.10	117
III	100(C)-1	P*	5	-	-	2.30	6.40	8.00	14.40	162											
	-2	A	5	11	16	2.25	6.88	7.61	14.49	178	3.70	3.55	1.07			x	2.40	1.20	2.00	1.10	114
	-4	A	6	10	16	2.20	6.88	8.00	14.88	151	3.80	3.60	1.06			x	2.10	1.30	1.62	1.00	129
	-5	A	6	11	16	2.10	6.10	7.50	14.60	169	3.80	3.30	1.15			x	2.20	1.20	1.83	1.00	110
IV	100(C)-1	A	5	9	14	2.30	6.08	7.50	13.58	158	3.60	3.40	1.06			x	2.20	1.20	1.83	1.41	115
	-2	A	4	10	14	2.10	6.24	8.80	15.04	183	3.60	3.40	1.06			x	2.50	1.20	2.04	1.12	110
	-3	A	4	10	14	2.10	6.50	7.40	13.90	168	3.80	3.40	1.12			x	2.40	1.25	1.09	1.10	115
	-4	A	4	10	14	2.30	6.30	8.95	15.25	197	4.00	3.40	1.12			x	2.40	1.10	2.08	1.20	130
	-5	A	5	10	15	2.25	6.30	7.90	14.20	188	3.50	3.30	1.11			x	2.30	1.10	2.09	1.10	103
V	100(C)-1	A	5	9	14	2.30	6.00	8.00	14.00	177	4.00	3.50	1.14			x	2.50	1.20	2.04	1.00	117
	-2	A	4	11	15	2.50	6.40	8.30	14.70	182	4.10	3.60	1.14			x	2.40	1.10	2.18	1.10	144
	-3	A	4	11	15	2.30	6.10	7.30	13.40	179	3.60	3.30	1.09			x	2.30	1.20	1.92	1.10	123
	-4	A	4	10	14	2.10	6.20	8.30	14.50	184	3.70	3.40	1.09			x	2.40	1.25	1.92	1.10	165
	-5	A	4	10	14	2.10	6.40	8.00	14.40	171	3.80	3.30	1.15			x	2.20	1.20	1.83	1.10	168
VI	100(C)-1	A	4	11	15	2.50	6.30	7.40	13.70	187	3.80	3.50	1.09			x	2.30	1.30	1.77	1.10	106
	-2	A	4	11	15	2.30	6.30	7.20	13.50	171	3.60	3.20	1.12			x	2.00	1.10	1.82	1.00	138
	-3	A	4	10	14	2.10	6.00	7.50	13.50	152	4.10	3.20	1.28			x	2.40	1.15	2.09	1.10	102
VII	100(C)-1	A	4	10	14	2.40	6.10	8.00	14.10	172	4.10	3.40	1.20			x	2.40	1.10	2.18	1.15	135
	-2	A	5	11	16	2.30	6.10	7.30	13.40	166	4.00	3.50	1.14			x	2.40	1.15	2.09	1.20	126
	-3	A	4	10	14	2.1o	6.10	7.50	13.60	186	4.00	3.50	1.14			x	2.20	1.15	1.19	1.10	112
	-4	A	5	9	14	2.10	6.50	7.50	14.00	188	4.00	3.60	1.11			x	2.30	1.20	1.19	1.10	151

*Pupal stage.

APPENDIX A

Table 3a. Measurements of Bees Reared by 200(C) Groups (1970)

Experiment No.	Replicate No.	Time of death	Days from grafting to capping	Days from capping to emergence	Total development time (days)	Pupal measurements: Tongue length (mm.)	Body length (mm.) a	b	total	Weight (mg.)	Adult measurements: Head (mm.) W	L	w/L	Mandibles W	I	Q	Basitarsus L	W	L/W	Dia. of spermatheca (mm.)	No. of ovarioles
I	200(C)-1	A	5	10	15	2.10	6.50	9.90	17.40	182	3.80	3.70	1.03			x	2.50	1.20	2.08	1.01	116
	-2	A	5	9	14	2.10	6.08	8.95	15.02	192	3.80	3.60	1.05			x	2.40	1.30	1.85	1.01	121
	-3	A	6	12	18	2.30	7.05	8.00	15.05	170	3.80	3.70	1.03			x	2.00	1.10	1.83	1.10	120
	-4	A	6	12	18	2.30	6.90	8.40	15.30	175	3.90	3.60	1.08			x	2.20	1.30	1.67	1.10	125
	-5	A	6	10	16	2.35	6.89	8.65	15.54	182	4.00	3.80	1.05			x	2.40	1.15	2.08	1.10	141
II	200(C)-1	A	5	12	17	2.30	6.14	7.90	14.04	177	3.90	3.60	1.08			x	2.50	1.40	1.79	1.10	150
	-2	A	5	9	14	2.10	6.50	7.50	14.00	188	4.00	3.60	1.11			x	2.30	1.20	1.91	1.10	151
	-3	A	6	7	13	2.30	7.51	8.40	15.91	161	3.60	3.21	1.23		x		2.00	1.10	1.82		89
	-4	A	5	9	14	2.35	6.08	9.10	15.18	188	3.70	3.40	1.23			x	2.30	1.25	1.84	1.00	116
	-5	A	5	9	14	2.10	6.08	8.70	14.78	172	3.50	3.20	1.10			x	2.20	1.20	1.87	1.00	97
III	200(C)-1	A	6	7	13	2.30	6.40	9.41	15.81	187	3.60	3.40	1.06			x	2.30	1.20	1.84	1.20	115
	-2	A	5	8	13	2.25	6.87	8.95	15.82	167	3.70	3.45	1.07			x	2.10	1.20	1.75	1.01	122
	-3	A	5	8	13	2.10	6.40	7.84	14.24	154	3.60	3.30	1.23		x		2.20	1.21	1.65	1.10	134
	-4	A	6	8	14	2.10	7.51	9.90	17.41	194	4.10	3.90	1.05			x	2.30	1.25	1.84	1.25	164
	-5	A	5	9	14	2.30	6.55	8.95	15.50	191.5	3.70	3.10	1.19			x	2.20	1.20	1.84	1.10	102
IV	200(C)-1	A	5	9	14	2.30	6.55	8.64	15.19	189	3.70	3.50	1.06			x	2.40	1.22	1.97	1.00	133
	-2	A	5	11	16	2.40	6.10	8.00	14.10	172	3.40	3.30	1.03			x	2.10	1.20	1.75	1.10	112
	-3	A	5	10	15	2.30	6.30	7.20	13.50	171	3.50	3.20	1.09			x	2.10	1.20	1.75	1.10	104
	-4	A	5	11	16	2.10	6.20	8.30	14.50	194	4.00	3.50	1.14			x	2.50	1.20	2.04	1.20	129
	-5	A	4	12	16	2.30	6.10	7.40	13.40	166	3.50	3.40	1.09			x	2.00	1.20	1.67	1.10	111
V	200(C)-1	A	4	12	16	2.10	6.10	7.50	13.60	186	3.50	3.20	1.09			x	2.00	1.30	1.54	1.00	135
	-2	A	4	12	16	2.50	6.30	7.40	13.70	187	3.50	3.10	1.14			x	2.10	1.30	1.62	1.00	121
	-3	A	5	10	15	2.10	6.00	7.50	13.50	152	3.40	3.10	1.10			x	2.00	1.30	1.54	1.00	107
	-4	A	5	10	15	2.60	6.50	7.50	14.00	188	6.00	3.00	1.11			x	2.30	1.25	1.84	1.00	118
	-5	A	5	10	15	2.10	6.40	8.00	14.40	191	3.70	3.30	1.12			x	2.10	1.00	2.10	1.20	142

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Table 3a (continued)

Experiment No.	Replicate No.	Time of death	Days from grafting to capping	Days from capping to emergence	Total development time (days)	Pupal measurements: Tongue length (mm.)	Body length (mm.): a	b	total	Weight (mg.)	Adult measurements: Head (mm.): W	L	w/L	Mandibles: W	I	Q	Basitarsus: L	W	L/W	Dia. of spermatheca (mm.)	No. of ovarioles
VI	200(C)-1	A	6	8	14	2.50	6.30	7.40	13.70	187	3.00	3.70	1.05			x	2.40	1.20	2.00	1.10	112
	-2	A	6	8	14	2.10	6.20	8.30	14.50	194	4.00	3.90	1.03			x	2.50	1.25	2.00	1.20	140
	-3	A	6	7	13	2.10	6.00	7.50	13.50	152	3.70	3.50	1.06			x	2.40	1.20	2.00	1.20	104
	-4	A	5	10	15	2.40	6.08	6.52	12.60	158	3.70	3.60	1.03			x	2.30	1.20	1.92	1.10	133
	-5	A	6	9	15	2.40	6.24	8.80	15.04	190	3.80	3.70	1.03			x	2.40	1.20	2.00	1.15	158

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Table 3b. Rearing Results of Bees Reared by 200(C) Groups (1971)

Experiment No.	Replicate No.	Time of death	Days from grafting to capping	Days from capping to emergence	Total development time (days)	Pupal measurements: Tongue length (mm.)	Body length (mm.) a	b	total	Weight (mg.)	Adult measurements: Head (mm.) W	L	w/L	Mandibles W	I	Q	Basitarsus L	W	L/W	Dia. of spermatheca (mm.)	No. of ovarioles
I	200(C)-1	P	4	11	15	2.30	5.92	11.63	17.55	243	4.00	3.48	1.15			x	2.58	1.29	2.00	1.13	140
	-2	A	5	11	16	2.30	5.92	12.89	18.81	211	4.13	3.74	1.10			x	2.52	1.29	1.95	1.01	139
	-3	P	5	10	15	1.76	6.56	10.55	17.11	225	4.19	3.42	1.23			x	2.39	1.29	1.85	1.13	120
II	200(C)-1	A	5	10	15	2.08	5.92	11.04	16.96	215	4.06	3.68	1.10			x	2.51	1.23	2.04	1.13	124
	-2	A	4	12	16	2.21	5.92	8.95	14.87	179	3.87	3.48	1.11			x	2.39	1.29	1.85	1.08	103
III	200(C)-1	P	4	10	14	2.58	6.62	9.45	16.07	220	4.13	3.76	1.10			x	2.46	1.23	2.00	1.13	140
	-2	P	4	10	14	2.31	6.93	8.92	15.85	227	4.19	3.68	1.14			x	2.51	1.29	1.95	1.13	143
	-3	A	5	12	17	2.835	6.30	10.185	16.485	198	3.87	3.55	1.09			x	2.46	1.23	1.85	1.08	112
IV	200(C)-1	P	4	11	15	2.415	6.93	8.925	15.855	223	4.13	3.68	1.12			x	2.45	1.23	1.99	1.08	139
	-2	A	5	11	16	2.940	5.46	8.40	13.86	198	3.87	3.48	1.11			x	2.32	1.09	2.13	1.13	124
	-3	A	4	10	14	2.940	6.30	9.975	16.275	235	4.13	3.74	1.10			x	2.38	1.29	1.84	1.08	113
	-4	A	5	10	15	2.940	6.09	9.345	15.435	230	4.06	3.48	1.17			x	2.45	1.23	1.99	1.08	116
	-5	A	4	12	16	2.310	6.405	8.505	14.905	231	3.94	3.68	1.07			x	2.46	1.23	2.00	1.13	132
V	200(C)-1	P	4	11	15	1.995	6.3	9.66	15.962	231	4.00	3.68	1.09			x	2.51	1.29	1.95	1.13	126
	-2	A	5	12	17	2.31	6.62	9.45	16.17	224	4.06	3.48	1.17			x	2.52	1.29	1.95	-	131
	-3	A	5	9	14	2.415	5.775	7.980	13.755	210	3.667	3.36	1.09			x	2.19	1.09	2.00	1.08	116
	-4	A	5	11	16	2.52	5.985	6.30	12.285	167	3.605	3.23	1.13			x	2.38	1.16	2.04	1.07	103
VI	200(C)-1	A	5	10	15	2.625	6.30	9.975	16.275	227	3.667	3.48	1.05			x	2.19	1.16	1.89	1.10	139
	-2	A	5	10	15	2.625	6.30	9.135	15.435	229	3.87	3.48	1.11			x	2.45	1.22	2.00	1.10	124
	-3	A	4	11	15	2.45	5.985	8.19	14.175	106	3.87	3.66	1.06			x	2.51	1.29	1.95	1.08	102

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Table 4a. Measurements of Bees Reared by 400(C) Groups (1970)

Experiment No.	Replicate No.	Time of death	Days from grafting to capping	Days from capping to emergence	Total development time (days)	Pupal measurements: Tongue length (mm.)	Pupal measurements: Body length (mm.) a	b	total	Pupal measurements: Weight (mg.)	Adult measurements: Head (mm.) W	L	w/L	Mandibles			Basitarsus L	W	L/W	Dia. of spermatheca (mm.)	No. of ovarioles
I	400(C)-1	A	5	9	14	2.5	6.87	8.95	15.82	222	3.88	3.7	1.05		x		2.4	1.2	2.00	1.20	136
	-2	A	4	10	14	2.4	6.55	8.95	15.50	230	4.00	3.7	1.03		x		2.50	1.2	2.08	1.20	129
	-3	A	5	10	15	2.6	6.40	9.10	15.50	232	3.91	3.8	1.03		x		2.50	1.25	2.00	1.10	126
	-4	A	5	10	15	2.6	6.08	8.64	14.72	211	3.97	3.76	1.06		x		2.50	1.2	2.08	1.20	118
	-5	A	4	10	14	2.4	7.51	9.41	16.92	236	3.88	3.7	1.05		x		2.30	1.2	1.91	1.20	120
II	400(C)-1	A	5	9	14	2.3	7.05	8.0	15.05	210	3.8	3.7	1.03		x		2.40	1.2	2.00	1.10	123
	-2	A	5	10	15	2.3	6.9	8.4	15.30	220	3.9	3.6	1.08		x		2.40	1.25	1.92	1.10	109
	-3	A	6	9	15	2.4	6.08	9.10	15.18	200	4.0	3.0	1.11		x		2.40	1.25	1.92	1.09	108
	-4	A	5	9	14	2.6	7.51	9.90	17.41	242	3.7	3.3	1.12		x		2.30	1.15	2.00	1.25	110
	-5	A	4	10	14	2.5	6.55	8.80	15.35	220	3.5	3.4	1.09		x		2.30	1.20	1.91	1.20	125
III	400(C)-1	A	5	10	15	2.3	6.20	8.3	14.5	119	3.88	3.73	1.04		x		2.40	1.20	2.00	1.20	104
	-2	A	5	9	14	2.2	7.05	8.65	15.70	120	3.85	3.7	1.05		x		2.40	1.25	1.92	1.20	106
	-3	A	4	10	14	2.3	6.50	8.95	15.45	125	3.90	3.8	1.05		x		2.40	1.15	2.08	1.10	117
	-4	A	4	10	14	2.4	7.05	8.80	15.85	220	3.90	3.7	1.03		x		2.50	1.20	2.08	1.10	114
	-5	A	4	10	14	2.4	7.05	8.65	16.70	235	3.90	3.7	1.03		x		2.5	1.20	2.08	1.20	231
IV	400(C)-1	A	5	9	14	2.3	6.3	8.30	14.60	193	3.6	3.4	1.06		x		2.4	1.2	2.00	1.10	123
	-2	A	5	9	14	2.3	6.2	8.30	14.50	189	3.5	3.2	1.09		x		2.5	1.2	2.08	1.10	105
	-3	A	6	10	16	2.3	6.1	8.80	14.90	201	3.7	3.5	1.06		x		2.5	1.2	2.08	1.00	102
V	400(C)-1	A	5	10	15	2.4	6.08	8.70	14.78	195	3.6	3.3	1.09		x		2.4	1.2	2.00	1.00	112
	-2	A	5	9	14	2.4	6.30	7.40	13.70	187	3.5	3.2	1.09		x		2.2	1.2	1.83	1.00	96
	-3	A	4	9	13	2.3	6.1	8.0	14.10	197	3.4	3.3	1.03		x		2.5	1.2	2.08	1.10	120
	-4	A	4	9	13	2.3	6.0	7.5	13.50	184	3.5	3.3	1.06		x		2.4	1.2	2.00	1.00	98
VI	400(C)-1	A	5	9	14	2.5	6.40	8.80	15.20	196	3.6	3.4	1.06		x		2.5	1.2	2.08	1.10	107
	-2	A	5	10	15	2.5	6.50	8.64	15.14	220	3.7	3.4	1.09		x		2.5	1.2	2.08	1.10	124
	-3	A	5	10	15	2.4	6.87	8.65	15.52	224	3.5	3.4	1.03		x		2.5	1.25	2.00	1.10	118
	-4	A	4	9	13	2.5	7.05	8.80	15.85	235	3.5	3.4	1.03		x		2.4	1.25	1.92	1.10	133
	-5	A	5	8	13	2.4	6.55	8.95	15.50	226	3.4	3.3	1.03		x		2.5	1.25	2.00	1.10	140
VII	400(C)-1	A	4	8	12	2.4	6.10	8.65	14.75	205	3.7	3.5	1.06		x		2.4	1.2	2.00	1.00	126

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Table 4b. Measurements of Bees Reared by 400(C) Groups (1971)

Experiment No.	Replicate No.	Time of death	Days from grafting to capping	Days from capping to emergence	Total development time (days)	Pupal measurements: Tongue length (mm.)	Pupal measurements: Body length (mm.) a	b	total	Pupal measurements: Weight (mg.)	Adult measurements: Head (mm.) W	L	w/L	Mandibles			Basitarsus (mm.) L	W	L/W	Dia. of spermatheca (mm.)	No. of ovarioles
														W	I	Q					
I	400(C)-1	A	4	10	14	2.31	6.93	8.93	15.96	217	3.87	3.67	1.65			x	2.45	1.23	2.00	1.08	146
	-2	A	5	10	15	2.84	6.30	10.19	16.49	238	4.06	3.67	1.11			x	2.52	1.29	2.00	1.11	132
	-3	A	4	11	15	3.15	6.30	8.93	15.23	205	3.87	3.42	1.13			x	2.00	1.10	1.82	1.11	120
II	400(C)-1	A	5	11	16	2.30	6.41	9.35	15.76	208	3.68	3.35	1.20			x	2.19	1.10	2.00	1.08	115
	-2	A	4	11	15	2.94	6.30	9.77	16.07	224	4.00	3.49	1.15			x	2.52	1.16	2.17	1.11	127
	-3	A	4	12	16	2.42	5.78	8.93	14.71	204	3.74	3.10	1.21			x	2.45	1.29	1.90	1.13	124
	-4	A	4	11	15	2.31	6.30	9.35	15.65	220	4.19	3.42	1.23			x	2.39	1.29	1.85	1.11	140
	-5	A	5	10	15	2.42	5.57	8.51	14.07	190	4.06	3.68	1.10			x	2.19	1.09	2.00	1.11	107
III	400(C)-1	A	5	9	14	2.52	6.09	9.45	15.54	228	3.87	3.67	1.05			x	2.45	1.23	2.00	1.08	151
	-2	A	4	10	14	2.42	5.78	9.05	14.83	200	3.87	3.42	1.13			x	2.06	1.16	1.78	1.11	139
	-3	A	4	9	13	2.52	6.41	9.95	16.36	234	3.74	3.67	1.02			x	2.50	1.42	1.76	1.34	140
IV	400(C)-1	A	4	9	13	2.63	6.30	9.35	15.65	219	4.19	3.48	1.22			x	2.45	1.35	1.82	1.11	128
	-2	A	4	10	14	2.42	6.41	9.05	15.46	215	3.87	3.48	1.11			x	2.39	1.29	1.85	1.11	130
	-3	A	4	10	14	2.42	6.30	10.19	16.49	229	3.87	3.48	1.11			x	2.32	1.29	1.80	1.34	132
	-4	A	5	10	15	2.31	6.93	9.05	15.98	235	4.06	3.67	1.12			x	2.26	1.23	1.84	1.11	138
	-5	A	5	9	14	2.52	6.20	8.93	15.13	198	3.67	3.35	1.20			x	2.45	1.23	2.00	1.02	97
V	400(C)-1	A	5	9	14	2.52	6.30	8.93	15.23	220	3.87	3.74	1.04			x	2.45	1.29	1.90	1.11	133
	-2	A	4	9	13	2.42	6.41	9.25	15.66	232	3.74	3.10	1.21			x	2.32	1.16	2.00	1.13	151
	-3	A	4	10	14	2.52	6.30	9.45	15.75	119	3.80	3.42	1.11			x	2.26	1.29	1.75	1.08	114
	-4	A	4	10	14	2.31	6.09	9.55	15.65	213	3.80	3.48	1.09			x	2.32	1.29	1.80	1.34	140
	-5	A	4	10	14	2.31	6.20	9.20	15.40	105	3.74	3.36	1.11			x	2.32	1.16	2.00	1.34	137
VI	400(C)-1	A	4	9	13	2.62	6.20	9.25	15.45	221	3.80	3.48	1.09			x	2.39	1.16	2.06	1.08	142
	-2	A	5	9	14	2.52	6.30	9.70	16.00	241	4.19	3.48	1.20			x	2.52	1.35	1.90	1.11	145
	-3	A	4	9	13	2.42	6.41	8.93	15.34	237	3.87	3.48	1.11			x	2.52	1.29	1.95	1.11	129
	-4	A	4	10	14	2.42	6.30	9.45	15.75	224	4.06	3.67	1.11			x	2.26	1.23	1.84	1.11	122
VII	400(C)-2	A	4	9	13	2.30	7.51	9.10	16.60	238	3.60	3.40	1.06				2.50	1.20	2.08	1.10	138
	-4	A	5	9	14	2.30	6.50	8.95	15.50	227	3.60	3.40	1.05				2.40	1.20	2.00	1.10	129

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Table 5a. Measurements of Bees Reared by Control Colonies (1971)

Experiment No.	Replicate No.	Time of death	Days from grafting to capping	Days from capping to emergence	Total development time (days)	Adult measurements									Dia. of spermatheca (mm.)	No. of ovarioles
						Head (mm.)			Mandibles			Basitarsus				
						W	L	w/L	W	I	Q	L	W	L/W		
I	(C)-1	A	5	9	14	3.87	3.48	1.11			x	2.45	1.29	1.90	1.39	174
	-3	A	5	9	14	3.94	3.29	1.20			x	2.52	1.11	2.17	1.41	149
	-4	A	5	10	15	4.00	3.48	1.15			x	2.65	1.42	1.86	1.41	133
II	(C)-2	A	5	10	15	3.94	3.55	1.11			x	2.58	1.23	2.11	1.36	177
	-3	A	5	8	13	3.74	3.55	1.05			x	2.52	1.23	2.05	1.26	152
	-4	A	6	16	16	4.06	3.55	1.14			x	2.45	1.16	2.11	1.39	164
III	(C)-1	A	5	10	15	3.87	3.48	1.09			x	2.52	1.23	2.05	1.34	125
	-2	A	4	10	14	4.13	3.87	1.19			x	2.52	1.42	1.77	1.39	145
	-3	A	5	9	14	4.19	3.55	1.08			x	2.65	1.29	2.05	1.26	172
	-4	A	5	9	14	4.06	3.55	1.15			x	2.65	1.23	2.05	1.46	162
	-5	A	5	8	13	4.13	3.35	1.23			x	2.58	1.23	2.11	1.31	158
IV	(C)-1	A	5	8	13	4.19	3.55	1.18			x	2.65	1.23	2.16	1.39	155
	-2	A	5	8	13	3.87	3.55	1.09			x	2.58	1.23	2.11	1.36	137
	-3	A	5	10	15	3.94	3.23	1.22			x	2.52	1.23	2.05	1.21	154
	-4	A	5	10	15	3.94	3.61	1.09			x	2.52	1.16	2.17	1.31	126
	-5	A	5	9	14	4.06	3.81	1.07			x	2.65	1.35	1.95	1.44	172
V	(C)-1	A	5	9	14	4.06	3.61	1.13			x	2.45	1.23	2.00	1.29	143
	-2	A	5	9	14	4.00	3.42	1.17			x	2.65	1.36	1.95	1.41	149
	-3	A	5	10	15	4.00	3.48	1.15			x	2.52	1.36	1.86	1.29	103
	-4	A	5	10	15	4.06	3.68	1.11			x	2.58	1.29	2.00	1.36	146
	-5	A	5	9	14	4.00	3.55	1.13			x	2.52	1.10	2.29	1.41	156
VI	(C)-1	A	5	8	13	4.00	3.61	1.11			x	2.65	1.36	1.95	1.41	161
	-3	A	5	8	13	4.06	3.29	1.22			x	2.58	1.16	2.22	1.34	119
	-4	A	5	8	13	4.06	3.61	1.19			x	2.52	1.29	1.95	1.36	135
	-5	A	5	9	14	4.06	3.74	1.07			x	2.58	1.29	2.00	1.26	140

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Table 5b. Measurements of Bees Reared by Control Colonies (1972)

Experiment No.	Replicate No.	Time of death	Days from grafting to capping	Days from capping to emergence	Total development time (days)	Adult measurements									Dia. of spermatheca (mm.)	No. of ovarioles
						Head (mm.)			Mandibles			Basitarsus				
						W	L	w/L	W	I	Q	L	W	L/W		
I	(C)-1	A	5	9	14	3.81	3.48	1.09			x	2.45	1.16	2.11	1.08	151
	-2	A	5	9	14	4.06	3.81	1.05			x	3.71	1.29	2.10	1.03	137
	-3	A	5	10	15	3.94	3.48	1.13			x	2.64	1.29	2.05	1.08	144
	-4	A	5	10	15	3.87	3.35	1.15			x	2.64	1.29	2.05	1.18	167
	-5	A	5	9	14	4.00	3.68	1.09			x	3.71	1.16	2.33	1.11	135
II	(C)-1	A	5	10	15	4.00	3.68	1.09			x	3.71	1.16	2.33	1.08	151
	-2	A	5	10	15	3.94	3.81	1.03			x	2.52	1.29	1.95	1.06	163
	-3	A	6	10	16	4.00	3.81	1.05			X	2.58	1.23	2.11	1.13	142
	-4	A	6	9	15	4.06	3.81	1.07			X	2.58	1.16	2.67	1.13	124
	-5	A	6	9	15	4.19	3.74	1.12			X	2.52	1.29	1.95	1.01	151
III	(C)-1	A	5	10	15	3.94	3.68	1.07			X	2.58	1.29	2.00	1.01	165
	-2	A	5	10	15	4.00	3.74	1.07			X	2.65	1.16	2.28	1.13	151
	-3	A	6	9	15	3.94	3.61	1.09			X	2.65	1.29	2.05	1.01	147
	-4	A	6	10	16	4.00	3.74	1.07			X	2.65	1.36	1.95	1.13	149
	-5	A	5	10	15	4.19	3.74	1.12			X	2.65	1.29	2.05	1.16	155

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Table 6. Records of Mating Experiments (1972)

Queen No.	No. of Nucleus	Test Group	Date of introduction	Date of first eggs seen	Dates of brood counts	Unsealed brood (in^2)	Sealed brood (in^2)	Total brood (in^2)	Sum of brood counts (in^2)
1	1	400(C)-1	14-7	30-7	14-8	36	111	147	
					26-8	29	229	258	405
2	2	400(C)-2	14-7	30-7	14-8	90	175	265	
					26-8	57	196	253	518
3	N-12	400(C)-2	11-7	19-7	12-8	73	215	288	
					24-8	32	210	242	530
4	N-13	400(C)-3	11-7	17-7	31-7			19	
					12-8			35	54
5	N-14	400(C)-1	11-7	17-7	31-7	160	142	302	
					12-8	26	223	249	551
6	N-3*	200(C)-3	1-7	6-7	17-7	28	126	154	
					-			-	154
7	N-4	200(C)-2	17-7	30-7	4-8			26	
					16-8			69	95
8	N-6	200(C)-4	1-7	15-7	31-7	121	209	330	
					12-8	32	175	207	537
9	N-18	200(C)-3	12-7	28-7	15-8	85	300	385	
					27-8	73	219	292	677
10	N-19	200(C)-4	12-7	28-7	12-8			28	
					24-8			54	82
11	N-3	75(D)-1	14-7	30-7	12-8	52	140	192	
					24-8	19	261	280	472

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Table 6 (continued)

Queen No.	No. of Nucleus	Test Group	Date of intro-duction	Date of first eggs seen	Dates of brood counts	Unsealed brood (in^2)	Sealed brood (in^2)	Total brood (in^2)	Sum of brood counts (in^2)
12	N-5	75(C)-2	2-7	15-7	3-7	66	110	176	
					12-8	70	181	251	427
13	N-15	75(C)-2	11-7	30-7	14-8	11	70	87	
					26-8	133	147	280	367
14	N-16	75(C)-1	11-7	28-7	12-8			150	
					24-8			111	161
15	N-17	75(C)-2	11-7	30-7	12-8			28	
					24-8			25	53
16	N-7	control	11-7	28-7	12-8	74	153	227	
					24-8	52	158	210	437
17	N-8	control	13-7	28-7	12-8	86	162	248	
					24-8	76	179	255	503
18	N-9	control	13-7	28-7	12-8	40	216	256	
					24-8	23	189	212	468
19	N-10	control	11-7	19-7	31-7	190	182	372	
					12-8	81	191	272	644
20	N-11	control	13-7	28-7	12-8			39	
					24-8			41	80

*The queen was injured and killed accidently.

APPENDIX B

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Table 7. Rearing Records of 30(Md), 30(S) and 30(C) Groups (1971)

Treatment No.	Experiment No.	Replicate	No. of larvae grafted	No. of larvae accepted	Age of larvae when introduced (hr.)	Time when* larvae grafted (hr.)	Capping Ability	Time of death
(Md)	I	30(Md)-1	2	0	6.50	30.00	-	L
			2	0	6.50	35.30	-	L
			2	0	16.00	46.00	-	L
			2	1	28.00	60.00	+	L
		30(Md)-2	2	0	6.20	32.67	-	L
			2	0	16.25	50.25	-	L
			2	1	20.25	74.25	+	L
	II	30(Md)-1	2	1	6.30	32.30	-	L
			2	1	16.30	46.50	-	L
			2	1	50.17	74.00	+	L
		30(Md)-2	2	0	6.25	36.00	-	L
			2	1	17.08	44.17	-	L
			2	1	24.00	75.10	-	L
(S)	I	30(S) -1	2	0	5.22	32.58	-	L
			2	2	25.42	78.00	+	L
			2	1	50.67	-	-	L
		30(S) -2	2	0	6.58	30.00	-	L
			2	2	25.18	49.50	+	P
			2	1	50.67	80.00	-	L
	II	30(S) -1	2	1	7.67	24.17	-	L
			2	1	10.42	50.83	-	L
		30(S) -2	2	0	7.55	24.00	-	L
			2	1	10.55	49.17	-	L

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Table 7 (continued)

Treatment No.	Experiment No.	Replicate	No. of larvae grafted	No. of larvae accepted	Age of larvae when introduced (hr.)	Time when* larvae grafted (hr.)	Capping Ability	Time of death
(C)	I	30(C) -1	2	1	6.50	25.00	+	P
			2	1	6.50	25.00	+	L
	II	30(C) -3	2	1	6.50	25.00	+	L
		30(C) -4	2	1	6.50	25.00	+	P

* Time following the dissection of the nurse bee and when the second, third, fourth, etc. larvae were introduced.

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Table 8. Rearing Records of 75(Md)* 75(S)** Groups (1971)

Treatment of nurse bees	Experiment No.	Replicate No.	No. of larvae grafted	No. of larvae accepted	Age of larvae when introduced (hr.)	Time when larvae grafted (hr.)	Capping ability	Time of death	Total development time (days)
(Md)	I	75(Md)-1	2	1	7.25	24.75	-	L	-
			2	1	28.75	47.55	+	L	-
			2	1	9.5	72.00	-	L	-
		75(Md)-2	2	1	7.25	19.50	-	L	-
			2	1	28.85	41.30	+	L	-
	II	75(Md)-1	2	1	10.3	22.5	-	L	-
			2	1	9.65	51.5	-	L	-
			2	1		72.00	+	L	-
		75(Md)-2	2	1	10.3	48.5	-	L	-
			2	1	29.0	72.0	+	L	-
			2	1	76.5				
	III	75(Md)-1	2	1	8.00	28.5	-	L	-
			2	1	25.50	45	-	L	-
		75(Md)-2	2	1	8.00	27.00	-	L	-
			2	1	21.00	67.50	+	L	-
	IV	75(Md)-1	2	1	7.25	28.50	+	P	-
		75(Md)-2	2	1	8.25	21.25	-	L	-
			2	1	28.00	45.5	-	L	-
	V	75(Md)-1	2	1	9.50	31	+	L	-
		75(Md)-2	2	1	9.50	28	+	Prepupal	-
	VI	75(Md)-1	2	1	9.50	27	+	Prepupal	-
		75(Md)-2	2	1	9.50	28.30	-	L	-
(S)	I	75(S) -1	2	2	10.50	25.60	+	A	17
	II	75(S) -2	2	2	10.80	23.90	+	L	-
	III	75(S) -1	2	2	9.58	25.58	+	P	-
	IV	75(S) -2	2	2	9.58	26.5	+	A	17
	V	75(S) -1	2	1	9.67	28.17	-	L	-
	VI	75(S) -2	2	2	9.5	27.17	+	A	18

* Nurse bees without their mandibular glands.
**Nurse bees which received "sham" operation.

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Table 9. Rearing Records of 75(Md)(L)*, 75(S)(L)** Groups (1971)

Treatment of nurse bees	Experiment No.	Replicate No.	No. of larvae grafted	No. of larvae accepted	Age of larvae when introduced (hr.)	Time when larvae grafted (hr.)	Capping ability	Time of death	Total development time (days)
(Md)(L)		75(Md)(L)-1	2	1	9.67	30.17	+	L	-
		-2	2	1	71.50	103.3	+	P	-
		-1	2	1	10.17	31.83	+	P	-
		-2	2	1	10.17	30.17	+	L	-
		-1	2	1	9.83	33.30	+	L	-
		-2	2	1	9.83	31.30	-	L	-
		-1	2	1	9.67	31.67	-	L	-
		-2	2	1	9.67	29.17	+	P	-
		-1	2	1	5.30	32.50	+	L	-
		-2	2	1	5.30	30.50	+	L	-
(S)(L)		75(S)(L) -1	2	1	9.75	28.92	+	A	17
		-2	2	1	9.83	25.	-	L	-
		-1	2	2	10.17	28.17	+	L	-
		-2	2	1	10.17	27.5	+	P	-
		-1	2	2	9.92	29.42	+	A	18
		-2	2	2	9.92	25.42	+	A	18
		-1	2	2	9.67	27.83	+	L	-
		-2	2	2	9.67	25.83	+	P	-
		-1	2	1	5.50	29.83	+	P	-
		-2	2	1	5.50	28.50	+	P	-

* Nurse bees which had their left mandibular gland removed.
**Nurse bees which had an "sham" operation on left gena.

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Table 10. Morphological Characteristics of Bees Reared by 75(S) Groups (1971)

Experiment No.	Replicate No.	Time of death	Pupal measurements					Adult measurements										
			Tongue length (mm.)	Body length (mm.) a	b	Total	Weight (mg.)	Head (mm.) W	L	w/L	Mandibles W	I	Q	Basitarsus L	W	L/W	Dia. Spermatheca (mm.)	No. of ovarioles
I	75(S)-1	A	2.31	6.30	8.40	14.70	141	3.94	3.35	1.18			x	2.26	1.09	2.07	1.07	105
III	75(S)-1	P	2.40	6.30	8.30	14.60	171	4.20	3.04	1.38			x	2.58	1.17	2.21	1. 7	-
	75(S)-2	A	2.52	5.88	8.82	14.70	147	3.42	2.00	1.71		x		2.00	1.07	1.07	0.88	112
IV	75(S)-2	A	2.63	6.09	8.30	14.39	138	3.48	3.23	1.08		x		1.29	0.88	2.15	0.68	92

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Table 11. Morphological Characteristics of Bees Reared by 75(Md)(L), and 75(S)(L) Groups (1971)

Experiment No.	Replicate No.	Time of death	Pupal measurements					Adult measurements										
			Tongue length (mm.)	Body length (mm.) a	b	Total	Weight (mg.)	Head (mm.) W	L	w/L	Mandibles W	I	Q	Basitarsus L	W	L/W	Dia. Spermatheca (mm.)	No. of ovarioles
I	75(Md)(L)-2	P	2.42	5.78	8.61	14.39	126											
II	75(Md)(L)-1	P	2.63	6.30	7.35	13.65	117											
IV	75(Md)(L)-1	P					95											
I	75(S)(L)-1	A	2.31	6.30	8.40	14.70	241	4.14	3.81	1.09			x	2.52	1.24	2.03	1.09	127
II	-2	P	2.42	5.78	9.45	15.23	184	3.94	3.38	1.17		x		2.26	1.03	2.19	0.84	71
III	75(S)(L)-1	A	2.63	5.67	7.88	13.55	179	4.00	3.58	1.12			x	2.47	1.16	2.99	1.13	109
	-2	A	2.31	6.93	7.88	14.81	181	3.87	3.10	1.24			x	2.32	1.05	2.21	1.13	121
IV	75(S)(L)-2	P	2.63	5.78	10.00	15.78	204	3.94	3.48	1.13			x	2.51	1.16	2.16	-	-
V	75(S)(L)-1	P	3.15	5.78	8.40	13.18	172	3.94	3.23	1.12			x	2.47	1.11	2.23	-	-
	-2	P	3.20	6.30	9.77	16.07	224											

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Table 12. Morphological Measurements of the Bees Reared by 75(Md) Groups for Different Test Periods* (1972)

Treatment of nurse bees	Test Period (hr.)	Exp. No.	Bee No.	Pupal measurements					Adult measurements											
				Tongue length (mm.)	Body length (mm.) a	b	total	Weight (mg.)	Head (mm.) W	L	w/L	Mandibles W	I	Q	Basitarsus L	W	L/W	Dia. Spermatheca (mm.)	No. of ovarioles	
75(Md)	45	I	1	-																
			2	-																
			3	-																
			4	-																
		II	1	-																
			2	-																
			3	2.31	6.30	7.98	14.28	144	3.87	3.48	1.11		x		2.58	1.29	2.00	1.03	98	
			4	-																
		III	1	2.21	6.41	7.88	14.29	150	4.13	3.66	1.13		x		2.32	1.16	2.00	1.01	110	
			2	2.10	6.30	7.98	14.28	154	4.13	3.05	1.35			x	2.06	1.16	1.28	0.98	96	
			3	2.31	6.72	8.19	14.91	169	3.80	3.29	1.16			x	2.25	1.09	2.06	0.96	102	
			4	-																
		IV	1	2.10	6.51	7.77	14.28	149	3.75	3.10	1.21		x		1.81	1.03	1.76	0.98**	72	
			2	-																
			3	-																(L)
			4	2.21	6.62	8.19	14.81	177	3.75	3.35	1.12		x		2.25	1.16	1.94	0.98**	98	(L)
	42	I	1	2.63	6.30	8.51	14.81	198	3.80	3.35	1.13			x	2.32	1.16	2.00	1.06	137	
			*2	2.52	6.30	8.40	14.70	174	-			-			-				-	
			3	2.42	6.41	9.56	15.97	185	3.80	3.35	1.13		x		2.32	1.29	1.80	1.13	108	
			4	-																
		II	1	2.31	6.30	7.56	13.86	192	3.87	3.35	1.16			x	2.48	1.16	2.14	1.01	112	
			2	-																
			*3	2.34	6.41	7.67	14.08	198	-			-			-			-	-	
			4	-																
		III	1	2.63	6.51	9.56	16.07	221	3.87	3.22	1.20			x	2.12	1.09	2.03	1.08	125	

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Table 12 (continued)

Treatment of nurse bees	Test Period (hr.)	Exp. No.	Bee No.	Pupal measurements					Adult measurements									
				Tongue length (mm.)	Body length (mm.) a	b	total	Weight (mg.)	Head (mm.) W	L	w/L	Mandibles W	I	Q	Basitarsus L	W	L/W	Dia. Spermatheca (mm.)	No. of ovarioles
75(Md)	42	III	2	-															
			*3	2.42	6.30	7.98	14.28	196	-			-			-			-	-
			4	2.52	6.72	8.40	15.12	212	4.06	3.22	1.26			x	2.25	1.35	1.67	1.03	146
		IV	1	2.52	6.41	8.82	15.23	190	4.06	3.66	1.11			x	3.32	1.16	2.00	1.06	140
			2	-															
			3	2.52	6.51	8.51	15.02	192	3.93	3.84	1.13		x		2.52	1.23	2.05	1.11	145
			4	-															
	40	I	1	2.52	6.51	8.51	15.02	192	4.06	3.35	1.21			x	2.12	1.16	1.83	1.11	154
			2	2.72	6.30	8.61	14.91	193	3.55	3.10	1.15			x	1.68	1.09	1.54	0.98	106
			3	2.31	6.51	8.40	14.91	195	4.25	3.66	1.16		x		2.58	1.29	2.00	1.08	95
			4	2.52	6.30	8.51	14.81	194	4.06	3.87	1.03			x	2.45	1.29	1.90	1.13	113
		II	1	2.63	6.41	8.72	15.13	182	4.06	3.62	1.12			x	2.58	1.16	2.05	1.01	124
			*2	2.31	6.51	8.72	15.23	190	-			-			-			-	-
			3	2.42	6.41	8.40	14.81	188	3.94	3.61	1.09			x	2.39	1.16	2.06	0.98	119
			4	2.42	6.51	8.30	14.81	197	4.13	3.87	1.07			x	2.32	1.23	1.89	1.01	134
	36	I	1	2.31	6.30	8.61	14.91	201	3.93	3.66	1.07			x	2.52	1.29	1.95	1.01	135
			2	-															
			3	2.52	6.51	8.61	15.12	192	4.06	3.66	1.11			x	2.52	1.29	1.95	1.06	126
			4	2.42	6.51	8.51	15.02	195	4.25	3.48	1.22			x	2.45	1.29	1.90	0.98	132
		II	1	2.42	6.41	8.93	15.34	221	4.06	3.48	1.17			x	2.58	1.35	1.91	1.16	127
			2	2.63	6.51	9.03	15.54	205	3.87	3.48	1.11			x	2.06	1.23	1.67	1.08	141
			3	2.42	6.41	8.72	15.13	188	3.81	3.55	1.07			x	2.06	1.23	1.67	1.01	120
			4	2.42	6.51	9.14	15.65	207	3.87	3.68	1.05			x	2.19	1.16	1.84	1.03	109

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Table 12 (continued)

Treatment of nurse bees	Test Period (hr.)	Exp. No.	Bee No.	Pupal measurements					Adult measurements										
				Tongue length (mm.)	Body length (mm.) a	b	total	Weight (mg.)	Head (mm.) W	L	w/L	Mandibles W	I	Q	Basitarsus L	W	L/W	Dia. Spermatheca (mm.)	No. of ovarioles
75(Md)	24	I	1	2.31	6.41	8.72	15.13	199	4.19	3.87	1.08			x	2.45	1.29	1.90	1.13	157
			2	2.42	6.83	9.14	15.97	223	3.81	3.61	1.06			x	2.39	1.16	1.44	0.98	140
			3	2.42	6.72	9.03	15.72	210	3.81	3.55	1.07			x	2.45	1.23	1.99	1.01	151
			4	2.31	6.93	8.82	15.75	203	4.06	3.94	1.03			x	2.26	1.23	1.84	1.03	137

* The Period of time in which larvae were fed by nurse bees received various treatments.

** Died in the pupal stage.

APPENDIX B

Table 13. Morphological Measurements of Bees Reared by 75(S) Groups for Different Test Periods (1972)

Treatment of nurse bees	Test Period (hr.)	Exp. No.	Bee No.	Pupal measurements					Adult measurements										
				Tongue length (mm.)	Body length (mm.) a	b	total	Weight (mg.)	Head (mm.) W	L	w/L	Mandibles W	I	Q	Basitarsus L	W	L/W	Dia. Spermatheca (mm.)	No. of ovarioles
75(S)	45	I	1	2.39	6.30	9.56	15.86	230	4.06	3.75	1.08			x	2.52	1.35	1.87	1.11	144
			2	2.13	6.09	8.51	14.60	170	4.06	3.87	1.05			x	2.45	1.29	1.90	1.13	113
			+3	2.31	6.30	8.72	15.02	189	4.06	3.29	1.23			x	2.52	1.29	1.95	1.13	128
			4	2.31	6.51	8.93	15.04	207	4.06	3.61	1.12			x	2.58	1.29	2.00	1.01	140
		II	1	2.21	6.72	8.40	15.12	200	4.25	3.55	1.20			x	2.58	1.29	2.00	1.03	132
			2	2.52	7.04	8.93	15.97	213	3.93	3.61	1.09			x	2.38	1.29	1.84	1.08	137
			+3	2.31	6.09	7.88	13.97	153	3.93	3.22	1.22			x	2.19	1.23	1.78	0.98	98
			4	2.42	6.41	9.35	15.71	210	4.06	3.61	1.12			x	2.38	1.16	2.05	1.11	126
	42	I	1	2.10	5.99	6.22	12.21	150	4.25	3.66	1.16			x	2.58	1.29	2.00	1.08	95
			2	2.31	6.93	8.40	15.33	186	4.25	3.66	1.16			x	2.52	1.23	2.05	1.08	140
			3	2.21	6.30	8.51	14.81	175	4.06	3.62	1.12			x	2.38	1.16	2.05	1.01	124
			4	2.31	6.62	8.51	15.13	183	4.06	3.66	1.11			x	2.64	1.29	2.05	1.11	152
		II	1	2.42	6.83	9.03	15.86	212	4.20	3.75	1.11			x	2.64	1.35	1.96	1.11	149
			2	2.21	6.41	8.82	15.23	191	4.40	3.66	1.20			x	2.64	1.23	2.15	1.11	152
			3	2.42	6.93	8.72	15.65	211	4.32	3.93	1.10			x	2.58	1.35	1.91	1.13	160
			4	2.42	6.83	8.61	15.44	204	4.06	3.55	1.14			x	2.58	1.29	2.00	1.13	158
	40	I	+1	2.21	6.51	7.88	14.39	176	4.13	3.29	1.26			x	2.19	1.23	1.78	1.06	122
			2	2.31	6.72	7.30	15.02	192	4.20	3.55	1.18			x	2.32	1.23	1.89	1.08	135
			3	2.31	6.62	8.40	15.02	199	4.06	3.22	1.26			x	2.38	1.29	1.84	1.08	140
			4	2.31	6.72	8.51	15.23	210	4.25	3.80	1.12			x	2.64	1.29	2.05	1.11	139
	36	I	1	2.42	6.83	8.40	15.23	211	4.06	3.55	1.14			x	2.58	1.29	2.00	1.13	128
			2	2.31	6.51	8.19	14.70	176	3.93	3.29	1.19			x	2.26	1.23	1.84	1.01	107
			3	2.52	6.93	8.51	15.44	205	4.06	3.66	1.11			x	2.32	1.29	1.80	1.16	159
			4	2.42	6.83	8.40	15.23	208	4.13	3.66	1.13			x	2.58	1.23	2.10	1.13	144

APPENDIX B

Table 13 (continued)

Treatment of nurse bees	Test Period (hr.)	Exp. No.	Bee No.	Pupal measurements					Adult measurements										
				Tongue length (mm.)	Body length (mm.) a	b	total	Weight (mg.)	Head (mm.) W	L	w/L	Mandibles W	I	Q	Basitarsus L	W	L/W	Dia. Spermatheca (mm.)	No. of ovarioles
75(S)	24	I	1	2.42	6.83	8.61	15.44	214	4.13	3.94	1.05			x	2.45	1.29	1.90	1.11	147
			2	2.52	7.04	8.51	15.55	210	4.06	3.68	1.10			x	2.52	1.16	2.17	1.01	151
			+3	2.42	6.83	8.61	15.44	213	4.00	3.68	1.09			x	2.45	1.16	2.11	0.98	93
			4	2.42	6.72	8.40	15.12	194	3.68	3.29	1.12			x	1.87	1.07	1.68	0.98	113

+ The adult measurements were done in the late pupal stage.

APPENDIX B

Table 14. Morphological Measurements of the Bees Reared by 75(C) Groups for Different Test Periods (1972)

Treatment of nurse bees	Test Period (hr.)	Exp. No.	Bee No.	Pupal measurements					Adult measurements										
				Tongue length (mm.)	Body length (mm.) a	b	total	Weight (mg.)	Head (mm.) W	L	w/L	Mandibles W	I	Q	Basitarsus L	W	L/W	Dia. Sper-matheca (mm.)	No. of ovarioles
75(C)	45	I	1	2.31	6.30	8.93	15.23	210	3.81	3.35	1.07			x	2.23	1.23	1.81	1.13	129
			*2	2.31	6.30	8.61	14.91	187	3.74	3.10	1.21			x	2.06	1.16	1.78	1.08	107
			3	2.42	7.04	9.45	16.49	236	4.06	3.68	1.10			x	2.45	1.23	1.99	1.18	187
			4	2.63	7.04	8.30	15.34	219	4.06	3.74	1.09			x	2.58	1.23	2.10	1.18	140
		II	*1	2.31	7.04	8.93	15.97	220	6.10	3.55	1.72			x	2.39	1.16	2.05	1.06	113
			2	2.52	6.51	8.72	15.23	224	4.00	3.61	1.61			x	2.00	1.29	1.55	1.06	117
			3	2.42	6.62	8.82	15.44	230	4.00	3.74	1.74			x	2.32	1.36	1.78	1.01	139
			4	2.63	6.30	8.40	14.70	232	4.00	3.81	1.81			x	2.06	1.16	1.78	1.18	125
		III	*1	2.42	6.83	8.72	15.55	217	4.13	3.87	1.07			x	2.65	1.29	2.05	1.03	124
			2	2.42	7.04	8.82	15.86	240	4.45	4.06	1.10			x	2.77	1.36	2.04	1.11	153
			3	2.31	6.51	8.30	14.81	210	3.74	3.55	1.05			x	2.26	0.90	2.51	1.11	142
			4	2.31	6.30	8.19	14.49	198	3.94	3.68	1.07			x	2.65	1.16	2.28	1.03	159
		IV	1	2.52	7.14	8.61	15.75	236	4.00	3.87	1.03			x	2.45	1.29	1.90	1.18	148
			2	2.42	7.25	8.40	15.65	239	4.00	3.55	1.13			x	2.35	1.23	1.91	1.16	153
			3	2.42	7.04	8.61	15.65	243	3.94	3.68	1.07			x	2.45	1.29	1.90	1.13	160
			4	2.31	7.04	8.82	15.86	239	4.00	3.48	1.15			x	2.39	1.03	2.32	1.16	168
	42	I	1	2.31	6.30	8.61	14.91	201	4.13	3.89	1.07			x	2.13	1.16	1.84	1.11	150
			2	2.42	6.20	7.98	14.18	187	4.06	3.55	1.14			x	2.32	1.10	2.11	1.13	139
			3	2.42	6.41	8.30	14.71	194	4.00	3.16	1.27			x	2.19	1.16	1.89	1.06	169
			4	2.42	6.72	8.72	15.44	210	4.00	3.42	1.17			x	2.39	1.10	2.17	1.16	164
		II	1	2.52	6.41	8.93	15.34	223	3.87	3.61	1.07			x	2.13	1.29	1.65	0.98	120
			2	2.52	7.04	8.93	15.97	215	3.74	3.55	1.05			x	2.45	1.36	1.80	1.06	130
			3	2.31	7.04	9.03	16.07	237	4.19	3.94	1.06			x	2.58	1.42	1.82	1.11	163
			4	2.31	6.93	8.51	15.44	186	3.81	3.61	1.06			x	2.32	1.23	1.89	1.06	103
		III	1	2.63	6.83	8.30	15.13	147	4.32	3.81	1.13			x	2.45	1.16	2.11	1.03	92

APPENDIX B

Table 14 (continued)

Treatment of nurse bees	Test Period (hr.)	Exp. No.	Bee No.	Pupal measurements					Adult measurements										
				Tongue length (mm.)	Body length (mm.) a	b	total	Weight (mg.)	Head (mm.) W	L	w/L	Mandibles W	I	Q	Basitarsus L	W	L/W	Dia. Spermatheca (mm.)	No. of ovarioles
75(C)	42	III	2	2.42	7.04	8.82	15.86	216	3.74	3.55	1.06			x	2.32	1.16	2.00	1.06	134
			3	2.31	6.41	9.35	15.76	209	3.74	3.42	1.09			x	2.26	1.10	2.05	1.11	161
			4	2.63	6.62	9.45	16.07	245	3.81	3.55	1.07			x	2.58	1.36	1.90	1.18	183
		IV	1	2.42	6.93	8.93	15.86	204	4.13	3.74	1.10			x	2.45	1.23	1.99	1.13	130
			2	2.52	7.04	8.72	15.76	197	4.00	3.74	1.07			x	2.71	1.36	1.99	1.01	121
			*3	2.31	7.14	9.14	16.28	189	3.94	3.68	1.07			x	2.58	1.29	2.00	0.98	114
			4	2.31	7.04	9.03	16.07	211	3.87	3.55	1.09			x	2.32	1.29	1.80	1.01	131
	40	I	1	2.63	7.04	9.45	16.49	215	3.94	3.68	1.07			x	2.39	1.29	1.85	1.01	129
			2	2.52	7.04	8.72	15.76	202	3.87	3.61	1.07			x	2.45	1.23	1.99	1.11	138
			3	2.42	6.93	8.82	15.75	210	3.87	3.55	1.09			x	2.58	1.29	2.00	1.06	113
			4	2.42	6.93	8.51	15.44	184	4.06	3.68	1.10			x	2.45	1.29	1.90	1.08	102
		II	1	2.42	6.83	8.82	15.65	211	4.00	3.74	1.07			x	2.39	1.36	1.76	1.01	125
			2	2.73	7.04	9.03	16.07	204	3.87	3.68	1.05			x	2.52	1.23	2.05	0.98	118
			3	2.52	7.14	8.82	15.96	196	3.61	3.23	1.12			x	2.26	1.10	2.05	0.98	96
			4	2.42	7.35	9.14	16.49	222	4.00	3.68	1.09			x	2.58	1.36	1.90	1.18	197
	36	I	1	2.63	7.04	9.03	16.07	204	3.74	3.42	1.03			x	2.39	1.29	1.85	1.11	141
			2	2.84	7.14	9.14	16.28	231	4.00	3.69	1.09			x	2.45	1.29	1.90	1.06	123
			3	2.52	6.93	9.03	15.96	213	4.13	3.61	1.14			x	2.58	1.29	2.00	1.08	140
			4	2.52	6.62	8.30	14.92	199	4.19	3.68	1.14			x	2.58	1.16	2.22	1.11	118
		II	1	2.52	6.93	8.51	15.44	186	4.00	3.29	1.22			x	2.13	1.23	1.73	1.11	107
			2	2.42	6.51	8.40	14.91	201	3.94	3.61	1.09			x	2.19	1.10	1.99	1.11	101
			3	2.31	6.41	8.72	15.13	194	3.87	3.55	1.09			x	2.52	1.23	2.00	1.13	122
			4	2.42	7.14	9.14	16.28	225	4.06	3.68	1.14			x	2.45	1.29	2.00	1.03	120

APPENDIX B

Table 14 (continued)

Treatment of nurse bees	Test Period (hr.)	Exp. No.	Bee No.	Pupal measurements					Adult measurements									
				Tongue length (mm.)	Body length (mm.) a	b	total	Weight (mg.)	Head (mm.) W	L	w/L	Mandibles W	I	Q	Basitarsus L	W	L/W	Dia. Spermatheca (mm.)	No. of ovarioles
75(C)	24	I	1	2.31	6.41	8.93	15.34	200	3.81	3.55	1.07			x	2.19	1.10		1.03	140
			2	2.42	6.41	8.51	14.92	201	3.94	3.74	1.05			x	2.26	1.29		1.06	122
			3	2.63	6.93	8.82	15.75	212	3.81	3.61	1.06			x	2.45	1.36		1.11	132
			4	2.31	7.04	8.93	15.97	224	4.00	3.48	1.15			x	2.26	1.29		1.13	147

* Pupa died in the pupal stage.

APPENDIX B

Table 15. Mortality of Nurse Bees of Groups of Thirty Bees within 7 Days (1970 and 1971)

1 9 7 0			1 9 7 1				
30 (Md) %	30 (S) %	30 (C) %	30 (Md)(X) %	30 (S)(X) %	30 (Md) %	30 (S) %	30 (C) %
80	26.67	10.00	23.33	13.33	16.67	16.67	10.00
60	36.67	5.33	10.00	10.00	13.33	13.33	13.33
66.67	23.33	6.67	23.33	10.00	6.67	6.67	4.00
66.67	30.00	23.33	16.67	20.00	4.00	4.00	4.00
43.33	16.67	3.33					
46.67	23.33	6.67					
46.67	36.67	0.00					
73.33	46.67	3.33					
66.67	40.00	6.67					
56.67	26.67	6.67					
20.00	26.67	6.67					
63.33	20.00	10.00					
70.00	16.67	3.33					
63.33	33.33	6.67					
66.67	20.00	6.67					
56.67	30.00	3.33					
53.33	20.00	3.33					
63.33	30.00	3.33					
20.00	23.33	3.33					
53.33	13.33	3.53					
43.33	13.33	3.67					

APPENDIX B

Table 16. Mortality of Nurse Bees of Groups of Seventy-Five Bees within 7 Days (1971 and 1972)

1971			1971			1972		
75(Md) %	75(S) %	75(C) %	75(Md)(L) %	75(S)(L) %	75(C) %	75(Md) %	75(S) %	75(C) %
20.00	13.33	14.67	9.33	9.33	9.33	6.67	4.00	1.33
22.67	5.33	12.00	10.67	8.00	8.00	9.00	8.00	4.00
16.00	21.33	5.33	12.00	4.00	4.00	4.00	4.00	1.00
13.33	14.67	13.33	9.33	6.67	6.67	7.00	3.00	3.00
16.00	17.33	5.33	10.67	9.33	5.33	7.00	4.00	2.67
10.67	6.88	4.00	14.67	8.00	13.33	8.00	4.00	1.33
13.33		10.67	8.00	10.67	10.67	8.00	5.33	2.67
12.00		9.33	16.00	12.00	9.33	9.33	4.00	1.33
13.33		10.67	14.67	6.67	5.33	4.00	12.00	0.00
16.00		10.67	13.33	5.33	12.00	6.67	2.67	1.33
18.67		13.33				6.67	12.00	0.00
12.00		8.00				12.00	10.67	0.00
						9.33	2.67	4.00
						8.00	1.33	2.67
						5.33	5.33	2.67
						9.33	2.67	0.00
						4.00	6.67	4.00
						6.67	2.27	2.67
						6.67		2.67
						1.47		8.00
						6.67	10.67	2.67
						8.00	8.00	1.33
						6.67	4.00	1.33
						5.32	12.00	8.00
						10.67	10.67	5.33
						8.00	6.67	2.67 4.00
						4.00	4.00	2.67 4.00
						12.00	12.00	10.67 1.33

APPENDIX C

APPENDIX C

Table 17. Ovary Development of Caged Worker Bees Which were Collected from Different Colonies (1972)

Experiment No.	Collection date	Treatment of nurse bees	BEE NO. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	0	1	2	Index
I	19-7-71	75(Md)-1	0	0	1	2	1	0	0	1	1	1	0	2	1	0	2	0	1	0	1	2	0	0	1	2	0	11	9	5	44
		-2	2	0	2	0	2	1	0	2	0	1	1+	2	0	1	0	2	2	2	2	2	1+	2	2	1	0	7	4	14	57
		-3	1	2	1	0	0	2	2	2	2	2	0	2	1	2	0	2	0	2	1	2	0	2	0	1	2	7	5	13	56
		-4	2	2	2	2	2	2	1	2	2	0	2	2	0	0	2	2	2	1	2	2	2	2	2	1	0	4	3	18	64
		75(C) -1	0	2	2	2	2	2	1	0	0	2	2	0	0	0	2	2	0	2	0	1+	2	1	0	1	2	9	3	13	54
		-2	0	1	2	0	2	1	2	1	1	2	2	0	1	2	0	2	1	2	2	0	1+	0	0	2	0	8	6	11	53
		-3	0	0	2	1	0	2	2	0	0	0	2	1	0	0	0	0	2	1	1	0	0	0	2	0	1	14	5	6	42
		-4	0	2	2	2	2	0	0	2	1	2	1	0	2	2	1	2	0	1	2	2	2	2	2	0	0	7	4	14	57
II	9-8-72	75(Md)-1	1	1	1	2	2	1	0	2	2	1	1	2	0	0	0	2	0	1	2	2	2	0	0	0	2	8	7	10	52
		-2	2	1	1	0	1	0	2	1	1	1	1	2	0	1	0	0	0	1	2	2	2	1	1	0	0	8	11	6	48
		-3	2	1	2	2	2	2	1	1	1	2	0	0	1	2	0	2	2	2	2	1	2	1	0	0	2	5	7	13	45
		-4	0	0	0	0	1	1	0	1	0	0	2	0	0	0	0	1	1	1	0	1	0	0	1	0	2	15	8	2	37
		75(C) -1	1	1	2	2	1	2	1	2	0	1	2	1	1	1	0	2	2	1	1	2	2	1	0	1	2	3	12	10	57
		-2	1	2	0	0	1	1	0	2	0	1	2	1	2	1	2	1	0	2	0	0	0	0	2	1	0	10	8	7	47
		-3	2	1	0	0	2	0	2	1	0	1	0	0	0	0	1	1	0	0	1	0	2	0	1	1	0	13	8	5	44
		-4	1	0	0	0	1	0	1	1	0	0	0	0	0	1	1	0	1	0	1	0	0	0	0	1	0	16	9	0	34

APPENDIX C

Table 18. Ovary Development of Worker Bees which Received Different Treatments (1972)

Experiment No.	Collection date	Treatment of nurse bees	BEE NO. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	0	1	2	Index
III	26-7-72	75(Md)-1	0	2	1	1	1	0	2	2	2	0	0	0	0	1	2	1+	1	0	0	1	0	0	1	1	0	11	8	6	55
		-2	2	2	2	1	1	0+	1	1	0	2	2	2	2	0	0	2	1	0	0	0	2	2	1	0	1	7	8	10	53
	3-8-72	-1	1	0	0	0	2	1	2	1	0	1	0	1	2	0	1	1	2	2	2	1	1	2	1	0	1	7	11	7	50
		-2	1	0	2	0	0	2	1	2	1	0	1	2	1	0	0	0	1	0	0	1	1	2	1	0	1	10	10	5	45
	26-7-72	75(C) -1	1+	1	1	1	0	0	2	0	0	0	0	1	1	2	2	1	1	1	1	1	1	0	2	0	1	8	12	5	47
		-2	2	1	0	1	1	0	0	0	0	0	0	0	0	1+	0	0	1	1	2	0	0	0	1	0	0	16	6	3	37
	3-8-72	-1	0	0	1	2	0	0	0	2	2	1	0	2	2	0	0	1	1	2	0	0	1	0	0	2	1	12	6	7	45
		-2	1	0	0	0	0	2	0	0	2	1	1	2	0	2	2	0	2	2	0	2	0	2	1	1	1	10	6	9	49
		75(G) -1	2	1	2	2	2	0	0	0	0	1	0	1	2	1	1	0	2	2	0	1	2	0	2	1	2	8	7	10	52
		-4	1	1	2	0	2	0	0	0	0	2	0	1	2	1	1	0	2	1	2	0	2	1	1	0	1	9	9	7	48
	3-8-72	75(G) -1	0	2	1	0	1	1	0	0	0	1	0	1	0	0	1	1	0	2	1	1	0	1	1	2	1	10	12	3	43
		-2	2	1	1	1	0	0	0	0	2	1	0	0	1	0	1	1	1	1	0	0	2	1	2	1	0	10	11	4	44
	24-8-72	75(C)(m)-1	2	1	2	0	1	1	1	2	1	2	1	0	1	2	0	1	0	1	0	1	2	1	1	0	1	6	13	6	50
		-2	0	0	1	1	1	1	1	2	2	2	1	1	2	0	0	0	0	1	0	2	2	1	1	1	0	8	11	6	48
		-3	1	0	2	2	0	1	1	1	2	1	1	0	0	1	0	0	1	1	2	1	0	2	0	0	1	9	11	5	46
		-4	1	0	0	0	0	2	1	2	0	2	0	0	2	0	1	1	1	0	0	1	0	0	0	2	1	13	8	4	41
	24-8-72	75(C)(m)-1	2	1	2	1	0	2	0	1	1	0	0	1	1	1	0	0	1	0	0	0	0	2	0	1	0	12	9	4	42
		-2	2	2	2	1	0	1	1	2	0	1	1	1	1	0	0	1	2	1	0	0	1	2	1	2	0	7	11	7	50
		-3	1	1	1	1	1	1	0	1	0	2	0	1	0	2	0	2	1	2	1	1	1	2	1	1	0	5	15	5	50
		-4	1	2	0	2	0	1	1	2	0	1	0	0	0	1	1	2	1	2	1	2	2	0	0	1	0	9	10	6	47