APIMONDIA MONOGRAPHS

UNDER THE DIRECTION OF PROF. DR. ENG. V. HARNAJ

QUEEN REARING

EDITED UNDER SUPERVISION OF PROF. DR. DR. F. RUTTNER

APIMONDIA PUBLISHING HOUSE

In memory of Hans Ruttner

Born 2.V.1919

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APIMONDIA MONOGRAPHS

UNDER THE DIRECTION OF PROF. DR. ENG. V. HARNAJ

QUEEN REARING

BIOLOGICAL BASIS AND TECHNICAL INSTRUCTION

EDITED UNDER SUPERVISION OF PROF. DR. DR. F. RUTTNER

APIMONDIA PUBLISHING HOUSE
Bucharest 1983

Photos on the outer cover by courtesy of E. MÜLLER

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QUEEN REARING

FOREWORD

This book is the first step towards attaining the long cherished desideratum of APIMONDIA, of providing the practical beekeepers and research workers in apiculture with a number of books, an encyclopaedia — a series of monographs on apiculture, to include the fundamentals required for efficient accomplishment in their everyday activity.

Professor RUTTNER was the first who met our appeal and promptly provided APIMONDIA with a work of utmost importance for world apiculture : Queen Rearing, *the first of a series of monographs which we wish to continue to publish, monographs in which the valuable experience and research results in various branches of apiculture will be brought together and be made available to all beekeepers.*

By collective experience and exchange of views concerning application of the most advanced technologies, we shall possibly be able to provide the beekeepers at large with what they need for a rational beekeeping using intensive methods.

Many such monographs have been initiated and are now being prepared ; difficulties are however occurring often, primarily in the establishment of contacts of experts, to make up a large and well-knit team, with a precise programme.

Because it is difficult to bring together the experience of each and everyone without the inherent differences of views in practice and research work.

In connection with this first book of the APIMONDIA monographs series, prepared by the remarkable team under Prof. RUTTNER's direction, we point out that all results of the research on queen rearing — now being extended the world over, have been taken into consideration in it, and that it attempts to provide the practical beekeepers and research workers with the methods having been developed. The authors have endeavoured, on the one hand to approach the problems at a high scientific level — with reference to the latest and most thorough research work, and on the other hand to give due attention to the methods of the leading

queen breeders who have been producing an ever increasing number of top-quality, selected queens.

The classical methods are also referred to, whenever required.

The book is a retrospective survey of the activity of queen breeders, of those who have made their contribution to the vast work for improvement of bee races by selection, to the benefit of beekeeping production.

We thank the team who, under the competent direction of Prof. Dr. F. RUTTNER, have supplied the beekeepers the world over with valuable knowledge, with approach of most delicate bee biology problems.

This book is the first step along a new road in research and publication of books to the benefit of the beekeepers everywhere.

Prof. Dr. Eng. V. HARNAJ
APIMONDIA President

PREFACE

Queen rearing is one of the most fascinating and economically most important aspects of beekeeping. Thorough knowledge of queen rearing methods is equally important for the biologist working in research, as well as for both the amateur and professional beekeeper. It is only in this way that the natural reproduction of bee colonies may be guided along previously determined lines, and queen rearing be integrated with systematic management. Dozens of methods have been developed for producing queens of top quality, by rational methods. Meanwhile, science has answered many questions underlying the success of the methods.

This book is intended to bring two domains together : the practical queen rearing based on the experience of several decades, and that of research, with its results on the complex problems of bee biology and the development of the two different female castes in the bee colony. The book tries to give detailed and clear indications about the "How" of the different rearing methods, and also to explain "Why". We therefore hope the book will elucidate interrelations not understood before, and that it will also eliminate previous prejudicial opinions.

The major aim is to provide a book to be useful in practical work. That is why we included in the book only the rearing methods which have been ascertained as efficient in current practice.

This concept, materialized for the first time in this book, has been prepared for many years. We have, in this period, been successful in obtaining the co-operation of many noted experts in the domain, and in acquiring considerable personal experience in many countries. Important parts of chapters VII, VIII and IX, that is of the "technical advises" resulting in a comprehensive survey of the essential methods practised round the world, were contributed by a number of colleagues as listed below.

We wish to express our sincere gratitude to them, as well to those who helped in the English translation.

Co-authors : Harry H. LAIDLAW, Davis, USA
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Three of the authors, Werner FYG, one of the best experts in anatomy and diseases of queen bees, Giulio PIANA, one of the best queen breeders in Europe, and Hans RUTTNER, who, most competently and thoroughly, completed the practical part of the book, died before this book was published. Their life's work will continue to live in this book.

Oberursel/Lunz am See, May 1983

Friedrich RUTTNER

CHAPTER I

The events which take place during the natural replacement of the queen in a colony of bees

F. RUTTNER

1. Introduction

The rearing of young qeens has its well defined place in the annual cycle of the colony. It does not occur haphazardly but is correlated to very distinct conditions and releasing factors.

A "normal" colony, being in a harmonious condition will not raise young queens, therefore the activities of queen rearing in apiculture are centred around the creation of optimal conditions for this and in using the releasing factors in a systematic way. In nature the rearing of queens as such, is the concern of the colony alone. In this sense each queen reared within the colony is "natural". The artificial rearing of queens in the laboratory is dealt with in chapter IV. Consequently all experiments and methods described in the following chapters will concentrate on the presentation of the sequence of the natural rearing of queens within the colony in detail. On this basis alone a technique can be developed for the best and most economic accomplishment of queen rearing in apiculture.

It will become evident that despite the amount of research done, we are far from being able to answer for certain, all the questions involved in the rearing of queens. The major difficulties are not concerned with the individual bee, but with the situations where the influence of the colony as a whole and the co-operation of all its elements, have to be considered. The principal question is : What is this colony which is to be understood as a "normal colony" ?

2. The social structure of the colony and its disturbance

According to the usual definition, a colony of bees is composed of the queen, a variable number of worker bees and during certain parts of the season a much smaller number of drones ; also depending on the season are the brood in various stages, the combs and the provisions. This is however a very superficial description, comprising only the visible facts. Its internal structure is extremely complex and despite all our en-

deavours is beyond our understanding in some respects. The crucial problems for our consideration are the manifold relationships of the workers with each other, with the brood and with the queen.

Fundamentally a "normal" colony contains worker bees of all ages divided into classes who accomplish certain functions according to their age. However this organization is not based rigidly on the development of the stages of the individual bee — it can vary — to a large extent it is based on the principle of supply and demand. The functions, physiologically programmed for a certain age class, may be shifted temporarily within certain limits, or even completely suppressed. As a consequence a high degree of flexibility results according to requirements.

The distribution of food from bee to bee results in the uniform nourishment of the colony including the brood and thereby achieves a total, common metabolism of the "super individual" bee colony. For the first days of life a young bee builds up her brood food glands and fat-protein-body. As a nurse bee she then feeds these protein reserves mixed with honey as brood food to the larvae. This leaves her a short life as a flight bee (MAURIZIO 1954). The decisive factors which direct the course of events within the colony are the preparation of brood cells, the provisions of food, the maintenance of the appropriate temperature for brood rearing and the feeding of the queen. These are achieved by the worker population. It is the workers who regulate and accomplish the vital functions: the extension of the brood nest by constructing new combs and cleaning existing ones, reduction of the brood by diminishing the food delivered to the queen or by removing eggs or larvae ; by increasing or reducing young queens and by the intensity of food collection.

The most important functions in the colony are carried out directly by the worker bees. However the queens' influence — at least as great as that of the workers — is achieved not directly, but indirectly through them. Only in the presence of a queen are the workers able to function

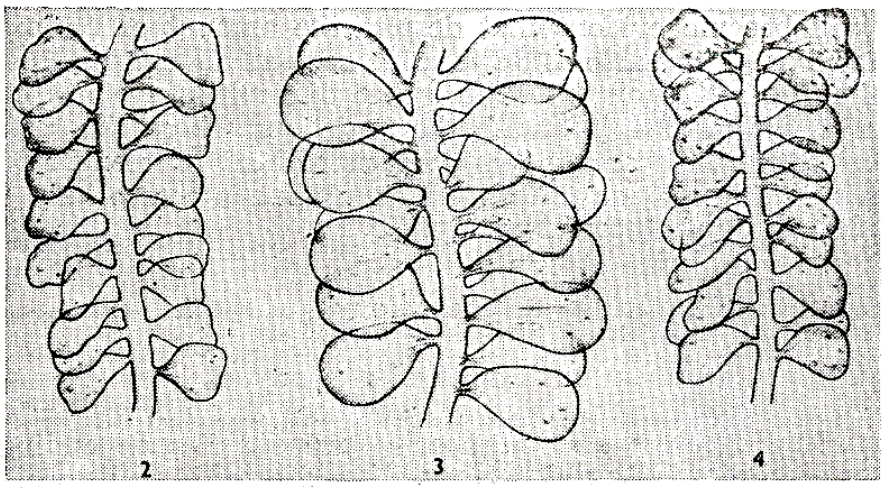

Fig. 1 — *Sections of brood food glands of worker bees : 2 — freshly emerged bee (undeveloped) ; 3 — nurse bee, 8 days old (fully developed) ; 4 — field bee (retrogressive).*
From Jordan and Zecha, 1958.

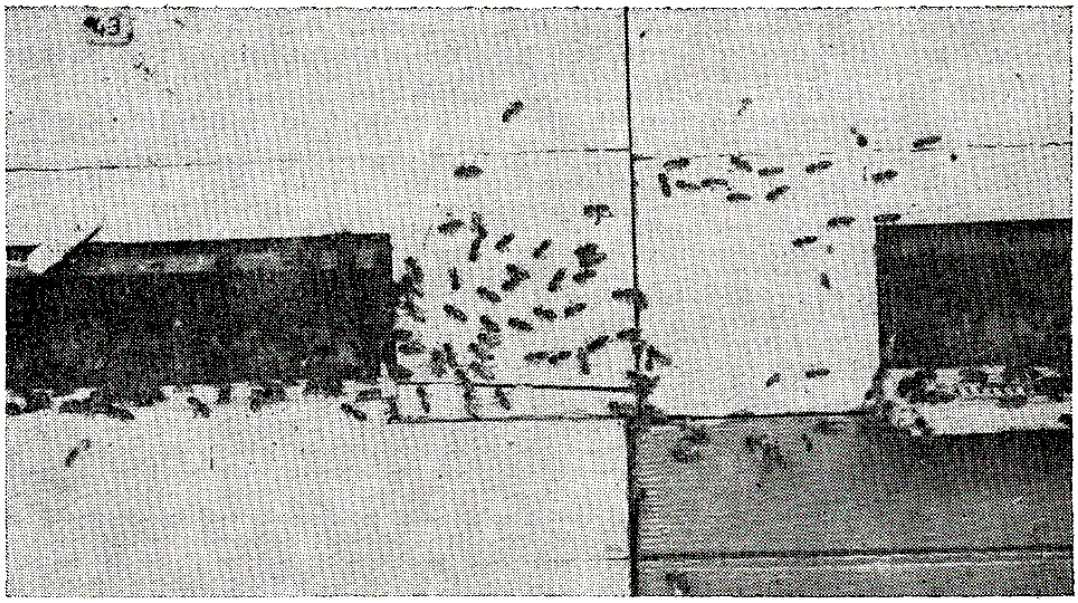

Fig. 2 — *Many bees abandon their colony after loss of their queen and join the queenright neighbour ("desertion").*

as an integrated part of the colony. The queen is the focal point necessary for the functioning of biological requirements. In a queenless colony comb building finishes, field activities and normal defense behaviour diminish as does co-operation within the colony. The relationship therefore between worker and queen is of major importance for maintaining the working of the colony.

The effects after the loss of the queen, that is a recently queenless colony, are really dramatic.

2.1. *Buzz of queenlessness*

About 1/2—1 hour after the loss or removal of the queen a state of unrest begins. This spectacular change in behaviour is frequently accompanied by distinctive sounds well known to experienced beekeepers. This is quickly followed by the cessation of comb construction and diminished field collection and flight activity. One consequence of diminished colony coherence is a phenomenon called desertion, the result of which is most clearly seen when the colonies are in close proximity to one another. Frequently veritable roads of fanning bees are observed deserting their own colony and emigrating to the queenright neighbour.

2.2. *Construction of queen cells*

A few hours after removal of the queen certain cells containing young worker larvae show a greater provision of larval food and the cell is enlarged to a queen cup. After 2—3 days these changes become

more conspicuous (fig. 3). This is why it is best to wait until the third day after adding a brood comb to check for queenlessness. The number of queen cells constructed by a given number of bees is a good indication of the quantity of queen substance present (BUTLER 1960).

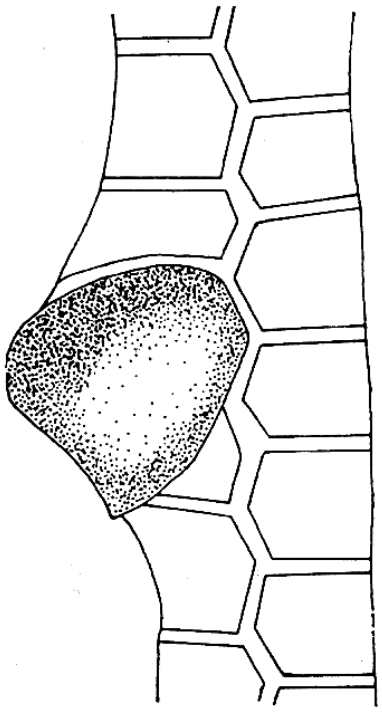

Fig. 3 — *Reconstruction of a worker cell to a queen cup.*

2.3. *Development of the ovaries in worker bees*

Three to four days after removal of the queen clear indications can be seen in the ovarioles of a number of workers of the development of egg cells (PAIN 1954 ; VELTHUIS 1970 a). After 10 days single completely developed eggs are present. After 30 days in European races, the first eggs are laid (in other races considerably earlier). With the decrease in open brood there is an increase in the storage of reserve substances in the food glands and in the fat protein body, similar to that of wintering bees (see Chapter IV). The presence of open brood on the other hand suppresses the development of ovaries even in queenless colonies (HESS 1944 ; MÜSSBICHLER 1952 ; MAURIZIO 1954). This suppressing effect of open brood is sometimes a stronger effect than that of the queen (JAY 1970 ; KROPAČOVA and HASLBACHOVA 1971).

Together with the development of ovaries the disintegration of the coloney proceeds. A colony with laying workers is "demoralized". There is hardly any storage of food (though sometimes the bees show a super nervous behaviour) or defense of the colony, and the acceptance of a new queen is achieved by certain tricks only. Such colonies are not

suited to rear young queens. With races where laying workers appear very soon after dequeening (eg. in the Tell bee) only a few days are available for queen raising.

Thus the consequences of the loss of the queen are manifold beginning with the buzz of queenlessness through to the fundamental disintegration of the structure and organization of the whole colony numerous changes occur which affect each individual bee. The absence of the queen is quickly perceived for within the hour the message has reached all bees of the colony. How is the presence or absence of the queen communicated within the dark hive ?

The answer to this crucial question was given almost simultaneously by two separate working groups ; C. G. BUTLER (summary 1959) in Britain and J. PAIN (summary 1961) in France. It was shown that the information is passed from the queen to the worker bees of the court and from these, to the workers of the colony. For this, immediate body contact of the queen → worker and worker → worker is necessary. Odours alone (for instance through a double wire mesh, where odours may pass, but not direct contacts occur), are not sufficient. The substances secreted by the queen are present also in a dead queen and in extracts of queens and exert the same effects on the worker bees as does a living queen : attraction, prevention of the construction of queen cells, suppression of the development of ovaries and egg laying in worker bees. This is why the secretions are collectively called "queen substance". The largest amount of this substance is found in the head of the queen

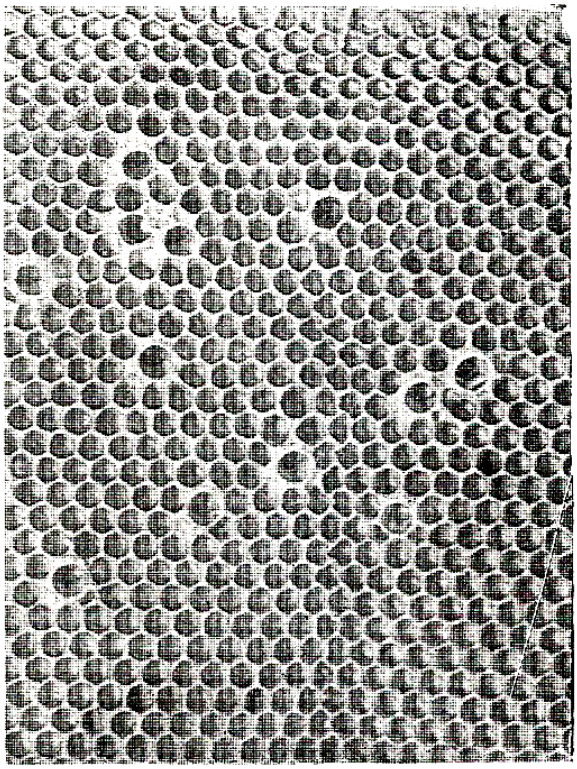

Fig. 4 — *Started queen cells on a half built comb, in a queenless colony (photo GONTARSKI)*

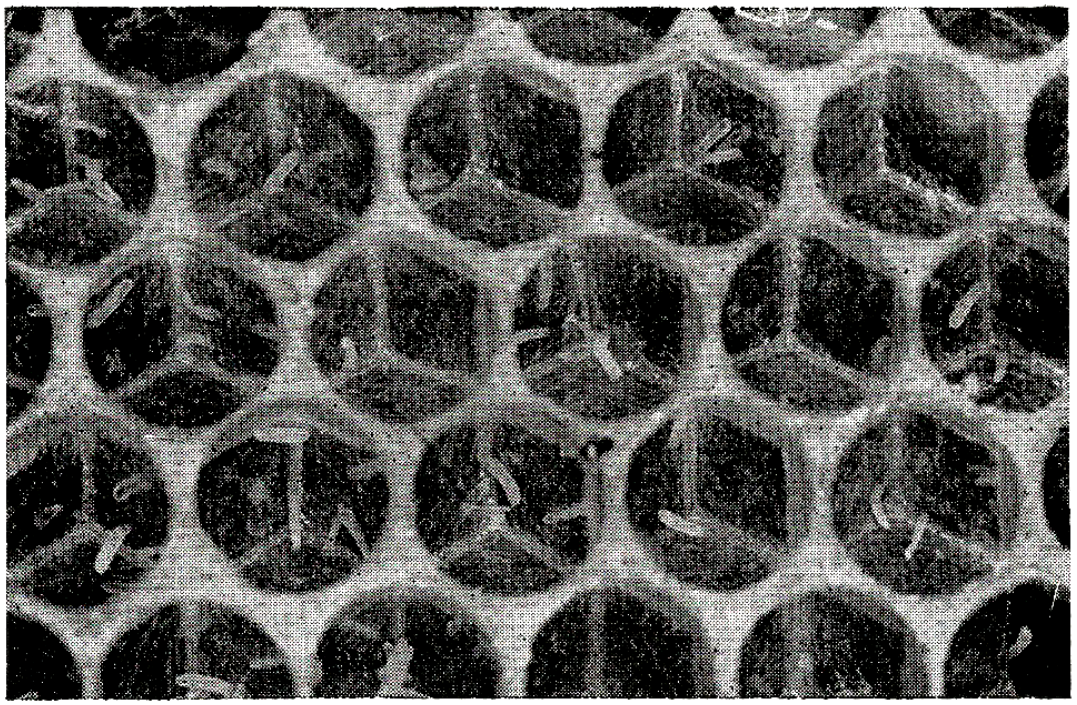

Fig. 5 — *Eggs laid by worker bees : Several eggs within one cell, some dried, some attached to the cell wall.*

particularly in the mandibular gland which in the queen is developed in a special way (see Fig. 13).

By chemical analysis it was shown that the chief factor in this substance and also having the greatest biological efficacy, is an unsaturated lipid, trans-9-oxy-2-decenoic-acid or (9-O-D) (CH_3-CO-$(CH_2)_5$-CH--CH-COOH" (BARBIER and LEDERER, 1960; CALLOW and JOHNSTON, 1960). The compound is very stable chemically and not very volatile. This is why a dead queen may influence a colony for a long time (MILOJEVIC and FILIPOVIC-MOSKOVLJEVIC, 1963). The substance produced synthetically suppresses ovary development of worker bees and the construction of emergency cells. A further effect was found by GARY (1962). In the open 9-O-D acts as a sex attractant for drones. A lure given 9-0-D and exposed at a height of 10 m, attracts drones nearly as much as a queen does.

During experiments it appeared that though there is an obvious effect of 9-O-D on worker bees it is far less than that of queen extracts or of living queens. The conclusion was that 9-O-D is only a fraction of the complex "queen substance". Later, CALLOW, CHAPMAN and PATON, were able to isolate quite a number more related substances in the mandibular glands of queen bees. Until now their significance has been only partly understood. Of major importance certainly is 9-hydroxy-decenoic acid, a volatile substance, which is evidently attractive to workers ("queen odour") and stabilizes swarms (BUTLER, CALLOW and CHAPMAN, 1964 ; BUTLER and CALLOW, 1968).

As queens were also attractive to workers when the mandibular glands were removed immediately after hatching, the search for activating glandular secretions was extended to the rest of the queen. VELTHUIS (1970) was able to demonstrate an effect similar to that of queen substance by the tergites of the abdomen. According to VIERLING and RENNER (1977) this effect is due to the secretion of glands in the tergite pockets. This secretion is also attractive to drones during the mating flight (RENNER and VIERLING, 1977).

The phenomenon "queen bee" cannot be reduced to a single common denominator. Also there are indications that the nervous system of the workers plays an important role in the interaction of queen and worker (VERHEIJEN-VOOGH, 1959). Further more, considering that this system is influenced also by the presence of larvae and by nutrition, some idea of its complexity results.

With the earlier observations on the division of labour related to age and the function of glands and the presence of brood, the discovery of substances permanently circulating in the colony explains its social strucure to a great extent. In the "normal" colony a state of equilibrium in tension exists between the influence of the queen and the worker bees, which are fixed to the social functions by the queen substance. A colony appears in this "harmonic" balance only during this state of equilibrium — a single organism with full integration of individual functions. Within this supra-individual totality is the queen, a source of queen substance, the central point of coherence. At the same time she is the producer of eggs, when stimulated by appropriate feeding and

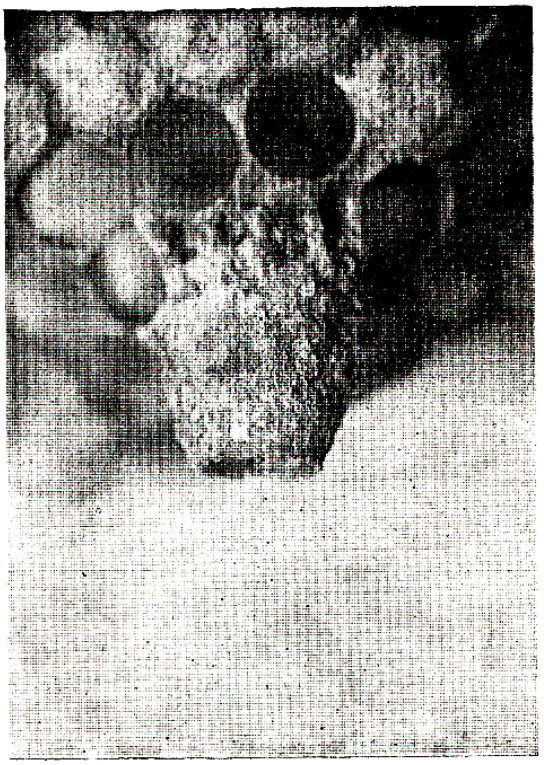

Fig. 6 — *The swarming queen cells are usually built at the lower or lateral margins of the comb.*

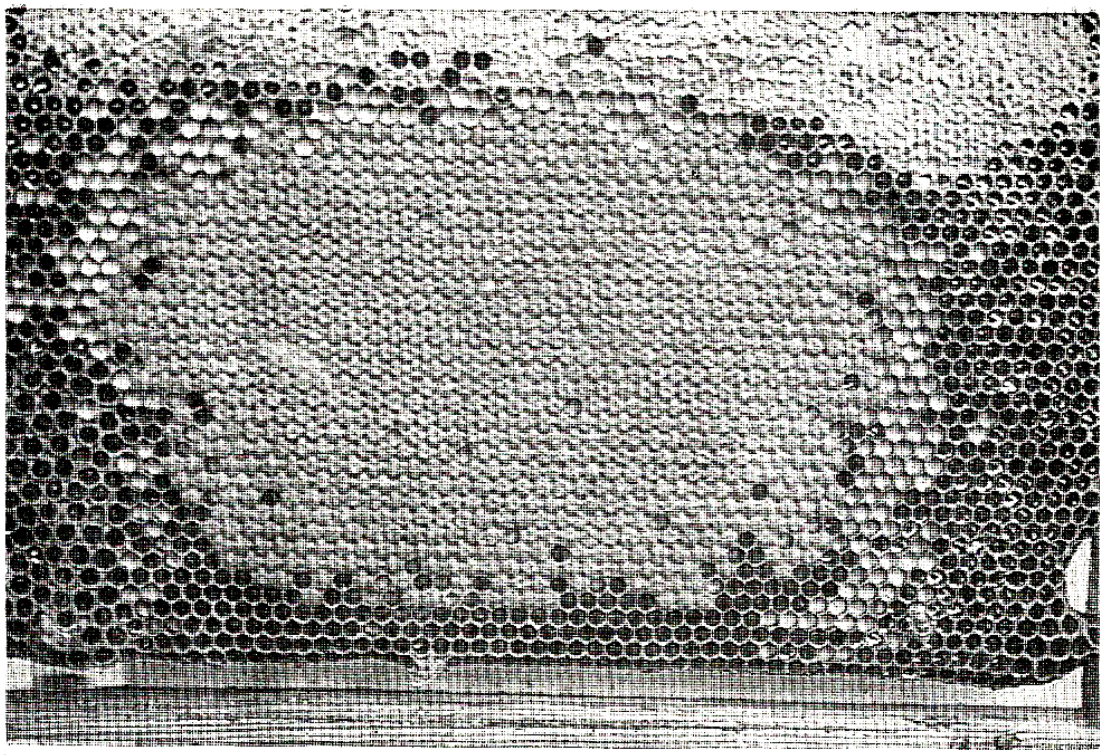

Fig. 7 — *The young, not inbred queens have compact brood.*

provided with the number of empty brood cells suitable to the situations, the offspring being reared by the workers. The workers functionally sterilized by the presence of the queen will efficiently accomplish all duties relating to their physiological condition and needs of the colony.

This "harmonic" condition of the colony may be disturbed by different causes ; by disease, by overcrowding of the hive, by a deficiency of the queen, by temperature and other external conditions. In connection with queen rearing only those disturbances of the equilibrium are of interest, which initiate the production of queens. Perfect young queens will result only during good overall conditions within the colony (strenght, food, provisions) when at the same time the dominant position of the queen is diminished or when she is missing.

In beekeeping books the reasons for the raising of natural queens are differentiated — swarm, supersedure and emergency queens. Frequently the opinion expressed is that these are fundamentally diferent events leading to different results in regard to the quality of the queens reared. In the following description we will make the same distinction, keeping in mind however it is one single process in respect of the physiological condition of the bees and their behaviour directed by instinct, leading to essentially identical results.

The common denominator in this process leading to the raising of young queens is a shift in the equilibrium within the colony in favour of the worker bees. This shift will start very quickly as soon as the

signal "queen" diminishes or vanishes. The changes which now occur with the worker bees were described earlier. All events and causes which are to be observed during the natural remplacement of the queen, are of importance for artificially started queen rearing also.

3. The rearing of queens during the life cycle of the bee colony

3.1. *Queen rearing for the propagation of colonies : swarm queens*

No other event in the bee colony has been as frequently described and analysed as the swarming of bees and the occurrences preceding it. In spite of this, there is not complete agreement as to the releasing factors involved.

Descriptions summarizing the swarming phenomenon are given by SIMPSON (1958, 1972).

3.1.1. Condition of the colony

The inclination to swarm is greatest at the time when the colony approaches the peak of its development. As a consequence, season is of special importance in the production of swarm queens ("season of swarming"). Frequently at this time a situation arises where the available space becomes too restricted for the multitude of bees produced. According to SIMPSON, 1972, this is one of the most important reasons for swarming. Apart from the general restriction of space an overcrowding of the brood nest with young bees develops without sufficient larvae for nursing. Many young bees are forced out of the brood nest and thus become swarm bees (TARANOV, 1974 ; HAYDAK, 1952). In this context it is important to note that the ovaries are developed in 40—60% of the worker bees in colonies preparated to swarm (TUENIN, 1926 ; MARTIN, 1963).

Other swarm inducing factors are, the ample supply of pollen, long lasting, but frequently interrupted nectar flows and warm weather (or a warm stand).

The natural swarming impulse can be released by the use of appropriate measures, to the extent that the factors (size of space, number of young bees, supply of pollen) can be manipulated experimentally. These measures are used to promote the "disposition to rear queens" in different methods of queen rearing (see Chapter VII).

In general the condition of the colony is evaluated by external signs only, that is quantity of bees and brood, provisions, supply of food for larvae, nectar flow in the period preceding etc. The physiological condition of the bee, as expressed in the stage of development of the internal organs (glands, fat-protein body, ovaries), is not to be seen from the exterior. Differences of this kind may be sometimes responsible for the fact that certain colonies give bad results in rearing queens. It has long been experienced that colonies with advanced swarming tendencies are not very efficient for raising queens. The reason may be that the ova-

ries are strongly developed in the bees of these colonies. Earlier at the beginning of the preparation for swarming, the ovaries are still mostly undeveloped, but the food glands are at their peak. This condition produces a very good "disposition for queen rearing".

3.1.2. **Condition of the queen**

The age of the queen is of great importance for the rise of the swarming impulse. Colonies with queens older than one year swarm several times more frequently than do colonies with young queens (SIMPSON, 1960). The same applies to queens with a body defect (SIMPSON, 1960 b). In general it can be stated that any decrease in the quality of the queen has a corresponding increase in the swarming tendency. BUTLER (1960) explains this phenomenon by a diminished secretion of queen substance.

3.1.3. **Genetic causes**

Certain races show a high innate swarming tendency even with small colony streagth (F. RUTTNER, 1975). This applies especially to certain African races (e.g. the Tellian bee) where the colonies may literally swarm until destruction (BROTHER ADAM, 1970). Other races

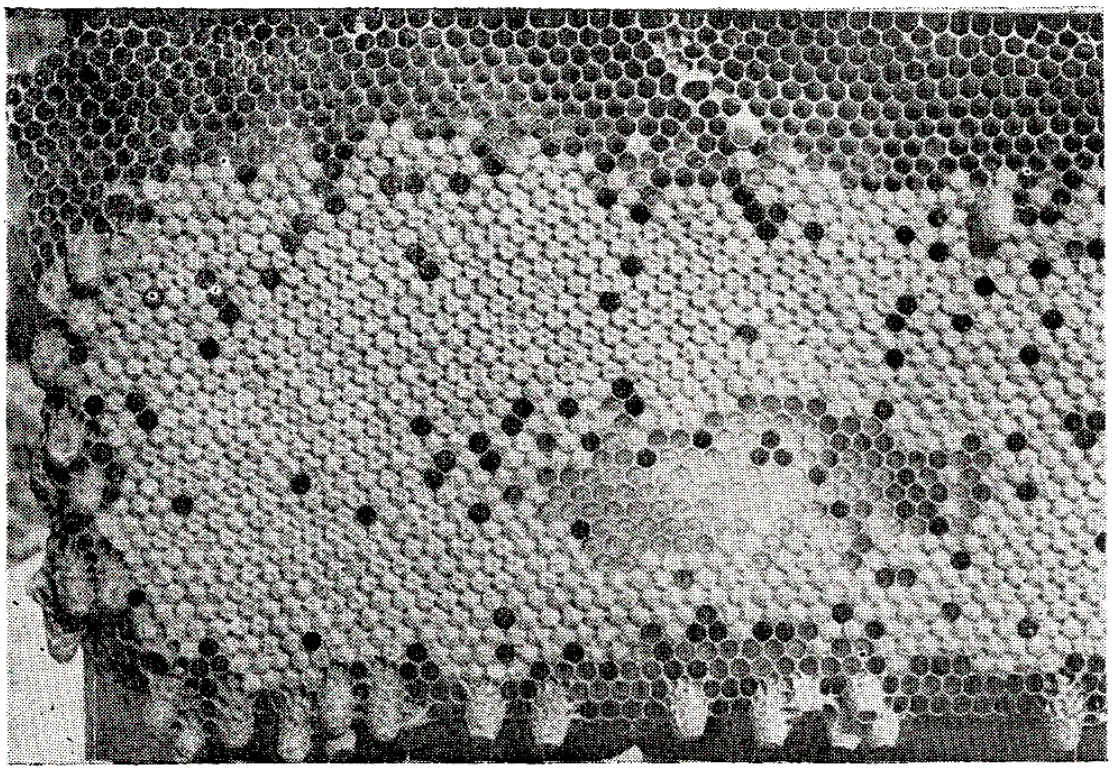

Fig. 8 — *Brood comb of a Tellian colony, ready to swarm. On this sector of a brood* comb 29 swarm cells are visible on one single side.

on the other hand show a very low swarming tendency, as *A.m. ligustica* or *A.m. capensis*. There exist however considerable hereditary differences within one and the same race. This is why by selection a rather quick shift in one or other direction can be achieved (F. RUTTNER, 1973). In the Heather bee of northern Germany and in the Carniolans in the South of Austria and in Yugoslavia "swarming beekeeping" for centuries resulted in strains with an extremely high swarming tendency ; on the other hand by constant selection the swarming tendency was considerably diminished, e.g. in Carniolans.

The number of queens produced during the process of swarming varies between 10—200, according to race. Races, or hybrids with a high number of cells are especially suited for nurse colonies (Tell bee, F. RUTTNER, 1975).

3.2. *Queen rearing to replace an inferior queen without propagation of the colony : Supersedure*

This event is observed when the conditions as enumerated in 3.1.4. exist, but without the conditions for swarming — that is during replacement of a queen at a time other than the swarming season, with weak colonies, during adverse external conditions or with a low genetic swarming tendency. According to WEISS (1965) supersedure is a kind of reduced swarming process.

Another observation is the supersedure of freshly introduced queens of foreign origin (e.g. another race or another strain). This happens though the queen was initially tolerated for a couple of weeks. In this case the reaction of bees to "foreign" is evidently the same as to "inferior".

The number of queen cells produced during supersedure is low (3—5). The changes in behaviour as observed during the preparations for swarming do not take place.

It is an important goal in apicultural selection to reinforce the genetically determined tendency for replacing the queen by supersedure only, without the preceding swarming.

3.3. *Rearing to replace a lost queen : Emergency queen rearing*

After the sudden loss of a queen the tendency arises among worker bees, apart from other changes in behaviour, to rear queens from larvae initially destined to become worker bees. To achieve this the narrow hexagonal worker cells are reconstructed to form large bell-shaped queen cups and the larvae are fed with royal jelly. There have been discussions as to which is the first action in this process, the reconstruction of the cell or the provision of royal jelly. According to GONTARSKI (1956) the primary action is the rebuilding of the cell; only the shape of the queen cup furnishes an adequate stimulus (releaser) to deposit royal jelly. On the other hand in a queenless colony of a few

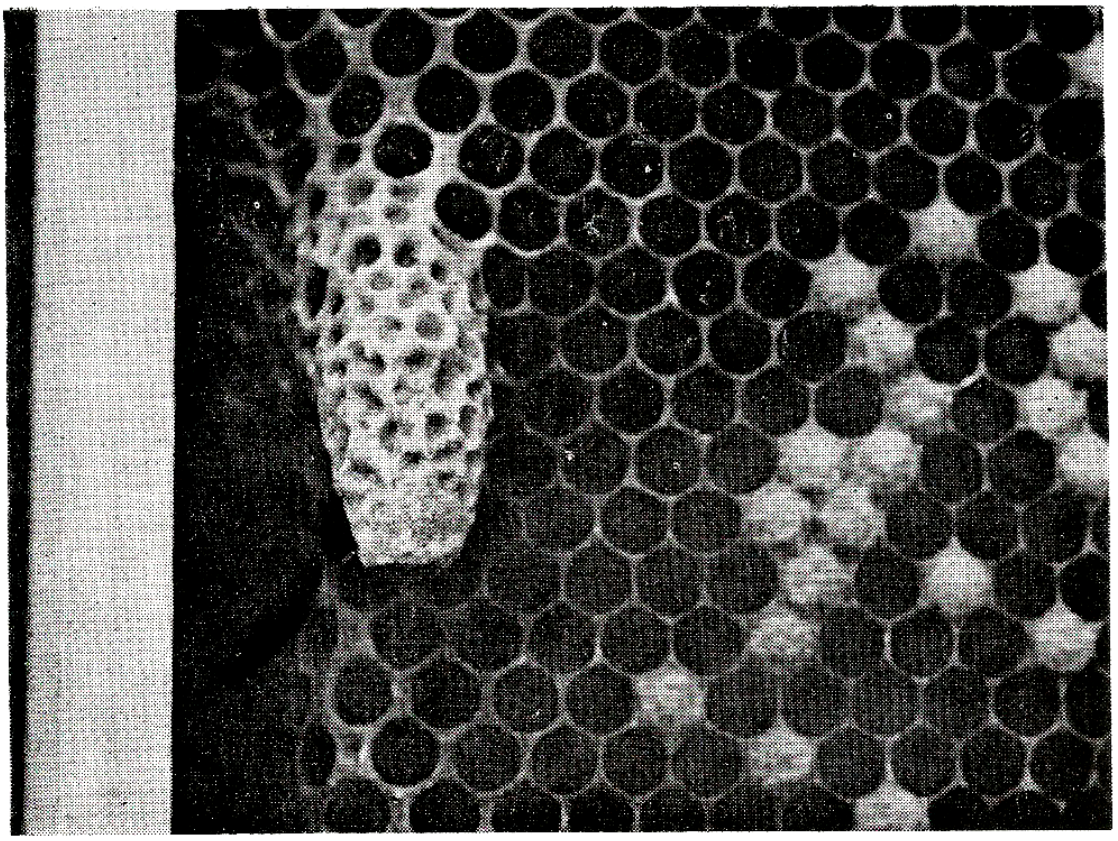

Fig. 9 — *Solitary queen cell for supersedure*

hours great differences are found in the provision of larvae with royal jelly without any visible changes in the shape of the cell. Thus it could be just the reverse — the cells that by chance have a better supply of royal jelly are selected to be constructed into a queen cell.

Unlike swarm and supersedure cells, mostly placed laterally near the sides of the frame or on the lower edge of the combs, emergency cells are found distributed everywhere on the surface of the comb. Their number may be two or three dozes even with European races, which construct only a few swarm cells.

Emergency is to a large extent independent of the season, as is supersedure. The number however (and also the quality) of the queens produced definitely depends on the overall condition of the colony (strength, state of nutrition) and on environmental factors. During queenslessness queens are produced as long as young larvae are present.

Also a colony with emergency queens may swarm provided it is of sufficient strength and the external conditions are favourable. Thus it appears that no fundamental difference exists between the different types of queen renewal.

At one point however the uncontrolled emergency differs from other types of queen renewal : The point in time when the larvae is taken into royal care. This varies considerably.

Worker cells with eggs only hardly ever show any change in queenless colonies (GONTARSKI, 1956). There is no rebuilding of the cell and normally no provision of food near the eggs. Larvae however quickly release the "stimulus of emergency". However between larvae of different ages no discrimination is made by queenless bees. Very young larvae receive the same royal care as do very old ones which are still just at the verge of possible queen determination. As a result in a colony left to itself to select the larvae the queen cells will be of very different ages. Thus in this kind of queen rearing the queens hatching first will be the smallest and worst developed as they originate from the oldest larvae.

If a "wild" emergency rearing occurs from a brood comb into a nursery colony which did receive a series of very young larvae, the "wild" queens will be ripe and hatch at least one day earlier than the queens from the grafted larvae. This has to be considered if no check of the nursey colony is done.

Frequently, it has been claimed that emergency queens are not of top quality since they originated from larvae which were not intended to become queens. From experiments quoted in Chapter V and from practical experience it becomes clear that this supposition is not valid — provided the emergency queens come from the youngest larvae.

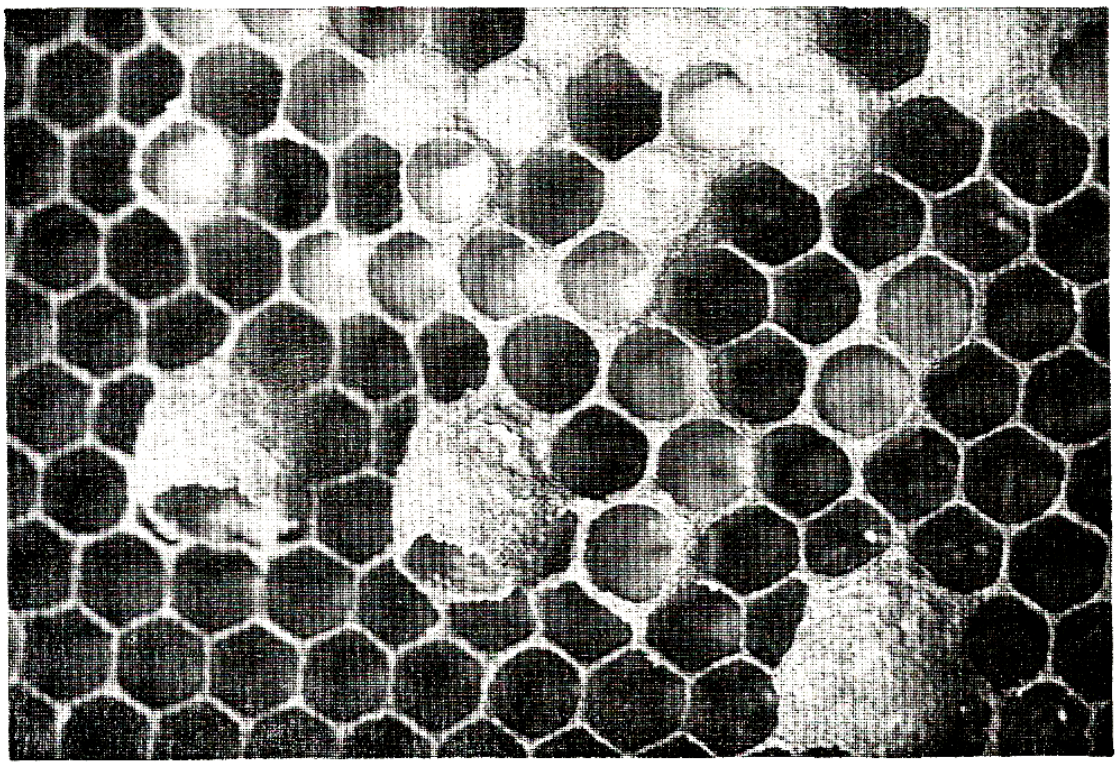

Fig. 10 — *Emergency queen cells on a brood comb*

Final remarks

All these types of queen renewal were used for the controlled production of queens. The intentional stimulation of the swarming processes were recommended in former times but this method was never important because it is too dificult to control events precisely and because the cells produced are very different in age.

The supersedure situation is used with all procedures where a method of queen rearing in a queenright colony is applied. The principle in this method consists of organizing the colony in such a way that the nurse bees rear the queen cells for though they do not feel completely queenless they stay in a state of strongly reduced contact with and diminished influence of the queen.

As in a colony with an old or mutilated queen, the signal "inferior" arises and with this a tendency to raise young queens. As in natural supersedure the number of queens produced is mostly small but the quality in general very high.

Finally, the events of an emergency are initiated whenever the start of queen rearing is made in a queenless colony or with queenless bees in a swarm box. It is not difficult to produce a high number of queens using an appropriate amount of bees. Once the larvae receive royal care the process of queen rearing is completed even in a queen right colony provided the queen has no access to the cells, the colony is of sufficient strength and the environmental conditions are not too adverse.

CHAPTER II

Royal jelly

H. REMBOLD

The honey bee larva is fed, after hatching from the egg, with larval food prepared by the young bees called nurse bees. This food contains all energy giving and biulding substances necessary for the development of the three bee castes — drones, queens, and worker bees. The queen larvae are fed on larval food throughout their larval stage, while worker and drone larvae only for the first few days, after which they are fed a variable mixture of larval food, pollen, and honey. The chemical composition of various larval foods has been described by several authors (ARMBRUSTER, 1960 ; HAYDAK, 1970 ; JOHANSSON 1955, 1958 ; REMBOLD, 1961, 1964, 1965, 1974 ; TOWNSEND, 1962 ; WEAVER, 1966).

The advantage of intensive brood rearing is obvious when we consider the rigorous labour division in the community of bees. At the height of the season, the queen lays over two thousand eggs daily. This means that food of high physiological quality must be provided for up to ten thousand unsealed cells and for as many worker larvae of various ages. This must be done by the young bees in which the pharyngeal gland is very well developed ; the gland atrophies after brood rearing is over. The larval food is produced by a combined function of the pharyngeal gland and honey sac, which will be discussed later. The pharyngeal gland supplies the two major constituents, lipids and protein, and the honey sac, the carbohydrates. In addition to providing nutrition to the brood, the larval food also has a key function in honey bees, i.e. the determination of the female castes. Contrary to drones, whose sex is already genetically fixed in the egg, the larva hatched frorm a fertilized egg, can either result in a worker bee or in a sexed individual, altogether different from the former in terms of functions and physiological characteristics, namely a queen bee. The determination of the female caste is the result of a complex synergic activity of the various signals, including chemical substances (REMBOLD, 1974) and starting with the very structure of the queen cell. While in the cell of a worker larva the volume of the larval food provided for is that of a pin head, the cell having a 3-day old queen larva contains 300 mg and even more larval food.

1. The food for the queen larva

By direct observations of the feeding process JUNG-HOFFMANN (1966) found that the nurse bees produce two types of secretions — one clear as water, and the other with a milky appearance. The average age of the nurse bees which produce the clear food, 17 ± 2 days, is significantly higher than that of the nurse bees which produce the white milky food of 12 ± 2 days. Both components are given to the queen larvae in equal proportions. The ratio depends to a great extent on the age of the nurse bees ; the older individuals produce smaller amounts of the milky component. As the larvae become older they are fed more often : the one-day old larva 13 times ; the three-day old larva 16 times ; and the four-day larva 25 times. The duration of feeding also increases ; it is estimated that a queen larva is fed, during the nursing period, about 1,600 times, which means the presence of the nurse bees for about 17 hours. In each and every queen cell about 1.5 g of larval food is introduced, most of which is consumed by the larva. In comparison with it, the care taken of the worker brood is very modest. It is estimated that a worker larva is fed by the nurse bees 143 times on an average during its development (LINDAUER, 1952).

During the first 3—4 days of larval stage, the sensitive period for caste determination, the weight of the queen larva increases slower than of the worker larva of the same age. Later on, the weight increase is steep, reaching finally 300 — 325 mg for the queen larva, while the weight of the same age worker larva is about 175 mg (WANG, 1965). However, within the same ranges of age, considerable differences in weight would occur. This is true both for the brood of one colony alone, and above all when the brood of several colonies is compared (REMBOLD *et al.*, 1980).

2. Composition of royal jelly

Royal jelly is usually obtained by the method used in breeding queens. In a dequeened colony, prepared for the purpose (SMITH, 1959 ; ZANDER, 1971 ; CALE, 1971), 40—60 woorker larvae of 1—1.5 day of age are introduced. The larvae are immediately taken care of by the worker bees, and 3 days later they are taken out together with the royal jelly to the queen cells. The queen cells of older larvae contain relatively less royal jelly ; about 250 mg royal jelly/cell, and 15 g/colony/batch. With a certain skill, 1 kg of royal jelly may be obtained from 8—10 bee colonies.

The "royal jelly" is a yellowish, milky and quite viscous liquid, the dry matter in it accounts for about one-third. Solid components may also include larval skins, various amounts of beeswax, and pollen grains. Of the total dry weight, sugar and albumin roughly account for 90%, and fat substances for 10% (Table 1). It is remarkable that 90% of the lipid fraction are free fatty acids.

Table 1

COMPOSITION OF THE LARVAL FOOD IN QUEEN CELLS (REMBOLD, 1960)

A. Water	60%
B. Dry matter	40%
1. Lipid fraction	10%
a. Strong acids	90%
b. Weak acids	2%
c. Neutral lipids	8%
2. Dialyzable components (invert sugar, amino acids, vitamins, etc.)	52%
3. Albumin fraction	38%
a. Water soluble	55%
b. Insoluble in water	45%

Just as with every biological substance, variations were also recorded in the composition of the larval food in queen cells. But they are within a narrow range and as such the rough estimated composition given in table 1 may be used for checking the purity of commercial products. In an analysis of seven commercial royal jelly samples collected in different countries and different years, REMBOLD and LACKNER (1978) demonstrated their very constant cation contents (Table 2) which reflects the high synthetic control of food composition by the nurse bees. However, the same authors found remarkable differences in the nucleotide pattern of commercial samples if compared with larval food collected from their own colonies. Differences in ADP, AMP, and adenosine might reflect stress situations of the nurse bees. Slight diffe-

Table 2

CATION CONTENTS OF DIFFERENT COMMERCIAL ROYAL JELLY SAMPLES, GIVEN IN μg/g FRESH WEIGHT (AFTER REMBOLD AND LACKNER, 1978).

Sample	K	Na	Mg	Ca	Zn	Fe	Cu	Mn
Bulgaria								
11/77	4440	221	304	131	26.5	10.58	5.40	0.60
Yugoslavia								
7/75 a	4620	190	283	122	24.5	9.55	5.55	
7/75 b	4160	209	271	122	26.5	10.28	5.20	0.48
Taiwan								
3/76	4460	278	287	124	25.0	9.62	5.23	0.75
5/76 a	4100	208	273	116	26.5	8.95	4.28	0.70
5/76 b	4180	239	284	135	27.5	9.75	4.80	0.80
5/76 c	4410	217	321	160	30.5	10.85	5.33	0.83
Average	4339	223	289	130	26.7	9.94	5.11	0.69

rences between the food supplied to larvae of various ages were found to exist when comparing the food for queen larvae to that for worker larvae. The food for worker larvae (age, 0 to 30 h) contained more protein, while the food for queen larvae of the same age contained more sugar. The worker bee larvae (age, 72 to 96 h) are obviously fed on more honey than the queen larvae which are fed on a well defined food of constant composition. However, the young larvae of both castes are fed, as shall be described later, a larval food of a very similar composition and with the same nutritive qualities.

3. The lipid fraction of royal jelly

Of the total dry matter of the royal jelly about 10% is soluble in organic solvents such as ether or benzene. The free acids can be extracted from the organic phase with a diluted alkali; they account for about 90% of the lipid fraction. Their biological function is not yet known, the findings according to which they are likely to have bactericidal action, and cancer inhibiting action require further investigations (REMBOLD, 1965). The results of the thorough investigation of fatty acids present in the queen food are given in table 3. The major component is 10-hydroxy-1-decenoic acid (I) identified by BUTENANDT and REMBOLD (1973). The other fatty acids in royal jelly are likely to be intermediary steps in the synthesis or decomposition of 10-hydroxy-1-decenoic acid.

Table 3

ALIPHATIC FATTY ACIDS SEPARATED FROM FATTY ACIDS FRACTION OF ROYAL JELLY (REMBOLD, 1965, 1974)

Hydroxycarbonic acids

$HOH_2C—CH_2—CH_2—CH_2—CH_2—CH_2—CH_2—CH=CH—COOH$ (I)

$HOH_2C—CH_2—CH_2—CH_2—CH_2—CH_2—CH_2—CHOH—CH_2—COOH$ (II)

$H_3C—CH_2—CH_2—CH_2—CH_2—CH_2—CH_2—CHOH—CH_2—COOH$ (III)

$H_3C—CHOH—CH_2—CH_2—CH_2—CH_2—CH_2—CH_2—CH_2—COOH$ (IV)

$HOH_2C—CH_2—CH_2—CH_2—CH_2—CH_2—CH_2—CH_2—CH_2—COOH$ (V)

$HOH_2C—CH_2—CH_2—CH_2—CH_2—CH_2—CH_2—CH_2—COOH$ (VI)

Monocarbonic acids

$H_3C—CH_2—CH_2—CH_2—CH_2—CH_2—CH_2—CH_2—COOH$ (VII)

Dicarbonic acids

$HOOC—CH_2—CH_2—CH_2—CH_2—CH_2—CH_2—CH=CH—COOH$ (VIII)

$HOOC—CH_2—CH_2—CH_2—CH_2—CH_2—CH_2—CH_2—CH_2—COOH$ (IX)

$HOOC—CH_2—CH_2—CH_2—CH_2—CH_2—CH_2—CH_2—COOH$ (X)

$HOOC—CH_2—CH_2—CH_2—CH_2—CH_2—CH_2—COOH$ (XI)

$HOOC—CH_2—CH_2—CH_2—CH_2—CH_2—COOH$ (XII)

The wide and most characteristic spectrum of the free fatty acids existing in royal jelly are a valuable analytical means in checking the quality of a commercial product. First, paper or thin-layer chromatography is done for qualitative identification of the fatty acids; for quantitative determination, gas chromatographic analysis is done, eventually with a coupled mass spectrometer. For more details the reviews mentioned in the introductory section of the chapter are recommended; other original papers can also be consulted.

4. Low molecular, water soluble constituents

The major fat free component with low molecular weight is the invert sugar which results from the honey obtained from nectar containing sucrose. Of the total dry weight of the queen larval food is 9.76% glucose, 11.32% fructose, and 0.95% sucrose. It also includes a number of amino acids and vitamins of the B group (Table 4). Significant differences in the amount of pantothenic acid present in royal jelly and worker larval food (110—320 μg/g vs. 24—26 μg/g of food) suggests its role in caste determination. The concentration of two other components is also much higher in larval food from queen than from worker cells; biopterin and neopterin, both being heterocyclic compounds.

Table 4

VITAMIN CONTENT IN LARVAL FOOD (REMBOLD, 1965, 1974)

	Royal jelly μg/g food	Larval food μg/g food
Thiamine	1.2— 18	1.2
Riboflavin	6— 28	10.8
Pyridoxine	2.2— 50	7.3
Nicotinic acid	48—125	52
Pantothenic acid	110—320	24—26
Biotin	1.6— 4.1	2.5—3.3
Folic acid	0.16— 0.5	0.11—0.52
Inositol	78—150	not determined

Just as pantothenic acid, both biopterin and neopterin are present in ten times higher concentrations in royal jelly as compared to worker larval food (biopterin, 25 μg vs. 4 μg/g of food; neopterin, 3 μg vs. 0.3 μg/g of food). The amount of biopterin can be determined most accurately by a bio-assay with *Crithidia fasciculata,* a flagellate. At a concentration as low as 2.5×10^{-5} g/ml, the growth response is half of the maximum thus enabling biopterin determination, even in the traces as present in certain glands.

The only differences found, by chemical analysis, to exist between the food for young queen larvae and that for worker larvae are the amounts of biopterin, neopterin, and pantothenic acid. Biopterin

and the pantothenic acid are most accurately determined by bio-assays, while both pterins can be detected by thin-layer chromatography. All three substances are recommended as indicators for royal jelly when checking the quality of products, certainly also taking into account the other analytic criteria already mentioned. Other markers for the quality of commercial royal jelly samples are percent dry matter, total protein, sugar, inorganic phosphate (Table 5) which are fairly constant (REMBOLD and LACKNER, 1978).

Table 5

COMPOSITION QUANTIFIED IN DIFFERENT COMMERCIAL ROYAL JELLY SAMPLES, GIVEN PER GRAM OF FRESH WEIGHT (AFTER REMBOLD AND LACKNER, 1978)

Sample	Dry matter	Protein	Sugar (Anthron)	Phosphate	Total Phosphate	Nucleotide
	%	mg	mg	mg	mg	E_{260}
Yugoslavia						
7/75 a	35.0	142.5	129	0.88		233
7/75 b	33.5	141.3	116	0.85		223
6/76 a	32.8	135.6	102	0.86		
6/76 b	33.8	132.5	120	0.75		
Taiwan						
3/76	34.8	145.0	116	0.97		243
5/76 a	32.3	129.4				225
5/76 b	35.0	141.3	124	1.06		218
5/76 c	34.0	140.0	143	1.00		227
Average	33.9	138.5	121	0.91	2.13	228

5. Albumin constituents

More than one-third of the dry matter in royal jelly is high molecular weight albumin. By dialysis in water, it is retained by a semi-permeable membrane, thus being separated, simply and accurately, from the low molecular weight material. Analytically, the protein in royal jelly can be separated by electrophoresis in five different fractions, of which two are not found in the food for older worker larvae. But the albumin in the food for young worker larvae is identical to that in the food for queen larvae.

6. Constituents characteristic of royal jelly

The problem of the quality control of royal jelly, or of its preparations often arises. In summary, the criteria pointed out in describing its constituents are given again.

1. Royal jelly roughly consists of a mixture of gland secretion with high contents of albumin, fats, and honey. The ratio of the two component parts varies within quite narrow limits as illustrated in table 1. Consequently, any extra amount of honey or water added to it can be easily determined.

2. For the usual checking of the quality of royal jelly, the specific fatty acids given in table 3 are used. Above all the main constituent, the 10-hydroxy-1-decenoic acid can be determined even when in traces, by enriching methods commonly used for fatty acids.

3. Much more difficult is the quantitative determination, by bioassays, of the two markers, the pantothenic acid and biopterin, because both are highly labile substances. For both determinations, a reliable test is necessary. Therefore, paper or thin-layer chromatography is sometimes used for determining biopterin and neopterin as an analytic criterion for determination of royal jelly. Both pterins can be identified by ultraviolet absorption as intense fluorescent, clear blue patches.

4. A most accurate method for checking is the rearing of young larvae on royal jelly in an incubator, as described in a separate chapter.

7. Production of the larval food

The food given to the young larva decides whether from the fertilized egg a queen or a workerbee will result. The question how the two types of food, with so diverse functions, are produced by the nurse bees, will be discussed in conclusion of this brief review. For a more thorough study, other publications are recommended (REMBOLD, 1973, 1974).

In the head and thorax of queens and worker bees, a number of secretory glands exist, of which the pharyngeal glands of the nurse bees which open into the pharynx, are most active. They also contain substances characteristic of the various kinds of larval food (proteins, 10-hydroxy-1-decenoic acid, biopterin, and purines). The function of the other glands (mandibular, occipital, and thoracic) has not yet been ascertained. HANSER and REMBOLD (1964) have investigated glands separated from queen and worker nurse bees, in order to determine the content of the royal jelly constituents in them. Considering the fact that the specific "indicator" or "marker substances", biopterin and pantothenic acid, are present in the mandibular glands of the queen nurse bees in significantly greater quantities than in those of worker nurse bees, the authors concluded that the mandibular gland has an important function in the production of the food for queen cells. It is likely to be the source of the elements in this larval food characteristic for feeding the queen larvae. The food for the larvae in queen cells consists, according to them, of honey, secretions of the pharyngeal and mandibular glands, while the food for worker larvae of honey and secretion of the pharyngeal glands. These findings, following chemical analyses, have also been confirmed by the afore mentioned observations of JUNG-HOFFMANN (1966).

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CHAPTER III

Developing of female caste in the bee colony

K. WEISS

In the bee colony, the female sex is represented by two types of individuals (phenotypes): the mass of worker bees and the unique queen. The two female types are designated as "castes", and because their external (morphological) aspect is different, the term dimorphism of bee castes is used. The third type of individual in the colony — with a completely other structure —, which appears in the colony during the season only, as drones, must not be considered a caste because it is of male sex, and has therefore a different structure of the body.

1. Caste differences

The presence in the bee colony of morphologically different castes is related to the distribution of the vital functions among the female individuals. It is therefore but normal that the queen, whose major function is to lay eggs, has a body structure differing from that of the sterile worker bees which must take care of the brood and provide for food. As the bee does not emerge from the egg directly as an adult but undergoes a "complete" transformation (holometabolous development) — traversing the stages of pupa and lava, it is likely that the first caste differences appear already in this period.

1.1. *Major caste differences in adult individuals*

The beekeeper distinguishes between the two female individuals, first by their size. When emerging from the cell, the queen weighs about twice as much as the worker bee ; when she starts laying eggs she gains considerably more weight. For appreciating its size a simple measure is used, the width of the thorax — visible with the naked eye. In more thoroughly considering the anatomy of the queen and of the worker bee, more than 50 morphological differences are recorded (ZANDER and BECKER 1925 ; LUKOSCHUS, 1956 a). For biological appreciation, in addition to weight, the shape of the head, development of mandibles

Fig. 11 — *Dimorphism actually exists in the bee colony. The female bee always appears as one single queen and several sterile workers. The picture shows the queen and her "court".*

and of hind legs, and the number of barbs on the sting are considered. Of the internal organs, special heed is paid to the ovary and spermatheca, and to the mandibular and pharyngeal glands (ZANDER and BECKER, 1925; WEISS, 1978). Some of these indices are given in Figs. 2—7.

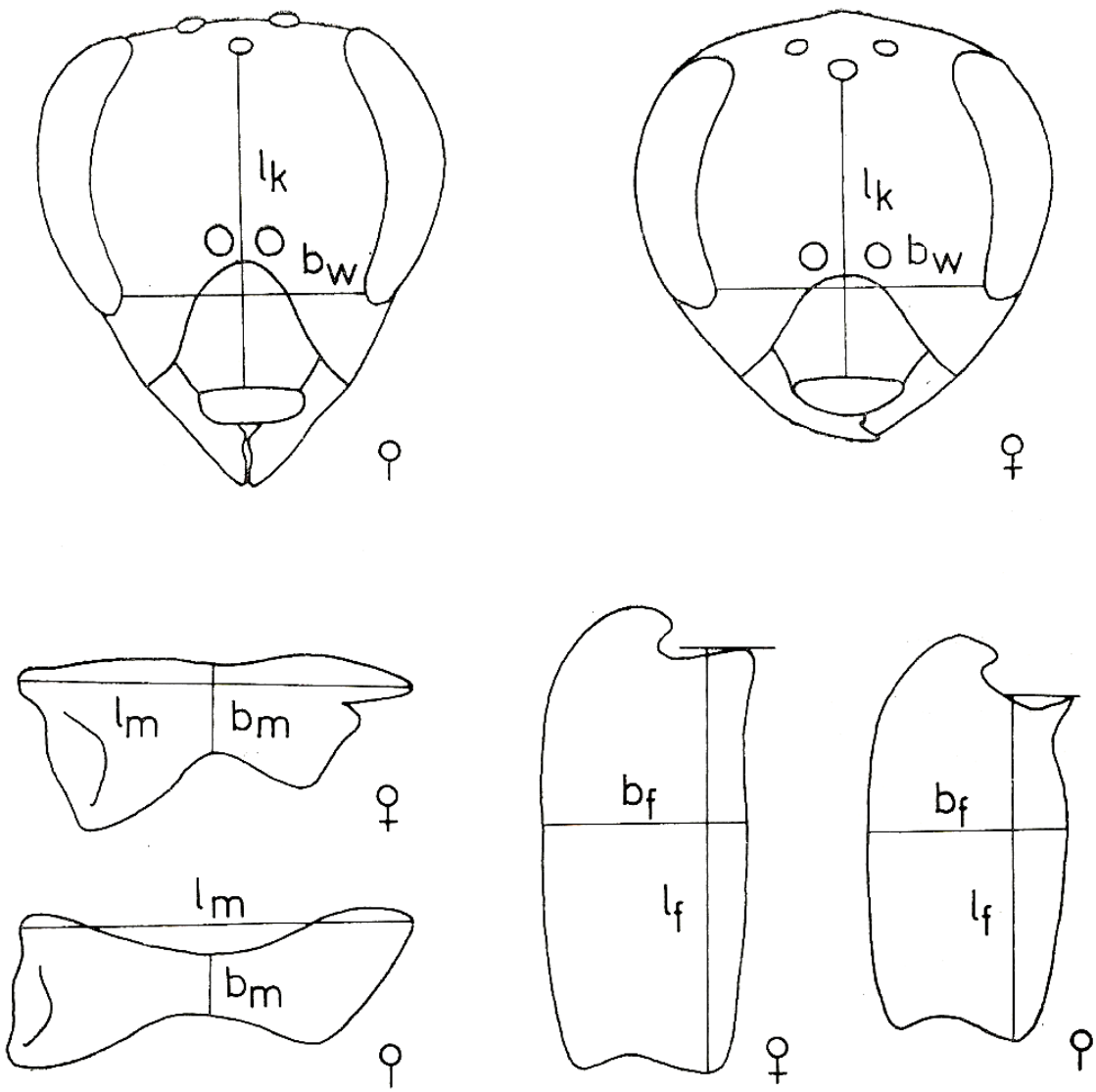


Fig. 12 — *For appreciating the differences in shape of certain external caste caracteristics, the following indices are mostly used in biological tests : the facial index* $\frac{l_k}{b_w}$ *for the head, the mandibular index* $\frac{I_m}{b_m}$, *and the tarsus index* $\frac{I_f}{b_f}$

From the summary of the most important caste characteristics (Table 4), it is obvious that all these differences result from the biological functions of the two castes being different. The organs for collecting food, building the nest, and for brood rearing are not developed in the queen, while the reproductive organs are fully developed ; in worker bees the reverse is true.

Table 4

CASTE DIFFENENCES IN THE HONEYBEE

Characteristic	Worker bee	Queen bee	Figure No.
Body length (mm)	12— 14	15— 20	11
Weight (mg)	70—120	220—320	11
Width of thorax (mm)	4.0—4.2	4.7—5.0	11
Tomentum	+	—	11
Head			
Shape	triangular	round	12
Gene index	1.27	1.07	12
Mandibular notches	—	+	12.21
Mandibular index	4.77	3.38	12
Pharyngeal gland	+	—	13
Mandibular gland	small	very large	13
Hind leg			
Pollen brush	+	—	14
Pollen basket	+	—	
Auricle (44)	+	—	
Tarsus index	2.10	1.75	12
Wax gland	+	—	
Nasonov's organ (scent gland)	+	—	15
Sting	straight	bent	17
Number of barbs (47)	10	3	17
Number of ovarioles/ovary	3—10	160—180	18
Spermatheca (diameter in mm)	0.1	1.2	18
Venom gland, furcula (362)	close to the end	within the basis one third	

An important difference between the two castes is their span of life. Worker bees would live for 4—6 weeks in summer, and for as many months in winter. The queen may live for 5 or more years.

1.2. *Caste differences during the larval stage*

All larvae being hatched from fertilized eggs have the same structure. Shortly after, physiological differences appear in the developing individuals : first in terms of breathing. SHUEL and DIXON (1959)

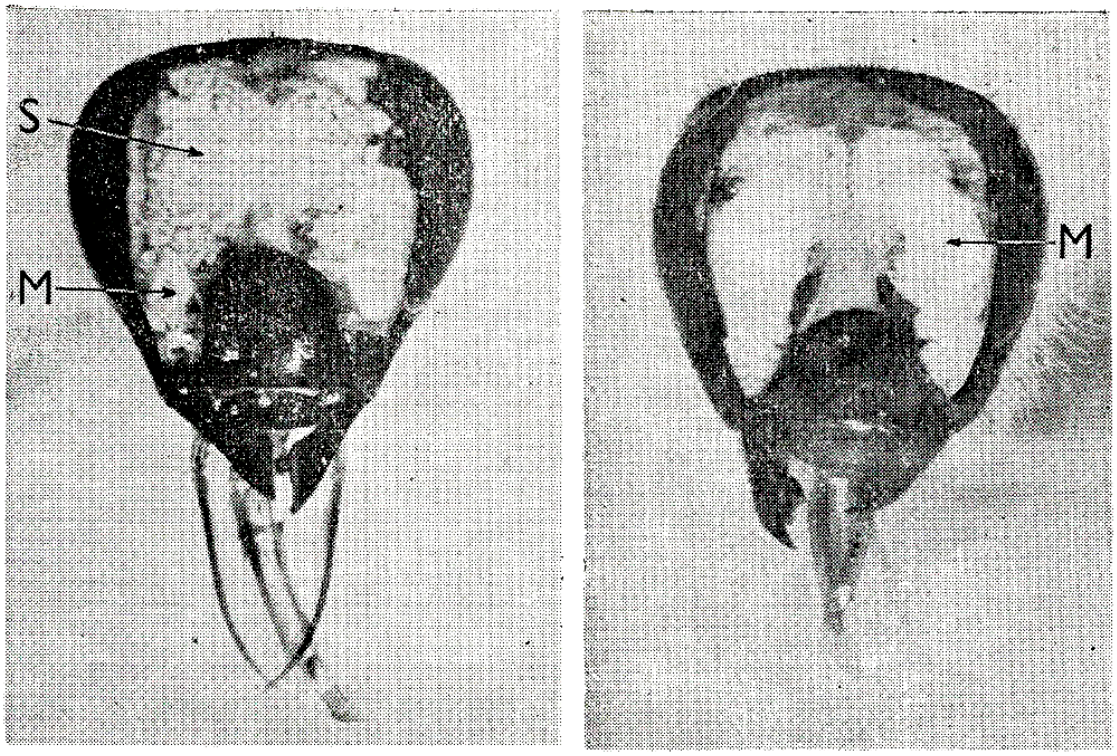


Fig. 13 — *Of the many differences between the two castes of female bees, some major ones are found in the head. The figure to the left illustrates the head of a worker bee, of triangular shape, smooth mandibles and long glossa. In the cut up section in the face, one can see the pharyngeal gland (S) with the many gland vesicles (on top), and below, to the right and left of the cephalic shield — the two small mandibular glands of the shape of a pouch (M). The figure to the right illustrates a queen head, of a rounder shape, with notched mandibles and short glossa (tongue). There are no pharyngeal glands, but the pouches of the glands of the upper maxilla are very developed.*

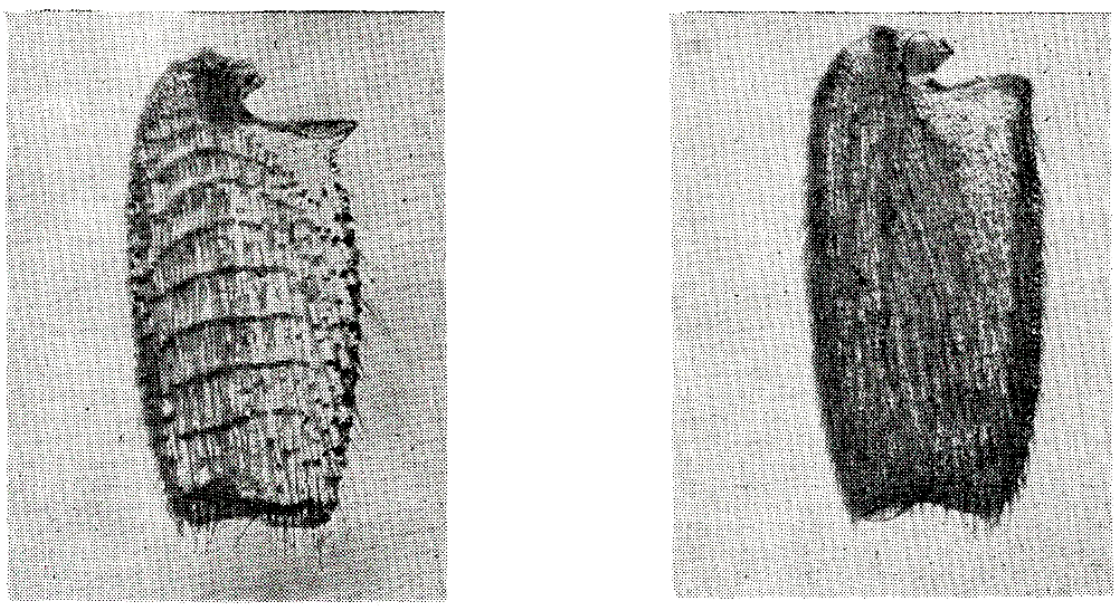


Fig. 14 — The metatarsus of a worker bee ***(left)*** *with its characteristic tarsus hook and 9 rows of hairs of the brush; to the right —* ***the queen*** *tarsus with no such pollen collecting device (See also Fig. 31).*

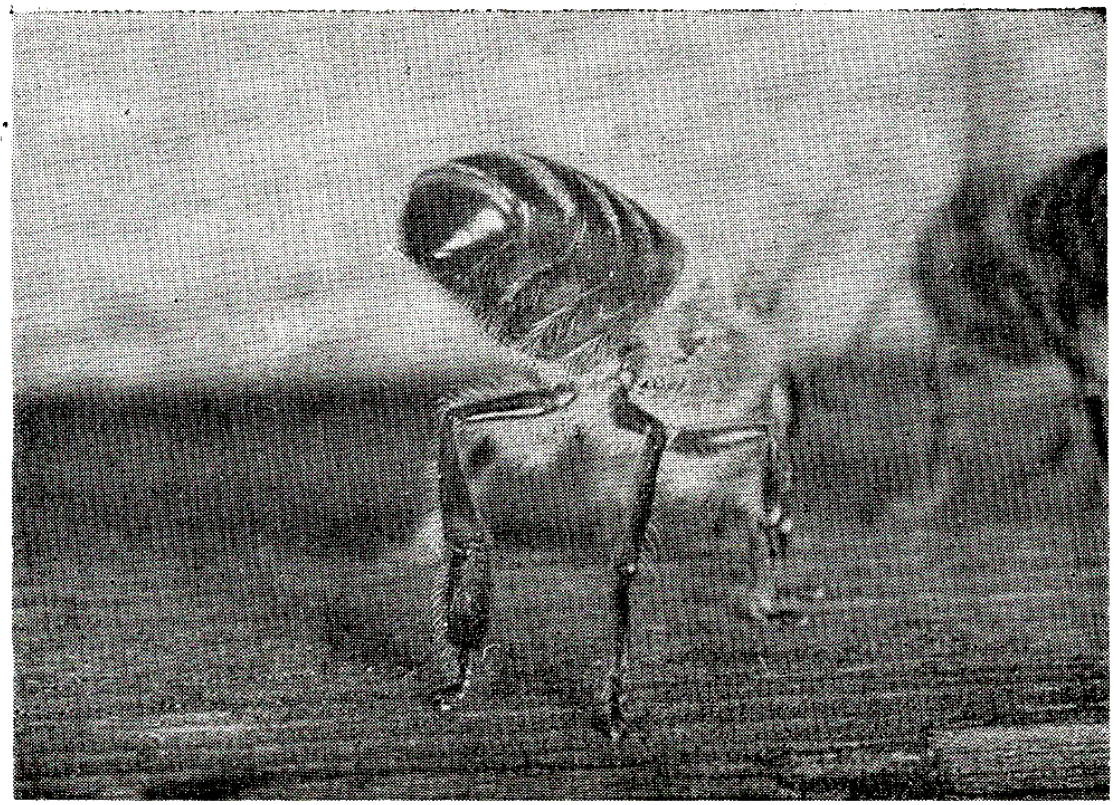


Fig. 15 — *As the queen has no wax glands, she is not able to draw out combs. Also, she is not able to expose her scent gland. Only the worker bees are provided with such a gland (Nasonov's organ) on the last tergite, which it exposes by lifting the abdomen and flapping its wings fast. With this gland it can attract individuals of its species.*

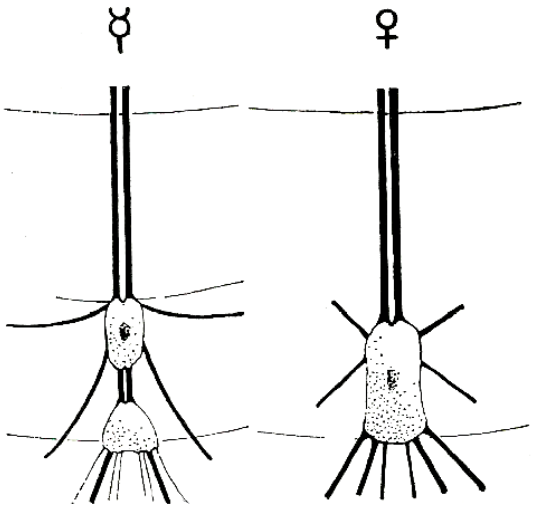
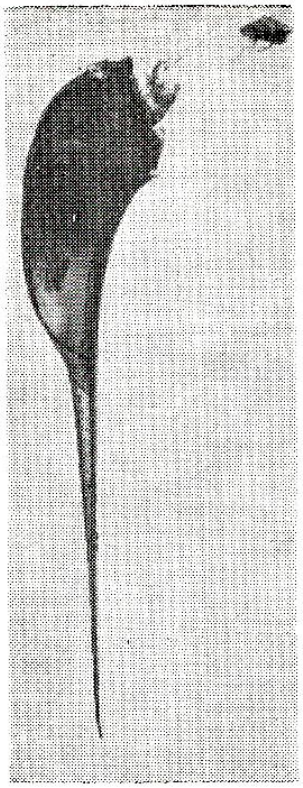
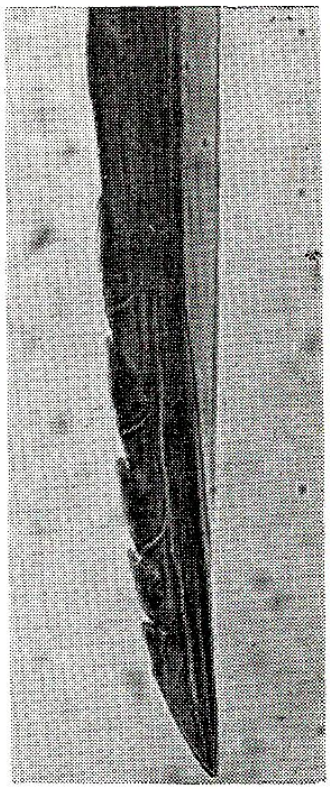
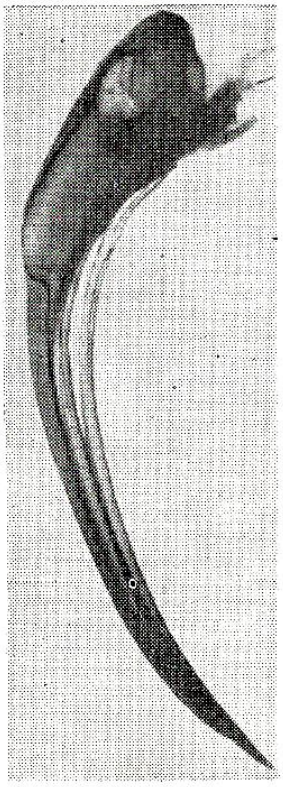


Fig. 16 — *The last two abdomen ganglions of the nervous system ; in worker bees they are seen as two individual parts (left), while in queens they are joined (right).*







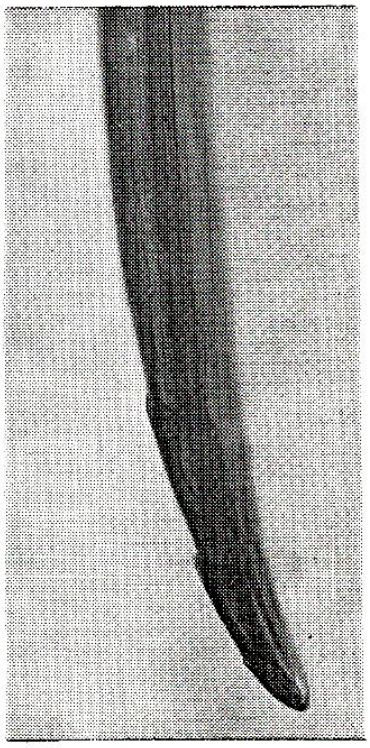


Fig. 17 — *The sting of the worker bee is straight and has 8—10 barbs (a, b) on it, while the queen sting has 3 barbs at the most on it, being longer and distinctly bent.*

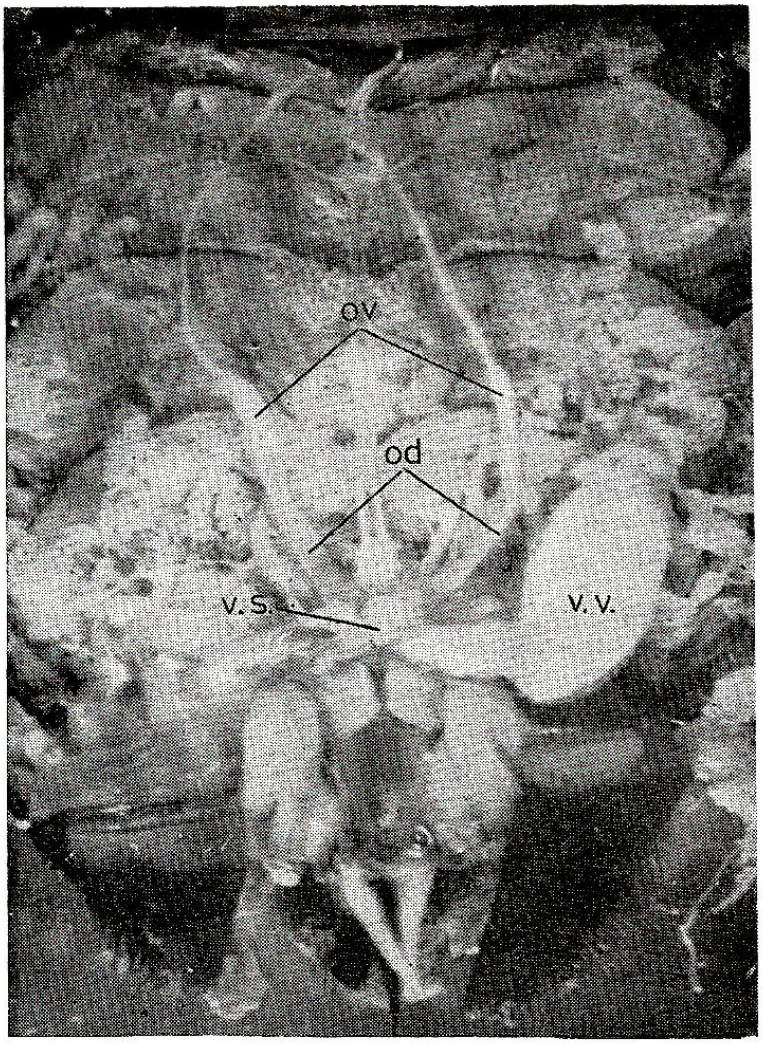
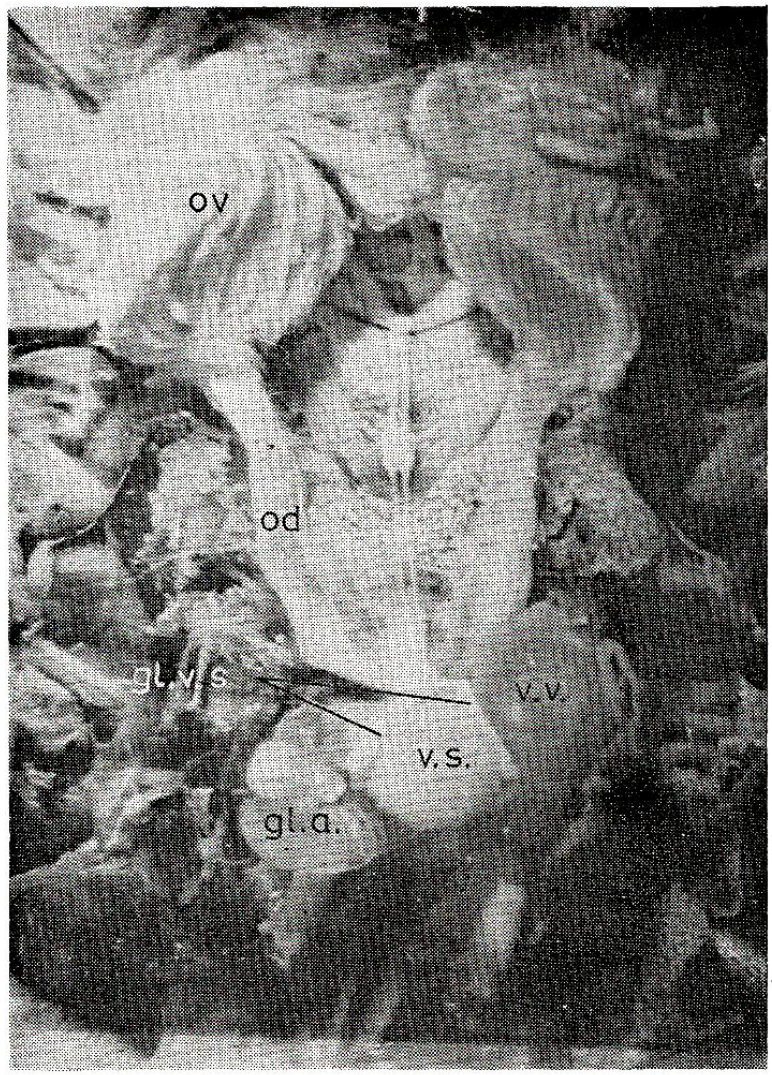


Fig. 18 — *The ovary of the worker bee consists of only two thin threads (left). The caudal section, consisting of oviducts (od) is the end of the ovary, with a few filiform ovarioles (ov). On top of the posterior nervous ganglion, the rudimentary spermatheca (v.s.) is seen. The large white patch is the venom pouch (v.v.). The digestive tract has been removed, just as in the preparation of a queen (right). In the two pyriform ovaries (ov) many ovarioles in bundles are seen. The spermatheca is a large spheric pouch*



completely covering the nerve ganglions and the vagina. Also, the two thin threads, very close to one another, of the bursal pouch (gl.v.s.) are seen ; to the left — the venom gland (gl.a) looking like a sausage, and to the right — the venom pouch (v.v.). The latter is in reality larger than that in worker bees, fact which must be kept in mind in order to have the proper ratio of sizes. The worker bee ovary was enlarged 0.5 times as compared to that of the queen.

report that with an equal intake of oxigen, the queen larva releases — already in its first 15 days of life — more CO_2 that the worker larva. Little before the 3rd day of the larval stage, the first differences in the metabolism of the energy-giving and plastic substances appear, while the difference in terms of structure of the body — only after the 3rd day. In respect of growth, the reverse is true, namely the worker larva grows faster than the queen larva at the beginning of their development, but by the end of the 4th day it is surpassed by the latter (Fig. 19).

With the development processes being governed by the hormonal system, it is not surprising that the histological structure also provides evidences of a different secretory behaviour, specific of the caste. Of primary importance are the *corpora allata* which release the juvenile hormone. In the queen larva, a clearly greater amount of juvenile hormone is released (Fig. 20). Recently, DOGRA, ULRICH, and REMBOLD (1977) have found that on the 2nd day of the larval stage, a more marked gowth took place of the projections of the neurosecretory cells in the brain of queen larvae which make up the chiasm ; in worker bees, this process began one day later.

Specific literature is available about the physiological processes of development in the formation of castes. More recent reports include those by SHUEL and DIXON (1973), REMBOLD (1973, 1974), and WEISS (1978).

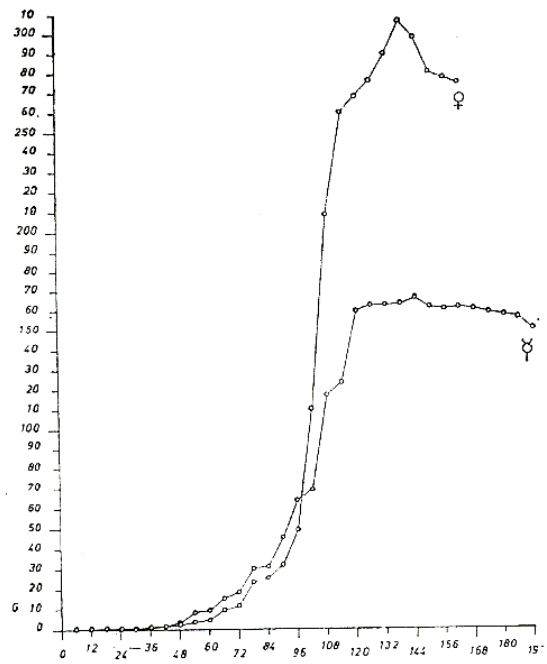


Fig. 19 — *The curves of growth of queen and worker larvae (according to the weight determinations of WANG, 1965) meet at the end of the 4th day of larval stage. Only from this moment on, is the queen larva taking weight much faster than the worker larva. E — time of development, in hours; G — weight increase in mg.*

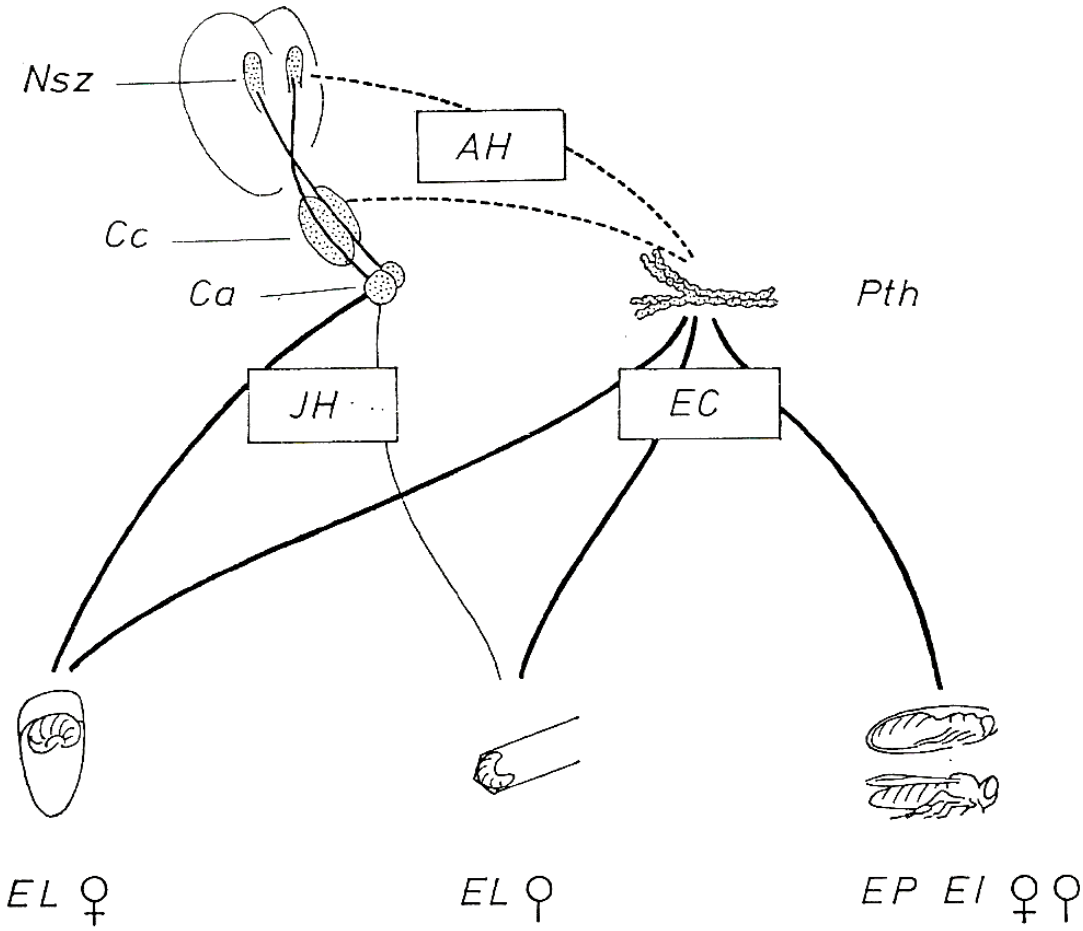


Fig. 20 — *The figure illustrates the possible action of the internal secretion system during the period of post-embryonic development, especially the moulting process in holometabolous insects (with complete metamorphosis). The influence of hormones on caste formation is shown by a limited supply of the worker larvae with juvenile hormone. Nsz — neurosecretory cells ; Cc* — Corpora cardiaca ; *Ca* — Corpora allata ; *Pth — prothoracic glands ; AH — adenotropic hormone ; JH — juvenile hormone ; EC — Ecdyson ; EL — larval moult ; EP — nymph moult ; EI — moult of imago.*

Each and every beekeeper knows the differences between the stages of development of the two castes of bees. Table 5 gives precise chronological data about the development process. The queen, which is heavier and has a longer span of life, reaches the adult stage faster — her development is completed in 16 days — than the worker bee which emerges as late as after 21 days. The decisive moments are the hatching (after a 3-day stage as egg, similarly long for all larvae), and sealing — of the worker bee larva after 8 days, and of the queen larva after $7\frac{1}{2}$ days. The great difference in the general development occurs during the nymph stage. The deadline between the 4th and the 5th day of life indicates to the bee breeder that up to that moment the young worker larvae may be turned into perfect queens.

Table 5

STAGES (IN DAYS) OF DEVELOPMENT OF LARVAE AND PUPAE OF THE TWO FEMALE CASTES IN HONEY BEES (MODIFIED AFTER BERTHOLF, 1925)

Days after hatching of egg	Days after hatching of larva	Worker bee	Queen bee	Days after hatching of larva	Days after hatching of egg
1					1
2	3	Egg		3	2
3					3
4	¾	Hatching of egg L 1		¾	4
		1st moult			
	1½	L 2		1½	
5		2nd moult			5
	2½	L 3		2½	
6		3rd moult			6
	3½	L 4		3½	
7		4th moult			7
	5	L 5		4½	
8			Sealing		8
		Sealing	Stretched larva	5½	
9	6	Stretched larva	Pupation		9
		Pupation	Prepupa	7	
10	8	Prepupa			10
			5th moult		
11		5th moult			11
12					12
13			pupa	12	13
14					14
15					15
16	17	pupa	6th moult		16
			emerging	12½	
17					
18					
19			Imago		
20					
	17½	6th moult			
21		emerging of imago			

2. Plasticity of caste determination

As mentioned above, the bipolar development of bee castes is obvious already in the first days of the larval stage. We may assume that it begins right after hatching of the larva. On the other hand, a number of factors involved in caste formation indicate a very great lability.

By grafting worker larvae of various ages into artificial cell cups, queens are obtained from larvae up to 3 days of age. Worker bees would result from 4-day old larvae. Of larvae of intermediate ages, often "intermediate individuals" would result. The first reports in this respect, of KLEIN (1904) and KOZHEVNIKOV (1905) were ascertained by the systematic tests made by ZANDER and BECKER (1925). The latter two have primarily demonstrated that the turn from worker to queen takes place within a short period of the larval stage, not gradually, while the grafted larvae become older.

This finding requires further discussion. If it is true that the food given to the worker larva is different from that given to the queen larvae, from the very moment of their hatching, and that already in the first days of the larval stage differences in metabolism and hormonal deviations can be identified, the adult individuals are also expected to be different depending on whether they resulted from eggs, or from younger or older worker larvae. Some research workers belived to be able to prove this idea. They assumed that perfect queens would result only from individuals reared from eggs (See Chapter V, 1.1). The The extensive experiments of WEAVER (1957) and WEISS (1971, 1978) contest the possibility of such an early determination of the caste characteristics, and ascertain the findings of ZANDER and BECKER as to the moment of the sudden turn of the caste characteristics — on the 3rd day of the larval stage. The only exception is the weight of the adult queen : starting from the stage of egg, the older are the worker larvae when they are grafted, the smaller is the weight of the adult. Given the fact that worker larvae develop faster than the queen larvae in the first 3 days, this seems surprising, but it has been accounted for (WEISS, 1974). The queen breeder must know that the difference in weight of the queens developed from larvae of different ages within a range of $1^1/_2$ days are statistically insignificant and therefore are not consequential for the breeding results.

Leaving aside the size of the body, it results that the worker larvae can perfectly be turned into queens in the first 3 days of life. We may designate this stage of life of the worker larvae as the *sensitive (bipotent phase)*. An indefinite stage of development follows. Transition individuals develop, with characteristics ranging between queen and worker bee (Fig. 21). It is true that sooner or later after grafting, such larvae disappear from the queen cells. It seems that the intermediate forms are not viable. This *critical phase* lasts until the end of the 4th day of the larval stage. The older worker larvae are stable in their development ; no breeding method can possibly change the caste. Only

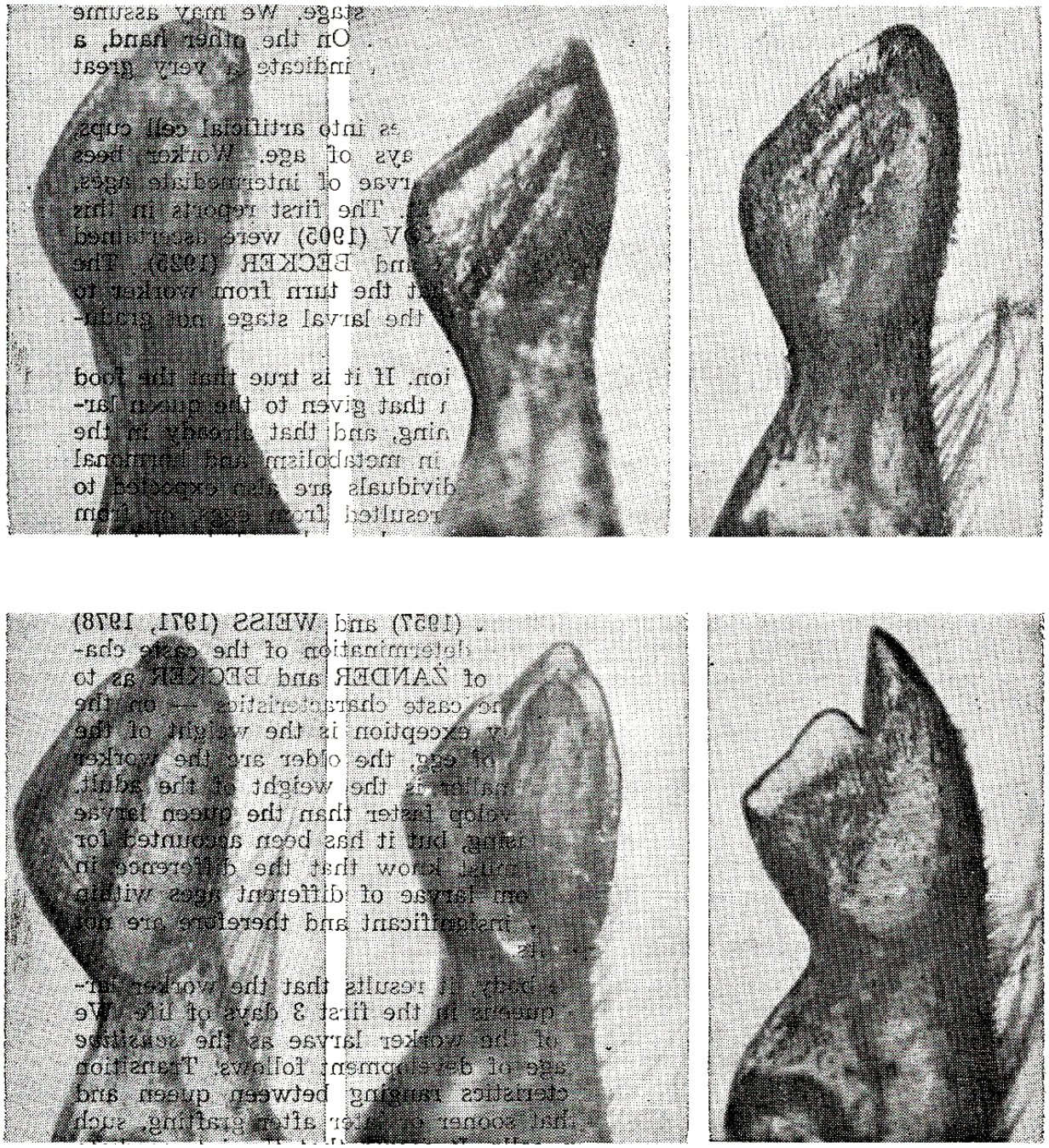


Fig. 21. *The range of upper maxilla shows various intermediary types between the pure worker (upper left) and the pure queen (lower right).*

typical worker bees would result. This is the *fixed phase* of the larval development, which lasts till the end of the nursing period.

Under natural conditions, the reverse situation — turning of a queen larva to develop into a worker bee — would never occur. After preliminary investigations by WEAVER (1957), WEISS (1978) made investigations to find whether the queen larvae have the same possibility of determination. Queen larvae may be grafted into worker cells just as the worker larvae into queen cells. Naturally, this is possible only up to the age of $3^1/_2$ days, because later they are too large ; to study later stages, queen larvae must be starved for various periods, previously to cocoon spinning (DONHOFF, 1859 ; von RHEIN, 1933 ; HAYDACK, 1943 ; SMITH, 1959, JAY, 1964). It was found that at the beginning of their development the queen larvae are perfectly bipotent just as the worker larvae. It seems that the caste morphological criteria (shape of the head, structure of the mandible and of the gathering leg) may be re-determined later. But the number of ovarioles in adult individuals is already increased in the queens obtained from larvae grafted at the age of $2^1/_2$ days, while the spermatheca paradoxically remains equal to that of worker bees. Contrary to the worker larvae — in which the critical period of incompleted re-determination is followed by another feeding period of at least one day — during which the development can no more be influenced (the fixed phase), the queen larva is liable to influences which change the caste up to the end of the nursing period. It is only when the larva completes the spinning of the cocoon in the sealed cell — at the beginning of the 6th day of larval stage, that it is 100% fixed for the specific caste. Only the number of ovarioles had already previously reached the level typical of the queen, with no possibility of regression. Fig. 22 illustrates the process of determination of the two female castes in bees.

The plasticity of caste formation in the honey bee is not a mere play of nature. The possibility to re-determine the worker larva, turning it into a queen, meets an important biological requirement. As a rule, the bee colony multiplies itself by issuing swarms or by quiet supersedure of the queen. But when a colony is deprived of the queen, which may happen because of external causes, the bees may "produce" queens from the worker larvae available. The bee colony is thus saved from extinction (See Chapter I). The bipotent phase, which is relatively long in the larval development, increases the efficiency of this wise provision by nature.

3. Causes of caste determination

Because both queens and worker bees result from the same kind of fertilized egg, the differences between the two bee castes cannot be genetically governed; they are more likely to be due to external factors. The external influences have a different effect on the gene pool of the identical phenotype of the bee egg, the hereditary bipotentiality coming thus to play its part in the formation of castes. Which are these factors?

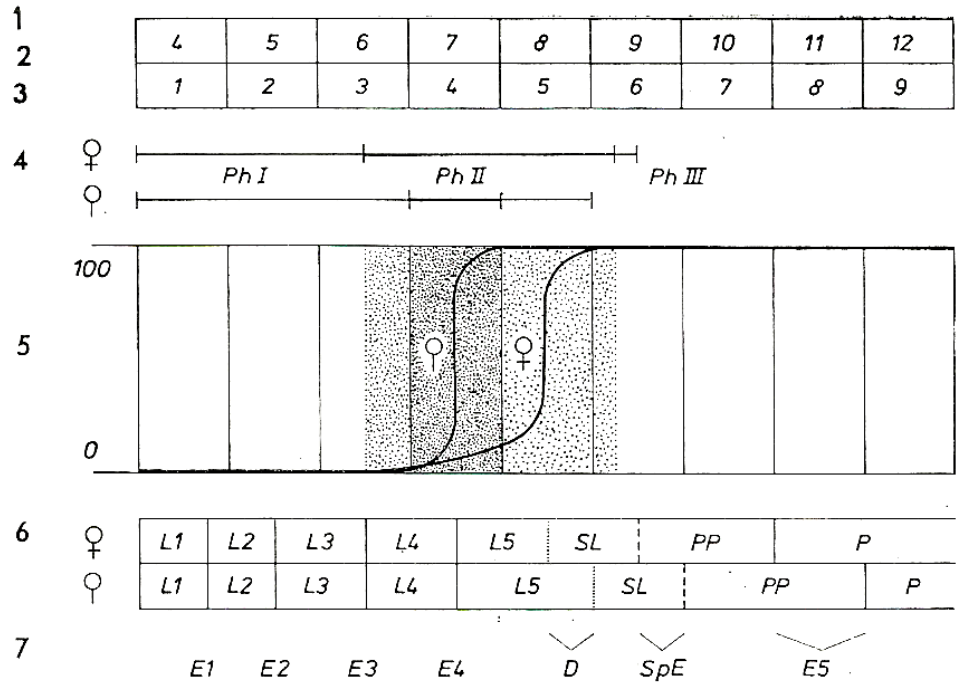


Fig. 22 — *Formation of castes when attempts are made to determine worker larvae of various ages to turn into queen development, and vice-versa. The critical period, during which an incomplete turning to the other caste is still possible with intermediary individuals resulting, lasts, for the worker larva, for a short interval on the 4th day. After this interval the development of the worker larva is determined precisely. In queen larvae, the period of non-determination lasts until shortly before preparation to receive food, namely the beginning of the 6th day of larval stage. This means that the queen larva may be influenced in its caste determination also during pupation in the sealed cell. Ph I — bipotent phase (liability to changes) ; Rh II — critical phase ; Ph III — fixed phase ; L 1—5 — larval stages ; SL — stretched larva ; PP — prepupa ; P — pupa ; E 1—5 — moultings ; D — sealing ; SpE — completion of pupation ; 1 — age of larva, days ; 2 — after hatching of egg ; 3 — after hatching of larva ; 4 — phases of determination ; 5 — change of caste development after change of food ; 6 — stages of change ; 7 — post-embryonal developments*

3.1. *Shape of the cell — change of food — amount of food*

Rearing of queen larvae differs from that of worker larvae primarly in two fundamental elements : the shape of the cell and the food supplied to them. In nature, the two elements are closely related to one another By rearing experiments in laboratory (Chapter IV) it can be easily seen that neither the shape, nor the position of the cell are decisive in the formation of the two female castes. Also, the temperature — slightly lower around the queen cells than around the other brood cells (LUKOSCHUS 1956 b, DRESCHER, 1968) cannot possibly be a determining cause. The only decisive factor is the food. We know from PLANTA (1888) that the worker larva is fed on larval food in the first 3 days, and later on a mixture consisting of larval food, pollen and honey. The queen larva is fed exclusively on larval food, throughout its life. In addition to the difference in terms of the quality of the food, there also is a difference in respect of quantity : while the queen larvae are permanently swimming into the extra food, the worker larvae are mostly living "from hand to mouth".

The question was whether the feeding with the mixture instead of larval food made the initially bipotent (two-way) larvae to develop into a worker bee, or it was simply the pollen added into the mixture ? The answer was easily found by an experiment, keeping the bee colonies caged and feeding them exclusively on sugary food. HIMMER found (1927) — and after him many other investigators — that of the small amount of brood which those bees reared feeding it on the initial stores of larval food, perfect worker bees emerged.

Neither the second possibility, namely that the greater amount of food with which the queen larva is fed is decisive in developing the queen attributes and qualities, can be taken for granted, at least not in this oversimplified manner. Under certain conditions, the worker larvae too would be fed on more food than usually, as for example when part of the unsealed brood is taken out from a colony with much brood, or when a strong swarm would build the new nest. Under such conditions, although food is supplied in excess in the worker cells, only normal worker bees would emerge (KUWABARA, 1947 ; GONTARSKI, 1953 ; WEAVER, 1956). On the other hand, we know that only small (dwarf) queens would emerge from the queen cells from which food stores would be removed immediately after sealing (Fig. 23). Often, these queens are not larger than worker bees but in principle they perform the functions of queens (for reference see JAY, 1964, and WEISS, 1978). For rearing queens from female bee larvae in laboratory, royal jelly in excess is usually supplied to them. Research workers are disappointed because, in addition to queens also a wide range of intermediate individuals and typical worker bees would be obtained again and again (See pag. 70).

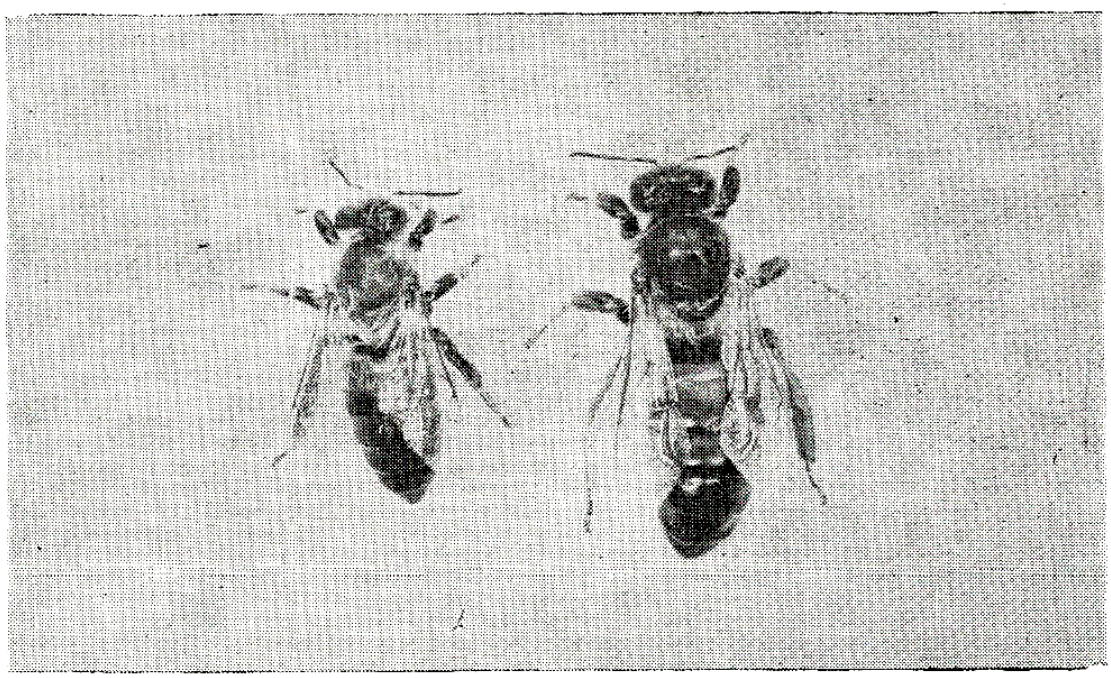


Fig. 23 — *The "hungry queen" (left) is not larger than a bee as it is seen by comparison with a normal queen right)*

3.2. *Oligoelements or combinations of substances ?*

The feeding of the two female bee castes differs not only in terms of type of food (larval food and mixed food), and of amount of food supplied, but also in terms of composition of the larval food. As reported by von RHEIN (1933), nurse bees would generally feed the young larvae with two different components of larval food : a "white" one, and a "clear" one. JUNG-HOFFMANN (1966) found that queen larvae are supplied with almost equal amounts of each of the two components, throughout the larval stage, while the young worker bee larvae with less "white" component. The worker larvae over 3 days of age are given almost no white component any more (See Chapter V 3.1.1.).

The composition of royal jelly is given in Chapter two, with reference literature reviewing the subject being specified. Going into further detail, it was found that the difference in terms of quality between the larval food given to worker larvae and that given to queen larvae is chiefly determined by the different amounts of the components in the two types of larval food.

Admitting that the quality of the larval food determines the formation of castes, the question arises whether a certain substance — or several substances, present in different amounts — is decisive.

After von RHEIN (1933), many authors have tried, more or less successfully, to rear queens in laboratory. As already mentioned above, in incubator, from young worker larvae fed with royal jelly they obtained not only queens, but also worker bees and intermediary individuals. Practice and experience in artificial rearing technique may reduce the percentage of imperfect sexed individuals but not avoid their occurence (See Chapter IV). Worker larvae were fed with larval food with certain substances — thought to contribute to the development of the queen, being either extracted from it or added to it. But both when potential components of the royal jelly and other substances were used, the percentage of the queens obtained was not increased. A hypothesis advanced by von RHEIN already in 1933 was therefore generally accepted : queen formation might be determined by a certain substance which, being volatile and present in very small amounts, is difficult to identify. Later on, in 1962, WEAVER found in royal jelly a fraction which determined development of queens under laboratory conditions. REMBOLD and HANSER (1964) have finally identified the active factor in royal jelly — by dialysis in water of the components with low molecular weight. Surprisingly, REMBOLD (1969, 1973) identified the same active factor also in analogous samples of larvae and adults of silkworms and of other insects. This would suggest that the queen determinant substance is not characteristic of the species ; that it is a higher hormone stimulating and controlling the processes of internal secretions which determine the castes ; and that it is present in very small amounts, this being the reason why it could not be identified. According to REMBOLD (1974) this substance is however not identical to the juvenile hormone whose significant role in the formation of female castes in bees is incontestable. The substance concerned has not yet been identi-

fied. And whether it will be identified, one will have to first prove that it is indeed secreted by the nurse bees. It might also reach the larval food during the shedding of the larvae fed on the food specific of the queen — and if so, it would no more be the determinant cause, but a (hormonal) product of the initial cause which induces queen development.

HAYDAK is the first investigator who, in 1943, made a different statement about queen formation : not a special substance present in royal jelly only, but the quantitative ratio of the different determinant substances, or the presence in trace amounts of these substances — which are also found in the larval food for worker bees —, would be decisive for queen formation. The laboratory experiments, during which young worker larvae were fed on larval food, seem to confirm HAYDAK's assessment : by supplying additional amounts of certain components or by eliminating certain components, development of queens or of worker bees respectively was induced. It was found that the physiological processes of larval development — growth, breathing, histochemistry, histology of the internal secretory glands, and ovary development — may be influenced specifically, to develop into one or another caste (SHUEL and DIXON, 1959 ; DIXON and SHUEL, 1963, WANG and SHUEL, 1965 ; O'BRIEN and SHUEL, 1972 ; TSAO and SHUEL, 1973). WEAVER (1974) points out, on the contrary, that although he made considerable changes in the ratio of the nutritive components of royal jelly, the caste specificity of adult individuals obtained by him could not be changed.

3.3. *New aspects in research on queen determination*

Noteworthy is that in spite of the fact that it distinguishes from the queen larva in terms of development physiology, the worker larva may still be turned to develop into a queen, for 3 days after hatching. The worker larval food and royal jelly must therefore be very similar, at least in respect of the properties which determine the caste. But when rearing larvae in laboratory, on pure worker larval food, unexpected difficulties would occur. Von RHEIN (1933) and SMITH (1959) fed larvae on such food but they would not reach the pupa stage. The larvae reached the pupa stage only when a mixed food was supplied to them, just as under natural conditions. The successful rearing, by REMBOLD and HANSER (1964), of worker larvae on larval food exclusively was possible because they used a dietetically modified larval food. We know today that the cause of the failures in rearing larvae on (worker) larval food was the lower sugar content of this food. The development of the worker larvae in the first days is based on less food — glycogen and fats with a higher water content — than the queen larvae, and therefore the former could appear as poorly nourished as compared to the queen (HAYDAK, 1943). When feeding larvae on mixed food, which is richer in sugars, glycogen stores increase and ensure their development into pupae. SHUEL and DIXON (1968) fed the worker larvae reared in la-

boratory on larval food to which sugar was added, and the larvae reached the stage of pupa. In 1975 WEISS, using worker larval food whose sugar content was similar to that in royal jelly — by adding to it a mixture of equal amounts of fructose and glucose, obtained worker bees, as well as intermediate individuals, and a typical queen. So the evidence was produced that royal jelly and (worker) larval food are not two different foods having different effect, specific of caste, but that they only distinguish themselves in terms of the amount of the determining factor they contain. The next step forward was made by ASENCOT and LENSKY (1976) who increased the sugar content of the worker larval food. They obtained a greater number of perfect adults, and also more queens — proportionally to the increase in the sugar content : with 16% additional sugar, 8% queens were obtained, 46% intermediate individuals, and 46% worker bees ; with 20% additional sugar — 50% queens were obtained, 41% intermediate individuals, and 9% worker bees.

At the first sight, from these experiments it could seem to result that sugar is the determinant factor having been sought after. But this fact has not yet been proven. Such high sugar concentrations as those used by ASENCOT and LENSKY do not exist in nature. Several years ago, DIETZ and HAYDAK (1971) thought they had found just as simple a solution to the problem of caste determination — the water content of the larval food. It is known that when larvae are reared in laboratory, feeding on diluted larval food would result in more queens that with undiluted larval food. And because in nature the water content of royal jelly would change with the age of the queen larvae, the authors thought that by merely providing for identical conditions as in nature, only queens could be obtained. But they still owe us the evidence.

Without considering sugar or water as being directly determinant factors, one may however assume that these food components could stimulate the larvae to consume more food and implicitly more of the actually determinant compound. The more recent findings of REMBOLD (1976) namely that adding of yeast extract (certain fractions only) into royal jelly would increase the growth rate and above all the percentage of queens obtained in laboratory, are also evidences in support of the above mentioned assumption. But this does not necessarily mean that the determinant principle must be a specific oligoelement. Since this substance has not been identified, that is since its origin in nurse bees has not yet been ascertained, the other, simpler, assumption is more likely to be true. According to this assumption, the content of each of the essential nutritive elements in the larval food, specific of caste, would be the determinant factor in caste formation. The important part, already demonstrated, played by sugar to this effect is not the only one coming in support of the "balance of nutrients".

Although new findings about the causes governing queen development are being constantly reported, much work is still necessary in order to fully elucidate all problems.

CHAPTER IV

Rearing queen bees in the laboratory

Gisela HANSER

1. History

W.v. RHEIN was the first who tried to read adult bees from larvae in an incubator. He used worker larvae of 2—4 days of age, and fed them, every four days, with fresh larval food taken from cells in the colony with larvae of the same age. Providing a temperature of 35°C and a humidity of 80—100%, he tried to supply conditions very close to the natural conditions existing in the bee colony (A. HIMMER, 1927). The relatively high air humidity prevented larval food from instant hardening. Under natural conditionsè nurse bees would permanently supply queen larvae with fresh royal jelly, by about 1,600 feedings (I. JUNG-HOFFMANN, 1966) ; certainly, such a high rate of feeding is not possible in artificial rearing. The high humidity in the incubator was found to affect the perspiration of larvae, as indicated by the drops of water on their bodies. Under normal conditions, the bees in the hive fan with their wings thus providing for good ventilation. In compensation, W. RHEIN used a small ventilator which controlled temperature and humidity whenever the incubator was opened for providing food to the bees, which means very often. With ventilation in the incubator, the larvae would develop normally and reach the stage of pupa — reared on gauze in an uncovered watch glass. W. RHEIN found that neither the position of the cells to be reared, nor their shape had a decisive influence on the development of larvae. This finding was later confirmed by other authors (N. WEAVER, 1974 ; M. V. SMITH, 1959 ; S. C. JAY, 1965 a). W. RHEIN failed to obtain worker bees by rearing worker larvae on larval food, but often 60% of the larvae fed with royal jelly reached the pupal stage. Because these pupae were always attacked by mould, in order to avoid great losses, he had to kill them on the 4—5th day of pupa stage — when chitin formation would just begin, in order to identify the indices of caste determination. In the best cases, huge worker bees with large ovaries and spermatecae would result. He has never obtained perfect queens, with typical characteristics — notched mandibles and missing pollen collecting brush on the hind leg.

To elucidate the part played by the age of larvae and by the specific food in caste formation, N. WEAVER (1955, 1957) conducted experiments of rearing larvae *within colonies*, under closest possible conditions to the natural ones. 1—1½ day old worker larvae were grafted into queen cells of queen larvae of the same age. By closing the queen cells with small cotton wool pads he prevented the contact of nurse bees with the larvae. In order to provide for the necessary larval food, he would move, every two hours, the initial worker larva on the fresh royal jelly in another queen cell which had previously contained a queen larva of the same age. In this way WEAVER managed to obtain a queen from a 1—1½ day old worker larva, rearing it in a breeder colony, and preventing contact with nurse bees.

After this success, WEAVER (1955, 1958) tried to rear worker bee larvae *outside the colony*, in the incubator, at 34 ± 1°C temperature,, and over 75% air humidity. The larvae were grafted into glass cell cups. Every two hours they were given fresh royal jelly, which WEAVER extracted with a pipette from corresponding queen cells from a nurse colony. So, perfect queens developed from several worker bee larvae. If however the royal jelly was stored for a period of time, the queens obtained were no longer perfect; intermediate individuals were obtained, with characteristics of both castes. At that time WEAVER (1955) already assumed that royal jelly was likely to contain one or more, highly labile substances, which induce and control the turn of a female larva into a queen.

Almost at the same time, I. JUNG-HOFFMANN (1956) managed to rear worker larvae in the incubator, feeding them with larval food. Her rearing method and the conditions of incubation were to a great extent similar to those used by V. RHEIN (1933). With fresh larval food always taken from cells with larvae of the same age, of the 1973 larvae with initial weight of 2.5 mg, only 22.6% reached the pupal stage; during another experiment of 45 larvae, 71% reached the pupal stage. For the same reason as v. RHEIN, she examined the animals on the fourth day of pupal stage and found a greater variation of the size of the ovaries as compared to the normal workers. In addition to this, she also found that the survival rate would not depend on the beekeeping season.

The experiments concerning temperature and humidity conditions inside the hive made up to now were based on the results of the experiments of A. HIMMER (1927) and A. BÜDEL (1948). Later on, M. V. SMITH (1959), by measurements of temperature and humidity inside the hive, obtained average values of 34.7°C and 64.3% relative humidity; the slightest disturbance, as for instance smoke, would entail a change in humidity. In queen cells he found, by measuring at the surface of royal jelly and of the queen larvae, 92—95% relative humidity, which — after the larva reached the pupal stage — would fall down to the usual average values inside the hive.

As compared to RHEIN (1933) and WEAVER (1955, 1958), SMITH obtained better results in rearing queens in the laboratory: he

would place ten larvae just hatched from the egg into porcelain cups (17 mm diameter, 3 mm deep) on 300 mg royal jelly. He then introduced the cups into an incubator, at 94.5°F (34.7°C) temperature, in an exsiccator with a certain concentration of sulphuric acid to control the humidity of the air. The air humidity for larvae was 63.4%. Every 24 hours, he would move the larvae on fresh royal jelly. About at the moment of elimination of faeces, the larvae weighing cca. 200 mg were washed with distilled water, dried on filter paper and each placed in a little cup with moist filter paper on the bottom. For the nymphal stage, the animals were placed into exsiccator with 80% humidity, and after this stage, up to their development into imago, they were kept in 60% air humidity. SMITH obtained a decisive improvement of the results of rearing by diluting the royal jelly with distilled water 1 : 2, with an about 30% content of dry matter. Although the dilution of royal jelly resulted in an equal increase in the water content in the haemolymph of aged larvae, they would grow much facter than the larvae fed with undiluted royal jelly. Of 1033 one-day old larvae, he obtained 65.4% aged larvae, 32.3% pupae, and 25.4% emerged adults, of which 8.3% bees similar to queens, 7.6% intermediate individuals, and 9.5% workers. Just as v. RHEIN, M. V. SMITH also failed in rearing 1—3-day old worker larvae fed with larval food for young worker larvae. All larvae would die before reaching the nymphal stage, after turning to a yellow-brownish colour. But when the larvae were grafted on royal jelly after three days of feeding with larval food, they continued their development, and emerged adults also included a number of queens. But when larvae were given royal jelly only as late as the 5th day, they would normally reach the pupal stage but looked like workers. SMITH has reached the same conclusion as v. RHEIN (1951) namely that the larval food for young larvae was likely to contain a substance which inhibits metamorphosis, and whose effect can be inactivated in an aged larva by feeding it with royal jelly.

M. ASENCOT and Y. LENSKY (1976) managed to rear worker larvae in an incubator by adding to the larval food 4% glucose and 4% fructose. Increase in the additional sugar up to 20% resulted not only in worker bees, but also in queens and intermediate individuals. N. WEAVER (1974) described a standard method of rearing bee larvae in the incubator, at 34—35.5°C temperature and 70—75% humidity. Royal jelly was diluted with water, just as M. V. SMITH did (1959) up to 30—35% dry matter. The best results were obtained with 2-day old worker larvae taken from a normal bee colony, which confirms the conclusions of I. JUNG-HOFFMANN (1956). As reported by G. KINOSHITA and W. SCHUEL (1975) in the incubator bee larvae would grow more slowly than under normal conditions in the hive. Any disturbance such as taking of larvae from the cells, their washing and weighing would slow down their rate of growth. Of the bee larvae reared by N. WEAVER, under certain conditions, mostly workers and intermediate individuals (between queens and workers) were obtained.

A. DIETZ and M. H. HAYDAK (1971) reported that by diluting royal jelly they obtained, during experiments of rearing in the incu-

bator, not only a higher rate of survival, but also a greater number of queens. Because, during these experiments, they used royal jelly stored for $1^1/_2$—$2^1/_2$ years, they assume that only the dilution of the royal jelly determined the change of larvae to become queens.

The investigations discussed above were concerned with artificial rearing of bee larvae by giving them natural food — larval food or royal jelly. A. S. MICHAEL and M. ABRAMOVITZ (1955) obtained good results in rearing larvae in incubator in another, very simple way: they used 3—5-day old worker larvae which they fed on an aqueous solution of 25% honey and 10% dry yeast, at 34°C. After the active feeding was over, the larvae were moved into Petri dishes which had a layer of beeswax on the bottom. The larvae would spin the cocoon, turn to pupae, and after 14 days adult bees would emerge as usual. But this nutrient solution of a simple composition is efficient only for worker larvae in a more advanced stage of development, a stage during which even in the colony they are fed only on mixed worker larval food. The latter consists of the gland secretion of nurse bees, honey, and pollen. This method of feeding was developed by A. S. MICHAEL and M. ABRAMOVITZ for investigations of infectious diseases in bees; it may also be used for elucidating some biochemical questions. By adding certain chemical compounds or radioactive tracers to this nutrient solution given to 3—4-day old larvae, the metabolism of those substances in aged larvae and in pupae can be studied (H. REMBOLD and Gisela HANSER; Gisela HANSER and H. REMBOLD, 1968).

Many attempts have been made to rear bees outside the colony, from the first larval stage, feeding them on artificial food exclusively, but no rousing success has been obtained as yet (see A. DIETZ, 1972, 1973).

S. C. JAY (1965) managed to demonstrate that temporary cooling to 21°C of aged brood (larvae in an advanced stage of development, prepupae, and pupae) during the period between its taking from the colony and its introduction into the incubator (35°C and 80% air humidity) does not damage the queens or the worker bees. The test larvae developed in the incubator in their comb cells, just as well, at various values of humidity: 80%, 60%, 40% and 20%, probably because of slight loss of humidity. On the contrary, when aged larvae were transferred from cells on filter paper, at values of humidity lower than 60—80%, individuals with malformations would often occur. Aged bee larvae or 0 to 1-day old pupae would develop better on vertical stands of gauze, cotton wool, or staple fibre than on similar stands but of wood or glass. S. C. JAY recorded the following anomalies: the larvae did not have normal motility, did not spin the cocoon as usually, and moulting of prepupae was incomplete: more time was necessary for prepupae and pupae to moult. It is true that bees would emerge sooner than from comb cells, but they would be injured more often and die.

During other series of experiments, S. C. JAY (1965 a) investigated the value of various types of artificial cells as for instance cups of gelatine for further rearing aged larvae, and found that the size of

the cell had no importance. Before moulting, the queen adheres to the wall of the cell, whereas the worker bee to the bottom of the cell. Kleenex paper was found to be excellent for covering the cell ; better than the gelatine or beeswax covers. Even when prepupae and pupae were facing other directions than normally in the hive, this had no influence on their further development.

2. Personal experiments

The method used personally in rearing bee larvae in the incubator, described below, is based on the experience accumulated during more than 1,500 tests, each with 60 bee larvae initially. These tests and the assessments made have been, for 15 years, the basis for the chemical processing and enriching of the determining substance by H. REMBOLD, B. LACKNER and I. GEISTBECK (1974).

The carnica bee colonies used were kept in summer in a balcony in open air, and in winter — in a flight room (for the rearing method in flight room see F. RUTTNER and N. KOENIGER, 1976). Bees were reared in an incubator, at 35°C temperature and 80—90% air humidity.

As recipients for rearing larvae, plastic thimbles were used as they were found, after a number of experiments, to be the most efficient. Both in shape and size they are most adequate for rearing bees ; and being cheap and easily available, one can afford discarding them after using them once.

The recipients are fixed into small stands (Fig. 24) made of two sheets of plastic 15 mm apart. The sheet above has orifices in it, 15 mm in diameter, into which the recipients are introduced, supported by the sheet below. In order to prevent hardening of the larval food in the incubator, the stand is covered with a plastic sheet. It is simply laid on and loosely folded by an elastic in order to provide for aeration. After pupation of the larvae, the recipients with the pupae are each intro-

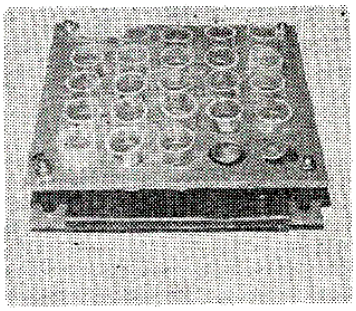


Fig. 24 — *Stand with plastic recipients for rearing larvae in laboratory*

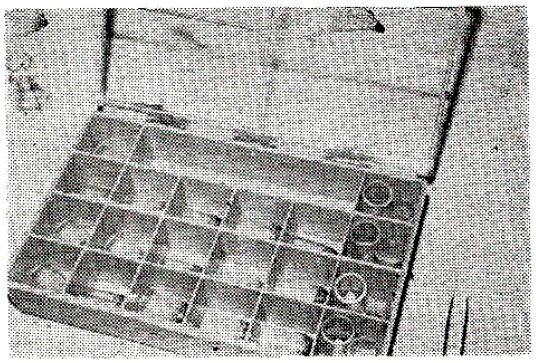


Fig. 25 — *Box for separately placing the bee pupae*

duced into compartments of a box (Fig. 25) in order to prevent the contact of the first queens emerged with the other, more developed queens because the latter may hurt or eventually kill the former. The cover of the box is perforated too. For grafting the young bee larvae from combs into the rearing recipients we use a very narrow grafting tool, only 2 mm wide, with a bent end.

2.1. *The food for artificial rearing*

A solution of 25 g of flower honey, 10 g of dried yeast *(Torula)*, and 0.3 g Nipagin (4-hydroxy-methyl benzoate, Schuchardt Inc. Munich) in 100 ml of bidistilled water is boiled fast and after cooling at room temperature it is centrifuged for about 10 minutes, at 3000 r.p.m. The supernatant is used as nutrient solution for diluting the royal jelly. It may be stored for one week, at 4°C. Adding of Nipagin prevents development of the moulds eventually brought into the food. It is worth mentioning that the food for bees is only rarely attacked by bacteria and fungi, in the humid and warm atmosphere in the hive and in the incubator. This is certainly due to the natural high content of 10-hydroxy-2-decenoic acid in all types of larval food (BUTENANDT and H. REMBOLD, 1957); its antibacterial effect is well known. Consequently, there is no need to add any antibacterial antibiotic into the larval foods used during rearing experiments. Because after collection the royal jelly, even when stored in the refrigerator, would change its consistency and harden very soon, for rearing young bee larvae it must be diluted (2 : 1) with nutrient solution, and for aged larvae 1 : 1. Even at 4°C royal jelly would change its consistency very soon; it become more viscous containing, in this state, greater amounts of insoluble components. For the feeding experiments, it is recommended to use royal jelly which was lyophilized as soon as possible after collection. The lyophilized royal jelly is relatively soluble, and after dilution it is in general less viscous than the fresh royal jelly.

N. WEAVER (1974) reported similar results of his rearing experiments; he found that the fresh royal jelly contained 1.14—1.19% insoluble substances, and the royal jelly stored in the refrigerator — 2.95—2.74%, while the lyophilized royal jelly — only 0.67—0.63%.

In royal jelly, a number of vitamins in higher concentrations were found as compared to the larval food (H. REMBOLD and C. CZOPPELT). After storage for a longer time, either in the refrigerator, or in lyophilized form, the vitamin content of the royal jelly would decrease substantially. It is therefore useful to add vitamins and some important amino-acids into the stored or lyophilized royal jelly — to reach the concentration in the fresh royal jelly. This is very important for the experiments with various types of royal jelly whose different fractions are chemcially separated, and subsequently brought together.

The vitamin solution has the following composition :

4.05 g Ca panthotenate

0.8 thiamin-HCl

0.36 riboflavin

0.10 pyridoxin-HCl

0.05 g folic acid

0.05 biotin

0.0004 g B_{12} vitamin

3.6 g inositol

3.6 g nicotinic acid

10.0 g Cholin-HCl

6.0 g α-lysin

6.0 g α-arginine

diluted in 100 ml of bidistilled water

0.4 ml of this vitamin solution is added to 100 g royal jelly.

For an experiment of rearing 60 larvae, 25 g royal jelly are necessary, diluted in 12.5 ml nutrient solution. If the amount of royal jelly is not sufficient, on the last day the larvae are fed with a solution of royal jelly administered in 1 : 1 dilution from the very beginning ; the same is recommended for various lyophilized types, which are highly viscous in solution.

2.2. *Rearing of larvae*

1st day: the worker larvae are taken from the comb when 1—2-days old. In order to obtain as many larvae of this age as possible the queen is kept separately, for 12—24 hours, on a broodless comb, 5 days before beginning the experiment. With a certain experience, the age of the larva can be accurately determined by its size and weight. The larvae weighing 1—2 mg are developing the best. Because of the great differences in weight between larvae of the same age, it is recommended to specify their weight, not their age. The bee larvae weighing less than 0.5 mg are more sensitive when reared in the laboratory ; they even grow more slowly. Of such small larvae — so called "egg larvae" — the number of queens (%) obtained is not greater than that of queens resulted from larvae weighing 1—2 mg. The small bee larvae are easily taken out from comb cells, with the very narrow grafting tool, without damaging the combs. When a little larval food is also removed together with the larva, the risk injuring the small and sensitive bee larvae is reduced. The larva is grafted into a rearing recipient into which 0.25 ml royal jelly (2 : 1 or 1 : 1 dilution, as mentioned above) was previously pipetted. Care must be taken to place the larva with the dorsal part

up to keep it away from the food; otherwise, its respiration will become obstructed and it will die. When too little royal jelly is available, on the first day of rearing, 4—5 larvae may be placed together into a recipient on 0.25 ml of royal jelly solution.

The test larvae are then introduced into the final recipients fixed into the stands described above and covered with a plastic sheet, in an incubator at 35°C temperature and 85—90% air humidity. It is recommended to begin the experiment in the morning, which allows for adding, late in the afternoon, 0.1 ml nutrient solution into each recipient — *i.e.* 2 drops, with a graduated or Pasteur pipette. By slightly inclining the recipients, the royal jelly would flow down against their wall and reach the underside of the larvae which will thus float, with no risk of being suffocated by the added royal jelly.

2nd day : 0.1 ml of royal jelly, prepared as described above, is given to the larvae in the morning and in the evening. If, on the first day, several larvae were placed into one recipient, in the morning each larva must be grafted into a separate recipient with 0.25 ml of diluted royal jelly in it ; in the evening, they are also given 0.1 ml royal jelly.

3rd day : the larvae are fed just as on the 2nd day. Fig. 26 illustrates the stage of development of larvae on this day.

4th day : only if necessary, namely if the royal jelly is highly viscous and larvae would not accept it, one drop of royal jelly solution is given to each larva in the morning.

In the afternoon, the unconsumed royal jelly is carefully removed (sucked up) by a pipette. A Pasteur pipette, with a rubber cup, is used for this purpose. When the number of test larvae is greater, connection of the pipette to a waterspout is recommended, to suck up the royal jelly with great care. With a certain experience, one can recognize — after the shape of the larvae — which of them will soon eliminate faeces and spin the cocoon. For the larvae whose active feeding is not finished, a little royal jelly must be left in the recipient.

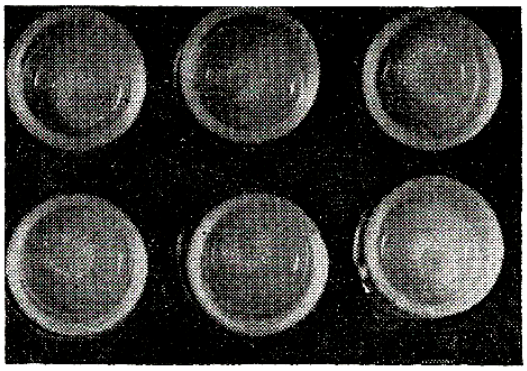


Fig. 26 — *Bee larvae on the 3rd day of rearing*

5th day : in the morning, the last royal jelly is removed from the recipient, to provide for dry ground for the start of pupation. Most of the larvae from which all royal jelly had been removed on the pre-

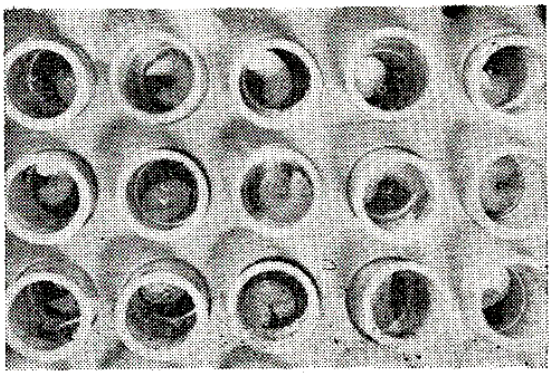


Fig. 27 — *Bee larvae on the 5th day of rearing ; they will spin the cocoon after the royal jelly has been removed*

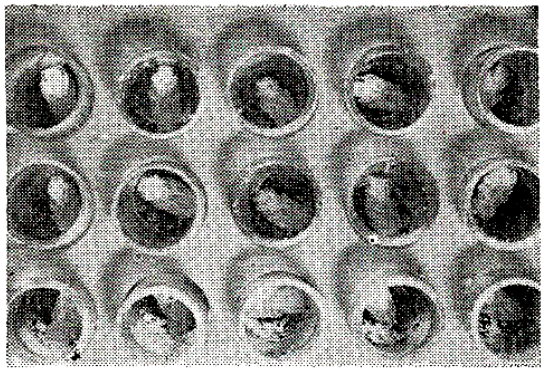


Fig. 28 — *Bee pupae with various colours of eyes, on the 8th day of rearing in laboratory*

vious day, have exhausted the food possibly left in the recipient and began to eliminate faeces. A few are already spinning their cocoon (Fig. 27).

As the plastic sheets covering the recipients prevent evaporation, they must be removed to provide for better ventilation and prevent accumulation of moisture.

6th day : the larvae are left in the incubator as undisturbed as possible.

7th day : several stretched larvae turn into pupae. It was found that queens resulted from all test larvae developing into pupae on that day. Thus as early as 7 days after the beginning of the test, the number of resulting queens can be appreciated.

The pupae in recipients face various directions, but this has no effect on their further development.

8th—9th days : the pupae determined to become queens or intermediate individuals with queen characteristics prevailing are distinguished from the worker pupae by the more marked pigmentation of the eyes and the early coloration of the chitin (Fig. 28).

10th day : the pupae with queen characteristics or of intermediate individuals (see above) must be separated, as they might kill one another. They are therefore placed, either with their recipient inside the compartments in the box — as described above (Fig. 25), or together with the cup, in a recipient with cover.

The stretched larvae which had not started pupation or were only semipupae (which happens more often on the 10th day) are dead and they are eliminated from the test.

11th—12th days : the first bees emerge ; they are true queens with the same period of development (16 days, including the egg and

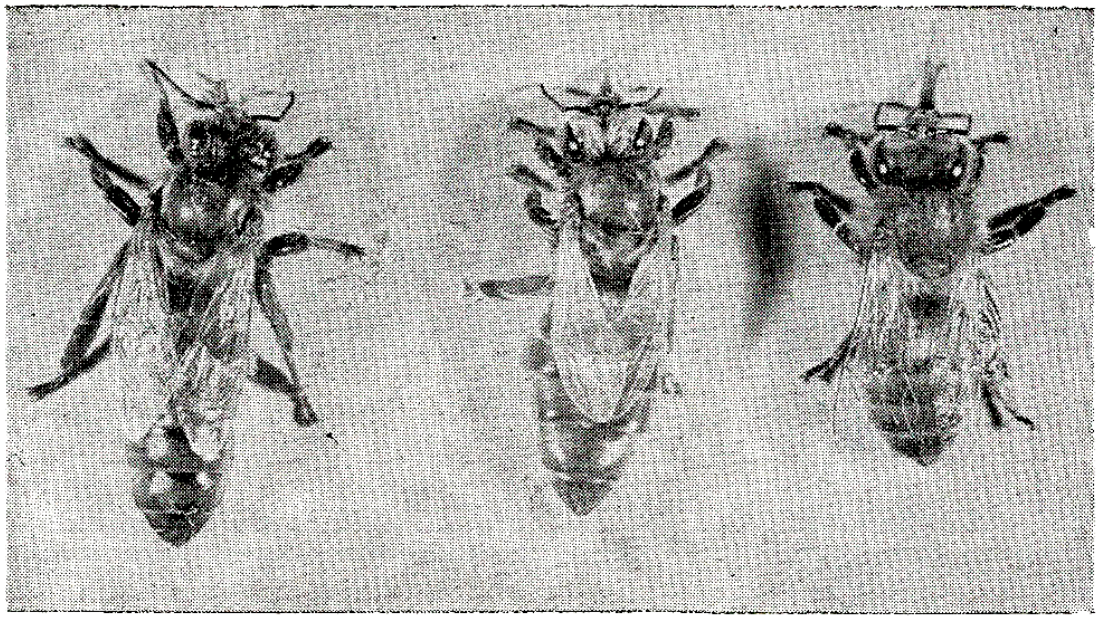


Fig. 29 — *Queen (♀), intermediate individual (Zwf), and worker bee reared in laboratory (☿)*

larval development in the hive before the test) as the queens reared in natural conditions in the colony (Fig. 29 ♀).

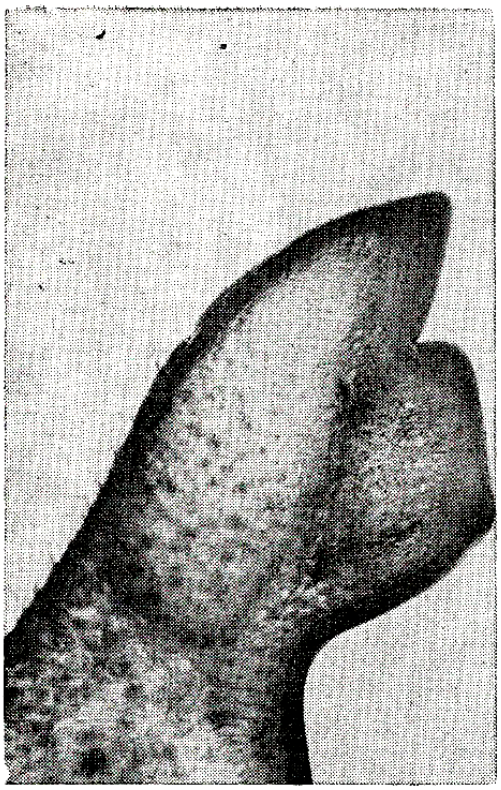
13th day : if very young larvae, weighing 0.5 mg or less, were used, queens may still emerge. Also, intermediate individuals, with more marked queen characteristics, emerge.

14th—15th days : mostly intermediate individuals emerge, with characteristics of both queen and worker bee, but often with greater similarity to worker bees (Fig. 29 Zwf). On the 15th day, also true worker bees emerge.

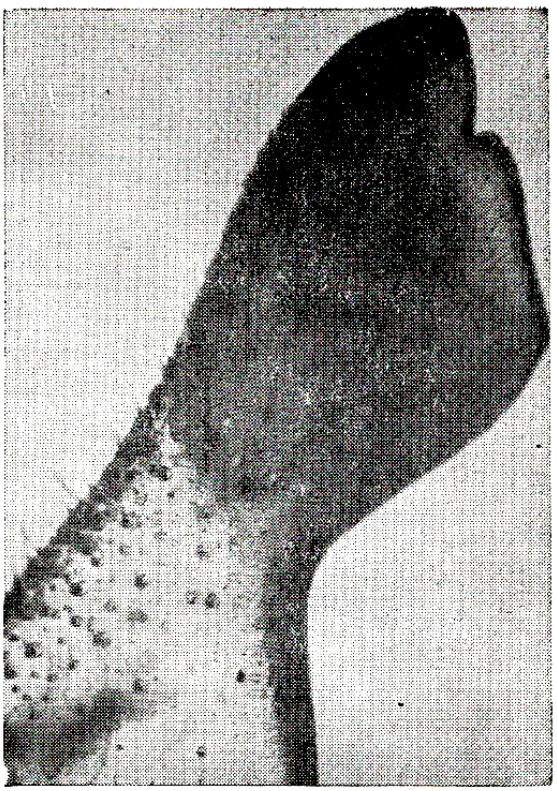
16th—17th days : during these two days, the other animals turn into adults too, worker bees exclusively (Fig. 20 ♀).

2.3. *Assessment of the individuals obtained*

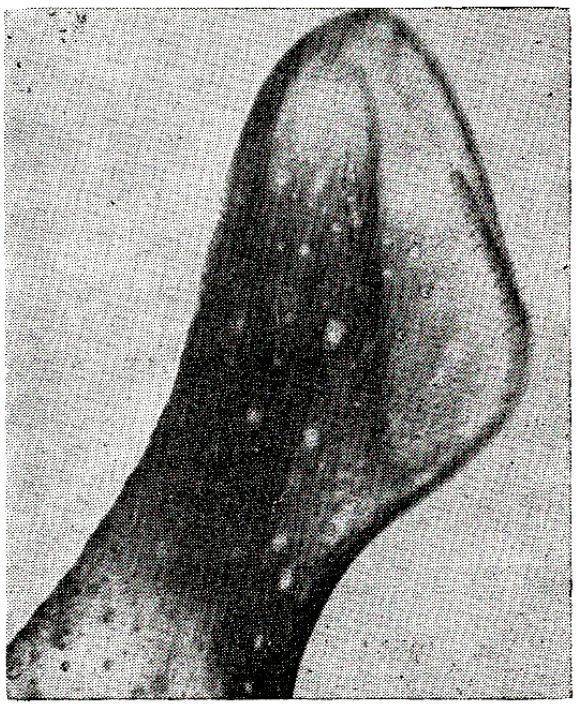
The queens and worker bees reared in laboratory are evaluated according to the caste specific characteristics. First to be examined are the weight, the number of ovarioles, size of the spermatheca, shape of the head and of the mandible, and the development of the metatarsus and of the sting. In terms of weight, number of ovarioles, shape of mandibles, metatarsus development, etc., "intermediate individuals", between true queens and worker bees were frequently obtained (Figs. 29—31). Because most of the different characteristics are correlated one to another in their specific caste formation, it is sometimes enough to evaluate a number of individual characteristics, easy to examine — such as shape of mandibles, and the metatarsus (Figs. 30, 31).



♀

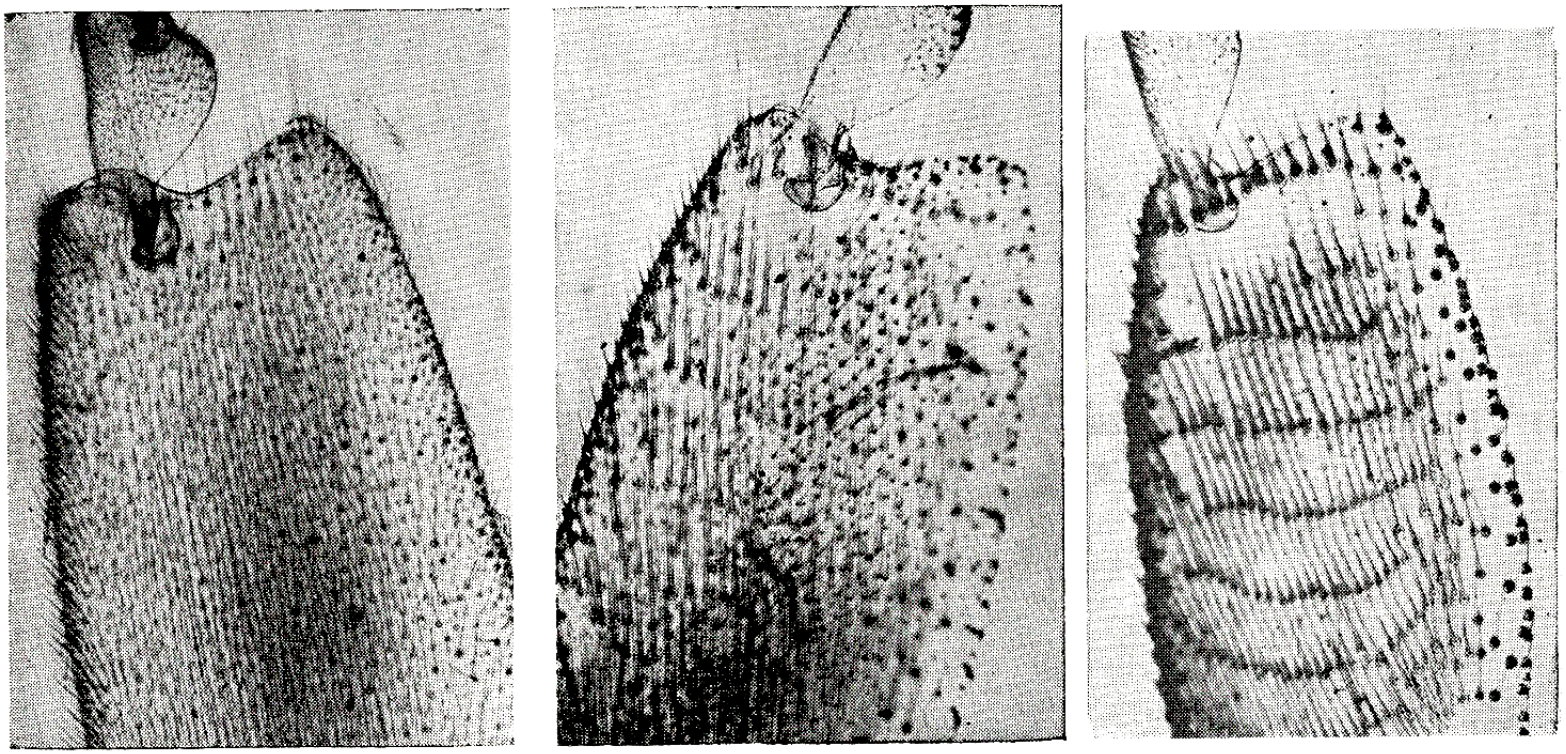


Zwf



☿

Fig. 30 — *The mandible of a queen* (♀), *of an intermediate individual (Zwf), and of a worker bee* (☿).



♀ *Zwf* ☿

Fig. 31 — *Position of hairs on the hind tarsus of a* queen (♀), *of an intermediate individual (Zwf), and of a worker bee* (☿) *(magnification (32×).*

In the bees reared in incubators, the ovaries are sometimes so deeply covered by the fat body that they are difficult to identify and evaluate by unexperienced investigators particularly in intermediate forms. The same observation was also reported by N. WEAVER (1957) and M. V. SMITH (1959). Therefore recently, H. REMBOLD and collab. (1974) have no longer examined the development of ovaries for determining the caste, during their experiments for isolating the queen determining substance. During his experiments of rearing queens in laboratory, M. V. SMITH also took into account only the external characteristics of the test animals; but he did so only after the statement that queenlike mandibles and hindlegs indicate a queenlike development of the internal organs.

2.4. *Results of rearing experiments*

Table 6 gives some results of the more than 1,500 rearing experiments, each of them with 60 bee larvae in general. The comparison of results show that when rearing larvae with freshly collected or with lyophilized royal jelly, the average rate of survival did not differ significantly (about 5%). Also, the percentage of adult bees with queen characteristics was in both cases almost the same. In a number of tests, lyophilized royal jelly stored in the refrigerator, for more than a year at —20°C, was used as well, and it was found that it had not lost its queen determining capability. Usually, when royal jelly contains components detrimental for larvae, this would be obvious as early as the first two days of experiment by the larvae turning brownish and by their slow development. Similar phenomena were recorded to occur when larvae were fed with formulae of royal jelly not including vital substances. During all tests the greatest losses were recorded when stretched larvae developed to the pupal stage, on average only 60—70% of the larvae turning to pupae. Often, because of incomplete moulting of pupae, semi-pupae would result, which, being unable to develop further, would die. This incapacity of normal moulting of pupae was most obvious during a test when larvae were fed with lyophylized royal jelly having been stored at —20°C for more than 3 years. Only cca. 30% of the stretched larvae reached the pupal stage, in some tests the percentage of pupae obtained being even lower. Moreover, from the larvae fed with the royal jelly stored for such a long time, worker bees were obtained almost exclusively. This shows that the queen determining effect of this royal jelly was lost.

It was strange that when using royal jelly collected in Israel, rearing of bee larvae in the laboratory failed, or only a small number of larvae survived. The reason might be the origin of the royal jelly concerned — from bee colonies of Italian race —, while the bees used for rearing were of carnica race. The royal jelly, a gland secretion of nurse bees, might have different chemical compositions in different races, in terms of protein substances for example. No investigation on the royal

Table 6

RESULTS OF EXPERIMENTS OF REARING BEE LARVAE FED WITH VARIOUS TYPES OF ROYAL JELLY, IN INCUBATOR, AT 35°C. SIXTY 1—3-day OLD LARVAE WERE USED IN EACH EXPERIMENT (WORKER LARVAL FOOD : 75 LARVAE)

Larval food	Stretched larva	no. pupae	no. adults	% survival	no. ♀	no. intermediate individuals	no. ♂	% ♀+intermediate
Royal jelly (fresh)	53	44	36	60	12	9	15	58
	57	51	50	80	19	20	11	78
	50	49	41	68	13	14	14	66
	58	39	39	65	6	4	29	26
	54	22	22	37	8	4	10	55
	272 (91%)	295 (75%)	188 (92%)	62,0±7,0	58 (31%)	51 (27%)	79 (42%)	56,6±8,6
Royal jelly (lyophilized)	33	21	21	35	15	3	3	86
	36	16	15	25	6	5	4	73
	43	43	43	72	16	4	23	46
	59	46	46	76	15	10	21	54
	43	27	27	45	5	7	15	44
	53	48	48	80	13	7	28	42
	52	42	42	70	21	7	14	67
	319 (76%)	244 (76%)	242 (99%)	57.6±8.4	91 (38%)	43 (18%)	108 (44%)	58.9±6.4
Royal jelly (lyophilized, stored for 3 years)	51	24	21	35	0	0	21	0
	53	16	14	23	0	3	11	21
	56	14	11	18	0	0	11	0
	39	8	5	8	0	0	5	0
	199 (83%)	62 (31%)	51 (82%)	21±5.6	0 (0%)	3 (6%)	48 (94%)	5.0
Royal jelly without determinating fraction (basic food)	54	37	35	58	0	3	32	9
	60	43	41	68	3	4	34	17
	59	43	36	60	0	2	34	6
	58	45	43	72	1	4	38	12
	53	33	30	50	2	1	27	10
	59	37	32	53	0	1	31	3
	59	39	39	65	0	2	37	5
	60	44	39	65	1	1	37	5
	56	38	36	60	0	1	35	3
	58	49	48	80	1	2	45	7
	54	43	42	70	4	3	35	17
	56	42	42	70	3	3	36	14
	52	32	32	53	0	1	31	3
	52	45	40	67	1	1	38	5
	53	46	45	75	1	2	42	7
	842 (94%)	616 (73%)	580 (94%)	64.4±2.2	17 (3%)	31 (5%)	532 (92%)	8.2±1.2
	60 (80%)	37 (62%)	32 (86%)	43	0 (0%)	0 (0%)	32 (100%)	0

In brackets : Per cent survival as compared to the precedent stage, resp. distribution (in per cent) of the surviving adults (= 100%) to the three types of differentiation (♀, intermediate, ♂).

jelly produced by different bee races has yet been made as far as I know.

Rearing of bee larvae was also successful when using royal jelly from which the component required for queen development had been chemically extracted. The average number of stretched larvae, pupae, and adult bees obtained with this royal jelly was not smaller than that obtained when feeding bee larvae with natural royal jelly. It is true that, as mentioned in the paragraph on the food used in artificial rearing of larvae, during these experiments we had to add some vitamins and aminoacids to the royal jelly of which it had been deprived by chemical processing. From these rearing experiments, using "basic royal jelly", the number of individuals with queen characteristics was very small, obviously due to the fact that the queen determining substance had not been completely eliminated.

From the bee larvae fed with pure worker larval food — taken from cells with 2—3-day old worker larvae, pure worker bees were obtained, with a satisfactory survival rate (43%); (H. REMBOLD and Gisela HANSER, 1964). This clearly shows that the larval food is distinguished from royal jelly in terms of quality. The young female larva is not determined to turn into a queen by a greater amount of food, but by at least one specific substance, constituent of royal jelly.

The question whether the queens reared in the laboratory are perfect queens also in terms of biological function has been raised many times in the course of the years. Queens reared in laboratory have often been introduced into mating nuclei. After the required period of time, these queens would start laying fertilized eggs. Unfortunately, observations of such queens could never last more than one year. The same was reported by M. V. SMITH (1959).

3. General conclusions concerning rearing in laboratory

When processing the results of the experiments, it is found that from the bees reared in the laboratory, on royal jelly, in addition to queens also intermediate individuals and typical worker bees often are obtained. Neither N. WEAVER (1955, 1958, 1974), nor M. V. SMITH (1959) have succeeded in rearing only queens in the laboratory.

In the development of the larva into queen, other factors are obviously involved. Decisive in this respect is not only the effect of the quality of the food, but also the stage of development when the bee larva is fed with the specific food and responses to it.

The success of rearing queens under laboratory conditions is decisively determined by three factors:

1. Appropriate *dilution* of the *royal jelly*: dilution of the royal jelly is an essential premise for rearing larvae in the laboratory. For a long time, this fact was not given the proper attention, and this might be the cause of all laboratory rearing exepriments having re-

sulted in more or less complete failure. In the hive, during natural feeding, the royal jelly is liquid being constantly supplied by the nurse bees. Royal jelly would become viscous only after a longer storage into the combs inside the hive, and sooner when stored in the refrigerator. The young larvae are not able to fully consume this royal jelly ; they would consume less food and implicitly less determining substance (N. WEAVER, 1966, 1974 ; H. REMBOLD, B. LACKNER, I. GEISTBECK, 1974), as well as less additional substances required for accomplishment of the determination, e.g. various vitamins, above all biopterin. In order to have a sufficient amount of these substances administered to the larvae, the dilution of royal jelly must not exceed a certain level.

2. *Age of bee larvae* at the beginning of the experiment ; an important premise for successfully rearing is the age at which the young bee larva is taken from the hive and grafted on royal jelly in the laboratory. The assumption that grafting the youngest possible larvae on royal jelly would be more advantageous — as it provides the larvae, the earliest possible, with the nutrient substances necessary for the development of the queen —, has not been ascertained. M. V. SMITH (1959) and A. DIETZ (1964) managed to rear bee larvae just after hatching from the egg in the laboratory, while our experiments and those of other authors (I. JUNG-HOFFMANN, 1956 ; N. WEAVER, 1974) have shown that the 2nd day of larval stage is the most favourable time for grafitng he worker bee larva on royal jelly. Replacing of food always means chilling of the young queen larva, which, together with the change of the food, means disturbance and consequently discontinuation of development.

As reported by N. WEAVER (1974) the moultings related to the development of the bee larva are succeeding very closely one to another, namely at the age of 3/4 — 1 day, 1 3/4 — 2 days, 2 1/2 — 2 3/4 days, and 3 1/4 — 3 1/2 days after hatching from the egg. It is known that insects are highly sensitive to all external factors during moulting, being easily injured. It is consequently most likely that the bee larva too is disturbed in its development whether it is taken from the comb cell in the hive in order to be grafted on royal jelly in the laboratory just during moulting.

It is likely that the food on which the larva was fed in the hive, in the first two days, has an influence on the results of rearing in the laboratory. Contrary to the data reported by I. JUNG-HOFFMANN (1956), it was found that the results of rearing often depended on the season when the experiments were started. The content of biopterin, pantothenic acid and B_6 vitamin in the larval food varies with the season (Gisela HANSER and H. REMBOLD, 1960 ; Gisela HANSER, 1971). In spring, in the phase of intensive increase of the bee colony and maximum care of brood, the content of these substances in the larval food is very low. In summer — July and August, when the amount of brood in the colony is smaller, and bees for winter — with longer span of life — are reared, these suvstances are present in the larval food in significantly higher concentrations.

The occurrence of one phenomenon was noted during the rearing experiments: the bee larvae taken from the colony late in summer are developing better on royal jelly in the incubator, and from them a greater number of queens and intermediate individuals are obtained than from the bee larvae taken from the colony in the first weeks of spring ; — these showed a much weaker response to the determinative effect of royal jelly. Contrary to the larval food, the contents of biopterin and pantothenic acid in royal jelly is much higher (G. HANSER and H. REMBOLD, 1964), as well as of B_6 vitamin (G. HANSER, 1971), and consequently we designated these substances as "indicators" for royal jelly, although they do not have a direct effect on the queen determination of the larva (H. REMBOLD and G. HANSER, 1964).

3. *The proper time for removing the royal jelly* at the end of the larval stage : another very important aspect of the artificial rearing of queens in laboratory is the removal of the royal jelly around the aged larva before it starts spinning the cocoon and prepares for pupal moulting. At that time, feeding of worker larvae in the hive normally ceases. But the queen larva will continue to eat ; she ceases ingesting food only on the next day, after the elimination of faeces (N. WEAVER, 1974).

According to my experience, as early as this stage of development estimations may be made on the basis of certain differences in structure : the queen larvae are slimmer, of a cylindrical shape, the cross section of their body being a circle, while the worker larvae have convex thickenings on both sides of the body, which makes the cross section rather angular. N. WEAVER (1974) also reported different shapes of larvae, but in his opinion these were by no means sure evidences of one or the other caste.

The larva must be separated from food at the right moment, namely just before pupation, so that the latter should take place at ease, in a dry cell cup. We have removed the royal jelly by suction, contrary to other authors, who, for this purpose, would take the larvae out of the rearing recipient. Only insignificant traces of royal jelly would be left on the walls of the recipient providing some food to the larvae in case of need ; this also reduced the risk of depriving the larvae of food too early. Thus pupation of larvae will be disturbed to the least possible degree, and the cocoon will be spun normally as a prerequisite of the pupa and imago moulting.

The greatest losses during rearing in the laboratory were recorded at the transition from the stage of stretched larva to the pupal stage *(see the table in page 76)*. Very frequently semipupae occur whose moulting of the front part of the body is incomplete. W. v. RHEIN (1933) assumed that these semi-pupae were intermediate individuals which, because of a malformation and a disturbed development cannot moult normally before developing into pupa. But our rearing experiments have not confirmed this assumption. Some other reasons are more likely to be the cause, as for instance weakening of the larva by insignificant feeding, or injury of the larva during spinning — in fact during the stage of

stretched larva, when it is very sensitive —, because the stretched larvae would often die before the incipient stage of pupa. The "wrong" position of the larvae in the rearing recipient however is certainly not the reason for incomplete moulting of the pupa, because during our experiments the pupae faced in most different directions the rearing recipients.

None of the rearing experiments with feeding with fresh royal jelly taken from cells with 1—2-day old larvae has resulted up to now in queens exclusively. Again and again, as we have already mentioned, intermediate and sometimes even worker bees would occur. Because of the complicated combined effect of the determining substance in the food, and of the response pattern of animals in certain, sensitive stages of their development, the results of rearing will hardly be possible to be improved. With sufficient royal jelly being available, it might be more advantageous to transfer the larvae, every day, on a new fresh royal jelly, instead of supplying them with royal jelly, every day. On the other hand, the frequent change of food might impair and delay the development of larvae. The experimental results of H. REMBOLD, Ch. CZOPPELT and P. J. RAO (1974) show that the abundance of the determining substanace administered together with the food may be of decisive importance during larval development. When a three times greater amount of determining fraction, than in natural royal jelly, is added to the test larval food, 98% queens are obtained, only 2% intermediate individuals, and no worker bee. The aim of the artificial rearing namely obtaining only queens from female bee larvae, is almost attained.

This method of rearing perfected along many years should first and foremost be an instrument for increasing the content of the determining substance in royal jelly. It also gives us a model picture enabling a more thorough examination of the mechanisms of differentiation, which in this case depend on external factors introduced together with the food. The switch of the differentiation specific of caste is turned by the determining substance only relatively late during the development of the bipotent female bee larva. Consequently, by specific food we can control the turning of larva either into a queen or into a worker bee. We also have the possibility to observe and induce, under controlled conditions, changes in structure and metabolic differences depending on time and stage of development. This model picture enables us to have an idea about the first phases of the processes of differentiation taking place in an organism. The generally valid problems of differentiation of the physiology of rearing — of fundamental importance for biology and biochemistry — are thus elucidated.

CHAPTER V

The influence of rearing condition on queen development

K. WEISS

The quality of a queen is determined by her parentage and by the way she had been nursed. In this chapter we will discuss aspects of nursing only. Optimum nursing implies that optimum conditions were provided during the developmental period of larvae and pupae.

Queen and worker bees originate from the same ontogenic breeding material and the different development of the two castes is exclusively determined by nutritional factors. It may therefore be assumed that the factors which influence the nutrition of larvae directly or indirectly will also have an influence on their development. This may be of great significance if we are rearing queens artificially when, in addition to the natural variability of the conditions for rearing, the consequences of any manipulations performed by the beekeeper can have an effect on the conditions within the cell raising colony. We must expect that we will obtain perfect female reproductives or females with malformations and that the emergence of either depends on the rearing conditions. The former can be assessed as "optimum queens" when their characteristics and properties are examined, while the latter diverge more or less from this ideal pattern.

It is not at all easy to define what we mean by the term "optimal queens". On one hand we may have queens which had been raised in the honeybee colony under absolutely natural conditions of swarming and yet we will find quite remarkable variations of characteristics. On the other hand, important differences can be due to the parentage of the queens. KOMAROV and ALPATOV (1934) reported that queens originating from southern Russia were smaller than those from central Russia. HOOPINGARNER and FARRAR (1959) found that evident differences exist between the weight of queens bred from various inbred lines and that of their hybrids. KOMAROV and ALPATOV (1934), ECKERT (1934) and BURMISTROVA (1965) counted different numbers of ovarioles in various races and strains. By rearing queens time and again in nursing colonies without replenishing the population of bees of nursing age in one colony of *A. m. mellifera* and another of *A. m. carnica*, I obtained significant differences in terms of number of ova-

rioles, width of head, length of tarsus and the duration of development of larvae and pupae (WEISS, 1972). It is therefore not surprising that the egg laying activities of queens of various races and lines shows much variability.

In order to assess the effect of various queen rearing techniques on queen development accurately, comparative rearing experiments are needed. Certainty over parentage and a large number of experimentally reared individuals is prerequisite for evaluation. Those characteristics which clearly distinguish queens from worker bees are of greatest interest to us in this evaluation. Additionally we must make allowances for any influences which are not related to caste formation.

We can group all possible influences on the development of queens under the following headings: 1. Living Factors; The Graft; 2. Mechanical Factors; The Queen Cell; 3. Nutritional Factors; Brood Food; 4. Environmental Factors; The Microclimate and the Weather. These are also the four sections of this chapter.

1. Living factors: the graft

Chapter VI explained that when we use the term "graft" we think of the early stages of honeybee females. Either fertilised eggs or the youngest worker larvae may be used as the starting point for rearing queens. It it also well known that the way the beekeeper treats his chosen breeding material influences the results of queen rearing.

1.1. *Age of graft*

Female bee eggs are identical in structure and genetic potential and are indistinguishable from one another except by their location. Worker bees will develop in worker cells, while queens would emerge from queen cells. Yet when a queen is lost, bees are able to change worker cells into queen cells. In such a case the conversion of the young worker larva into a queen is simply a matter of feeding, the composition of larval food playing the decisive role (see Chapter III). The honeybee colony's ability of changing young worker larvae into queens by altering the brood food provided has been exploited widely in the artificial rearing of queens.

Rearing from larvae is relatively simple and good results are obtained in the acceptance of grafted queen cells. Following the thorough investigations by ZANDER and his students, (1916, 1925) concerning the quality of the individuals obtained, this method has been beyond doubt. Recently however, the method was questioned when the results of more detailed investigations showed that even the very young worker larvae and queen larvae are fed different food. JUNG-HOFFMANN (1966) reported that nurse bees give young queen and worker larvae two types of brood food components — one "white" and one "clear" — in significantly differing quantative ratios. STABLE (1930) and WANG (1965) recorded differences in nursing from a very young larval

age. In addition, metabolic differences and serological particularities appear already at a very young age in the larvae of both castes (MELAMPY and WILLIS, 1939 ; SHUEL and DIXON, 1959, 1968 · DIXON and SHUEL, 1963 ; LIU and DIXON, 1965 ; LUE and DIXON, 1967 ; OSANAI and REMBOLD, 1968 ; TRIPATHI and DIXON, 1968 ; CZOPPELT and REMBOLD, 1967). The differences of the components of the two types of royal jelly still require chemical analysis — (see Chap. II) at least in terms of quantitative assessment (see JOHANSSON and JOHANSSON, 1968 ; SHUEL and DIXON, 1960 ; TOWNSEND and SHUEL, 1962 ; REMBOLD 1964 ; HAYDAK, 1968). Following such observations it was expected that differences in characteristics and performance might be discovered in adult queens which may depend on the age of the chosen larva. A queen would be expected to be nearer the "ideal type of the perfect female", the younger the larva had been when grafted. Extrapolating from this point of view, queens reared from eggs should be the very best ; so lately many insistent attempts have been made to develop a more practical method of "rearing from the egg". Practical aspects of rearing queens from larvae and from eggs are described in Chapter VI. In this chapter we will only investigate the problem whether the use of eggs instead of larvae is necessary at all, and which larval age is the best for rearing queens.

1.1.1. The age of graft and acceptance

It has been mentioned before that when bees rear emergency queens they usually change worker cells with larvae into queen cells rather than use those with eggs in them. It seems as if the age of the larva is unimportant to them. Even if the colony has only larvae of the same very young age available when it is dequeened, the bees will not only build queen cells over these, but also over other larvae as they grow gradually older. When I introduced tough old brood combs with eggs to nursing colonies, almost half of all queen cells were drawn out 1—2 days after hatching and 10% of the total number of cells chosen were built even later over 2—3 day old larvae (WEISS, 1962). After making a similar experiment, OROSI-PAL (1960) reported that he too obtained queen cells which had been raised over older larvae of 3—4, 4—5 and even 5—6 days of age. On the other hand, I have also found some queen cells which had been constructed in new comb over freshly laid eggs (Fig. 32). Though in this case too most of the cells were built later over older larvae. There was no difference when a cut brood comb with scallopped edges was given to a cell raising colony. Only when cells were introduced to the nursing stock with their cell mouth downward (Rearing from egg, Erlangen method), did the first queen cells appear either over eggs or immediately after hatching (see Chapter IV).

The question now arises ; Do bees have a preference for any of the different larvae ages ? Many investigators believe that bees would accept older larvae by preference. ZANDER also reported (1925) that "many more one-day old larvae are chosen for rearing than half-day old larvae". VUILLAUME (1959) on the other hand found that the

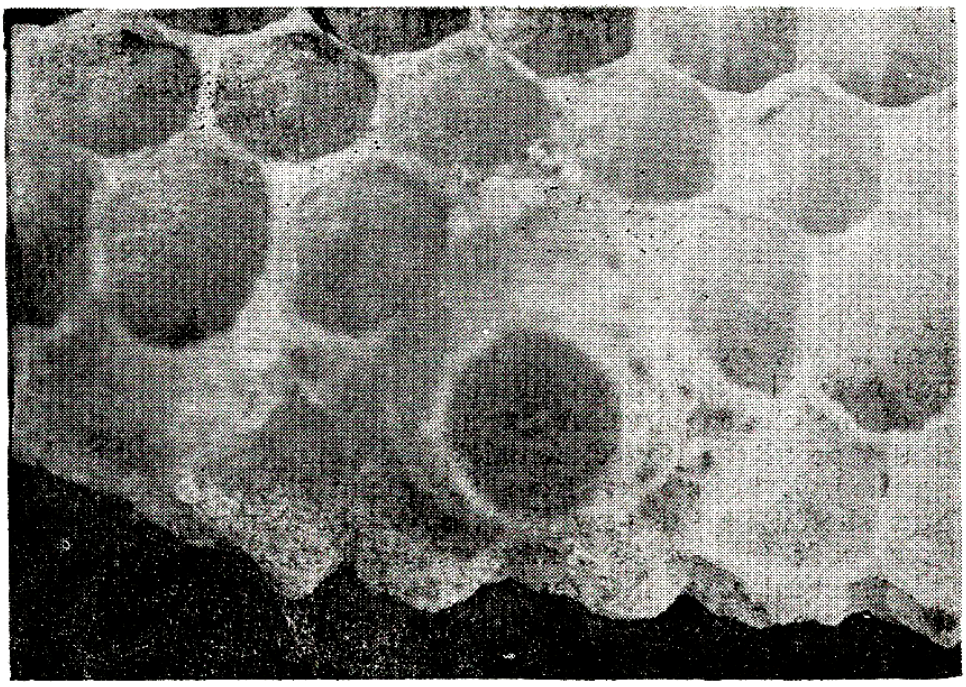


Fig. 32 — *Emergency cells are often erected over eggs on comb which had not been used for brood rearing before.*

age of larvae does not have a great influence on acceptance, even though in his experiments larvae a few hours old were less readily accepted than those 2 or 3 days old. WAFA and HANNA (1967) recorded no difference in the acceptance of one-day or two-day old larvae. KOMAROV (1943) reported that he found that the older the nursing bees are the less they are capable of telling the age of larvae and consequently they will accept older larvae for rearing. Young hive bees, on the other hand, were said to prefer younger larvae. He points out that the true age of the nursing bees need not be conincidental with their physiological one, and this also applied to the true age of larvae and their size.

These last findings were confirmed by many other investigators. The reason for this may be found in the variability of the food supplied to the larval stages, which, — to use GONTARSKI's words — seems to be based on the "shot gun principle". Some larvae may be poorly provisioned. Occasionally this may be due to its position near the edge of the comb, and the lower temperatures prevailing at this point may also play an important role. I myself have found larvae of different sizes occurring when batches of equal age had been grafted. The age of bees in the nursing colony may also be important in this respect.

My experiences concerning acceptance of larvae of various ages gave me several surprises. During one investigation into this problem BÖTTCHER and WEISS (1962) recorded the acceptance of larvae grafted at various ages.

Nine batches of grafts were started in five nursing colonies, in four of these 2 batches were reared one after the other. The ages of the

larvae used ranged at half-day intervals from half day old to $3^1/_2$ days. The grafted cell cups were fastened alternatively to the cell bars of the frame. Of 286 larvae grafted altogether, the bees accepted 151. In these experiments the overall acceptance of young and older larvae was almost equal. Only the older larvae over three days old were accepted in smaller numbers. With such wide experimental results in mind it seems that the frequent complaints by beekeepers about poor acceptance of very young larvae must be due to the grafting difficulties involved with this age group. It is also true that in subsequent queen rearing experiments I discovered that bees have a preference for older larvae (though not older than 2 days !) (WEISS 1974 a). It is fairly certain that in these cases the age of the nurse bees had not been of any importance. A colony-specific tendency could be involved in this behaviour of nursing colonies.

During all the above experiments nurse bees had been able to choose from larvae of different ages. It is even more difficult to prove a preference when only one larval age is supplied for rearing as is usually done in queen rearing practice. Recalling the many rearing experiments which I have conducted in the course of years gives me no right to think that in practice the sole use of larvae of a certain age is likely to influence the rates of acceptance. It is much more important to discover if there is a correlation between the age of graft and the quality of the queens obtained from it and what kind of relationship it will turn out to be.

1.1.2. **Age of graft and the development of caste characteristics**

Initially the morphological development of worker and queen larvae is identical. During a number of investigations into the post-embryonic development of vee larvae, KOZHEVNIKOV, (1905), and Zander and his students LÖSCHL and MEIER (1916), found no difference in structure and histology between worker and queen larvae in the first two days of life. MEIER reported that the first differences in the development of ovaries appear on the second day of larval life, but this was subsequently not confirmed by WANG and SHUEL (1965). According to the latter, differences in one development of the *corpora allata* begin to appear on the 3rd day. MICKEY and MELAMPY (1941) reported to have discovered differences in the cytological development of fat cells on the same day.

The results of the above mentioned investigations on the development of larvae are similar to those of the rearing experiments which were conducted in 1904 by Rev. KLEIN from the Alsace and later more thoroughly by ZANDER and BECKER (1925). Their results showed that the essential characteristics which distinguish queens from workers (ovaries, spermatheca, mandibular and pharingeal glands) are fully developed in queens which had been reared from larvae up to 3 days of age. Caste determination takes place on the 3rd and 4th day of larval life and occurs suddenly. However, ZANDER recommends the use of $1^1/_2$ day old larvae for grafting on the strength of the results of his

weighings. He reported that queens obtained from $2^1/_2$ day old larvae were lighter than those raised from younger ones. Most subsequent investigations also proved that there is a clear dependence of the weight of queens on the use of larvae in their very early stages of development.

However, completely diverging results have been reported when other characteristics were investigated. ECKERT (1934) had not found any vast differences in the number of ovarioles between queens raised from larvae 12, 24, 36, 48, and 60 hours old when grafted. WEAVER (1957) repeated the investigations of ZANDER and BECKER and extended them to cover external characteristics. He too could not discover differences, nor in the spermatheca, between queens obtained from 1—2 day old larvae. In 19 queens which had been obtained from 1-day old larvae he recorded a smaller number of ovarioles (335 $\pm$ 8) than in 19 queens raised from 2-day old larvae (341 $\pm$ 7). In addition, the latter were also found to have a very short glossa, which does not confirm the generally accepted statement that queen characteristics diminish in step with the age of the larvae used for queen rearing. He could also find no difference in the structure of the basitarsus, which is generally a most specific caste characteristic. Only in queens obtained from 3 day old larvae or older did he find a reduced number of ovarioles and a smaller diameter of the ovaries and the spermatheca. In these queens the tarsal index was smaller while the glossa was longer and the number of barbs on the sting was greater. In line with BECKER's results the queens from larvae a little over $3^1/_2$ day old showed obvious transition towards worker type, although not always equally in all characteristics. They were generally predominantly either queens or worker bees. About half of them died before reaching the adult stage. In queens raised from $^1/_2$, 1, and 2 day old larvae, VAGT (1955) found no difference in the shape of the tarsus when he compared with those of swarm queens ; but he reported changes in the pollen brush and the pollen basket of the tibia of adults obtained from 2 day old larvae. Deviations in the characteristic shape of the head, which in queens in more round and in workers more triangular, were reported to have been found already in queens raised from 1 day old larvae, as were the first changes in the round shape of the spermatheca. These results are unfortunately not reliable because far too few queens were examined in this respect. JORDAN's report (1955), that queens reared from the egg by ÖRÖSI PAL's method are superior in the number of ovarioles to those queens reared from larvae, was based on fewer observations still (2 queens in each experimental group). The results of previous experiments by JORDAN (1955), showing a constant decrease in the number of ovarioles in queens obtained from larvae just hatched, and 1, 2, and 3 days of age, were based on averages obtained from 4 queens only. In his work SOCZEK (1965) reports a greater average number of ovarioles, namely 349 (325 — 374) in swarm queens, as compared with 313 (200 — 357) in emergency queens and 312 (289 — 341) in queens reared from one day old larvae. He based his report on his examination of 12 swarm queens, 82 emer-

gency queens and 41 grafted queens. Origin and time of formation of the test groups was not the same for all. This may also apply for the greater number of queens of the experiments made by VOLOSIEVICH (1954). MAUL reported that when comparing queens obtained from single grafts and double grafts with swarm queens, VOLOSIEVICH found a greater number of ovarioles and larger spermatheca in the swarm queens.

Examining 400 queens of the same parentage, ÖRÖSI-PAL found that the number of queens in which the number of ovarioles exceeded a certain level increased the younger the larvae had been when they were grafted. More than 300 ovarioles were counted in 80% of queens reared from eggs ; next followed the queens reared from worker larvae 18—20 hours old — 51% ; those obtained from larvae 42—54 hours old — 39%, and finally those reared from larvae 66—78 hours old with only 12% above the critical threshold. Larvae grafted by WOYKE (1971) did not come from the same breeder queen but from the same line. He found that queens reared from eggs and those reared from 1, 2, 3, and 4-day old larvae showed in addition to the decrease of body weight a reduction in the volume of the spermatheca and in the number of ovarioles. Analogue to this he found that the number of spermatozoa in the spermatheca of both naturally mated and instrumentally inseminated queens decreased.

Summarising these experiments it becomes clear that the development of queens characteristics depends on the age of the larvae used for grafting. But it is doubtful if this is true for the very young larvae. In order to elucidate this problem I made thorough experiments (WEISS, 1971). I paid special attention to using larvae of the same colony and to providing equal rearing conditions. I also used pupae instead of emerged queens for weighing in order to standardise conditions for comparison (WEISS 1967 a), and I counted the ovarioles under a binocular stereomicroscope instead of using microtomy for sections which produces doubtful results (Fig. 33, 34). I reared only from young larvae as is done in practice, and I examined only the characteristics which distinguish queens from workers most obviously : weight, number of ovarioles, shape of head, mandible and the pollen gathering hind leg.

I found that the queens which had been reared from eggs or young larvae — up to $1^1/_2$ days of age — hardly differed at all from one another. Especially the disputed number of ovarioles showed no reduction in step with the use of older larvae. Only the weight of adult queens seemed to decrease with the age of the larvae and this was confirmed by a subsequent investigation. (WEISS, 1974 a). When larval stages younger than $1\ ^1/_2$ days were used, no statistically significant differences were found. Paradoxically, the queens reared from eggs were even slightly lighter than those obtained from 1-day old larvae. However, this was not due to the different age of the breeding material used, but was due to the size of the queen cup. "The queens from eggs" had been started from freshly drawn worker comb, while the "grafted queens" were reared in artificial queen cups 9 mm wide. Large queens are

born from large queen cells (see 2.1.3.). However, the differences in weight between queens reared in this way and those reared from larvae are so slight that they can be neglected in practical rearing.

1.1.3. Age of graft and performance of the colony

Because no clear relationship between weight of queen and the number of ovarioles has yet been established, it is no wonder that a relationship between size of the queen and the amount of brood had been discovered by some investigators (BOCH and JAMIESON, 1960), yet has been denied by others (VESELY, 1968) ; nor has a correlation been ascertained between the number of ovarioles and the egg laying capacity of a queen. Investigating 38 queens ECKERT (1937) discovered no connection, while AVETISYAN and TIMIRIAZEV (1961) found a certain positive correlation between the number of ovarioles and the amount of brood. Confusing findings have also been reported about a relationship between the size of the queens body and honey production. While the investigations of AVETISYAN and TIMIRIAZEV (1961) and AVETISYAN (1967) show that such a correlation exists, the work of SKROBAL (1958) and VESELY (1968) invalidate such a correlation. Still more difficult is the question whether the performance of a queen depends on the age at which the larva was grafted. The work done by the beekeeper KOFER (1960) regarding the requeening of colonies with either queens raised from larvae or queens raised from the egg in the first four years of their lives, spoke in favour of the queens raised from the egg. Yet the production records reported by KRASNOPOIEV (1949) do not agree with that result. Whenever the production records of queens from the egg is highly praised in the bee press, we are usually given no comparisons with other queens raised from larvae. In spite of the difficulties involved in seeking a conclusive comparison of productivity, I attempted to make such an investigation. Three Strain Testing Stations were at my disposal for this work. These provide special facilities for comparative tests of colony performance. My tests comparing queens reared from larvae with queens reared from the egg were made over 10 years in two year evaluation cycles. 72 queens from egg and 74 queens from larvae were involved. The graft queens were obtained from 1 — 1 $^1/_2$ day old larvae and, exceptionally, from $^1/_2$-day old ones. Eggs and larval grafts of the same origin were reared in the same or in similar colonies, and were of equal batch size. They were also taken together to the same mating yard. They were introduced to nuclei of the same strength, whose productivity was then recorded from the following year onwards. No difference could be found between queens obtained from egg transplants and those reared from larvae. Mating results were similar, losses following introduction of mated queens were almost the same and queen losses during the 2 year testing period were also equal. All in all, the colonies of queens from the egg and those reared from larvae produced the same amounts of honey (WEISS, 1971).

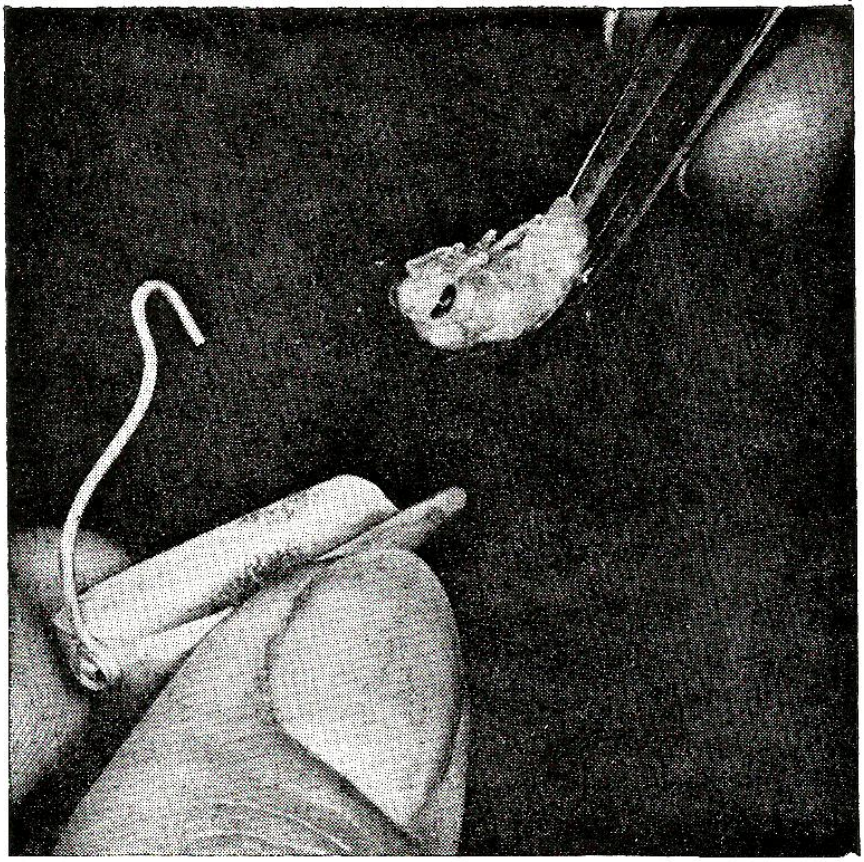


Fig. 33 — *For comparative determination of queen weight, they were weighed during their pupal stage.....*

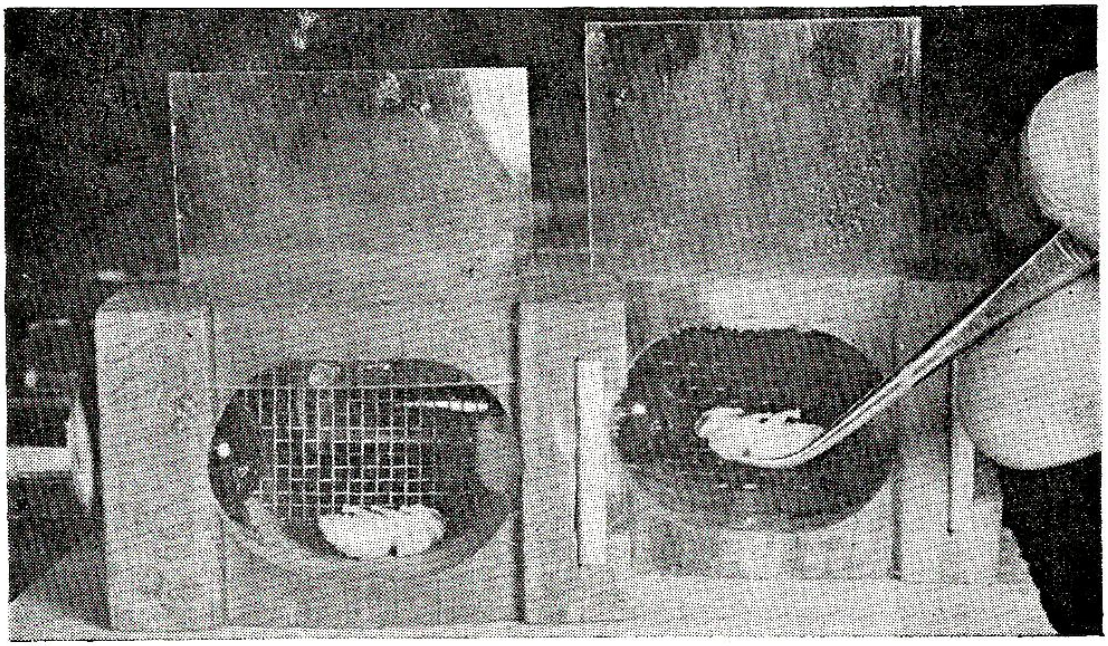


Fig. 34 — *... they were then returned to the incubator in individual nursery cages* ***without their cocoon.***

1.1.4. **Conclusions**

No clear cut differences could be shown to exist between queens reared from the egg and from larvae up to 1 $^1/_2$ day old. But we must expect that queens which had been reared from older larvae may diverge in their characteristics from those of the perfect type, say swarm queens. They may also show different behaviour and it may affect the productivity of their colonies. We find that when using larvae older than 3 days of age we obtain some intercaste types (ZANDER and BECKER, 1925 ; v. RHEIN, 1933 ; WEAVER, 1957).

During its early period of development (1 $^1/_2$-day) the young worker larva its 100% undetermined. The development of caste characteristics starts later. In fact, the same is true for the young queen larva as I have demonstrated by grafting many larvae of accurately recorded ages back into worker cells following the earlier experiments by KLEIN (1904) and WEAVER (1957).

The weight of the queen is the exception — it seems that this may be influenced very early in larval life. From the practical point of view it is of minor importance whether the weight of an adult queen is a true caste criterion ; we can occasionally find typical queens as small as workers ; while huge workers may be obtained by supplying larvae with food in excess — mixed larval food or denatured royal jelly (v. RHEIN, 1933 ; HAYDAK, 1943 ; WEAVER, 1955 ; WEISS, 1978). Nutritional factors in the brood food seem to be of supreme importance in determining size in queens and workers. These same factors are probably responsible for the caste-specific differences in the metabolism of the very early larval stages, even though they may have no influence at the beginning on the subsequent development of either caste.

This must not be taken to mean that the differences in the weight of queens are of no importance in queen rearing practice. When offered two queens of the same origin, he breeder would generally prefer the larger one. This is instinctively considered to be the most productive one. Russian researchers have found that heavier queens are accepted more readily by mating nuclei and that mating and egg laying starts sooner than with lighter queens (from a report for this book by TARANOV).

However, for the practical breeder it is of no importance if small differences are recorded in the weight of queens in large experimental rearing batches, provided larvae younger than 1 $^1/_2$ day old had been used. Differences are hardly significant statistically and in practice may be ignored.

We may therefore conclude that both rearing methods — from egg or from larva — are equally good. He who chooses rearing from larvae can be sure than by using those up to 1-day old he stays well within the safety margin for obtaining optimum queens.

Viewed biologically, both methods of rearing are of equal value, — provided a satisfactory technique is used. The use of larvae or eggs and the choice of method of rearing must remain the decision of the breeder according to his inclination and skill (See Chapter VI).

1.2. *Survival of grafting material outside the honeybee colony*

Artificial queen rearing necessitates that eggs or larvae as well as the later stages of development must be removed temporarily from their natural environment and that they become exposed to external and foreign influences. How long can they survive such exposures ?

1.2.1. **Viability of eggs**

Rearing from eggs is successful if young or old eggs are taken from the colony and are used immediately. But if eggs must be kept outside the care of the colony for some time, possibly during shipment we must remember that all reports in old bee books or other sources of reference are wrong when they state that eggs can survive for several days, even weeks, otuside their natural environment (e.g. WITZGALL, 1906 ; ALFONSUS and MUCK, 1929 ; DÄCHSEL, after ZANDER, 1947 ; SPITZNER, 1950 ; HEROLD, 1956 ; SCHULZ-LANGNER, 1956 ; EHRICH, 1958 ; etc.). These observations were based on wrong interpretations of facts because systematic trials in this respect gave differing results (WEISS, 1960). I cut comb with eggs to pieces and stored them unedr a variety of conditions. The age of the eggs had been established precisely. After different intervals I grafted the pieces in their respective order back into the frame and returned them to the care of the colony. I allowed some of the eggs to hatch in an incubator which had been adjusted for brood nest temperature and humidity. Under these conditions I have never been able to keep eggs alive outside the colony for longer than 3 days. One prerequisite for optimum survival was a minimum age of the egg of 1 $^1/_2$ days. Results : only few eggs survived outside the colony for 3 days ; after 2 days of storage outside the colony half of them survived at the most ; after one day storage practically all eggs survived the treatment. It proved immaterial whether the comb had been stored in a cellar (15—18°C) or in a room (18—22°C), in humid places (up to 100% RH) or in dry ones (20—25% RH) ; in well lit places or in the dark ; lying or standing up. Only in the refrigerator (5°C, 60% RH) eggs would not keep well. Eggs younger than 1 $^1/_2$ days old and, so it seemed, those which were ready to hatch, died after short periods of exposure. No larvae or only few would hatch when such eggs were stored in a cellar or room even for one day only.

Sometimes bee colonies remove eggs which according to the above trials should be capable of development. We may be fairly sure, for example, that bees remove eggs from supers in which no other brood is present. Eggs seem to "belong" into the brood nest. But there too failures have been recorded in queenright colonies and even in hopelessly queenless ones, which were really dependent on eggs for their own survival. These are puzzling exceptions.

1.2.2. **Viability of larvae**

In contrast to the generally optimistic opinion of beekeepers about the possibility of storing eggs outside the bee colony, most of them think that unsealed and sealed brood is very vulnerable. Yet there are reports in beekeeping literature which contradict such opinions (HIMMER 1927), and we may recall the practice of heather beekeepers who used to recommend the "driving" of all bees from a skep and storing the brood nest denuded of all bese overnight (LEHZEN, 1880). There are no precise data about cold resistance of brood and these could only be obtained after exhaustive trials (WEISS, 1962). I tested resistance to cold of brood in the same way in which I had tested eggs ; I stored pieces of comb with larvae of various ages for various periods in various places. Afterwards I returned them to the colonies for further development. 65—100% of the very young larvae (0, $^1/_2$ and $^1/_2$—1 day old) survived 24 hours in every place of storage. 12—70% of the 1—2 day old larvae continued to develop ; and 16—73% of those 2—3 day old. No relation could be found to exist between survival rate of these stages of brood and the place of storage (cellar or room) ; only in the refrigerator did older larvae not survive their ordeal. After 48 hours only a small percentage of the very young and the very old larvae had survived, but none of the latter survived in the refrigerator. Very few larvae which had been close to being capped over were alive after 3 days, and these could only develop if stored at room temperature. Adults obtained from surviving larvae were fully developed when left to emerge in the incubator.

The viability of the very young larvae is of special interest in queen rearing. In spite of the good survival rates achieved in the trials, it seems that larvae which had been stored outside the colony for 24 hours are useless for practical purposes. Acceptance by the nursing stock is quite unacceptable even after 12 hours of neglect, at least with the grafting method used by me. Losses for unknown reasons were recorded in addition to the predictable ones which were arising from storage. On the other hand larvae were well accepted if stored for only 6 hours. Repeatedly I grafted larvae which had been taken from the brood nest as well as others of the same age which had spent 6 hours in a comb stored in cellar or room under variavle conditions of temperature and humidity. Grafted into the same queen rearing frame and returned to a nurse colony no difference in acceptance could be observed. It is possible that we can extend the period of storage without damage even further if the environment is not too dry. This

was not investigated. Queens reared from larvae which had been stored for some time always developed perfectly. 1—2 day old queen larvae, once accepted and provisioned with royal jelly are just as insensitive to cold as young worker larvae. The possibility of storing young female bee larvae outside the bee colony is of great importance for the distribution of selected genetic material to other breeders.

2. **Mechanical factors : queen cells**

Prefabricated queen cups instead of natural queen cells are used as a royal craddle in artificial queen rearing, although some methods utilise natural worker or drone cells for this purpose. The use of artificial cups poses questions of acceptance of the graft by the nursing colony and about the supply of larval food to the growing larva. Two problems ; yet both are decisive for the successful outcome of queen rearing.

2.1. *Properties and positioning of queen cups*

We should not expect bees to accept just about everything we give them in substitution for their natural queen cells. It stands to reason that any foreign material or wrongly constructed shapes which may differ greatly from the natural thing may be rejected. But the responses of bees are unpredictable.

2.1.1. **Material : wax**

How do bees behave towards material from which queen cups are formed ? Until recently it was believed that these had to be made of pure beeswax and the choicest quality at that. It was considered important whether the wax had been rendered from new or old comb, from scrapings or from cappings. In the relevant literature we find recommendations for the use of the finest, lightest beeswax. French beekeepers prefer cappings wax, in some German beekeeping circles the opinion prevails that cups must be formed of "virgin" wax, which is that obtained from the wild comb constructed freely in the "building" frame in which no brood has been raised (ZANDER, 1944). To discover the truth I made queen cups of the same size and shape from beeswax rendered from newly drawn wild comb and others from wax from old, tough brood frames. I fastened both kinds alternately to the same grafting frame given to a nursing colony (WEISS 1967 a). During three such tests there was not the slightest difference in acceptance. (I should

add that the beeswax had been extracted with a wooden wax press and had been refined twice. It is possible that when beeswax with impurities or even foreign matter is used the results could be different). VUILLAUME (1957 a) recorded differences in acceptance when using a variety of wax and comb raw materials for forming queen cups. He is convinced that the propolis content in beeswax reduces acceptability.

"2.1.1. **Material : plastic**

However, bees also accept queen cups made from other materials, especially plastic substances (Fig. 35). BOGNOCZKY (1967) even managed to force queens to lay the eggs into queen cups of plastic material. VUILLAUME (1957) obtained good acceptance of cell cups made from paraffin wax and a number of artificial resins as well as of some made from glass, — provided no other cells made of beeswax were present in the hive at the same time. SMITH (1959) and WAFA and HANNA (1967), who used plastic queen cups for the production of royal jelly, reported no differences in acceptance when compared with those made of beeswax. They found that cups made from the wax obtained from sugar cane would not be accepted. RAZMADZE (1976) reported that no cups made from polyethylene had been accepted over the previous few years. I myself have introduced many queen cups made of various synthetic materials as well as some made from wax for additional choice by the nursing bees. Bees accepted the cups made of polystyrol and perspex just as well as they accepted those made of beeswax. But they rejected those made of Hostalen * and Lupolen. This proves that the choice of the material is very important indeed. I discovered an interesting fact of minor importance during my test. Platsic cups constructed with an integral base plate for attachment were not accepted. When I dipped them into liquid wax so that the base plate was covered in beeswax, acceptance was very good. It is possible that bees could not get a grip on the smooth surface and therefore neglected the offered graft.

SMITH (1959) recommends that a second batch of larvae is grafted immediately after the removal of the remains of royal jelly if the plastic cups are to be used all over again. Acceptance is poor when remnants of old brood food have dried up. In such a case it is best to dip the grafting frame into water and let bees clean it out in the hive before using it again. (There is another, simpler way for anyone who maintains wax moth colonies for research at suitable temperatures. They will clean cups up in a few days.). On the other hand, the plastic cups should be so cheap that they can be discarded after being used once.

*) *Hostalen : a proprietary brand of polyethylene*

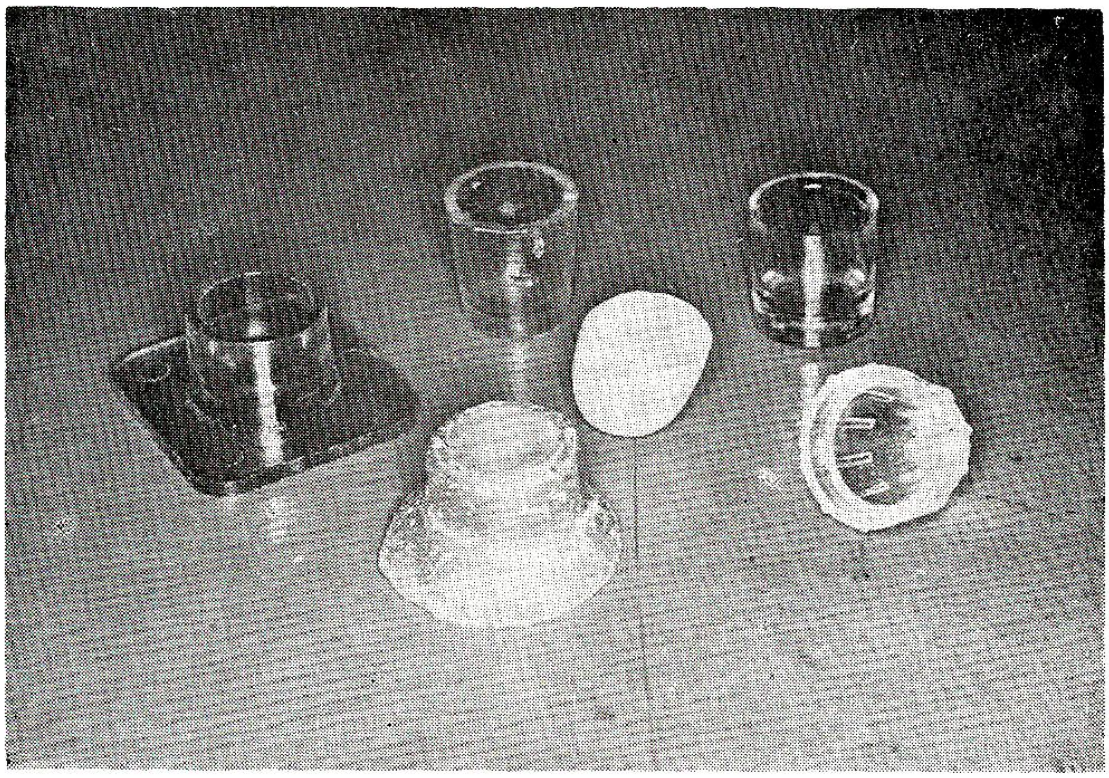


Fig. 35 — *Selection of queen cups of various materials and forms*

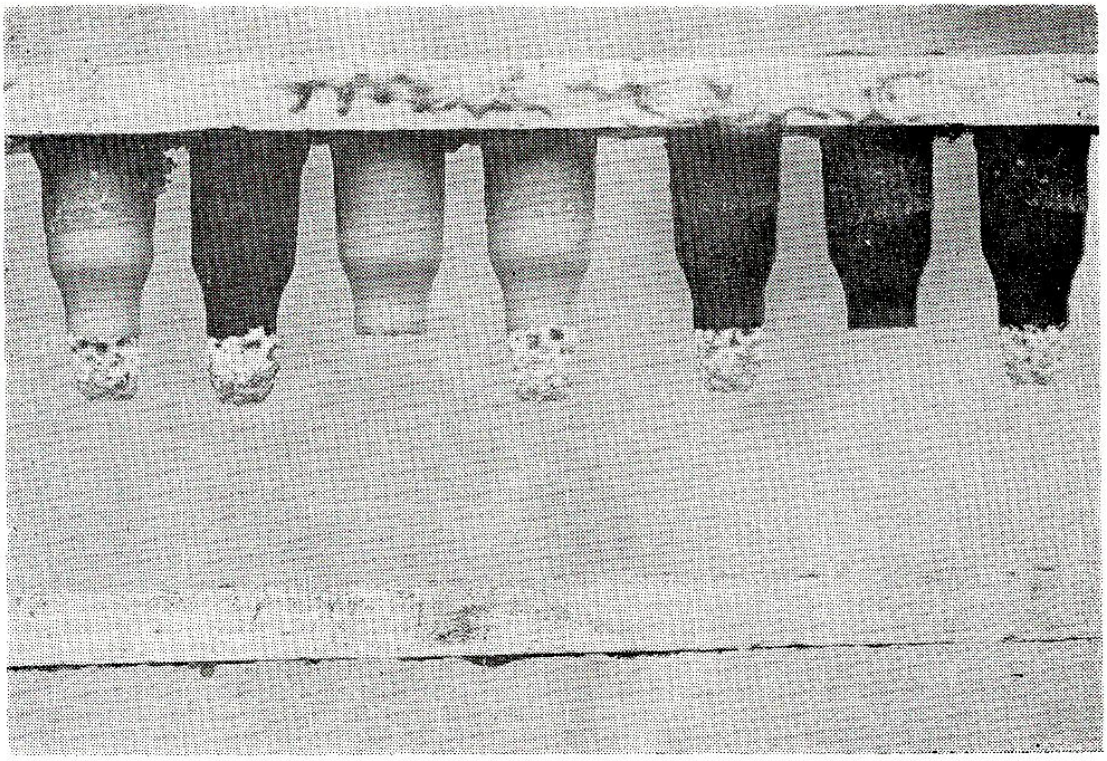


Fig. 36 — *Australian queen cups made from plastic have a deep base. Here they are shown two days after grafting.*

2.1.2. Size and shape of queen cups

Bees accept cells of various sizes and shapes ZANDER (1944) established that natural queen cups are 7.8 mm in diameter and are 8—10 mm deep. VUILLAUME (1975 b) states the natural diameter to be 8 mm. RUTTNER (1965) reports the size of a completed queen cell to be 8.5—9 mm at the level of the rim of the cup. It is possible, of course, that the size of the queen cup depends on race and is variable within limits.

As can be expected, queen cups are well accepted if they fall within the range of natural sizes. That bees accept occasionally smaller cups is shown by the fact that they can convert worker cells and drone cells into queen cells. Queen cells may either be in a vertical or a horizontal position. VUILLAUME (1957 a) stated that the vertical one is preferred by bees.

Given the choice between worker cells with larvae and artificial cups made of beeswax, bees preferred the latter (ÖRÖSI-PAL, 1960). ZECHA (1959) reported the opposite, but she had used punched worker cells for her comparative trials and the brood food already in these may have affected their attractiveness. During a number of comparative trials which I made myself with drone and worker cells and with dipped queen cups made from beeswax of an inside diameter of 8 and 9 mm, bees would always accept the drone cells in preference to the worker cells, and the queen cups with a diameter of 9 mm gave better results than those measuring 8 mm across the mouth of the cell. There was a tendency towards poorer acceptance in step with the reduction in the size of the receptacle for the graft. During tests made by VUILLAUME (1957 a) a starter colony initially accepted the larger cups of 9 mm less than those of 8 mm; but a cell finisher colony sealed more of the larger queen cells.

The cups with a diameter of 9 mm are at the borderline of the natural range. Bees would probably accept still larger cell cups, but they would soon be forced to make unaccustomed changes to them and this would bring about more and more rejections. (VUILLAUME, 1967 a, b).

VUILLAUME found that bees would prefer cylindrical cups to those with angles. They did not accept cups when the rims touched one another. Very short (0.5 mm) and very long cells (20 mm) were accepted with slightly greater reluctance than the 8—10 mm size. On the other hand, they seemed to prefer the cups with a rounded bottom to those with a flat one. VUILLAUME and NOULLEAU (1957) also reported that bees made repairs to cups with a 1.5 mm hole; but larger tears at the rim were not closed so easily.

The thickness of the wax of the cups seemed quite unimportant for acceptance. For years the Research Institute of Erlangen had been using cups with relatively thin walls. These were made by dipping the queen cup moulding tool only twice into the molten wax; only the bottom was reinforced by dipping a third time. Yet many breeders use cups

successfully which have walls twice as thick or thicker still and obtain theirs by compression moulding. The top edge of the cell cup need not be smoothed over as bees accept cups with a burred edge just as well as those with a smooth rim.

We must note that differences in acceptance in these comparative tests are also important in practice. Bees are usually offered an abundance of cells to choose from in these trials and they then can show their preference for one type of cell cup or another. It has not been investigated if bees would accept fewer cells if they were given a type only which they had neglected while they had the choice.

2.1.3. Size of cell-cup and weight of queen

More important than mere acceptance is the problem of the weight of the queens which had been reared in cell cups of different shapes and sizes. We know that the weight of worker bees depends on the size of their cells. The more generations of brood had been reared in a comb the smaller becomes the diameter of the cell and the smaller are the bees which emerge from it (see JAY, 1963 ; GLUSHKOV, 1964 ; ABDELLATIF, 1965).

Bees which had been reared in comb constructed from foundation with a larger cell diameter had a weight which exceeded the average. Could this fact not be applicable to queens too ?

There are reports in literature that bees provisioned larger queen cups with more food than the smaller ones. (VUILLAUME, 1957 a ; BURMISTROVA, 1960 ; WAFA and HANNA, 1967). WEAVER (1957) reported that he obtained queens from cell cups made of glass and with a 6.5 mm diameter which were hardly different in queen characteristics from their sisters which he had reared in normal queen cups made from beeswax. Yet the same was also true about 3 individuals which he had reared in glass cups with a diameter of 10 mm. During my own tests in this respect (WEISS, 1967 b), I compared the weight of queen pupae. These had been reared in cell cups with a diameter of 8 and 9 mm or from drone and worker cells. I used 2—3 types of cups or cells at the same time in each nursing colony. Usually the drone and worker cell cups were supplied as cell strips into which I grafted larvae every 20 mm apart. I want to give here a few comparative data : the average weight of 102 pupae which had been raised in 9 mm cups was 284.7 mg and was about 10 mg heavier than the average of 85 queens from cups with 8 mm diameter. 20 pupae from the 8 mm sized cups were on average 25 mg heavier than 44 pupae which had been reared in converted drone cells. 51 pupae of the last kind were weighing in the mean 21 mg more than 34 pupae which had been raised in converted worker cells. These differences in weight were usually statistically significant. Consequently we can say that queen weight decreases with the reduction in size of the cells in which they are reared, just as with worker bees. It has not been established

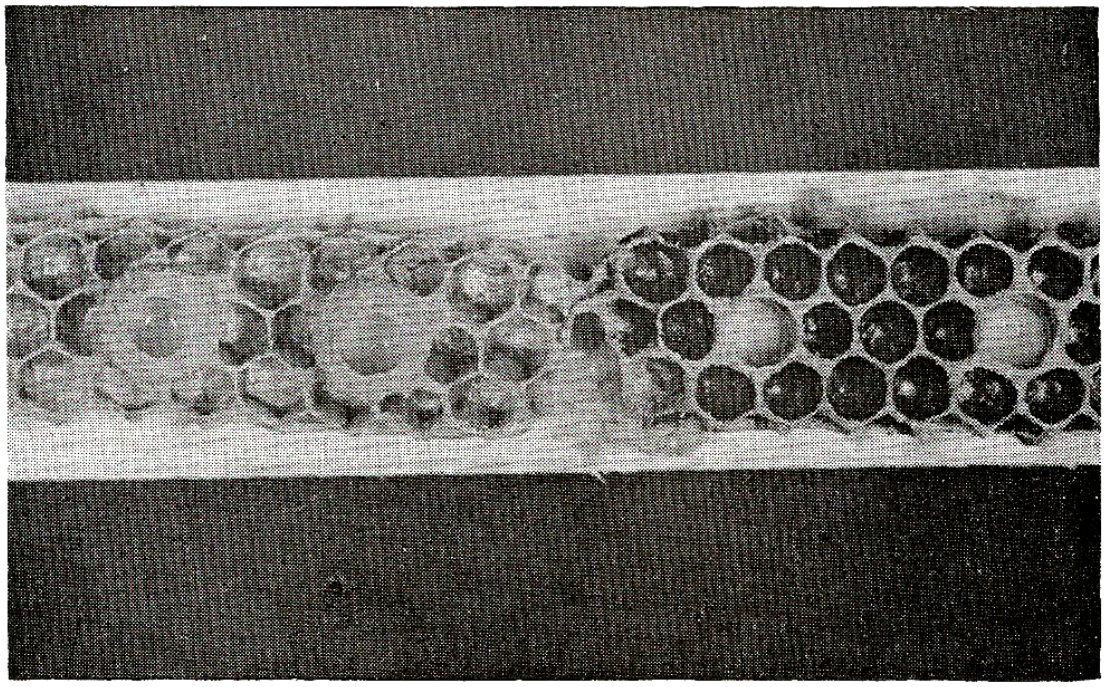


Fig. 37 — *When strips of comb are used for grafting, bees will begin to mould the accepted cells much sooner if it had not been used for brood rearing (left). Tough old brood comb on the right.*

if we can expect a further increase by using cups over 9 mm in size, because bees begin to reject such cell size.

During the trials with drone and worker comb cut into strips I found that the queens which had been reared in unused comb were clearly heavier than those queens from comb in which brood had been reared before. The photograph of the early changes on one strip of used comb and one which had contained brood clearly shows that bees are prepared to commence work on the fresh comb much sooner and will enlarge them by moulding the wax further back towards the base. Yet they would never go right back to the base of the cell itself with this work. The more cocoon layers there are in an old cell the more difficult it becomes for workers to alter the shape of the original cell (Fig. 37). A queen cell drawn from a worker cell is narrower than one which had been constructed over an artificial queen cup. During the drawing period both types of cells show an equally large mouth. When queen cups are used, bees actually reduce the leading opening, yet take care not to reduce the cell size so that the initial diameter is maintained behind the mouth. Therefore we will have larger cells grow from a larger cup, and larger queens can develop in these.

Bees provision large cells with more royal jelly than they do with smaller cells. This observation can be confirmed by weighing the amount of royal jelly remaining in the base of queen cells of various sizes after pupation of the queen larvae (Fig. 38). In commercial production

of royal jelly it is well known that more can be harvested from cells of the 9 mm size than from the ones of 8 mm diameter (VUILLAUME 1957a). Whether the excess of food in the larger cells also plays its part in increasing the size of queens, apart from the larger room they provide for development, is not known for certain.

Some conclusions may be drawn from the trials of rearing queens in cell cups of various sizes and these may be useful for practice. Only large cups should be used for grafting ; those cups with 9 mm diameter are best. When using cell strips of empty worker or drone comb for grafting we cannot recommend the use of old comb because of the net decrease in the weight of any queens reared from them. For this very same reason I see only disadvantages if the cell punching method is used to obtain worker cells with young larvae in them, unless freshly drawn comb is used. Then larger queens may be obtained, even though the lack of cocoon layers, which lend greater strength to the cell walls, will make the work more difficult. (Rearing queens from the egg uses groups of cells and thus takes the weakness of freshly drawn comb into consideration. See Chapter VI).

Another question needs clarifying : are larger queens more prolific and do they produce more honey ? In general, the answer must be : Size in queens is not determined by rearing factors alone, their

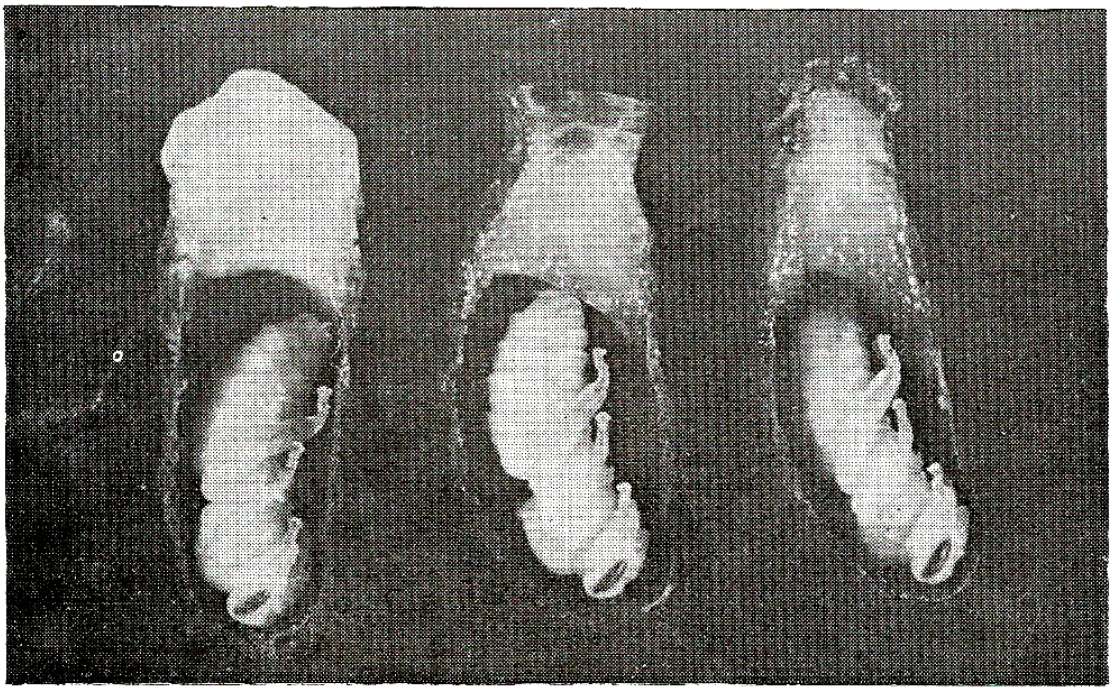


Fig. 38 — *The residual amount of royal jelly in sealed cells depends on the size and the properties of the cell cup chosen for the graft. From left to right : An artificial queen cup has been used with a ∅ of 9 mm ; a larva was grafted into a worker cell drawn from foundation not used before for brood rearing ; a worker cell cut from tough comb had been used in this instance.*

genetic endowment contributes greatly. Any animal with an inheritance tending to produce a large body may be a failure in terms of productivity. This is possibly the reason why no conclusive evidence has been supplied regarding a relationship between a queen's size and her prolificacy, never mind the honey production of her colony. On the other hand, when we are dealing with identical characteristics, then the rearing conditions alone determine their size in a queen. So we may assume that greater body weight and quality of a queen are correlated to a certain degree. As we can influence the size of a queen by choosing a cell cup adequate enough for full development, there is no reason why we should not make the best choice from the start.

2.1.4. **Positioning of cell cups within the nursing colony**

In a colony building emergency cells, we can often find them so near one another that they touch. Other cells are to be found over half a frame away or are even spread over several combs. The choice of location appears to have been made at random. In artificial queen rearing we usually arrange queen cells in rows along a cell bar of a grafting frame. VUILLAUME (1957 a) made trials with various distances between artificial cell cups. He came to the conclusion that a distance of 2 cm between centres of neighbouring cups will give optimal acceptance and supply of brood food.

Although the emergency cells can appear anywhere within the brood nest and at any height on a frame, breeders are constantly worried about adopting the position where optimum conditions prevail. Some believe that grafts placed high up in the grafting frame are more likely to be accepted, while others think the position mid-way or lower is the better one. VUILLAUME (1957 a) and SOCZEK (1965) have found no difference. I am convinced that many factors such as hive type, size of frame, distribution of stores on the comb, even race may be involved, if it is not in most cases simply a matter of chance. It seems to me that the location of the cups along the cell bar, either too far forewards or backwards, is of greater importance than the positioning of the cell bar in the grafting frame itself. Actually, acceptance of peripheral cells was no worse during my tests than of those cells placed near the middle. It appeared to me though, that from such cells occasionally some extremely small or large queens emerged. Quite often the larger queens had developed more slowly than the rest. Such findings were usually recorded when I had used weak colonies for rearing or when several batches of cells had been reared already in the nursing colony. On one hand, bees might have neglected some peripheral cells (small queens) ; on the other hand, it may have been the lower temperatures in that area slowing down the development and extending the period of active feeding, which resulted in the emergence of obviously large individuals.

When considering the distribution of cell cups within the nursing colony, we should give a thought to spreading the load over two grafting frames several combs apart. Especially in the very strong nursing

stocks this will help to distribute the larger number of nurse bees more evenly so that they can work more effectively and that more cells will be provisioned optimally (see 3.2.7.). DREHER (1960) also thought on such lines. TARANOV (1974) believes that is it of advantage, if the gap which will take the grafting frame is opened up 4—6 hours before its insertion. He is also convinced that when successive batches are reared every 5 days in the same nursing stock, it will help, if the grafting frames are inserted in the same place every time as bees would have become accustomed to attending to the unsealed cells here. Queens reared this way are said to be heavier.

2.2. *Familiarisation*

To familiarise means to become acquainted. In queen rearing we use this term to describe the period during which queen cups are left in the nursing stock, or when the larval brood to be grafted is introduced to their nursing colony before the work of queen grafting actually begins. This is expected to improve the acceptance of the graft.

2.2.1. **Familiarisation of artificial cell cups**

When VUILLAUME (1957 a) offered queen cups made from two different samples of wax to one nursing colony, he found that they accepted one kind less well than the other. He then introduced the neglected cups, still empty, to another colony for 24 hours before he began his next test. He found that their acceptance had been enhanced. VUILLAUME assumed that the queen substance which circulates throughout a harmonious colony, and which suppresses the drawing of queen cells (BUTLER, 1954), also adheres to combs and is retained by the wax rendered from them. Cell cups made from this would also contain a trace. He suggests that a substance of acceptance acts in competition. This is added to the cups during the period of familiarisation. This latter substance he suggests to be highly volatile in contrast to the persistent queen pheromone.

The experiments made at Erlangen by BÖTTCHER and WEISS (1962), could not support a hypothesis of two antagonistic substances. We provided bees with both adapted and unfamiliar cell cups to choose from. One day before grafting we placed two grafting frames, each containing only one cell bar, into a nursing colony. The cell bars had been put alternately, one high, the other low, into the grafting frames. The next day a second cell bar was added to each frame, filling the unused space. Both batches of cell cups were now grafted together with larvae of the same age and origin and the frames were returned. Out of 68 grafts one colony accepted 48, the other 39. The ratio of adapted cups and unfamiliar ones was 31 : 27 and 18 : 21 respectively.

The rate of acceptance of the unfamiliar cups was slightly better than of those which had spent a period of familiarisation in the hive ; the results though are probably due to chance. Things were different, when we used strips of comb rather than cell cups. For this experi-

ment we cut the strips from comb which had been used once for brood rearing. They were cut three cells wide and were fastened to the cell bars with their cell openings downwards. Again, each nursing colony received one cell bar only in each grafting frame for a period of familiarisation of one day ; next day another cell strip was added and all strips received grafts at 2 cm intervals. 6 such batches with 24 larvae in each were given to moderately strong nursing colonies ; Only 25 larvae were accepted. 24 cells were accepted on the familiarised strips of combs and only one cell was accepted on the unfamiliar ones. It is clear that bees preferred the larvae in the familiarised strips of comb.

While the results of the first experiment offer no proof of the existence of one factor of inhibition and another one of acceptance, as suggested by VUILLAUME, one could see the results of the second test as confirmation. Yet it may also be explained simply by saying that bees preferred the prepared cells to the fresh ones, or that some unpleasant scents had been eliminated during the period of familiarisation. The truth is, that the combs from which the strips of cells had been cut, came from the fumigation chamber and had been treated with brimstone. A similar scent factor could have influenced VUILLAUME's experiments when he found that cell cups formed from wax extracted with acetone were not accepted as readily as those made from wax which had been rendered in water. The method of wax extraction could have removed some of the attractive materials. The same applies to the tests which VUILLAUME made by washing previously familiarised queen cups in alcohol, acetone and water. Improvements in the rate of acceptance similar to that which he had obtained through familiarisation in a colony for 24 hours, were achieved simply by storing the cups in an incubator for one day, or after exposure to the rays of the sun for 2 hours.

Allowed bees a free choice in our experiments no difference resulted in the rate of acceptance between larvae in strips of comb which had been familiarised and those which were fresh. We formed 2 groups of four colonies each, with roughly equal colony strength. One group was given the strip of comb 24 hours before grafting; the other group received freshly cut and grafted strips. All grafts were done on the same day and rearing was interrupted 5 days later. Now we reversed the arrangements and the colonies which first had the familiarised strip of comb received the fresh piece and the others were given the accustomed strip. In all 16 experiments bees accepted 130 larvae out of the 168 which had been grafted. 66 queens were reared in the familiarised strips of comb and 64 in the unfamiliar comb material. There is practically no difference. Rates of acceptance were very variable from colony to colony and ranged from 1 to 22 cells per graft. Colonies with good results, when given familiarised comb, were also good nursing stocks when they received freshly grafted strips. Poor colonies did not improve when the test was reversed.

As it is unlikely that in practice different cell cups are used competitively in the same nursing colony, I feel it does not matter about familiarising them before the grafting takes place. There is not doubt, that optimal acceptance can be achieved without the process of familiarisation, provided that the best conditions for queen rearing are achieved in the first place. It is of course feasible, that if forced, bees will finally accept cell cups which they had rejected initially because of strange scents or because it is foreign material ; but such cells should not have been used from the start.

2.2.2. **Familiarisation of eggs and larvae**

In days gone by there was a firm conviction that a nursing colony had to become familiar with the eggs and larvae it was to rear into queens. The old "Zander" grafting frame had a space between the top bar proper and the uppermost cell bar. This was designed to take a piece of comb. One or two days before the grafting was to be done, a piece of comb containing eggs only was cut from a comb from the breeder stock and was fastened into this gap. This grafting frame, already equipped with wooden cell holders, was now given to the nursing colony. Grafting was done after the eggs had hatched and the larvae were 1—1½ days old.

We have made a number of experiments this way. As soon as the larvae had hatched we transferred them into every second cell cup. The cups in between received unfamiliar larvae from a fresh comb. The results showed that nurse bees did not prefer the previously familiarised grafts. This provided the proof that there is no need to leave eggs for some time in the nursing colony in order to improve the rate of acceptance.

2.2.3. **Grafts from nursing colony ?**

When we are rearing queens in a queenright colony, there is the possibility to utilise larvae from its very brood nest. This raises the question : will bees accept larvae of their own queen more readily than strange ones ?

BÖTTCHER and WEISS (1962) made 3 trials during which the nursing colony was rearing larvae of their own queen as well as those from another stock, the different grafts alternated in the grafting frames. We found that bees do not prefer the larvae of their own blood. Therefore it does not matter in the least, whether the breeder uses the breeder colony as nursing colony or gets another stock to rear his queens. In general practice both breeder colony and nursing stock belong to the same race. This was also the case in the above mentioned experiments. No one has tried to see if a vast difference in racial characteristics could influence acceptance. After all, deviations from the character of the queens reared, towards the type of the nursing colony are quite feasible. (see 3.3.1.).

2.3. *Priming the cell cups with royal jelly*

Many breeders follow old recommendations and prime all cell cups with a small drop of royal brood food before grafting. They usually save this royal jelly from colonies in a swarming mood and keep it for use at a later date in well stoppered glass bottles and in the cold. This practice is called "wet grafting". Similarly we speak of "double grafting" when the breeder transferes into dry cups some larvae from any colony and removes them, usually after 24 hours, from the cells. He now grafts fresh larvae from the desirable strain into well provisioned cell cups. Both methods are widely used by queen breeders. The first mainly for the reason that larvae are easily grafted into a bed of fluid, and because better acceptances are expected from it. Both methods — especially double grafting — are thought to give many advantages during larval development and result in a better quality of the end product, the queens. Are we justified in raising such hopes ?

2.3.1. **Grafting "wet"**

The danger of injuring larvae is reduced when we can slip off the tiny grub into a bed of fluid. In practical work this may be the real reason why "wet grafting" gives better rates of acceptance. Whether the nurse colony has a "preference" for such larvae on royal jelly is still a disputed question. While FREE and SPENCER-BOOTH (1966) thought that priming cell cups with royal jelly is not necessary, VUILLAUME (1957a and 1959) is convinced of the opposite. He found that larvae which had been grafted into cells after they had been primed with undiluted royal jelly, were accepted as readily by swarm box bees as those larvae which had been transferred into cells primed with diluted royal jelly and others with just plain water. On the other hand, he found that more of the first kind were sealed by the finisher colony. He also reported that the larvae which had received the pure royal jelly were provisioned with more brood food than those which had been given the diluted food. He also stated, that when more royal jelly was provided initially, the production of royal jelly increased. Royal jelly which had been stored in a refrigerator for one year was just as effective as the freshly obtained material. Together with BÖTTCHER I have been able to make my own experiment in the practice of grafting wet when using queenless nursing colonies. In 5 competitive trials we provisioned every second queen cup along the cell bar with a drop of freshly extracted royal jelly, the size of a hemp-seed. This royal jelly had been obtained from queen cells with roughly two day old larvae. The cups in-between remained dry. In each trial we grafted 12 larvae "wet" and 12 larvae "dry" and used only the youngest larvae. While in three of the trials the acceptance of the wet grafts was better, (4 : 2 ; 7 : 3 ; 8 : 1) the results in the other two were slightly in favour of the dry graft (10 : 12 ; 8 : 11). It is surely wrong to draw the conclusion from the totalled results (29 acceptances "dry", and 37 grafted "wet") that larvae grafted on royal jelly are accepted preferentially. The condition of the nursing colony is more likely to play the decisive role : it is quite obvious, that in general acceptance

altogether had been poor in those stocks in which bees had preferred the larvae which had been bedded "wet".

We obtained similar results when we reared queens in a subdivided part of the brood chamber in queenright colonies. In contrast to rearing queens in a queenless hive, one can raise only a few cells at one time with this method. Under these conditions bees also clearly preferred the "wet" grafts. It is possible that bees consider such cells "started", and started cells are accepted by a queenright stock quite readily while neglecting dry grafts. The customary method of "starting in a queenless colony and finishing in a queenright stock", has been developed from this experience.

VUILLAUME had already pointed out that it is not just the moisture factor but the royal jelly which plays a decisive role in acceptance. (see above). Our own experiments with two nursing colonies confirmed this. We gave those colonies some cell cups grafted wet in a drop of water while the others had been grafted dry. As the colonies were old and had been without brood for some time, one would have expected a preferential acceptance for wet grafts. The low rate of acceptance of only nine cells consisted of 6 cells grafted dry and 3 cells which had been grafted into a droplet of water. In a furher experiment, in which we filled some cell cups with an excess of royal jelly (half full), bees accepted the dry grafts more readily in a ratio of 10 : 1. Yet VUILLAUME (1959) had reported better acceptance when 0.1 g food had been placed in the cups instead of 0.033 g.

Finally we must consider that another factor could have an important bearing on acceptance. This is the age of the royal larvae in the cells from which the royal jelly had been removed. TARANOV (1972; 1974) has found differential acceptance due to this, as well as differences in the weight of queens — and this is of greater importance still. When he grafted larve into cells provisioned with royal jelly taken from cells with 3—4 day old queen larvae, he obtained clearly smaller individuals than those which had been grafted into honey. But he obtained larger queens with more ovarioles when he grafted on royal jelly which had been taken from cells with larvae only 12 hours old. These queens also mated sooner and began to lay eggs much earlier. Further experiments showed that royal jelly should be taken from queen cells with larvae 24 hours old at the most. Such royal jelly could be stored for one week at 3—5°C without detriments to the quality of the queens obtained.

Again the question of the queens is brought to our notice. When we come to appreciate it in connection with the method of priming queen cups with royal jelly we never can be cautious enough. This has been shown primarly by all investigations of double grafting — and double grafting is after all the perfection of the wet grafting method.

2.3.2. **Double grafting**

ÖRÖSI-PAL ponted out in 1952 that within 10—30 minutes royal jelly dries up in queen cups in the colony if it is not topped up regularly. The nurse bees on the other hand will not begin to take care of the

larvae until after this time, occasionally hours later. In 1963 he made tests and labelled the brood food with a colour. He did this by feeding bees with sugar solutions containing a red dye. From these experiments he was able to conclude that larvae are fed much sooner after the double grafting technique than after grafting on a small drop of brood food.

All investigators who experimented with double and single grafting techniques were impressed by the long queen cells, which were the result of the large amounts of royal jelly within them. WEAVER (1957) compared queens which had been reared by double grafting in one weak, and one strong colony, with queens reared by simple grafting in a stock of medium strength. He stated that he found obviously larger spermathecae in the queens which had been reared by double grafting in the strong colony. Between the various queens he could not detect any other differences in length of abdomen, diameter of ovaries, number of ovarioles and length of mandibular gland. In a test run by MONTAGNER (1962) the queens obtained from double grafts had developed into larger ones with more ovarioles than the queens from single grafting. The results were significant statistically ; but in 6 individuals of each group the differences were not convincing. MÂRZA (1965) reported heavier queens obtained by double grafting. WOHLGEMUTH (1933) reared queens by double grafting and compared them with others obtained from single grafting on a drop of royal jelly. Rearing was done in various nurse colonies. 13 queens from double grafted larvae weighed 220 mg on average 1—1$^1/_2$ day after emergence. This average was by cca 7 mg heavier than that for the single grafted queens (19 and 8 queens from two other nursing colonies). Such a small difference can not be said to be statistically significant when the variation of the results is 46 mg within the groups, and especially not, if the differences had vanished on reweighing after matings. H. MAUL mentions that VOLOSIEVICH (1954) obtained queens by the double grafting technique which were superior in size and development of their sexual organs to those which had been grafted on a drop of honey. The best time for the second graft is said to be between 10—14 hours after the first graft. After 5 hours he found too little royal jelly in the cells, while after 24 hours the royal jelly was "too old". This latter comment was also raised by JORDAN (1956) against the technique of double grafting. The larva grafted after removal of the first one, when following the usual routine, will not find the type of royal jelly which matches its age. When WEAVER (1955), SMITH (1959), and WOYKE (1963) reared queens manually in an incubator by feeding royal jelly obtained from queen cells of the matching age group, they had better results than when they fed the larvae with royal jelly taken time and again from queen cells with 3-day old larvae. The above mentioned experiments by TARANOV with "wet grafting" gave similar results. He also found, that when he grafted larvae on royal jelly from 24 hours old queens cells, the queens were equally as good as those obtained by VOLOSIEVICH when using the double grafting technique. The quality of these queens was judged by size, diameter of the spermatheca, number of ovarioles and length of poison gland, as these criteria are all corre-

lated to one another (The author of this chapter has certain doubts in connection with the count of ovarioles).

TARANOV quotes SULTANOV to have discovered that bees will accept shiny larvae more readily than larvae of "dull, matt" appearance. This must be related to ecdysis, moulting of larvae.

Because of the contradictory reports in literature about the value of double grafting, I have investigated this problem thoroughly over several years (WEISS, 1974b) At first, I too came to so many varying results, that I could not recognise a principle. Only after grouping all results according to the ages of larvae used for rearing, and constantly adding new findings according to age grouping, did I see the light. I made all tests with queenless nursing colonies. Each colony received one grafting frame with single grafted and double grafted larvae alternating on the cell bar. All larvae used for comparison were of the same age, even though their ages differed in the various trials ($^1/_2$; 1 ; $1^1/_2$; and 2 day old).

Results : 1. When bees were made to choose in a critical test between single grafts and double grafts, they accepted as a rule more of the double grafted larvae (%). Yet the total rate of acceptance was no worse than in the initial trial with exclusively dry-grafted larvae. Adding double grafted larve for choice did not result in an improvement of acceptance. This means that for practical purposes, single grafting without another choice will not result in lower rate of acceptance when compared with all double grafting.

2. Queens obtained by double grafting were indeed reared in larger queen cells (Fig. 39) and a greater amount of royal jelly was left behind

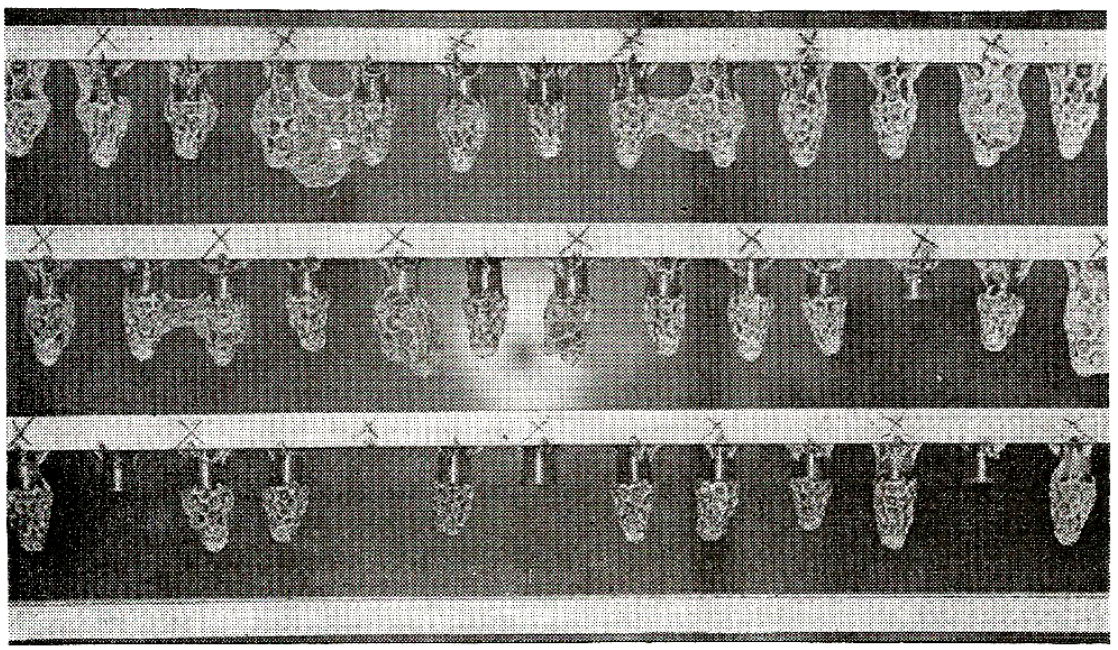


Fig. 39 — *A grafting frame with completed queen cells after using single grafting and double grafting techniques. The double grafted cells are marked X and are longer ; they do not contain heavier queens.*

after emergence. Yet they were not heavier in every case. Often lighter queens developed when 1 day old larvae were used for the first graft, and larvae of the same age were used for the second one after 24 hours. Indeed, they were often lighter than those queens reared by dry grafting. It seems that the excessive amount of royal jelly in these cups — occupied by the now 2 day old larvae — had a negative effect on these fresh and younger grubs. Maybe the queen larvae were neglected for a while because of the excess of food around them. Thic may have affected their rate of growth. Because when I used for the first graft larvae which had just hatched and replaced these after 24 hours with one day old larvae on royal jelly, which suited their age in quality and quantity, the negative effect on growth could not be observed any longer. Indeed, when totalling the results I now found a slight advantage in weight as compared with single grafting. It was only after I used $1^1/_2$—2 day old larvae for the second graft that an obvious, statistically significant difference in weight could be established in favour of double grafting. And it also seemed immaterial now whether these second grafts were placed on royal jelly which suited their age. No differences, apart from their weight, could be discovered between the queens originating from double grafts or those grafted once with larvae of slightly older age. All caste-specific characteristics, such as number of ovarioles, shape of head, the mandibular notch and the tarsus of the hind leg, were typically queen-like. An explanation for the different development in weight of the older stages in this experiment with single and double grafting techniques could be, that because of their greater need for nourishment, the older larvae in the dry grafts suffer more from the delay in provisioning.

For practical queen rearing we can draw the conclusion from the above experiments, that double grafting has no advantages if larvae under one day old are used. The excessive amount of royal jelly in relation to the larval age of the second graft may actually have a negative effect on the weight of the queen. The breeder should not worry unduly about the fact that queens had weighed heavier when $1^1/_2$—2 day old larvae were used for double grafting, as compared with queens raised from larvae of the same age, but grafted once only. He will gladly give up using such older stages of development, especially as the younger larvae are handled much more easily.

3. **Nutritional factors : the brood food or royal jelly**

The nutrition of queen larvae is of greater importance for the quality of the developing queens than anything that has been discussed so far. When royal nourishment is in short supply ; for example when there are too few nursing bees in the colony or when these are not willing to rear a queen, then the best technical preparations for queen rearing are all in vain. The capability and the disposition of the nursing stock to adopt and to take care of the selected larvae are decisive factors for successful queen rearing. There are great differences in this respect. In this chapter we want to examine the nursing colony closely and disco-

ver its innate nursing capacity. Data resulting from simple observations by experienced breeders and the researcher's opportunity to alter rearing conditions experimentally will be helpful in our search.

3.1. *Biological facts*

A close study of nursing behaviour in a colony of honeybees begins necessarily with the producers and the distributors of the brood food and royal jelly ; the nursing bees. Of prime interest in this study are the origin of the brood food, any physiological peculiarities of the nurse bees, as well as their feeding activities and responses if they are exposed to special experimental conditions.

3.1.1. **Origin of brood food and physiology of nurse bees**

In a normal bee colony the bees which take care of the brood are the young bees : the house bees during the first two weeks of their lives (ZANDER — WEISS, 1963). They produce the glandular secretion which is given to worker and drone larvae initially, and to queen larvae as their exclusive food. This brood food is mainly a product of the hypopharyngeal glands, also called brood food glands, which are located in the head. According to HAYDAK (1975a) they develop to their greatest size by the 5th day if the bee can eat much pollen. During this time their content is at first translucent, milky, and will later become white-opaque before turning yellow. The brood food glands atrophy during the second half of the period of hive duties and are said to produce only a number of enzymes finally. Other glands known to produce food for larvae are the mandibular glands. They are well developed from the day of emergence. Their initial fullness shows oily vacuoles from the 7th day onwards and these increase in numbers as the bee ages. KRATKY stated (1931) that the secretory cells shrink after the 14th day but were likely to remain capable of functioning throughout the life of the bee.

It is certain that the proteins in royal jelly originate mainly from the hypopharyngeal gland (KRATKY, 1931 ; PATEL, *et al.* 1960). A free fatty acid is also present in quantity and this was identified by BUTENANDT and REMBOLD (1957) as 10-hydroxy-2-trans-decenoic acid. Royal food is also enriched with a pteridin derivate and REMBOLD and HANSER (1960) have confirmed the presence of this biopterin in the hypopharyngeal gland. The fatty acid has also been discovered in the mandibular gland (BARKER *et al.*, 1959 ; CALLOW *et al.*, 1959), where it is likely to be produced. Its quantity increases with the age of the house bees (BOCH and SHAERER, 1967). HANSER and REMBOLD (1964) examined the same glands of nurse bees which were found to be feeding queen larvae and detected an enrichment with biopterin. They also found pantothenic acid, the vitamin known to be present in quantity in royal jelly.

It is also possible that two further glandular systems contribute to the production of royal jelly : the postcerebral glands and the thoracic glands ; even though these glands only come to full development towards the end of the nursing period. Fatty material in the royal jelly were

said to originate in the postcerebral glands, according to WETZIG (1964). The same author reported to have found carbohydrates in the hypopharyngeal glands. But it should be beyond doubt, that the major part of the sugars in brood food come from the honey sac.

All chemically identifiable elements which have been found in royal jelly, have so far been shown to be present in the brood food given to worker larvae, even if the quantities were in different ratios to each other. We must therefore assume, that the different glands make quantitatively different contributions to the composition of the two types of brood food. When JUNG-HOFFMANN (1966) observed nurse bees feeding worker and queen larvae, she was able to confirm the observations of von RHEIN (1933), that brood food contained two secretions of different colours, and she also discovered varying ratios of the two components "white" and "clear" as given to worker and queen larvae. In queen cells the ratio was 1 : 1 throughout the feeding period ; Worker larvae, on the other hand, received less "white" secretion and often it was not quite as white as that given to queen larvae. The quantity of the "white" component varied with the age of the larva and the time of the year. During summer larvae received about 20% "white" on their first day and 27% on the second day ; during autumn the percentages were a little higher. The share of the white component decreased rapidly on the third day. Larvae older than three days hardly received any white food ; instead it was found that at 2/3 of all feedings the "clear" component was added to the cell and a "yellowy" material containing pollen grains was fed. Although no detailed observations were made, it is quite clear that drone larvae also received clear and white components at first and later the yellowy material.

JUNG-HOFFMANN found by chromatography and electrophoresis that the whitish component consists of a secretion of the pharyngeal and mandibular glands, while the clear secretion is likely to come from the pharyngeal gland and the honey sac. The whitish component consists of 14% protein and has a low sugar content; the clear material has a high sugar content and contains only 10% protein.

In a normal queenright colony the fat body of young bees remains undeveloped, apart from a slight opaqueness of these cells, the first few days of their lives during summer work.This colouration disappears together with the shrinking of the brood food glands (MAURIZIO, 1954). Ovaries also remain underdeveloped. Nurse cells and immature eggs were reported to have been found in oviducts in this period by HÜSING and ULRICH (1938). These structures dissolve when the bee begins its life as a forager from cca. 20th day onwards. Only winter bees retain these cells until spring or show an increased development, according to MAURIZIO (1954) and PAIN and VERGE (1950). Winter bees are furthermore an exception ; their brood food glands remain capable of functioning and their fat bodies become fully developed and enriched with reserves (see ZANDER-WEISS, 1963). During the summer colonies may contain older workers with fully developed brood food glands and one can observe others, mostly young bees with developed ovaries (HÜSING and ULRICH, 1938 ; HESS, 1942 ; PAIN and VERGE, 1950 ; LEVIN

and HAYDAK, 1951; KROPAČOVA and HASLBAKHOVA, 1969). Swarming bees usually have greatly atrophied pharyngeal glands, according to HALBERSTADT, (1966). Yet KROPAČOVA and HASLBAKHOVA (1970) report that the number of protein fractions of these bees, as well as of those who had stayed at home, is smaller than before the issue of the swarm, because much brood remained to be fed in the hive. These two authors and KOPTEV (1957) could not confirm the findings of TUENIN (1926) and PEREPELOVA (1929), that worker bees with developed ovaries could be found in colonies in a swarming mood. Instead, they discovered better developed ovaries in the worker bees which had stayed behind after swarming. A positive correlation could be established between development of pharyngeal glands and ovaries. This can be explained as the result of a diminished demand for brood food by the shrinking brood nest giving in turn rise to increased storage in the two organs.

We should also be interested in the conditions within a dequeened colony such as we use as a starter colony in artificial queen rearing. Indeed, the interrelationships between the development of the three organs : brood food gland, fat bodies and ovaries will become more obvious in such a stock. Dequeening a colony results in ovarian development in an increasing number of workers, until their ovaries are capable of functioning. MÜSSBICHLER, (1952), ALTMANN (1950) and DRESCHER (1956) state quite clearly, that all laying workers with functioning ovaries have also proud brood food glands and strongly developed fat bodies (MAURIZIO, 1954). The physiological condition of the bees in a queenless colony is approximately like that of winter bees, apart from the development of their ovaries, which can be a consequence of lack of queen substance (PAIN, 1954 ; DE GROOT and VOOGD (1954) : or may be due to the absence of brood (JAY, 1970). The length of life is extended considerably in such bees, and MAURIZIO (1954) has established a clear relationship between such a physiological condition and length of life in bees.

The appearance of functioning ovaries in the workers of a dequeened colony has often been considered detrimental to queen rearing by artificial means. GONTARSKI (1948) discovered enlarged ovaries in 80% of all workers 9 days after a colony had been dequeened. For this reason he was against the method which had been recommended by ZANDER (1944) in Germany : "Rear your queens in a colony which had been queenless for nine days". Yet there is no proof that bees with developed ovaries are unsuitable for nursing. Even the appearance of laying workers — there are probably only a few of them — does not seem to influence the inclination of a stock to rear brood (WEISS, 1971). If no other technical reasons can be raised against this time-consuming and laborious "Zander" method, then the aspect of the ovarian development should be neglected.

3.1.2. **Feeding behaviour and distribution of food**

Every feeding of larvae is preceded by an inspection, during which the nurses investigate the larvae and the brood food already surrounding them thoroughly with their antennae (LINDAUER, 1952). These inspec-

tions last about 2—20 seconds, and not every one of them if followed by feeding. During feeding the mandibles of a bee open and begin to vibrate ; after 1—2 seconds a minute droplet appears on the lower maxillae. This is then expelled onto the bottom or sides of the cell or onto the larvae itself. The mandibles then spread it evenly around the larvae. Sometimes the nursing bee cannot detect the larva precisely and the droplet of food may be placed on its back. While feeding, the larvae move constantly in a circle and the movements speed up the longer a larva has to wait for its next feed. Even older larvae are not provisioned by direct mouth to mouth feeding ; they have to search for their nourishment, which had been deposited near the hind-end of the larva or along the cells wall. Only one tenth of all visits results in feeding. Inspection and feeding together take scarcely half a minute ; under exceptional circumstances it can take 2—3 minutes. The average time taken for feeding increases with the age of the larva. In their early stages larvae are fed every few hours ; later on — several times an hour. GESCHKE (1961) reports 4 feeds in 6 hours during young stages and 25 feeds for older larvae. LEVENETS (1959) reports that drone larvae were fed between 5—14 times per hour, but there were always more visits of inspection, especially during their advanced age.

Provisioning of royal larvae is in principle similar to that for worker larvae ; though immediately after hatching their cells are supplied with more food than they require. While worker larvae have an excess of brood food only during the first 2—3 days, (NELSON *et al.*, 1924), queen larvae receive a surplus during their entire period of development. SMITH (1959) found 147 mg of royal jelly on the second day after grafting ; 235 mg on the third day and only 182 mg of royal jelly on the fourth day — the larva consumes now more food at all times. LINDAUER (1952) reports that in contrast to the provisioning of worker cells, nurse bees keep on adding more and more food without long inspections — at least from the 3rd day onwards, even if considerable stores are already in the cell. While provisioning of worker larvae took on average 50—60 seconds, that of queen larvae took only 10—19 seconds. Despite this speed the total time spent over feeding exceeded the time dedicated to workers. JUNG-HOFFMANN (1961) found no difference in the number of feedings per hour between queen larvae of various ages, it varies between 3 and 26 and the average was 14.3/h. According to her reckoning, the queen larva is fed 1600 times before capping. The 143 feeds recorded by LINDAUER (1952) for worker larvae look sparse in comparison, and of these an estimated 50 feeds fell into the initial 3 days when pure brood food is given to worker larvae. According to JUNG-HOFFMANN (1966) worker larvae are fed 20 times less frequently up to the 3rd day of larval stage than queen larvae. Until point of sealing, worker larvae are fed 10 times less often and require only a tenth part of the time which nurses dedicate to the care of royal larvae. According to KUWABARA (1947) the feeding patterns change in a queenless colony and worker larvae are provisioned more rapidly and are capped sooner. The great number of feeds reported by KUWABARA can be assumed to also include visits.

JUNG-HOFFMANN estimates that the total weight of food deposited in queen cells amounts to 1 g, and the total time taken up by feeding is 17 hours. Visits without food deposition were not included in this count. It is true that queen larvae consume only part of all food provided, while worker larvae eat nearly all food given to them. When using radioactively labelled food in their artificial rearing experiments, DIETZ and LAMBREMONT (1970) found that queen larvae consumed 13% more food in the first three days than those larvae from which workers developed. During this period the former consumed 9 mg on average, the latter consumed 7.9 mg. Unfortunately the total amount of food consumed by worker and queen larvae cannot be determined by this method because of the losses of radioactivity during the elimination of faeces, but the authors are of the opinion, that it amounts to between 2—3 times the weight of the prepupae, which is about 295 (258—315 mg) in normally developed queens. SASAKI and OKADA (1972) calculated a total food consumption of 360 mg for the development of a queen weighing 200 mg, by using the metabolic conversion factor for royal jelly in their calculations.

In a normal colony preparing to swarm or thinking of supersedure nurse bees have enough time to get ready to take care of queen larvae. This does not seem necessary. Bees which are capable of rearing brood can begin at once rearing a new queen if they are suddenly deprived of their old one. It seems that the royal food is permanently available, or at least they are able to secrete it whenever queen substance is diminishing. Apart from this fact, it is obvious that the considerable indifference of the young female larva in its response to royal food will permit a certain period to elapse before the production of royal jelly becomes a vitally pressing need.

The supply of larval food to worker larvae in the bee colony proceeds at random. Because of the great number of nurse bees an even distribution is achieved. The choice of worker cells to be used for rearing queens in a colony building emergency cells also seems to be made at random. Many cells are richly supplied with brood food long before bees begin to alter the shape. According to JUNG-HOFFMANN (1966) this food already is royal jelly.

Chance is also a factor in the supply of royal food during artificial queen rearing. If one gives a superabundance of dry-grafted larvae to a nursing colony, one can already find varied amounts of food in the queen cups after one day. When fewer cells are offered or there are many excellent nursing bees in the stock this is not quite so apparent. ÖRÖSI-PAL (1960) made systematic trials in order to discover the time-lag before larvae are supplied with their first feed in queenless colonies. After 10 minutes the first larvae were found to lie in brood food ; many larvae had to wait 30—54 minutes, in the worst cases even hours. Bees occasionally reared queens from larvae which had been starving for hours, yet they ejected later some larvae which had initially been supplied with an abundance of food and had apparently developed well.

When we are weighing queens obtained by artificial queen rearing, we can often find considerable variations among queens which had de-

veloped side by side along the grafting frame. Occasionally we find queens whose period of development was extended by 1—2 days ; yet there seems no relationship with their weight. Such differences cannot be explained genetically, they occur with grafts from pure blood lines in different nursing colonies with different frequencies. But they occur most frequently in weak nursing stocks. It is obvious that differences in nourishment play the major role. Yet it does not seem as if the total quantity of food deposited in the individual cell played the decisive role in the development of the queen contained in that cell. The residual amount of food left in the cell after pupation is often variable. I discovered in the many measurements that there is no correlation between the residual amount of food and the weight of the emerging queens reared in the same nursing colony (WEISS, 1974a). Normally, food is available in excess right from the beginning for the queen larva and throughout its period of development. We simply have to imagine that the recorded differences in the development had been caused by differences in the consistency of the royal jelly, or in minor differences in the distribution of the brood food components from the various food glands as well as the honey stomach. The special situation of the larva in the exposed cell along the cell bar had been mentioned before.

3.1.3. **Age of nurse bees**

It had been reported already, that in a normal colony the great number of nursing bees, and that includes those bees taking care of queen larvae, belong to the house bees. Because young worker larvae receive brood food differing in quality from that given to larvae older than three days, we may assume that the provisioning of the two age groups is the task of groups of nurse bees which are differing from each other. RÖSCH (1925) reported that very young bees with glands still in the process of development were feeding older brood with food mixture, while the older bees with proud brood food glands took on the care of the younger larvae. DREISCHER (1956) observed the same sequence but there was much overlap.

FURGALA and BOCH (1961) observed bees 1—10 days old mainly over young workerbrood, while the older ones between 11—20 days old were distributed evenly over both old and young brood. FREE (1960) discovered no such organisation and it seems that in small colonies especially all age groups are participating in the care of both age groups of larvae (LINDAUER, 1952 ; SAKAGAMI, 1959 ; GESCHKE, 1961) According to JUNG-HOFFMANN's (1966) observations nursebees covering a range from 3—30 days old were distributed evenly over the varied age groups of worker larvae. The average age of the nurse bees was between 11—13 days, depending on the composition of the ages of bees within the nursing colony.

Taking all these observations into account we can say, that if there is a division of duties into nurses for young brood and nurses for older brood, than the dividing line is a floating one and any dissolution of such an organisation is easily done according to the colony's needs. After

wintering only old bees are available for the care of the first batch of brood. It is also not difficult to make bees of an age well beyond the nursing stage rear brood under experimental conditions. RÖSCH (1930), who provided us with the classical picture of the division of labour within a colony, found that in colonies without young bees the older bees (17—33 day old) would rear the brood. ALPATOV reports that when MIKHAILOV (1928) made an artificial swarm, the bees reared by the old foragers of this swarm were larger, had broader tergites and larger wings than those bees which had been reared in the part of the colony which contained all nurse bees. On the other hand, the bees reared by the latter had longer tongues. In this case we must take into account, that many young bees which had made orientation flights, will return to the original stand and strengthen the artificial swarm. Removing all sealed brood constantly from a colony, HIMMER (1930) forced bees to rear brood for 42 days without a break. In similar experiments conducted by MOSKOVLJEVIĆ (1939) the oldest nurses were 73—75 days old ; by BUCHNER (1953) — at least 107 days old and by HAYDAK (1963) — 138 days old. Brood food secreted by nurses older than 30 days was more watery and less opaque than in the beginning. With increasing age the nurse bees reared smaller bees and their life expectancy was not as good. Such bees had a fragile intestine.

All this shows, that the brood food glands can remain capable of function for longer time than is usual when the survival of the colony demands such activity. Contrary to KRATKY's (1931) assumptions, fully atrophied brood food glands can be made to function again perfectly, and old foragers can begin to nurse brood again in an emergency. This was shown by the experiments made by KRAMER, 1896 ; HAYDAK, 1930 ; FREE, 1961 ; JORDAN, 1963.

In view of the wide variability in the ages of the bees rearing worker brood, it must be of interest to us how this affects drone rearing and queen raising itself. Drone larvae are fed first with brood food and later receive a mixture of brood food, pollen and honey just like the worker brood does (HAYDAK, 1957b).

We may possibly assume, that the same nurse bees which feed worker brood will also take care of drone brood. No attempts have been made to my knowledge, to prove the existence of nurse bees specialising in the care of drones. Queen rearing is a different matter as this requires specialised feeding altogether. Under natural conditions a colony will rear both worker larvae and queen larvae side by side ; for example when preparing to swarm, when superseding or rearing queens in an emergency. Such a colony produces both worker brood food and royal jelly at the same time. Is every nurse bee capable of both duties? Or are specialists required for each job ?

Some authors think it possible that certain nurse bees specialise in rearing queens. GONTARSKI (1958) believes that the existence of groups of nurse bees specialising in the production of specialised food has been proven, and he rests his opinion on the findings of ALTMANN (1950), who discovered in royal jelly a component with gonadotrophic action (stimulating ovary development). This fraction was not

found in all nurse bees, but only in those feeding queen larvae. GONTARSKI extends this hypothesis and is convinced that the specialists who are taking over the care of queen larvae, age at the same rate and thus deliver at all times a food suited to their charges (1958). TOWNSEND (1965) refers to the discovery by HABOVSKI, that nurses of workers and queens showed differences in the development of their hypopharyngeal glands. This would mean that the differential glandular food given to worker and queen larvae is not due to an intentional act of the nurse bee, but is a consequence of her physiological condition. FURGALA and BOCH (1961) found over open queen cells (age of larvae not determined, but mostly of advanced ages), more nurse bees of age 11—20 days, than of the 1—10 day age group. JUNG-HOFFMANN reports that nurse bees secreting white food components are younger on average than those bees which feed the clear component. This would point to a predominance of young bees as royal nurses. But there must be wide overlapping in this respect, especially as a nurse bee can secrete either component or, rarely, a mixture of the two. Her findings of a high average age (17 days !) for the nurse bees secreting clear food, were explained by the author to have been due to the unnatural conditions within the nursing colony, in which the younger nurses were fully occupied in elaborating the white component, so that older bees had to assist in taking care of the brood. WAHL-BUCHGE (1964) discovered in experiments with caged bees, that bees were capable to rear queen larvae only if more than 5 days old — and that best results were obtained with 9—12 day old bees. 13—20 day old bees reared some perfect queens as well as some intercaste individuals ; while older nurses were only capable of rearing dwarf queens, intercastes and workers.

Our question, if in nature queen rearing is done by specialist bees, has therefore not been answered to our satisfaction in spite of the many experiments quoted. Seen from the biological point of view, there seems no need to stipulate special groups of bees for nursing queens. Neither does nursing seem confined to restricted age groups. Just as nurse bees can extend their brood rearing activtiy considerably when this is required of them, so the nurses of queen larvae can do the same well beyond their normal period of nursing activity. This was demonstrated in experiments in which queenless nursing colonies without fresh supplies of emerging generations of bees were made to rear queens constantly. GOETZE (1952) after his first experiment came to the opinion that old bees can not rear queens. In this first test he had raised three batches of queens, each one month apart. In his second attempt he reared fresh batches 14 days apart and obtained queens right to the end, even if some were showing anomalies and malformations. GONTARSKI (1948) concluded after some experiments — which he did not describe further — that the food secreted after raising 12 batches had no more queen-determining effect. When making experiments in continuous brood rearing in colonies with open brood only, BUCHNER and HAYDAK (1953) found that nurse bees were able to rear queen-like individuals up to the age of 107 and 105 days respectively. True enough, in the last phases of these experiments by HAYDAK *et al.* it was found that bees constructed

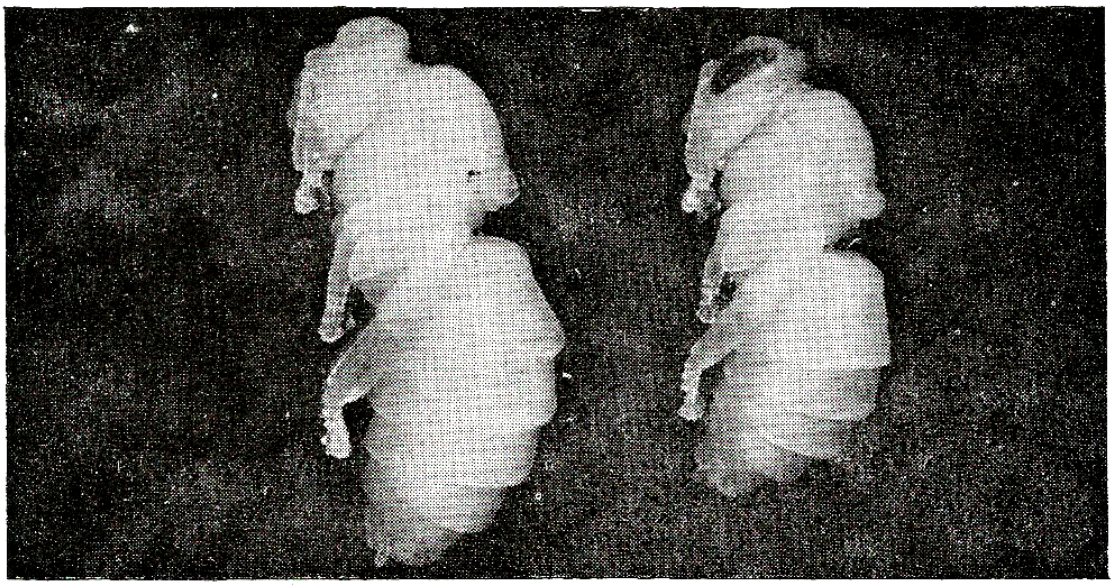


Fig. 40 — *A "normal" queen pupa baside one which was raised as 13th batch in an experiment, during which the colony was not re-inforced with young bees.*

queen cells over empty worker cells, or worker or drone larvae were found in the royal cells. I myself succeeded in rearing 20 batches of queens successively in a dequeened colony which had been very strong at the beginning and had all brood taken away. Each new batch was given as soon as the previous one was sealed. During this time the nursing bees attained an age of at least 112 days. 24 grafts were given in each batch ; but only after the tenth batch did the number of completed cells drop. Queens developed more slowly and their body weight decreased, depending on the number of acceptances (see Fig. 40). Towards the end they grew up in cells which were getting more and more crooked (Fig. 41). Strangely, the number of ovarioles was not dimished

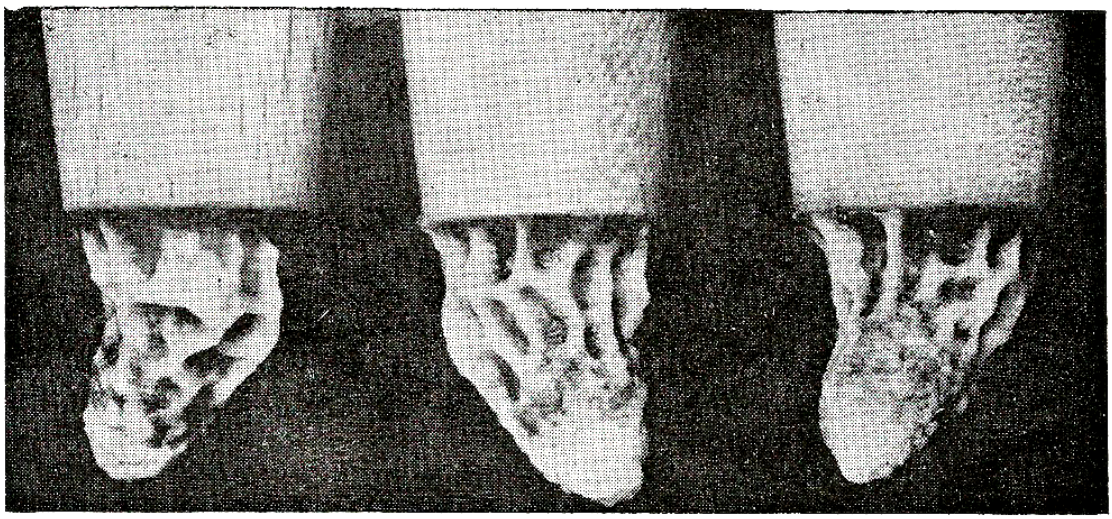


Fig. 41 — *Crooked queen cells obtained in the same experiment, in which successive batches were reared in one nursing colony. The shape indicates declining nursing abilities of the colony.*

even in the last queens. As time went by I found greater or lesser deviations from the optimal queen type in the indices of head, mandible and tarsus. But right to the last a few individuals still showed the characteristics of typical queens. This proved convincingly, that as long as bees can elaborate brood food and nurse workers they are also capable of producing royal jelly (WEISS, 1972). The caste-determinating effect of any royal jelly produced by old bees persists, even though HAYDAK (1961) and HAYDAK *et al.*, (1964) reported that it contains less of the vitamins of the B-group, and that it differs considerably in consistency and colour from that produced by bees of normal brood rearing age.

3.2. *Basic principles of queen rearing*

For the practical and efficient use of nursing bees in queen rearing we must set out a number of basic principles on which the acceptance of cells and the quality of the queens we intend to produce will depend a great deal. Only too often these important factors are simply summed up and we speak of "the right condition" of the nursing colony. What do we mean by that ?

3.2.1. **The health of the nursing colony**

The first basic condition for ensuring perfect care is a healthy stock. Diseased colonies are unsuitable for queen rearing because their vitality is diminished. The specific action of the disease itself may have an additive effect. Heading our list in this respect is nosema disease ; this is common the world over. The parasite consumes much protein and inhibits the full development of hypopharyngeal glands in young bees until we find them to be malformed and atrophied. (LOTHMAR, 1936 ; HASSANEIN, 1952 ; BÄHRMANN, 1965 ; WANG and MOELLER, 1969). This reduces the capacity of secreting brood food, and normal brood rearing activity of a colony is directly affected. It must be obvious that optimal queen rearing is not possible in a colony suffering from nosema. In practical queen rearing the bees of the nursing colony are often used for making mating nuclei after the emergence of the young queens. This intensifies the danger of the spread of the disease to other colonies.

Queens are not infected with nosema disease as often as worker bees, but it can happen. The danger is increased if the queens emerge among infected bees and spend the first weeks of their lives among them. The infection with nosema has an indirect effect on the ovaries of a queen ; it results in the heavy deterioration and decomposition of this organ according to FYG (1945). Infertility appears soon and is followed quickly by early death. The beekeeper then notices the early supersedure of his still young queens.

It is not known to what extent acarine disease will affect the activity of nurse bees, but because it spreads easily, it creates similar conditions in a breeders yard as nosema disease. The same is true for septicaemia.

A further danger is that queen larvae may get infected with brood diseases as easily as worker larvae. We can firmly state that any disease of brood or adult bees which can weaken a colony is a heavy burden for a queen raiser's out-fit. The breeder's prime concern must be to have healthy colonies throughout.

3.2.2. Colony strength and its composition

There is no doubt that the strength of the nursing colony has an important effect on the outcome of queen rearing. Even though we have to be careful before integrating into queen rearing the results of several authors who reported on larger bees or workers with a longer tongue from stronger stocks (MIKHAILOV, 1926, 1927; LEVIN and HAYDAK, 1951, NURIEV and MISRIKLANOV, 1960) — the size of the worker cell has a limiting effect — there is just no doubt that the greater capacity for nursing of a numerically stronger colony must have an effect on acceptance and the supply of food of the royal cells. In the vast majority of cases the stronger stocks will take better care of their charges than will weak ones. It is true, that it is not just the sheer number of bees either which plays the important role, it is the composition of the colony in terms of groups of bees of the right ages which is important. In general we find large numbers of nurse bees in a strong colony and this is decisive when rearing queens. In spite of all flexibility in adjusting the organisation of labour within the colony, normally the young house bees still do brood rearing according to the development of their brood food glands. Unfortunately we can not recognise nurse bees by their appearance. We can draw some conclusions of their numbers by judging brood rearing qualitatively and quantitatively. But brood rearing is strongly influenced by weather and nectar flows ; it depends on the condition of the queen and is affected by manipulations. While the breeder is usually not fully aware of all internal processes within the colony ; he is able to take stock of the amount of brood and colony strength accurately. A large brood nest is a useful sign, but it must not necessarily indicate optimal conditions for queen rearing. It is just possible that at this point in time there is an acute shortage of nurse bees ! Again, a mass of nothing but nurse bees may not create the best situation for rearing queens either. We can observe this occasionally when we use the swarm box method to initiate queen cells. The bees have to take care of the grafts for 24 hours while being confined. The results depend very much on having the right mixture of bees. When JUNG-HOFFMANN was observing the behaviour of nurse bees, she found that the amount of royal jelly given to larvae was less, if either young or only old forager bees were in the swarm box. Here too the optimal conditions depended on the right mixture.

A swarm box contains only a fraction of the bees of a full colony at its peak of development, and yet we can achieve excellent acceptances by this method. But other stocks have to take over the finishing of cells. It is possible to rear queens successfully in relatively weak colonies. Such bees often accept more cells than they can effectively rear to perfection. For this reason it is up to the breeder to limit the number of cells. It

appears that acceptance itself does not depend on the absolute strength of a colony. It seems rather, that a crowded condition is more important. We mislead bees about colony strength by overstocking the box with bees (SIMPSON, 1973). But this method is only effective if we do not exaggerate it. Overcrowding a naturally very strong stock may drive bees out of the hive and may lead to smaller queens.

3.2.3. **The state of development of the colony**

Within colonies of honeybees the generation of young queens is the central pivot for all natural developmental and reproductive activities. The timing for this last action is usually summer and it is this time which is the most suitable period for artificial queen rearing. Yet we must ask the question : how and to what extent will swarming preparations influence the results of our efforts.

PAIN reports that SINYAEVA (1953) noticed colonies preparing to swarm, or those undergoing supersedure, were placing more royal jelly into their queen cells than other colonies. PESCHETZ (1966) believes that the stocks in a swarming mood are specially suited for queen rearing. STRÄULI (1915) writes that DOOLITTLE found superseding colonies will adopt grafted larvae readily and will supply them with an unusual amount of brood food. Many such reports may be found in literature on beekeeping.

On the other hand, it is well known that swarm cells are often provisioned arbitrarily with food and that the weight of queens emerging from them is very variable. Quite often swarm cell queens weigh less than those produced by artificial queen rearing (ZANDER, 1925; LEVICHEVA, 1961). Many breeders therefore reject colonies in a swarming mood. Most beekeepers think, just as RUTTNER (1965) does, that those colonies are the most efficient nursing stocks, which are strong, yet are still in an expanding mood. It is possible that in many cases their judgement is right for the very reason that in such colonies the removal of open brood will release an abundance of young bees for nursing duties. Disregarding this aspect, we find that a colony preparing to swarm — or thinking of supersedure — has the same possibilities, especially when we want to rear queens in a queenright stock and the cell raising is done in a compartment separated from the queen by means of a queen excluder. At swarming time this method of "queen rearing in a queenright colony" could actually lead to improved acceptance, as the preparations to swarming and to supersedure are interlinked with the reduction of the amount of queen substance, and this is said to be the factor which inhibits swarming and cell building (SIMPSON and BUTLER, 1960). Finally we can report that GOETZE (1954) observed increased acceptance at those times, when less drone comb is being constructed by the bees of a colony. He also believes that the intensity of the drive to reproduce is inversely proportional to comb building activity. Colonies which had not got over their urge to construct drone comb do not accept grafts as readily. The question if the presence or absence of drones in a nursing colony has an influence on successful queen rearing has not been investigated.

3.2.4. **The effects of dequeening**

In the section "Biological Facts" I stated that the bees of a colony of honeybees do not require a period of preparation before they can nurse queen larvae. If this were only a matter of producing the brood food suited to the respective age of larvae, the queen rearer could insert the grafts immediately after dequeening. But we will never achieve optimal acceptance this way. After we have removed the queen from the stock, we have to wait until the bees know they have lost their mother, or, to express this a little more scientifically, until the effect of the queen substance has began to wear off. A waiting time of 2 hours is usually enough for this to happen. In beekeeping literature we find variations of up to 24 hours. Every breeder thinks his timing is the best. Unfortunately I have yet to discover such an ideal time in spite of my many experiments. I have only discovered this : it is essential that we await the period of restlessness after dequeening. Should we rashly give the grafts too early we may find that the bees will remove some or all larvae from their cups.

3.2.5. **The starter colony — queenright or queenless ?**

The absence or presence of a queen is an important factor influencing the number of queen cells which may be nursed. The chances of acceptance are certainly greatest in a queenless stock. In a queenright colony the presence of the queen prevents unlimited queen rearing. The conditions are different again in the method used widely in Germany and known as the "simulated queenlessness method". In this method cell acceptance is achieved in a separate compartment which is divided from the queenright stock by means of a wire screen. The bees nursing these larvae are truly queenless and acceptance varies according to the strength of the bees in this compartment. After 24 hours the wire screen is replaced with a divider made of queen excluder and the finishing of cells is continued in a queenright colony.

When FREE and SPENCER-BOOTH (1961) evaluated all results obtained in two large professional apiaries in England over 3 previous years, they were able to establish that acceptance by nursing colonies decreased in the following order : "queenless and broodless"; "queenless but with brood" (open or sealed ?); "queenright". The presence of open queen cells had no influence on acceptance.

The following point is occasionally discussed or disputed : are there differences in size, or otherwise, between queens which had been reared in queenless and queenright colonies ? TARANOV (1975) thinks that queens from queenright stocks are better. MÂRZA (1965) observed that the queens from queenless colonies were heavier, while VELICHKOV reports (1971) that queens from queenright colonies were laying more eggs. Colony-specific differences in nursing capabilities do not allow an objective evaluation of this problem.

3.2.6. The presence of open brood in the nursing colony

After Soviet publications had first raised the question about the influence of open brood on the quality of the queens in nursing colonies, it was discussed on a world-wide basis. SINYAEVA (1953) reported to have found more royal jelly at the time of sealing in queencells which had been nursed adjacent to open brood, than in cells reared in a colony without any brood ; figures quoted are 110—566 mg as against 45—120 mg. GUBINA-OSHMANN reported that BILASH (1963) divided strong colonies into two halves each ; one half had only open brood. while the other part had only sealed brood. In both groups he raised queens. He states in his report : "After 3 days cells of the first group contained 422.5 mg royal jelly, those of the second group 360 mg. Queens of the first group had a greater weight (214 mg) and more ovarioles (372.2), as compared with queens of the second group (weight : 200 mg, ovarioles : 334.2).

If these observations withstand critical examination, then we have to ask ourselves, why do queen larvae receive better care in a nursing colony with open brood than in one without any — providing of course, that their nursing capacities are equal ? We would expect the opposite to be true. Yet it is possible, that when we rear several batches of queen larvae in the same nursing colony, the presence of open brood has then a stimulating influence on the nurse bees present and those which emerge continuously. Queen larvae will then benefit from this. KROPAČOVA and HASLBAKHOVA (1971) report also that the presence of open brood prevents ovary development in worker bees. ZHDANOVA (1963) mentions that the temperatures vary less near open brood than they do near sealed brood.

I am not convinced that these reports justify the unconditional use of open brood in a nursing colony. This problem is directly related to the very method of queen rearing adopted by the breeder. If he gives queen cells from a starter to a queenright colony for finishing either behind or above a divider of excluder material, he may find that insufficient nurse bees will move over to take care of the cells on the grafting frame. Nurses may stay in the queenright portion near the open brood and in the vicinity of the queen. It is therefore important to transfer some open brood into the separated compartment together with adhering bees before the grafting frame is inserted. The same advice holds for starting queen cells in a queenright colony, where this is done in a compartment separated by queen excluder. This method is usually associated with constantly renewed batches of grafts and this requires that fresh, open brood and an adequate supply of young nurses is repeatedly added to the breeding compartment. Only this way can we guarantee optimal acceptance as well as good after-care for our queen cells.

These considerations are of no importance when we are rearing in a queenless stock, which must nurse the cells from the beginning to sealing. On the other hand, the beekeeper must expect that bees will rear some emergency queen cells on the brood frames if large areas of

open brood are present. The acceptance of cells on our grafting frame may well be reduced for that reason. These use of open brood can only then be recommended, if it can be shown, that "better queens" are really obtained this way.

3.2.7. **Size of batch**

There are different opinions about the number of cells which may be given to a nursing colony at one time, or, as the breeder says : the size of the batch. There are the defenders of the small batch with 10—15 cells at the most, and others who like to see a nursing stock taking care of almost unlimited numbers of grafts. We can never settle the question of batch size by a simple statement. The number of cells which a nursing colony can look after well depends largely on its strength, its condition and the way it is managed by the breeder.

We must never think that a colony "knows" how many grafts it can accept in order to ensure optimal development for these reproductives. There are some poor nursing colonies which could accept a multiple of the number they actually take on, when judging it by its strength and composition alone. On the other hand, we have also colonies which accept so many cells that finishing is inevitably affected. This sometimes occurs in weak colonies, and not rarely when several batches are reared in one stock. Many of the initiated cells will vanish before coming to completion ; but more often than not too many of them are nursed along; many more than the colony can really cope with. This is the reason for diminishing queen weights with increased batch sizes. Yet any such observations must not be taken as gospel truth (KOMAROV, 1934 ; SVOBODA, 1949 ; KRASNOPIEV, 1949 ; VUILLAUME, 1957 ; BARES, 1963 ; PUȘCĂ, 1970), and also my own observations, even though I could never discover a correlation between batch size and the number of ovarioles. When GOETZE (1954) tried to evaluate all queens reared in a number of colonies with good and indifferent rates of acceptances, he could not find a correlation between the number of cells and the development of emerging queens. A colony with poor acceptance need not rear larger queens. Instead we must accept the fact, that for any rearing technique, modified by the prevailing, various influences on nursing, there is a maximum number of cells which can be reared into optimal queens.

When rearing queen cells in a queenright colony, the batch size is already limited by the low rate of acceptance in such a stock. Many breeders who use this method reckon with a batch size of 15 at best ; but at intervals of a few days they can repeat grafts of this number if they transfer some young brood at the same time. When rearing queens in a queenless stock it is up to the queen rearer to limit acceptance. In the presence of unsealed brood it is not recommended to exceed that number of cells which is equal to the number of swarm cells raised in such a stock under normal conditions. In the case of the Carniolan bee under Central-European conditions the number of swarm cells is around 30. But I believe that 50—60 cells is not exaggerated if we remove open brood before the first graft. While it is uneconomic not to utilise the

nursing capacity of a strong colony to its fullest, it would equally be wrong to overtax a weaker colony. A dequeened swarm or a small nucleus with brood could nurse only a correspondingly smaller number. It is up to the breeder to find the right answer.

Although queen cells are supplied from the start with more food than worker larvae receive, they will also be given more feeds as they grow older. JUNG-HOFFMANN (1966) gives the following figures for the number of feeds per hour, and the total time taken up by feeding per hour (in brackets) : for freshly hatched larvae : 3.3 (2 min 25 sec) ; for one day old larvae 13.1 (7 min 53 sec) ; for two day old larvae 15.7 (11 min. 49 sec.) and for four day old larvae 25.3 (15 min 0.3 sec). This shows that the resources of a nursing colony are not taxed as heavily as later on nearer the time when cells are at point of sealing. Therefore one can give twice the number of cells if a colony is used only for 1—2 days as a starter colony. These cells can then be given to queenright finishers by accommodating them in the supers. Swarm boxes too can cope with a relatively large number of cells depending on size and strength, of course. But we must add that CALE reports in "The Hive and the Honeybee", (1963), that WHITCOMB and OERTEL (1938) achieved only 56% acceptance when they offered a swarm box 120 grafts, but had 82% acceptance when they offered 60 grafts. Many of the queens reared in large batches were eliminated from colonies by supersedure after they had been introduced in the field.

In sharp contrast to the starter colony, it is the finisher which carries the main load of nursing. As a rule it can only spare a part of its nursing force for rearing queens as much open brood needs looking after. It should therefore never receive more queen cells than we would give to a queenright colony. On the other hand we can always add more initiated cells after the previous batch is sealed. Even though the capacity of a finisher depends on its strength and its physiological condition, one can never rely on the assumption that the bees themselves will reduce the number of cells to that number, which suits their nursing capacity best.

It is a tempting exercise to calculate the nursing capacity of a colony in spite of all the problems involved in this. Let's just try : Even though the number of feeds given to a queen cell — 1600, as well as the total time involved in feeding — 17 hours, seems enormous (given by JUNG-HOFFMANN, 1966), the work taken up by rearing worker brood is also considerable. JUNG-HOFFMANN calculates that of the 143 feedings, as reported by LINDAUER (1952), 50 fall into the period during which glandular food is administered. This amounts to 320 000 feeds given to 6400 worker larvae — an area of comb measuring 20×20 cm with brood on both sides. To rear 30 queen cells some 48 000 feedings are required. Now we must recall, that worker larvae receive 25% of their food as "white" component, while queens get a 50% share as white food. This brings the resultant white feeds for each caste into a comparable range with 80 000 feeds to these worker larvae and 24 000 feeds to the 30 queen cells. If we assume that these calculations correspond

somewhat to natural conditions, then we can be assured, that queen rearing will not exhaust the nursing capacity of a normal nursing colony. But we should always remember, that rearing queens in a colony is not exclusively a problem of its unexploited reserves of glandular food production.

3.2.8. **The number of batches and their intervals**

There are queen rearing techniques which provide for continuous cell raising in the same colony. One of these had been mentioned frequently before ; queen rearing in a queenright stock. The grafts are given at regular intervals of 2 or 5 days, depending on whether the cells are transferred to the finishers as sealed or open cells. The same system may be adopted for a queenless colony ; but in this case we must add brood or young bees each week.

We can also raise more than one batch in a colony which is not re-inforced further after we had dequeened it. If such a colony had some sealed brood of all ages at the beginning, then more young bees will continue to emerge for another 2 weeks. In theory we would therefore have young bees of nursing age available for 4 weeks. But as we pointed out in section "Biological Facts", colonies are quite capable of nursing and producing queens for much longer periods. The problem is, are such queens high-performance queens ? We can be certain that the extent of acceptance by the nursing colony is a vital factor. Sometimes the number of cells accepted in the repeats is greater than that of the first batch. This seems to be the case whenever we rear in a queenright stock, and it looks as if bees had to learn the job of queen rearing. Yet in this case we need worry least about a fall-off in the quality of the queens produced, as there is continuous rejuvenation of the colony. It is different though in the queenless stock without re-inforcement : here the will to accept and initiate is disproportionate to capacity to nurse royal larvae; the latter diminishes as a rule in spite of steadily emerging brood. An extreme example for increased acceptance with repeated grafts is provided by KOMAROV (1934). Although he did not further describe the nursing colony, he obtained in 4 batches given 8, 4 and 8 days apart, acceptances of 16, 16, 69 and 82 respectively. 65 and 74 queens developed fully in the third and forth batch respectively. But he does not supply us with queen weights ; yet we can take it for granted, that the queens of these batches were smaller than those of the first. WAFA and HANNA (1967) could establish, that the age of the nurse bees did not influence the rate of acceptance. I have already mentioned my own experiments in which I raised queens in succession in colonies which had been deprived of all brood from the start. Initially they were given grafts every 5 days, later the interval between batches increased to 6 and 7 days. When batches of 24 grafts were given, the rate of acceptance decreased only after the 13th and 14th batch (WEISS, 1972). Surplus of royal jelly remaining in the cell after the emergence of queens diminished rapidly right from the start. For the first batch the remnants of food weighed 25.5, and 17.0 mg per cell (acceptance : 14 and 19 cells) ; in the two second batches the average food left behind had gone back to 14.3 and

9.6 mg with an acceptance of 15 and 22 cells in each stock. Values for the two third batches are 10.7 and 4.5 mg and 20 and 22 cells respectively. In the first of these two colonies only a thin, dry skin of royal jelly was left in the cell after the 7th batch (19 cells), in the second stock this occurred after the 4th batch of grafts (22 cells accepted) (WEISS, 1974a).

Corresponding with the above results it is well known from commercial producers of royal jelly, that the amount of material harvested regularly every 3rd day diminishes rapidly with the third batch, unless the production colony is re-inforced all the time (SINYAEVA, 1953 ; MÂRZA and BARAC, 1961 ; H. SCHLÜTER, pers. communication). This reduction in the amount of royal jelly takes place even though the colony has hardly weakened and much emerging brood is present. But we must remember, that in the production of royal jelly twice or four times the number of grafts is given as compared with artificial queen rearing (VUILLAUME, 1957 ; DADANT, 1957 ; SMITH, 1958 ; MÂRZA and BARAC, 1961 and others). This can contribute to the rapid exhaustion of its capacity to produce royal jelly.

In spite of the rapid depletion of the measurable residue of royal jelly in repeated grafts, we must not be forced to expect that this has an influence on queen quality. During the many attempts to rear queens successively, I had been able to find only a few cases in which a serious loss of weight of queens had occurred. This was usually the case in weaker colonies, although these usually showed good rates of acceptance. In most instances the weight of pupae did not change in the first 3—4 batches ; sometimes more were needed before this occurred. Yes, occasionally the weight increased in the 2nd and 3rd batch. Only after this number of batches did the weight drop below that which I had obtained at the beginning. No change in the caste-specific characteristics for queens (number of ovarioles, shape of head, mandibles and tarsus) could be observed at least in the first 5 batches.

I am inclined to draw the conclusion from these experiences, that for practical purposes we can expect at least three satisfactorily reared batches given with an interval of 5 days, from a queenless, strong nursing colony, without having to strengthen its population. If the condition of the cell raiser allows, we can risk a further graft or so, especially if smaller number of grafted cups are given from then on. Should the nursing stock be used solely for starting cells we can double the number of batches easily, providing that the grafting frame is exchanged every two days.

Maybe it is important to force a nursing stock to elaborate royal jelly constantly in order to maintain its inclination to look after queen cells. This is what happens in a stock which is used solely for starting cells. When we leave cells in a colony until all of them are sealed, we should endeavour to hang in the next frame with grafts as we remove the completed batch. TARANOV (1974) suggests that this should always hang in the same place. Continued stimulation is said to keep nurses very efficient. Experiences made in the commercial production of royal jelly (VUILLAUME, 1959 ; SMITH, 1959) show, that colonies will accept new cells constantly and open or sealed queen cells excert no influence

on acceptance itself. Yet TARANOV (1974) stated that queens tended to be smaller if the sealed queen cells of the previous batch were left with the bees ; the rate of acceptance was also said to diminish.

It has already been mentioned, that we can turn a queenless colony into a permanent cell raiser if we add emerging brood every 8 days. Of course, in a queenright colony there is brood at all times. We can also strengthen it with more brood or bees should this prove necessary. Whenever we give a new grafting frame we should transfer open brood to the rearing compartment (LAIDLAW and ECKERT, 1954). Even when we start queencells in a confined shaken swarm it is possible to give more than one batch. At the Institute in Erlangen we give two batches and leave them for 24 hours each. LAIDLAW and ECKERT (1950) speak of 2—3 grafts with 90—120 cells in each batch. Their swarm box contains about 2.5 kg bees. The first batch is introduced after 2—5 hours. As a rule the bees are returned to a finisher colony afterwards. The number of batches can be increased if the bees of the swarm box are given free flight. I feel that in this case it would pay to add sealed brood as well as the obligatory frames of food. After emergence these frames should be exchanged for new ones every 7 days. It is better still to add more young nurse bees from the start. This creates the permanent starter colony (LAIDLAW and ECKERT, 1950; VUILLAUME, 1957).

3.2.9. **Choice of method**

There are many methods for rearing queens, and beekeepers the world over apply them more or less rigorously. They vary by degrees from rearing in queenless colonies via the starter colony and the starter in the swarm box to the queenright cell raiser. The influence these methods have on the development of queens depends a great deal on the way the graft and the equipment is utilised. A comparison of the methods is useless, unless the same conditions are applied to them all. For example, the tests made by SCHRAMM (1956) have no significance for us, although he was most thorough in every detail. He compared the old method recommended by ZANDER (1944) of rearing queens in a colony which had been queenless for 9 days, with another one by HEINECKE and ZIEGLER, which differed only slightly from the first. The same is true to a far lesser extent for the Russian experiments reported by TARANOV (1974). For these trials "artificial" nursing colonies were created by using brood and bees from 3 stocks. They were constantly re-inforced with more brood from two other queenright colonies. The queens emerging from successive batches given at 5 day intervals were said to have been smaller than the queens which had been reared in "normal" nursing colonies in 3 successive batches which had been introduced over 15 days.

Speaking generally I would like to say that optimal queens may be reared by using about any of the well known methods, provided we pay attention to their specific possibilities and limitations. Our first commandment should be not to overtax the nursing capabilities of the bees

we use. It is true though, that the race of bees and the prevailing climatic factors may make one mthod more suitable under certain conditions, or it may ensure better results than another one. Finally it is the beekeeping methods and the personal inclination of each beekeeper which will decide which of the methods of rearing queens will become his "own" method.

3.3. *A question of race and character*

Besides the factors which influence nursing and which may be analysed in our nursing colony, we must also make allowances for its "character"; a word which covers all biological and physiological individualities and also the way in which it is preparing itself for rearing queens. We must differentiate between its inheritance arising from its genetical background, and its "personal" character.

3.3.1. **Race**

There are races of honeybees which are supposed to be more suitable for raising queens than others, which are then condemned as "unsuitable". In this connection it is usually the rate of acceptance and the production of royal jelly which is being assessed. The opinions about the suitabilities of the races are usually varied. VUILLAUME (1957) reports that the results obtained by using the Caucasian race for queen rearing were better than those in which the black, local race had been used. He also mentioned that the French breeders consider bees of the Italian race to be poor nurses of queens. PHILLIPS (1905) and LAIDLAW and ECKERT (1950) of America report that the Carniolan bees are superior as nurse bees when compared with Italian and Caucasian bees. KLINK (1956) states in one paper, that in France the Carniolan race is better for the production of royal jelly for commercial purposes than the European black bee. He assumes that the Cyprian bee would be the best for this purpose. The beekeepers of West Germany do not think that the Carniolan race, widely used in that country, is exceptionally suited for rearing queens. On the other hand, the colonies obtained by crossing the Carniolan bee with the European race are believed to be very good nursing stocks. I am tempted to believe, that this also applies to other crosses between races. Hybridisation usually brings about a heterosis effect which can also be beneficial to the artificial rearing of queen bees. Anna KROL (1976) reports a rate of acceptance for the "local" race of 26%; for a cross between the Caucasian bee and the local strains 76% and for a Carniolan cross with the local bee a value of 83%.

I am convinced, that one can not assess the suitability of a race of bees for queen rearing without considering environmental influences. In a different locality and a different climate the behaviour of a colony changes. This may also affect its innate drive for propagation, which of course plays a vital role in queen rearing. Any evaluation of the bee's nursing capabilities must make allowances for such a change of habitat. Aparat from that, I feel that the world "race of bee" covers too much

ground when we want to evaluate the suitability of a bee for artificial queen rearing. Looking simply at the two races of bees which predominate in Europe, the Carniolan bee *A. m. carnica* and the black European bee *A. m. mellifica*, we find within these races so many strains of different origin. All show considerable variations of patterns of behaviour which are influenced by the environment or by selective breeding efforts. The same variation applies for their patterns of reproduction or swarming. In the two regions which each race inhabited naturally we find a few strains which are well known as either "swarmy" or "non-swarming" bees. The bee of the Lüneburg heath, also called the "heath-bee", belongs to the racial group of the black European bee, even though it is by now slightly mongrelised with some Italian blood. This bee was selected for its readiness to swarm over centuries and is still known as an excellent bee for queen rearing. I am convinced on the other hand, that the fame of the Carniolan bee as a good race for queen rearing is due to the fact, that from the beginning of this century onwards a certain strain of this bee from Austria became known in many parts of Europe and the world. This was the bee from Carinthia, a part of Austria. The heath-bee had been selected towards a swarmy type so as to make best use of the local flora, the late flowering heather *(Calluna vulgaris)*. The same trend was followed in Austria in the interest of exporting swarms. The very small bee hives used in Carinthia, the "farmer's box", forced the local race to develop into a bee which "enjoyed" swarming. As we nowadays select this race with an eye on reduced swarming tendencies by means of artificial queen rearing, we will have to make a fresh assessments of its reputation as a good nursing bee. But as breeders place

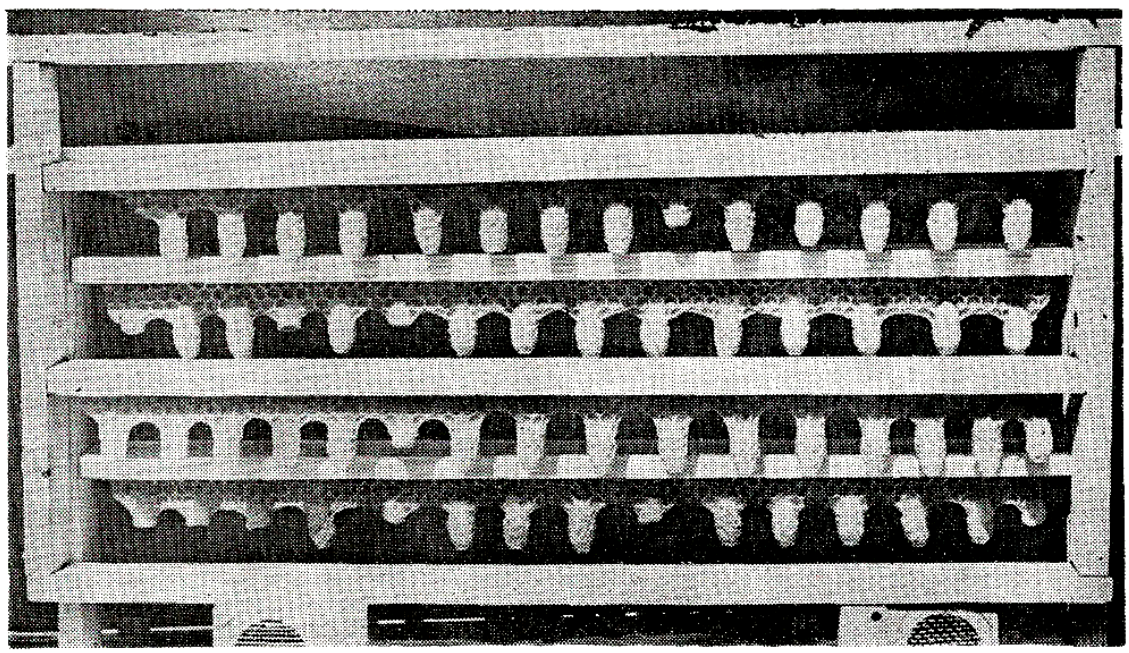


Fig. 42 — *Two grafting frames from a queenless colony of the Tellian bee. These frames may be passed on to another queenright stock for finishing after 10 hours and can be replaced by another batch. That way a nursing colony can "start" about 100 cells per day ; but only for about 3 days, when the first laying workers become active.*

a heavy selection pressure on its explosive development in early spring, it may be that this drive for expansion itself will bring many advantages for queen rearing in its wake.

Finally we must examine the possibility that race itself will influence the products of rearing. There is the possibility that the nursing colony will exert some race-specific influence on its charges and will leave its mark on the characteristics or the behaviour of the queens it has reared. Especially some Soviet researchers have discovered over the last few years, that the bees which bad been reared within a racially unrelated nursing colony, showed that some characteristics were approaching those of the host. Length of proboscis is one aspect which seems to get investigated preferrentially. Bees of a long-tongued race, the Caucasian for example, have shorter tongues if they are reared in a colony of a short-tongued race from Central-Russia, the black European race, *A. m. mellifica*. The reverse also seems to be true. The adaptation towards the host type applies also for various other characteristics, such as size of sternites and tergites, measurements of wings, wax glands and tarsus, the cubital index, the width of tomentosae, even length of hairs. (MIKHAILOV, 1929 ; SINYAEVA, 1952 ; KOLESNIKOV, 1959 ; DUBROVENKO, 1960; SHVEDKOVA, 1960; SMARAGDOVA, 1960, 1964; AVDEYEVA, 1965a). Behaviour patterns too are said to be influenced by the host colony's character, such as the way honey cells are capped with either white or moist cappings (GUBIN and KHALIFMAN, 1950); or the ability to winter well and the production of wax and honey (VINOGRADOVA, 1955). In West Germany F. RUTTNER (1957) at least has been able to establish a minute change in the cubital index towards that of the host colony.

After reading all the above results it must not surprise us, that Soviet scientists have reported a morphological influence by the host on the queens it reared from grafts. According to BURMISTROVA (1963), after a few years the number of ovarioles will approach the number which is typical for the host race. Yet this was observed only when central-Russian queens were reared in a colony of Caucasian bees; the reverse was not true. It seems very strange, that these morphological and behavioral changes towards the host type were observable even among the workers of these queens — in the second generation, so to speak, as well as in repeated generations of daughter queens, when reared by the other race (KOLESNIKOV, 1959 ; AVDEYEVA, 1961, 1965a ; BILASH, 1962 ; MELNICHENKO, 1962 ; SHAKIROV, 1963). MEYERHOFF (1957) examined in East Germany cubital index and length of tongue of worker bees of some Carniolan queens, which had been reared in colonies of the North-European bee and vice-versa; he could discover no difference in these characteristics from those of the original race.

Nowadays it is probably no longer questioned that in certain cases characteristics and the behaviour patterns can be influenced by the nurse bees of another race. One explanation is to be found in the racially influenced differences of the royal jelly, and SMARAGDOVA, (1963), and MELNICHENKO and BURMISTROVA (1963) have found differential spectra of amino acids in royal jellies from Caucasian bees

and that taken from colonies of the Central-Russian bee. On the other hand, our present level of knowledge can not concur with the reports, that any such changes in the characteristics and the behaviour are passed on to subsequent generations. "Hereditary transmission of acquired characteristics" is a disputed point and has yet to be demonstrated for animals less complicated than the honeybee.

The breeder should never overestimate this racial influence of the nursing colony on queen rearing in all its practical aspects. If we want to change our bees genetically we will achieve much more by programmed selection and planned matings then we will ever obtain by utilising the race of a nursing colony. We also need not fear that queen rearing in a nursing stock belonging to a different race will ever influence future generations genetically. We should have no hesitation in letting another race be the host for our queens ; nursing capabilities alone should be the criteria for our choice.

3.3.2. **The nursing colony as an individuality**

If we allow, that the race of a nursing colony may have some influence on the results of artificial queen rearing, we will have to admit that this applies more so to individual colonies regardless of their origin. I myself was confronted by this fact time and again over the years and I can confirm the experiences of many breeders and beekeepers. Colonies which rear brood readily and which may be considered as specially suited for queen rearing, can be found in any race or strain of bees. But the reverse is also true ; we also find colonies with small brood nests, yet much honey, which are less suitable for nursing. Apart from these two types we will find stocks which will make poor or good nursing colonies without taking into account their brood rearing capacity. These are colonies, in which the reproductive instincts are either poorly expressed or well pronounced. One can make parallel experiments and give colonies at the same time, in the same apiary, and of the same race, equal numbers of grafted cells. There will always be some colonies with good rates of acceptance and others which can be termed complete failures, which only accept a limited number of cells only and will rear small queens. As with acceptances, we will find that the production of royal jelly is variable from colony to colony. Commercial producers of this product will know that there are colonies with "huge yields" and others with tiny quantities (DADANT, 1958 ; MÂRZA and BARAC, 1961). Even the average time lag before the first feed is given to dry-grafted larvae is variable from colony to colony (ÕRÖSI-PAL, 1960). Poor nursing stocks do not improve with a succession of grafts. These latter colonies are simply unsuited for artificial queen rearing. Yet again, poor acceptance of the first batch does not imply that this is a poor nursing colony; the second attempt will be a better indication of its performance.

Every colony of honeybees has an individual character. This character is composed of many single traits. All factors are interlinked and are often dependent on one another. Honey production is only one such example. But occasionally one single character trait is more pro-

nounced than usual and we all know this about aggressiveness. It comes therefore as no surprise for the experienced breeder, that the reproductive instincts of a colony may also be expressed to a variable degree.

4. External factors : microclimate and macroclimate

After discussing at length any influences which the presentation or the age of the grafts may exert, and after looking closely at nutritional aspects of rearing, only the environmental effects on queen rearing need our further attention. This study must cover various microclimatic factors, especially those of internal and external temperatures on the growth and development of the queens ; the influences of nectar flows or feeding before and during queen rearing, and last, not least, any indirectly acting, multifactorial aspects of weather, climate, environment and the season of the year.

4.1. *Microclimatic factors*

The colony of honeybees usually is able to control all microclimatic aspects required for queen rearing within its brood nest and the queen larvae develop under largely optimal conditions. But occasionally the last stages of "ripening" takes place in an incubator and sometimes the cells are exposed to unusual environmental conditions for some time. What are the effects of these conditions on developing queen ?

4.1.1. Control of the conditions within the colony

Humidity and temperature are fairly constant within the brood nest of a colony in spite of constantly changing, ambient conditions. BÜDEL (1948) recorded relative humidities of cca 40%, in Munich, West Germany. OERTEL (1949) did the same in Baton Rouge, USA, and measured 40—60% R.H. The average brood nest temperature has been found to be cca 35°C by HIMMER (1927) and many other researchers under widely varying, external conditions. While brood seems to be fairly resistant to variations of humidity, it will develop normally only within a narrow range of temperatures between 32°—37°C (HIMMER, 1927). It rarely happens that temperatures rise beyond 37°C ; but a drop below the lower threshold is quite possible. Temperatures seem to stay very constant in the vicinity of eggs and open brood (BÜDEL, 1955). Variations of up to 4°C do occur over sealed brood and in those places where sealing of brood is taking place. ZHDANOVA reports a comparatively low brood nest temperature for early spring when only small areas of brod are present. Her figures of 31—32°C were also said to vary considerably and near the edge of the brood nest she was able to measure drops in temperatures to 26 and 24°C of short durations. Even later on when these colonies were developing rapidly, temperatures near the edge of the brood nest were clearly lower (32—33°C) occasionally down to 30°C, than those of the centre, where 35—36°C were the rule. The temperature gradient from the central regions to the marginal ones occurs

also in observation hives with one single comb. DRESCHER (1968) has blamed this variation of temperatures for the lengthening of the developmental period of worker brood from 19 days in the centre to 21 days at the periphery of the brood nest. It is also likely, that the disappearance of open brood from the edge of the brood nest, observed by FUKUDA and SAKAGAMI (1968), is linked to these lower temperatures.

After studying these reports it makes us wonder why queen cells are constructed near the edge of a brood nest and not in the centre when a colony is preapering to swarm. That way the cells are exposed to widely varying temperatures, instead of being in constant warmth. This makes us contemplate if this behaviour pattern dates back into evolutionary periods, when the perfect female developed as the original female form within a society, in which the temperatures were not controlled to such a fine degree.

What conditions prevail when we rear queens artificially ? We are probably wrong in believing that the queen cells on the grafting frame in the centre of the brood nest enjoy a constant temperature. Here we find wider passages between the combs and these often give rise to considerable variations of temperatures. BÜDEL (1955) found between unusually wide passagers variations of as much as 5°C. When the grafting frame had been inserted between two combs with open brood, ZHDANOVA (1963) measured air temperatures between 30 and 35°C between the queen cells. When the frame hung between sealed brood the variations ranged between 24 and 34°C.

During good honey flows the average temperatures of the brood nest are a bit lower than usually (ZHDANOVA, 1963). On the other hand, they may rise to nearly 38°C when external temperatures are high (LENSKY, 1964). These temperatures have also been measured exceptionally near the grafting frame (ZHDANOVA, 1967). Under these conditions lighter queens were said to have emerged than any bred before or afterwards. However, it is not possible to say whether this is related to temperature alone. We all know that with high external temperatures many bees leave the broodnest and this can mean that brood is not fed at optimal levels. It is equally possible, that when smaller queens are reared in early spring, and breeders often find this to be true, the underdevelopment may be a consequence of undernourishment rather than of lower temperatures.

It is also tempting to assume that the prolonged periods of development of a few queens is due to the position of the cells near the ends of the grafting frame, where the temperatures may not be as favourable. But here too we must weigh up the possibility that some nutritional factors could be involved beside these of temperatures. The same may apply for whole batches of grafts which occasionally seem to emerge belatedly. This may have been the case with repeated grafts given experimentally in order to study the nursing ability of ageing bees without rejuvenating the population. GOETZE (1924) and BUCHNER (1953) found that temperatures fell as the amount of brood dwindled through emergence. BUCHNER actually connected delayed emergence of queens with this fact. On the other hand we should not forget, that in queen rearing

in the swarm box, or when we rear queens in a colony after the removal of all its frames with brood, no such delays in emergence seem to appear, even though similar conditions of temperatures may prevail. When I successively reared queens time and again in a colony without adding any young bees until 17 and 20 batches had been reared respectively, I also observed delayed emergence of queens, and the average time increased until it neared that time which is typical for workers. The temperature factor must have played a secondary role in this experiment, as right to the last some queens emerged with normal periods of development. In any case, all stages of development were affected : that of the small, coiled larva, the fully grown one as well as that of the pupa — and during pupation period all sealed cells were exposed to equally optimal conditions in an incubator.

4.1.2. **Conditions in the incubator**

Within the colony the conditions of temperature and humidity are constantly monitored by bees and are adjusted to remain within the range which provides the biologically optimum condition for development of the queens we rear. Without bees to regulate environment any microclimatic disturbances can have a serious effect on our charges. Many breeders place their queen cells either immediately after sealing or later into an incubator in which they can "ripen" and emerge. A controlled temperature of 35°C and a relative humidity of between 50—60% is maintained, just as we find it in the brood nest.

Irregularities in relative humidity seem to affect the growing queens very little. I have had queens emerge under the extremes of 30%, as well as 80% relative humidity. But it seems that values below 40% are not without danger. RUTTNER (pers. communication), found, that cell walls dry up and turn hard and that pupae die before emergence. For a start, the storage conditions of cells forbid extremes in humidities, as they are usually held in nursery cages which are supplied with some honey or candy. When the air is too dry the candy will harden and queens can starve ; when the air is too damp, the food will liquefy and queens will get sticky all over and may also perish.

In sharp contrast to humidity, some minor variations of temperatures can be fatal for our queens. As long as these irregularities do not exceed 37°C they are fairly harmless.

Slightly higher temperatures than normal will result in a lighter colouration of the body (in queens and workers) (MÜLLER, 1940; SOOSE, 1954). The death rate of worker brood during pupation is very high if temperatures rise above 37°C (HIMMER 1927 ; SOOSE, 1954). If any bees still emerge, they will do so with delays, and such individuals usually die after a few days, according to KRESAK (1972).

Under the constant influence of slightly lower temperatures on sealed brood, emerging worker bees show deviations in the length of appendages of the body. Tongue and wings are reported to get shorter when temperatures of 30°C prevail (MIKHAILOV, 1929). HIMMER (1927) reports that this occurs already at temperatures of 32°C. SOOSE (1954) measured a clearly reduced cubital index and found some malfor-

mation of wings at 32°C. Malformations were also found by HIMMER (1927) at 32°C. Brood which had been held constantly at 30 and 32°C emerged with delays. MIKHAILOV (1927) speaks of a 3 day delay, SOOSE (1954) mentions delays of up to 12 days. It must be obvious, that changes in development in terms of delays and malformations must be greater, the longer the lower temperatures act on sealed brood.

We can assume, that queens developing in an incubator will react to the ambient microclimate like worker brood. Maybe they are a little more robust, but we can not say that for certain. SOOSE (1954) thinks that workers show a degree of toughness which is race-specific. However, the conscientious breeder will endeavour at all times that the queen cells in an incubator will be exposed to conditions which are as similar to those in a nursing colony as is possible.

4.1.3. **Viability of queen cells**

So far we have discussed only those influences, which either show minor variations from natural conditions, or are of longer duration such as we can find in an incubator. In artificial queen rearing the cells must be taken occasionally out of their microclimate, even if for only short periods. This is the case when they are transferred from the nursing colony to the incubator or are given immediately to a nucleus. The queen breeder of course, would like to know the viability of the queens in their cells under the external conditions.

Chilling is usually the reason given when such cells do not emerge. This explanation should be correct in only a few cases. I have kept queen cells of various ages for up to 3 hours in rooms, cellars and refrigerators. They hatched nearly all, and all queens had developed normally. Only the refrigerated cells did not survive a full day in their cool place. Most of the others emerged, especially the cells which had been in the living room. The period of least resistance to cold appears to be the time of pupation and shortly afterwards; between 10th and 12th day after the egg had been laid. More damage is caused by mechanical shocks. A minor shake of the grafting frame can reduce the rates of emergence considerably when the grafting frame is transferred 5 days after grafting — that is 9 days after the egg was laid or immediately after the cell was sealed over. This sensitivity to mechanical shock continues until the 14th day, although it is decreasing all the time. After that day mechanical shocks do not seem to affect the cells any longer. We can put such cells on their sides, even on their heads while we are working; queens will emerge undamaged just the same. (WEISS, 1962).

These experiences show, that the beekeeper need not be too anxious about damaging cells 1—2 days before their emergence. He can take his time over introduction. Cold weather should not prevent work. Of course, he must make sure that the cells are returned to an environment with optimal temperatures. The cells can withstand a brief cooling and development will be interrupted during this time; but they will not tolerate a permanently reduced climate for development. It is possible, that in practice neglegence in this respect must carry the blame for most failures to emerge.

The toughness of older queen cells opens up some practical possibilities. Queen cells may be used to requeen mating nuclei repeatedly in their mating yards. They will withstand the transport to these places in a small box simply lined with soft cloth or tissue without additional source of heat. We can even post or mail such cells. If they are returned to a suitable environment within hours they will emerge normally, even though emergence will be delayed by the period they had spent away from the colony. The Bee Research Station at Lunz am See (Austria) sends thousands of cells annually in blocks of expanded polystyrene or extruded styrofoam with holes which hold the queen cells firmly.

4.2. *Supply of food*

Any good nursing stock must have honey and pollen reserves at all times. But apart from these reserves we must ask ourselves the question : Will a nectar flow, or a simulated one of sugar syrup, influence the queen rearing programme ?

4.2.1. **Nectar flows**

All breeders are agreed on this : good nectar flows mean poor queen rearing results. Rates of acceptance drop and the care of larvae is neglected. Often bees will ignore cell cups which they had readily accepted earlier. Especially in queenright nursing stocks many sealed cells are then destroyed. BURMISTROVA (1963) speaks of smallr queens with fewer ovarioles under such circumstances. It seems that good honey flows are incompatible with reproductive processes ; even swarming instincts are suppressed at such times.

Yet modest flows are thought to be of advantage for artificial queen rearing. A little trickle works like magic : the queen lays many eggs and nurses are busy. But we should pause and consider, if it is not simply an opinion, that such intense activity leads to better acceptance and better nursing, as compared with those days, when bees just stay at home. In reality bees will continue to nurse any cells which they had accepted as long as sufficient pollen stores are present. The expansion of the brood nest is then the only activity which slows down. I myself had never noticed that bees will neglect initiated cells. I also found it impossible to discover a correlation between acceptance and nursing capacity on the one hand, and the prevailing conditions of flows on the other, — honeydew flows excepted. Periods without nectar flows will make themselves felt much later in their impact on queen rearing. That is the case when the reduction in brood rearing finally results in fewer nurse bees within the stock. And this can occur at a time when the environment is favourable again.

The conditions are different after a good honey flow, which at the same time provided an abundance of good pollen and also fell into the period of intense development. These three conditions can occur during the flowering of oil seed rape in Germany, for example. This crop promotes brood rearing activity and afterwards bees are ready to swarm. This provides us with an excellent opportunity to rear queens.

Maybe it is the availability of much pollen, which encourages bees to consume much protein, which in turn favours the production of the glandular food.

4.2.2. **Feeding**

The speculation that a minor flow will improve artificial queen rearing simply because it puts new life into the colony, makes beekeepers simulate such flows during a period of dearth by giving small feeds of sugar syrup. As a rule, these gifts of sugar syrup or honey solutions begin a few days before the grafting frame is inserted and will continue until cells are sealed. At that point feeding usually stops in order to avoid that all cells are encaged in a welter of wild comb. This method of stimulating breeder colonies is so widely used, that I feel a heretic when I doubt its effectiveness. I myself used to follow this recommendation years ago, but I changed over to using candy later. Nowadays I omit all stimulative feeding before and during the nursing period. Ever since I have been unable to discover a correlation between stimulation and acceptance, I have stopped feeding colonies even when a complete dearth sets in. Cells are no smaller when nectar flows cease, as one hears so often. Cells may be less sculptured externally and this is connected with poor constructing activity. But in contrast to the opinions of many beekeepers there is no relation whatsoever between the external patterning of a cell and its contents. I have seen some smooth cells with very large queens and others with beautiful sculpturing but containing queens of low weight. (Fig. 43).

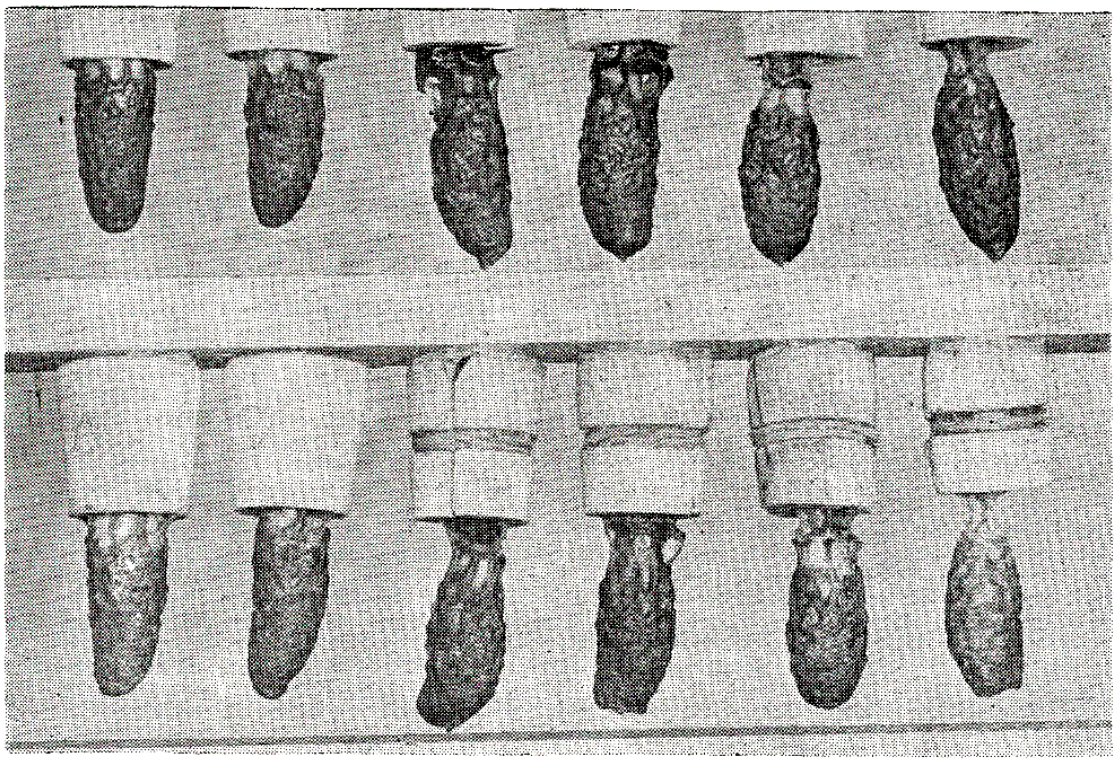


Fig. 43 — *When the flow is scarce queen cells are not sculptured*

Only for rearing out of season ; either very early or very late, or when the weather is very cold, can I see some advantage in stimulative feeding. But in this case feeding brings about an invigoration of the colony, which will result in better temperature regulation within the stock. But even that requires confirmation. The need for food containing much protein also needs confirming. CHAUVIN (1962) cites BURTOV (1954) who fed syrup with 5—10% yeast or pollen added and reported that queens had improved in weight and quality. WEAVER (1957) writes that more royal jelly was found in queen cells if pollen was fed during a lack of pollen. Giving a comb full of pollen stores did not have the same effect. RUTTNER (1965) on the other hand had been unable to reach a decisive conclusion by feeding a candy containing additional protein, but he recommends its use during long periods of bad weather.

There is a possibility that we achieve the opposite of what we expect when we stimulate. The intake and elaboration of food may absorb some of the young bees and prevent them doing their real work ; looking after the queen larvae. The value of continued large quantities of syrup (up to 1 liter of sugar or honey solution daily), seems questionable to me. It is possible that heavy feeding and queen rearing are just as incompatible as heavy honey flows and optimal queen production.

In contrast to stimulation just before and during queen rearing, there could be some value in providing stimulative feeds long before the work begins. We must encourage colonies to expand early in the year if we want to rear early batches of queens. In addition to having strong colonies after winter and to uniting stocks if the arises, the feeding of honey, sugar solution or soft candy 4—5 weeks before our planned date can help. Provided that enough pollen is available, the colonies will then expand and those larvae will be nursed which will later be the nurses of our queens.

It is obvious that our nursing colonies must have reserves of food at all times. Pollen is of special importance ; no brood can be raised without it. There must be a pollen reserve in the nursing colony and especially so, if it is to be used for successive batches. Bees can replenish their stores only during good weather. It is true, that at such times queenless colonies may store excessive amounts. When we are checking that adequate pollen stores are available, we must not forget our finshing colonies. And finally we must ensure that also sufficient stores of carbohydrates (honey or sugary feed) are present in the nursing colonies. This should not mean that the stock should be honey-bound ; clogged up with stores. There is no evidence that such a condition ensures better queen rearing in such a colony ; indeed, the opposite is likely to be true. On the other hand it goes without saying that we cannot expect optimal queen rearing in any colony which is suffering from starvation — and it makes no difference if the hunger is due to lack of pollen or carbohydrates. Every breeder will take care that all nursing colonies have adequate supplies of food.

4.3. *Factors beyond our control*

Every beekeeper knows that weather, environment, climate and season exert strong influences on bees and beekeeping. That the same factors influence artificial queen rearing will not come as a surprise.

4.3.1. **Weather**

Breeders are of the opinion that bad weather is not suitable for rearing queens. Of course, bad weather affects bees very much and the honey crop will suffer from it. But must it have an unfavourable effect on queen rearing itself? I suspect that this opinion is based on an unacceptable analogy. VUILLAUME (1957) reports to have had reduced rates of acceptance, sometimes giving him only 50% of the usual cell numbers, when rainy days prevailed. He assumed it to be due to a shortage of water within the colony, as bees cannot fly for this when it is raining. Yet we do know, that bees have a water depot within their bodies and make use of this reserve in times of need. Besides, rainy periods without any chance of flight are rare. I prefer to believe, that the drop in temperatures accompanying such rainy periods has a deeper influence on reproductive instincts. DREHER (1960) says that it amounts to a "catastrophy", if a rainy period (or dearth) begins at the wrong time when the cells are being sealed. He believes that a nursing colony will loose its inclination to take care of its charges within a few days and will destroy all cells but one. In another report (1948) he writes that "large numbers of weighings have shown clearly that the weight of queens decreases rapidly, if bad weather sets in when queen rearing is in progress". H. RUTTNER (1969) also stated that one cannot rear queens successfully in bad weather. I myself had never found that weather had such a bad influence on the behaviour of queen rearing stocks, especially not during the summer months which favour such work. Acceptance especially did not seem to suffer from short-term, even long-term periods of variable weather. Although I must admit, that in spring and autumn the weather will influence rates of acceptance and nursing unfavourably. This may be due to external temperature variations, which have a greater effect on the colonies which as yet have not much brood and few nurses. There is also no doubt, that any brood stop caused by long periods of bad weather and accompanied by a dearth of nectar, will be the cause for poor queen rearing results later on.

Finally, we find many reports in apicultural literature, that excessive heat can harm queen rearing efforts. ZHDANOVA (1967) blames the hot weather for the lower weight of queens reared during June ; many bees were forced to leave the brood nest. We can well believe, that bees must neglect their nursing duties in order to lower internal temperatures somewhat, when the initially crowded colonies form thick clusters of idle bees at the entrance. Under such conditions we must provide shade and leave entrances wide open.

4.3.2. **Climate and environment**

In some parts of the world queen rearing is possible over a longer period and larger batches may be raised here than in other areas. Primarily this is due to the climatic conditions of the region and their influence on the development of colonies of honeybees. Geographical location and climate form a unit : the environment. Both are inseparable in their influence on queen rearing.

We can state a rule of thumb : queen rearing creates fewer problems in areas where beekeeping is naturally favoured than in those where the keeping of bees is less successful. Mild climates are better than mountainous, harsh ones; southern countries are more suitable than the cold North. We can illustrate this with an example : rearing queens in a queenright colony is possible in West Germany, but there are many difficulties. Because of the poor acceptancies achieved, a wire screen had to be inserted a few hours before the introduction of the grafts in the compartment without the queen. 24 hours later this screen is replaced with queen excluder material and full contact is re-established between the two compartments. In Israel it is not difficult to succeed with this method, and LENSKY (1971) states that no screen is required between the brood chamber and the queen rearing compartment. To what extent racial factors are involved — the Italian race is used there — needs investigating further.

There is a possibility, that the special character of the geographical-climatic environment has an influence on anatomical and behavioural peculiarities of the growing bees. Even if we cannot any longer take the findings of ALPATOV (1928) and of others for granted, that there is a change of size of bee and length of tongue in a direct relation to the geographical latitude and the elevation, it does not mean that external influences like these have no influence at all. KRESAK (1964) feels he had found a lengthening of the tongue and faster growth when he moved colonies of the Carniolan race from the mountainous regions of the lower Tatra (740 m above see level) to the milder climate of southern Czechoslovakia (147 m). When he reversed the experiment and moved Carniolan stocks into the mountains, he could detect only a shortening of the tongue, but no extension of developmental period. Contrary to this, it seems that behavioural characteristics may be more stable. When LOUVEAUX (1966) transported colonies of different race and origin into other regions of France with different climatic conditions, he found that their productivity suffered in the process. Yet there are also reports of increased productivity, especially when bees from the south were moved north (FOTI, 1956 ; BILASH, 1958 ; LOPATINA and RAGIM-ZADE, 1962; KRESAK, 1963; MELNICHENKO, 1965; BARAC, 1971 etc.).

If geographical-climatic conditions can have an influence on the worker bees, then the same could apply for queens reared in the same conditions. On the other hand, such changes can never become inheritable, as our considerations of rearing queens in colonies of a different race have shown. For practical queen rearing both factors are of no importance.

4.3.3. **Seasonal influences**

Brood rearing in colonies of honeybees depends very much on the season of the year. Not only does it influence the amount of brood, but the morphology of the workers is also affected by it. MIKHAILOV (1927) has shown, and many other workers have investigated the problem after him, that many bodily measurements show seasonal changes: weight at emergence ; size of body ; length of tongue and wing ; and width of the last. LEVIN and HAYDAK (1951) established that ovarian development depended on season. So it need not surprise us that we can find reports in literature, which maintain that seasonal changes influence the development of queens. The rate of acceptance by nursing colonies is the main point of reports ; but the development itself appears to be influenced too. Sometimes the differences reported can be explained by the latitude of the countries making the report. In Egypt ABDELLATIF (1967) reported poorer acceptance for March than during April and May. Queens were said to have been of equal weight, and he concludes that bees would accept only the number of cells which their condition permitted. During March cell size could be related to queen weight; this was not the case for April and May. In a later report ABDELLATIF, EL GAIAR and MOHANA (1970) gave the rates of acceptance for March as 46% ; for May as 60% and for July as 72%. The reason for the low acceptance is given as high external temperatures, but the authors also mention that nectar and pollen supplies were influential. ȘERBĂNESCU (1971) also reports reduced numbers of queens reared in April as compared with May, but he could find no differences in the weight of queens. WAFA and HANNA (1967) found in Egypt, that acceptance is better in spring and summer than in autumn and is worst in winter. Their reports concern the commercial production of royal jelly and their harvest was greatest during the spring period. ROBERTS (1965) reports from the South of Louisiana (USA), that acceptance dropped after "midsummer" when he was breeding queens in a queenright stock. The reason for this was the decline in brood rearing at that time. It is therefore surprising, that FREE and SPENCER-BOOTH (1961) could not establish a seasonality of cell acceptance under various conditions of queen rearing, when they evaluated the results obtained for April, May, June, July and August by 2 bee farmers in Oxfordshire, England, over three years. We can assume that the various batches had been adjusted in size to the conditions of the nursing colonies. WEAVER (1957) reports on queen rearing in Texas (USA) that anatomically the best queens were those reared during June ; quality declined progressively during July and August. But the "worst" were produced in early May. WEAVER also observes, that many changes occur over the months and it is difficult to pick out certain factors which may be responsible for his observations : strength of colony, the physiological condition of the bees and honey flows may all have contributed. AVETISYAN *et al.* (1967) have reared queens in central Asia for three years in the months March, April, May and June. They examined and compared the queens in respect of cell size, queen weight, size of tergite and number of ovarioles (by section!). According to these criteria the best queens emerged in Tadjikistan

during April, in Uzbekistan during May. Queens born during March and June were not as good. ZHDANOVA also found May-queens to be smaller than those reared in June. May had been warm and a good flow was on ; June had been very hot. During July a flow occurred from the lime trees and queens were larger again. AKOPIAN and MARKOSYAN (1967) measured the fat content of queen larvae just before sealing and found this to be greater in June and July than during May and August. Larvae were lighter in May and August and had lower nitrogen content than those queens born in June and July. Queen weight as well as the weight of a worker increases in Czechoslovakia during spring and summer and decreases in autumn, according to a report by SKROBAL (1958). MÂRZA *et al.* (1967) observed on the other hand, that among the native bees of the steppes of Romania heavier queens were obtained during August as compared with June. They reported that queens can be reared from 15th April to 31st August, especially if the nursing colony is insulated when this becomes necessary and is fed when no flows exist. SHIMANOVA (1966) states that there are good and bad years ; in good years queens are heaviest at the period of first blossom and get smaller towards autumn ; in poor years their weight increases towards the end of summer.

Many more examples could be given, but a clear pattern of seasonal influences on queen rearing can be recognised. It is obviously not the time of the year itself, but the condition of the colony, which is decisive for good results in queen rearing. The season controls the weather, and the flow conditions affect the conditions within the colony. Especially in the temperate regions we experience considerable variations in the weather from year to year, and because of these we must expect variable results in queen rearing. The quoted differences can be explained by these phenomena.

In general we can say, that the best time for queen rearing is the time of continued expansion and intense brood rearing activity. At that time we have many nurse bees in our colonies and these can be employed to rear queens. In West Germany this is the period from 15th May to end of July, roughly speaking. H. RUTTNER, (1969), suggests 15th May as a starting date for Lunz in Austria. But it is quite possible to extend the queen rearing period both ways in West Germany. Especially the later batches can turn out very well as late as August. Earlier batches make many difficulties ; without additional heat and continued early stimulation we can achieve little under the Central-European conditions. It would also be advisable to keep batch size to a minimum, as otherwise starved individuals would emerge.

In other countries and continents of the world the yearly brood rearing seasons are distributed differently throughout the year and there may well be two definite peaks of development in some regions. Other timing must be observed there for optimal results. But there too the generalised rule applies : The periods with minimal brood rearing activity are not suitable, nor is the very beginning of a seasonal up-swing, leaving aside any annual periods of dormancy of course. All other time is "best" time and the breeder must make full use of it.

SUMMARY OF RESULTS

1. The graft

1.1. Age of graft

1.1.1. It is not true that nursing colonies prefer older larvae to younger ones. No difference was found to exist in the acceptance of larvae of different ages ($^1/_2$—3 days old), when several batches of grafts were reared experimentally for comparison. It is very difficult to graft very young larvae and this may lead to heavy losses.

1.1.2. Queens showed only minor differences when eggs and very young larvae (up to approx. $1^1/_2$ day old) were used for rearing. Weight of queens decreased when older larvae were used. These queens showed no statistically significant changes in other characteristics — including the number of ovarioles — apart from proportional reduction in size when weight was lower. When using larvae older than 3 days of age for rearing, intercast individuals may suddenly occur.

1.1.3. Length of life and the productivity of their colonies showed no differences for queens reared from eggs or young larvae (up to $1^1/_2$ day old).

1.1.4. By using 1 day old larvae we stay within a safe margin for obtaining optimum queens.

1.2. Survival of grafting material outside the honeybee colony

1.2.1. Eggs are relatively hardy when they are removed from the colony at the age of $1^1/_2$ day old to point of hatching. They will continue to develop after their return to the brood nest. Eggs survived after having been outside the colony for 1 day 100%
outside the colony for 2 days 50%
outside the colony for 3 days ..... very few
Very young eggs (point of lay to $1^1/_2$ day old) are very sensitive and survive for only a few hours when removed from the hive.

1.2.2. Larvae at the age suitable for grafting (0—24 hrs) survived to 65—100% when removed from their colony for up to 24 hours. Removal of such larvae for short periods (6 hours) did not affect their chances of acceptance.

2. Queen cells

2.1. Properties and positioning of queen cups

2.1.1. Queen cups formed from fresh wax, from wax refined from old comb, and those moulded from polystyrene (of certain manufacture) were equally acceptable to bees.

2.1.2. There are limits to shape and size of cell cups which are acceptable to bees. Thickness of wall of cup was immaterial.

2.1.3. Larvae reared in cell cups of 9 mm ∅ received more food and emerging queens were heavier than those reared in cups of 8 mm ∅ or those grafted into cells of workers or drones. Strips of worker comb used for queen rearing should be of freshly drawn

wax. Lighter queens emerge from comb which had been used previously for brood rearing.

2.1.4. Rearing conditions are equal at any level of the cell bars within the grafting frame. It is better to spread the load and insert the graft on two frames a few combs apart.

2.2. Familiarisation

2.2.1. It is immaterial for acceptance whether cells are inserted into the colony before grafting for a period of familiarisation, or not, provided optimal rearing conditions exist in the nursing colony.

2.2.2. There is no need to familiarise eggs or larvae by inserting a piece of comb from the genetically desirable stock before grafting larvae into queen cups. Acceptance is not improved by this practice.

2.2.3. Nursing bees will accept larvae from their own brood nest or from other colonies equally well.

2.3. Priming queen cell cups with royal jelly

2.3.1. Grafting "wet" on a small drop of royal jelly gives no advantage over grafting "dry". It has not been clarified if wet grafting gives better acceptances when rearing in a queenright colony.

2.3.2. As long as young larvae are used for grafting, the double grafting technique results neither in better acceptance nor in heavier queens. Only when using larvae older than $1^1/_2$ days old can dobue grafting be contemplated as a technique ; but using larvae of that age is not advisable in queen rearing practice.

3. Royal jelly

3.1. Biological facts

3.1.1. Bees in colonies preparing to swarm and those in dequeened colonies have well developed brood food glands. This is a prerequisite for good nursing.

3.1.2. Time lag before the first feed is given to grafts, the number of feeds and the amount of food given to royal larvae varies considerably. This must affect the growth and the final size of the queen. The quantity of residual food left in the queen cells after the spinning of the cocoon has no relation to the body weight of the queen.

3.1.3. It is not known for certain, which bees will feed queen larvae. We do know for certain though, that there is great flexibility in the duties performed by workers. Very old nurse bees are still capable of rearing queens. (Up to 20 batches have been reared in succession in one colony). Acceptance and quality of the queens suffer as nurses age.

3.2. Basic principles of queen rearing

3.2.1. The colony must be a healthy one. Any disease of adult bees will affect nursing capacity.

3.2.2. Best results are achieved in a colony with a well balanced mixture of all age groups.

3.2.3. Colonies in an expanding mood are specially suited. It can not be confirmed that swarming preparation increases the inclination to nurse queen larvae.

3.2.4. The best time for the insertion of the graft is when bees become restless after dequeening. A period of waiting of 2 hours is sufficient.

3.2.5. More queen cells are accepted in a queenless colony than in a queenright one in which a compartment had been screened off to improve acceptance and queen rearing.

3.2.6. A hypothesis has been advanced recently, that the presence of open brood in a nursing colony will improve the quality of queens reared in it. This must be viewed in connection with the technique employed. When rearing in a compartment within a queenright stock, for example, the presence of open brood will act as bait for more nurse bees. The same applies when we initiate queen cells in a queenless "starter" and transfer them into a queenright finisher. Open brood in the super acts as bait.

3.2.7. The inclination to nurse queen cells varies between colonies. The following figures of batch sizes are given as a suggestion only and should be guide lines for profit-oriented queen rearing with quality and perfection as its aim.

Queen rearing in a queenright colony	15 cells (per batch)
Queen rearing in a queenless stock with reduced amount of open brood	30 cells
Queen rearing in a queenless stock without open brood	45—60 cells

This applies when using the Carniolan bee in Central-Europe and when cells remain in the colony until sealed. When the above colonies are only used as "starters" for 1—2 days, the batch size may be doubled.

3.2.8. A queenless colony can be used to rear several batches in succession. This should not affect the quality of the queens. 3 batches given at 5-day intervals will be reared satisfactorily. More batches are possible, if young bees are added to the nursing colony. The big advantage of a queenright colony is, that it may be used as a permanent queen rearing colony.

3.2.9. Method is far less important in queen rearing than the nursing capacity of the queen rearing colony. All methods are good methods if the capacity of the colony is not overtaxed.

3.3. Race and genetics

3.3.1. Nursing abilities are influenced by genetic factors. These differ between races. Many racial hybrids are specially suitable for queen rearing.

3.3.2. There are differences in the nursing ability of colonies which cannot be explained by race or environment. There are "good" and "indifferent" nursing colonies by inheritance.

4. External factors

4.1. Microclimate

4.1.1. The relative humidity of the air in the brood nest of a colony of bees is maintained between 40—60%. Temperatures vary much more than had been thought. Even though larvae and pupae do not seem to suffer much from short term variations, it is known that continued undercooling to 30—32°C will result in extension of the period of development and can cause abnormalities in many cases.

4.1.2. Optimal temperature for keeping sealed queen cells in an incubator is 35°C. Relative humidity should be regulated around 50—60%. When this drops below 40% for longer periods, the cell walls harden and queens may die before emergence.

4.1.3. Queen cells with older pupae may be kept outside the colony for several hours and up to 1 day without causing harm. Prepupae (5 days after grafting 1 day old larvae) are more sensitive, and for a few more days afterwards. Besides these variations in temperature it is light mechanical shock which can cause damage during this time. Cells are least sensitive during the last two days before emergence.

4.2. Supply of food

4.2.1. As long as good reserves of honey and pollen remain in the colony, we need not worry unduly about short term periods of lack of flow before or during the nursing period. Periods of shortages 1—1½ month before our planned date for queen rearing influence colony population at the later date and queen rearing itself suffers indirectly. Heavy flows can depress results in inverse proportion.

4.2.2. Stimulative feeding does not influence acceptance or quality when given just before and during the period of queen rearing. This is the experience of the author and is in contrast to general opinion.

4.3. Weather, climate and season

4.3.1. Unfavourable weather influences queen rearing only when it lasts a long while and has an influence on brood rearing activity itself.

4.3.2. It is obvious that climatic factors influence the out-come of queen rearing, just as they influence timing and the length of the period in which it can be done successfully. This provides a satisfactory explanation for the contradictory reports by some authors, who were working in different regions and under different climatic conditions.

4.3.3. In general we can say : queen rearing time — for acceptance and quality — coincides with the period of greatest expansion of the brood nest.

CHAPTER VI

Preparing the graft

K. WEISS

The rearing of queens from an egg or from a young female larva right up to the ripe queen cell is one integral biological process ; for technical reasons work may be divided into two separate aspects :

1. All work which prepares the young stages of brood for optimum acceptance by the nursing colony ;

2. All other manipulations which ensure optimum nursing care within the colony after acceptance and initiation.

Separate chapters deal with each of these aspects in our book. This chapter will first consider the graft and its preparation for utilisation for queen rearing.

At the beginning we should define the term "graft" as used in this book. We must take it to mean those very young stages of development from which we can rear perfect queens, provided the right care is given to them by the nurse bees. These stages are either the fertilised eggs which are destined to become female individuals, or they are worker larvae which have not yet reached a certain age, a point of no-return from the worker caste. As we can read in Chapter V, this border-line is the age of $1^1/_2$ days after hatching. Although we can obtain female reproductives even from $3^1/_2$ day old worker larvae when they are fed on royal jelly, we will find that most of these "queens" show certain external deviations from the perfect queen type. Often the bees themselves will reject such advanced stages of brood.

In artificial queen rearing both eggs and larvae may serve as graft; but the use of young larvae is practised so frequently when compared with the use of eggs, that we will concern ourselves with this method first.

1. Rearing from larvae

Queens are usually reared in a queenless colony, or in a queenright colony in which a separate compartment is cut off from the rest by means of a screen of queen excluder material or by a temporarily inserted wire screen. Such queens develop from worker larvae under an emergency impulse ; in this case we have selected them according to

their age and origin and we have prepared them for optimum acceptance by nursing bees.

We must also present the young larvae in such a way, that the ripe queen cells can be handled easily by the breeder. It is not good enough simply to hang a frame with young worker brood into a queenless nursing colony ; bees would rear emergency cell on such a comb, provided that no other young brood is available. But such a method can not be recommended because the resulting queen cells can rarely be removed from the comb without damage or difficulties, and because pupae do not develop equally well even though they were of equal age originally. Experience has shown that bees do not begin to feed all larvae destined to become queens at the same time. Often they let days pass by before making up their minds. Even though the time of development may not be extended by this delay, we must expect that unsatisfactory queens may be reared from older larvae and that these queens cannot match the performance of queens reared from the youngest stages (see Chapter V). This can lead to early supersedure. Nowadays we also are aware, that queens reared from larvae in worker cells, and especially in cells of tough old comb, will clearly be smaller than those queens which had been nursed from the start in larger queen cups. We therefore have quite a number of reasons for rejecting this simple method. In order to avoid these disadvantages, it is necessary to prepare the graft in a special way for acceptance. A number of methods are used with variable success.

1.1. *Raising cells along the edge of cut comb*

For a long time this method has been recommended as the simplest one and as specially suited for the beginner. It is similar to the Miller method (LAIDLAW 1979), but it is simpler by omitting the preparation of inserting tapered strips of foundation into the graft supplying colony, several days prior to the start of queen rearing, a freshly drawn comb with eggs and young brood is used for this, and all comb is cut away below the lower edge of a curved line which follows the stages of youngest brood. Because the queen has laid her eggs in concentric rings or spirals starting from the warm centre, and because the oldest stages of larvae are now found in the centre of this comb, this cut follows a crescent shape along the lower edge. When wired foundation had been used, our knife must be withdrawn whenever it reaches such a wire. Each part of comb must be removed separately and care must be taken, that the edge of the remaining comb is not damaged. Heating the knife makes the work easier ; dipping it into hot water gives even temperatures, but in an emergency the flame of a candle may be used.

In addition to cutting along the lower edge of the brood nest, a further cut may be made along the upper rings of the brood nest, where again larvae of the right ages may be found. This cut removes the older larvae and leaves a hole in the comb.

Bees raise their queen cells along this edge of comb. We must avoid the fusion of many neighbouring cells, as this will create many

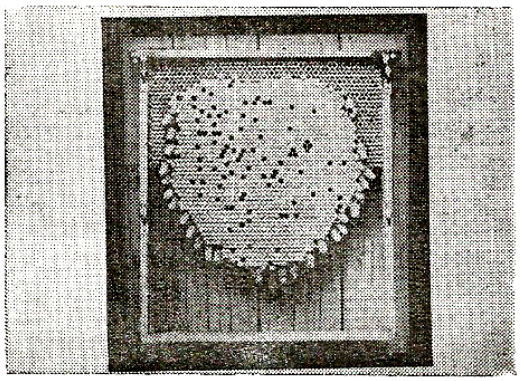


Fig. 44 — *Raising cells along the edge of cut comb. No brood should have been reared in this comb.*

difficulties later on when we want to secure separate queen cells. Gaps between individual cells are easily created by destroying every other larva along the edge on each side, or by leaving one cell out of three. A match stick or pencil is used for this purpose. The cells left intact should be placed alternately on the two sides of the comb. Should some of the cells be fused in spite of our efforts, then we can separate such twins with a cold knife. A small piece of foundation may be used to close any hole made inadvertantly to a side of one cell, — the development will be normal provided the contents of the cell were not damaged in any way.

It is unfortunate, that the crescent cut along the edge of the comb does not guarantee the uniform adoption of youngest larvae. Bees will raise additional queen cells over older larvae well after accepting the first "batch". This can also occur if the cut is made along an edge with eggs only. Bees enlarge cells over eggs in exceptional cases only (WEISS, 1962).

If a beekeeper wants to use this method in spite of these objections, the cut should be performed on freshly drawn comb only. Unused worker cells are enlarged into queen cells more easily and more rapidly than cells which had been used for brood rearing. Tough old comb takes longer still. Experiments have confirmed that when queens were reared from larvae in such old comb under the emergency impulse, they were clearly lighter than those queens which had been raised in unused cells. But queens weighed heavier still when they were reared from an early start in natural or artificial queen cups. (Chapter V).

The comb cutting method is therefore not the best method of preparing for queen propagation.

1.2. *The use of strips of comb, and of individual cells*

When we use strips of comb or individual cells, or when we graft larvae (described later) we must have a special frame for this purpose, often called the "grafting frame". The grafting frame is a standard frame which may be subdivided by 2 or 3 horizontal "cell bars". These cell bars may be loose laths resting in the notched side bars, or they may be fixed with a nail or screw on both ends so that they can be turned along their longitudinal axis.

The use of cell strips comes nearest to the method just discussed : the cutting of comb. A comb containing young larvae is placed on a level surface and is divided with a hot knife into cell strips with one or several rows of cells. These strips are inserted into the nursing colony so that cells point downwards. The American TOWNSEND (1880) originally fastened these strips to the sides of an empty comb by means of needles. Later the strips were fastened by means of liquid wax to the lower edge of a comb which had been cut to receive it. This method became known as the Alley-method in the United States. An American beekeeper named BROOKS used a special frame with 3 cell bars as early as 1880 and fastened the strips of comb with liquid wax after he had cut back to the mid rib all cells on the reverse side. This method is still used in many parts of the world, although the method of fastening has changed. Wire may be used to attach the strips to the cell bar ; stong wire is bent to form U-shaped staples. One leg of the U is hammered into the side of the cell bar and the other leg supports the cell strip. Or specially designed cell bars may be used : they may be grooved so that the reverse cells are secured with a wedge, or the cell bar may have hinges and the reverse row of cells is clamped tightly between two bar faces as in a vice (Fig. 45).

In these cell strips it is also advisable to destroy most larvae so that cells may be formed cca 1—2 cm apart.

Cell-strips can also be cut up into individual cells. They are really groups of cells consisting of one undamaged cell with its larva and others on the reverse side. By means of these latter cells the chosen cell can be fastened directly to a cell bar as JORDAN did with his bar with steel clips (ZECHA, 1960). Tongue-like steel clips hold the reverse cell tightly against the vertically supported cell bar (see Fig. 46). Wooden

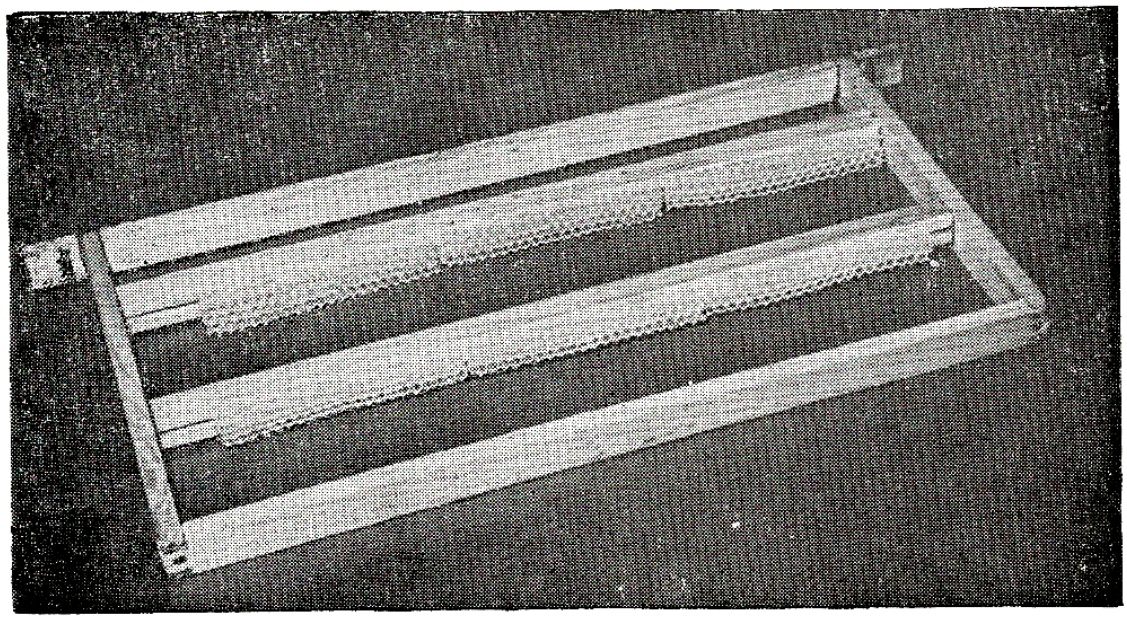


Fig. 45 — *Queen rearing frame with special clamping bars to hold cell strips*

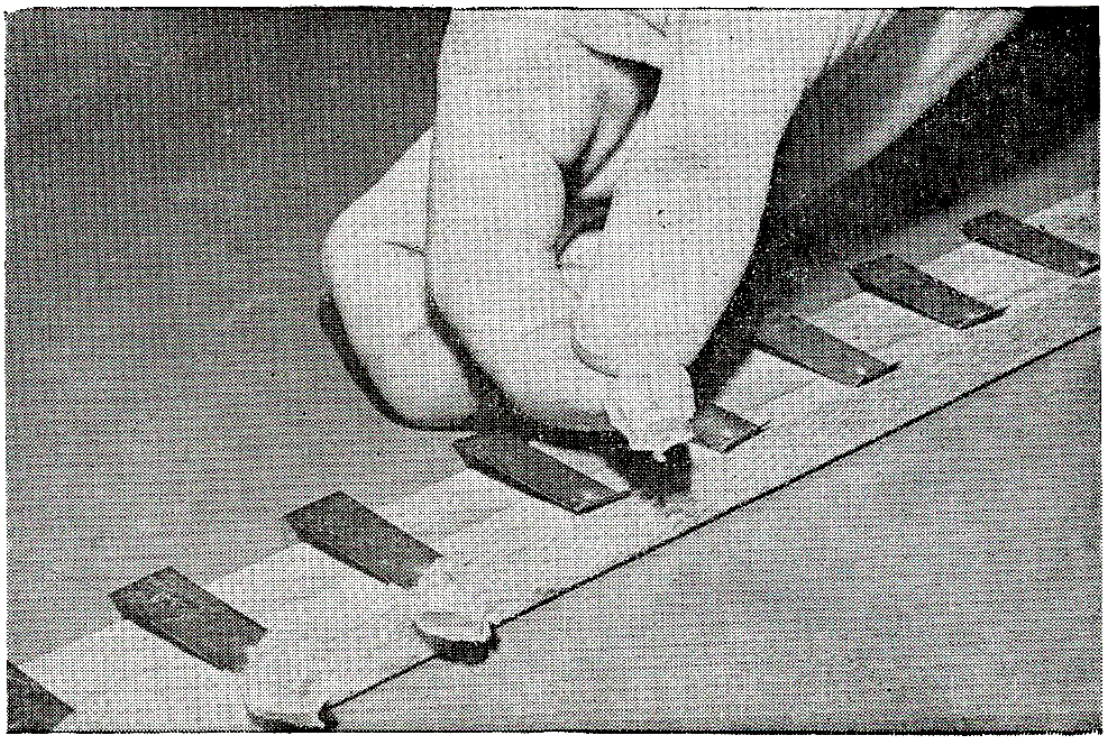


Fig. 46 — *Punched cells are attached to this cell bar by means of simple springs*

cell holders incorporating a simple clamping device are used more frequently nowadays. These resemble the wooden cell cup holders but are vertically divided. A narrow groove is cut around the middle and a rubber ring holds both parts together. The central cut is bevelled on the upper half and this allows the beekeeper to apply gentle pressure here, thus opening the bottom split to accept the reverse cell still attached to the cell to be used. The rubber ring closes the split cell holder and the cell is held firmly. The conical cell clamps are fastened to a cell bar with a little liquid wax (see Fig. 53, lower left).

The optimum distance between centres of queen cells is about $2^1/_4$ cm. A grafting frame with 3 cell bars can take 45—48 cells according to frame size. A nursing colony is able to rear this number of queen cells if the right method is used. There are several rearing methods which are unable to cope with this number. (see Chapter V).

Cell punches may be used to cut out individual cells instead of a knife. Of several punches available through the trade channels, the best one is the Swiss pattern (Fig. 47).

This punch consists of two symmetrical half-cylinders which are attached to each other by means of a spring handle. When punching cells, the two halves are pressed together to form a tube. This tube is forced into the comb over the cell chosen while rotating the punch. After withdrawal from the comb the punch opens and the cell is released without

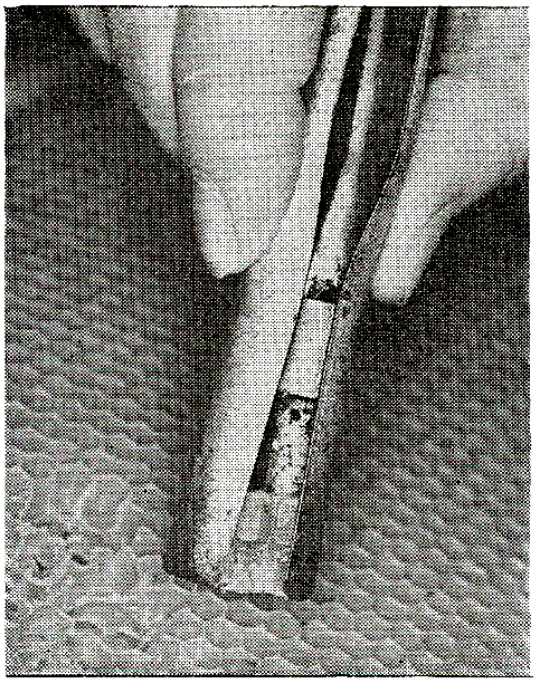


Fig. 47 — *The "Swiss Cell Punch" allows the cutting of individual cells from comb.*

damage to the contents. Dipping the punch into hot water before attempting to cut cells eases the work considerably.

The use of cell strips and punched cells has many advantages over the crescent cut on a full comb. Bees will accept these cells soon and will provision them readily. They are unable to rear queens from older larvae as an afterthought, by this method.

But all methods which use worker cells have the inherent disadvantage, that queens reared in them cannot attain optimum size. When discussing the crescent cut it had been pointed out that the cocoons in worker cells limit larval growth and cell size considerably. Even when the worker cells are cut back to $^2/_3$ or $^1/_2$ of the original size this makes little difference. Only the use of freshly drawn comb can improve matters sometimes. This creates difficulties again apart from the labour involved in getting such combs drawn and laid out by a queen. Such soft cells are difficult to punch and handle. All these reasons have contributed to the decline in the popularity of these methods.

1.3. *Grafting*

This method involves the transfer of young larvae from worker cells into natural or artificial queen cups (rarely into natural drone cells). These queen cups are then attached to a grafting frame and are given to a nursing colony. This method has a long history. François HUBER, the Swiss blind investigator of the life of the honeybee, was probably the first man to have done this for scientific reason. He describes in 1791 in his 4th letter to the naturalist Charles BONNET that he had dequeened a colony and had discovered that bees began to construct queen cells

over worker larvae on the same day. "I asked to have 5 of these larvae taken out and be replaced by 5 others which we had observed hatching 48 hours previously. The bees did not seem to notice this change, because they took care of these new larvae as well as of those they had chosen themselves. They continued to enlarge these cells and seal them with proper care". Hundred years had to pass before the parson WEYGANDT in Germany attempted to graft larvae, this time as a breeder. He reported his successes to the 25th meeting of the German and Austrian beekeepers at Cologne in 1880. The Swabian watchmaker Wilhelm WANKLER heard about this method and improved it. He is still esteemed as the father of modern queen rearing in Germany, also because of his valuable book, "Die Königin" (The Queen Bee), published in 1903. The American D. M. DOOLITTLE in America developed grafting around the same time independent from the work done in Germany, but — and he admits this freely, — basing his work on that of his own contemporaries. His book "Scientific Queen Rearing", published in 1889, popularised the method of transferring larvae into artificial queen cups. DOOLITTLE worked with these artificial cups from the start ; WANKLER had tried this method but was unsuccessful ; he remained content to use drone cells.

1.3.1. **Artificial queen cups and their manufacture**

The transfer of larvae into empty drone or worker cells, either singly or as strips, should be a thing of the past. We know now that smaller queens emerge from used cells and freshly drawn comb is too tender and makes grafting difficult. We should not even attempt this technique after we had learnt in Chapter V that the use of freshly drawn comb does not produce the best of queens.

Natural queen cups are often used for rearing queens. We find these "play cups" in variable numbers in nearly all colonies during summer. We can cut them out and keep a supply of them at hand. Their use is restricted to a relatively limited quantity of queens.

Artificial queen cups are more widely used nowadays. They may be obtained through trade channels or they can be produced by the beekeeper himself.

The dipping process is probably used throughout. The "cell mandril" is the most important tool. It is made from hard wood and the dipping end is rounded. Occasionally the tip is made of darker wood to indicate the depth to which the queen cell moulding tool should be inserted into the wax. Cell depth should be cca 8—10 mm. The diameter of the dipping end — this gives the internal diameter of our cell — should be 9 mm accurately. In Chapter V we can find that this cell size provides conditions for the optimal development of queens.

Before use, the moulding tool is soaked for $^1/_2$ hour in cold water. The wax is heated in a water bath in the meantime. An enamel bowl supported by a grid in a cooking pot will do the job well. The temperature of the wax should be around 70°C. At lower temperatures the cups will be too thick ; when the wax is hotter than 70°C cell walls are too thin and are removed with difficulty. A thermometer can be used to find the

right temperature, but with growing experience the right temperature will be found without it. Thermostatically controlled water baths or boiling rings may be used with advantage.

The cups are produced as follows : take the mandril from the water and shake off any surplus droplets. Dip it qickly into the wax to the right depth. A this coating will form when we withdraw the mandril (Fig. 48). Shake off surplus wax. Dip the mandril a second time to thicken the coating. The third dipping is done to half the original depth only and this will re-inforce the bottom of the cup. Cool the cup in cold water and pull off with a twisting motion.

We must insert the mandril into the cold water before each renewed dipping process or the queen cup cannot be removed. Such a mishap can also occur if the mandril had not been soaked long enough initially or if the water is too warm. In this case it is important that all wax is carefully removed. A solvent can be used for this purpose ; the non-flammable carbontetrachloride is excellent. Moisten a small rag with it and wipe the mandril hard until all traces of wax are gone. After dipping once more into cold water it can be used again. A potato may be used if difficulties are experienced. Cut the potato in half and push the mandril into it as deep as when dipping into wax.

The manufacture of queen cups takes up much time. The work can be speeded up by using two mandrils alternatively. The work can be done more economically if many mandrils are lined up into batteries of mandrils. (Fig. 49). These are dipped into a suitable vessel in one operation. WEAVER uses batteries of 15 mandrils fastened to a bar. Two are in use ; one is dipped into wax while the other remains in the water. A special stop on the bar ensures that all cells are dipped to the same level. Other beekeepers mount their mandril on a disc for dipping into circular vessels (Fig. 49).

In some countries, especially the U.S.A. it is possible to buy queen cells ready made. These are usually manufactured by compression moulding. They are thicker than the home-made cups. While self made cups have side walls $^1/_2$ mm thick, the pressed ones may have sidewalls 1 mm

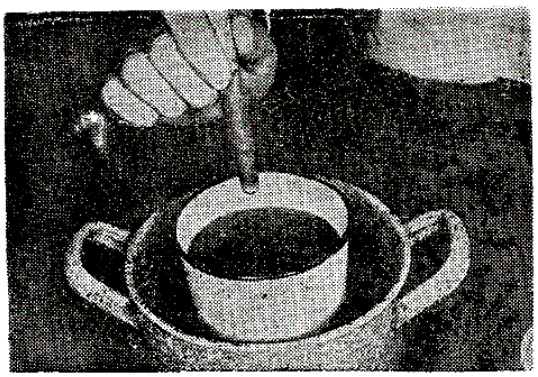


Fig. 48 — *Artificial cell cups are obtained by dipping a mandril into liquid wax.*

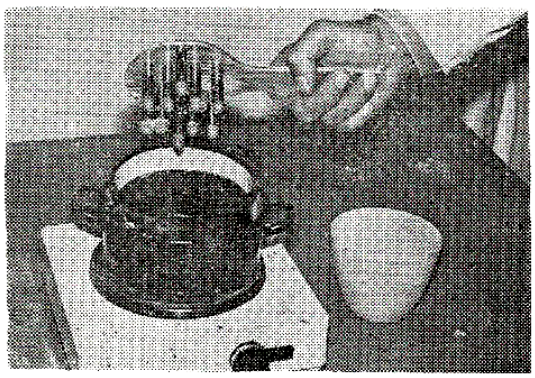


Fig. 49 — *Fastening 10 mandrils to one holder speeds production.*

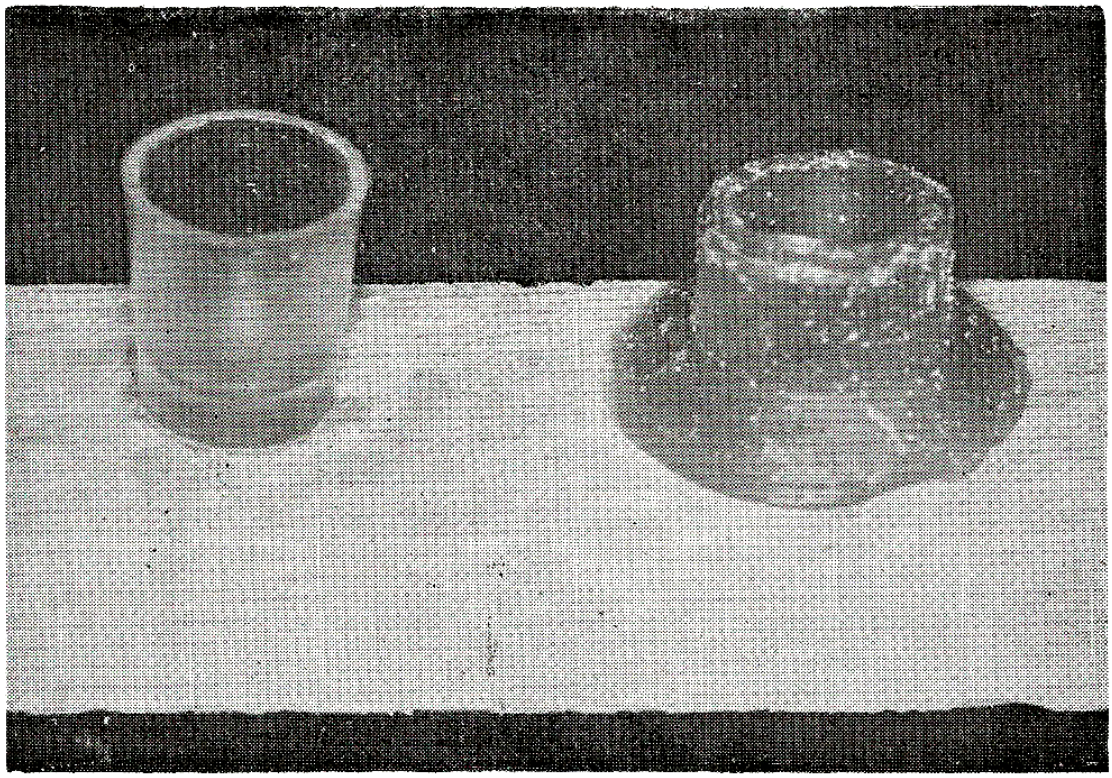


Fig. 50 — *Plastic cell cup made of polystyrene (left) and a wax cell cup produced by compression moulding*

thick or thicker still as a rule. These cups often have a heavy, wide base so that they can be fastened to the cell bar quite easily.

An alternative to wax cups are those made from plastic material and they are widely used in America, France and Australia. The choice of material is important if good acceptance is to be obtained. The form can be variable within limits. These cups are much thicker than wax cups. Occasionally they are much deeper than 8 mm, but the cell opening is usually around the optimum 9 mm (Fig. 50).

1.3.2. **Preparing the grafting frame**

Queen cups made from wax or plastic have to be fastened to the cell bars of the grafting frame. Most breeders attach cells directly to the cell bar, some of them use a wooden cell holder, a wooden square or a disc of wax between cup and bar. Much depends on the way the sealed cells are used. If the cells can be introduced directly to nuclei or to mating colonies, there is no need for additional holders. For certain types of nursery cages their presence is unnecessary. Other cages are designed to be closed by the cell holders and it is then convenient that the cups are attached to their base before grafting work commences (see Chapter VIII).

In order to fasten the home made queen cups to the cell bar it is advisable to place small pieces of wax foundation on the cell bar. The wax is heated with a hot knife, wire hook or soldering iron and the thin walled cell cup is held in position. Fingers need not be used for this delicate job ; the mandril can be used dry and can be withdrawn when the wax has set again. The distance between cell cups is usually 1—2.5 cm. To maintain even gaps, the cell bar is marked beforehand.

When wooden cell holders are used it is best to fasten these to the cell bar first. As has been mentioned before, we dip the base of the holder into liquid wax and attach them with gentle pressure to the cell bar. Cell holders usually have an indentation for cups on their free end. This is filled with additional wax before cups are fastened. A good tool for this purpose is a wax syphon or wax pipette (Fig. 51). This is a metal tube with a conical tip with a small hole in it. At the other end another small hole can be closed with the finger tip after inserting the syphon into a container of liquid wax and letting the wax rise in the tube. Air can enter the tube again when we raise the finger slightly, thus letting wax flow into one cup holder after another.

Cells may be pressed into the soft wax in one working process. It is also possible to reheat the wax gently by means of a soldering iron or a heated wire rod. Some cell holders have a conical hole ; no additional wax is required for these. The wax cell cup is lifted with a wooden rod slightly smaller than our mandril and the cup is gently pressed into the conical opening until firmly held.

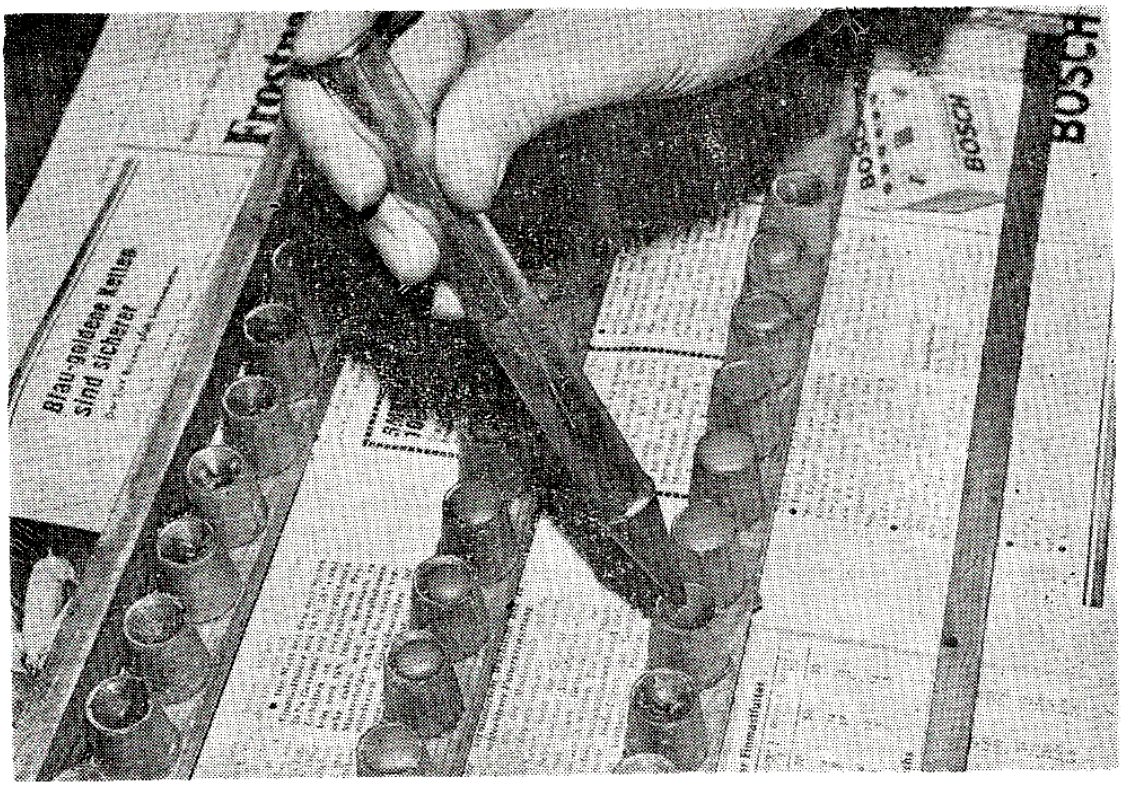


Fig. 51 — *Dimpled cell holders are filled with liquid bees wax.*

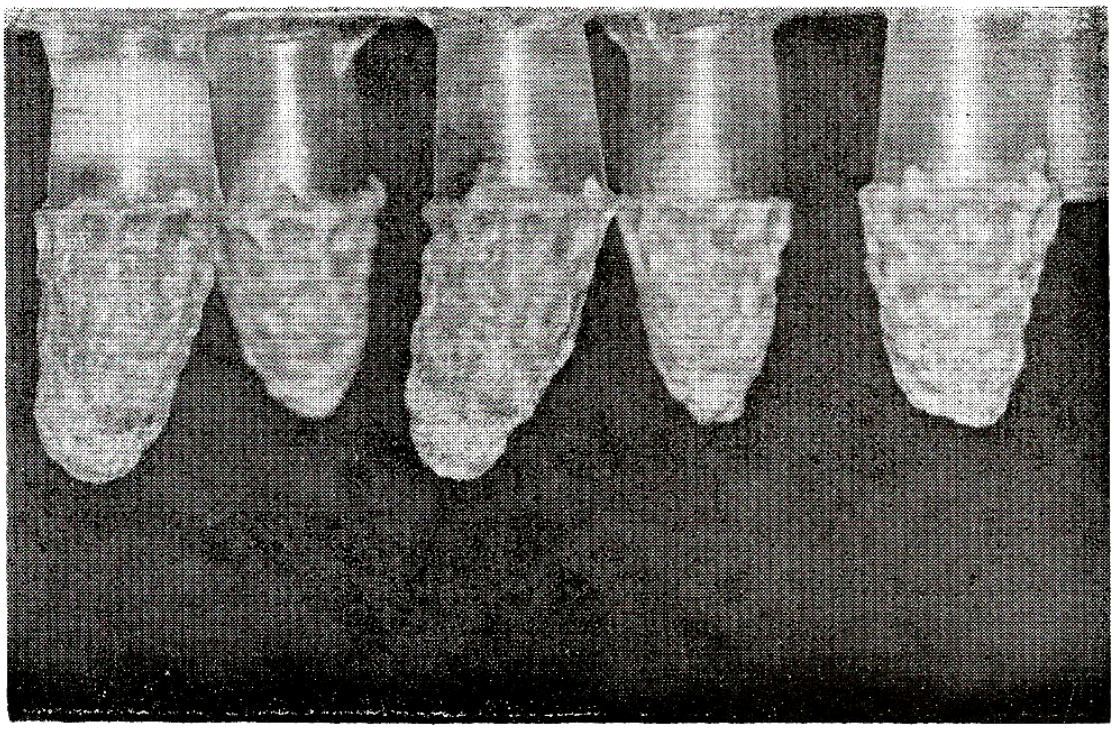


Fig. 52 — *Plastic queen cells are becoming very popular in queen rearing*

Ready made waxen cups and those made from plastic material available through trade channels are usually not fastened to cell holders, although this can be done if it is desired. The heavily moulded cups with a hollow base can be fastened by pressure to a cell bar coated with bees wax. Smaller plastic cell cups can be attached to the cell bar by means of liquid wax. Figure 53 shows a number of different types of cells cups attached to the cell bar in various ways.

1.3.3. **Grafting**

Young worker larvae must now be transferred into the cell cups. In Chapter V we discussed the problem at great length. Some royal jelly may be used to prime the cells before grafting. In that chapter it was shown that in a queenless, well prepared nursing colony the priming of cups with a little brood food did not influence acceptance or the quality of the queens. Acceptance was improved by this technique when queen rearing was done in queenright stocks.

Royal jelly for priming cups is harvested from open queen cells in a colony preparing to swarm. Occasionally we find recommendations to obtain brood food from queen cells in colonies specially managed to produce it for this purpose. This is said to be more suitable (TARANOV, 1972). Royal jelly can be stored in the refrigerator in glass vessels with air-tight lids at a temperature of 5°C. It is customary to dilute the royal jelly slightly before use. A metal grafting tool or a pipette (syringe without needle) may be used to place a small quantity of food in the bottom

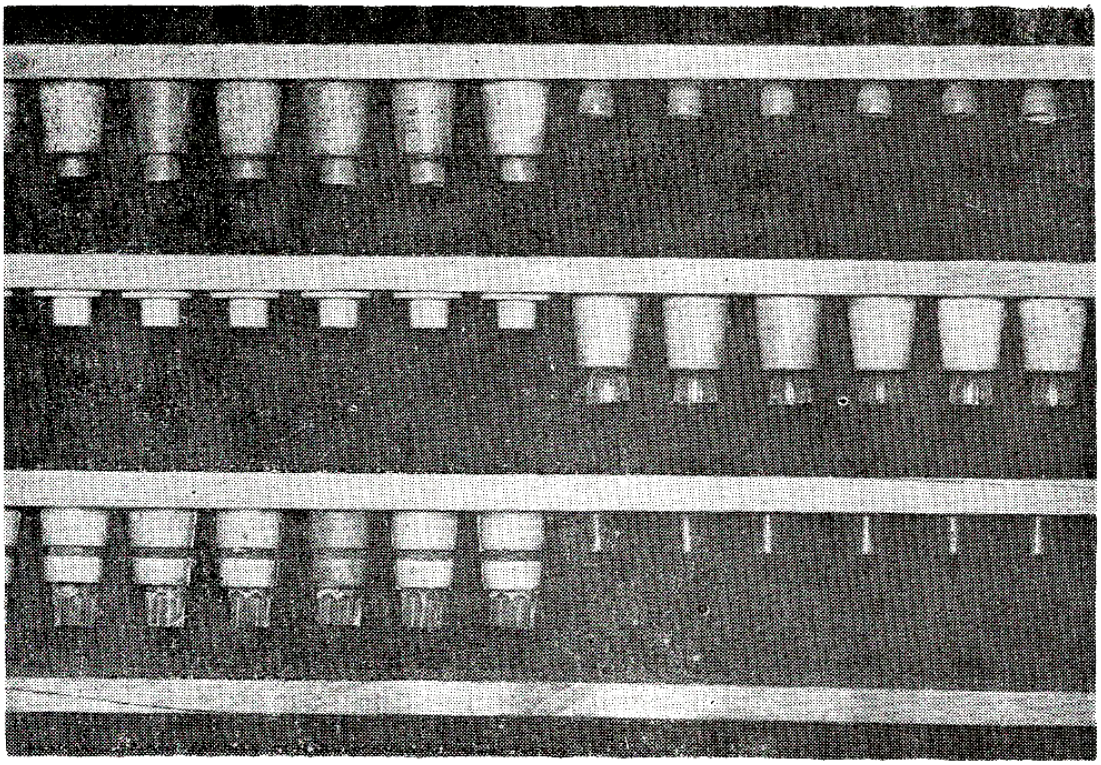


Fig. 53 — *A grafting frame with a variety of cell cups fastened in a number of ways to the cell bars*

of the cell. Grafting larvae into primed cell cups is called "wet grafting". But, as mentioned before, we can save ourselves this work. "Dry grafting" is equally successful.

We need a suitable tool for grafting larvae (Fig. 54). Beekeepers have used various tools for transferring larvae : fine hair brushes, quills of bird feathers, matchsticks with a thin, flat end which is moistened slightly to make it soft and pliable. Some complicated equipment has been developed too ; in America the "Master queen grafting tool" has been available for some time. In this instrument a lever can extrude a thin, curved metallic tongue from a long, hollow, flat tube, picking up the larvae without damage on the spoon shaped tip. Releasing the lever pressure withdraws the tongue and leaves the larva in the cell. But the same work can be done with a very simple grafting needle. It its simplest form it is a handy rod of wire which has a gently curved, flattened spoon at its tip. The tip is rounded and about 1 mm wide. One excellent version is the "Swiss" grafting tool. This has been cranked slightly cca 2 cm from the spoon end and does not obstruct the view, when the beekeeper peers into the bottom of the cell.

In order to pick up the larva the spoon glides gently under the curved grub so that it supports its middle and both head and tail are free (Fig. 55). This technique automatically picks up some of the larval food. The larva is left behind in the cell when the spoon is drawn backwards with gentle pressure on the bottom. This is very easy in cells which

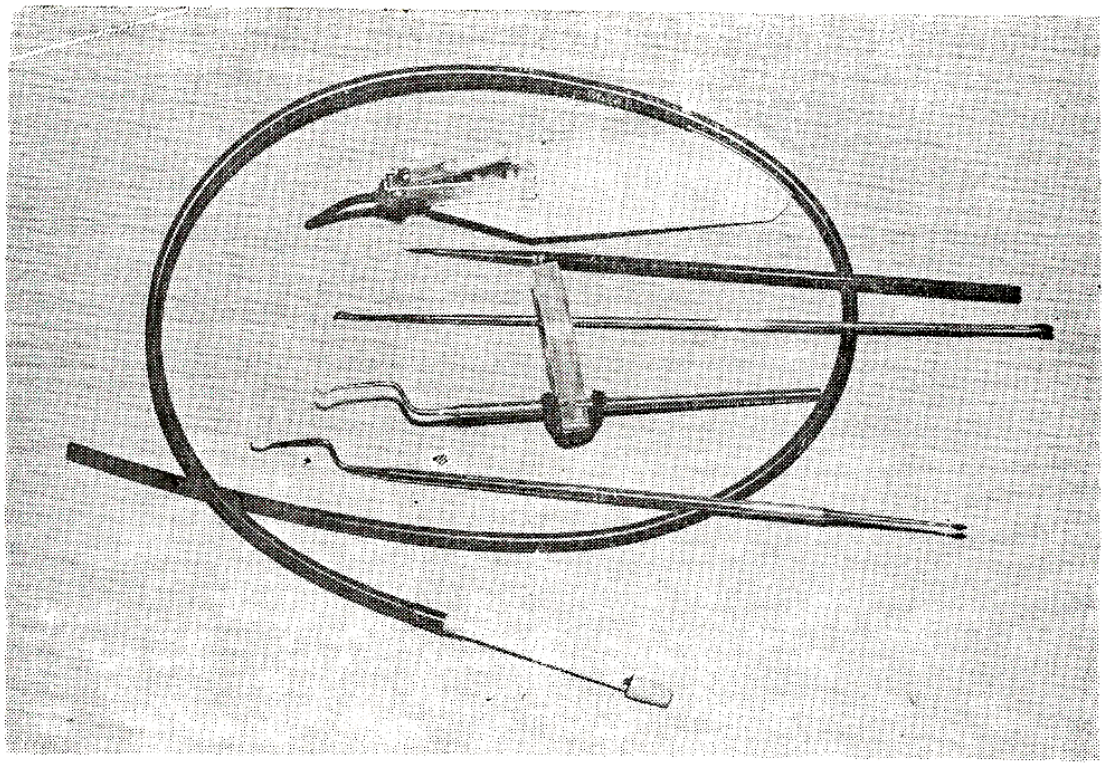


Fig. 54 — *A number of grafting tools. Any one may be suitable, but it seems to go quickest with the cranked "Swiss grafting spoon", with or without the magnifying glass (lower centre).*

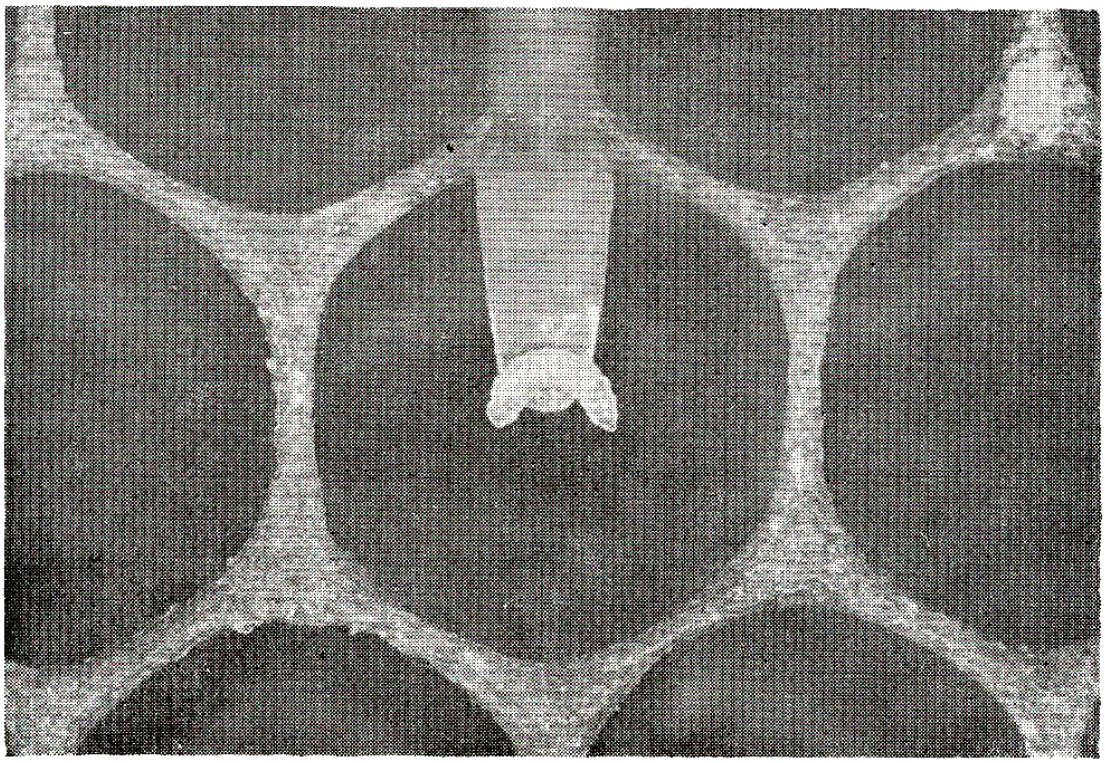


Fig. 55 — *A one-day old larva on the Swiss grafting spoon*

contain a little brood food. With a little practice one can soon graft into dry cups or plastic ones.

"Double grafting" has been though to give better results than either dry or wet grafting. When double grafting is practised, the cups are first grafted dry with larvae of any origin. After 24 hours at the latest these grubs are removed from their bed of royal jelly and are replaced with fresh, young larvae of the breeder strain. Recent work has shown, that the additional work involved is not compensated by an improvement in the quality of the queens obtained. "Double grafting is of doubtful value and far too costly when rearing queens commercially" (Roy WEAVER).

A good eye and a steady hand are the prerequisites for success when transferring larvae. Poor eyesight can be corrected by the use of a simple magnifying glass or by one with its own light source built in. Stereoscopic magnifying glasses are also useful under these circumstances. Magnification should not be greater than 2.5—3 times. If the above conditions cannot be met it is probably better to adopt a less reliable method of queen rearing, e.g. cutting along the crescent shaped edge of a brood comb or punching cells. It is also possible to learn to master a completely different technique ; queen rearing from eggs.

Good illumination penetrating to the bottom of the cells is essential. Generally daylight may prove adequate if the comb is slanted in the right direction. Improvements have been devised where much grafting must be done at any time of the day. At the Institute directed by J. WOYKE in Warsaw all grafting is done with the aid of a stereomicroscope under vertical illumination. At the Erlangen Institute at Erlangen we use a source of cold light with a flexible conductor of glass fibres with a terminal lense focussing directly on the worker larva. This has been highly successful.

2. **Rearing from egg**

Rearing queens from the egg stage is not a new idea. When beekeepers began to cut comb along the edge of the brood in the shape of a crescent, they thought that they would achieve it that way, especially if only one comb with eggs was used. But as we had seen earlier, this did not result in rearing from eggs. Bees usually waited until after larvae had hatched before they converted the cells into queen cells. And when old comb had been used, they waited much longer.

Many specialised attempts have been made lately in order to achieve queen rearing from the egg stage. When research showed, that the brood food given to very young workers already differed from that given to very young royal larvae(Chapter III), breeders thought, that truly royal conditions must be provided right from the point of hatching in order to obtain the best possible queens. This line of thought has been proven to be wrong (Chapter V). But a lot of effort had been put into the development of techniques suitable for "rearing from the egg" and some successes have been achieved, before the theory had been discredited.

2.1. *Historical facts*

Transferring eggs from worker cells into queen cups will always remain a hopeless undertaking. Eggs are so delicate and fragile, that very high losses must be expected even when the greatest care is exercised. The Hungarian scientist ÖRÖSI-PAL discovered, that the school master REIDENBACH attempted to transfer eggs in order to breed queens from them just before the turn of the century (1883). He used a needle which had been bent to form a hook and he slipped this underneath the egg, hoping to lift it off the bottom of the cell. On the bottom of the queen cup he pressed the needle into the wax and withdrew it from under the egg which was then left standing. DICKEL (1898) used the same kind of needle in his futile attempts to solve the problem of sex determination in bees. DIETZ (1964) has tried this system in more recent times, and TABER (1961) tried to improve the instrument by constructing "egg-forceps". This was a pair of tweezers with their tips angled and a tiny opening which avoided all damage to the egg. These methods are not suitable for practical queen rearing. Manipulating eggs demands a high degree of skill and much patience. This takes up much time which a commercial queen rearer can ill afford.

Eggs can not be handled because of their delicate texture. This fact stimulated interest in getting the queen to lay her eggs into queen cells. Such cells could then be allowed to develop in the same colony or could be given to a nursing stock. This idea was tried time and again. (ÖRÖSI-PAL, 1960 ; SIMPSON, 1961 ; RUNKIST, 1962 ; BOGNOCZKY, 1967). By fastening cell cups to regular brood comb or directly to a cell bar, the breeder queen was expected to deposit her eggs in these cells. The difficulty seemed to be that the colony had to be in a swarming mood first before the queen showed any interest. Even then she was laying only the odd egg here and there and not all at once, as would have been desirable. The method was too uncertain and it involved too much work to be of any value for breeders.

The attempts gave rise to the idea to try rearing from egg stage by using strips of comb or punched cells. But experiments showed, that the easy way did not work with single cells. Bees did not accept cells with eggs instead of larvae or reared only the odd one. (WEISS, 1962). Even if acceptance could be improved by the use of older eggs near point of hatching and familiarised beforehand (PUHLHORN, 1959) or if acceptance was more certain in a colony in a swarming mood (MALY, 1959), the method would still be unsatisfacory. Acceptance must not depend on chance Acceptance must be secured by using the right methods.

2.2. *Örösi-Pal's method*

The Hngarian bee scientist developed a new method for rearing queens from the egg stage ; this involved a kind of "egg grafting". The delicate eggs are not injured by touch, but are transferred together with a small disc of wax. An "egg-punch" is used to achieve this. Such a tool

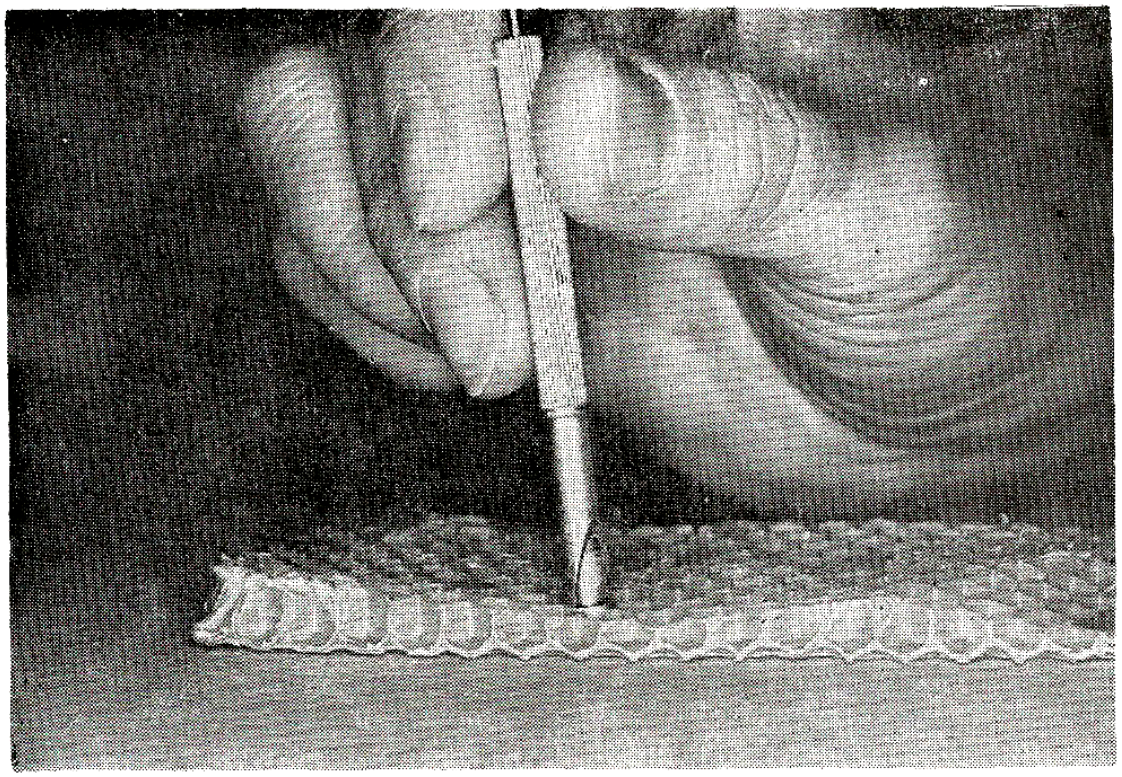


Fig. 56 — *Punching eggs with disc. The obliquely cut window in the punch can be seen clearly.*

is available in a variety of forms through trade channels. It usually comprises a narrow tube—smaller than a worker cell—within which a hollow piston is held by a spring. Near the working end the tube has an opening through which the egg can be seen at all times when the punch is used. The sprung, hollow piston is used to eject the wax disc with the egg from the base of the tube (Fig. 56). ÖRÖSI-PAL believes that a retracting pencill is much better. The device which holds the lead in the pencil is a tube split at its end into three parts. The wax disc can easily be removed from this, while it is often quite difficult with other types of punches.

The comb to be used for punching eggs must be freshly drawn comb. It is best to remove a handy piece from the frame. Cells should be trimmed back with a sharp knife ; this should never be hot. Eggs and discs which have been removed from the comb should be saved on a piece of paper for further use.

All queen cells should be prepared for acceptance and be ready before this work can start. An adhesive is needed to attach the wax disc to the cell. The simplest is royal jelly. Instead of collecting brood food and priming cells with it, ÖRÖSI-PAL first grafted larvae of any origin into dry cells. 24 hours later, when all larvae were swimming in accumulations of royal jelly, they were removed with tweezers and were replaced with discs of wax with eggs. It is necessary to widen the opening to each cell a little for easy access. The wax disc adheres to the tip of the tweeezers when this touches the edge of the wax. It is then

transferred into the dimple in the food, which the removal of the larva had left behind (Fig. 57). Acceptance is not affected by closing the enlarged cell opening or leaving it. On the other hand it can be recommended to tap the cell bar gently in the open palm with the cells pointing upwards. This will settle the cell discs correctly and nurse bees will do the rest.

It can be shown that the old royal jelly plays no role in nourishing the larva after hatching. The wax disc prevents contact with this food. Nurse bees will deposit fresh food suitable for the age of the larva on the disc. Soon this disc will be lost in the masses of the accumulating royal jelly and the larva will be swimming on its suface.

ÖRÖSI-PAL points out, that one does not require artificial cell cups for his method. Cell strips or punched worker cells may be used instead. The discs with eggs are then used to replace larvae after their removal, when workers will have enlarged the cells. In order to obtain cells with a wide opening the use of freshly drawn comb is recommended. ÖRÖSI-PAL also recommends the use of eggs at least 2 days old as this will improve rates of acceptance. When judging the age of an egg we cannot rely on the angle of inclination which the egg adopts. This angle is less dependent on age, but rather on the position which queen adopted in relation to the vertical comb when she deposited her eggs (ÖRÖSI-PAL, 1930). Eggs of the right age can be obtained by caging the queen over suitable comb. This will be discussed under section 4 of this Chapter.

It may be of historical interest, that the Russian parson and beekeeper Epifanii Savich GUSEV in the village of Sernur, Viatka District, already used a similar technique for grafting eggs 100 years before ÖRÖSI-PAL. He received in 1860 a silver cup for his invention at an apicultural exhibition. It seems that neither DOOLITTLE nor WANKLER were the first to use artificial cell cups made from bee's wax. The same

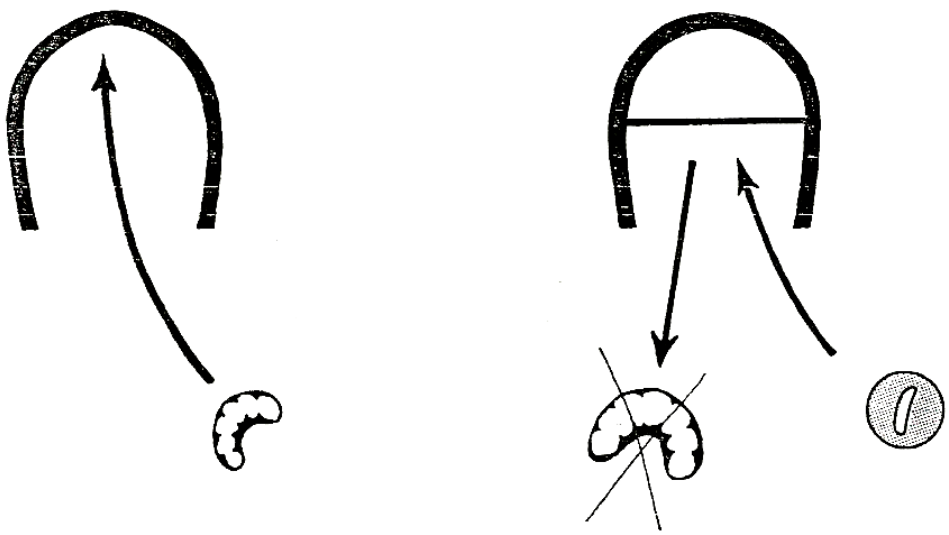


Fig. 57 — *The principle of ÖRÖSI-PAL's system of grafting eggs*

parson moulded cell cups from warm wax by hand around the bone handle of his egg punch. The punch consisted basically of a metal tube with an opening at the side. His way of fastening the wax disc to the base of the cell was interesting : he pierced the base of the cell cup with a needle and inserted the cell punch with its wax disc and egg. Placing his mouth over the hole in the back of the cup, he drew in his breath sharply and withdrew the disc from the punch, fastened it to the base of the cell at the same time. It is unfortunate, that no success rates were mentioned when A. G. BELYAVSKI reported this forgotten technique to the western world.

2.3. *The cell cluster method (Erlangen)*

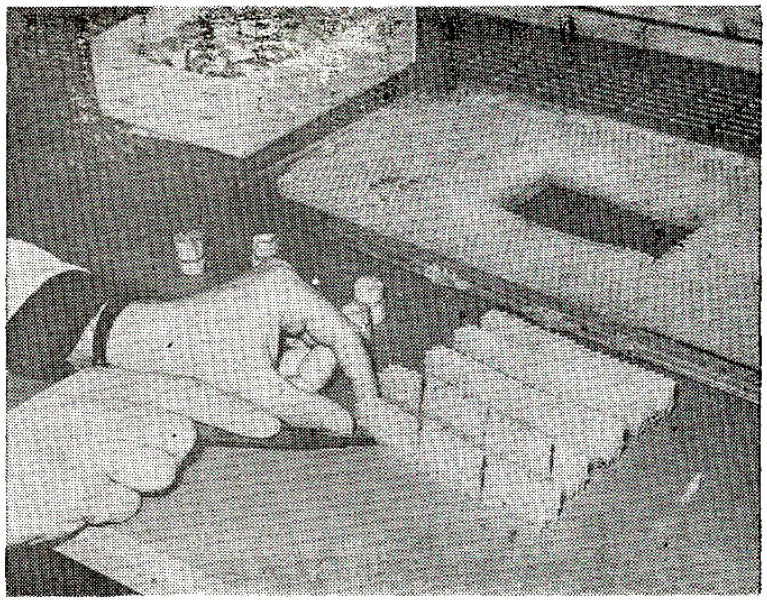
Grafting eggs with a wax disc had often resulted in poor acceptance when it was used on a commercial scale. The reason for this may be found in the technical difficulties of punching eggs without harming them. Even ÖRÖSI-PAL reported moderate successes obtained occasionally. In addition to these difficulties we must account for those incurred in raising queen cells in preparation for grafting. No wonder beekeepers tried to find another, simpler method for rearing queens from the egg stage. The Bavarian Bee Research Institute developed one such technique (WEISS, 1962 ; 1964).

Bees hardly ever accept punched cells containing eggs. Better results were obtained when cell strips were used. These were fastened to a cell bar with the opening pointing downwards. The method is not new ; MEIER-MARQUARD (1957) only awakened fresh interest in it. Americans had used it towards the end of the last century. When O. H. TOWNSEND (1880) used strips of comb with a single row of cells, he apparently used eggs and not larvae for his work. It is also known that H. ALLEY used not only larvae but also eggs near hatching in his method. Undisputable successes in acceptance, when rows of single cells were used, raised the question : why the failures with single cells ? We can only assume, that bees may damage the eggs when they are enlarging such cells for queen rearing. It seems that they have a better foothold when they are working on strips of comb and damage is less likely. This assumption appears confirmed, when we find even better results are obtained after using wider strips of comb with two or three rows of cells. Therefore the method required only improvement in the tehnique to become a practical proposition. Rearing from the egg stage by using clusters of cells was developed.

a) To start with we require freshly drawn comb with eggs of known, equal age. Laying the comb flat on a firm base we cut the comb vertically into strips two cells wide. The cut passes through each third row of cells. The strips are now cut diagonally into diamond-shaped clusters of 5 or more cells. At the ends some pieces may have fewer cells than that number, but should never have less than 3 cells (Fig. 58).

b) The cells on the reverse side have been cut at random and they are now squeezed together to form a sharp edge. This edge is held

Fig. 58 — *Cutting cell clusters for the Erlangen method of rearing from eggs*



firmly in a split cell holder (Fig. 59 ; fig. 43, lower centre). All eggs but one are now destroyed by a match stick or a similar tool in each cluster of cells. The cell with the remaining egg must not be damaged in any way ; especially cuts in the lower part must be avoided, as bees will tear it down. On the other hand we must reduce the number of eggs in each cluster so that bees cannot fuse several cells together Acceptance of too many cells can also lead to queens of lower quality.

c) After surplus eggs have been destroyed in each cluster, the cell holders are attached to the cell bars. Wax has proved best for this purpose. While dipping the holders into the liquid wax, we press the two halves of the cell clamp together firmly, so that later the clusters can

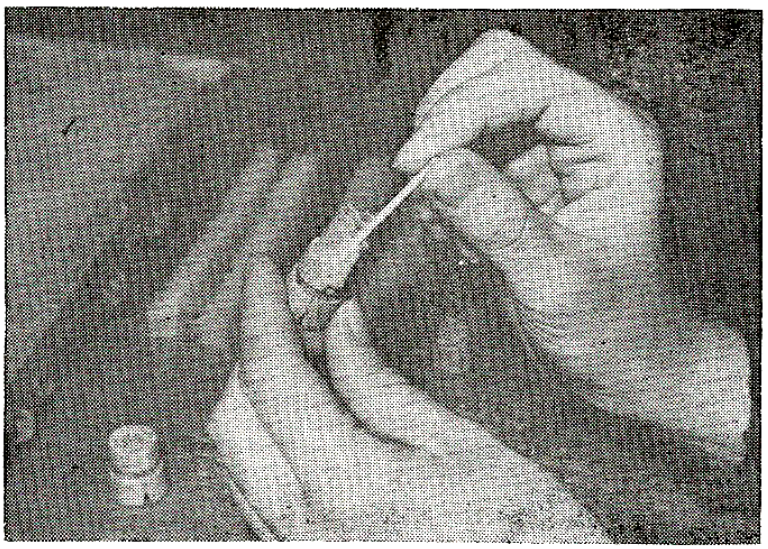


Fig. 59 — *All eggs but one are distroyed in the cell clusters.*

bear the weight of the nurse bees hanging from it (Fig. 60). Fallen cell clusters are ignored and useless.

d) Cells of these clusters may be trimmed back by half with a sharp knife which had been warmed slightly. It is possible, that such cells with eggs are enlarged sooner ; but this has not been proven and acceptance was not influenced in any way. The age of the eggs used in this method seemed unimportant. While it must be admitted, that eggs under $1^1/_2$ day old are relatively sensitive to chilling and that they survive for shorter periods only outside the colony than elder ones (see Chapter V), this does not affect queen rearing in any way. It is unlikely that these cell clusters remain in the cold for longer than 2 hours, and even the very young eggs can withstand that much exposure. However, the use of older eggs is in the breeder's own interest; he can check acceptance sooner and the period of rearing is reduced. In order to have all queens emerge at same time, it becomes imperative that all eggs are of the same age.

I would like to stress once more the need for using freshly drawn comb when rearing from eggs. The piece of comb used for cutting should never have been used for brood before. The reason for this is the same as when strips of cells or punched cells are used : nurse bees can enlarge fresh cells sooner and easier ; this will improve queen weight. However, it his been found, that not all cells enlarged into queen cells are started over egg stages, although in the case of fresh comb it is done over a number of them. This percentage varies from colony to colony. ÖRÖSI-PAL (1974) found that this took place over eggs in 23%, and over larvae in 31% of all cases. The other eggs were removed by bees. I myself worked with colonies which constructed queen cells over 70% of the eggs offered. We cannot explain this difference ; it may a genetic factor of the bees or the physiological condition of the nursing colony. Important

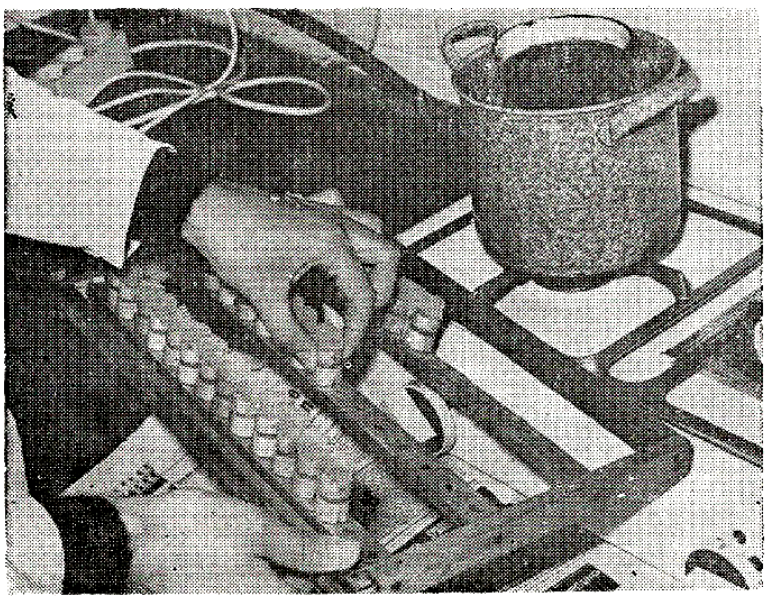


Fig. 60 — *The two halves of the split cell holders are squeezed together as they are fastened to the cell bars with liquid wax. This prevents the clusters from dropping out under the weight of bees.*

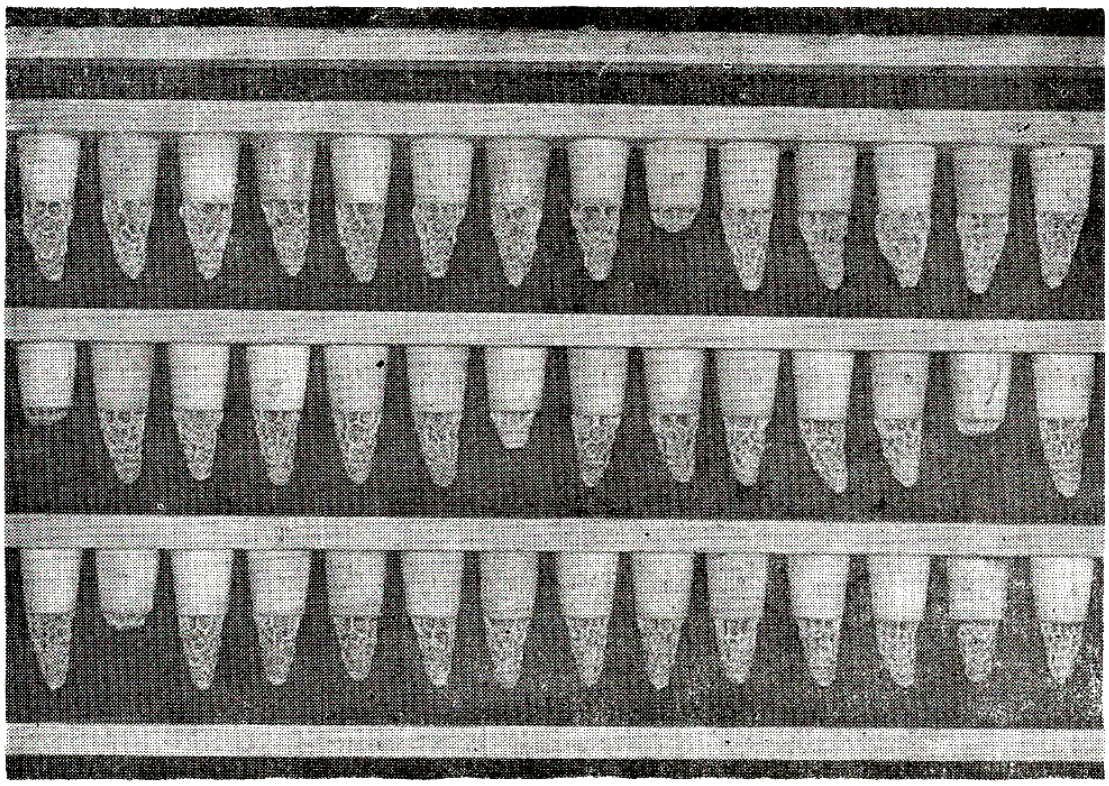


Fig. 61 — *A successful batch : Queen cells reared from larvae...*

is this : that bees will enlarge all remaining cells immediately after the egg hatches, where they have not done so before this occurs, and not in the stages of advanced larval growth. Thus we can use the term "rearing from the egg" in its literal sense when we speak of this method.

Yet we must point out, that queens reared from eggs by means of cell clusters are somewhat inferior in weight than those queens reared from day old larvae in 9 mm wide cell cups (WEISS, 1971). The difference in weight is so slight, that in reality it makes no difference. The technical simplicity of the above method, and the fact that the precious eggs are not handled at all and can not be damaged in any way, may be of great advantage to many beekeepers. Therefore we may see this method as a valuable alternative to grafting. (Fig. 61 ; 62).

2.4. *Securing eggs or larvae*

One important advantage of all methods of grafting is the economical use of the stages of brood. No larvae are wasted. Young larvae required for one or several batches may be obtained at any time during the season from the breeder colony. Neither are combs damaged in any way by the removal of larvae. Nor does it matter whether old comb or freshly drawn cells are available. It also is immaterial when other stages of brood are present in these brood combs.

It is a different matter when we rear queens from larvae in cell strips, single cells, cell clusters or when we rear from egg stages. In

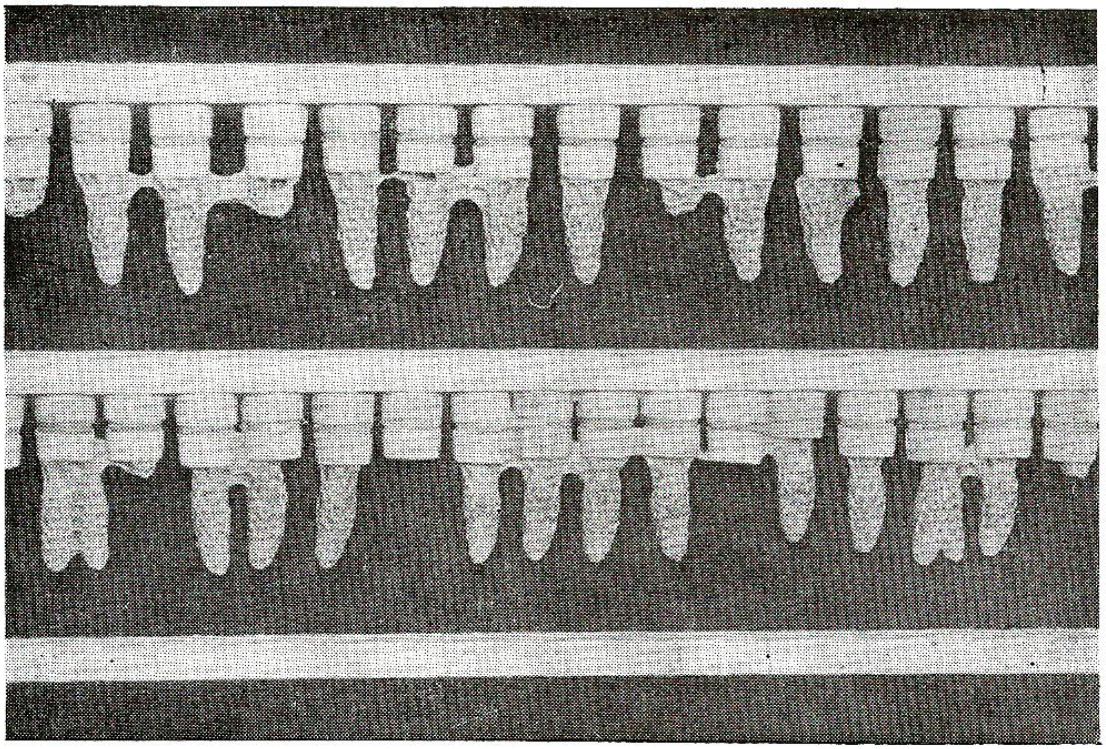
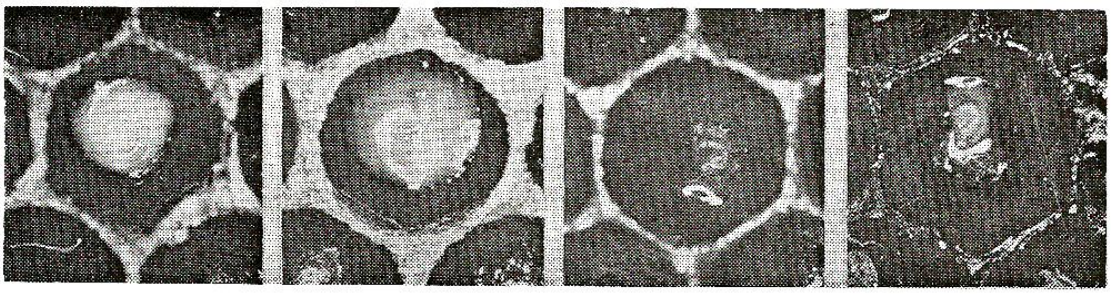
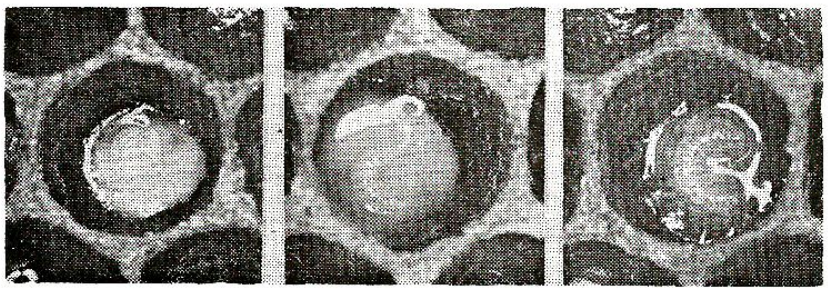


Fig. 62 — *... and queen cells reared from eggs*



0—2 4—6 18—20 22—24



28—30 46—48 48—50

Fig. 63 — *Larvae of known ages (±1 hour)*

this case we need much brood potential. This must also be of the same age and must be found over a concentrated field. Furthermore, the comb should not have been used before for brood rearing. We can obtain such combs during the period of spring development in good colonies when a stimulating flow occurs. We hang a drawn, but unused comb into the centre of the brood nest after moistening it with honey solution or when honey had been extracted just before. If the queen is slightly short of room for egg laying in the rest of the hive, it will increase our chances, that by the next morning larve areas of cells have been filled with eggs. Occasionally we have to wait much longer than this.

For this reason it is much better if we can force a queen to deposit her eggs into suitable comb at our convenience. In order to achieve this we need to have a cage able to hold a whole frame. made from queen excluder material ("isolator"). Generally this cage consists of two frames of the same size as the brood frames and covered with excluder material. At their base both screens are hinged to a strip of thin timber, and a brood frame can be held safely between these two screens. A piece of tin may be used instead of hinges ; nailed to the two frames it makes a flexible bottom which is easily prised apart to take the brood comb. At the upper end the two screens are held by simple hooks. We need only place the queen on the brood frame within the cage ; soon she will be joined by bees passing freely through the queen excluders.

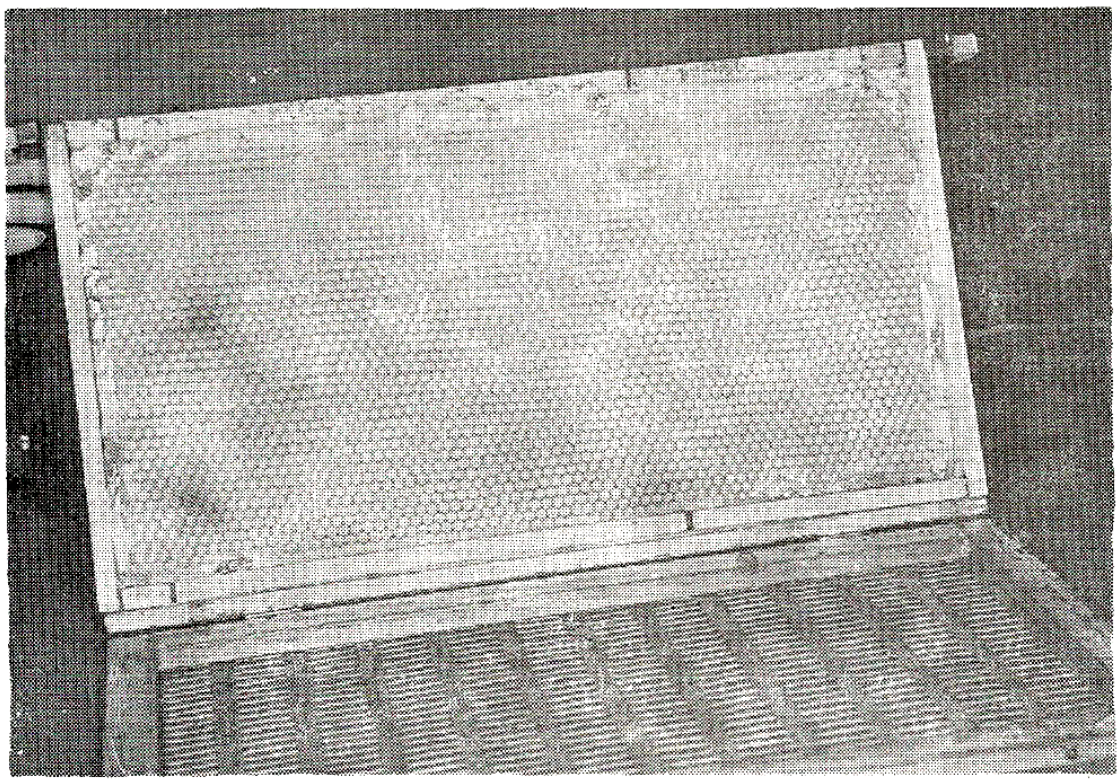


Fig. 64 — *A frame cage with two hinged sides made with excluder material (isolator). This one is being used for a frame of freshly drawn comb. Notice that passages along the edges have been closed with aluminium foil.*

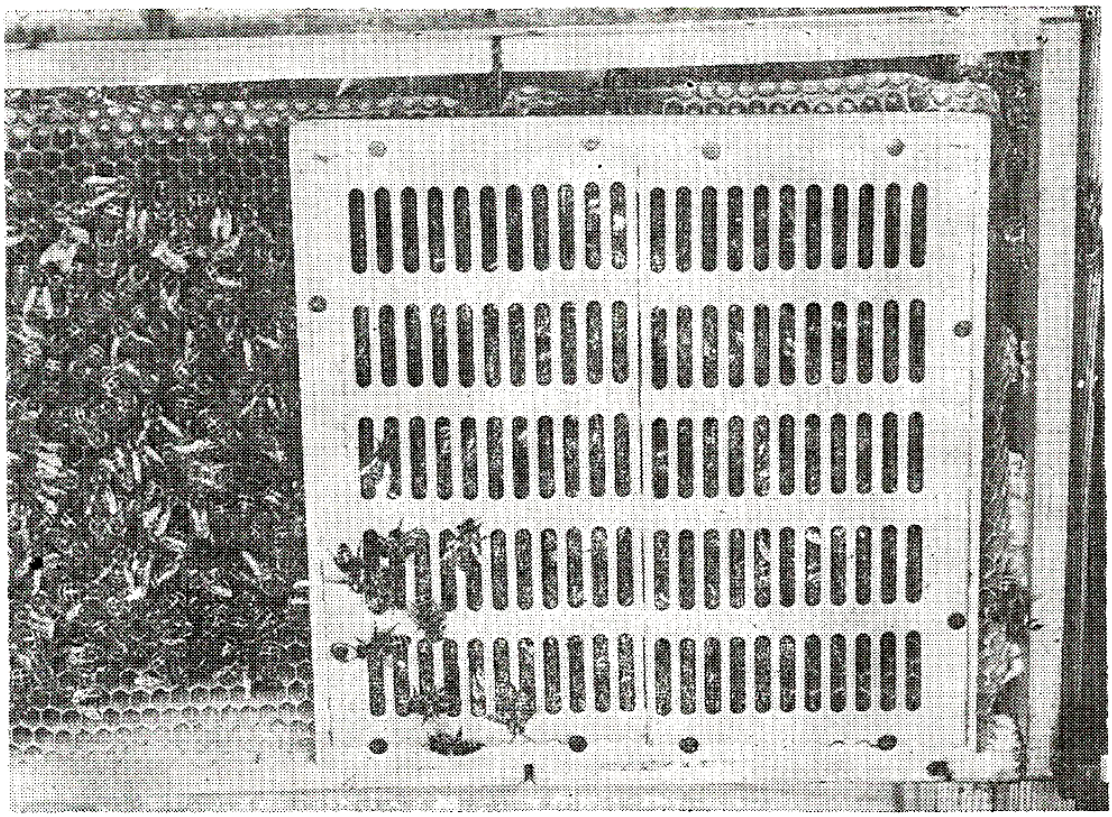


Fig. 65 — *In order to obtain eggs or larvae of known ages, a queen can also be confined over a part of one comb by means of a large push-in cage.*

When strips of comb, punched cells or cell clusters are to be used, there is no need to permit the queen to lay on both sides of the brood comb. In that case it is easy to block all passages to the other side of the comb by means of aluminium foil. Such foil is not destroyed by bees and the queen can lay on one side only. This will also enlarge the area of comb which can be filled with eggs. When the queen is in a highly productive phase we may find enough eggs after 12 hours of confinement to provide material for several cell strips and batches. As a rule we leave the queen confined for 24 hours and release her after that. We leave the frame within the frame cage until such time when we need the comb. When rearing queens from eggs in cell clusters the time of removal is $1^1/_2$ day later. Eggs are $1^1/_2$—$2^1/_2$ days old by then. When rearing queens from larvae in cell strips or in punched cells the comb remains in confinement for 3 days.

If small quantities of breeding material are required the queen can be confined over some comb by means of a smaller cage which is pressed into the side of a comb. Fig. 65 shows such a cage. This design can also be used with Hoffman frames, which cannot be placed in an isolator of the type described.

3. **Distribution of valuable eggs and larvae**

3.1. *Mailing eggs*

Experiences have shown (Chapter five) that when eggs are to be sent for distribution, it is important, that only those over $1^1/_2$ day old, but not yet ready to hatch, are used for this purpose. As already mentioned before, we cannot determine the age of egg by its angle of inclination. The breeder should never forget to make absolutely sure he knows the correct age. During my observations I often found $2^1/_2$ day old eggs standing upright within their cells. Usually they bend more strongly just before hatching.

In order to obtain eggs of known age we confine the queen or cage her on a good piece of comb which had been used once for rearing brood towards evening. We release her towards evening of the following day. The comb may remain in the hive or can be placed into an incubator at a temperature of 35°C and at 40—80% relative humidity. Eggs will develop naturally. After a further $1^1/_2$ days, that is on the morning of the day after the next, the youngest eggs will be $1^1/_2$ days old, the oldest will be $2^1/_2$ days, provided the queen had started to lay eggs as soon as she was placed on the comb. During the development period of a colony this is usually the case.

Before its despatch the piece of comb is cut from the frame with warmed knife. We wrap it gently in tissue paper and carefully put it among some crumpled newspapers into a card board box of sufficient size (Fig. 66 ; 67). Small boxes made of expanded polystyrene are even

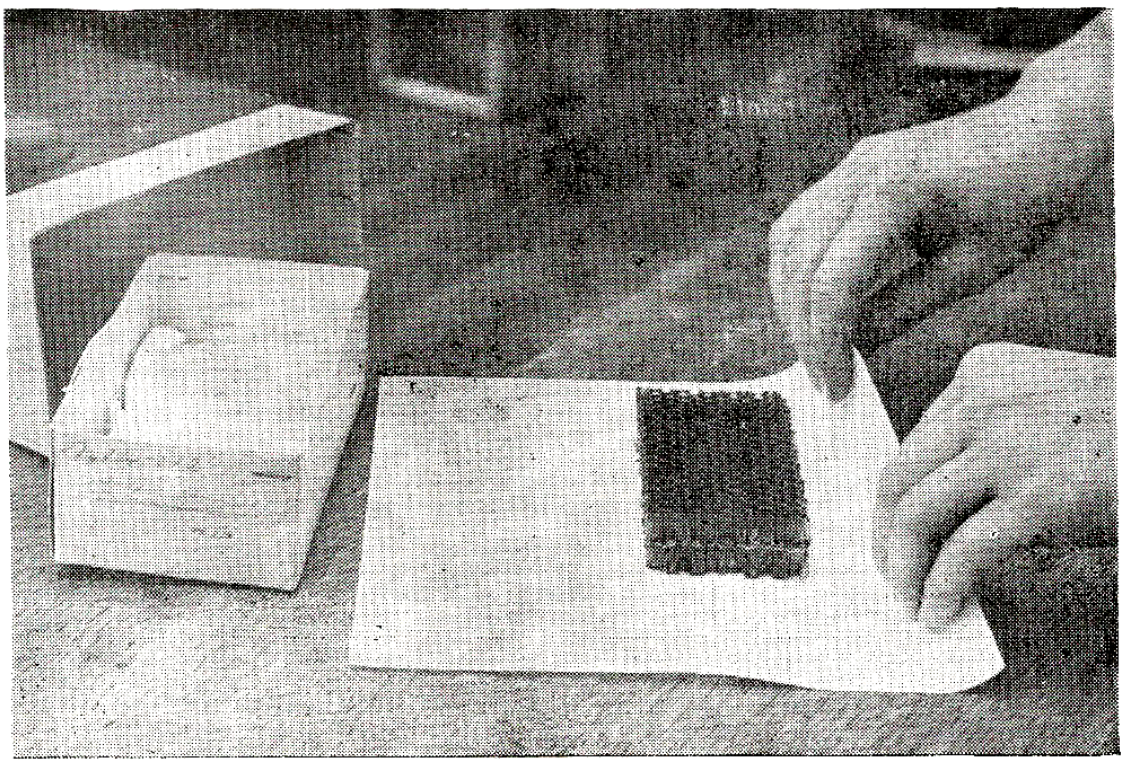


Fig. 66 — *The piece of comb with eggs is first wrapped in tissue paper...*

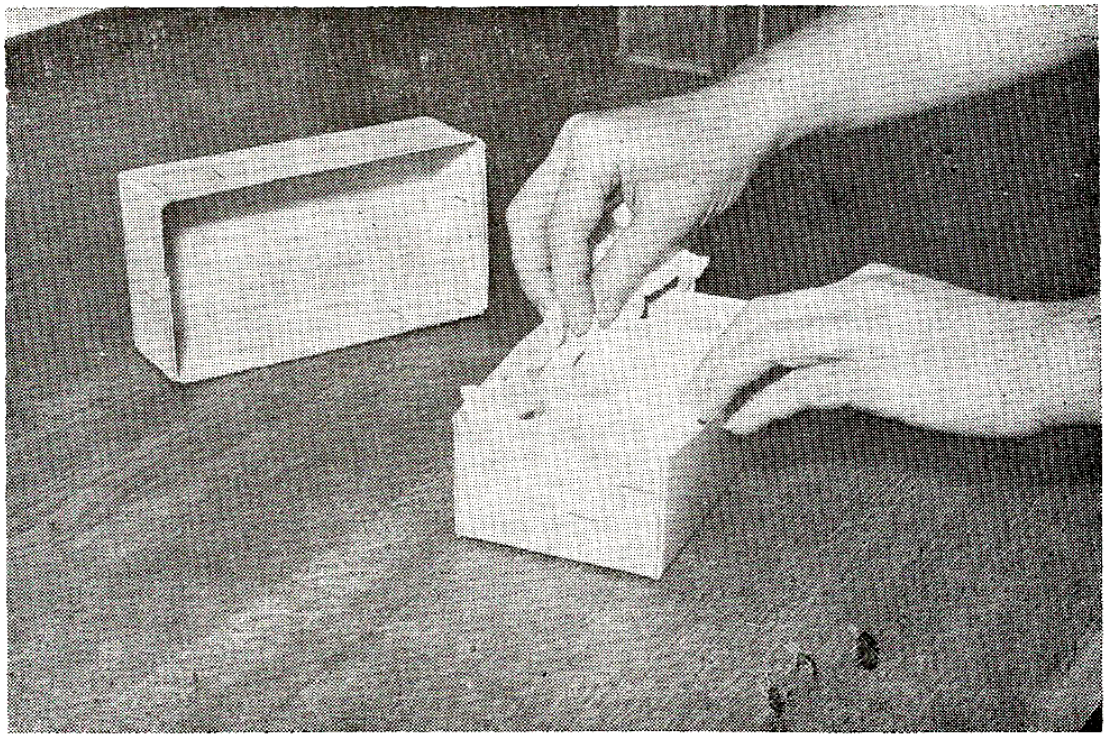


Fig. 67 — *Crumpled newspaper buffers against mechanical shock in a carton of ample size*

better. Eggs are protected against shocks sufficiently when sent in this way. Besides, eggs of the age suitable for transportation are more firmly attached to their base than the younger ages.

The box and the wrapping material should have no obvious scent. The consignment is best sent by express post.

Such a piece of comb with eggs should never be used for rearing queens from the egg stage. Apart from the small quantity of cells available, we can never tell which and how many of these eggs had suffered during transportation. It is much better to return the comb to the care of a nursing colony at once. We can either fit it temporarily into a nursing frame or we can fit it into a comb with a hole of the same size. Either may be given to the prepared nursing colony or can be placed in any colony for temporary care. Make sure the comb is placed between comb with brood, as otherwise bees may remove many eggs. Any queen in such colonies must be kept away from this special comb. On the following day we inspect the comb to see if hatching has taken place and we now prepare for grafting.

Although eggs mailed can stand chilling to 15°C (and probably beyond that point) without suffering damage, I would not like to guarantee their survival during periods of dry heat. The sender and the receiver should see to it, that such pieces of comb are not exposed to the sun for any length of time. Experiments must yet be conducted in hot countries to see if this way of sending eggs is possible. When external

temperatures resemble those of the brood nest the development is not arrested and any larvae hatching may then starve to death. But mailing eggs under normal conditions is quite possible and this fact has been proven when eggs were sent from Erlangen, West Germany, by air to the USA. In the state of Maryland queens were reared from such consignments and were used for experimental purposes.

3.2. *Mailing larvae*

While comb with eggs may be mailed over great distances, larvae are more suitable for rapid distribution from apiary to apiary. In Chapter five we mentioned experiments which had been made in order to discover survival rates of open brood of different ages. It was discovered, that larvae in the right age for grafting may be removed from the hive for 6 hours without paying heed to the weather conditions ; after that time every one was accepted and all were nursed normally. All developed into perfect queens. Now it is normal practice in our Institute to remove a frame with brood from a colony in an out-apiary, to return with it to the home apiary some hours later and still use the larvae successfully on arrival. Such transport of larvae in the comb is to me an easy, simple way of distributing selected qualities within an association of breeders. A keen beekeeper collects young larvae in a comb or piece of comb from a breeder or breeding centre at a previously agreed time ; when he gets home he grafts immediately into prepared cell cups and rears queens from this batch. The comb can be transported without bees or other protection against chilling. Though eggs as well as these larvae must be protected against sun and heat. When the weather is very dry we will probably increase survival rates even further if the comb is wrapped in a moist cloth. The recommendations made in old beebooks are a thing of the past now. No longer need we wrap the frame in warm cloth and do our grafting in heated rooms only and in a great hurry.

Many breeders who come to us from further away arrive with a swarm box (Chapter VII, 3.2.3.) and graft at our breeding centre. Many larvae die if the return journey begins immediately afterwards. This does not happen if a few hours are allowed to pass between grafting and travelling home.

Our research into the survival of larvae and eggs has shown that that both stages can withstand transportation for several hours without nursing care by bees. This raises the question if the need for labourious travelling with swarm boxes is not also a thing of the past ?

Certain methods of rearing queens require that the grafting frame is transferred from a started colony to a finisher (Chapter seven). This may be done in a few minutes, but the batch will not be harmed if it takes a little longer. Early experiments have shown, that initiated queen cells with larvae between 2—3 days old can withstand at least 6 hours' exposure to variable room temperatures without taking harm. This means that such started queen cells may be transported without protection over

great distances to their finisher colony, in case none of these are in that same apiary. This opens the way to the distribution of selected grafts taken as open queen cells by the members of a breeder group. When these cells are nursed to completion in an upper chamber of a queen-right colony after some frames of open brood have been raised above queen excluder to draw nurse bees, they may be inserted into nuclei. This system can ease the effort of establishing pure mating zones quickly and easily (HEROLD, 1972). SCHÖNUNG, (1972) uses blocks of foamed plastic for the transport of such cells. He drills holes 15 mm deep and with 15 mm diameter to take the cells attached to their cell holders. He is convinced that a drop of water in the bottom of the hole can not harm in hot weather. The Bee Research Institute Lunz (Austria) has had good results with this equipment when day-old larvae were taken on journeys lasting half a day.

Success is even possible with freshly grafted larvae in either wax or plastic cell cups. There seemed no need for "acceptance" before commencing the journey. Recent work at Erlangen shows, that grafting either wet or dry seemed to make little difference.

3.3. *Mailing semen*

Semen survives for much longer periods than either eggs or larvae can live outside a colony. When it is sealed under sterile conditions in glass capillaries it can be stored for more than 14 days without noticeable damage. (TABER, 1961) ; with the use of antibiotics and stored at temperatures of 13°C the time may be much longer (POOLE and TABER, 1970). This gives sufficient time to mail semen to just about any place on earth. More information on this can be found in the book on Instrumental Insemination published by APIMONDIA (F. RUTTNER, 1975).

The method has been simplified tremendously with the use of glass tips for microsyringes. The semen is drawn in the customary way into this glass tip, which is capable of holding 10—50 µl according to need. Both ends are sealed with vaseline. On arrival the same tip may be used to inseminate after sterilisation. Cylindrical capillaries without tip may also be used. In order to draw semen or to inject it, a short tip of a syringe can be attached to the capillary by means of a tube of PVC.

The importance of the distribution of semen through the mail has increased lately with the wide use of instrumental insemination in the breeding of the honeybee. The fear of the spread of bee diseases adds to the extended use of semen in breeding techniques. F. RUTTNER and many others have achieved excellent results from it.

SUMMARY

1. Rearing from Larvae

1.1. Raising cells allong the edge of cut comb. A crescent shaped cut is made on comb with very young larvae or eggs. This can only be an expedient ; it does not guarantee acceptance from youngest larvae only. When tough comb is used for this, smaller queens will be the result. Anybody trying this method should make sure, freshly drawn comb is used.

1.2. The same applies for strips of comb or punched cells. When old comb is used for this method, queens will weigh less. Freshly drawn comb is recommended also for this work, even though it requires more work and makes it difficult.

1.3. Grafting one-day old larvae is the most economic and efficient method for queen rearing. They may be grafted into natural play cups cut from comb, artificial cell cups or those made from plastic material Cell cups may be fixed directly to cell bars by means of liquid wax or can be fastened first to cell holders of various designs.

1.4. A grafting tool is needed for this purpose. The best available is cranked close to the spoon-shaped end. Others may not quite be so convenient. A magnifying glass helps. It is not essential to prime cells with a little royal jelly, but it does facilitate the sliding off of the larva.

2. Rearing from Eggs

The grafting of eggs has been shown to be impossible as a practical method. Eggs are delicate and can easily be injured in the process. Bees do not accept cells with eggs when they are punched out individually. When a crescent shaped cut is made along the line of eggs, most cells are formed into queen cells after larvae have hatched, sometimes even long afterwards.

2.2. ÖRÖSI-PAL has developed a method which transfers eggs together with a small disc of wax from the base of the cell, using a special punch for this. Freshly drawn comb must also be used in this case. Queen cells must be initiated by the grafting method one day ahead of the transfer of the eggs. First batch of larvae are removed from the pool of royal jelly and the disc of wax with the egg is placed into the dimple in which the larvae had been swimming. This double procedure makes it an elaborate method, it takes a long time and much work.

2.3. The Erlangen method of rearing from eggs uses small clusters of approximately 5 cells. These clusters are cut from strips of freshly drawn comb. Clamped between special cell holders which in turn are attached to cell bars with liquid wax, all eggs but one are destroyed in each cluster.

Rearing queens from eggs in clusters of cells is done in a queen-right colony. The work can be done by unskilled persons.

2.4. The unavoidable loss of larvae or eggs is the biggest drawback in this process of preparing comb for rearing from the egg stage. A frame cage or large push-in cage is used to confine the queen on freshly drawn comb in order to obtain eggs of predetermined age at the right time. In large frame cages the queen may be confined to one face of the comb by blocking all passages with aluminium foil. Smaller quantities of eggs are obtained by caging the queen on one face with a large push-in cage made of excluder material.

3. Mailing, transporting and distributing eggs and larvae

3.1. Eggs should be at least $1^1/_2$ day old for distribution. Absolute certainty over the age of eggs used for mailing can only be achieved by caging the queen on comb for short period.

The piece of comb is cut from the frame and is wrapped in tissue paper first. It is then placed in to a larger carton, with crumpled newspaper filling surplus space and absorbing shock. The addressee should receive his consignment within 2 days at the latest (eggs do not survive for longer periods outside the hive). He will cut a piece of comb as large as the one with the selected eggs from one of his own frames and replaces it with the piece at once. It is immediately inserted in the centre of a brood nest so that nurse bees can take care of it. The queen in this colony should not have access to this comb. She may be removed altogether, if the colony is to be used for raising the cells. Grafting is done when larvae have hatched. Mailed eggs should not be used for rearing from eggs.

3.2. Young larvae of the right age for grafting will not take harm if removed from the hive for 6 hours. This allows transportation of selected brood comb from apiary to apiary and provides opportunity for wide distribution of selected genes. It also gives us the chance to do all grafting at a breeding centre and to distribute larvae in their cell cups. Larvae and eggs must be protected against great heat. Cold seems not to damage them to the same extent. A moist cloth wrapped around the comb will help to keep larvae alive during dry periods.

Not only these young stages of royal brood are capable of surviving journeys of 6 hour duration ; more advanced, open queen cells 1—2 day old and well provisioned may equally be removed from the hive for similar periods. Distributing initiated cells to beekeepers can assist in the creation of pure line breeding zones (mono-strain areas).

3.3. Semen collected from drones may be stored at room temperatures in well sealed capillaries without loosing its ability to fertilise. This permits its mailing over vast distances.