STUDIES ON SOME ASPECTS OF THE BIOLOGY OF THE HONEYBEE, *Apis Cerana indica* (*Fab*).

By

RIGAPHY SYNTEM

DEPARTMENT OF ZOOLOGY
SCHOOL OF LIFE SCIENCES

Thesis submitted in fulfilment of the requirement of the Degree of

DOCTOR OF PHILOSOPHY

To

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C E R T I F I C A T E

I certify that the thesis entitled "Studies on some Aspects of the Biology of the Honeybee, _Apis cerana indica_ (Fab.)" submitted by Miss. Rigaphy Syntem for the Degree of Doctor of Philosophy of the North-Eastern Hill University, Shillong embodies the record of original investigation carried out by her under my supervision. She has been duly registered and the thesis presented is worthy of being considered for the award of the Ph.D. degree. This work has not been submitted for any degree of any other University.

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(RIGAPHY SYNTEM)

LIST OF FIGURES

GENERAL INTRODUCTION

Apiculture or bee-keeping is quite an interesting job based not only on the bee being economical but specially on their social life which is said to have attained perfection.

'Working on an apiary where the air is saturated with the sweet perfume of flowers in the bee-hive garden, along with the spicy odour of bee bread and honey is quite beneficial for one's health, relaxing the nervous system and enhancing one's physical powers', is what a bee-keeper of Meghalaya said.

The bibliography of bee is extensive starting right from the time of Aristotle, Cato, Varro, Pliny, Columella, Palladius, who studied them for years together varying from twenty to fifty years. The real history of bees began in the Seventeenth century, but of course to talk of its economical life, one of the worker who really toiled hard on the improvement of rearing system was L.L. Langstroth (1851). He invented a wooden box with movabla frames for the bees to live in; and thus this box came to be known as 'Langstroth's hive'. With time specially in Western countries more improvements came in that one can say that there is no need for the bees to construct their hives, everything is readymade for them; their only work is manufacture of honey. The different inventions apart from Langstroth's hive was the beewax foundation in 1857 by

Johannes Mehring, a German, and the extractor in 1865 by Major Hrushka, an Austrian. Under the influence of the industrial revolution; enterprising individuals further developed these ideas. In United States, Root A.I., produced a roller press that made wax foundation commercially, and Langstroth, C. Dadant and M. Quinby (1875) modified the extractor to suit the needs of the commercial bee-keeper.

The life of a bee-colony is diversified and intricate. They exhibit social organisation. The remarkable qualities of social life are mass phenomena that emerge from the meshing of these simple individual patterns by means of communication. Wheeler in his tract: "Emergent Evolution and the Social (1927)" anticipated this property of the subject - "Owing moreover to the loose and primitive characters of the integration and size of the components even the densest societies, it is possible to ascertain the behaviour of the parts and to experiment with them more extensively than with chemical and organismal wholes. Since the part of the latter are either microscopic or ultra microscopic and are always compactly integrated that analysis becomes very difficult and involves a considerable of statistical inference."

In a colony or hive an overwhelming majority of individuals are females. But during their course of evolution they completely lose their ability to mate rendering them incapable of continuing the species. They cannot reproduce because neither their body size, nor their strongly degenerated sexual organs will permit it. "Organs in rudimentary condition plainly show that an early progenitor had the organs in a fully developed condition; and this in some cases implies an enormous modification in the descendants". (Ref. Charles Darwin (1859) Origin of Species). However, these bees preserved their maternal instinct, as can be observed in their case of progeny, and on emergency one of the female workers can even lay eggs; (in the absence of queens accidently) when there are no more eggs (brood cells) in the hive, for a successor. At the same time they acquired certain biological properties which subsequently becomes highly developed, which are of great importance for the survival of the colony like building their nest, foraging for food, feeding innumerable brood cells, maintaining temperature and guarding their nest. In brief, they began to accomplish all the jobs necessary for their life and activity of their community. Thus they came to be called - worker bees.

Among the great number of the colony of worker bees, it is only the queen that has preserved her sexual instinct and is capable of reproducing, a function which has become the most vital one in the queen's life. The morphological transformation and physiological changes which the bees (workers and queen) have undergone during the course of evolution is highly expedient. They marked a biological process which resulted in the further prosperity of the species.

The queen is constantly in the nest, except for mating and swarming flight. She is the most important individual in a colony. The entire population of the hive depends on her. Nevertheless, in many ways the life and activity of the queen is determined by the colony as a whole.

Female bees (fertile queen and worker bees) are the foundation of a bee colony; it is biologically complete, it can make its own nest, procure its food reserves, and continue its race by reproduction.

Drones are considered to be seasonal residents of the hive. They are male bees and the bee species cannot multiply without them. They are apart from mating, helpless individuals where they cannot even feed themselves and have to depend on the worker bees for their bee-bread.

The honeybees, like many other social insects, live in nests/hives, constructed by themselves, where all the vitally important processess of the colony's life takes place. These hives are made up of wax secreted by the worker bees. A unique feature about the bee-hives is that it is constituted of a large number of wax cells, which are in the form of regular hexagonal prisms. The three sides of the bee cell pyramid are set, in plane projection, at co-equal angles at 120° to one another, but the three apical angles are co-equal angles of 109° (cf. Thompson, 1966).

These hives are made up of brood. Brood is a term commonly use to designate the young of the bees that has just emerge from the cell. It may be young bees just before they come out from the cells, the larvae in various stages of growth or even eggs. Sealed brood is of light to dark brown colour depending on the age and colour of the comb itself. The cappings are made of wax and fibrous material, smooth and slightly convex, with around four cells to an inch. The cappings over honey are white, bluish white or yellow and are more or less irregular and somewhat flattened. The honey may be either in worker or drone cells. The presence or absence of brood reveals the real condition of the colony. It is the presence of eggs or young larvae that shows that the bees have a queen and are beginning to rear brood. This usually occurs during the early spring, but with the progress of season brood are usually present in all stages of growth. But things can happen the other way round too, that is, the presence of unsealed brood and specially absence of eggs indicates a queenless colony. The amount of brood and the manner in which the eggs are laid - whether scattered or irregular patches gives one a fair idea even without seeing, of the quality of their queen. As goes the saying, "By their fruits, ye shall know them".

A healthy colony of bees in their hives looks more dead than alive, their movements are slow incoherent and incomprehensible. But the fact is that, everyone of the little almost motionless groups in the hive is incessantly working each at a different trade. The bee is above all even to a greater extent than the ant, a creature of the crowd. She can only survive in the midst of a multitude. When she leaves her hive which is so densely packed that she will dive for an instant into the flower filled space as the swimmer will dive to the sea filled with pearls, but under pain of death it behoves her at regular intervals to return and breathe the crowd as the swimmer must return for air. Isolate bees and however abundant the food or favourable the temperature, they will expire in a few days, not of hunger or cold, but of loneliness. From the crowd, from the city, she derives an invisible aliment that is necessary to her as honey. This craving will help to explain the spirit of the laws of the hive. For in them the individual is nothing her existense conditional only; and herself,for one indifferent moment, a winged organ of the race. Her entire life is a sacrifice to the manifold everlasting being whereof she forms part (Maeterlinck 1958).

Bees apart from having a highly social organised pattern of living are also very sensitive creatures. Their learning capacity is impressive in several respects. They are able to learn signals in every known sensory modality. Honeybees can learn quickly to orient with respect to attractive odours but not at all with respect to repellants (Von Frisch, 1919).

Insects are provided with a great variety of external sense organs having characteristic structural differences in both the cuticular and the cellular parts. It is very difficult, however, to isolate the various types of organs for experimental purposes, and for this reason we can, in most cases, only form an opinion as their probable function based on a study of their structure; and the structure is often so widely different from that of any organ of known function in other animals that many insect sense organs cannot yet be satisfactorily identified as receptors of any group of stimuli. The sense organs of insects, therefore, are generally classified on a purely anatomical basis. The receptor complex formed of the cuticula the sense cells or group of sense cells and the associated chitogenous cell is called sensillum.

Von Frisch (1919,1921), Ribbands (1953), Schwarz (1955), Fisher (1957) and Martin (1964, 1965) have established that the sense of smell of the honeybee worker is closely comparable to that of man. Even though the anatomy of the organs of olfaction is entirely different, in bees and men, it is surprising that their olfactory relation are nevertheless so nearly the same, (Von Frisch, 1919). The sense of smell is located in the antennae (Von Frisch 1921). A glimpse through the microscope shows that the antennae of bees are densely covered with sense organs some of which are organs of touch and others of smell.

Another interesting phenomena exhibited by bees is swarming. What is swarming? Swarming is applied to the act of a family of bees leaving their home to establish a new home elsewhere; i.e., reproduction of a colony. This term is also applied to a colony after it has established itself in its new home to distinguish it from a parent colony (A.I. Root; 1966).

There are several prevailing conditions which lead to this very act apart from reproduction. Usually the exact time of issuing of the swarm that is normal and prosperous is during the spring time. But at other times even though the colony is ripe with excess population and surplus honey yet it stays as such without swarming; whereas at other times a colony stubbornly persists on carrying out this programme even though nothing is ready. This condition at one season might effect only one colony and at other seasons a majority of the colonies. Depending on the flow of nectar in spring, just preceding the main flow, may also start furious swarming (Demuth; 1958).

Earlier it was taken to believe that it is the queen who decides every task which takes place in the hive (thus the name). But with time and more investigations this was not found true. She is not the colony's queen, she issues no orders, she obeys as meekly as the humblest of her subjects, the masked power which was considered by some workers as the "spirit of the hive", but she is considered as a unique organ of love, the mother of the city. She founded it, she peopled it with her own substance, all who moves within its walls - workers, males, larvae, nymphs and the young princessess - whose approaching birth will hasten her own departure; all these have issued from her flanks (Macterlinck 1958).

Swarming is also influenced by photoperiod, genetics, physical crowdings, and the age of the queen. Old queens have a greater tendency to swarm than young ones.

Never is the hive more beautiful than on the eve of its heroic renouncement, where it takes off to its new home. This is not of course the end of swarming, where happiness resides, but an understanding among themselves is essential about the new nest site. Lindauer (1961) first showed, a similar 'democratic' process which is employed by honeybee swarms in choosing nest sites.

Keeping all these in view, certain biological aspect of the bee (Apis cerana indica Fab.) are worked out.

The area of research which has been selected is mainly Cherrapunji, but some experiments were also carried out in Shillong and few others in Umroi of Meghalaya (which lies between latitudes of 25°N and 26°N and longitudes of 90°E and 92°E).

The aim of this research programme is to gather some more basic knowledge on the life of this particular insect, the honeybee (Apis cerana indica Fab.); and also information on some of its peculiar behavioural pattern which has intrique mankind.

A glimpse through the literature shows that a major work has been carried out on the individual components by a number of workers, but this insect being or leading a social life that makes up a colony; it would be interesting to study its socialization and behaviour which makes it form a colony; or the different activities which involves the colony as a whole.

Thus, with all this in mind, efforts are being made to carry out the work, with the available facilities.

CHAPTER I

SOCIAL ORGANISATION AND BEHAVIOR OF THE DIFFERENT CASTES OF HONEYBEE, *Apis Cerana Indica* (Fab.)

INTRODUCTION

Insects are classified as solitary, communal, social and so forth. Such terms are generally applied to the most complex type of organisation attained during the life cycle of the species. Such usage is appropriate and useful, but when precision of reference to a particular colony or interaction is needed, it often does not suffice to allude to the organisational level attained by the species. Only the highly eusocial bees remained permanently at that level. Nearly all other forms change during their life-cycles, usually from solitary to some social patterns. Such is the case of honeybee species too.

A social insect has been defined as "an insect which lives, in a society, each society consisting of the two parents, or at least the fecundated female and their offsprings, and with the two generations living together in a common abode or shelter and exhibiting a variable degree of mutual co-operation". All true insect societies are in fact families. This being so it can be concluded that the insect social life in any highly developed state, such as that found in the honeybee colony has evolved through numerous stages. These stages show an increasing degree of intimacy between the mother insect and her offspring, and with it an everincreasing degree of mutual co-operation.

Social organisation with division of labour among insects, specially among Hymenoptera and Isoptera has evolved four times with the independent evolvement of social system.

The society of honeybees may be compared to individual organs possessing group attributes that collectively indicate a biological unit. An individual, cellular or multicellular organism is an open system with orientation, adaptation and interaction with its environment (Emerson, 1964).

The social life of the honeybees, *Apis cerana indica* (Fab.) is far more complex as was indicated by the discussion of its rather elaborate communication system.

Comparing the social life of the different species of bees it is only in colonies of *A. mellifera* and *A. cerana indica* (Fab.) that we find the highest degree of social development amongst bees. In these species a very distinct worker caste is present consisting of sterile females and that each colony normally, only possesses a single queen who is the mother of all the bees forming her colony. Queens of all other four species of honeybees (*Apis* species) have no trace of pollen collecting apparatus; wax secreting and special scent producing glands and their hypo-pharyngeal glands are also vestigial. These queens have become immensely fertile and capable of producing many thousands of eggs, in the course of their lives which may last for several years.

The colonies of all true honeybees are perennial and the development of new colonies takes place by swarming.

Honeybees (A. cerana indica Fab.) are remarkable amongst the higher social insect in exhibiting all stages of development of sociality as mentioned. Differentiation of the honeybees into castes has intrigued biologists for many years. There are only two castes - the workers and Queen. While a worker's duty changes with age and colony needs, this one caste is able to take care of such necessities of life as feeding, thermo-regulation, foraging and defence. Caste determination in the honeybee which presumably results from selective feeding of the female larvae by the nurse bees, has been subject to much discussions (Haydak, 1968; Pain 1968).

While differentiation has been researched extensively, there are unanswered questions. It is a complex process not controlled by a specific factor or factors but apparently several working together. One hypothesis tested was that workers are the result of deliberate undernourishing of larvae, and the queen is a product of lavish feeding all during the unsealed portion of larval development, which then interferes with endocrine function of ovaries.

The caste of worker and queen show considerable differences in their morphological, anatomical and physiological features (Lukoschus, 1956; Snodgrass, 1956).

The division of labour between distinct castes of honeybees within a supraorganismic social population related with the systematic arrangement of many hundreds of species of insect provide material for the detection of phylogenetic order and evolutionary trend.

Learning in insects has been explored in various ways (Markl and Lindauer, 1965). The learning ability of honeybees, in particular, has been the subject of a number of learning experiments (Von Frisch, 1965; Ribbands 1953; Wenner and Johnson, 1966).

The division of labour among worker honeybee varies with external environmental differences ánd differences in hive 'economy'. Kolmes (1985) showed that in different environmental and economic conditions this behaviour appeared to be performed differently.

There appear to be many factors involved in the way worker honeybees divide the labour of the colony among themselves. Usually young bees perform hive duties and when older they undertake foraging duties. This pattern of behaviour applies not only to honeybees but to other species of bees as well (Michener, 1974). Seasonal differences is also associated with differences in the division of labour among workers of various age.

The strength of temporal variability of specific behaviour patterns differs from study to study. (Rosch, 1925; 1927; 1930; Lindauer, 1952; Sakagami, 1953; Sekiguchi and Sakagami, 1966; Winston and Punnett 1982; Seeley, 1982; Kolmes, 1985).

The prediction that increased task specialization will generate greater overall efficiency in a complex social insect nest (Oster and Wilson, 1978), and can therefore, be examined in the light of different challenges presented to honeybee colonies and some insight into adaptive behavioural responses in such a complex society may be gained.

Honeybees do not appear to pass through a temporal sequence of task while performing hive duties; there does appear to be a strong behavioural demarcation between young hive bees and older field bees.

Lindauer reported (1952) the type of flexibility with a number of task performed side by side on the same day.

Rosch (1925,1927,1930); Sakagami (1953); Sekiguchi and Sakagami (1966) and Seeley (1982) have concluded that worker bees pass through a definite sequence of task in the hive, with cell cleaning, brood nursing, pollen manipulation etc. occuring at different ages. They reported somewhat different timings of portions of this sequence and gave different sets of names to the proposed stages.

Apart from the exhaustive labour carried on by the worker bees for maintaining their social life the queen's sole function is production of eggs. The queen honeybee has become so highly specialized as an egg-producing machine that she is quite unable to collect food for herself or her offsprings or to build a nest. She cannot even survive for very long on her own. If one removes the queen from the colony, neither she (queen) can survive long nor her associates (workers). The condition of the colony becomes disorganised. But when the queen is replaced in the hive, her associates began to cluster and examines her with their antennae, they feed and lick. Also if some of the workers are taken and kept in the place where the queen was kept earlier and is later emptied, their behaviour shows as if she is still there, as they try to examine the cage carefully; its as if she left some scent behind. With this and more experiments carried on it seems highly probable that the queen leaves some kind of scent and one is tempted to suppose that bees habitually recognise the presence of their queen by her scent and realise when she is absent because of the lack of her scent. With an innumerable numbers of experiments conducted by several workers none could deny the fact that there is some substance (scent-like) given off by a queen bee in a colony, and thus, they term this substance as 'Queen Substance' and it was Verheijen-Voogd (1959) along with Butler and Simpson (1958) who showed that the principal source was the queen's head. Later on the name 'Pheromone' was introduced and it is believed that the different glands (mandibular glands) are responsible for this. This substance have several functions like inhibition of OOgenesis, swarming and sex attractant.

The next caste the drones, being specialist designed only for mating; they have no pollen basket, stinger or wax gland. They are seasonal residents in the hive; that being only during the mating season.

MATERIAL AND METHODS

Description of the bee-boxes used

Two types of boxes have been used in this study. The first box resembles that discovered by Rev. L.L. Langstroth (1851), whereas the second one is a natural wooden bark-type box.

1. In Meghalaya, the government has one institute, viz: Village Industries and Development Agency (VIDA), from where these ready made boxes are available. This has been done to popularise apiculture, and to make people realize the importance of honey in these areas; rearing of bees being not a difficult task be it as a full time, part time or just as one's hobby; either way its equally profitable.

   These ready-made boxes are of two types, one of them having ten frames, the other eight frames. They have been constructed according to the agreement with the Indian Standard Institute. The boxes have two chambers - the brood chamber - where egg-laying, larvae and pupae nursing takes place; and the super chamber - for storing of nectar and pollen. (Fig. 1a).

2. In some other rural areas of Meghalaya like Cherrapunji, in some places viz., Nohkalikai - another type of box is in use. This box is made up of a wooden

bark, which is hollow inside with an outlet on both ends; these outlets are covered with specially made lid covers (Fig. 1b).

These types of hives are of great help to a researcher as the lids covering the bark are easily removable without disturbing the hive any time an investigation is to be made.

Experiment No. 1

To study the life cycle of the various castes of the honeybee, *Apis cerana indica* (Fab.) in its natural state.

The different stages involved in the life cycle of the different castes, the time period and other marked differences with their significance are observed with the help of photographs where ever necessary and possible.

This particular experiment was carried on, in Cherrapunji in the hollow bark type box, where the different stages of development could be studied easily.

Experiment No 2

To study the behaviour of the different castes with a special reference to social organisation.

Fig. 1(a) (i + ii) A Langstroth type of beehive box, showing the external and internal set up

PLATE 1

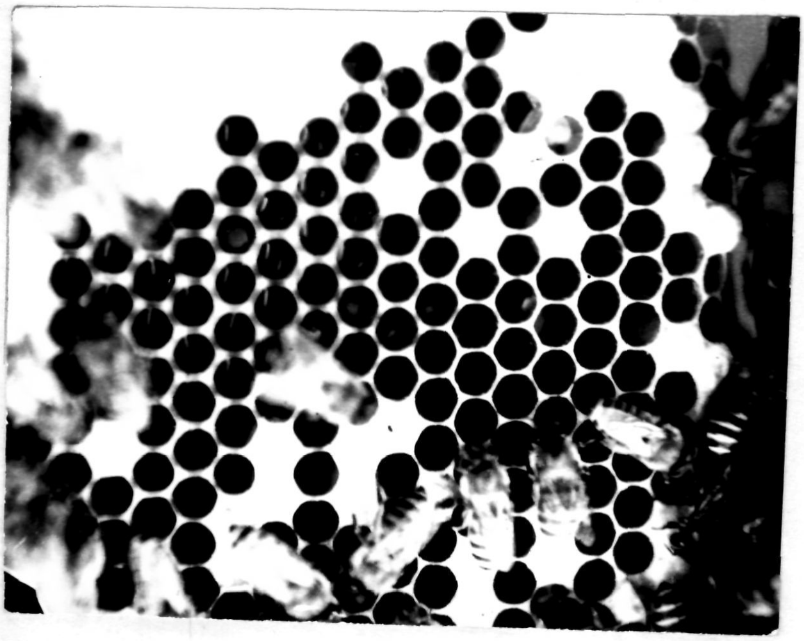
Fig. 1(b) (i + ii) A wooden bark type of beehive with clusters of bees and also showing a part of the hanging comb in the box.

PLATE 2

Fig. 2(a) A worker honeybee Apis cerana indica (Fab.) inspecting a worker cell.

Fig. 2(b) Workers honeybees Apis cerana indica (Fab.) guarding their nest.

PLATE 3



(A) **Behaviour of the workers in a colony** – Division of labour is one of the most important behaviour of the workers of a colony. This involves comb building, foraging, maintenance of hive temperature, nursing, cleaning etc.

This experiment can be carried on in both the types of boxes. This was done in Cherrapunji (Mawsmai and Nohkalikai) as well as in Umroi and Shillong. Investigations were carried on for different functions at different times mostly on bright sunny days (27°-30°C).

To observe the different acts of labour of the bees, Ribbands (1953) suggested that food supply some how directly influences workers activity and division of labour, while Lindauer (1952,1961) emphasizes the long periods that workers spend moving about the nest, apparently finding out what needs to be done, such episodes often ending when the bee does something seemingly useful. (Fig. 2a,b) shows some of the activities of the bees in the hive. Photographs were also taken regularly wherever necessary to represent the different acts of labour, the workers have to undertake for maintenance of their social living.

(B) **Behaviour of the Queen** – The most important role of the queen is that of reproduction for the continuity of the race. She is also responsible for uniting the of the race. She is also responsible for uniting the colony.

Experiments were conducted to note the reaction of the colony with and without the queen (Butler, 1964; Bulter, Callow, Simpson, 1973) behaviour of two queens in a colony,longevity and egg laying capacity of a single studied queen-right colony, and also role of 'queen substance' in social organisation, (Bulter et.al. 1954,1957,1960,1961).

(C) **Behaviour of the drone** - The drone is a male member, responsible only for mating the queen. Being a seasonal resident, observations were made to note down the maximum appearance of this particular caste in a year.

Investigations on drone massacre were also made.

RESULTS AND DISCUSSION

Life cycle of *Apis cerana indica* (Fab.) Table 1.1

The honeybees, *A. mellifera* are considered the highest form attained by the Anthophilous division of the Hymenoptera. The differentiation of three forms - males (drones); females (queen and workers); is carried to a greater degree of perfection than in the other bees. The life history of *A. mellifera* and its anatomy and physiology have been discussed elaborately by a number of authors; but there is no information on Indian domestic honeybees, *Apis cerana indica* (Fab.).

A colony of bees consists of the queen bee (female) and a number of workers, the latter being in fact, the surplus population that has been produced in a hive; and not forgetting the male components, the drones.

Workers -(Fig.3) The queen lays an egg in the cells (Fig. 4a) provided by the workers and as these soon hatch, great labour is alloted to the workers which then have to feed the young, this they do by eating nectar, honey and pollen, which being formed into a sort of pap by a portion of their digestive organs is then regurgitated and given to their young, a quantity of it being placed in the cell, so that the larvae are bathed by it, and possibly may absorb the food through the cuticle as well as the mouth.

TABLE I.1

SHOWING TIME INTERVAL IN DIFFERENT STAGES

OF Apis cerana indica (Fab.) LIFE CYCLE

	Stages	Days		
		Worker	Queen	Drone
1.	Egg	2-3	4	4
2.	Inmature larvae	4	3	4
3.	Mature larvae gorging food	2	2½	3-4
4.	Pre-pupal	2	1	4
5.	Pupal	10	5	8-9
6.	Sealed brood	11-12	7½ -8	13-14
7.	Egg adult	20-21	16	23-24
8.	Life expentancy	Around 3 months	2-4 years	1-2 months

FIG. 8 DIAGRAMATIC REPRESENTATION OF THE COURSE OF DEVELOPMENT OF WORKERS (FEMALES)

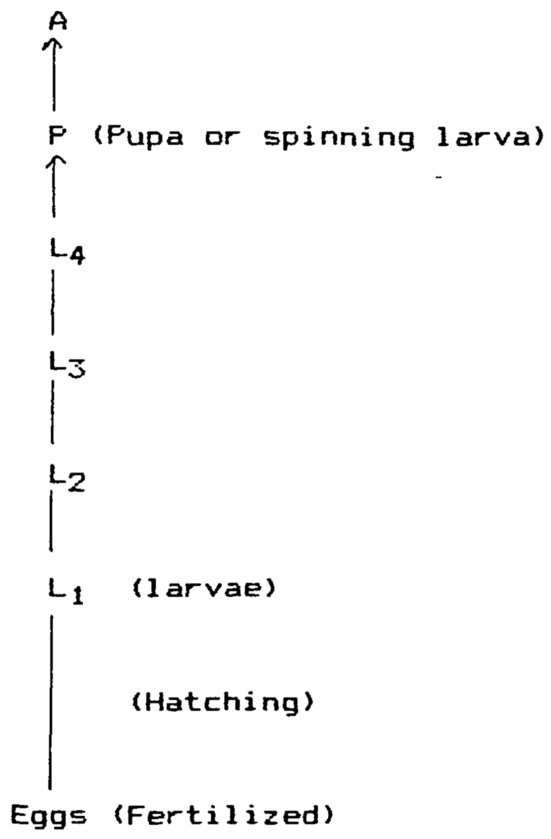
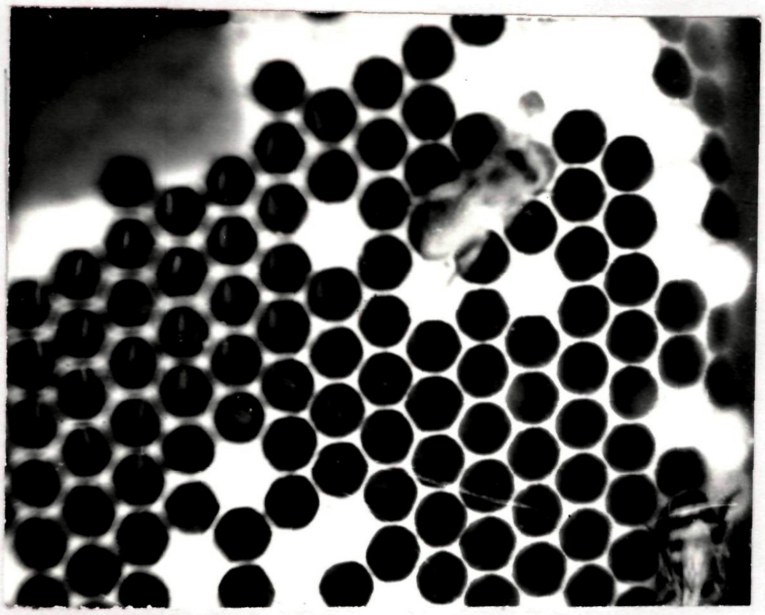


Fig. 4(a) Workers cells of honeybees <u>Apis cerana indica</u> (Fab.)

Fig. 4(b) Sealed workers cells of honeybees <u>Apis cerana indica</u> (Fab.)

PLATE 4



The larvae in the cell increase in size and shed a delicate skin (excurvae) four times.

The larvae usually grow rapidly. The number of moults is difficult to determine because the cuticle is so delicate, but in various Apidae there are five. (Oliveira, 1960).

When the larvae have reached its full size no more food is supplied, but the worker bee sealed up the cell by means of a cover formed of pollen and wax (Fig. 4b) in such a manner as to pervious to air : sealed up in the cell the larvae moult into pupa (Fig. 4c) and thereafter bites its way out through the cover of the cell, and appears for the first time, as a new being in the form of a worker bee, the whole process of development from egg state to the perfect condition of the worker bee (Fig. 4d) takes about three weeks.

<u>Queens</u> - (Fig. 5) When the denizens of a hive are about to produce another queen, one or more royal cells are formed. These are much larger than the ordinary worker cells and of a quite different form. (Fig. 6). In this cell is placed an egg not differing in any respect from the egg that if placed in an ordinary cell produces a worker. When the egg has produced a larvae this is tended with great care and fed throughout its life with royal jelly secreted by the hypophanyngeal glands of workers (Free, 1961; Rosch, 1930). The queen is produced more rapidly than the workers, about

Fig. 4(c) A cross-section of the combs showing the developing workers larvae and pupae inside.

Fig. 4(d) Adult workers honeybee, _Apis cerana indica_ (Fab.)

PLATE 5

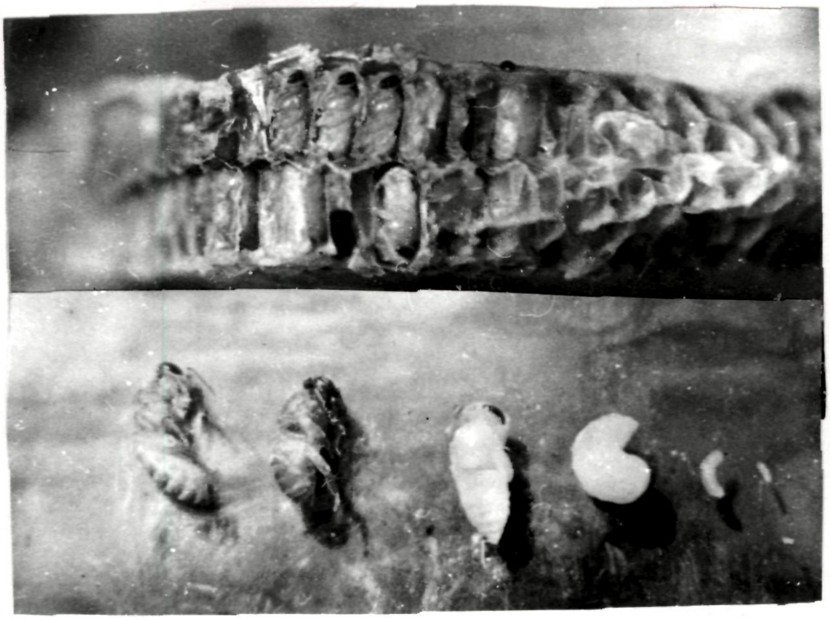


FIG. 5 DIAGRAMATIC REPRESENTATION OF THE COURSE OF DEVELOPMENT OF VIRGIN FEMALES (QUEENS)

A

↑

P – (Pharate adult)

|

P – (Pupal)

|

L_4 – (Spinning Larvae)

|

L_3

|

L_2

|

L_1

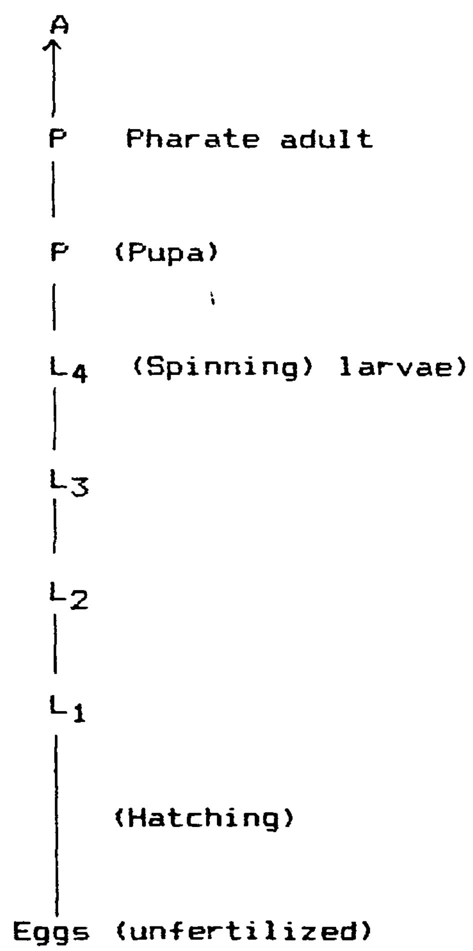
| Hatching

Eggs (Fertilized)

Fig. 6 A queen cell of the honeybee *Apis cerana indica* (Fab.).

PLATE 6

FIG. 7 DIAGRAMATIC REPRESENTATION OF THE COURSE OF DEVELOPMENT OF MALES (DRONES)



16 days being occupied in the process of development. Only one queen is normally allowed in a hive at a time. So that when several queen cells are formed, queen larvae nurtured in them; the first one that is developed into a perfect queen goes round and stings the royal nymphs to death while they are still in their cells.

Drones -(Fig. 7) The production of drones is supposed to depend chiefly on the nature of egg laid by the queen it being considered that an unfertilized egg is deposited for this purpose. The drone cells are somewhat larger than the ordinary worker cells, but this is probably not of much importance and it is said that the larvae intended to produce drones receive a greater proportion of pap (drone jelly), than worker larvae do. About 24 days are required to produce a drone from the egg.

From the above observations it can be concluded that the type of development of the honeybee, Apis cerana indica (Fab.) is ontogenic and holometabolous.

(A) DIVISION OF LABOUR AMONG WORKERS

A good point of social organisation among a colony of honeybees starts with the different behaviour patterns of the worker bees in its different acts of labour.

A new colony of bees in its selected home (artificial or natural) starts their life with the construction of combs. The combs of the bees, Apis cerana _indica_ (Fab.) are made up of wax; they hang vertically and have cells on both sides separated by a wall in the middle. They serve for rearing of the brood and for storage of honey and pollen. Within the area of nest, the queen bee lays her eggs in the central areas of the combs; only the pheripheral parts are used as containers of honey and pollen. The larger cells for rearing of drones are usually placed in the lower regions of the combs.

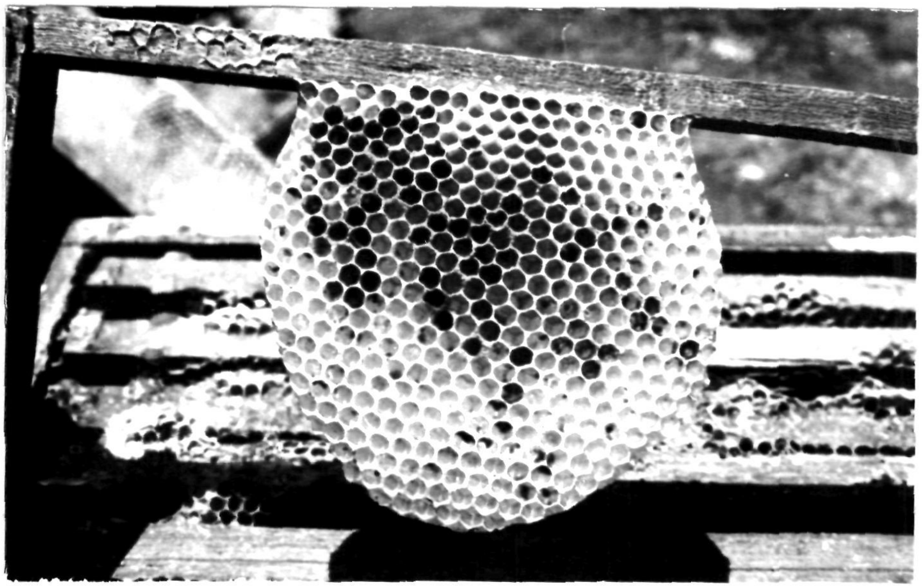
The walls of the main body of the cells which are connected form regular hexagonal prisms. The worker bees might build their cells with rounded walls as the bumble bees do or they build cradles (queen cells) of their queens. They base their architectural style on some other geometrical configuration. If the cells are round, octagonal or pentagonal, there are empty spaces between them (Fig. 8). This would compel the bees to build separate walls for all or part of each cell and entail a considerable amount of wax material.

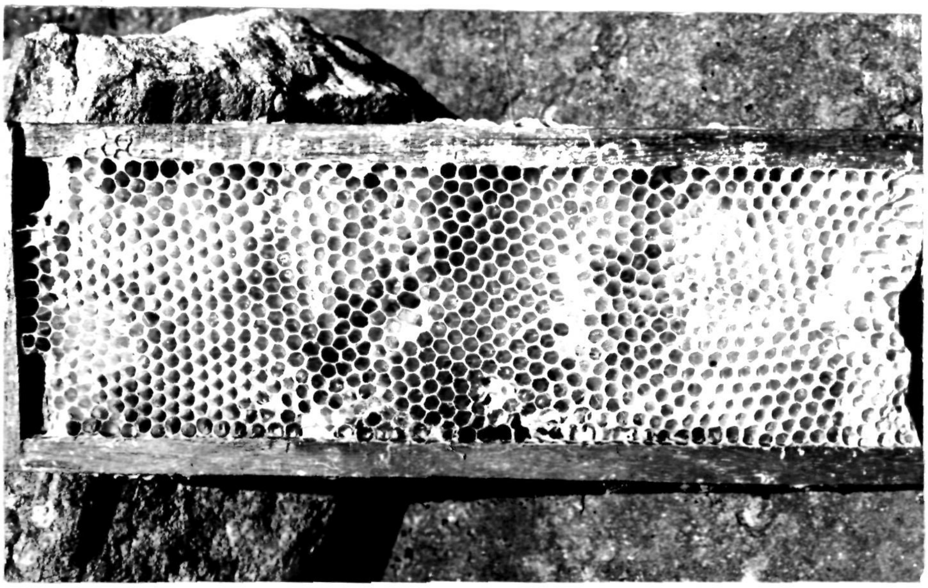
In most combs, one edge of the hexagonal prism points to the top, and another to the ground. occasionally combs are found in which there are horizontal walls at the top and bottom of the cells (Fig. 9). The geometry of the shape and depth of the cell bottoms and the manner in which they do into each contributes a great deal to the stability of combs.

Fig. 8 The development of the honeycomb of the honeybees, *Apis cerana indica* (Fab.) from a horizontal support.

Fig. 9 Horizontal walls at the top and bottom of the honeycombs of *Apis cerana indica* (Fab.).

PLATE 7





Construction of combs proceeds quite rapidly in the newly established colony; where the bees first attach themselves to each other in chains, soon they form themselves into a dense ball, the building cluster within which they maintain a temperature of 30°-34°C, the temperature needed for the secretion of wax. In bees, wax is formed only in glands on the under side of four abdominal segments, when these segments meet, wax appears in the shape of two small flakes between the ventral scales. To pick up a flake the bees use greater enlarged first tarsal joint of her hind leg, which carries on its inner side a small brush for the collection of pollen. The workers unpeel the little flake on the last two rows of bristles, pulls it out of its integument pocket and passes it forward to be taken over by her front legs and mandibles (Fig. 10). Holding it in front of her mouth the bee kneads and mixes it thoroughly with a secretion of her salivary glands. This treatment combined with the right temperature gives it the necessary homogeneous consistency and that degree of plasticity at which it can be moulded.

Building starts at the ceiling, which in a normal hive means at the top of the frame. Bees usually begin at two or three different places at once and construct sections that tapers downwards. They donot build one complex cell after another separately. While the lateral walls of the first cells are added to, new adjoining cells are being started lower down. As these triangular are enlarged laterally, they gradually coalesce from top down.

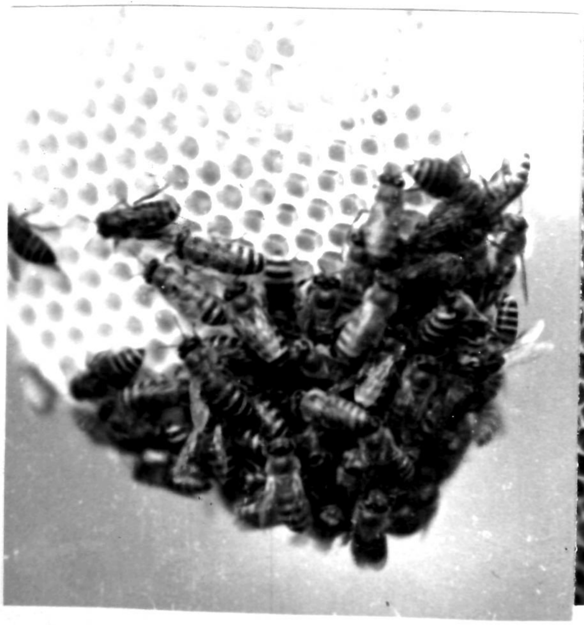
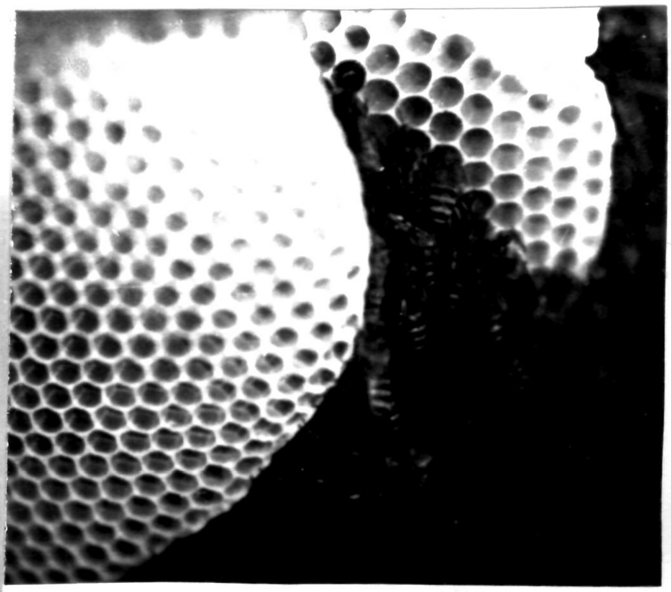
The joints are skillfully made that no traces of the separate beginning remain visible. This is more remarkable when one considers that many bees are employed in the building of each individual and that they often relieve each other at intervals of no more than half a minute or so. Apparently each bee immediately comprehends the stage of construction which has reached at the place where she starts to work and continues accordingly. When the pace is fast, the individual sections grows rapidly both vertically and laterally so that a contiguous building is reached in a small time (Fig. 11).

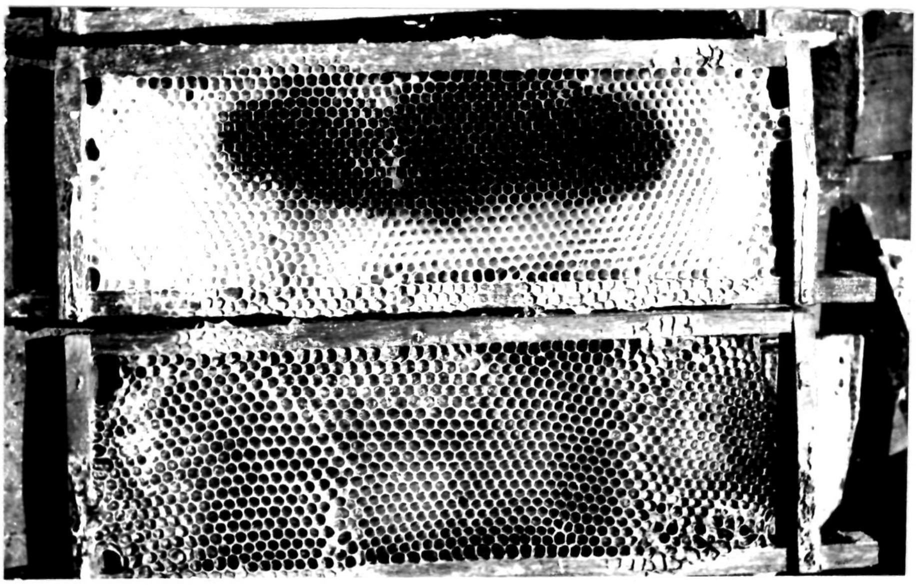
If artificial walls with stamped-on hexagonal patterns into the frames were put, they want their bees to fill with combs, the building programme partly speeds up because the bees need less wax. However, the cells built without such aids are no less regular. It has been observed from the beginning, that, the cells are built in the usual shape. Normally a rhomb-shaped section of the base is made first, followed by the first parts of two adjoining cell-walls. Next another rhomb is added and two more cell walls are started; thereafter the third rhombic section is erected which completes the hexagon, and the last two walls. From the start, the cell walls meet at the correct angle of 120°. Admittedly, the regular hexagonal shape is hidden at the top by a ring of wax which contains material for further use, but as soon as this bulky wax ring is carefully removed, it is noticed at once. This pattern

Fig. 10 Workers honeybees, *Apis cerana indica* (Fab.) in the process of building their combs.

Fig. 11 A completed frame of comb is shown, which is removed from one of the hive of the honeybee colony, *Apis cerana indica* (Fab.)

PLATE 8



seems to be imprinted in the minds of the bees by the influence of heredity. Sometimes, the worker bees even apply it as useless ornamentation to the outer surfaces of queen cells in the course of their modelling work. The hexagonal shape resembles the combs of the western honeybee (Apis mellifera) (D. Thompson, 1966; Von Frisch, 1973; Butler, 1967) and of the paper wasps.

The worker bees of A. cerana indica (Fab.) make not only the exact shape of the cells; they seem to have skill also to vary the size of the cells for workers and drones to manufacture such extraordinarily the walls, and to orient them accurately in space. These activities donot just "happen" but they are the result of work directed to a purpose.

The cell walls are built with a gradient of about 13° from base to opening, which is sufficient to prevent the honey from exuding out. The distance from the cell wall to that opposite is 5.2 mm in a worker cell, and 6.3 mm in a drone cell. The thickness of the cell walls is 0.073 mm, with a tolerance of about 0.002 mm. Parsimony in the use of building material seems to be taken to the utmost limit.

The storage cells for pollen are left open. Honey cells are closed with a wax lid when they are full. The wax necessary for this is kept ready for use as a thick ring around the lip of the cell, and can be rolled over the opening from all sides quickly, when it is required. The breeding cells, too, are covered with a domed lid of wax for the 15 days of pupation. Below the wax lid, the larvae themselves spin a dense cap of silk threads.

Within this two fold cover, the metamorphosis of larva into winged bee proceeds unmolested.

Foraging Behaviour

One of the note-worthy attributes of social bees is their ability to bring considerable amount of food to a nest from vast numbers of minute sources flowers. Gary (1976) has estimated upto 163,00 trips from a single colony of Apis Mellifera in one day. Free(1955) found that after 24 hours of starvation, workers of Bombus would drink amount of syrup averaging about 50 percent of their body weights, but sometimes attaining 90 percent and furthermore that they could fly with such loads in their crop. Large workers could fly with such loads in their crops than small ones. Pollen loads of Bombus average about 20 percent of the body weight but reaches 60 percent.

Almost the same figures have been obtained for nectar with Apis cerana indica (Fab.), the average load being about 50 percent, the maximum being 92-5 percent, of the body weight of a worker. For pollen loads, weight varies greatly for pollen of different plants, probably with density, from 10.5 percent to over 36 percent of the workers weight, these figures being means for heather and maple.

Foraging and Orientation

The food of bees is nectar and pollen. The highly eusocial bees, as well as bumble bees, convert the former into honey for storage, through invasion of the sugar and partial dehydration. Many Apidae use much food of glandular origin but in such cases the ultimate raw food resources are exclusively nectar and pollen.

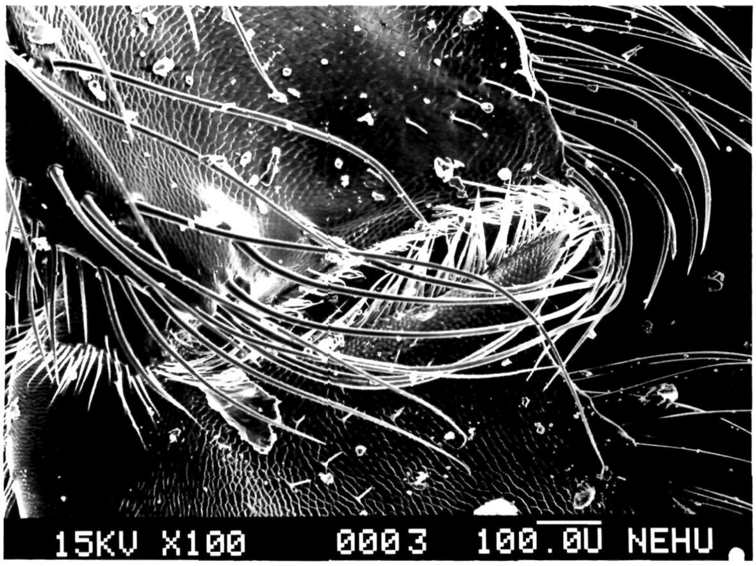
Bees obtain food by flying to suitable flowers specially orchids where they collect nectar. Female bees making nest or working in them take excess quantity of these foods beyond the immediate needs of the individual. These excess are used to provision cells or to feed larvae or are stored for future use. Female bees must have appropriate harvesting and carrying equipment for both nectar and pollen; as well as often small and hidden nest entrance. The structures used in having harvesting and carrying foods are illustrated (Fig. 12).

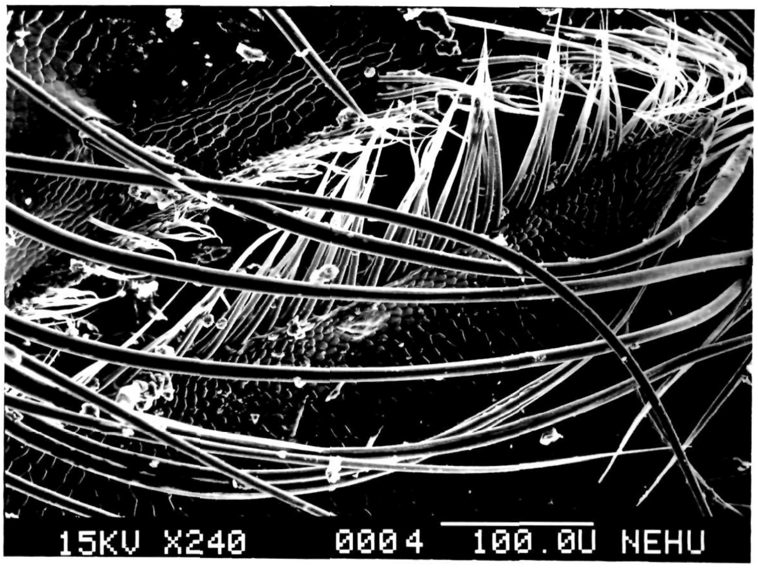
This chapter has been prepared to give a substantial amount of information about how individual bees get about in the environment outside their nests and find food. Such bees can be at any social level. Success in their foraging flights, of course, is ultimately related to pollen respectively. Probably, the size of the pollen, quantity rather than its weight, stops a bee from collecting and stimulates it to return home.

Fig. 12(a) Scanning Electron Micrograph (SEM) picture of the pollen basket of honeybee workers *Apis cerena indica* (Fab.) x 100

Fig. 12(b) SEM picture of the pollen basket at a higher magnification showing the corbicula x 240

PLATE 9





The size of the nectar loads of Apis cerana indica (Fab.) and probably other bees, like Apis florea and Apis mellifera is influenced by various factors. Nunez (1966, 1970) in a study using sugar syrup found that the size of the load increased with sugar concentration and with air temperature. Moreover, when the flow of syrup to the feeder is slow, the bees takes smaller loads, took longer to collect them, spent more time in the hive between trips, and abandoned the effort at a flow varying inversely with sugar concentration. Similar results have been obtained by Pflumm (1969). Most studies of these variables have dealt with sugar concentration because it is easily controlled in experiments.

The number of flowers which a bee visits to get a quantity of nectar or of pollen varies greatly accordingly to the kind of flower, time of day, soil moisture etc.

Observations showed that workers of Apis cerana indica (Fab.) seems to prefer nectar gathering. Changes from pollen gathering to nectar gathering are much common than the reverse. There is no ontogenetic sequence in types of foraging such as occurring for their extensive foraging hives, others only nectar. Most foragers however undertake both, simultaneously or in sequence. The mixture takes many form, even among bees in the same colony; at the same time. Some collect both pollen and nectar on each trip. Some individuals observed by Ribbands (1953) collected pollen each morning from poppy flowers that produced little nectar, but later each day visited other flowers, where they collected nectar only, or both nectar and pollen. Still other individuals collect nothing but pollen for days, then change to nectar, less commonly the reverse change occurs. The individual differences are related to conditions in the hive to different experiences of the individuals, and, among the various studies to differing outside environmental conditions.

Thermo-regulation Within the Hives

One of the principal attributes of social behaviour is the ability of honeybee colonies of organism to control the conditions under which they live better than related solitary organisms. Such social homeostasis involves virtually all of the functions of the colony, including food supply and defence against natural enemies as well as control of the physical conditions within the nest is remarkable among bees.

Emerson (1950) has argued that evolutionary progress generally consists of or can be described as improved homeostasis. Of course, it is easy to argue that social progress involves improved social homeostasis, for there is a direct relationship, on the average between colonial size, complexity of colonial integration, and homeostatic control of conditions in the nest. At least, *Apis* sps. has reached the stage at which, while it is individually poikilothermic, it is socially meaning as homeothermic as are birds and mammals, and successful brood rearing is dependent upon this homeothermy.

By contrast the allodopine bees, including eggs, larvae and pupae are peculiarily resistant to fluctuation in temperature and humidity; their nest dried often in the sun, must be characterize by remarkable seasonal and diurnal fluctuations in these factors. However, some species of the same group, are probably less tolerant, their nest only in the shade (Michener, 1970) Allodopine bees have evolved to greatly increase their tolerance to temperature and probably to humidity fluctuations. Over these factors their social homeostatic control is minimal.

In Apis cerana indica (Fab.), its ordinary nest sites, the common honeybees combs are not covered by blankets of bees like those of Apis dorsata. The same exposed combs may be covered by similar protective blankets. Moreover, the bees that cover brood areas and the outer layer of bees around a swarm seem comparable to the blankets of A. dorsata and A. florea. For ordinary colonies of Apis mellifera, the walls of the nest cavity serve the protective function of the blanket.

Colonies of the common honeybee maintain the temperature of the brood near 35°C irrespective of the outside temperature (Ribbands, 1953; Steiner, 1967). Brood survives from about 32°-36°C and the brood rearing area is always kept within this range, but usually between 34.5°

and 35.5°C, just below human body temperature (Lindauer,1961).

Cooling

Cooling is accomplished by fanning, which brings in outside air not warmed by metabolic activity and which also causes air circulation which increases the evaporation rate from most surfaces - the bees themselves, their larvae, uncapped honey, etc. At times of extreme heat Apis cerana indica (Fab.) brings water in quantities from outside the nest. This occurs when the outside air approaches the optimum brood temperature, say 34°C. The water is placed in drops within brood cells, or elsewhere in the nest. At the same time, many workers in the brood area repeatedly expose films of water by probosci's movements, producing large water surfaces for evaporation. These movements are the same as those that are used; also for evaporation (Fig.13).

(B) BEHAVIOUR OF THE QUEEN

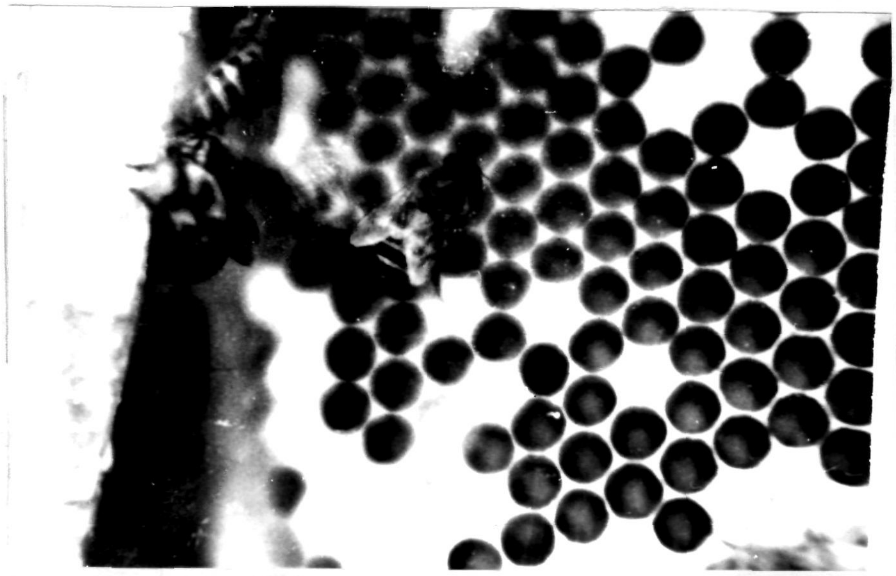
The queen is always considered to be the essential element in every honeybee colony. Without a mated queen a colony dwindles away as its members die of old age with no replacement.

Few simple experiments which were conducted in Cherrapunji (Mawsmai), have proved successful regarding the essentiality of a queen in a colony.

In the beginning the queen was removed from the colony and thus it was observed that the state of the

Fig. 13. Worker honeybees, _Apis cerana indica_ (Fab.) bringing in drops of water and placing them in brood cells and nest surfaces for cooling

PLATE 10



colony changes from an organised to a disorganised state of restlessness. Some of the bees began to run excitedly here and there, and mostly around the entrance of their hive, others began to stand near the exit and to expose their scent glands - thus stimulating other bees along its line of flow to behave in the same way.

The queen which was removed and kept in seclusion also behaved almost in the same way as if she missed her multitude, she crawls up and down, searching for the exit. After one hour or so, the queen was later returned to her hive, and the bees nearest to the queen and those having first contact with her stops their abnormal behaviour and instead began to examine her, grooms and feeds her, while others expose their scent gland and drive currents of air over them with swiftly fanning wings.

In the second experiment after another week or so as we have reared two colonies nearby, we tried to isolate the queen from one of the colony and later introduced a strange queen to the queenless colony; i.e. a queen from another colony. The bee's behaviour was again observed to be in a restless condition, some bees licked her after examining her, but this did not effect the colony and instead after sometime we observed that they were stinging her instead, the term known as "balling". The queen was taken away and their queen (original) were introduced again. A behaviour which was mentioned earlier again was observed, the acceptance of the queen.

In this particular observation the reason of non-acceptance could be that she (queen) even though a mated queen; is still quite young compared to their own queen, thus the "balling" behaviour.

In the above observations one can simply conclude that the age of the queen alters the behaviour of her associates. But this is not true in several other cases; where even though the same age queen was introduced yet some abnormalcy is still observed.

All the above observations focussed in what several workers termed as the "Queen Substance". As the honeybees's social organisation has evolved, they have developed the practise of caring to different castes. And it was observed that the proportions of bees in "courts" that examined and licked their queen, in contrast to merely examining them with their antennae and that feed other bees shortly after leaving a queen, were abnormally high. This shows that the presence of the queen in the colony is very essential. The manner as to how the queen makes known of her presence in the colony has been a controversial subject for many years. This "Queen Substance" also known as "Pheromone", the secretion of which in case of bees is a mixture of chemicals and its action is varied apart from communication and colony cohesion. It acts olfactorily both as an attractant for drones in mating, for aggregation of workers in a swarm; also orally it acts to inhibit ovarian development in worker bees and queen cell production (Butler, 1954).

In one of the colony in the same place (Cherrapunji) apart from our reared colony it was observed that due to the death of a queen there is [redacted] production of emergency gyne cells. It is learned that as the inhibitory substance which is suppose to come from the queen has stopped thus this enabled the workers to replace their queen, by building gyne cells for the production of a new queen.

Various authors independently (Butler, 1954; Pain, 1955; Voogd, 1955) have postulated a substance or substances on the queen's body that inhibit gyne cell construction and ovarian development of workers. Actually all three, in Britain, France and Holland, published their first notices in 1954. As summarized by Allen (1965) and Gary (1970), these, and other authors showed that extracts of queen whether presented in drinking water or on various objects (pith, dead workers, and the like) had an inhibitory effect similar to that of the queen herself. Even the daily addition of six workers that had recently licked a queen to a queenless group of 60 workers reduced ovarian development in that group (Butler, 1959). This was also observed in our colony in Umroi, where there were only around 100-200 workers, the queen before removing from the colony, her head was rubbed in most of the workers, by picking around 6-7 workers for almost 2 weeks or so, (i.e. letting the workers lick the queen and release them back in the colony). For so long the colony behaved as a queen-right colony. Verheijen-Voogd (1959) and Butler and Simpson (1958) showed that the principal source was the queen's head, and more specifically the mandibular glands and their reservoirs. This inhibiting substance was isolated and identified as trans-9-keto-2-decenoic acid, synthesized, and the inhibitory effect of synthetic material tested (Butler, Callow and Johnston 1961; Barbier and Lederer, 1960; Barbier, Lederer and Nomura, 1960).

This material is relatively non-volatile, although sufficiently so to be an attractant for drones in the field high in the air (Gary, 1962). In the nest however, it is seemingly not attractive to drones or workers. The same material is available in similar quantities in the heads of queens of Apis cerana indica (Fab.), A. mellifera, and A. dorsata - (Shearer, Boch, Morse and Laigo, 1970).

Although produced in the mandibular glands, it appears to become distributed over the body of the queen by her grooming (Velthius, 1970). According to Butler and his co-workers, it is then licked from her body by the court of workers that always surrounds her. Velthius (1972); however gives good reason to believe that it is mostly not licked from the queen but adheres to the bodies of workers that contact her. The queen is continually moving on the combs; and different bees from different parts of the nest form the courts and either feed or lick the queen or both, as well as contacting her with their antennae, legs etc.

Even in a small colony, it would not account for the very prompt response to loss of queen. Therefore, it has been postulated that worker bees that obtain "Queen Substance" from the queen's body pass it on to others via their food exchange, and that this process continues until every bee has a more or less uniform amount of the material.

Such dispersal of a non-volatile phenomena is consistent with the finding that more phenomena is needed to inhibit a large number of workers than a small number (Butler, 1954)l as would hardly be true for action of an odour. Moreover, this kind of dispersal seems reasonable in view of our knowledge of the extensive food transfer in an Apis colony. Kaissling and Renner (1968), have identified olfactory receptor cells in the antennae that are specialized for perception of trans-9-Keto-2-decenoic acid. Velthuis (1972), in observation of the responses of queenless groups of workers to workers that had been with a queen, obtained behavioural support for anntenal perception of trans-9-Keto-2-decenoic acid.

With all the tremendous responsibility of a single queen, to maintain the social organization of her colony, heading her colony for 2-5 years in certain cases even to 8 years (as was noted in Cherrapunji); she also has the capability of laying as many as 6,00,000 eggs (more or less) during this period. Thus the queen bee is worth being named a queen.

(C) BRHAVIOUR OF THE DRONE

Nothing much has been studied regarding the drones life by any worker so long. It is responsible for mating the queen, and dies off after the process as its sperm sac is being torn from its body.

Drone cells are usually built by the workers in mid-spring where the climate is getting warmer, and the colony is ready to be active and a virgin queen if present is ready for mating. Their maximum appearance being in summer from April-September or lesser. Again in the autumn, the workers of all queen-right colonies refuse to either feed or to allow them to the stores of honey. Soon the starving drones are pulled outside by the workers and left to die. Later it is also seen that those workers responsible for cleaning the hive entrance, carry them off and throw them. This, further shows, that the drones are very much dependent on the workers for being fed. Thus, for the workers, to get rid of them, they let them starve. This is referred to as "Drone Massacre".

CHAPTER II

EXTERNAL MORPHOLOGY AND ULTRA STRUCTURE OF THE DIFFERENT CASTES OF HONEYBEE

INTRODUCTION

Bees partake of all the ordinary morphological and physiological features of insects. Detailed account of morphology and physiology of the honeybees have been written by Snodgrass (1910, 1925, 1956); Zander , (1951). External or skeletal morphology of certain other species of bees has been treated in some details by several other workers.

The body of adult bees of all the castes presents a jointed structure which is termed segmentation, and is divided into a series of successive rings variously known as segments, somites or metameres.

The organisation of the body into metameres each bearing a pair of appendages presents the arthropods with a remarkable opportunity to take advantage of any available ecologic situation. At first all appendages probably were used for walking, but with legs to spare, some could be assigned other functions without interfering with the animal's locomotion. The legs of some insects play an important role in grooming behaviour (Trembrock, 1982).

The cuticle which is the exoskeleton of the insects also exhibit localized areas of hardening which are sometimes delimited by sutures. Typical appendages are segmented tubes invested with a dense cuticle. Between each pair of segments the cuticle remains membranous and becomes infolded to form the articular membrane. On account of its jointed structures, the whole or part of an appendage is movable by means of its muscles.

In addition to true segmental appendages numerous other out growths of the body wall are found in bees (Apis); like wings which are always confined to the meso- and meta-thorax and attain their full development in adult insects.

The body segments of an insect are grouped together to form three usually defined regions - the head, thorax and abdomen. In each of these regions certain of the primary functions of the organisms are concentrated. The head carries the mouth parts which are concerned with feeding and other organs of special sense, like the antennae, eyes, etc. The thorax bears the locomotor organs, i.e. legs and wings.

In most insects there are three pairs of legs and their functions are usually/primarily organs for running or walking and are well represented in their normal condition. They exhibit, however, a wide range of adaptive modifications in different families. Each leg consists of the following parts - coxa, trochanter, femur, tibia and tarsus together with certain basal or articular sclerites and a terminal pretarsus.

Arthropod appendages are hollow evaginations from the ventral part of the lateral body wall. They are divided into sclerotized segments separated by flexible joints.

Certain functions in addition to locomotion sometimes are performed by legs, functions that require special structures or special adaptations of existing structures. Among the Hymenopterans, the forelegs are frequently used as antennal cleaners, with structural modification of the tarsal surface, leg hairs and tibial spurs for this purpose; (Snodgrass, 1956).

Bees transport pollen to their hives by means of special equipment on their hindlegs. The pollen basket (corbicula) is formed on the dilated hind tibia by a smooth area, ringed by high, curved setae. The enlarged first tarsal sub-segment of each hindlegs has a row of stiff setae serving as the pollen comb (scopa). Pollen adheres to the hairy bodies and legs of bees. It is transferred to the pollen basket on one side by use of the comb of the other side.

Regarding the antennae there was a controversy whether they are true appendages or not. It was Snodgrass (1935) who showed that the antennae are not segmental appendages as they have not arisen from a segment but from the acron.

The antennae are a pair of very mobile appendages which are articulated with the head in front of the eyes. In case of bees the antenna is divisible into scape, pedicel and flagellum, where the scape is the first or basal segment of the antenna and is often conspicuously larger than any of the succeeding segments. The pedicel is the segment which immediately follows the scape, and the flagellum forms the remainder of the antenna.

In case of honeybees the mouth is that of sucking and chewing type, as bees (Hymenoptera) feed on flower nectar and accordingly have developed an extensible proboscis. The tongue of the bee is a highly complicated organ consisting of two portions; the tongue proper to suck up the smaller particles in the bottom of the flowers and the others a combination of the mouth parts folded up so that the coarser part of the food can be drawn into the honey sac.

The insect maxilla is to be regarded as the highly modified derivative of a walking limb, whose main shaft is represented by the palpus and base by the cardo and stipes. Hansen (1930) and also Crampton (1925) claim that a reduced third element, the palpifer, enters into the formation of a limb base. On this interpretation the galea and lacinea are masticatory lobes, or endites of the palpifer and stipes respectively. Börner (1921) and Snodgrass (1928) regard the palpifer as a secondarily demarcated portion of the stipes and of little morphlogical importance. On Snodgrass's theory the galea and lacinea are subdivision of a single endite of the stipes.

The presence of wings is one of the most characteristic features of insects, and the dominance of the latter as a class is to be attributed to the possession of these organs.

Although, in the greater number of insects the wings appear to be naked, in many cases microscopical examination reveals the presence of fine hairs.

Among other orders, the Hymenoptera (Apis), have a hamulate type of wing coupling in which a row of small hooks (hamuli) on part of the coastal margin of the hindwing catch in a sclerotized fold along the hind margin of the forewing.

The stinger is another interesting structure in the honeybee, Apis; it is similar to the ovipositor on other Hymenopterous insects; but here it has been modified to inject venom. The sting is made up of three separate pieces. Apically the sting is visible as a slender lancet. And it is only in the female caste that it is functional.

So in this way, each individual contributes its share to the success of the colony, by the evolvement and specialization of some of its unique structure.

MATERIAL AND METHODS

A colony of bees was raised one each in Shillong, Cherrapunji and Umroi. All these three colonies were kept without any disruption or barriers for their in and out flights in attending to all sorts of labour the different castes has to undergo.

The bees for study were taken at random, kept in a specimen tube and to maintain their freshness till needed they are placed inside the refrigerator of moderate temperature.

Secondly, for Scanning Electron Microscopical (SEM) study the insect is taken, cleaned with a brush and the different appendages, wings and head were detached from the body of the insect separately and kept aside. Later the organs to be observed were secured horizontally on the brass stub (10 mm x 32 mm) with Dotite electroconductive paint. They are then coated with gold in fine coat ion sputter JFC-1100 and later they were inserted in the SEM(JOEL) and viewed.

RESULTS AND DISCUSSION

Studying the morphology of the honeybees (Apis cerana indica) (Fab.) in the optical microscopes, did not give any satisfactory results due to some drawbacks like low resolving power and depth of field. Whereas the scanning electro-micrographs, not only show us a well defined shape of the different organs/appendages of the different castes of the honeybees, but also more interesting ultra structure on the body of the insect - the sense organs or the receptors. In the optical microscopes these receptors can only be detected like small hairs but in the Scanning Electron Microscope (SEM) it can be noted that they are of different size and shape, and each type has got a specific name and function. Also in SEM study these different organs do not have to be processed before viewing as, insect cuticular parts are sclerotized structures. They can withstand the vacuum of the microscope because of their structural rigidity. (Wooley and Vossbrinck, 1977).

It has been reported repeatedly through natural selection and evolution, that the honeybees (Apis) have developed into an efficient, highly specialized creatures.

Very conspicuous and unique to bees are body hairs with short side branches. While their functions are not completely understood, they are connected to nerves, undoubtedly serving as mechanical or tactile receptors, since the exoskeleton is rather insensitive to touch. These hairs have varied functions. Some serve as organs of balance so that the bees are able to know their position in relation to gravity. Old worker bees often lose many of their hairs, but whether this affects their performance is still unknown.

EYES - Like many other insects, honeybees have large compound eyes. Each is composed of hundreds of small visual units called Ommatidia; with their own lens and light sensitive tissues. Vision through a compound eye produces a mosaic. The Ommatidium directly in line with the object is clearly seen, but those on edges less distinctly.

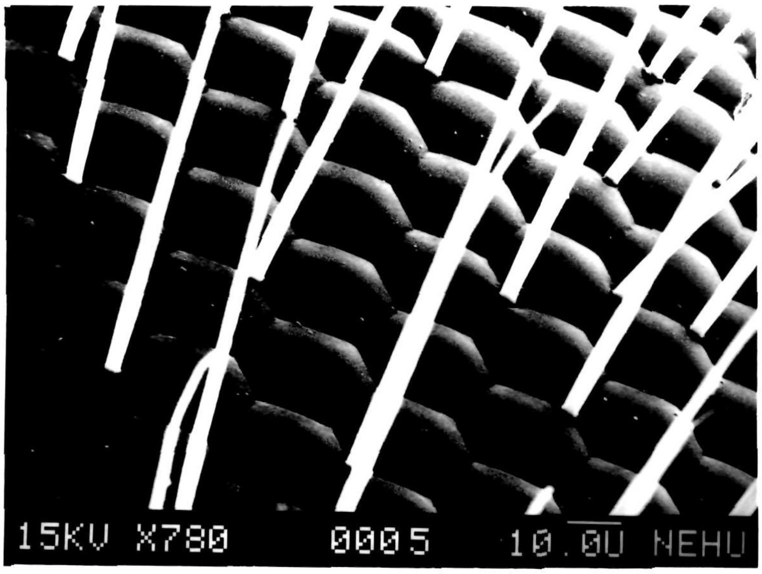
Bees also have three ocelli. Althought their function is not fully understood it is believed that they are sensitive to changes in light intensity and other activities.

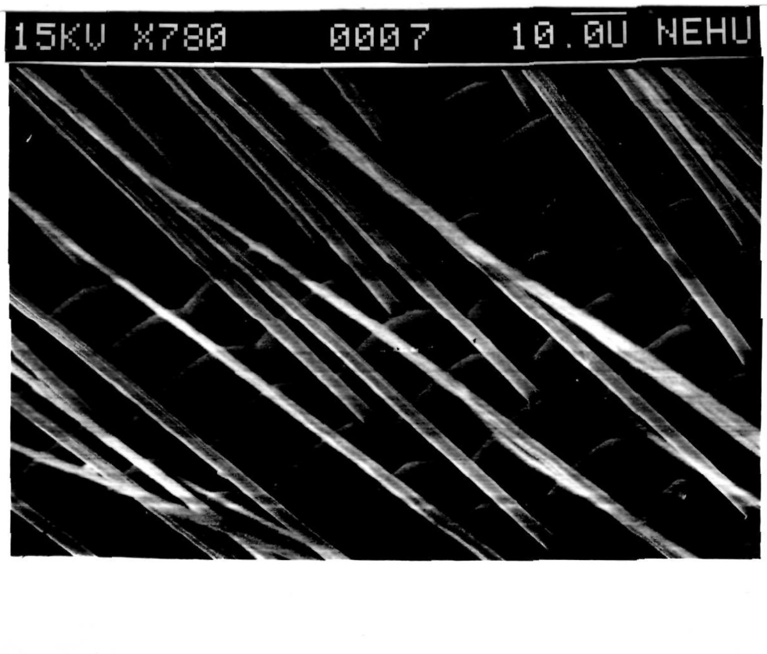
Electron micrographs of the rhabdomeres (Fernandez-Moran, 1958; Goldsmith 1962) show that each is packed with microvilli, finger - like invagination of the cell membrane approximately 400°A wide. The rhabdomeres appear to be fused into pairs so that each rhabdom actually consists of only four distinct morphological units. Within each unit the microvilli are parallel to each other and aligned in such a way that the central ones pass radially out from the longitudinal axis of the rhabdom. As a result the microvilli of each pair of rhabdomeres are parallel to the

Fig. 14 SEM photograph of the eye of worker honeybee, *Apis cerana indica* (Fab.) x 780,

Fig. 15 SEM photograph of the eye of drone honeybee, *Apis cerana indica* (Fab.) x 780.

PLATE 11





microvilli of the pair opposite them and at right angles to the microvilli of the two adjacent pairs (Fig. 14 and 15). Goldsmith (1958) reported the existence of retinal in the honeybee eye, but its exact location has not been ascertained. (Goldsmith and Fernandez, 1966).

ANTENNAE - The antennae of honeybees are important sensory organs which are covered with minute sensory receptacles. Even though it is quite difficult to determine the exact function of each type or variety, undoubtedly these receptacles respond to such common stimuli as odour, taste, touch etc. A whole picture of the antenna of the worker bee of Apis cerana indica (Fab.) is shown in Fig. 16. The antenna as mentioned is made up of the scape, the pedicel and the flagellum. The different sensillae/receptors detected in the antenna will be dealt in detail in the following chapter.

MOUTH - (Fig. 17 and 18) The mouth-parts as mentioned earlier are a combination of the chewing/biting and sucking types the labrum and mandibles being attributable to the former and labium to the latter. The mandibles are the principal tools used in nest making, and thus are of major social significance - e.g. to manipulate wax. Mandibles are also used for cutting open corollas to get at nectar in flowers, too narrow and deep to be robbed without such mutilation, for gnawing pollen out of cells, also as gras-

Fig. 16 Scanning Electron Micrograph of the antenna of the worker *Apis cerana indica* showing the scape, the pedicel and the flagellum x 48

PLATE 12

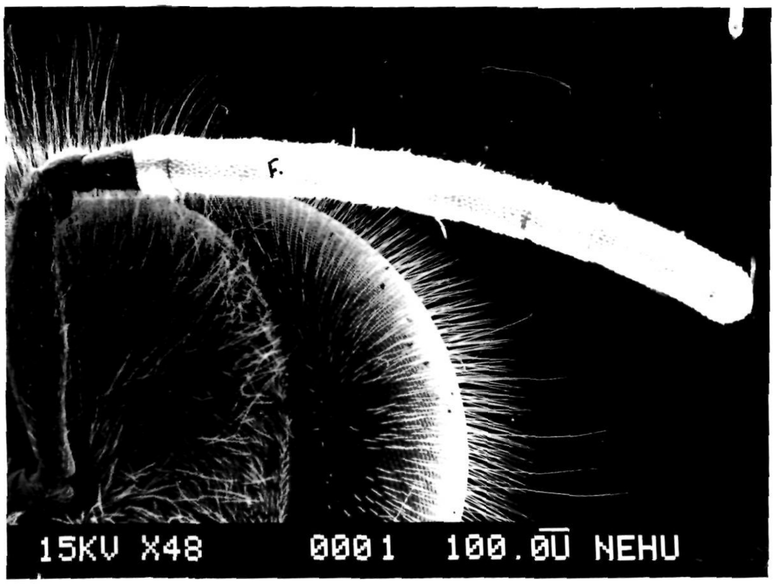
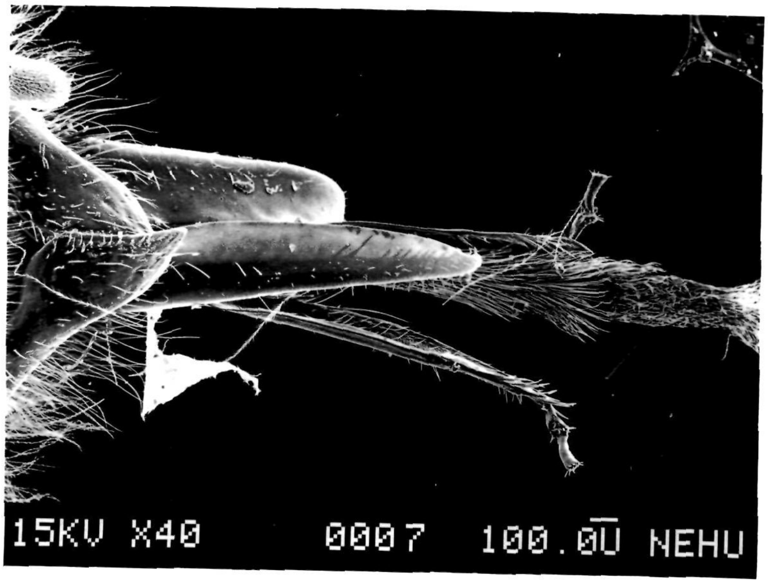
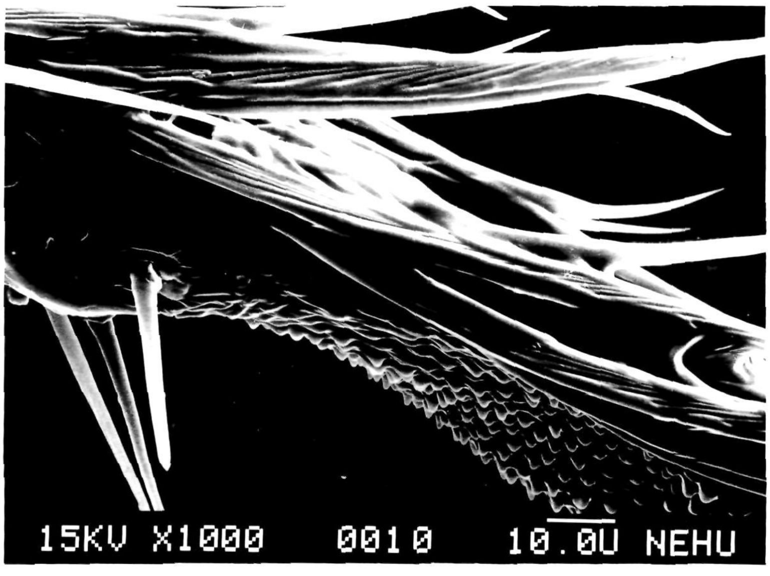


Fig. 17 SEM picture of the mouth parts of the worker honeybee, *Apis cerana indica* (Fab.) x 40

Fig. 18 A magnified SEM picture of the labial palps of the worker honeybee, *Apis cerana indica* (Fab.) x 1000

PLATE 13





ping instruments for general house-keeping chores and such as cleaning its colony.

Bees are rather unique in having both chewing and sucking mouth parts. The maxillae and labium together form the proboscis, (Fig. 19 and 20); which is a retractable tube through which they can ingest nectar and water. When not in use, it retracts automatically by curving backwards. Honeybees are not able to eat coarse solid materials, unless like sugar, which can only be dissolved and actually ingested as liquids. Bees also use their proboscis for a number of activities other than sucking liquids, such as food exchange among workers, "licking" of substances such as pheromones from one another's body, and spreading and smoothing the cell linings. The function of the proboscis in case of Apis has also been studied in detail by Snodgrass (1956).

In case of the drones the mandibles are smaller and differ slightly in shape from that of a queen or worker.

LEGS - Honeybee legs are divided into six principal parts or segments attached to each other by flexible joints. The names given to the different segments have already been mentioned in the introduction. The last segment of each leg is further subdivided into five parts, the basi-tarsus, which is the longest and sometimes called the planta. While the joints are flexible they can move only in one plane.

THE FORELEGS - The forelegs/first pair of appendages of the honeybee (Apis cerana indica Fab.) have something in common with all other insects, the antennal cleaner (Fig. 21). This structure is seen in both the female castes, the queen and the workers, in between the tibia and the tarsus; on the inner margin, equipped with a deep notch and attached with innumerable hairs as a row of small spines. These hairs are spoon-like in structure (Fig. 22). When the antenna becomes covered with pollen, the bee merely pulls it through this notch for cleaning. In addition the bee can also remove pollen and debris from her head, eyes and mouth parts with the help of its fore-leg.

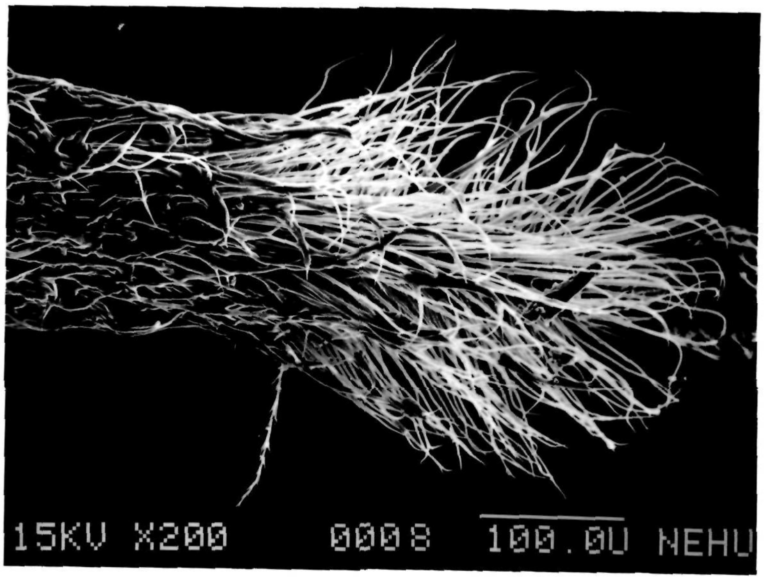
An electromicrograph picture shows that the antennal cleaner of the worker bee is more dense and longer hairs can be seen when compared to the queen or that of the drone which is negligible. This is because neither of the two does any foraging, (Fig. 23).

The tip of the fore-leg of the male caste (Drone) is lotus-shape, (Fig. 24 and 25). An enlargement of one of the lobe (Fig. 26) shows an irregular cuticular pattern and very few sharp pointed hairs, the trichoid sensilla; being responsible for the sense of touch. This could help the drone in its mating behaviour as it has to grasp the queen. In the segment after the tip (tibia) (Fig. 27) there are a number of branched sensillae, their function of which is still unknown. The dorso-lateral part of the femur also show innumerable branched hairs (Fig. 28) whereas the

Fig. 19 A SEM picture of the proboscis of the worker honeybee, _Apis cerana indica_ (Fab.) x 200

Fig. 20 A magnified SEM picture of a portion of the proboscis of the worker honeybee _Apis cerana indica_ (Fab.) x 440

PLATE 14



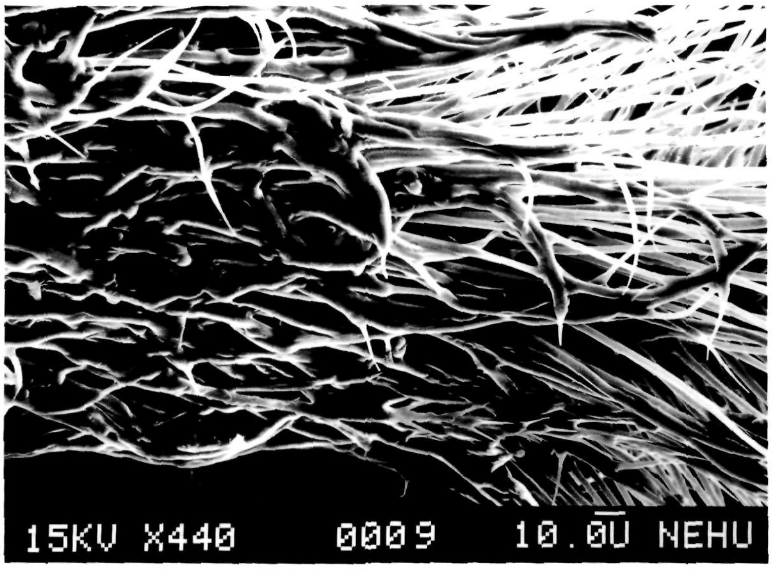
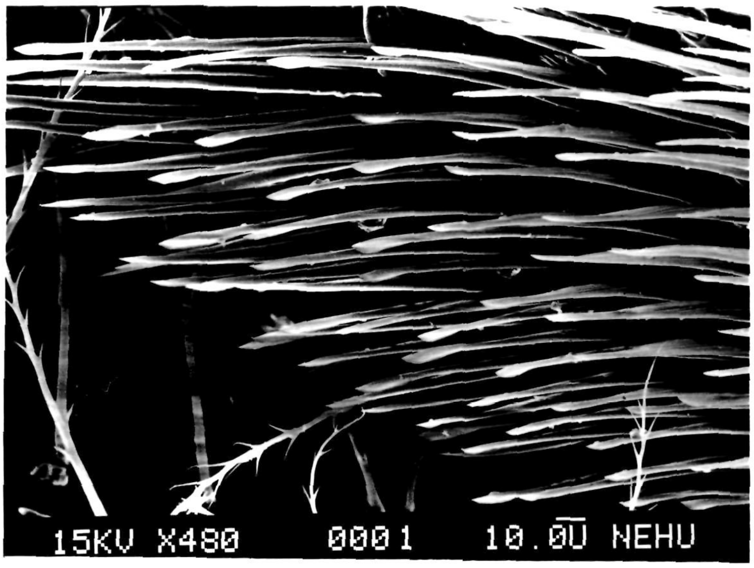


Fig. 21 Scanning Electron Micrograph of the antennal cleaners on the fore-leg of the worker honeybee, *Apis cerana indica* (Fab.) x 480

Fig. 22 A highly magnified SEM picture of the antennal cleaner of the worker honeybee, *Apis cerana indica* (Fab.) showing a spoon-shaped structure x 5400

PLATE 15



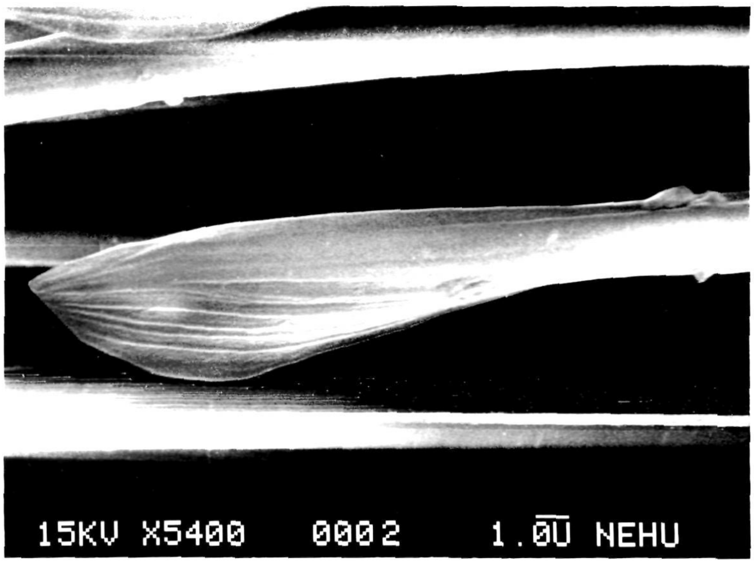


Fig. 23 SEM picture of the antennal cleaner of the queen honeybee, *Apis cerana indica* (Fab.)

PLATE 16

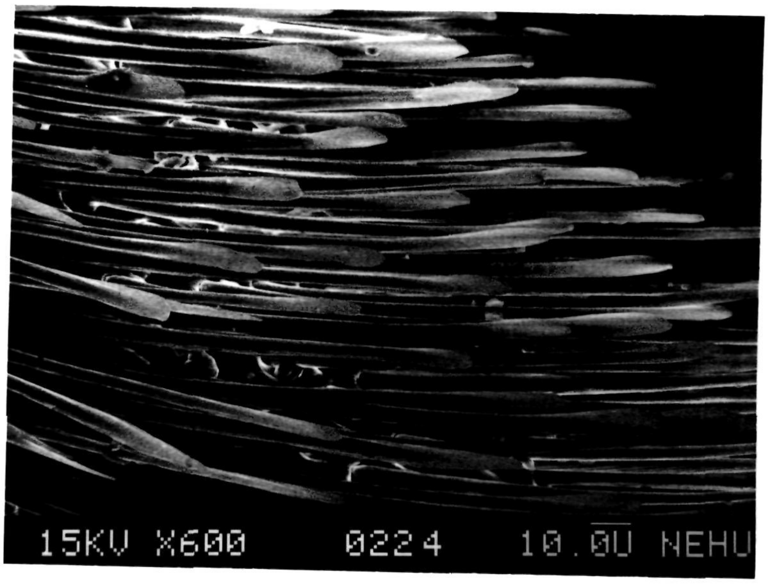
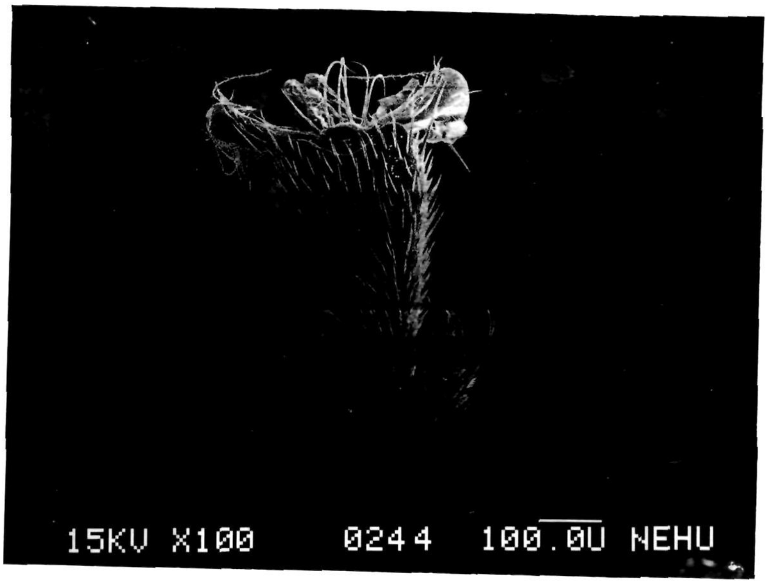


Fig. 24 SEM picture of the bifurcated of the tarsus of the drone honeybee's Apis cerana indica (Fab.) fore leg x 100

Fig. 25 A magnified SEM picture of the Fig. 24 x 260

PLATE 17



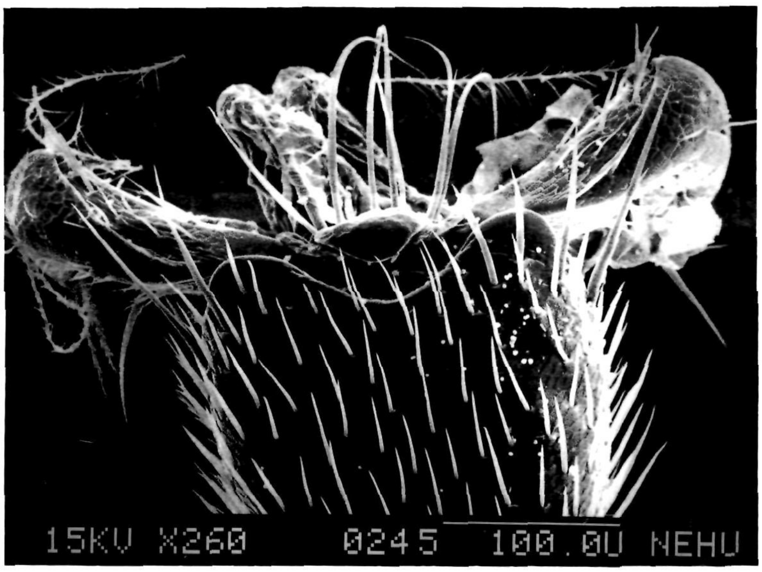
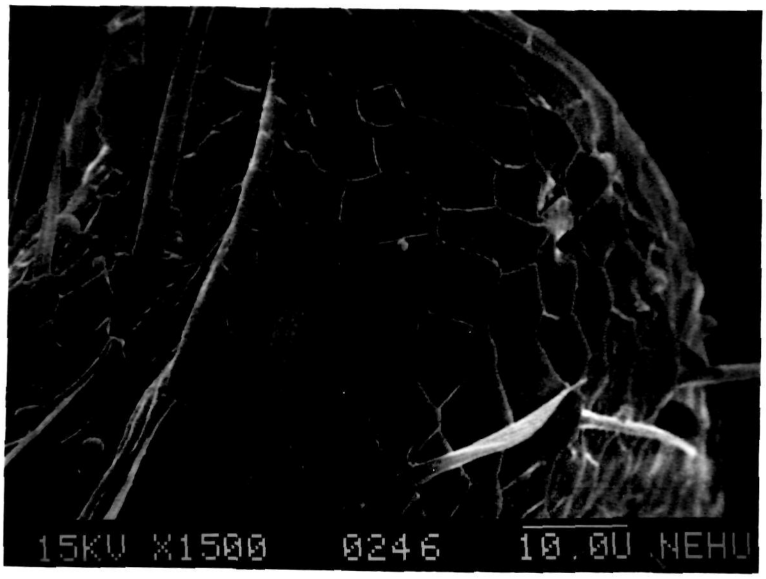


Fig. 26 A magnified SEM picture of one of the lobe of the fore leg tarsus of the drone honeybee *Apis cerana indica* (Fab.) showing irregular cuticular pattern x 1500.

Fig. 27 SEM picture of the tibia of the fore leg of drone honeybee *Apis cerana indica* (Fab.) x 160.

PLATE 18



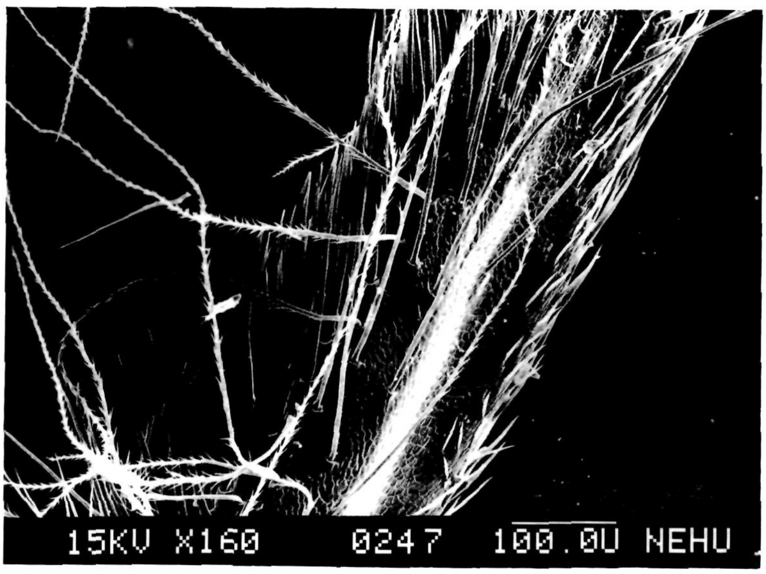
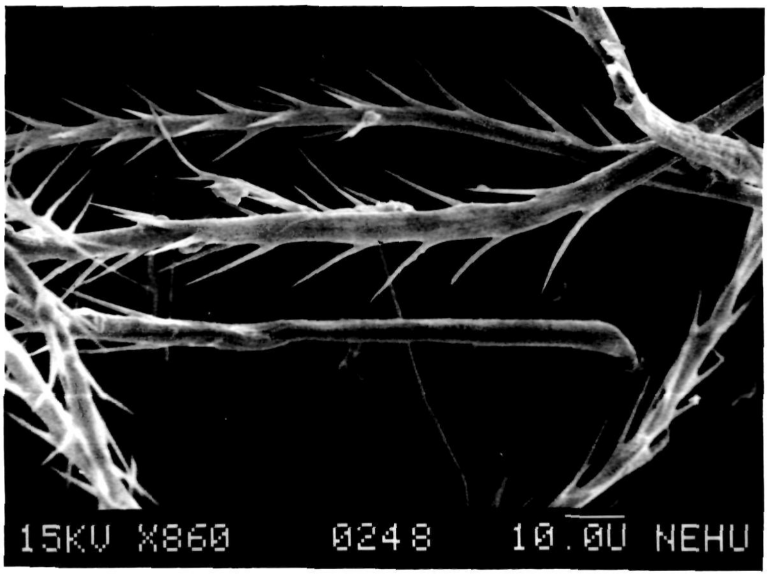
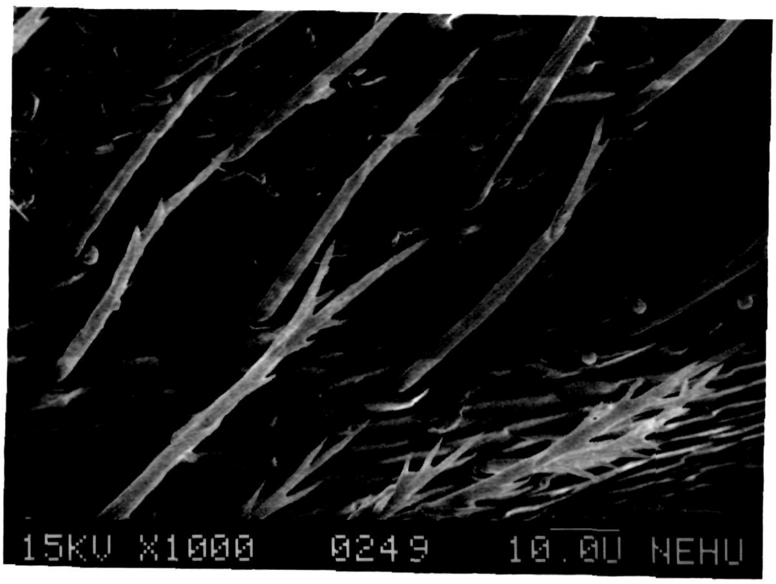


Fig. 28 SEM picture of the branched hairy sensilla on the femur of the fore-leg of the drone honeybee, Apis cerana indica (Fab.) X 860.

Fig. 29 SEM picture of the trochanter of the fore leg of drone honeybee, Apis cerana indica (Fab.) showing striped cuticular pattern with some hairs slightly branched x 1000.

PLATE 19





trochanter shows striped cuticular pattern with some sensilla slightly branched. (Fig. 29)

In the fore-leg of the queen, it is seen that the tip is slightly curve with a number of sharp pointed trichoid sensilla (Fig. 30). A part of the cuticular sculpture near/adjacent to the tip of the fore-leg is also shown (Fig.31 and 32). Apart from what was mentioned above, there are no other special features worth mentioning here.

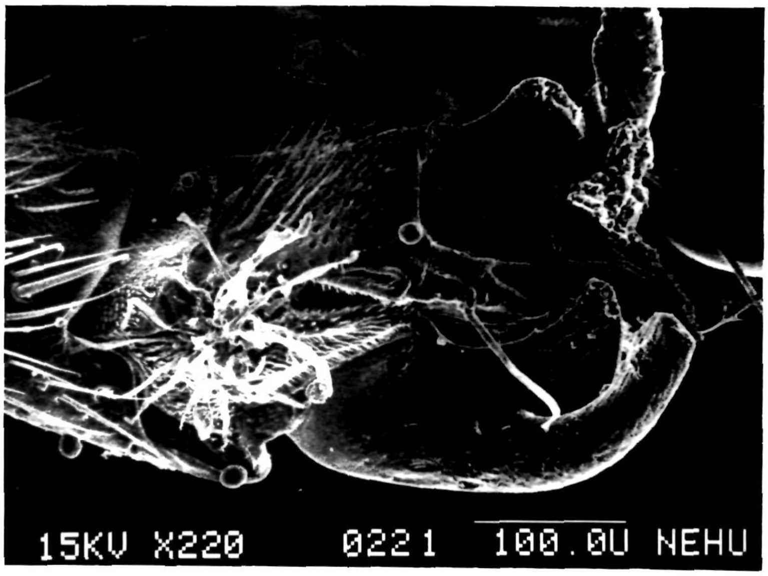
SECOND APPENDAGE - (Fig. 33) The second pair of leg in case of the worker is made up of a pointed tip and on the basal appendages i.e. - trochanter and femur on the dorso-lateral side is covered with dense branched sensillas. It is learned that this appendages helps in cleaning the thorax from pollen. The tibial spur is also shown, (Fig.34).

In that of the drones the hairs are inconspicuous on the different segments but nearing the base a number of pit sensillae (Fig. 35,36 and 37) were observed. Their occurrence being 150-200 μm^2 about four. These pit sensillae were learned to be sensilla ampullacea and sensilla coeloconica. Lacher (1964) discovered that they are responsible for humidity, temperature and for perceiving carbon-dioxide, i.e., thermoreceptors. Also at the base of the second appendage, the coxa, there are a number of branched sensilla, the origin of one of them is shown, (Fig. 38). The basal segment of the second appendage of the drone bee showed innumerable long and branched hairs

Fig. 30 SEM picture of the tarsus of the fore leg of queen honeybee. *Apis cerana indica* (Fab.) x 220

Fig. 31 SEM picture showing cuticular sculpture of the tarsus of fore leg of queen honeybee, *Apis cerana indica* (Fab.) x 1500

PLATE 20



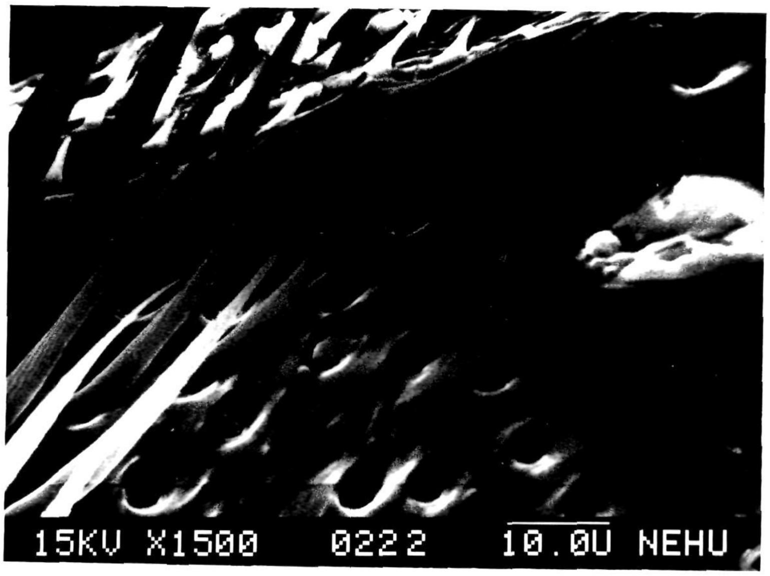
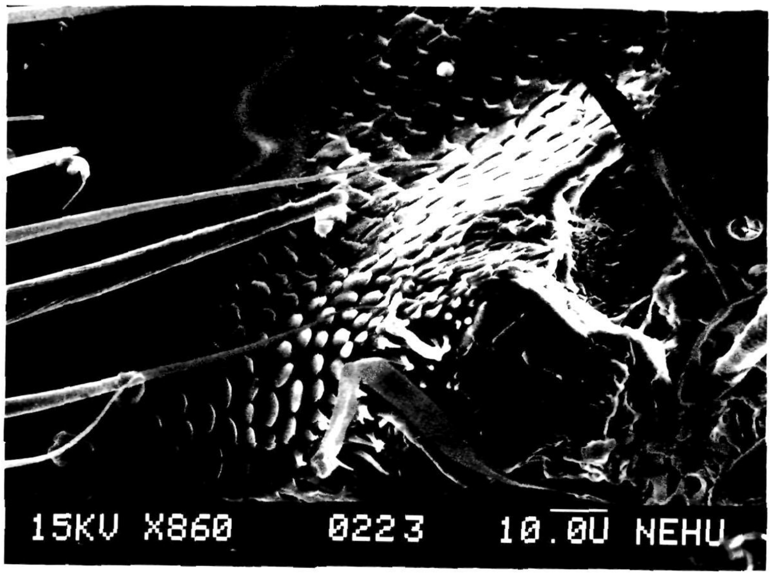


Fig. 32 SEM picture of the tarsus of fore leg of queen honeybee Apis cerana indica (Fab.) from another view at a lower magnification x 860

Fig. 33 A Scanning Electron Micrograph of the whole second appendage of the worker honeybee, Apis cerana indica (Fab.) x 26.

PLATE 21



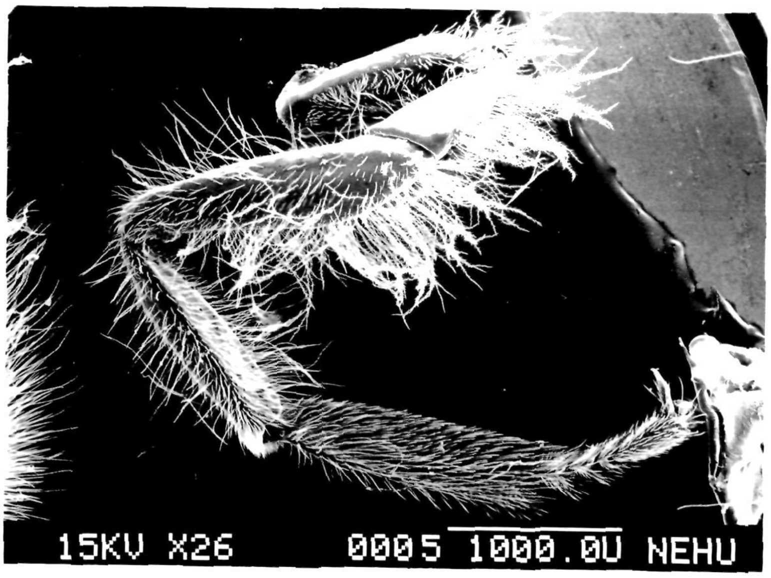


Fig. 34 SEM picture of the tibial spur of the workers honeybees, <u>Apis cerana indica</u> (Fab.) seen on the second appendage x 220

Fig. 35 SEM picture of the whole second appendage of the drone honeybee <u>Apis cerana indica</u> (Fab.) x 20

PLATE 22

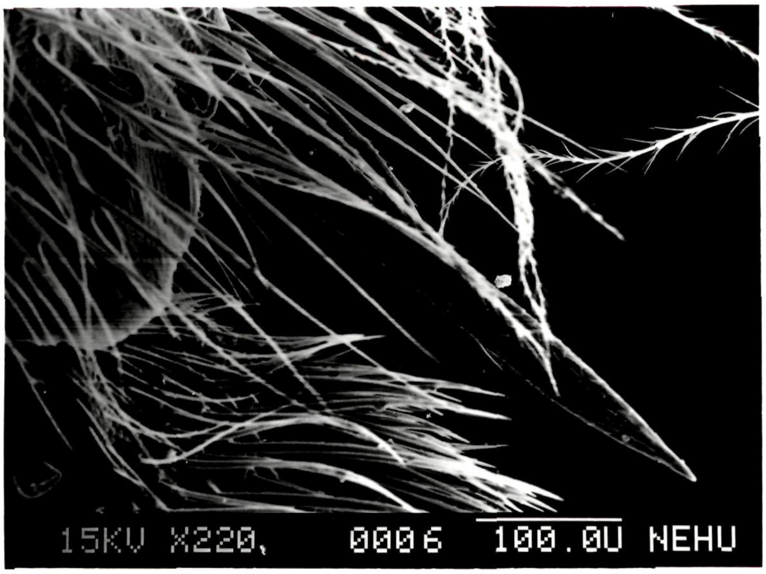
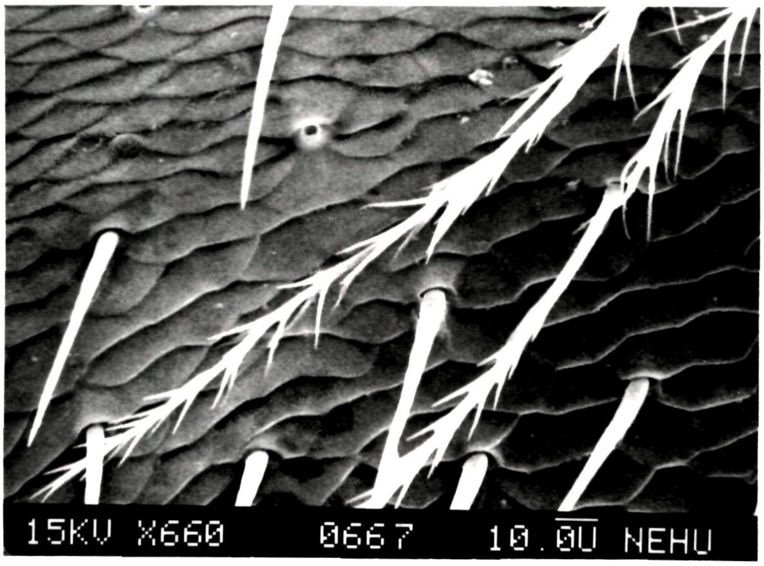


Fig. 36 SEM picture of the femur of second appendage of drone honeybee _Apis cerana indica_ (Fab.) showing pit and branched sensilla x 66

Fig. 37 A magnified SEM picture of the pit sensilla on the femur of second appendage of drone honeybee _Apis cerana indica_ (Fab.)

PLATE 23



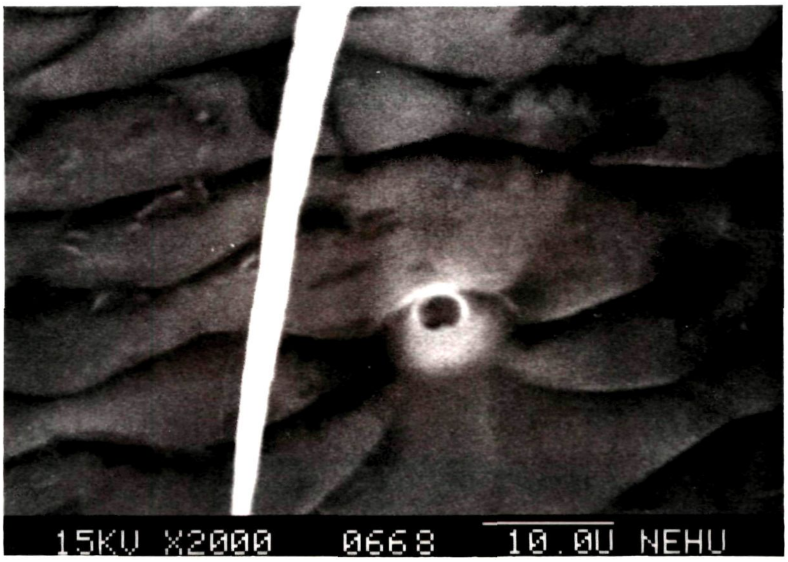
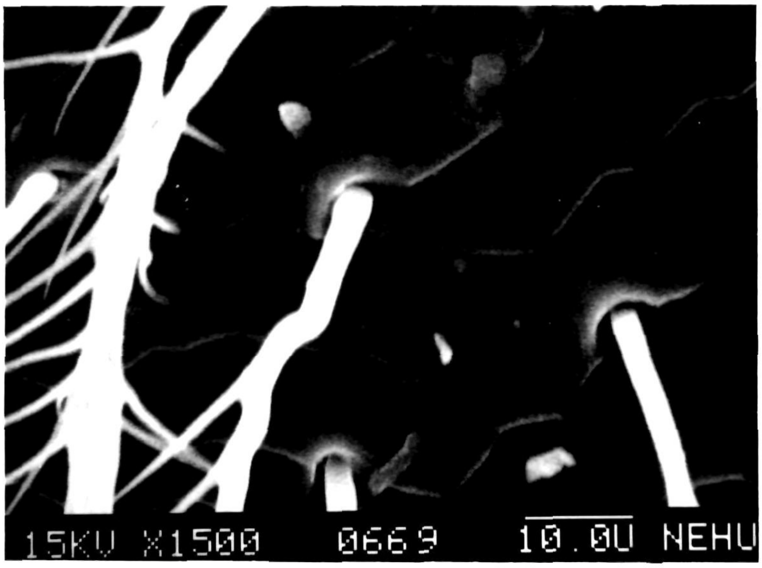


Fig. 38 A SEM picture of the origin of the branched sensilla seen on the femur of the second appendage of the drone honeybee, *Apis cerana indica* (Fab.) x 1500

Fig. 39 SEM picture of the basal segment (coxa) of the second appendage of the drone honeybee, *Apis cerana indica* (Fab.) showing innumerable branched hairs (sensilla) x 150

PLATE 24



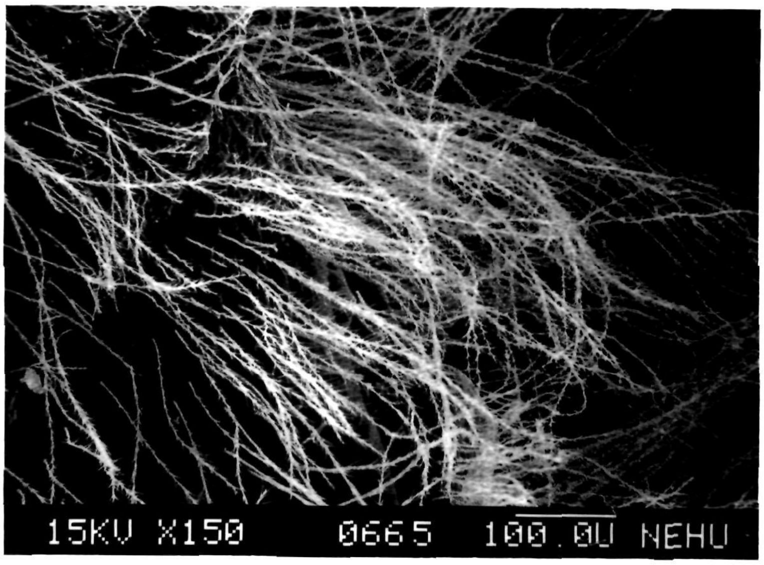
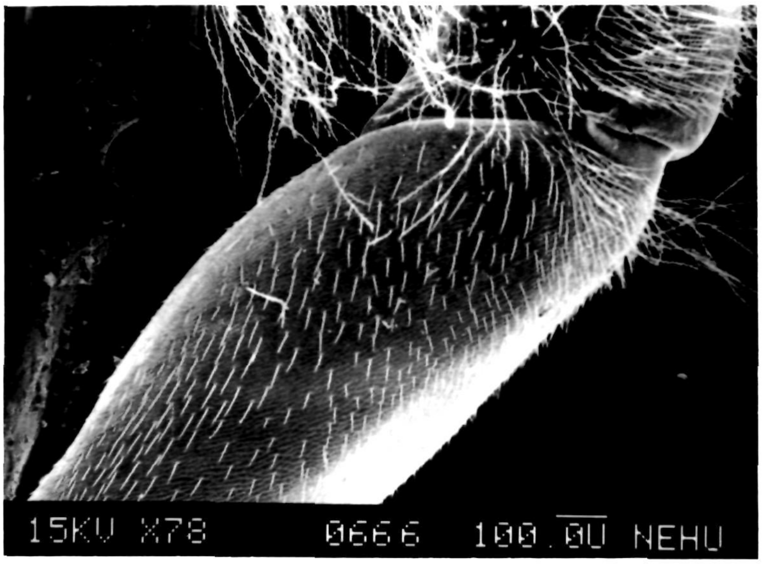
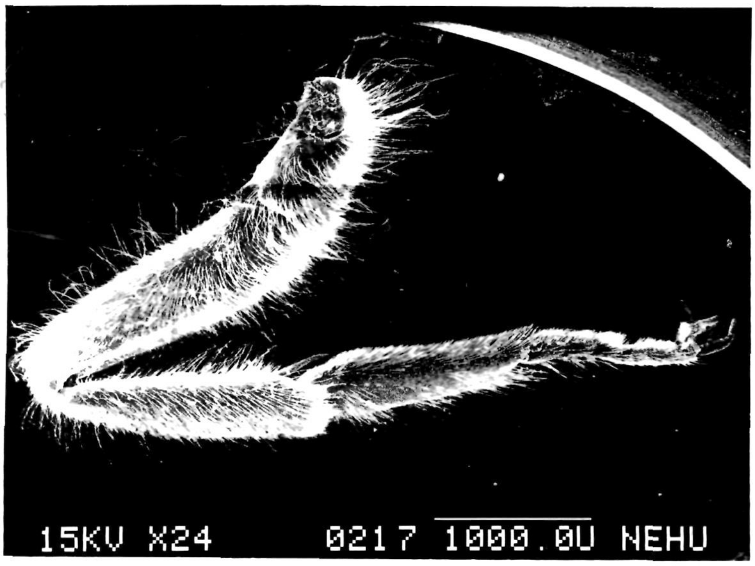


Fig. 40 SEM picture of the trochanter of the second appendage of the drone honeybee *Apis cerana indica* (Fab.) showing smooth regular arrangement of short trichoid sensilla x 78

Fig. 41 SEM picture of the whole second appendage of the queen honeybee, *Apis cerana indica* (Fab.) x 24

PLATE 25





(Fig. 39) whereas the trochanter region shows smooth and regularly arranged trichoid sensillae. (Fig. 40)

In another female caste, the queen, the second appendage is made up of a bifurcated and curve tip. The bilobate structure claw-like tip is distinct in Apis (Snodgrass 1956; Borror et. al. 1976). A number of sensilla trichodea was viewed at the portion adjacent to the tip, and coming to the second segment, the tarsus, the number of trichoid sensilla is increasing along the dorso-lateral rim of the appendage. (Fig. 41 and 42)

HINDLEGS - The hindlegs of the honeybee (Apis cerana indica Fab.) in workers is beautifully transformed into a pollen basket, where the scopa forms a carbicula on the hind tibia (Fig. 43). The pollen is brushed towards the mouth parts and some of it moistened with a little regurgitated nectar or honey before being passed into the corbicula. After becoming well dusted with pollen the bee usually hovers and while on wings, or less commonly at rest brushes her body rapidly with the hairs of the basi-tarsi; the head, appendages and front part of the thorax are brushed by the fore-legs, whereas the middle part of the thorax by the middle-legs, the abdomen by the combs of hair on the inner surface of each hind basi-tarsus. When enough pollen is accumulated there, the inner surface of these basi-tarsi are rubbed rapidly together in a pumping lengthwise motion. At the downward stroke of one leg, the tibial comb or rake across the end of the tibia, removes pollen from the opposite basi-tarsus into the space between the auricle and the comb. Movement of the basi-tarsus with respect to the tibia then pushes the sticky pollen mass upward unto the smooth surface of the corbicula. Pollen from the inner surface of one basi-tarsus is in this way transferred to the outer surface of the opposite leg.

At the time of unloading, the bee presses the sticky load against the wall of the nest and then helps to separate the material from the corbicula by pushing the basi-tarsus of the second leg along the smooth surface of the corbicula from the proximal to the distal end. The process is then repeated on the other side; unloading is thus accomplished in a few seconds. At other time unloading is also helped by the house bees.

A number of contact chemoreceptors were observed on the hind-leg of the worker caste, but on the edges of these appendages a number of spade-like receptors were also seen, there function is still not yet known, (Fig. 44).

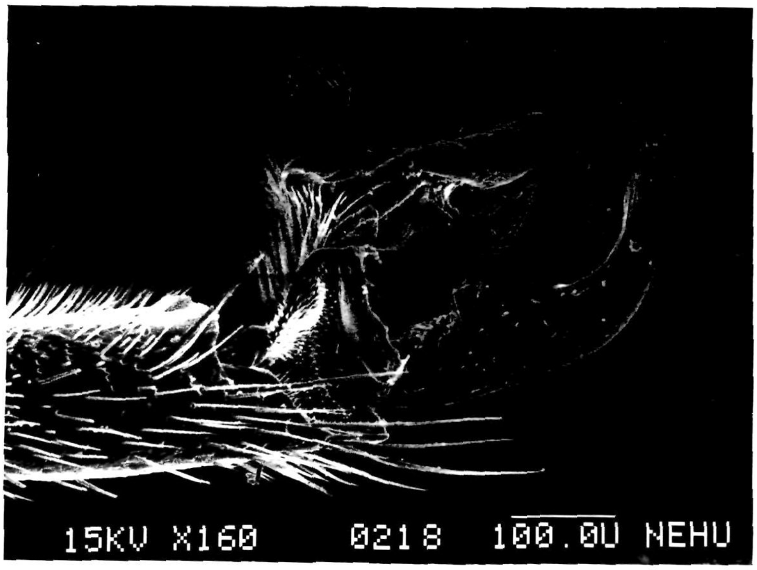
On the hind-leg of the male caste, the drone, nothing significant is observed, except for trichoid sensillae scattered all over its last appendage, whereas on the basal segment (coxa) there are again dense arrangement of branched sensillae, (Figs. 45-48).

The type of receptors observed on the hind-leg of the queen resembles that of the workers, being spade-like in shape. The cuticular pattern on the hair-less region of

Fig. 42 SEM picture of the tarsus of the queen honeybee *Apis cerana indica* (Fab.) x 160

Fig. 43 A Scanning Electron Micrograph of the whole pollen basket on the hind leg of the worker honeybee *Apis cerana indica* (Fab.) x 54.

PLATE 26



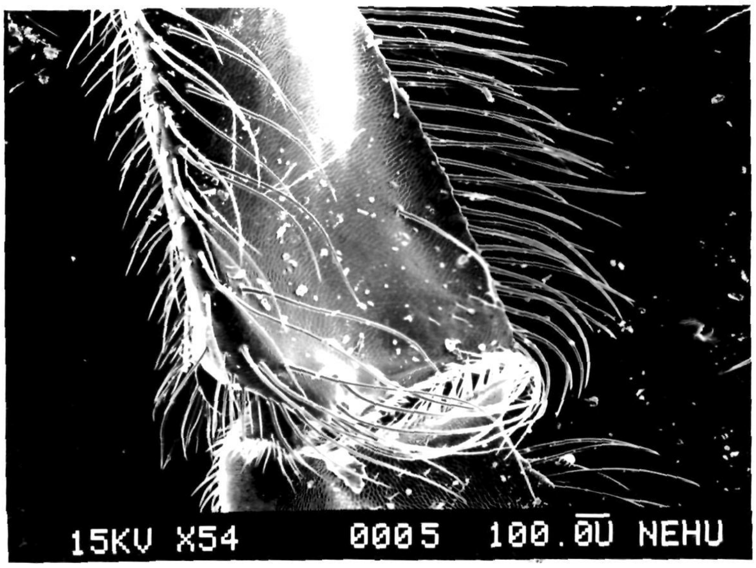


Fig. 44 SEM picture of the spade-like receptors on the rim of the femur of the third appendage of the worker honeybee, Apis cerana indica (Fab.) x 540

Fig. 45 SEM picture of the tarsus region of the third appendage of drone honeybee Apis cerana indica (Fab.) showing sharp clawed bifurcated tip x 110.

PLATE 27

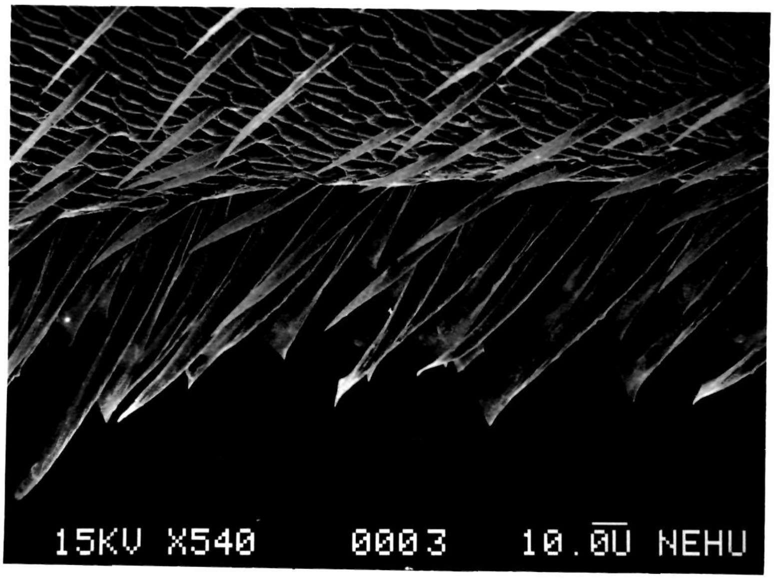
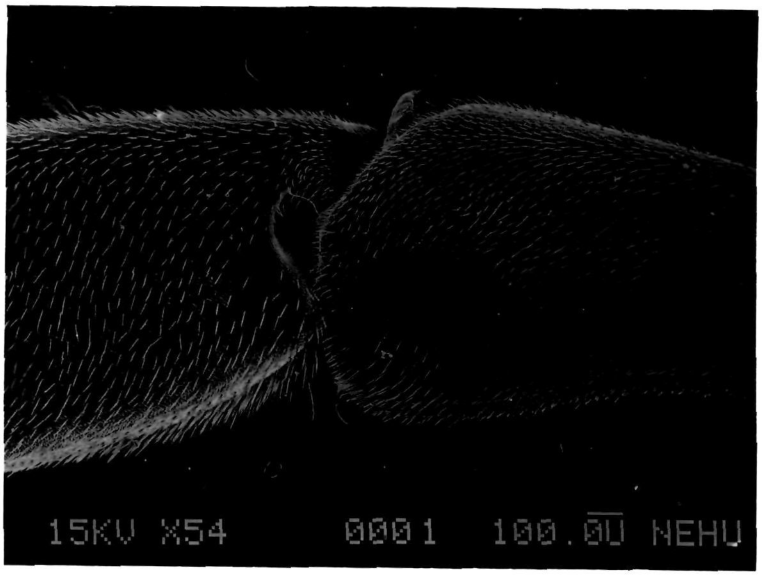


Fig. 46 SEM picture of the joint of the trochanter and the femur of hind leg of drone honeybee, _Apis_ _cerana_ _indica_ (Fab.) x 54.

Fig. 47 SEM picture of the cuticular pattern on the tarsus of the hindleg of drone honeybee, _Apis_ _cerana_ _indica_ (Fab.) x 540

PLATE 28



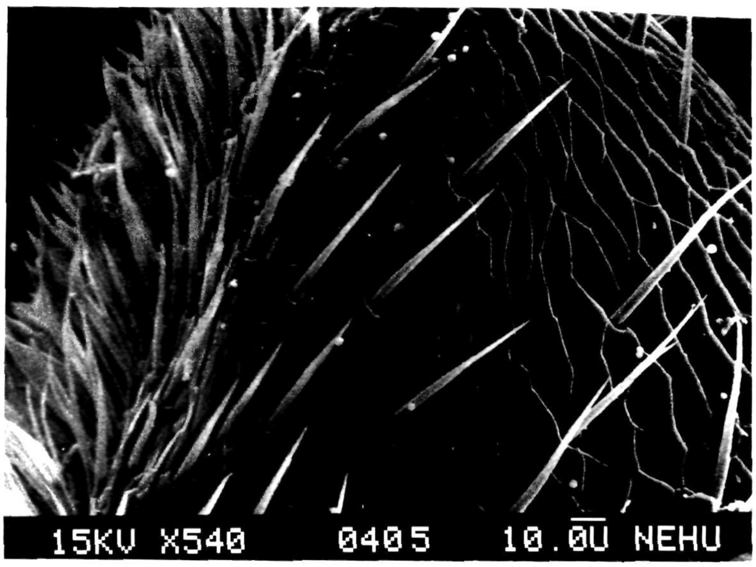
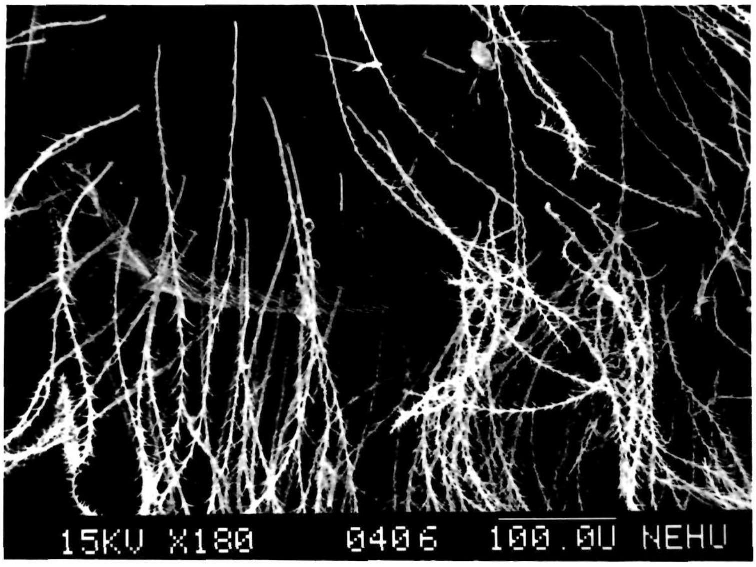


Fig. 48 SEM picture of the basal segment (coxa) of the hind-leg of the drone honeybee, *Apis cerana indica* (Fab.) where long branched hairs (sensilla) is seen x 180.

Fig. 49 Scanning Electron Micrograph of the spade-like receptors on the femur of hind-leg of queen honeybee, *Apis cerana indica* (Fab.) x 100.

PLATE 29



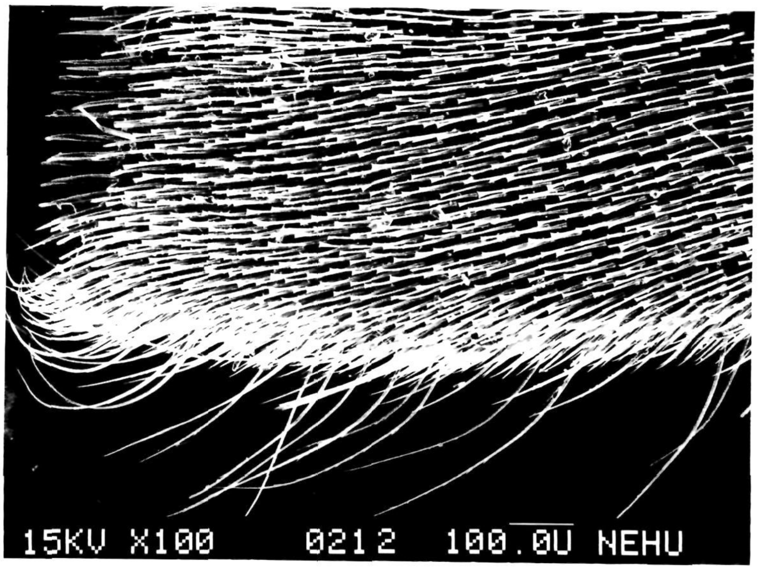


Fig. 50 A magnified SEM picture of the spade-like receptors on the femur of hind-leg of queen honeybee, *Apis cerana indica* (Fab.) x 940.

Fig. 51 A Scanning Electron Micrograph of the cuticular pattern on the hairless region of the tarsus of hind-leg of queen honeybee, *Apis cerana indica* (Fab.) x 1800

PLATE 30

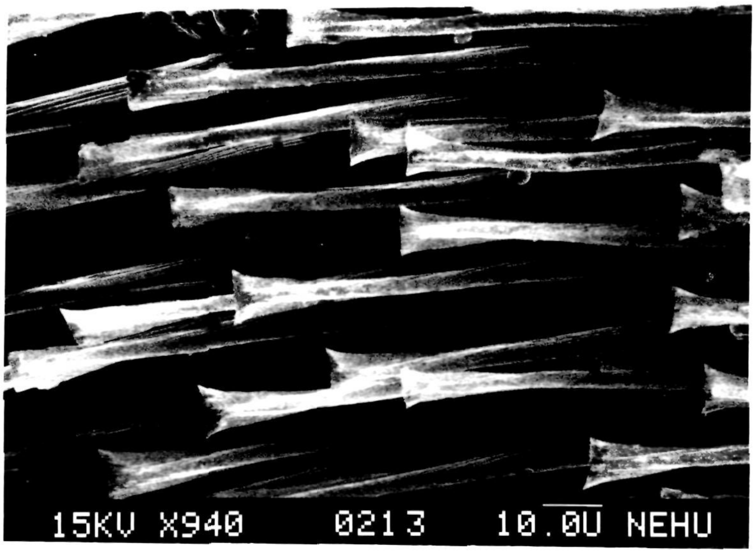
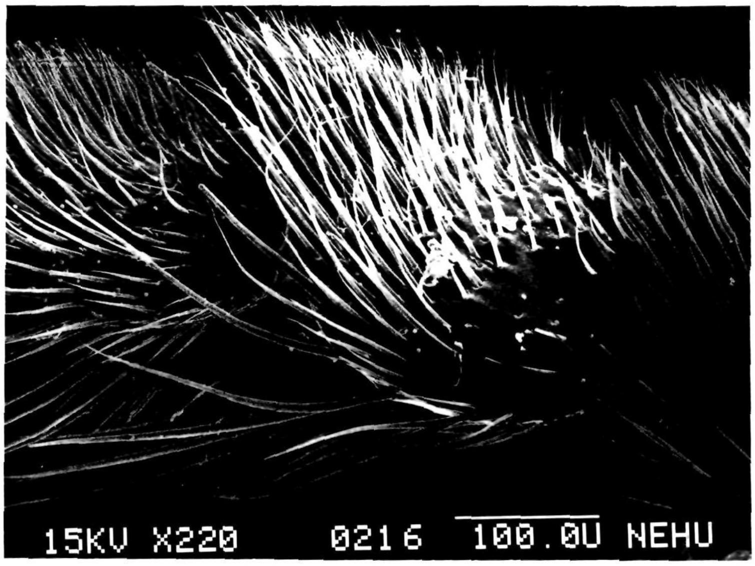


Fig. 52 SEM picture of the basi-tarsus region of hind-leg of queen honeybee, Apis cerana indica (Fab.) showing dense hairs x 220.

PLATE 31



the hind-leg is also shown, (Figs. 49-51). Dense arrangement of contact chemoreceptors (trichoid sensilla) on the basi-tarsus region of the leg is also observed. (Fig. 52)

WINGS - Wings of honeybees (Apis) are adapted for agile maneuverability, rapid flight, and strength for carrying heavy load. Each is hinged to its base and free to move up and down, forward, backward and can undergo a twisting and partial rotation. All wing movements are controlled by a complex system of muscles in the thorax.

Wings are thin plate-like expansions of the integument which are strengthened by a frame-work of hollow sclerotized tubes - the veins or nervures. Figs 53-56 shows fore wings and hindwings of both worker and drone bee (Apis cerana indica Fab.) respectively.

In Apis cerana indica (Fab.), a hamulate type of wing-coupling is observed, with a row of hooks known as hamuli, on the coastal margin of the fore-wing. In both the caste, the worker and the drone; the hamuli are clearly seen. (Figs. 57-59) A number of hairs are also marked in both the castes, but in case of the female castes the hairs/receptors are longer and pointed compared with that of the male caste, where they are quite small, short and somewhat blunt. On observation of the whole wings, both fore- and hind-wing - there are around four to five branched sensillae. (Figs. 60-68) elaborates more about the different arrangement of sensillas and other structures on

Fig. 53 A Scanning Electron Micrograph of the fore-wing of worker honeybee, Apis cerana indica (Fab.) x 20

Fig. 54 A Scanning Electron Micrograph of a portion of the fore-wing of the drone honeybee, Apis cerana indica (Fab.) x 44

PLATE 32

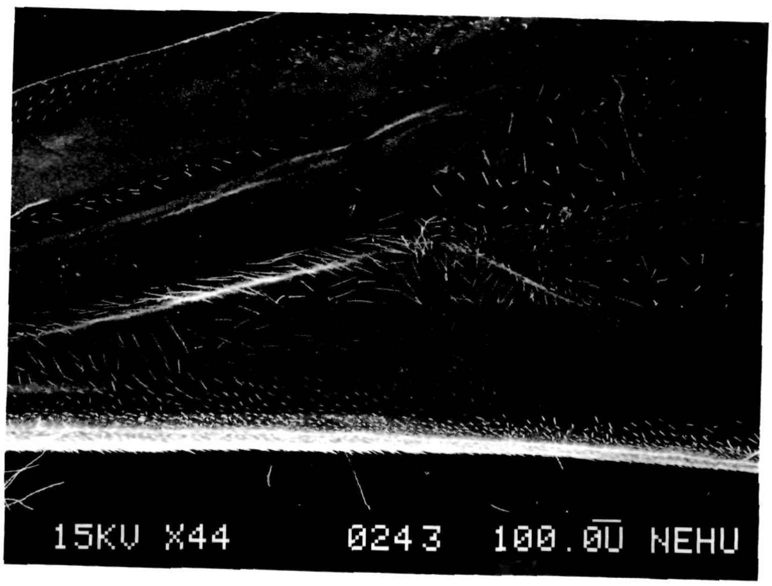
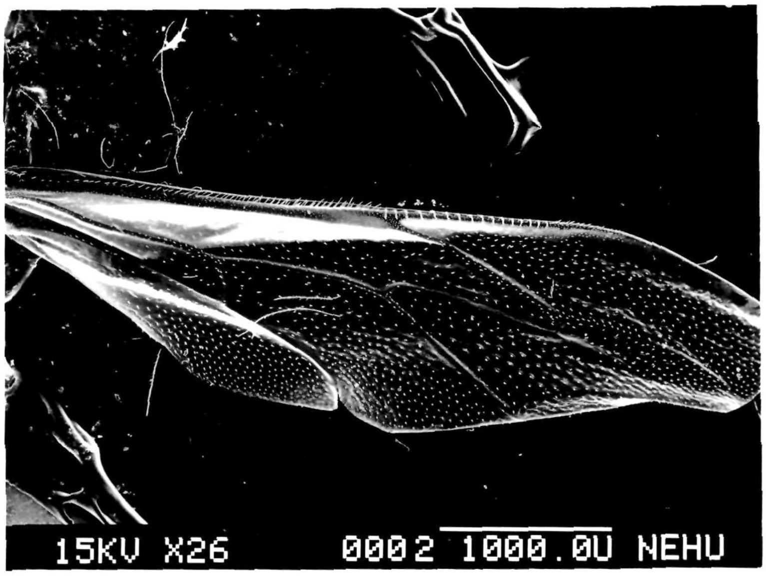


Fig. 55 SEM picture of the hind-wing of the worker honeybee, <u>Apis</u> <u>cerana</u> <u>indica</u> (Fab.) x 26.

Fig. 56 SEM picture of the hind-wing of the drone honeybee, <u>Apis</u> <u>cerana</u> <u>indica</u> (Fab.) x 20

PLATE 33



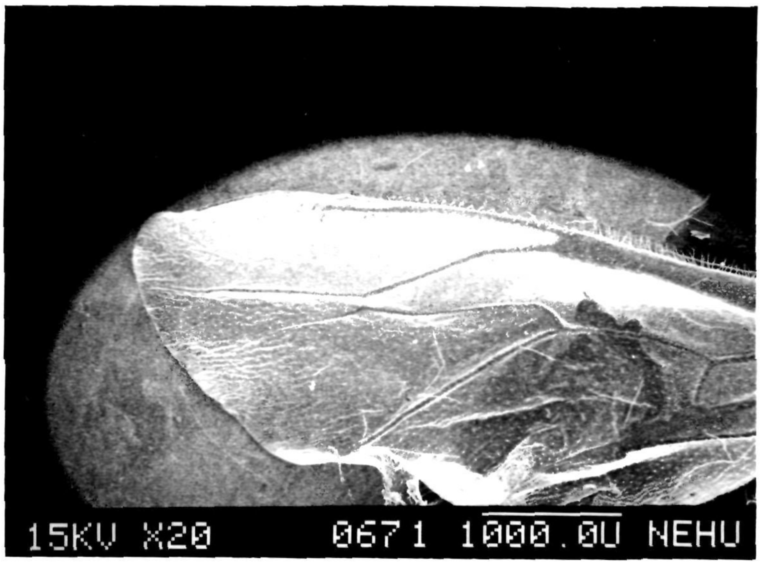
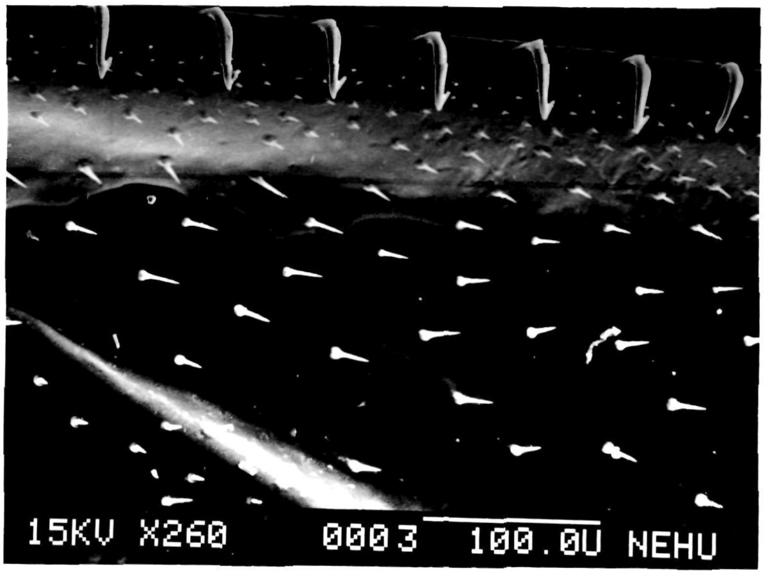


Fig. 57 A SEM picture showing the rim of the upper part of the hind-wing of worker honeybee, *Apis cerana indica* (Fab.) x 260.

Fig. 58 A magnified SEM picture of the hooks (hamuli) on the upper rim of the ventral side of the hind-wing of worker honeybee, *Apis cerana indica* (Fab.) x 660

PLATE 34



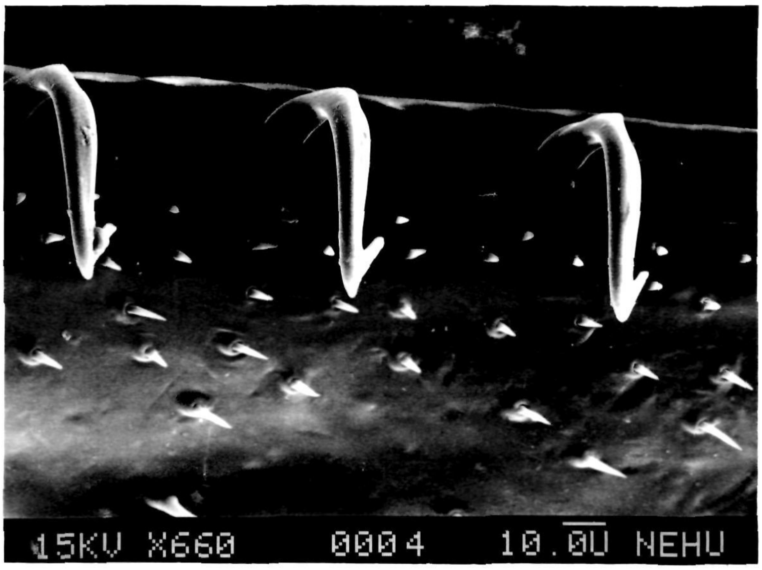
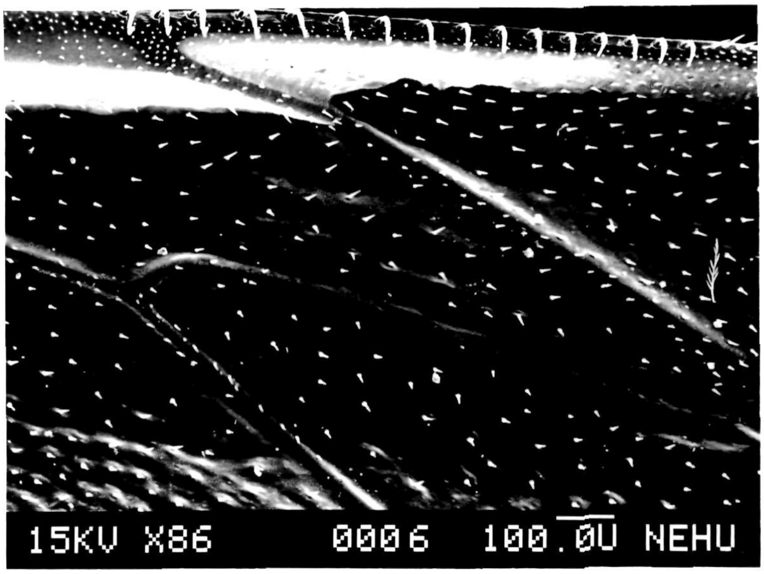


Fig. 59 SEM picture showing the margin and mid-portion of the hind-wing of the worker honeybee *Apis cerana indica* (Fab.) x 86

Fig. 60 SEM picture showing arrangement of hair plates on lower portion of hind-wing of worker honeybee, *Apis cerana indica* (Fab.) x 440

PLATE 35



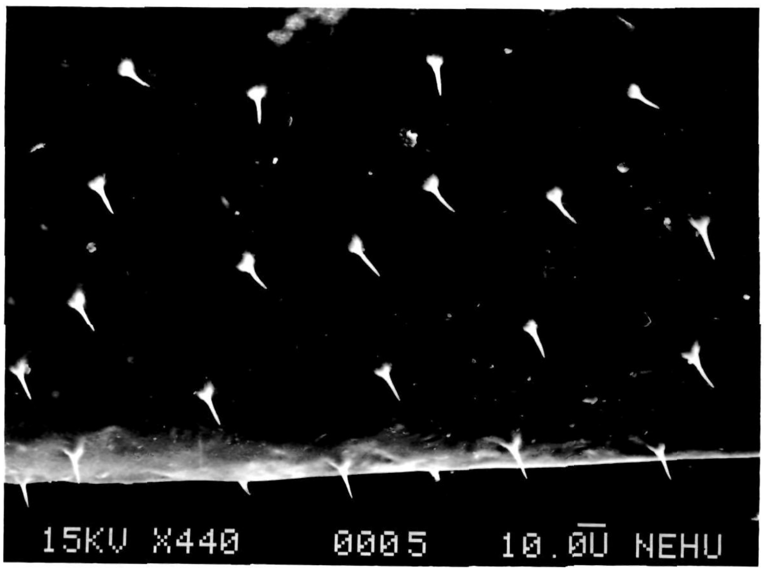
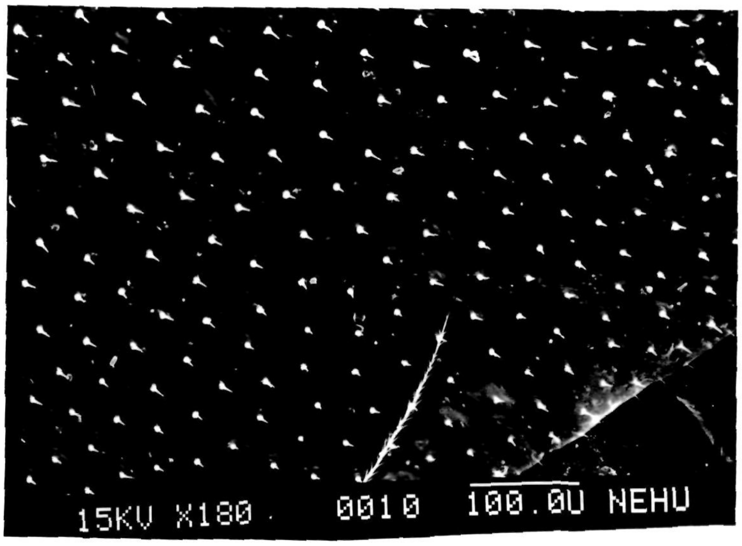


Fig. 61 SEM picture showing lower and mid-portion of fore-wing of worker honeybee, *Apis cerana indica* (Fab.) x 180.

Fig. 62 SEM picture showing margin of the upper portion of the fore-wing of worker honeybee, *Apis cerana indica* (Fab.) x 1200

PLATE 36



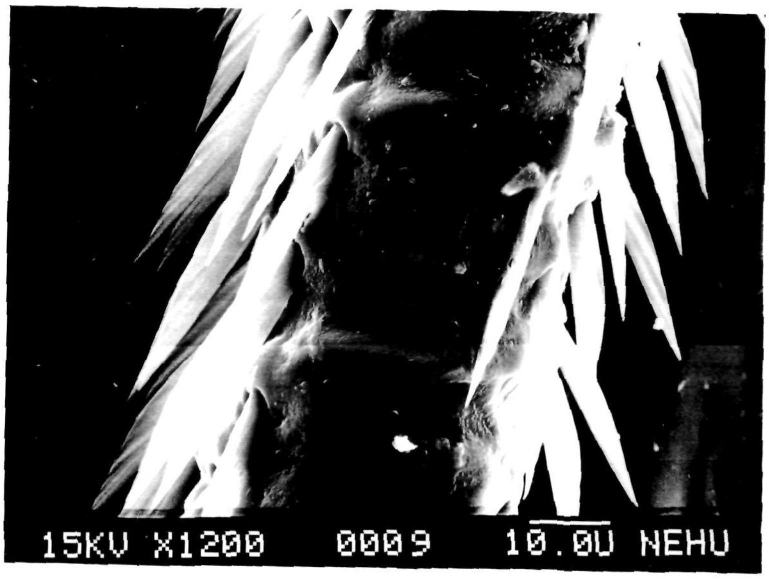
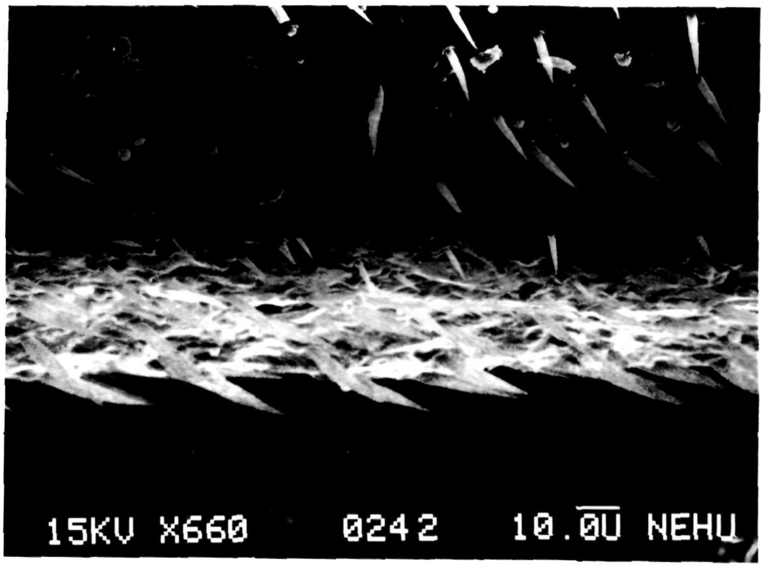


Fig. 63 SEM picture showing lower margin of fore-wing of drone honeybee, Apis cerana indica (Fab.) x 660

Fig. 64 SEM picture showing the rim of the upper portion of the hind-wing with hooks (hamuli) of drone honeybee, Apis cerana indica (Fab.) x 150.

PLATE 37



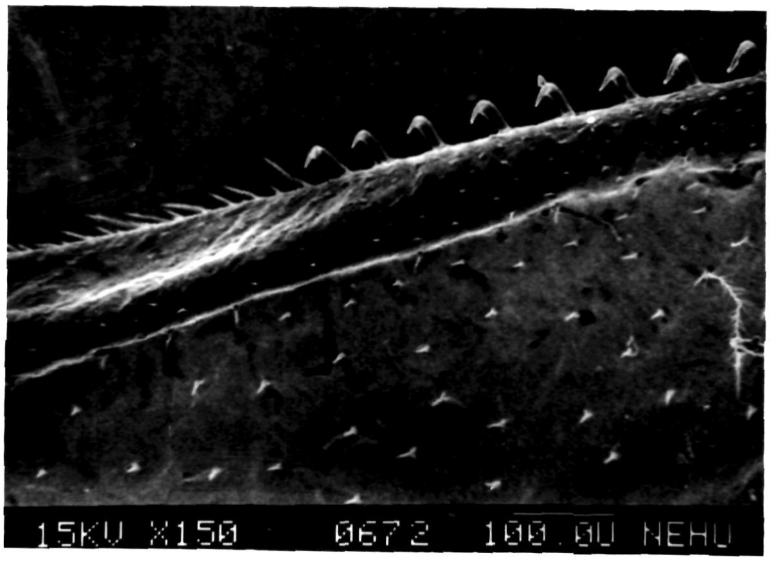
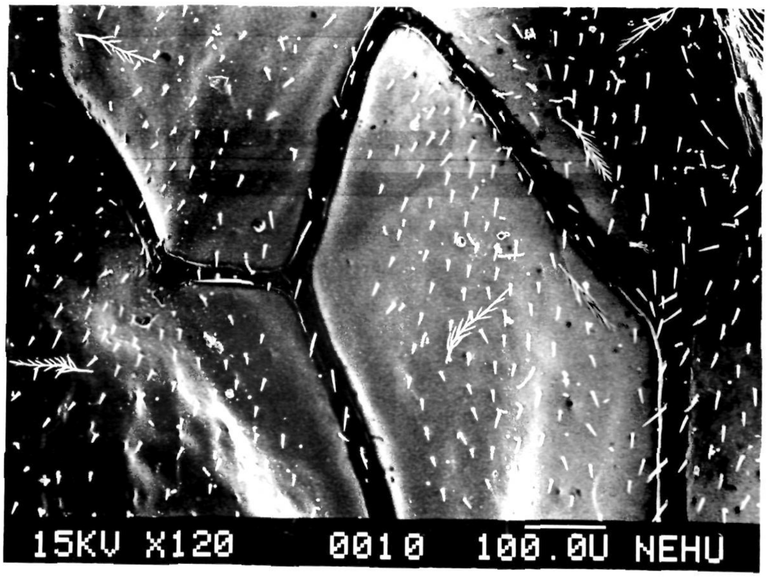


Fig. 65 SEM picture showing a portion of the fore-wing, where few scattered branched hair sensilla are seen on the fore-wing of worker honeybee, *Apis cerana indica* (Fab.) x 120

Fig. 66 SEM picture showing a portion of the fore-wing of a drone honeybee, *Apis cerana indica* (Fab.). The hairs (receptors) are somewhat blunt x 180

PLATE 38



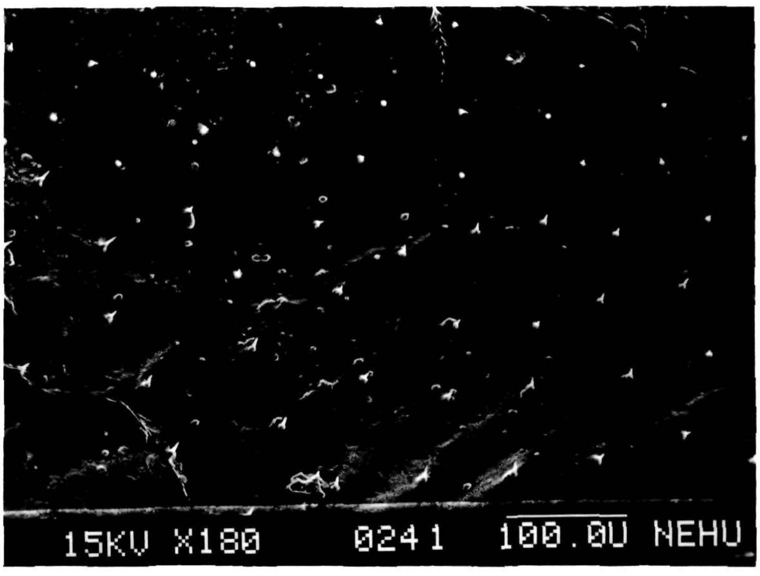
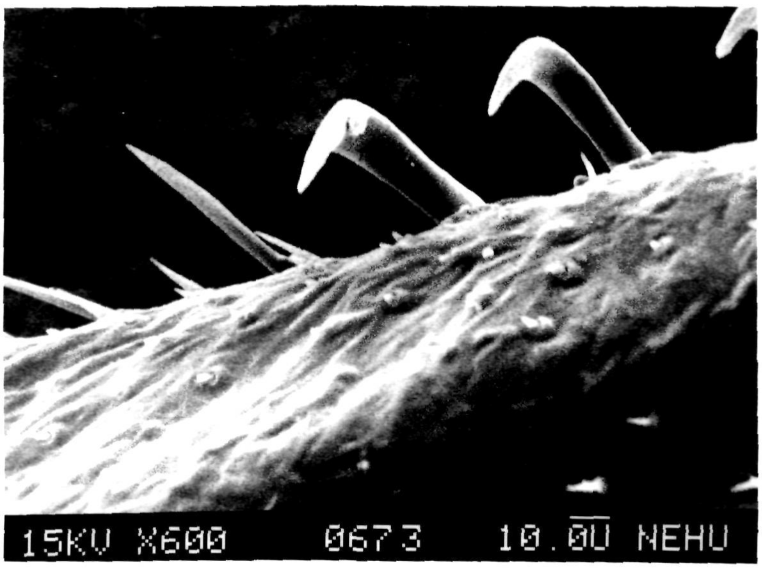
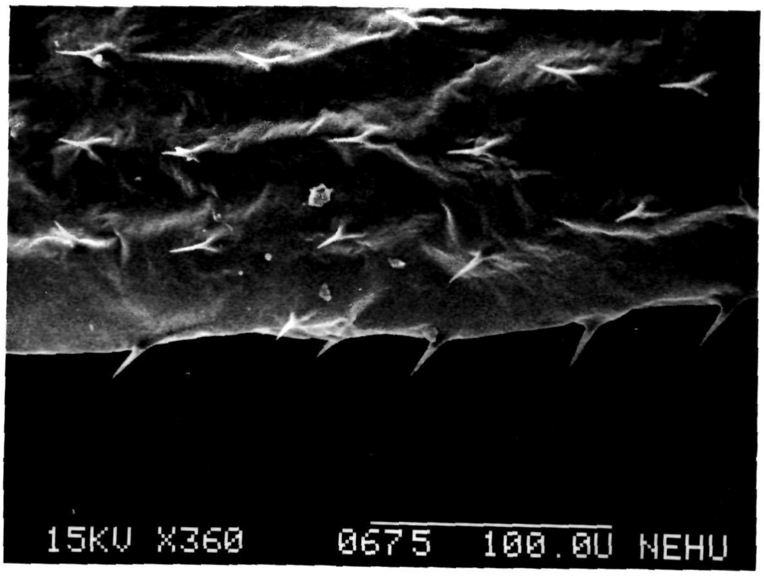


Fig. 67 A magnified SEM picture of the upper rim of the hind-wing of drone honeybee, *Apis cerana indica* (Fab.) x 600

Fig. 68 SEM picture of the lower rim of the hind-wing of drone honeybee, *Apis cerana indica* (Fab.) x 360.

PLATE 39





the surface of the wings of male and female castes of *Apis cerana indica* (Fab.).

An enlargement of one of the branch sensilla in the hind-wing of the drone is shown. (Figs. 69-71). The exact function of these branched sensillas has not been discovered but its presence in many parts of the body of the bee has led one to conclude that it must be responsible for some important function.

Over all it has been observed that tactile hairs are probably scattered all over the body and appendages. The tactile sense seems especially well developed on the mouth parts and antennae as well.

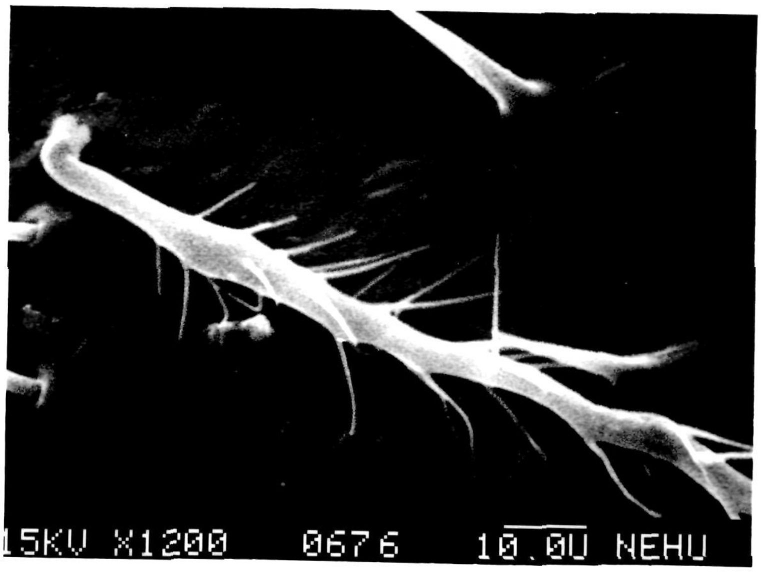
Tarsal sensilla of a wide variety of insects in different orders have been shown to be contact chemo-sensilla (Kaestner 1972; Wigglesworth 1972; Horn 1972).

Campaniform sensillae are also quite common and are widely distributed among all insect orders (Pringle 1938; Mc. Iver 1985) and are found in many locations including wings, legs, ovipositor, antennae and mouth parts (Snodgrass 1935, McIndoo 1914, 1922; Pringle 1938a; Mc. Iver 1985). Campaniform sensilla respond to cuticular stress resulting from compression and stretching of their surroundings (Pringle 1938; Pearson 1972; Spinola and Chapman 1975; Zill and Moran 1981). These cuticular stresses can arise from both internal and external forces including muscular contracted frictional resistance between

Fig. 69 A SEM picture of the branched hair of hind-wing of the drone honeybee, *Apis cerana indica* (Fab.) x 480.

Fig. 70 A magnified SEM picture of the origin of the branched hair (sensilla) on the hind-wing of drone honeybee, *Apis cerana indica* (Fab.) x 1200

PLATE 40



body parts, gravity, horizontal impedance due to substrate friction or loading and contact with external objects (Hustert et. al. 1981; Mc. Iver 1985). In most cases these stress forces concentrate in the cuticle of joint regions and the majority of campaniform sensilla tend to be associated with the articulations of legs, wings and mouthparts (Pringle 1938a).

Groups of sensilla on the legs of Apis were reported by Mc. Indoo (1914), he also reported a total of over 600 campaniform sensilla on the legs of Apis workers, some of which must have been located on the tibia and femur.

The external caps of the campaniform sensilla can vary greatly in form (Mc. Iver 1985) and the cuticular structure and shape of the domed portion its "buttress supports" and the socket rims are important factors in the mechanical coupling of the sensillum receptor to the cuticle (Moran and Rowley, 1975; Spinola and Chapman 1975; Gettrup 1973). The directional sensitivity of campaniform sensilla was first described by Pringle (1938b) and it has been demonstrated repeatedly in a variety of preparations (Chapman 1965; Heinzel and Gewecke 1979; Thurms et. al. 1975; Zill and Moran 1981).

Hair plates are composed of groups of trichoid sensilla which are located in the joint regions of insect legs, antennae, neck, wings and abdomen (Pringle 1938a; Markl 1962; Wright 1976; Schmidt and Smith 1985). Hair plates function as proprioceptive position detectors : the sensillae are situated such that movement of the joint brings them into contact with either the arthrodial membrane of the joint or the surface of the opposing limb segment (Pringle 1938b).

Hair plates are very widely distributed among the orders of insect (Markl 1962; Thurm 1964; Mc. Iver 1985). The most comprehensive morphological description of hymenopteran hair plates is that of Markl (1962), which includes representative of the families Apidae, Vespidae, and Formicidae.

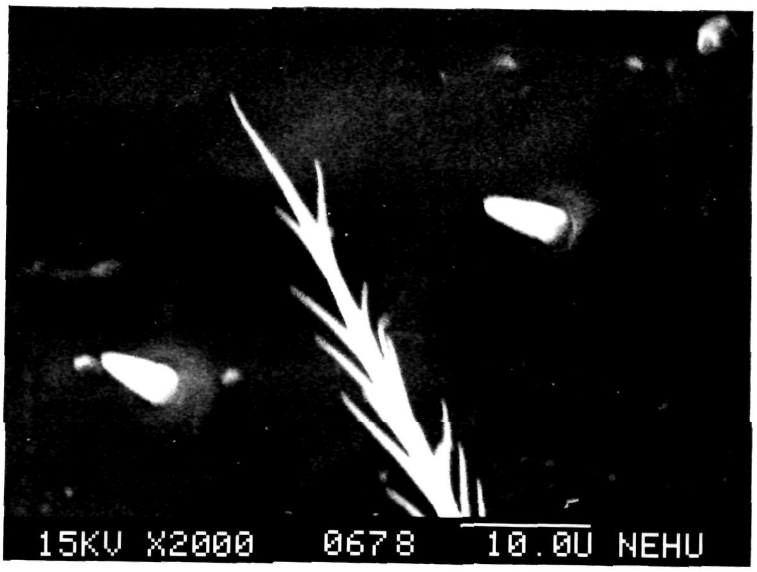
- Sensilla trichodea, devoted to the reception of simple touch, occur over the entire body (Ribbands 1953; Thurn 1964). It is difficult to touch the body surface of a bee with an object, the size of a needle without bending at least one of these hairs and arousing the bee.

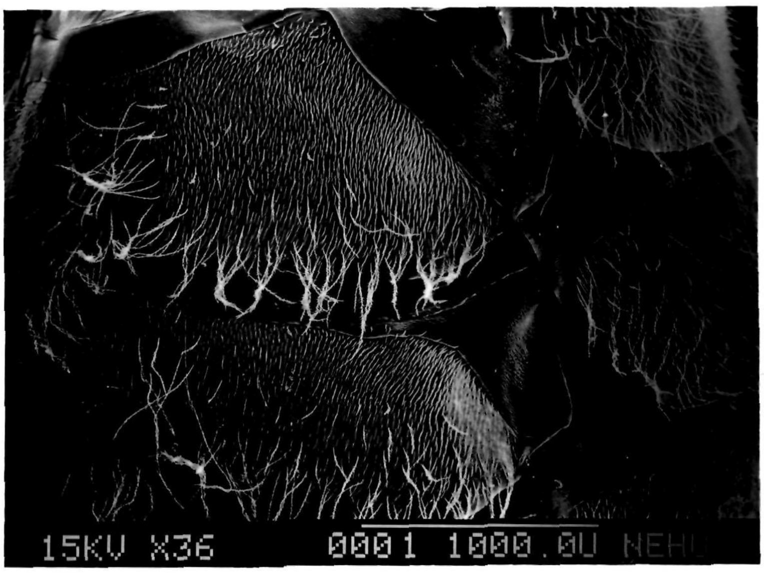
It is also learned that clumps of hairs at the base of the appendages, doubtless serve as proprioceptors to indicate the position of the appendages. These sensillae also serve as a part of the gravity detecting apparatus and perhaps serve to detect vibrations in the substrate or of objects being touched by the antennae. Since bees donot appear to respond to airborne sound, but only to vibrations in the substrate and surfaces being touched these sensillas presumably provide the basis for responses to vibrations. As probably, no bee could construct a proper nest without knowing the direction of the pull of gravity. There are

Fig. 71 A magnified SEM picture showing the pointed tip of the branched sensilla of the hind-wing of drone honeybee, <u>Apis cerana indica</u> (Fab.) x 2000

Fig. 72 SEM picture showing a portion of the dorsal abdominal cuticle of the worker honeybee, <u>Apis cerana indica</u> (Fab.) x 36.

PLATE 41





small groups of hairs at the neck and the base of the abdomen (Fig. 72) they are minute tactile hairs, but they normally touch only nearby parts of the body being deflected by any tendency of the head or abdomen, to hang downward. They thus serve to perceive the direction of the gravitational force; so that the bee knows how to behave appropriately.

Tactile hairs on the eyes of Apis as well as antennal positions perceived by the chordotonal organs (Johnston's organ) in the second antennal segment, provide information on air resistance in flight. These information enables a bee to respond to wind velocity and direction; which is important for orientation, i.e. to enable the bee to return back to its nest.

CHAPTER III

CHEMICAL SENSES OF THE HONEYBEE

INTRODUCTION

A sense organ is a part of an organism that is especially sensitive to a particular form of energy and relatively insensitive to others.

The receptor cells of all invertebrate sense organ are primary sensory neurons and, therefore may consist entirely of the dendrite terminations of the cell. Many of these, specially in the Arthropodas, end in association with a sensillum; that is, an architectural modification of the cuticle with its associated epidermal cells. Also a number of these, are innervated and approach the more elaborate vertebrate organs in structural complexity.

A necessary characteristic of a chemoreceptor is that the cuticle over_lying it must be thin enough, and of such a nature, as to allow the passage of at least some of the materials to be detected. Chemoreceptors in the insects appear to be variations of tactile receptors and, in some cases probably serve both functions.

A number of different morphologic types have been described; but they seem to fall into two series of increasing modifications, one series derived from the tactele seta, the other campaniform sensillum. The sensory dendrites penetrating chemoreceptors characteristically contain small refringent bodies.

Three kinds of chemical senses are recognized by physiologists - A general chemical sense and those of smell and taste. The distinctions between these seem to be relatively clear in most terrestrial insects.

Common chemical sense - It requires a high concentration of an irritant, which must be votatile at ordinary temperature. It can also be recognized as leading to an avoiding reaction to high concentration of irritant substances. (e.g. ammonia, vapours of essential oils etc.). It has been little studied, and these sense organs responsible though not satisfactorily identified occur on all parts of the body.

Olfactory Sense (smell) - It requires only very low concentration of compounds votatile at ordinary temperature. Olfactory stimulation has been extensively studied through the research for attractant and repellent substances in economic entomology (Dethier, 1947) but while behavioural aspects of the problem have received much attention, little is known of the mode of perception beyond the identification in a few cases of receptors on the palps and antennae, like sensilla placodea in Apis. In case of Apis mellifera it is a well investigated case where it reacts to the odour of essential oils, the threshold are similar to those of man with a close qualitative similarity between the olfactory sense of the two species (Von Frisch, 1919).

Gustatory Sense (taste) – The sense of taste requires direct contact with materials that volatalizes at physiological temperatures, but which need not be in such high concentration. In case of honeybees, these receptors are not identified with certainty but they are probably basiconica and short trichoid sensilla; which are known to occur on the antennae and also on the tarsus and distal parts of tibia (Frings and Frings, 1949). Experimental work on the sense of taste have consisted largely of behavioural studies of the acceptability or otherwise of various pure chemical substances (Dethier, 1953).

In general, the common chemical sense serve as a protection against dangerous substances. No receptor specialized exclusively for the common chemical sense has been identified; but receptors responding to it are thought to be scattered over the whole body surface not only localized in the antennae.

The sense and its reaction may be the result of a violent assault on all chemoreceptors.

The sense of smell gives notice to the bees of something either desirable or undesirable in the environment, generally at a little distance. It is used not only in food selection but also in locating a member of the opposite sex or the correct place for depositing the eggs.

The physiology of the chemical sense and the receptors involved are also discussed by Dethier and Chadwick (1948); Frings and Frings (1949) and Dethier (1953).

Bees can get a much more minute picture of the origin of odours than we can because its organs of smell are located on external projections (antenna) which it can direct over the surfaces of objects even into crevices, etc. Thus, as it moves about, a bee can make a sort of topographical olfactory analysis and can learn and respond to a sequence of odours on a substrate.

In case of the honeybees antenna, the sense of smell, i.e. the receptors are located usually on segments 5-12 in case of workers and queens, and segments 5-13 in case of drones.

MATERIALS AND METHODS

EXPT. 1 On an experimental table some cardboard square cards were kept. On top of these cards petri-dishes were placed; and only on one dish sugar water along with a fragrant flower were placed; whereas on other dishes plain water was kept. The position of this card containing food was frequently changed to avoid training the bees to a certain place; for it is wished that only the odour should guide them to the food.

Later on after several hours all the soiled petri dishes were replaced with a set of new and clean petri-dishes. Into one of them a little drop of the scent that has been used for training purposes is added, but like the rest devoid of food.This is to watch whether they can smell the odour and thus use it as a guide to the source of food.

EXPT. 2 To find out whether bees can distiguish as many different scents as a human being can :- The bees were therefore, first trained to an essential oil (olive oil). After one day of training, a number of petri-dishes were placed on the table with different scent, and later other new odours were added to the previous ones.

EXPT. 3 – This experiment was performed to observe the honeybee's senstivity to smell. The honeybees *Apis cerana indica* (Fab.) were trained to a certain odour. And later, on diluting the scent, more and more, it is wished to observed whether they could distinguish between a scented and an unscented dish or not.

According to Von Frisch (1919) – Even though the anatomy of the organs of olfaction is entirely different in bees and men, it is surprising that their olfactory reactions are nevertheless so nearly the same. We may be sure, therefore, that the scent of most flowers is insufficient to attract bees from a great distance.

Thus, there must be something else which must be responsible for attracting bees other than the sense of smell.

EXPT. 4 – Scanning Electron Microscope (SEM) study of the antenna of the different castes: The materials and methods for this experiment is same as in the previous chapter

This would enable us to study the different chemoreceptors, present in the different castes of bees, *Apis cerana indica* (Fab.)

RESULTS AND DISCUSSION

Usually a honeybee when foraging restricts its visit to a single species of plant. It is quite difficult to ascertain how bees recognize one species of flower among a variety of others nearby. Colour could be the answer, but investigations showed that bees can distinguish only four colours : yellow, blue, blue-green and ultra-violet. Apart from colour, flowers also emit some scent which is quite specific from species to species. So this might have something to do with their foraging behaviour.

Thus, the experiment we have performed showed quite interesting results.

Based on the first experiment it is learned that bees do perceive odour; because the bees after hovering around for sometime over the experimental table were able to fly to the petri-dish having sugar water, this is due to the fact that a fragrant flower was there to guide them. Even though the position of this dish was changed several times, yet they were successful in locating it. Later on when the rose flower, out of which whose fragrance has guided them to the place of food, was crushed and a few drops of the rose water dropped in one of the dish without any food, in a new set of experiment, it was again observed that the bees without mistake do perceive the odour and were seen to hover over the scented petri-dish. So it is evident that they perceive this colour and they use it as guide to a source of food.

As mentioned earlier that the bees are very particular of the type of blossoms they forage, be it for nectar or pollen; where they usually tend to localize themselves to one species at a time. In this case, bees should be able to distinguish among a variety of scent produced by a number of flowers.

To prove further to this, (Fig. 73b) the bees were trained first to an essential oil scent, in this case olive oil, after one to two days it was found that the perception of the bees were satisfactory. Thus more new scent were introduced in different petri-dishes placed randomly on the experimental table. The different scent used in this purpose are those which we have crushed from flowers of different types and species - roses, asters, zinnias, and a number of local orchids; also from skin of local oranges.

It was observed that bees, Apis cerana indica(Fab.), in Cherrapunji, where the experiment was carried on; preferred the scent of orchids, as when counted, around eighty percent of bees tend to hover around these scented dishes, than those compared with other scent, whereas in Shillong bees preferred roses and orange skin scent; and while performing these experiments an interesting observations was noted and, that is, that the bees were seen on an orange tree quite often as the tree was just

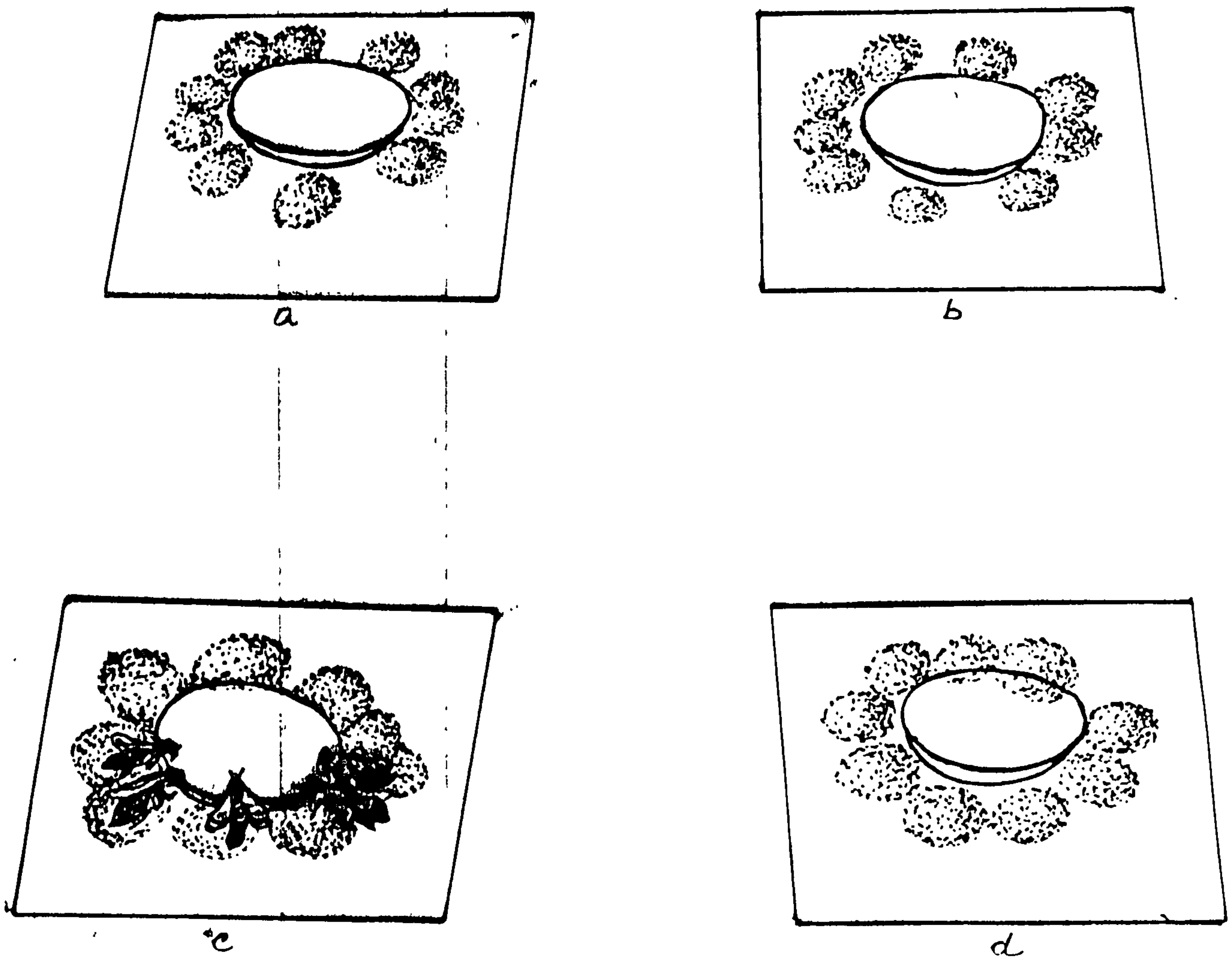


Fig. 73 : Worker bees (Apis cerana indica Fab.) are fed from a petri-dish surrounded with olive oil (c). The other glass-dishes are empty having different scents.

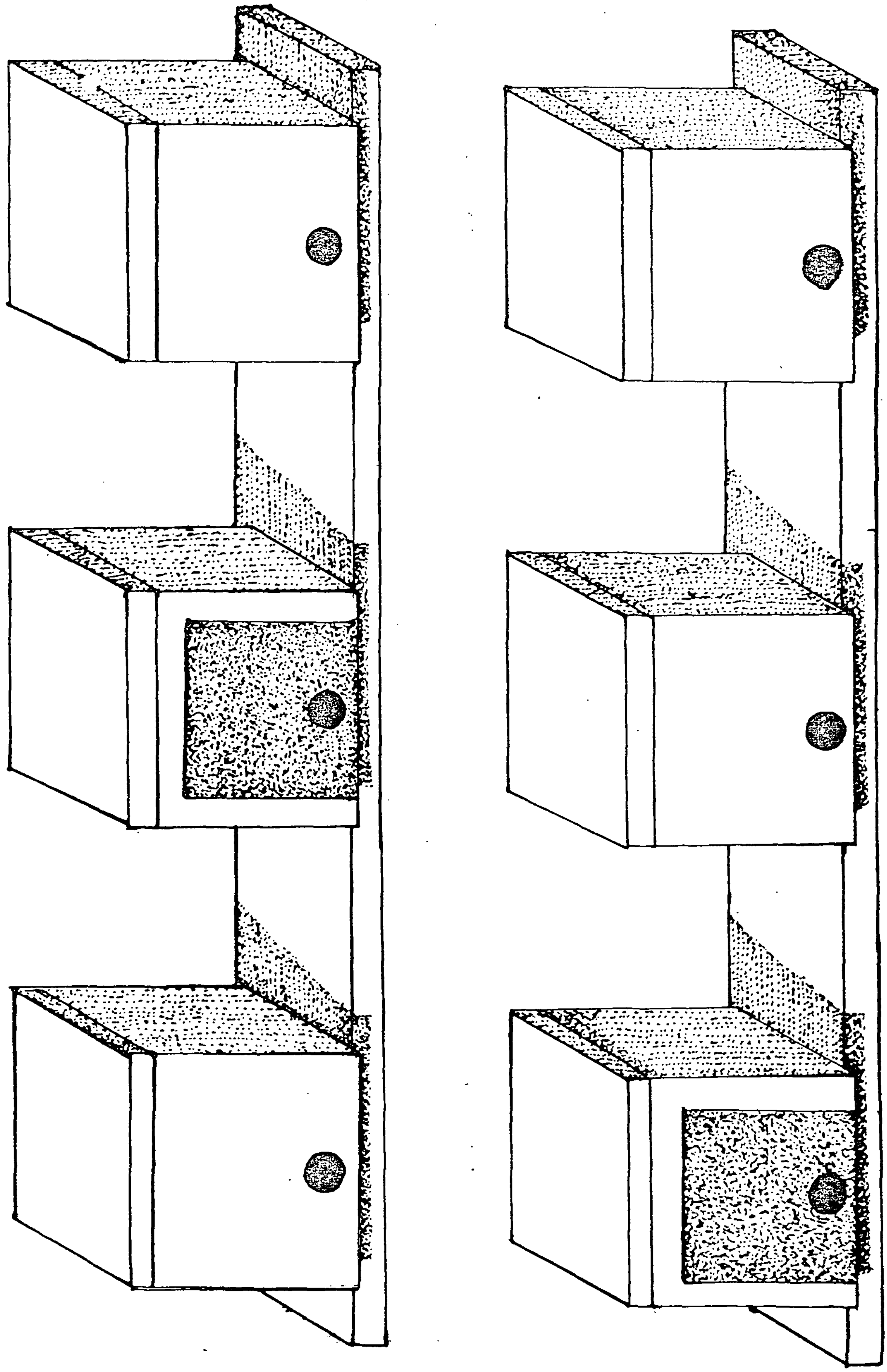
adjacent to the hive; which was not noted earlier. For bees which were fed on scented-sugar water seek diligently for the flowers whose scent was used at the feeding place, and they collect water more industriously than bees which have been fed on unscented sugar (Von Frisch, 1947).

From these and other experiments we can/may conclude that bees can distinguish between different qualities of odour just as well as a person whose sense of smell is very well developed. It also seems clear that odours which are similar for human nose are also similar for bees; and substances without odour to us are likewise odourless to bees (Von Frisch, 1919).

Bees are also very sensitive to the sense of smell; as when they are fed to a particular odour and on diluting the odour more and more, they are unable to distinguish between the odour and odourless site. When dilution had reached a stage where odour was no longer perceptible to us, we found that bees too had lost the ability to perceive it (Von Frisch, 1919); thus the olfactory sensitivity of bees is roughly the same as that of human beings. It can also be sure, therefore, that the scent of most flowers is insufficient to attract bees from a distance. According to Von Frisch, (1919) for bees to be attracted to any type/kind of scent colour is also essential.

On another experiment (Fig. 74), where six card-board boxes were kept, on the middle one, both scented and

Fig. 74 Worker honeybees _Apis cerana indica_ (Fab.) are trained to a scented box with blue cardboard surrounding the hole (above, middle box). Afterwards, during the experiment they find the blue color on left and the scent on the right of the former feeding place (below). They fly towards the colour from a distance of few meters, but they enter only the scented box.



coloured blue, the bees from a distance flew directly towards it. Later on the scent was kept in another box without colour and the coloured box on another side. It was observed that the bees are first of all attracted to the colour, but on approaching one hesitant, apparently seeking for the scent. And on perceiving it they alighted and went inside.

Apparently the colour was visible from a great distance, but the scent seemed to be more convincing of the two. Likewise the colour of flowers has the advantage that they can attract bees from a great distance, while the scent is specific for each species and thus permits the definite recognition of the flowers at close range (Von Frisch, 1919).

For bees, to be able to perform such interesting and wonderful behaviours, some special sensory organs must be present somewhere in the body. Von Frisch (1921) observed that the sense of smell is located in the antennae. Entomologists have known for a long time that insects can no longer react to scents if their antennae or 'feelers' are cut off. The antennae were, therefore, believed by some to be the organs of Olfaction.

Above all the association of touch and smell is very useful to bees as they visit flowers. Often they bring their antennae close to the flower, almost in contact with it, so that they can probably perceive even quite feeble odours.

An experiment to prove that the receptors for smell are located in the anntenna, similar to that of Von Frisch (1921) was conducted, where, bees were trained to a rose scent, later other scents were kept close by, but the bees recognized the scent kept earlier. Afterwards their feelers/antenna were cut off. When freed the bees still search for the trained scent but could no longer find it. It was only discovered by chance they were no more likely to visit the card with the training scent than any of the others. Such bees did not behave as though they were suffering from shock; they continued their search for food with vigour and persistence.

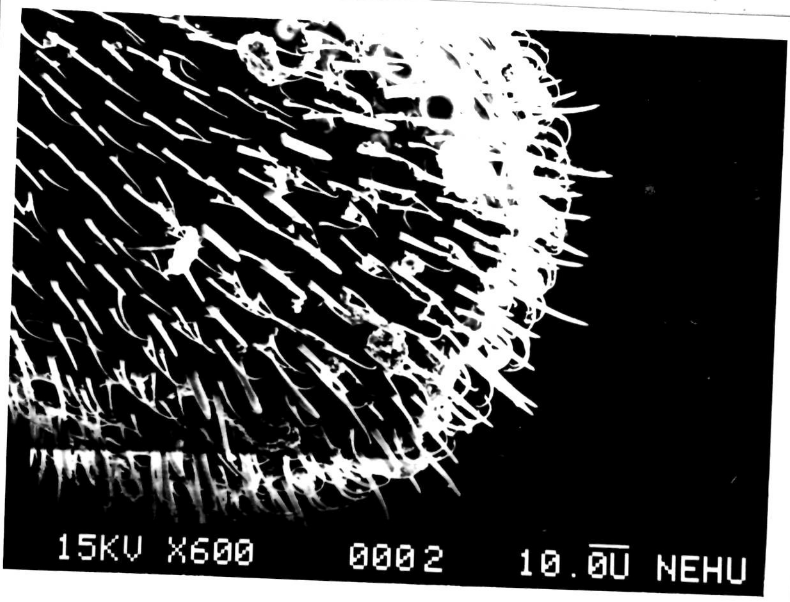
Some workers contradicted saying that by cutting the antennae the bees might have suffered from shock, but when bees were first trained to a yellow card over where a food dish was kept and later their antennae/feelers removed or cut, the bees found the yellow card without hesitation and never confused it with other colours. This proved that the operation did not cause any severe shock, for the bees still remember what they had learned but could not recognize a scent. Thus, the sense of smell is really located on the antennae.

An electromicrograph picture of the antennae shows that they are covered with a number of receptors on all the three castes (Figs. 75-77). Thus, it is now proposed to

Fig. 75 Scanning Electron Micrograph of the tip of the antenna of worker honeybee, *Apis cerana indica* (Fab.) x 600

Fig. 76 Scanning Electron Micrograph of the tip of the antenna of the drone honeybee, *Apis cerana indica* (Fab.) x 600

PLATE 42



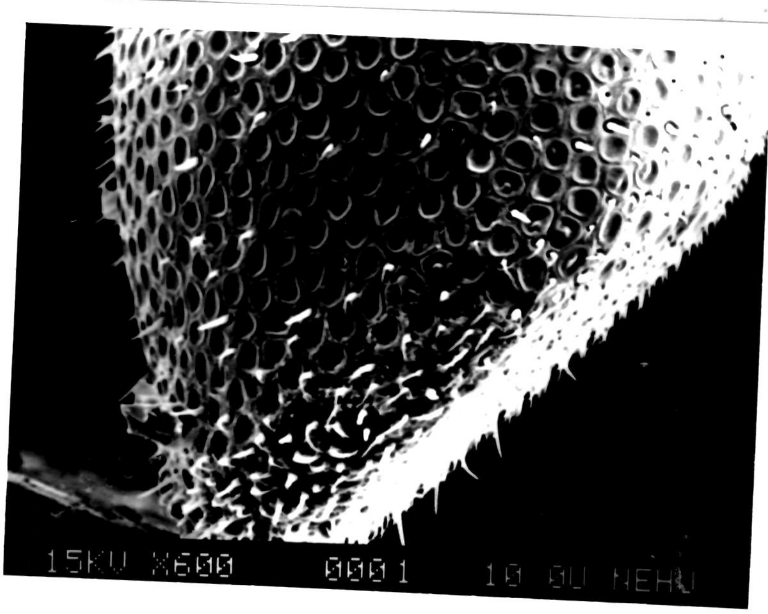
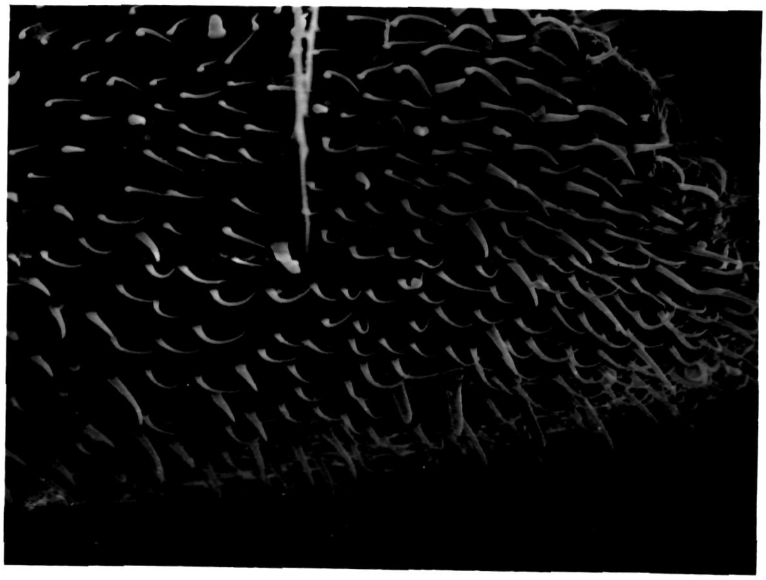
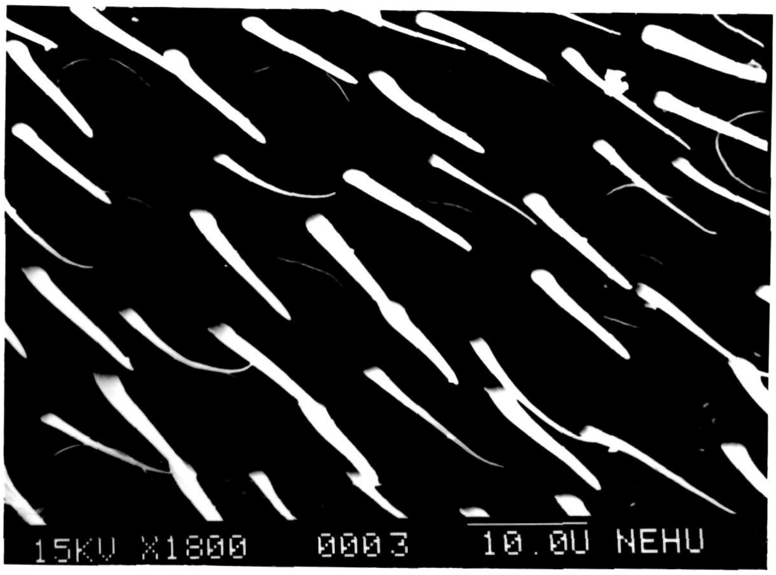


Fig. 77 Scanning Electron Micrograph of the tip of the antenna of the queen honeybee, Apis cerana indica (Fab.) x 600

Fig. 78 A magnified SEM picture of a portion of the tip of the antenna of worker honeybee, Apis cerana indica (Fab.) x 1800

PLATE 43





study the arrangement of these receptors of different types on the antennae of the workers, the queen and drones, and also why is it that the chemical senses is restricted to the workers caste only.

The different receptors detected in the antennae of the honeybee*, Apis cerana indica (Fab.) gives us a clear cut idea of caste differentiation based on the colony's behaviour as an individual component. The antennae of the three castes showed marked difference in the arrangements of the different sensilla on the corresponding segments. In the workers and queen the tip is almost rounded in shape with a maximum number of sensilla trichodea; but in case of the drone the tip is somewhat tapered with distribution of sensilla trichodea appearing maximum at the rim and sensilla basiconica and ampullacea scattered adjacent to the tip. In sub-sequent segments of the antennae also several differences in the arrangement of the receptors were marked which are discussed here individually.

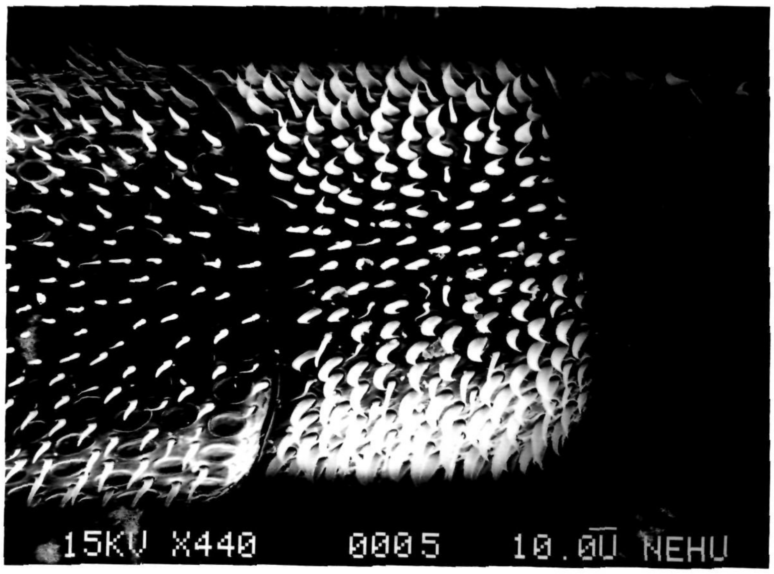
In the honeybee workers, at the first segment of the antennae, uniformly scattered sensilla trichodea of different shape was seen at the mid-portion, the density being more at the apex. Some of them have slim pointed shape, others are slim but curved at the tip, and few others with a broad base but blunt tip. Sensilla placodea was also marked, which was made up of a sunken pit with a double layered margin of negligible number, in the first segment. This arrangement is almost same till the eighth segment. But on the ninth segment, a marked difference was noted, where sensilla trichodea's density seems to dominate the other sensilla when viewed dorso-laterally. On both the lateral sides broad-based but curved and pointed tip sensilla trichodea were detected in huge number almost serially on the whole segment. Whereas on the dorsal side of the same segment other sensillae (basiconica and ampullacea) were also viewed in small numbers. Coming to the basal segment, the sensillae have reduced in number, all but one, sensilla trichodea exist till the last segment (Figs. 78-83).

In the other female caste, the queen, even though the shape of the apex is almost the same as that of the workers, yet it is not so smooth. The type of receptors in this segment is also like that of the workers, but out here the shape of the sensilla trichodea is mostly tiny even though slim and pointed and a maximum of these sensilla are curved, few broad based sensilla trichodea were noted at the rim. The mid-portion has a combination of all the sensillae mentioned above (i.e., trichodeum, placodeum and basiconica also very few ampullacea). In the subsequent antennal segments of the queen, the arrangement is more or less the same as that of the mid-portion. A negligible number compared with the other castes, or a uniformly scattered arrangement comparing different segments of the same. (Figs. 84-85).

Fig. 79 . SEM picture of the arrangement of the sensilla receptors on subsequent segments of the worker honeybee <u>Apis cerana indica</u> Fab.) x 440

Fig. 80 SEM picture to show receptors on the second segment from tip of the antenna of worker honeybee, <u>Apis cerana indica</u> (Fab.) x 2000

PLATE 44



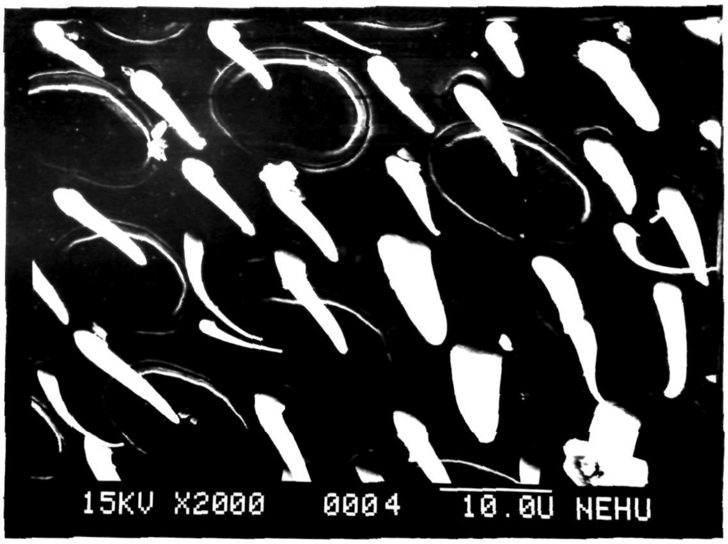
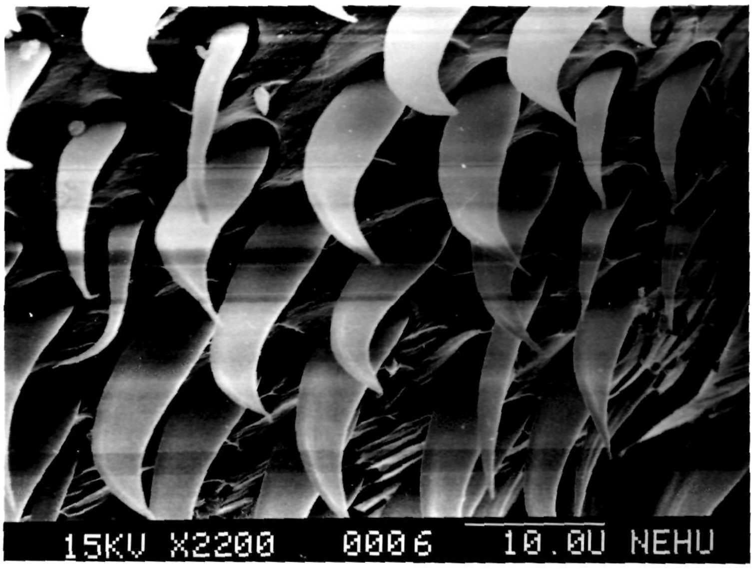


Fig. 81 SEM picture to show dominance of trichoid sensilla on the eighth segment of worker honeybee *Apis cerana indica* (Fab.) x 2200.

Fig. 82 SEM picture showing segments tenth, eleventh and twelfth from tip of the antenna of worker honeybee, *Apis cerana indica* (Fab.) x 300.

PLATE 45



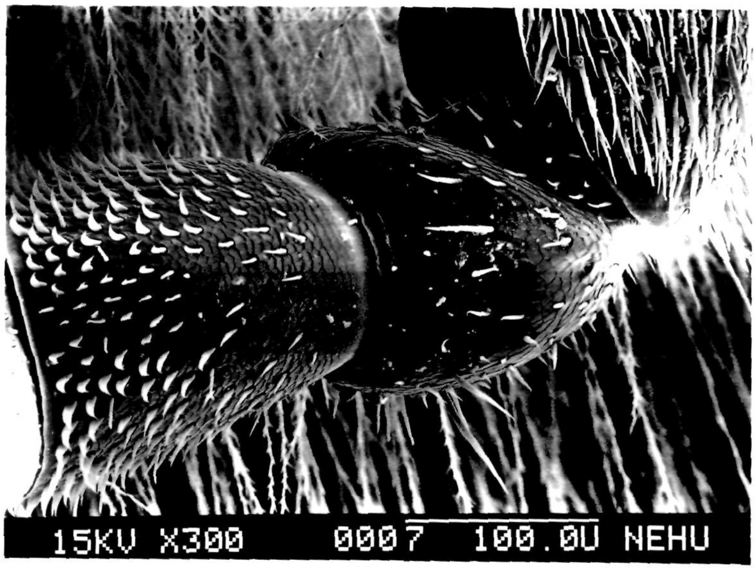
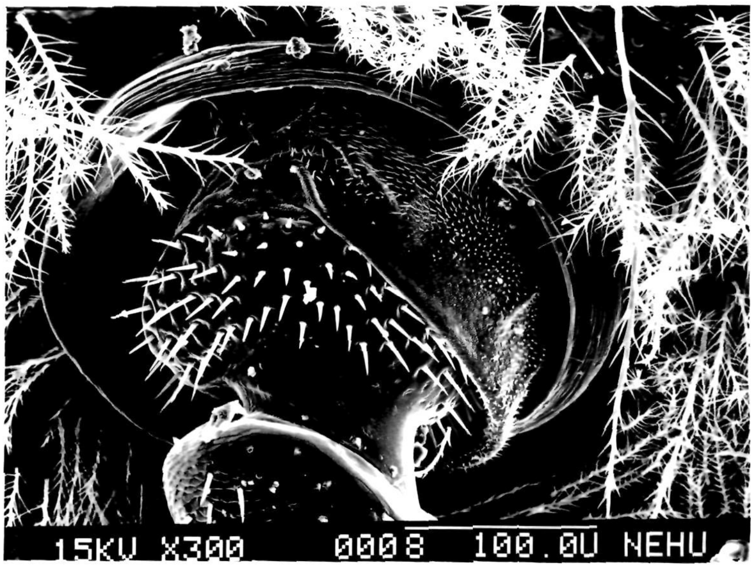


Fig. 83 SEM picture to show the basal segment of the antenna of the worker honeybee, <u>Apis cerana indica</u> (Fab.) x 300

Fig. 84 A magnified SEM picture of a portion of the tip of the antenna of queen honeybee, <u>Apis cerana indica</u> (Fab.) x 1800

PLATE 46



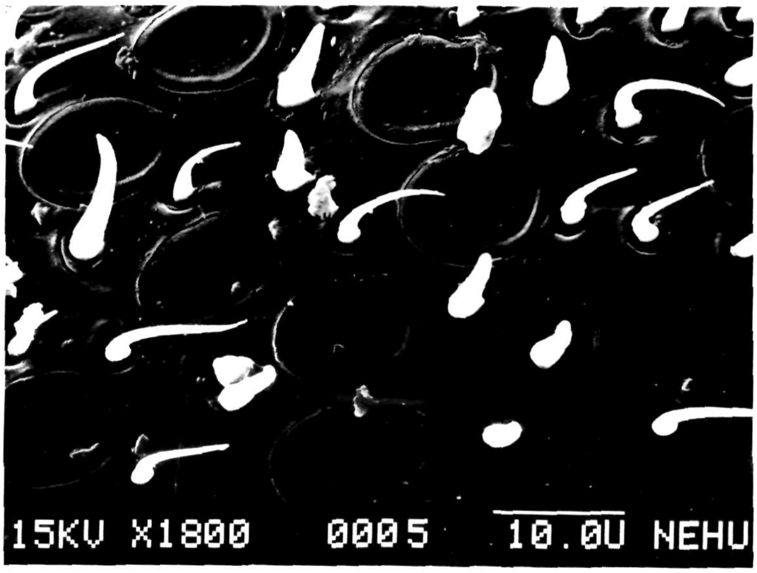
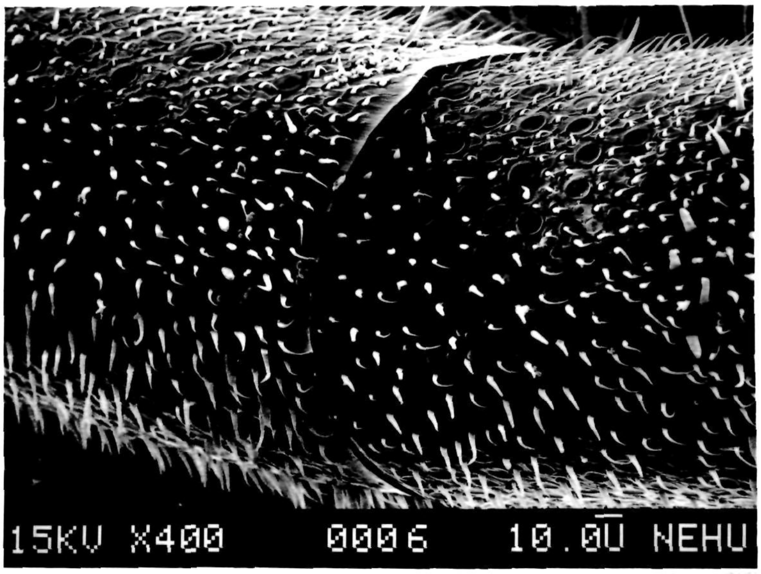
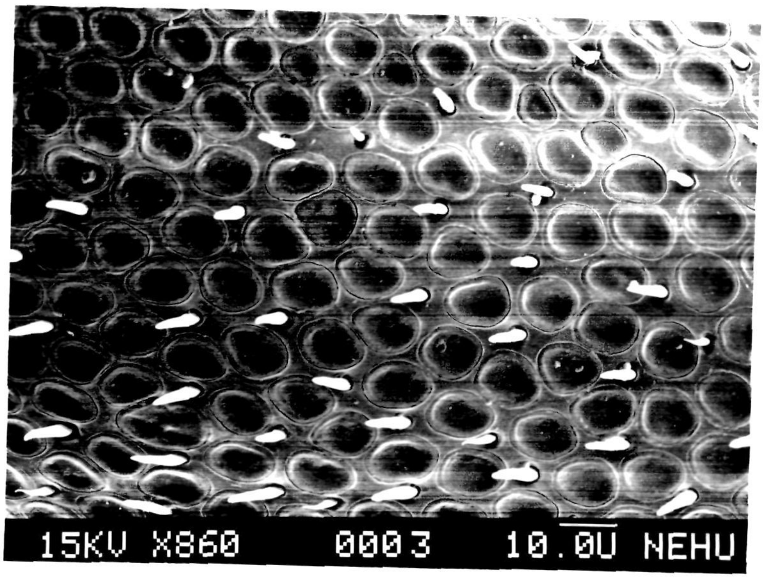


Fig. 85 SEM picture of the arrangement of the sensilla/receptor on subsequent segments of the queen *Apis cerana indica* (Fab.) x 400

Fig. 86 SEM picture to show the dominance of sensilla placodeum on the apical segment of the antenna of the drone honeybee, *Apis cerana indica* (Fab.) x 860

PLATE 47





Coming to the male caste, the drones, as mentioned earlier, sensilla trichodea is dense only at the rim, differing in shape, some long and slim, others long but curved with pointed tip, and few others curved right from the base having pointed tip. Sensilla basiconica is also scattered around 1/10 of trichodea, bulb-like in shape. But as one views the mid-portion of the tip, sunken pits, irregularly shape (almost rounded) double layered sensilla placodeum were seen [redacted] to have dominated the whole area being well dense. This nature of arrangement or of the dominating density of sensilla placodeum continues till the tenth segment. (Figs. 86-87).

On reaching the eleventh and twelfth segments, the reverse arrangement is seen, where out here, sensilla trichodea, having broad base with a bit of curved pointed tip dominating over sensilla placodeum whose presence is almost nil/ (Fig. 88).

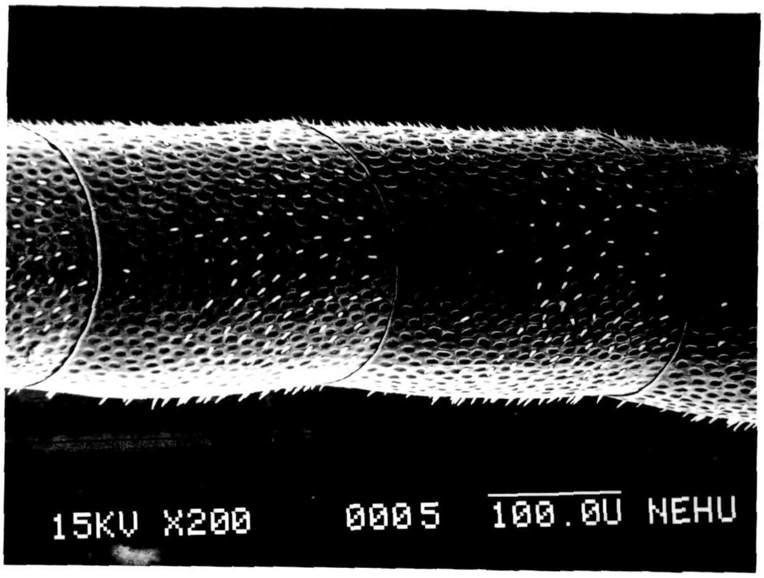
In the light of the above findings it is obvious that based on caste differentiation as well as on the division of labour even the arrangement of the different sensillas, their density, number, shape etc. differs in the different castes.

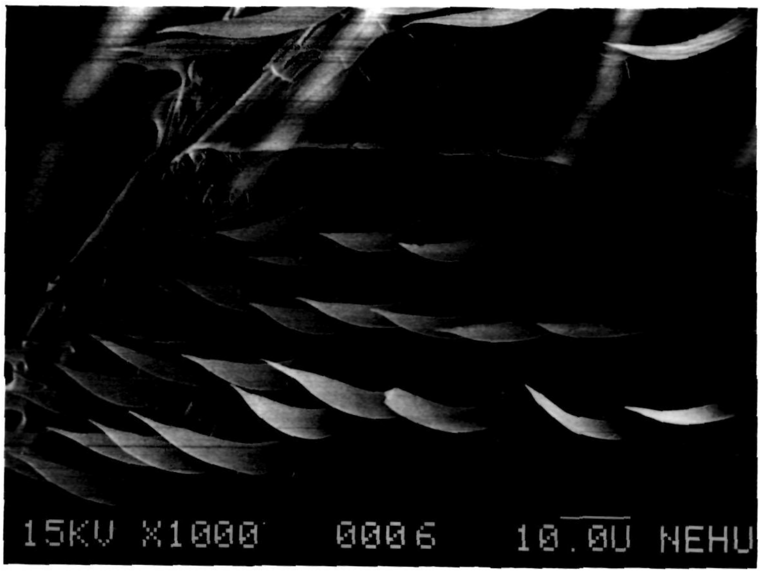
In the workers and queen being female castes, the arrangement of the sensilla is somewhat similar, but due to caste differentiation marked differences in the density were noticed.

Fig. 87 SEM picture to show arrangement of the sensilla receptors on the subsequent segments of drone honeybee, _Apis cerana indica_ (Fab.) x 200

Fig. 88 SEM picture showing segments eleventh and twelfth from the tip of the drone honeybee, _Apis cerana indica_ (Fab.) showing marked sensilla trichodeum x 1000

PLATE 48





On further investigation in one of the female caste, the queen, the presence of sensilla trichodea on the terminal segments helps the queen in finding a suitable cell for depositing an egg; its (queen) major function in he hive being that of laying eggs. It also helps the queen in coming in contact with a male caste (drone) for mating outside the hive. Thus these sensillae even though quite numerous are tiny and slim as the queen is not expose out much, compared to the workers. Sensilla placodeum helps the queen in uniting her colony and recognizing her own troupe; be it inside the hive or outside, while mating or swarming. Sensilla basiconica helps the queen in distinguishing between royal jelly and worker jelly mostly; where as sensilla ampullacea helps her intimate the worker regarding the temperature of the hive.

In the male caste, the drone, the maximum occurrence of sensilla placodea with a number of sensilla trichodea helps the drone in mating, the former one being responsible for the sense of smell and the latter one for the sense of touch. Specially on the eleventh and twelfth segment where the latter is quite dense. Sensilla basiconica was also marked at the tip of the first segment. Its function is not clear, it might have something to do with their food, as they are fed by the workers on *bee-bread alone (*stored pollen in comb).

Coming to the next major caste, the workers, it was observed that being an important caste of the colony and also a responsible individual of its home, the chemoreceptors are well marked viz., sensilla trichodea, which is responsible for the sense of touch (Ribband,1953 Thurm, 1964); sensilla placodeum for the sense of smell, (Boeckh, Kaissling and Schneider, 1965); Sensilla basiconica for the sense of taste (Minnich, 1932 and Von Frisch, 1934) etc.

Sensilla trichodea were seen to be well distributed, being a foraging insect, the bee, is quite particular in collecting pollens and nectar restricting its visit to a single species at a time, thus the sense of touch (trichodea) and smell (placodeum) is very essential. Sensilla basiconica also play a part in this case being responsible for the sense of taste. Thus the results of the different experiments conducted in this chapter would had ended blindly, had it not been for the works of the different scientists in the discovery of the functions of the different receptors individually.

As mentioned earlier that the chemo-reception of the bees is based on three types of senses, this has been proved true with the detection of these receptors responsible with the various senses respectively.

Most other insects also have the organs of smell confined to their antennae. But in several cases there are olfactory organs on other appendages of the head as well, i.e., on the palps of butterflies and of the water beetles, <u>Dysticus</u> (Von Frisch, 1921).

Insects can distinguish four qualities of taste (sweet, bitter, salty and sour). This experiment has been worked out with some water beetles sps. (Hydrous, Dysticus and Cybister). These insects when seeking their food in the water can perceive the taste of food as it diffuses for some distance. Hence one can train them to find sources of food by the sense of taste. Water beetles trained to find food along with salty-, sour-, sweet-, or bitter-tasting substances can distinguish any one of these taste with certainty from the other three qualities (E. Ritter, 1936; L. Bauer, 1939).

Some animals have sense organs of taste not only in or near the mouth but on other parts as well. Professor D.E. Minnich (1922) found that butterflies could taste with the tip of their legs. If a butterfly steps into a sweet substance it instantly stretches out its proboscis, and often begin to feed. In this case the sense of taste is adapted to detect food as well as to regulate its intake. Therefore a low threshold seems to be useful, for even a small amount of sugar can serve as a guide to a source of food. As a matter of fact, the taste receptors on the legs of butterfly are the most sensitive ones known to date. In flies, and other insects too, a well developed sense of taste is present on the tips of the legs (D.E. Minnich, 1922, 1929; F. Hastinger, 1935).

Same is the case with bees, as when fed with a sugar solution and added enough salt so that the bees rejects it.

At another time, the bees where fed with a mixture of sugar and quinine, bitter enough that they reject it. These two substances if they have the same taste for bees, they would have aroused the same sensation of taste. However, this was not the case, as when starved bees, were released they took to that bitter mixture even though it is six to seven times concentrated, but in another case, bees cannot take in a higher concentration of salt. Bitter and salty, therefore cannot be the same quality of taste for bees. In a similar fashion it is proved that sour is also distinct quality of taste (Von Frisch, 1934).

Thus, Von Frisch (1934) concluded that honeybees are a little more sensitive to salt than human beings are. The same is true for materials which taste sour. But to bitter substances bees are much less sensitive than human beings. They seem to enjoy a mixture of quinine and sugar which is so disgusting to the human taste that anyone would spit it out at once.

Bees also have sense organs of taste located on the mouth parts in order to examine food when it is taken up. Bees can recognize a sweet solution only when they come in contact with it, but they are rather fastidious about sweetness.

It is also significant, biologically, that bees do not collect for storage in the hive, solutions with a low concentration of sugar, although they may use them for their own nourishment, as, if stored in the honey-combs such solutions would not be kept until winter.

Thus it is always the worker who is playing an important role in carrying out the different activities and labour of the colony as a whole. And that is why nature has endowed something extra and special to this one caste of honeybee compared with the other castes. Out here Lamarck's theory (1809) of:- "use and disuse cause variation in a structure" applies well as in case of bees, among the three castes, it is only the worker who is playing a major role for the existence of its colony, so it possesses all the important characters which the other two castes do not possess since they do not need them; not overlooking/neglecting, of course, that they are specialized in other aspects, like the queen, being an egg-laying machine, and the drone a mating element; making them essential individuals of the hive, in their own way.

CHAPTER IV

COMMUNICATION IN WORKERS HONEYBEE OF *Apis Cerana Indica* (Fab).

INTRODUCTION

The biological significance of communication with respect to food supplies in highly eusocial bees is considerable. First, it takes no imagination to recognize the advantage to the colony in the use of distant food resources if the finder can guide or direct inexperienced colony members to the source, or can notify experienced bees that a resource with which they are familiar is again productive. Secondly, the colony rather than the individual becomes the foraging unit. This promotes distinctiveness of the colonies, because even nearby colonies commonly take in different foods or the same foods in different proportions. Communication, however, leads to distinctive colony odours and mutual recognition, which permits defense against robbing (Michener, 1974).

In general, biological communication can also be defined as action on the part of one organism (or cell) that alters the probability pattern of behaviour in another organism (or cell) in an adaptive fashion. By adaptive it is meant, that the signalling or the response, or both, have been genetically programmed to some extent by natural selection. This broad definition of communication still leaves some kind of interaction in nature ambiguously classified, particularly various predator-prey relations among species, for the moment, however it is adequate for the social insects (Wilson, 1971).

The modes of communication found in the social insects are diverse. There exist the expected tappings, stridulations, strokings and grasping; antennations, tastings, puffings and streakings of chemicals which evoke various responses from simple recognition to recruitment and alarm.

According to Wilson (1971) three generalizations are useful in gaining perspective on this subject. First, most communication systems in the social insects appears to be based on chemical signals. The known visual signals are sparse and simple. In some groups, such as termites, they play no role in the day-to-day life of the colony. Airborne sound is only weakly perceived by these insects and has not been definitely implicated in any important communication system. Some social insects, on the other hand, are extremely sensitive to sound carried by the substrate, but they evidently employ it only in limited fashion, chiefly during aggressive encounters and alarm signalling. Modulated sound signals evidently play a role in recruitment in the advanced stingless bees of the genus Melipona and in the honeybees, which have incorporated them in the waggle dance.

In contrast, chemical signals, or pheromones as they are now generally called, have been implicated in almost every category of communication. Pheromones, as the chemical releasers were first called by Karlson and Butenandt (1959), may be classified as olfactory or oral according to the site of their reception. Also, their various actions can be distinguished as releaser effects, comprising the classical stimulus-response mediated wholly by the nervous system; or primer effects, in which endocrine and reproductive system are altered physiologically. In the latter case, the body is in a sense "primed" for new biological activity, and it responds afterwards with an altered behavioural repertory when presented with appropriate stimuli (Wilson and Bossert, 1963).

The second generalization is that most of the communication system have parallels in behaviour patterns already present in some form or other in solitary and presocial insects. Nest building is a case in point. The primitive ants, termites, and social wasps build simple nest compared with their solitary relatives. Elaboration of nest structure occurred in certain phyletic lines after the eusocial state was attained. The dominance hierarchies that play a key role in bumble bees and wasps societies have a precedent in the territorial behaviour of many solitary insect species, including a few hymenopterans. Elaborate brood care, a hallmark of higher sociality, has its precursor in progressive larval feeding in a multitude of subsocial species belonging to several insect orders. Even the elements of the honeybee waggle dance, the distant apex of insect social evolution in the eyes of most biologists, have precursors : the modulated rocking behaviour of saturniid moths, which varies in duration according to the length of the flight just completed and thus resembles the straight run of the bee dance; the oriented "dances" of hungry Phormia regina flies which have been given a small drop of sugar water; and the ability of some solitary insect to shift from light to gravity orientation when placed in dark vertical surfaces.

The third generalization about communication in insect societies regarding the remarkable qualities of social life are mass phenomena that emerge from the integration of much simpler individual patterns by means of communication. If communication itself is first treated as a discrete phenomenon, the entire subject becomes much more readily analysed. In highly eusocial bees the colony size is usually relatively large, and the problem of maintaining the biomass of the colony on food brought in minute quantities from dispersed sources must be considerable.

There is in general a positive relationship between biomass of colonies, probable foraging distance, and effectiveness of communication. Thus, among the highly eusocial bees, and particularly among those living in large colonies consisting of moderate sized bees, good communication concerning food sources exists. The greater the flight range the greater the need for such communication. Hence communication is better developed in larger Apinae than in the minute ones. Poor communication in minute species, considered to be primitive by Kerr and Esch (1965), quite possibly represents instead a loss associated with small size and the adequacy of olfaction for finding food in forms with limited flight ranges.

In *Apis*, communications concerning the distance and direction to food sources is in part by means of the famous dances first elucidated by Karl Von Frisch, (1967) whose great work on the subject explains the dance communication in detail. Lindauer (1967) summarizes some of the recent findings. The mechanism serves not only to alert or recruit potential foragers and get these new foragers to food supplies that have been discovered by other members of the colony; but also serves to get them there in approximately the right numbers to exploit those supplies (Nunez, 1971). Honeybee dances appear to communicate information for vector orientation and sometimes locality knowledge (by odour). Although the effectiveness of the dances in transmitting vector information among bees has recently been questioned, as is explained,the existing of some sort of communication is not in doubt. It is perfectly plain that the number of bees going to a food source after a bee has found it and returned to the hive varies with the concentration and accessibility of the syrup and inversely with the density of bees already exploiting it. Thus the number of been that goes to a food sources is not vastly in excess of the number that can been accommodated there. No one questions that a food source may go unobserved by bees for days and then, when a bee finds it or is artificially introduced to it and is allowed to return to its hive, more bees soon appear there. The only question is whether the physical features of the dances provide vector information. The alternative is that odours, providing for object orientation and locality knowledge, are responsible for such behaviour, (Michener, 1974).

MATERIALS AND METHODS

This experiment has been dealt in detail for *Apis mellifera* by Karl Von Frisch; but very less information is available regarding the Indian bee, *Apis cerana indica*, (Fab.).

According to him if one wishes to attract honeybees he has to supply some food to them within the vicinity of the hive. And this chapter deals with the communication in bees mainly regarding their nutrient; so it is very essential to train bees to a particular spot where food (sugar water or honey) is provided.

Works performed by some scientists : Wolf, Santschi and Frisch have enlightened us profusely regarding communication in honeybees. These include their language in the form of dances like the round dances for short distance availability of food (below 100 metres) and the waggle dance for longer distance availability of food (above 100 metres). Also the help of polarised light like the angle of earth"s rotation and the apparent motion of the sun; and also the direction of gravity where the sun is not available.

In the first set of experiment we have attracted honeybees to a feeding spot which is within the vicinity of the hive and have mark them with coloured paints so that we can recognize them. When they return to their multitude, and to observe how they communicate with their colony members. This experiment was performed several times by keeping the feeding dish near a bed of flowers which emit scent, also by spraying some scented oil to the background of the experimental table. A specific timing is also kept to observe the timing memory of the honeybees.

In our second set of experiment the feeding site is kept quite far from the hive, and as this experiment take a longer time for the honeybees to identify or to spot the feeding place as spotting happens accidentally, so to enhance this experiment, we have collected some honeybees from the colony and gently allowed them to alight on the feeding site and freed them to find their way home. The bees when returning home do communicate with their co-members and after several days of observation it is observed that maybe they learned more about the distance and timing by the help of the sun along with the direction of gravity. This experiment was also repeated several times to get a better idea regarding the aspects a honeybee requires in imparting the news to its fellow members.

RESULTS AND DISCUSSION

The importance of floral scents in *Apis* communication has been known for years, as indicated in the reviews of Ribbands (1953) and Frisch (1967). Returning foragers closely approach, contact, and even feed bees that are ready to go foraging. The latter thereby learn the odour and taste of the returning foragers, probably are stimulated to leave promptly, and once in the field are frequently attracted by the odour and taste learned from the returning foragers.

Bees usually attract other foragers to a feeding spot not only by scent or odours but also by the secretion secreted by their nasanov gland, they leave trails of odour on the feeding area and emit some at the hive also. This guides as a pathway for their fellow members to go in search for food at or to a specific place.

But this information is not sufficient where the food is at a longer distance from the hive, for if this is the only sign of communication then it would take days, weeks and even months for a particular bee to locate the feeding site and it could happen that by this time the food is already exhausted. Mere random searching by *Apis* workers should get only some of them close enough to food sources that the chemical as well as visual cues would prove effective.

Wolf (1927) has demonstrated that bees do have a "distance" scent. Bees being social insects living in a colony, When their hive is shifted to a new place we have noticed that the first thing in the morning they would be hovering around the hive and later fly around the whole neighbouring site so as to be accustomed of the new surrounding area. Wolf has also shown that bees learn the position of visual land marks in their surrounding area and use them for orientation.

This experiment was performed as Wolf suggested, that is, by keeping a blue coloured tape as a guide to the feeding site; for a short distance. It is observed that honeybees (Apis cerana indica Fab.) follows it.

Bees do not always find their way by means of visual landmarks; they make use of several different sensory impression.

To learn further about how bees locate the feeding site and return back home and to note whether only the landmark or other impressions have helped the honeybees (Apis cerana indica Fab.)in successful foraging, we have first of all trained bees to a feeding site east of the hive, a distance of about 50-60 metres from their hive. Later when the honeybees are well trained, i.e., they are seen to fly to and fro from their hive to the site; we marked some of them with different coloured paints (bright coloured nail polish has been used for the paint as it dries off very fast (Root, 1966); and liberated them individually at other different points, other than the east (north, south and west). It was observed that they returned back home at different time intervals. The once from the original feeding site (east) took about half-a-minute, the others to the west took more than five minutes, while others north and south took an intermediate time. This happened because, as the honeybees have been already trained, they have somehow memorise their way home, but due to liberation at new places, they got confused and were lost for sometime, specially in the honeybees due west flew further thinking that they are in the east, for locating their nest.

Honeybees has a rudimentary form of odour trail which is employed for short distance orientation in special circumstances (Lecomte, 1956; Butler, 1966 b; Butler, et al. 1969). The most suggestive evidence has been reported by Butler in his initial article on the subject, when he changed the position of the hive entrance of one of his colonies, the first forager to return managed to find the new entrance only after random searching. When a few had succeeded in walking the whole distance from the former location of the old entrance to the new entrance, however, new comers were able to follow the trail on foot. The "foot print pheromone" has not yet been identified, deposited around the rest and food dishes and makes them both attractive. This particular experiment has also proved effective in the case of the Indian honeybee, Apis cerana indica, (Fab).

Von Frisch (1967) has shown that foraging honeybees also measures "distance" or "time spent in flying" between hive and source of food. This is done by appreciating the amount of energy she had spent on her journey. The honeybee on returning to her hive after a successful foraging expedition, i.e., after several to and fro visits, give this information to her members, by means of dance language when recruiting them to exploit the rich source of food that she has found.

In 1923, Von Frisch published his work which shows that it is by means of particular dances of successful foragers on the combs in the hive that other honeybees are recruited to work on particular crops. The two main dances of which a foraging honeybee is familiar with are, the "round dance" which indicates a food distance around the vicinity of the hive, and the "waggle dance" when the food distance is far off from the hive. These dances are not performed in flight; but the honeybees walk or run over the surface of the combs of cells, that is, they dance on vertical surfaces in the dark nest surfaces also supporting thousands of other colony members.

The "round dance" communication indicates a food source only a short distance away from the hive. A successful forager usually when returning back home from a shorter feeding site promptly offer her crop contents (unless she has collected pollen) to other members waiting in the comb. (Fig 89). These are mostly young workers who accept the nectar and either pass it on or process it into honey and place it in cells where it will be stored. As is in a majority of cases these workers honeybees are not ready to forage, they usually help in household duties. Others however, are older workers which are ready to forage, as is seen that such bees not only accepts bits of syrup from the incoming successful foragers but they also follow her on the combs, maybe due to the attracted odour her body emits. This particular honeybee worker thus dances her dance.

The round dance is similar to the waggle dance except that it lacks a straight run, the worker honeybee (Apis cerana indica Fab). runs round in a small circle (Fig 90) reversing and going in the opposite direction after every turn or two, for about half-a-minute or even less, this intensity depending on the amount of food availability and its percentage of sugar content. Peculiarly it was also observed that the other young household operator worker honeybees are somewhat repelled by the dance, they simply move away, as if making space for it, whereas, the forager worker honeybees touch the dancing honeybees and maybe recruited to the food source about which she is dancing.

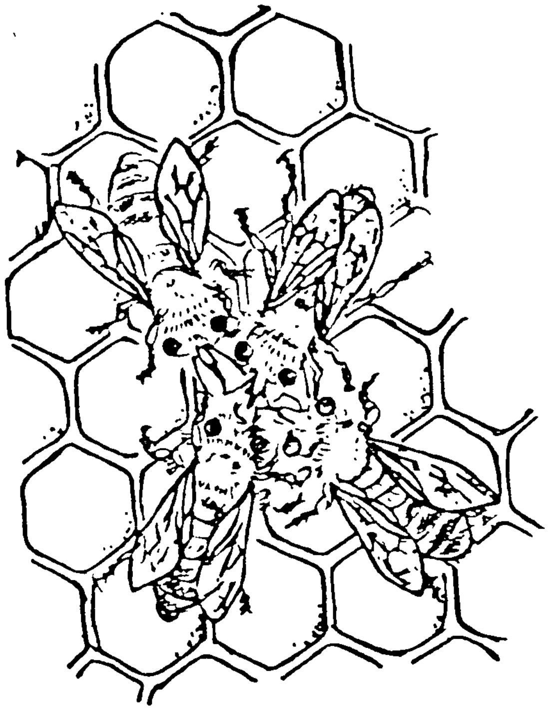


Fig. 89 - Diagrammatic representation of liquid (nectar) transfer in Apis cerana indica. The lower left bee is giving nectar to other three workers

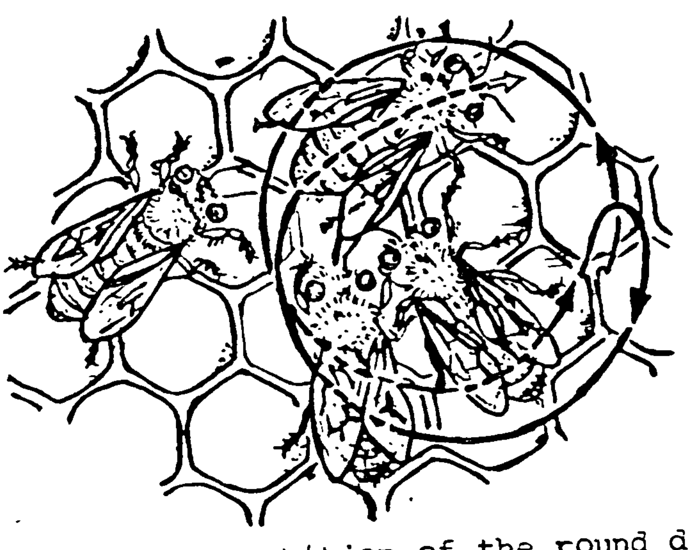


Fig. 90 - Diagrammatic representation of the round dance of Apis cerana indica. The upper worker is dancing along the arrow and is followed and antennated by other workers.

As this particular dance indicates nothing of the distance to the outgoing foragers; those incited by it who have had no previous foraging experience in the nest vicinity search at random close to the nest. Those who have earlier foraged in the nest vicinity may recognise the scent of the flowers adhering to the dancer's body and head; was from the spot where they have previously encountered the same type of flowers.

Other honeybees in the colony stimulated by the dancer touch her by their antennae, (Fig. 91) often trailing after her as she dances or cuts across the circle to catch her up. Her followers pay special attention to the pollen she carries if she has not unloaded them. The recruited foragers later grooms themselves engorge some honey for energy needed for the prospective trip and within a minute, leave the hive.

Learning through the odour of the nearby source from antennating the dancer herself and from tasting some of her nectar, the recruited bees, even if unfamiliar with this particular source are able to find it.

Another interesting point to note with regards to dancing of bees, is that, it occurs only when food is abundant, attractive, and the site not already crowded with other bees. The more desirable it is, the more likely are the dances, and more vigorous and long-lasting it will be. And this of course would result to recruitment of more bees to the food source.

Fig. 91 Photograph showing the workers following a dancing worker bee after returning from a food source.

Fig. 92 Photograph showing a worker bee inspecting a source of food, in this case nectar and pollen of aster flower

PLATE 49

Vigour or liveliness of the dance is not easy to measure but according to Von Frisch (1967) it increases with sugar concentration and is easily recognized by an observer at its extreme, it leads side to side with abdominal vibrations similar to those that occur in the straight run of the tail wagging dance (Wittekundt, 1960).

Presumably, vigour adds to the communicative effectiveness of the dance. Interestingly there is a social control of vigour. Vigour is also related to promptness/eagerness with which house bees react to the loads of food being brought in. For the same food source, bees will vigorously dance if few are returning from it. When many bees are returning, there is competition among them for the attention of house bees, thus vigour diminishes (Steche, 1957).

Another type of communication by dances of honeybee, is the famous "tail wagging dance". In German it is known as Schwanzeltanz. It is quite a famous type of all the forms of animal behaviours. Its fame stands both from the uniqueness of the mode of communication involved and the thoroughness are craftsmanship of the work of Karl Von Frisch. This dance permits transmission of chemical information like floral odours, exactly as the round dance, facilitated at extraordinarily rich food sources by the secretion of the nasanov's glands, and has the same control based on food richness, abundance, and exploitation, so that about the right number of foragers is recruited for each food source. In addition to these, it contains information on distance and direction to the food source. It can be said, that it is a signal constructed from a ritualized and miniaturized imitation of the journey that the signalling bee has taken in the past and upon which some of the colony sister members are about to embark. By following the dance, the receiving bees rehearse the journey in miniature and prepare to translate it into a real flight. when they really take off, it can be said that they were sent and not led to a goal. The difference in this (Waggle) particular dance is that, it is a truly symbolized message that guides a complex response after the message has been given. In almost all known forms of animal communication individual signal, usually contains very less information, than a single waggle dance and, unlike the dance are effective only while in existence. Even in most acts of chemical communication the behavioural effects cease very soon after the active pheromonal spaces disapear.

A typical tail-wagging dances consist of a short straight run, with the bee turning to one side and returning by a semi-circle on the opposite side of the straight run. Thus making is a roughly circular dance consisting of two halves. The straight run is emphasized by vigorous shaking of the abdomen from side to side, approximately around 14-15 wags per second, and accompanied by a buzzing sound made by the flight musculature and skeleton but without noticeable wing beating.

As in the communication by the waggle dance in Apis mellifera examined by Von Frisch (1964) in the fields, the waggle dance of workers of Apis cerana indica Fab. were examined in the fields of Cherrapunji near Shillong.

A scout worker bee after discovering a rich source of food of about the distance of 250 m., from the hive, of which in most cases, be a cluster of flowers (Fig. 92) bearing nectar and pollen, the usual food of the honeybee; on entering the hive mounts on one of the vertical combs, and crawls to a position that it will be able to communicate its findings to its fellow members. The bee is next observed to regurgitate nectar from her crop to the surrounding nestmates and then begins the dance in the midst of her multitude. She runs through a figure-eight pattern; a straight run, then a turn to the left and circle back to about the original position; takes another straight run, a turn and circle to the right and so on (Fig. 93).

The straight run is the most distinctive and informative element of the dance. The vibration of the bee in this dance is greatest at the tip of abdomen and least marked at the head. The duration of the straight run also coincides approximately with the duration of the buzzing sound emitted by the flight mechanism of the dancer; it is quite likely that the outstretched antennae of bees

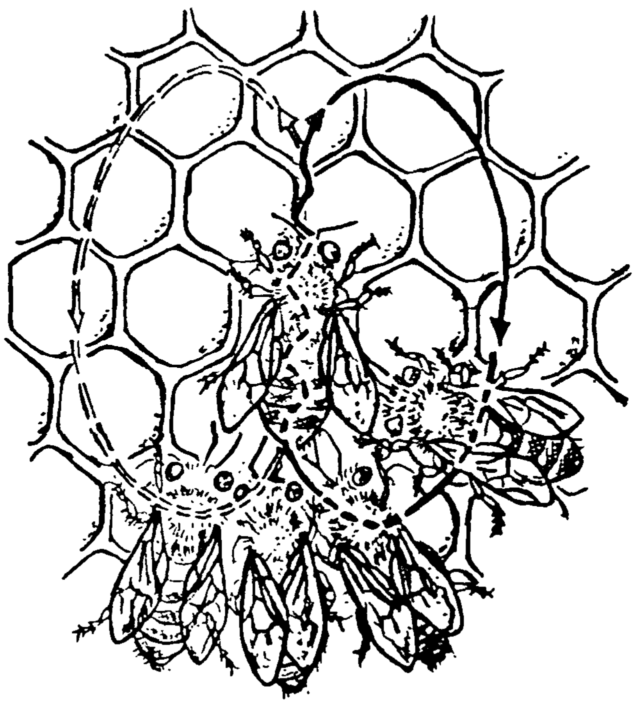


Fig. 93 - Diagrammatic representation of the tail wagging dance of <u>Apis cerana indica</u>

following the dancer are as much to perceive the vibration as the odour of the bee (Esch, 1961; Esh,Esh and Kerr, 1965). Increasingly the belief is developing that the duration of the vibration is likely to be the most important distance-indicating cue in the dance because, silent dances do not recruit foragers. Eskov (1969) places emphasis both on duration of the sounds and the number of impulses, which, however, is directly related to the duration.

Bees are living organisms not precision machines (Frisch, 1967); thus all features of the dance of a given individual somewhat vary. Moreover, workers of a given colony are not identical in their indication of distance. Age could be one of the factor which diminishes tempo; temperature should also not be neglected.

Distances indicated by dances are not actually measured as distances, instead the dance indicates the amount of energy required for the outward flight, or some factor correlated with this amount, somewhat modified by the requirement for the homeward flight.

A mere knowledge of distance to a food source would not be of great use except close to the nest. But it is interesting to note that the tail-wagging dance also indicates direction; which have made this type of dance communication an astounding accomplishment of nature.

Apis workers at times dance on horizontal surfaces, at times, just outside nest entrance. This can also be observed experimentally by turning the vertical combs on their sides, on a clear and sunny day, the bee maintains the same angle relative to the Sun as on the flight to the source.

Usually, dances occur on vertical surfaces in the dark of the nest. Here the dancer changes the angle with respect to the Sun, to an angle with respect to gravity. This is perceived by the help of sensillae present in the legs, head and abdomen (Fig. 94).

The dancer varies her tail wagging run according to the direction of the food source. A straight-on-run means "fly towards the sun"; down run means "fly away from or 120° from the sun"; and 60° to the left of the direction towards the sun". (Fig. 95).

The information to the recruit is obtained by touch and odour on a dark, vertical oriented surface. In some cases this dance is repeated outdoor just at the nest entrance. But this is rarely noticed in case of *Apis cerana indica*. The recruits then fly in approximately the direction with respect to the sun indicated by the dancer.

Minor variations in dance are always a regular phenomena. If recruits follow single dances and left they would often fly in somewhat different directions. Esch and Bastian (1970) showed that recruits follow atleast 6-7

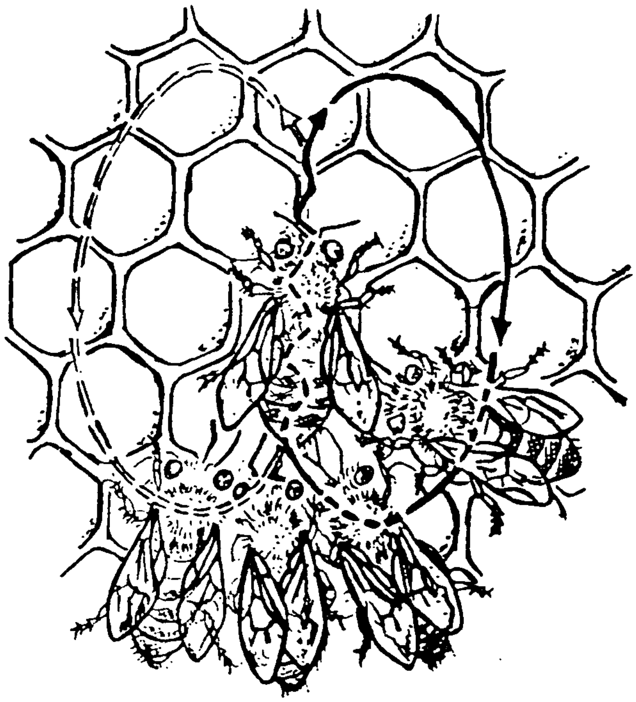
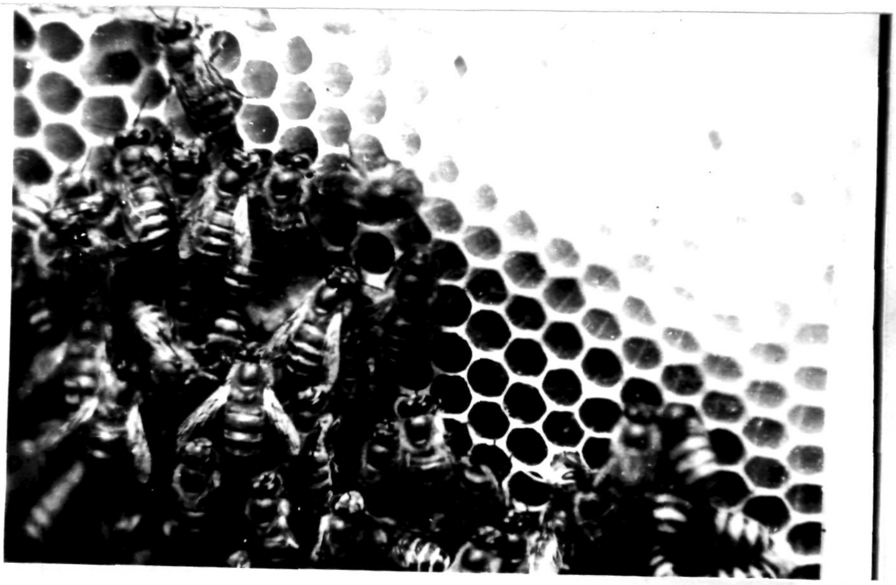


Fig. 93 - Diagrammatic representation of the tail wagging dance of <u>Apis cerana indica</u>

Fig. 94 Photograph showing the worker bees perceiving the waggle dance from their fellow worker bee who has discovered a rich source of food

PLATE 50



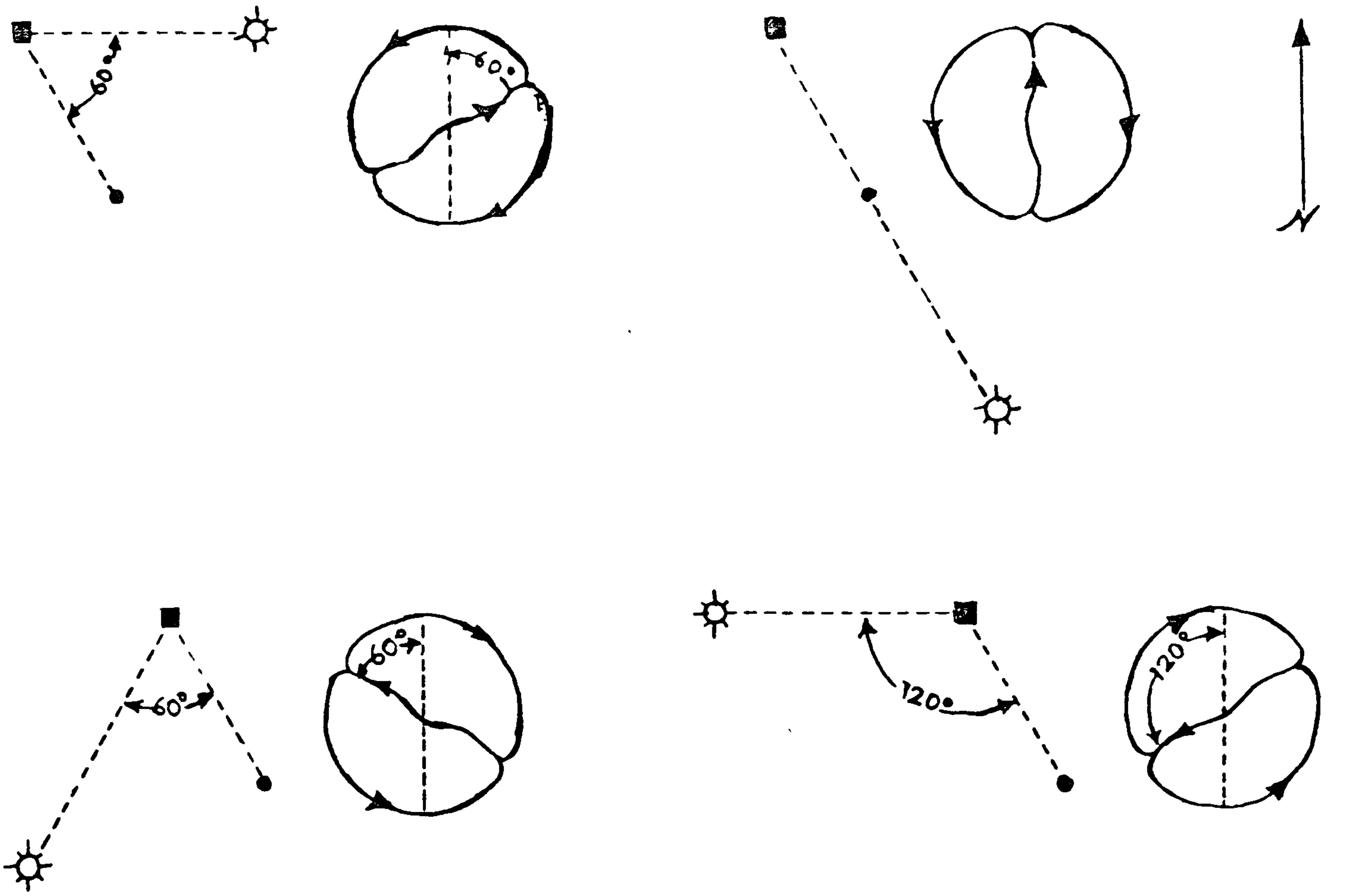


Fig. 95 - Diagrams showing the varying orientation of 'waggle dance' of *Apis* workers on a vertical surface at different relative positions of hive (square), food source (dot) and Sun. The dance orientation changes during the day at a single hive with one food source.

dances and Mautz (1971) found them following from 6.9 to 12.8 dances; before they successfully fly to a food source. Foragers that make fewer dances on returning probably stimulate less bees to activity but does not direct to the accurate position of food. Thus the significance of many dances direct the recruits to have a variety of idea and possibility to reach the food source.

A sickle dance is also noticed for distances more than 90 metres but lesser than that needed for a wagging. The flight direction elicited by sickle dances is the bisector of the angle formed by the two waggle runs. This was not observed by us in the case of Apis cerana indica. But the fact cannot be denied that it might be there.

In natural situation foragers returning from different food sources presumably ordinarily have different odours, so that recruits can easily follow sequential dances by bees returning from the same place. But under conditions where bees from two different artificial food source carry identical odours, bees that follow dancers may follow first a dancer from one, then a dancer from the other. Such bees integrate information from the two dancers and fly in an intermediate direction, (Lindauer, 1971).

The evolution origin of the waggle-dance and its incorporation of gravity cues are not really so mysterious as they might at first seem. Ritualization of functional motor patterns is a common place in communication systems of other animal species. In fact, almost all signalling among members of the same species consists of a re-enactment of the desired response, a "follow me" invitation often in the form of an incomplete intentive movement or imitation. Esch (1967a,b) has discovered a communication form in the stingless bees, Melipona quadrifasciata that not only illustrates this principle in graphic manner but also reveals the intermediate stage in evolution of the waggle dance of Apis mellifera.

Although the communicative function of the waggle dance is well established with several experiments, it is still a remarkable and disturbing fact that the sensory modality in which communication occurs remain to be known.

In conclusion, one can do nothing but still appreciate the work of Karl Von Frisch, and it would be worth to quote from him, (Frisch, 1967) two paragraphs:

'It is worthwhile to devote a little thought to the chain of accomplishments that takes place between the flight of harvesting bee and the directed flight of the newcomer. In her dance the harvester transposes the solar angle that is proper to the goal into the gravitational angle-even when with a side wind she has to set herself obliquely against the lateral drift and hence sees the sun at a different angle. The dance followers understand the angle correctly. In doing so their eyes are no help to them. In the dark hive the eyes are functionless, and the senses of smell and touch take over the transmission of information. The dances followers grasp the proper angle with astounding precision, although most of them stand oblique to the dancer-especially when she has a large following. Beyond this they take note of the floral scent that cling to the dancer - and of her announcement of the distance by means of the dance tempo; in doing the latter they observe several dances and average the result. If then they fly out they go in search of this scent at a corresponding distance, transposing the gravitational angles back into the visual angle and scarcely letting themselves be borne aside from the direction striven for even by a violent cross wind. As for anyone to whom the feeling of reverence for natures creation is foreign - it might well dawn upon him here, unquote.

Though we may be pleased that we understand the dances so well, at the same time we must be impressed with how fussily and clumsily we make use of the information given by the bees. We must possess a stop-watch in order to determine the tempo of dancing. We need a distance curve pieced together from many single observations in order to estimate the distance with some degree of reliability. We need a protractor to determine the direction of dancing, the azimuth curve for reading off the position of the sun for a given place for instant. Finally, we must have a compass, a surveyor's tape, and assistants in order to apply in the open country, the result we have calculated. How elegant this task is mastered by the bees themselves, who need follow only a few dance circuits in the dark hive;

then according to their innate behaviour set forth in free flight, guided by the sun, and steer towards the goal.'

Another remarkable feature of the dance communication is that it can be used not only to indicate the locations of food sources, but also to direct bees towards nesting sites, water, and to supplies of resin (propolis). Such multiple use of a single sort of communication in comparable to the multiple use of many pheromones which have different meanings in different situations and concentrations. A given communicative element, once evolved can develop a repertoire of functions and thus considerably enhance the interactions and the integration within a colony.

Lastly, the round dance and waggle dance and the forms intermediate between them are not the only stereotyped locomotory forms performed in honeybee hive, (Wilson, 1974). As some of the other 'dance forms' have communicative functions they deserve closer study. Most of these dances are not adequately understood. Wittekindt (1966) studies a hive that was closed for long periods. After such a period, when scouts first found a food source, they did not take time to fully fill their crops or corbiculae but returned immediately to the colony with small loads; penetrating through the most crowded part of the comb, pushed among the other bees, and following a winding course, offered food to them. Unoriented tail-wagging dance were frequent. All this seemed simply to alert bees and start them foraging. In spasmodic dance, orientation is proper, but there is doubt as to whether they are effective as signals or comprise anything more than incomplete intention movements preliminary to the performance of complete waggle dance (Heir, 1954). A Jostling run serves to excite other workers, and to draw attention to the forager. It has its exact parallel in the charging and bumping behaviour of trail laying ants and termites, (Schmid, 1964). In a grooming or shaking dance, the worker shakes her body rapidly back and forth, and from side to side while attempting to comb her thoracic hairs with her middle legs. Often, but not always, this behaviour induces a nearby worker to approach and employ her mandibles to groom the hairy coat on the petiole and base of the wings; as these are the parts of which a bee is unable to clean herself. (Haydak, 1945; Milum, 1955).

Another communicative movement is the buzzing run, used in starting swarming, subsequent flights of swarms and absconding flights, (Esch, 1967; Lindauer, 1955). Occasionally a worker bee touches her nestmate with her antennae, or move, typically seizes its body with her forelegs, or climbs on top of it, then make 7-8 rapid-up-and-down stroke, with her abdomen. Milum (1955) has referred to the abdominal movement as the 'dorso-ventral

abdominal vibration (D-VAD)'. The [illegible] abdominal vibration (D-VAV)'. The function is still unknown (Milum, 1955, Allen, 1959; Von Frisch, 1967a); and is sometimes termed as the jerking dance.

CHAPTER V

SWARMING BEHAVIOR IN HONEYBEE

INTRODUCTION

Swarming is the method by which colony reproduction as distinct from the reproduction of individual honeybees, takes place. Just as reproduction of individual bees within a colony is essential to the continued existense of the colony, so reproduction of colonies is essential to the continued existence of the species; for without it any increase in the number of colonies would be impossible as would replacement of any colonies destroyed by disease or other causes.

The origin of swarming behaviour in primitive eusocial bees such as Bombus, young gynes establish new colonies alone, or sometimes enter existing young colonies; usually of their own species and take over or attempt to do so. In stingless bee the situation differs in that the existing young colony was established by workers from the parent colony and is called a swarm. However, in Apis, there is a sharp difference where no intermediate conditions are known, so that understanding of the origin of Apis swarming is not easy. It is the old queen that leaves, and she is accompanied by workers which have not prepared a nest in advance.

The term 'swarming' is applied to the act of a family of bees leaving their home to establish a new home elsewhere, as mentioned; 'swarming out' usually applies to migration of the entire colony as in the case of lack of food (hunger swarms); recently hived swarms that are dissatisfied (Absconding swarms) and small nuclei that swarm out with the young queen when she takes her mating flight or because the little colony is dissatisfied.

The migrating family of bees is called a swarm though this term is sometimes applied to the colony after it has established itself in its new home, to distinguish the new colony from the parent colony.

According to Ribbands (1953), swarming in Apis arose, in the whole colony in response to unsuitable conditions. Such migration, or absconding as it is called in honeybee literature is extremely common in tropical Apis (cerana, florea, dorsata) and relatively uncommon only in the presumably, derived cool temperate zone races of A. mellifera, where large and nonmovable stores of food are vital to survival because of winter conditions. Absconding, however, does not lead to colony division, for this reason various authors contend that it has nothing to do with swarming (Kerr and Laidlaw, 1956). But the similarities are so impressive that a relation, at least the evolution of common behaviour patterns for both swarming and absconding seems evident. For example, for both, the bees fill their crops with honey before leaving the nest. Smoke causes the same response even in the northern races of A. mellifera, which do not usually abscond. Presumably this response much used by bee-keepers in handling bees, arose because of the improved probability of survival of bees carrying food in the event that they are forced from their nest by fire. (Newton, 1968).

An interesting aspect of swarming in all the highly eusocial bees is the problem of changing the orientation of foragers to a home site. They must "forget" the old site. As is well known, if a beehive is moved a short distance to a new location within the area already familiar to the foragers, many or most of them after a trip will return to the old site instead of the new one. This can result in decimation of the foraging population of a colony that has been moved.

When bees swarm they establish a new site, Taranov (1955) has considered this problem for Apis. He notes that return to the old site is much more likely for bees with empty crops. Swarming bees fill their crops with honey before leaving the old site, hence more easily return to the new one.

Swarming usually occur in late spring at a time of plentiful food supply. This makes biological sense, for the swarm must establish itself, build combs, store food, rear young ones, all before the onset of dry (flowerless) or cold weather. Simpson (1963), however, found lack of space for adult bees to be the critical factor for such circumstances. Certainly, crowded colonies do swarm apparently because of crowding.

Lindauer (1955,1961, full reviewed by von Frisch, 1967) found that at this time foragers, unable to continue foraging, start flying and walking about dark holes, cracks, empty boxes and the like. They become scouts and are already house hunting. The scouts having changed their behaviour in view of circumstances, play an important role in the swarm activities. They still dance, but the dances are not associated with food exchange, for the bees are not returning from food source. The dances indicate positions of possible nest sites.

MATERIALS AND METHODS

For this particular study six healthy colonies of bees which have been separated artifically, i.e. when the mother colony have constructed (mother) queen cells, till they have come back from their mating flight was kept under observation, to get a brief idea of the age of the queen.

The colonies were later on, left to themselves without any disturbance, like artificial feeding and the sort.

As the colonies grow, multiply etc., monthly to weekly study/observations were made for each colony regarding their health. Later on behaviour of the different hives; like constructing of queen cells, overcrowding, slowing down of field work were all taken into account. Another reliable symptom to be notified is that in a colony preparing to swarm there is lack of usual flight at the entrance due to many of the field bees staying at home. When this is noticeable it is also essential to look into the supers and if the crowded bees are wedged into every nook and corner, it indicates some sign as this is not seen in case of normal conditions in supers.

RESULTS AND DISCUSSION

After quite a number of observations it is noticed that swarming apart from meaning that it is the reproduction of the colony it also means that a colony who deserts its home for a new habitat fully does not in the true sense mean reproduction but as any colony of bees old or new is considered to be a 'swarm' thus the term might be misleading if we mean only reproduction of the species.

Thus it seems much more probable that the type of swarming which results in colony reproduction has evolved from so called 'mating swarms' than for 'absconding swarms'. Mating swarms occur frequently among *Apis indica* (and probably *Apis florea* and *Apis dorsata*) and much less frequently among *Apis mellifera*.

Based on our observations regarding the true sense of the word swarming, i.e. for reproduction of the species; it is noted that when the swarming impulse of a colony of honeybees has been aroused, the worker bees proceed to construct a number of special cells, the queen cells. These cells are cone-like in shape, (Fig. 96) and the queen starts laying eggs on some of these cell cups. These cell-cups are usually built on the lower sides of the comb, in the lower part of the brood nest. The construction of these special cells does not ring true for swarming in a majority of cases as we have observed that although many other colonies build cup cells yet they will not swarm.

It is also noted that a certain number of these 'queen cell cups' are present in almost every time in summer but it is when the colony is making preparations to swarm that their number is seen to increase. The queen lays eggs in some of these cell cups perhaps in a dozen or more. The worker bees then feed the resulting larvae with brood food secreted by their hypopharyngeal glands and the cell walls are quickly enlarged to accommodate the rapidly growing larvae and their normally very abundant food supplies. The old mated queen of a colony in such case produce fewer eggs daily and later stop laying altogether. Her abdomen has shrunken considerably, being devoid of eggs. The moment after the first of the 'swarm queen' cell is then scaled, the old queen of the colony leaves the hive accompanied by many worker bees, mostly young and middle aged and a few drones. This swarm usually settle for sometime on a tree or any support and later shift themselves to a suitalbe site. This type of swarm is known as the 'prime' or 'first' swarm. (Butler, 1957).

About a week or so after the 'prime swarm' has taken off the virgin queen emerges and it is observed that she goes on inspecting the presence of other existing queen cells where she tries her best to tear and to destroy them, unless she is prevented by the workers. She later takes off on a 'mating flight' which is termed as a 'mating swarm', where, thereafter, she returns back to her original hive and starts her duty of life, that is, laying eggs.

The main aspiration of a colony at the time of swarming is to provide for its future existence and the continuation of the species.

The instinct of swarming makes the bees get together in a compact mass so as to attract the queen and along with the queen they will calm down as if communicating among themselves about the next step of their adventure. By this time some scout bees have already taken off in search of a new site or others have already found one, as groups of scouts goes in search to different directions.

In this manner for sometime, the swarm takes off and after making a final inspection of their old home as to say goodbye; whereafter it follows the path of the scout bees. The bees in this process will forget their old home forever. This shows that during swarming, bees lose their instinct for their old home or dwelling place.

The swarm usually settles in an open branch of tree for quite sometime waiting for the scouts information of a proper dwelling home. In this process, as mentioned earlier, different scout bees goes in different direction and later they meet together and inform their colony of the findings by a particular dance, and thus the swarm decides which scout bees to follow. In this case the type of dance is again the so called 'tail wagging' but due to the difference in the behaviour of the colony it is understood that it infers about their new dwelling home, and the greater the intensity of the dance the better is the new homes position; so this is left to the swarm in general to decide.

At times even if the scouts fail to find an appropriate dwelling place yet in a majority of the cases the swarm will never go back to their old dwelling place. And if the food they carry with them gets exhausted they might even construct combs outside, on branches of trees.

Even if the swarm finds an appropriate home near the old apiary, yet it usually does not stop there. The aspiration of the swarm to fly as far as possible away from their native roof reveals nature's great wisdom, because it is thus that the species of the honeybees can be preserved from degeneration.

Distant settling of swarms precludes so called 'interbreeding', that is, mating between close relatives; where young queens annually mates with their drone brothers. Generations from closely related parents are known to be of lower vitality and weakened working capacity. Such generations have practically no resistance to external negative factors and various other infections. Prolonged interbreeding inevitably leads the offspring to complete sterility and subsequently to the degeneration of the whole species.

It is learned from Cherrapunji (the experimental place) that it is difficult to find two colonies (specially relatives) living close to each other in neighbouring tree hollows in a natural environment. It is only through artificial rearing methods by man that we see bees of different colonies living close together. But special methods have to be worked out so that inbreeding can be prevented among bees.

Nature has endorsed swarming as a good example to prevent the extinct of the species from famine. If every new colony establishes itself close to its parental one then in no time apart from inbreeding etc. overpopulation would occur and the bees would be threatened with starvation.

So the biological meaning of honeybees 'swarming' lies not only in the birth of new bee colonies but also in providing the uninterrupted existence of the bee species.

In another case, where the worker bees prevent the first emerging virgin queen in destroying other developing/growing queen cells, it is observed that, the virgin queens after emerging they take off with a small swarm of bees and this is known as a 'cast' or 'after swarm'. Several such cast may leave the parental hive one after another each with a virgin queen, and two or more cast will sometimes join together, in which case the virgin queens fight to kill one another until only one remains to head the new colony. Swarms containing mated queen will sometimes join together too, and in such cases several queens may continue to live in one, multiple colony for sometimes.

Environmental conditions also has some effect on the swarming behaviour of bees. It is noticed that at times the worker bees of a colony tears down queen cells, even though they are in advanced stage of development.

Whenever a swarm queen cell is constructed there is always a reduction in the colony's foraging activity and a reduction in the egg-laying and hence in brood-rearing of a colony (Fig. 97).

Queen supersedure is another major cause which can be mistaken for swarming. It was in 1954 that two Dutch workers, Prof. A.P. de Groot and Dr. S. Vogd found that there must be some substance in the queen which controls the behaviour of the worker bees. And after several experiments they have concluded that development of laying workers in a colony is normally prevented, like queen rearing, by material produced by the queen, i.e. the 'queen substance'. Later in the same year (1954) Butler and Miss D. Gibbon also discovered this same material. Soon afterwards Butler and Simpson demonstrated that the principal source of this substance (Queen's) are the queen's mandibular glands and two large glands situated in her head just below her eyes.

Fig. 96 A magnified photograph of the queen cell of Apis cerana indica Fab.

Fig. 97 Photograph showing a colony of honeybees, Apis cerana indica (Fab.) getting ready for swarming

PLATE 51

Thus after learning more about 'Queen substance' it is understood that queen supersedure is due to an insufficiency of this substance and this is often the direct cause of the emergence of a swarm from a hive. However, this type of swarm is merely a form of mating swarm which may or may not return to the parent hive. Several such swarms may issue separately from the same hive and only one return with a mated queen, who may live quite happily alongside her mother for a while but eventually supersedes her as head of the colony. Although it is very probable that a deficiency of 'queen substance' plays a direct and most important part in the development of both the supersedure impulse and the swarming impulse, these two impulses almost certainly have quite different origins and should not be confused with one another. The major factor in case of queen supersedure is a reduction in the amount of 'queen substance', available to the bees due to ageing or illness of the queen. So it is necessary that she be replaced for the survival of the colony.

There are also a number of hypothetical factors worked out by some workers but the two chief theories are, the 'Gerstung' or brood food theory and the 'Demuth' or over crowding theory.

Gerstung's hypothesis deals with the amount of brood-food that is required to feed the larvae and in cases where there is surplus quantity the worker bees tend to construct more royal queen cells so that the surplus amount may be utilized.

He believed that worker bees passes through a stage when their brood-food glands secrete abundantly and thus the outlet for this is to rear royal cells which needed a large quantities of it. However, even this usually proved insufficient to dispose off the surplus brood-food and swarm queen cells were started in order that the bees might relieve themselves of their unwanted surplus of brood-food by feeding queen larvae lavishly with it; So eventually swarming resulted.

In 1930, Morland also reviewed and elaborated this theory, and it is still more widely accepted in Western countries. But Gerstung theory even though it threw some light on swarming yet it still has a number of discrepancies/defficiencies regarding division of labour in worker bees; specially regarding nurse bees, where he said that every worker bee becomes a nurse bee at some stage in her life and there is no way in which the balance between nurse bee and larvae requiring to be fed can be redressed when necessary. Actually bees can forego hive duties as mentioned in the earlier chapters and can forage even when its very young (4-5 days old) depending on the need of the colony. Thus there are still lots of question's left unanswered.

According to the next theory, the Demuth or overcrowding theory, the only factor which is common to all

Fig. 98 Photograph showing a swarm of honeybees, trying to establish itself in a thatch roof in Umroi (few bamboo covers removed to expose the swarm).

Fig. 99 Photograph showing another swarm smaller in number than the previous one, in another hut in Umroi.

PLATE 52

Fig. 100 A swarm of honeybees in a bark of tree in the Botanical Garden, Ward's Lake, Shillong spotted by a passer accidentally; (the bees cannot be focussed, except for the entrance)

PLATE 53

colonies is that of congestion as the name suggests. This crowding might effect the brood area and swarming might ensue. Idle foragers confined to the hive by bad weather can also lead to overcrowding, and same is the case with shortage of storage space. Over heating due to either direct heat from the Sun or perhaps due to insufficient ventilation is only one of the factors which promote swarming (Figs. 98-100).

GENERAL DISCUSSION

The complex and wonderful organization of the honeybees has fascinated scientists as well as a common man.

Many early writers believed that bees are generated in a rather surprising way, such as gathering their youngs from certain kinds of flowers. It is also interesting to note here that servius (Ref. Butler : The world of the honeybees, 1974) attempted to explain seperate origins for drones and worker bees and Dr. Fraser quotes him as writing that, 'bees are said to be derived from oxen, drones from horses, hornets from mules and wasps from asses. Also in continuation, queens were described as kings; sex of the three kinds of bees were not taken to consideration. Rev. C. Bulter of Magdalen College, Oxford who published his book, The Feminine Monarchie in 1609 which said that the moment the sex of the drones, the male bees, was established; simultaneously, the statement was made that queens are females. But he made a mistake, however in believing that the workers mate with the drones and lay the eggs of which both workers and drones are produced.

His (C. Bulter) description of the drone is well worth quoting : "The drone, which is a gross hive bee without sting hath been always reputed a greedy lazer for howsoever he braves it with his round velvet cap, his side gown, his full paunch, and his loud voice; yet he is but an idle companion, living by the sweat of others' brows. For he worketh not at all, either at home or abroad, and yet spendeth as much as two labourers; you shall never find his maw without a drop of the purest nectar. In the heat of the day he flieth abroad, aloft and about, and that with no small noise, as though he would do some great act; but it is only for his pleasure, and to get him a stomach, and then returns he pleasantly to his cheer".

But with much work having carried out for so long by various scientists it is found that Charles Butler's, statement , 'as idle as a drone' does not ring true because without the presence of the drone, the only male member to fertilise the queen, the colony would dwindle away.

The highest degree of socialization/socialism is found in the colonies of A. mellifera and A. cerana indica among bees.

Investigation's showed that with respect to brood rearing and overwintering the ability to regulate nest temperature is highly beneficial. The precision with which honeybee colonies do this is truly remarkable but it would not be possible if it were not for the type of nest site selected and the way it was modified and utilized. The actual thermo-regulatory process, however is accomplished by the bees themselves rather than physically, as with termites. During the months when a brood is being reared the brood-chamber is maintained at a temperature of 34.5°-

35-5°C, even though the outside air temperature may reach more than 40°C. In winter the cluster temperature ranges from 20°-30°C and is never permitted to drop below 17°C; regardless of outside temperature. When the outside temperature drops, the temperature in the hive is maintained by the metabolic heat generated by the workers; whose behaviour changes as the temperature declines. At first the workers form a loose cluster towards the centre of the hive. As temperature drops the cluster tightens. The workers at the centre consume small amounts of honey and generate heat by vibrating their muscles; those forming the outer layers of the cluster act as an insulating blanket. As time passes, the outer bees moves towards the centre of the cluster, while those near the centre move into the outer layer. During hot weather, the temperature of the brood area is maintained by a circulation of air in the hive created by workers that fan with their wings at the hive entrance. When this activity proves to be inadequate, other workers carry water into the hive and distribute it over the brood cells; which are then cooled as the water evaporates.

The odour of food brought to the nest can also influence the behaviour of nestmates, and therefore serve in a primitive form of recruitment of communication. Honeybee workers recognize the odour of food source both from the smells adhering to the bodies of successful foragers and the scent of the nectar regurgitated to them.

If they have had experience in the field with flowers or honeydew bearing the same odour, they will then revisit the site searching for food.

Honeybees as we know now are eusocial insects. As such, they have "a reproductive division of labour in the colony, that is, a worker caste cares for the young of the reproductive caste". Also, "there is an overlap in generations so that offspring assist parents" (Wilson, 1972). Thus honeybee colonies replaced their individual in succession without its colony getting dissolved. Furthermore, the queen honeybee is not able to raise brood without workers.

Honeybees also possess interesting learning instinct. Bees inherently learn certain characteristics of a flower more easily than they learn others. Perhaps even more significant, once bees have acquired knowledge about a flower the ways in which they organize and refer to that knowledge are entirely instructive.

Bees recognize flower like objects instinctively; they land spontaneously on small, brightly coloured objects that have a high spatial frequency e.g. petals.

When bees are sure that the flower or object they have landed on rewards them with food, the flower's specific characteristics may be learned and imprinted as conditioned stimuli. Randolf Menzel (1978) showed that even one training visit is enough to teach the bee to choose the same odour 90 percent of the time in later visits; after only three training visits the rate of success is higher than 98 percent.

Investigations also show that honeybees along with odour also learn the colour of any object (flower). Around ten training trips shows that bees choose the correct colour 90-95 percent of the time. In addition, though this work was not included in our research, yet it cannot be denied that honeybees also learn shapes and colour pattern of flowers.

The cues bees do remember about, such as odour, colour and pattern, are not of equal weightage. As training also shows that when two objects have same odour then bees pay much attention to colour or shape. Honeybees stress more on odour as it is usually constant, whereas colour can fade with time, by damage from external environment.

Honeybees have also got a specific timing and knowing which flower provides nectar at what time of the day; even though this is imprint on the bee's memory more gradual/slowly, but once they have learned, it is really memorised in their minds like a computer.

Wilhelm et. al. (1984) showed interesting investigations and provide further evidence that bees control the duration of her scent marking behaviour at the food source only according to the concentration and rate of supply of the sugar solution and the state of the hives general food supply.

Ageing bees are selective in their choice of food. Barker (1974) showed that preference for honey might decrease which will lead to the honeybees foraging out.

Regarding the orientation of their comb, honeybees being very sensitive insects responds well to gravity. When their comb is tilted from its original position, for example, a swarm is placed in a cylindrical box having no mechanical cues, like frames they will use the earth's magnetic field to orientate the combs in a fixed compass direction (Lindauer, 1973).

Swarms which have recently moved into bait hives (empty boxes placed in trees to attract feral swarm) tend to maintain the previously used comb direction when removed and forced to build new combs, whereas swarms which have occupied the bait hives for a longer period (more than 9-10 days) do not. Recent swarms predictably alter the comb building direction within the influence of an applied earth strength magnetic field, indicating that honeybees are able to use the earth's magnetic field as a reference at the commencement of comb construction in a new hive. (De Jong, 1982).

When honeybees are ready for swarming, they also have to maintain their food balance, for it is needed for survival, as in some cases it takes days for a swarm to spot a new site for their further settlement. Thus the food load of the workers in a swarm few hours (3-4) before issuing weighed 4 times as much as that of workers in colonies not preparing to swarm. All workers in a swarm were not equally engorged, and different engorgement did not appear to be responsible for colony seggregation at the time of swarm issuance. Workers engorgement occurred gradually during the 10 days prior to swarm issuance. (Combs, 1972).

The sense organs or receptors also play a wonderful role in the life of the honeybees. They are associated with the integument and.ends up in a sensillum. The types of sensilla differ with the functions they are responsible for. Sensilla trichodea, Placodea, Basiconica, Campaniform etc. are present in different quantities on the body and appendages of the three castes of the honeybees (Apis cerana indica Fab.). This arrangement also varies with different species of the honeybees (dorsata, florea, melifera). In other words these sensillae are also known as chaemoreceptors, photoreceptors, mechanoreceptors depending on their respective functions in the animal. Honeybees's (Apis cerana indica Fab.) behaviour mainly depends on the alertness of these receptors. Thus it is said, that the body of bees are covered with innumerable hairs, that it is difficult to touch the animal without bending some of these hairs, which will lead to the response and alertness of this animal. That is why they are considered to be very intelligent due to their sensitiveness and their fast response to any stimuli applied to them.

REFERENCES

REFERENCES

Allen, M. Delia, 1959. The "shaking" of worker honeybees by other workers. *Animal Behaviour*, 7(3-4): 233-240.

______, 1960. The honeybee queen and her attendants. *Animal Behaviour*, 8 : 201-208.

______, 1965. The role of the queen and males in the social organization of insect communities. *Symposium of the Zoological Society of London*, 14 : 133-157.

Altner, H., 1977. Insect sensillum specificity and structure : *An approach to a new typology*, pp. 295-303. In J. Lemagnen and P. Mcleads (eds). Olfaction and taste VI. Information Retrieval, Washington.

Barbier, M. and E. Lederer, 1960. Structure chimque de la 'Substance royale' de la reine d'abeille (Apis mellifera L.). *Compte Rendu de l'Academie des Sciences Paris*, 250(26) : 4467-4469.

______, E. Lederer and T. Nomura, 1960. Synthese de l'acide ceto-9 de'cene-2-trans oique (Substance royale) et de l'acide ceto-8 nonene-2-trans oique *Compte Rendu de l'Academie des Sciences, Paris*, 251(10) : 1133-1135.

Barker, R.J. and Y. Lehner, 1974. Food choice changes in ageing honeybees (A. mellifera). *Ann.Entomol.Soc.Am.* 67(4) : 717-718.

Bauer, L. 1939. 'Geschmacksphysiologiche untersuchungen an Wasserkafern.*Zeitschrift fur Vergleichende Physiologie*, 26 : 107-20.

Boeckh, J., K.E.Kaissling, and D. Schneider, 1965. Insect olfactory receptors. *Cold Spring Harbour Symposia on Quantitative Biology*, 30 : 263-280.

Bee Research Association, 1967. Bibliography on honeybees other than Apis mellifera. *Bee World* 48(1) : 8-15, 18.

Beetsma, J., 1979. The process of queen, worker differentiation in the honeybees. *Bee World*, 60 : 24-39.

Bertholf, L.M., 1925. The moults of the honeybee. *J.Econ. Ent.*, 18(2) : 380-384.

Borner, C., 1921. Die Gliedmassen der Arthropoden In : *Lang's Handb.Morphol.Wirbellosen*, Tiere, 4 : 649-694.

Borror, D.J., D.M. Delong and C.A. Triplehorn, 1976. Introduction to the study of insects. 4th ed. Holt, Rinehart and Winston, New York.

Brian, A.D., 1952. Division of labour and foraging in Bombus agrorum (Hym). *Jour.Anim.Eco*, 21(2) : 223-240.

Breed, M.D. 1983. Nesmate recognition in honeybees (A. mellifera). *Anim.Beheviour*, 31(1) : 86-91.

Butler, C.G. 1954a. The world of the honeybee *New Naturalist Series*, Collins, London, XIV+ 226 pp.

------,1954b. The method and importance of the recognition by a colony of honeybees (A. mellifera) of the presence of its queen *Transactions of the Royal Entomological Society of London*, 105(2): 11-29.

------, 1954c. The importance of 'queen substance' in the life of a honeybee colony *Bee World* 35, 169-176.

------, 1960. The significance of queen substance in swarming and supersedure in honeybee (Apis mellifera L.) colonies. *Proceedings of the Royal Entomological Society of London*, (A) 35 : 129-132.

------, 1961. The scent of queen honeybees (Apis mellifera L.) that causes partial inhibition of queen rearing. *Jour.Int.Physiol*, 7(3-4) : 258-264.

------, 1964. Pheromones in sexual processes in insects. *Symp.Roy.Ent.Soc.*, London, 2 : 66-77.

------, 1967. Insect pheromones. *Biological Reviews*, Cambridge Philosophical Society, 42(1) : 42-87.

------, 1969. Some pheromones controlling honeybee behaviour. *Proceedings of the Sixth Congress of the International Union for the Study of Social insects*, Berlin, 1969 pp. 19-32.

------, R.K.Callow, and C.G.Simpson, 1973. Perception of the queen by workers in the honeybee colony (Apis mellifera L.) *J.Apic.Res.*, 12(3) : 159-166.

Butler, C.G.,and J.B. Free, 1952. The behaviour of worker honeybees at the hive entrance, *Behaviour*, 4(4): 262-292.

------, and D. Gibbons, 1954. The world of the honeybee *New Naturalist Series*, Collins, London, XIV.

------, and J. Simpson. 1958. The source of the queen substance of the honeybee (Apis mellifera L.) *Proc.Roy.Entomol,Soc.London* (A) 33 : 120-122.

------, R.K. Callow and W.C. Johnston. 1961. The isolation and synthesis of queen substance, 9-oxo-trans-2-enoic acid, a honeybee pheromone. *Proceedings of the Royal Society*, (B) 155 : 417-432.

------, P.N. Paton. 1962. Inhibition of queen rearing by queen honeybees (Apis mellifera L.) of different ages. *Proceedings of the Royal Entomological Society of London*, (A) 37 : 114-116.

Caron, D.U. and C.W. Greve, 1979. Destruction of queen cells placed in queen right (Apis mellifera L.) colonies *Ann.ent.Soc.Am.*, 72 : 405-407.

Carron, D.M. 1970. A study of swarming and behaviour of swarming in honeybees (Apis mellifera L.) Cornell University. Ph.D. Thesis.

Chapman, R.F. 1964. The structure and wear of mandibles in some African grasshoppers. *Proc.Zool.Soc.Lond.* **142** : 107-21.

-------, 1982. Chemoreception : The significance of receptor numbers. *Adv.Insect.Physiol.* **16** : 247-356.

Combs, G.F. 1972. The engorgement of swarming worker honeybees. *J.apic.Res*, 3 : 121-128.

Crampton, G.C. 1925. A phylogenetic study of the labium of holometabolous insects, with particular reference to the Diptera. *Proc.ent.Soc.*, Washington, 27 : 68-91.

Darwin, C.R. 1859. The origin of species by natural selection. Murray, London, ix + 502pp.

De Jong D. 1982. Orientation of comb building by honeybees (A. mellifera L.) *J.comp.Physiol. A. sens Neural Behav*, 147(4) : 495-502.

Deans, A.S.C. 1963. Bee keeping techniques. Oliver and Boyd Ltd. London.

Delcomyn, F. 1985. Factors regulating insect walking *AReview Ent.*, 30, 239-256.

Dethier, V.G. 1953. Vision. In K.D. Roeder, ed. *Insect Physiology*, John Wiley and Sons, Inc., New York, pp. 488-522.

------, 1957. Communication by insects : physiology of dancing. *Science*, 125 : 331-336.

------, 1963. The physiology of insect senses. Methuen and Co., London, ix + 266pp.

------, and L.E. Chadwick, 1948. Chemoreception in insects *Physiol.Rev.*, 28 : 220-254.

Dietz, A. and M.H. Haydak, 1967. Caste determination in honeybees : the significance of moisture in larval food. *XXI Int.Beekeep.Congr.Prelim.Sci.Meet Summ.*, Paper 7 : 56.

Emerson, A.E. 1949. The organisation of insect societies. In W.C. AMee et al, eds. *Principles of animal ecology* W.B. Saunders and Co., Philadelphia, pp. 419-435.

------, 1950. The supraorganismic aspects of the society. In Colloques Internationaux du Centre national de la Recherche Scientifique, Vol. 34, Structure et Physiologie des Societies Animaux, Paris, pp. 333-353.

------, 1959. Social insects. Encyclopedia Britannica 20 : 871-878.

Esch, H. 1961. Uber die Schallerzeugung beim Werbetanz der Honigbiene. *Zeitschr.Vergl.Physiol.* 45 : 1-11.

-------, 1967a. The evolution of bee language. *Scientific American* 216(4) : 96-104.

-------, 1967b. Die Bedeutung der Lauterzeugung fur die verstandigung der Stachellosen Bienen. *Zeitschrift fur Vergl.Physiol.* 56(2) : 199-220.

-------, and Bastian, J.A. 1970. How do newly recruited honeybees approach a food site. *Zeitschr.Vergl.Physiol.* 68 : 175-181.

Eskov, E.K., 1969. Evidence of the informative role of the sound components in honeybee mobilization drones. *Zh.Obshch.Biol.* 30 : 317-323.

Fernandez-Moran, H. 1958. Fine structure of the light receptors in the compound eyes of insects. *Experimental Cell Research, Suppl.* 5 : 586-644.

Fischer, W. 1957. Untersuchungen uber die Ruchscharfe der Honigbiene. *Zeitschrift fur Vergleschende Physiologie*, 39(b) : 634-659.

Free, J.B. 1955a. Queen production in colonies of bumble bees. *Proc.Roy.Entomol.Soc. London (A)*, 30 : 19-25.

------, 1955b. The division of labour within bumble bee colonies. *Insectes Sociaux*, 2 : 195-212.

------, 1955c. The behaviour of egg-laying workers of bumble bee colonies. *Brit.J.Anim.Behav.*, 3 : 147-153.

------, 1955d. The collection of food by bumble bees. *Insectes Sociaux*, 2 : 203-311.

_______ 1957a. The food of adult drone honeybee (Apis mellifera L.) *British Jour.of Animal Behaviour*, 5(1) : 7-11.

------, 1957b. The transmission of food between worker honeybees *British Jour.of Animal Behaviour*, 5(2): 41-47.

------, 1959. The transfer of food between the adult members of a honeybee community. *Bee World*, 40(8) : 193-201.

Free,J.B. 1960. The distribution of bees in a honeybee (Apis mellifera L.) colony. *Proceedings of the Royal Entomological Society of London*,(A), 35 : 141-144.

------, 1961. Hypopharingeal gland development and division of labour in honeybee (Apis mellifera L.) colonies. *Proceedings of the Royal Entomological Society of London (A)*, 36 : 5-8.

------, 1965. The allocation of duties among worker honeybees. *Symposium of the Zoological Society of London*. 14 : 39-59.

------, 1967. Factors determining the collection of pollen by honeybee foragers. *Animal Behaviour*, 15, 134-144.

------, 1969. Influence of the odour of a honeybee colony's food stores on the behaviour of its foragers. *Nature*, London, 222 : 778.

------, and J. Simpson. 1968. The alerting pheromones of the honeybees. *Z.Vergl.*, 61: 361-365.

------, and Ingrid H. Williams. 1972. The transport of pollen on the body hairs of honeybees (Apis mellifera L.) and bumble bees (Bombus Spp. L.) *Journal of Applied Ecology*, 9 : 609-615.

------, and A.W. Ferguson, 1979. Production of a forage marking pheromone by the honeybees. *Journal of Apicultural Research*, 18 : 128-135.

------, J.A. Pickett, A.W. Ferguson and M.C. Smith. 1981. Synthetic pheromones to attract honeybee (Apis mellifera L.) swarms. *Journal of Agricultural Science*, Cambridge. 97 : 427-431.

------, 1983. Scent marking of flowers by honeybees. *Journal of Apicultural Research*, 22 : 86-90.

------, A.W. Ferguson et al., 1983. Effect of honeybee rasanov and alarm pheromone components on behaviour at the nest entrance. *Journal of Apicultural Research*, 22(4) : 214-223.

Frisch, K.Von. 1919. Uber den Geruchsinn der Bune und Formensinn der Biene. *Zoologische Jahrbucher.Abt. Allgem.Zool.Physiol. Tiere*, 37(1-2): 1-238.

------, 1921. Uber den Sitz des eruchsinnes bei Insekten. *Zoologische Jahrbucher Abt. Allgem.Zool.Physiol. Tiere* 38(1) : 1-68.

------, 1934. Uber den Geschmacssin der Biene *Zeitschrift fur Vergleichende Physiologie*, 21(1) : 1-156.

------, 1954. The dancing bees : an account of the life and senses of the honeybee (tr. Dora IIsc.). Methuen and Co., Ltd. London, xiv + 183 pp.

------, 1967. The dance, language and orientation of bees. Cambridge, Massachusetts : Harvard University Press,

------, 1968. Bees, their vision, chemical senses and language. Great Britain, Chaucer Press.

Fox, R.M. and J.W. Fox. 1966. Introduction to Comparative Entomology. Reinhold Pub. Corp. New York.

Frings, H., and M. Frings. 1949. The loci of contact chemoreceptors in insects. *Amer.Midl.Nat.*, 41 : 602-658.

Gary, N.E. 1962. Chemical mating attactants in queen honeybee. *Science*, 136 :773.

------, 1970. Pheromones of the honeybee (Apis mellifera L.) In : Control of natural behaviour by natural products pp. 29-53. Academic Press, New York.

Gettrup, E. 1966. Sensory regulation wing twisting in locusts. *Jour.Exp.Biol.*, 44 : 1-16.

-----, 1973. Stimulus transmission in cuticular mechano-receptors. *Naturwissenschaften*, 60 : 52-53.

Gogmerac, W.L. 1980. Bees, beekeeping, honey and pollination. University of Wisconsin, Madison, Wisconsin.

Goldsmith, T.H. 1958. The visual system of the honeybee *Proceedings of the National Academy of Sciences of the USA*, 44(2) : 123-126.

------, 1962. Fine structure of the retinulae in the compound eye of the honeybee. *Journal of Cell Biology*, 14(3) : 489-494.

------, and H.R. Fernandez, 1966. Some photochemical and physiological aspects of visual excitation in compound eyes. In C.G. Bernhard, ed., *The functional organization of the compound eye*, Pergamon Press, Oxford, Eng. pp. 125-143.

Goodman, L.J. and P.T. Haskell. 1959. Hair receptors in locusts. *Nature*, London. 183 : 1106-1107.

Gould, J.L. 1985. How bees remember flower shapes. *Sciences*, (Washington D.C.) 227(4693) : 1492-1494.

Groot, A.~~D~~P.de, and S. Voogd. 1954. The world of the honeybee (*The New Naturalist*) Collins, London.

Hansen, H.J. 1930. Studies on Arthropoda III, The comparative morphology of the appendages in Arthropoda, Copenhagen, pp. 376.

Haslinger, F., 1935. 'Uber den Geschnacssin von Calliphora erythrocephala und uber die verwerting von zuckern und Zuckeralkoholen durch diese fliege.' *Zeits fur Vergleichende Physiol.*, 22 : 614-40.

Haydak, M.H. 1945. The language of the honeybee. *American Bee Journal*, **85** : 316-317.

------, 1968. In : Traite de biologie de l'abeille by R. Chauvin, Paris, Masson and Cie, 302-334.

Hein, G., 1954. Der Rucktanz als Wessentlicher Bestandteil der Bienentanze. *Experientia*, 10(1) : 23-24.

Imms, A.D. 1957. A general textbook of entomology, Dutton, New York, pp.866.

Ioyrish, N. 1977. Bees and people. MIR Publishers, Moscow.

Janzen, D.H. 1971. Englossine bees as long distance pollinators of tropical plants. *Science*, 171 : 203-205.

Johnson, C.G. 1966. A functional system of adaptive dispersal by flight. *Annual Review of Entomology*, 11 : 233-260.

Kaissling, K.E. and M. Renner, 1968. Antennale rezeptoren fur queen substance and sterzelduft bei der honigbeine. *Zeitschift fur Vergleichende Physiologie*, 59(4) : 357-361.

Karlson, P., and A. Butenandt, 1959. Phermones (ectohormones in insects). *Annual Review of Entomology*, 4 : 36-58.

Kerr, W.E. and N.J. Hebling, 1964. Influence of the weight of worker bees on division of labour. *Evolution*, 18(2) : 267-270.

-------, and H. Esch. 1965. Comunica cao entre as abellas sociais brasileiras e sua contribuicao para o entendimento da sua evolucao cuincia e cult [Sao Paulo] 17 : 529-538.

Koeniger, N. 1969. Experiments concerning the ability of the queen (Apis mellifera L.) to distinguish between drone and worker cells. *XXII Internat.Beekeeping Congr.Sum.*, p. 138.

------, 1970. Factors determining the laying of drone and worker eggs by the queen honeybee. *Bee World*, 51 : 166-169.

------, and S. Fuchs, 1975. On colony defence in the Asian honeybees (Apis). *Z.Tierpsychol.*, 37(1) : 99-106

Kolmes, S.A. 1985a. A quantitative comparison of observational methodologies for studies of worker honeybees. *J.Apic.Res.*.

------, 1985b. A quantitative study of the division of labour among worker honeybees. *Z. Tierpsychol.*, 68 : 287-302.

Lacher,V. 1964. Elektrophysiologische untersuchurge an einzelner rezeptoren fur geruch, Kohlendioxyd, luftfeuch tigkeit und temperatur auf der antennen der arbeitsbiene und der drohne *Z. Vergl.Physiol.*, 31 : 348-412.

Langstroth, L.L. 1890. The hive and the honeybee. Rev.ed. Hamilton, III. Dadant.

Lecomte, J., 1956. Uber die Bildung von 'Strassen' durch Sammelbiener, desen stock um 180° gradreht wurde. *Zeitschrift fur Bienenforschung*, 3 : 128-133.

Levin, M.D. 1961. Interactions among foraging honeybees from different apairies in the same field *Insectes Sociaux*, 8(3) : 195-201.

Lindauer, M. 1952. Ein Beitrag Zur frage der Arbeitsteilung in bienenstaat.*Zeitschrift fur Vergleichende Physiologie*, 34(4) : 299-345.

---------, 1955. Schwarmbienen auf wohnungssuche. *Zeitschrift Vergl.Physiol.* 37(4) : 263-324.

------, 1961. Communication among social bees. Harvard University Press, Cambridge, Mass ix + 143 pp.

------, 1967. Recent advances in bee communiction and orientation *Ann.Rev.Entom.* 12 : 439-470.

-------, 1971. The functional significance of the honeybee waggle dance *Amer.Naturalist*, 105: 89-96.

Lukochus, F. 1956. Zur kasten determination bei der honigbiene. *Zeitschrift fur Bieneforschung*, 3(8) : 190-199.

Maeterlinck, M. 1958. The life of the bee (English tr. by A. Sutro), Dodd, Mead, and Co., NY, pp. 427.

Markl, H. 1962. Borstenfelder an den gelenken als schwere-sinnesorgane bei ameisen und anderen Hymenopteran. *Zeitschrift fur Vergleichende Physiologie*, 45(5) : 475-569.

------, and M. Lindauer, 1965. Physiology of insect behaviour. In M. Rockstein, ed., The Physiology of Insecta. Vol II. Academic Press, NY, pp. 3-122.

Marshall, J. 1935. On the sensitivity of chemoreceptors on the antennae and fore-tarsus of the honeybee (Apis mellifera L.) *Jour.ecp.Zool.*, 12 : 17-26.

Martin, H. 1964. Zur nahorientierung der biene im duftfield. Zugleich ein nachweis fur die osmotropotaxis bei insekten. *Zeitschrift fur Vergleichende Physiologie*, 48(5) : 481-533.

------, 1965. Leistungen des topochemischen sinnes bei der honigbiene. *Zeitschrift fur Vergleichende Physiologie*, 50 (3) : 254-292.

Mautz,D., 1971. Der Kommunikationseffekt der Schwanzel tanze bei Apis mellifica carnica (Pollm). *Zeitschr.Vergl.Physiol* 72 : 197-220.

McIndoo, N.E. 1914. The olfactory sense of the honeybee. *J.exp.Zool.* 16, 265-346.

------, 1922. The auditory sense of the honeybee. *J.comp. Neurol.*, 34, 173-199.

McIver, S. and R. Sciemicki 1982 Fine structure of maxillary sensilla of larval Toxorhynchites brevipalpis (Diptera : Culicidae) with comments on the role of sensilla in behavior. *J.Morphol.* 171 : 293-303.

Menzel, R. and J. Erber. 1978. Learning and memories in Bees. *Scientific American*

Michener, C.D.1944. Comparative external morphology phylogeny, and a classification of the bees. (Hymenoptera). *Bulletin of the American Museum of National History*, 82(6) : 157-326.

------, 1969. Comparative social behaviour of bees. *Annual Rev. of Ent.*, 14 : 299-342.

------, 1974. The social behaviour of the bees. Belknap Press of Harvard University Press, Cambridge.

Milum, V.G., 1955. Honeybee communication. *American Bee Journal*, 95(3) : 97-104.

Minnich, D.E. 1932. The contact chemoreceptors of the honeybee, (Apis mellifera L.) *Jour.of exp.Zool.*, 61(3) : 375-393.

Morland, D.M.T. 1930. The world of the honeybee. (*The New Naturalist*) Collins, London.

Newton, D.C. 1968. Behavioural response of honeybees to colony disturbance by smoke. I. Engorging behaviour. *J.Apic.Res.*, 7 : 3-9.

Nunez, J.A. 1966. Quantitative beziehungen zwischen den eigenschaften von futterquellen und den verhalten von Sammelbienen *Zeitschrift Vergl.Physiol.*, 53 : 142-164.

------, 1970. The relationship between sugar flow and foraging and recruiting behaviour of honeybees (Apis mellifera L.) *Anim.Behav.*, 18 : 527-538.

Nunez, J.A. 1971 Bcobachtungen an sozialbezogenen Verhaltensweissen von Sammelbienen. *Zeitschr. Tiersychologie* **28** : 1-18.

Oliveira, B.L. de. 1960. Mudas ontogeneticas em larvas de Milipona migra schencki, Gribodo. Biol.Univ.Parana, *Zoology* No. 2 : 1-16.

Oster, G.F. and E.O. Wilson, 1978. Caste and ecology in the social insects. Princeton NJ., USA : Princeton University Press.

Pain, J. 1968. In : Traite de biologie de l'abeille by R. Chauvin, Paris, Masson and Cie, 45-99.

Pflumm, W. 1969. Stimmungsanderugen der biene wahrend des aufenlhath an der futterquelle. *Zeitschrift fur Vergleichende Physiologie*, 65 : 299-323.

------, 1985. Consecutive antenna grooming as displacement activity of the honeybee in collecting differently concentrated sucrose solution *Insectes Soc.*, 32 (4) : 435-444.

Pringle, J.W.S. 1938a. Proprioception in insects II. The action of the campaniform sensilla on the legs. *J.exp. Biol.*, 15 : 114-131.

--------, 1938b. Proprioception in insect III. The function of the hair sensilla at the joints. *J.exp.Biol.*, 15 : 467-473.

------, 1974. Locomotion : Flight. In the physiology of Insecta. (ed. M. Rockstein) Vol.B, Academic Press, NY, pp. 433-476.

Ribbands, C.R. 1949. The foraging methods of individual honeybees. *J.Anim.Ecol.* 18 : 47-66.

------, 1952. Division of labour in the honeybee community. *Proc. Royal Soc.*, London (B), 140 : 32-43.

------, 1953. The behaviour and social life of the honeybee. *Bee Res.Assoc.Ltd. Lond.*, pp. 352.

------, 1955. The scent perception of the honeybee *Proc. Royal Soc. Lond.*, (B) 143 : 367-379.

------, and Nancy Spiers. 1953. The adaptibility of the home coming honeybee. *British Jour. of Anim.Behav.*, 1(2) : 59-66.

------, H. Kalmus and H.L. Nixon. 1952. New evidence of communication in the honeybee colony *Nature*, London 170 : 438-440.

Richards, O.W. 1965. Concluding remarks on the social organization of insect communities. *Symposium of the Zoological Society of London*, 14 : 169-172.

Ritter, E. 1936. Untersuchungen uber den chemischen sinn bein schwarzen kolbenwasserka fir hydrous piceus. *Z.Vergl.Physiol.*, 23 : 545-570.

Rodionov,V.V. and I.A. Shabarshov. 1986. The fascinating world of bees. MIR Publishers, Moscow.

Root, A.I. 1966. ABC and XYZ of bee culture. Medina, Ohio.

Rosch, G.A. 1925. Untersuchungen uber die arbeitskilung im bienestaat. I. Teil die taligkeiten im normalen bienenstaate und inhre beziehungen zum alter der arbeitsbienen. *Zeitschrift fur Vergleichende Physiologie*, 2(6) : 571-631.

---------,1927. Uber die bautatigkeit im bienenvolk und das alker der baubienen. Weiterer beitrag zur frage nach der arbeitsteilung im bienenstaat. *Zeitschrift fur Vergleichende Physiologie*, 6(12) : 264-298.

------, 1930. Untersuchungen uber die arbeistelung im bienenstaat. 2. Teil. die tatigkeiten der arbeitsbienen unter experimental veranderten bedingungen. *Zeitschrift fur Vergleichende Physiologie*, 12 (1) : 1-71.

Sakagami, S.F.1953a. Untersuchungen uber die arbeitsteilung in einem zwergvolk der honigbiene. Beitrage zur biologie des bienenvolkes, Apis mellifera L., I, *Jap.J.Zool.*, 11 : 117-185.

------, 1953b. Arbeitsteilung der arbeiterinnen in einem zwergvolk, bestehend aus gleichaltrigen volksgenossen *J.Fac.Sci.Hokkaido Univ.*, (6 Zool). 11 : 343-400.

------, 1954. Occurrence of an aggressive behaviour in queenless hives, with considerations on the social organization of honeybee *Insectes Sociaux*, 1141 : 331-343.

Schmid, J. 1964. Zur Frage der Storung des Bienengeotachatnisses durch Narkosemittel, Zugleich ein Beitrag zur storung der sozialen Bindung durch Narkose. *Zeitschr.Vergl.Physiol.* 47 : 559-595.

Schmidt, J.M. and J.J.B. Smith. 1985. The ultrastructure of the wings and external sensory morphology of the thorax in female Trichogramma minutum (Hym : chalicidoidea, Trichogrammatidea). *Proc. Royal Soc. Lond.*, B. Biol.Sci. 224(1236) : 287-314.

Schneider, D. 1964. Insect antennae *Ann.Rev.Entomol.* 9 : 103-22.

Schoenitzer, K. and M. Renner. 1984. The function of the antennal cleaner of the honeybee. (A.mellifica). *Apidologie*, 15(1) : 23-32.

Schwarz, R., 1955. Uber die riechscharfe der honigbiene *Zeitschrift fur Vergleichende Physiologie*, 37(3) : 180-210.

Seeley, T.D. 1986. Social foraging by honeybees. How colonies allocate foragers among patches of flowers. *Behav.Ecol.Sociobiol.*, 19(5) : 343-354.

Sekiguchi, K. and S.F. Sakagami. 1966. Structure of foraging population and related problems on the division of labour in bee colonies. *Rep.Hokkaido natn.agric. exp.str.*, 69 : 1-65.

Shearer, D.A., R. Boch, R.A. Morse and .M. Laigo. 1970. Occurrence of 9-oxodec-trans-2-enoic acid in queens of Apis dorsata, Apis cerana and Apis mellifera. *J.Insect Physiol.*, 16 : 1437-1441.

Simpson, J. 1959. Variation in the incidence of swarming among colonies of Apis mellifera throughout the summer *Insectus Sociaux*, 6 : 85-99.

------, 1963. The factors that causes swarming by honeybee colonies in small hives. *J.apic.Res.*, 2 : 50-54.

Singh, S. 1962. Beekeeping in India. "Bee posture". ICAR Publication, India.

Sladen, F.W.L. 1902. A scent-producing organ in the abdomen of the bee. *Gleanings in Bee Culture*, 26 : 639.

Sndograss, R.E. 1935. Principles of insect morphology. McGraw-Hill, New York.

------, 1956. Anatomy of the honeybee, Comstock Pub. Assoc. Cornell University Press, Ithaca. NY., xiv + 334pp.

Steche, W. 1957. Soziale steuerung des Alarmiwortes der Bienentanze *Naturwissenschaften* **44** : 597-598.

Steiner, A. 1947. Der warmehaushalt der einheimis chen sozialen dautflugler buh. schweiz. *Bienen-Zeitg.* 2 : 139-256.

Szabo, T.I. 1972. Behavioural studies on queen introduction in honeybees (Apis mellifera) University of Guelph : Ph.D. thesis. 113 pages.

------, 1975. Behavioural studies on queen introduction in the honeybee. Effect of age and storage conditions of virgin queens on their attractiveness to workers. *J.Apic.Res.*, 13(2) : 127-135.

------, 1975. Behavioural studies on queen introduction in honeybees. Introduction of queen models into honeybee colonies (Hymn. Apidae). *Am.Bee J.*, 114(5) : 174-176.

Taranov, G.F. 1955. On the biology of swarming in bees (in Russian) Pchelovodstvo 32 : 32-35.

Thompson, D. 1966. *Growth and Form*, Cambridge University Press, England.

Thurm, U. 1964. Mechanoreceptors in the cuticle of the honeybee : Five structure and stimulus mechanism, *Science*, 145 : 1063-1065.

Tillyard, R.J. 1931. The wing-venation of the order isoptera. Introduction and the family Mastotermitidae. *Proc.Lin.Soc.New South Wales*, 56(4) : 371-390.

Velthuis, H.H.W. 1970. Queen substances from the abdomen of the honeybee queen. *Zeitschr.Vergl.Physiol.*, 70 : 210-222.

------, 1972. Observations on the transmission of queen substances in the honeybee colony by the attendant of the queen. *Behaviour*, 41 : 105-129.

Verheigin-Voogd, C. 1959. How worker bees perceive the presence of their queen *Zeitschr.Vergl.Physiol.*, 41 : 527-582.

Vidano, C. 1987. Bees and beekeeping in India. *Apic.Mod.*, 78(3) : 91-101.

Visscher, P.K. 1983. The honeybee way of death : Necrophoric behaviour in A. mellifera colonies. *Anim.Behav.*, 31(4) : 1070-1076.

Voogd, S. 1955. Inhibition of ovary development in worker bees by extraction fluid of the queen. *Experimentia*, 11 : 181-182.

Weaver, N. 1966. Physiology of caste determination *A. Rev. Ent.*, 11 : 79-82.

Wenner, A.M. and D.L. Johnson. 1966. Simple conditioning in honeybee *Animal Behaviour*, 14 : 149-155.

Wheeler, W.M. 1927. *Emergent evolution and the social*, Kegan Paul, Trench, Tribner and Co.Ltd., London, xviii, 378 pp.

Wigglesworth, V.B. 1972. The principles of insect physiology, London : Chapman and Hall.

Wilde, J.De, and J. Beetsma, 1982. The physiology of caste development in social insects *Adv.Insect Physiol.*, 16 : 167-246.

Wilhelm, K.T. and W. Pflumm. 1984. Influence of flower like fragrances at the food source on the scent marking behaviour of foraging honeybees (A. mellifera). *Apidologie* 14(3) : 183-190.

Wilson, E.O., and W.H. Bossert. 1963. Chemical communication among animals. *Recent Progress in Hormones Research* **19** : 673-716.

------, 1968. The ergonomics of caste in the social insects. *American Naturalist*, 102, 41-66.

-------, 1971. The insect societies. Cambridge, Mass : Harvard University Press.

Winston, M.L. and E.N. Punnett. 1982. Factors determining temporal division of labour in honeybees. *Can.Jour. Zool.*, 60 : 2947-2952.

Wittekindt, W.O. 1960. Schwanzelbewegungen als Ausdruck gesteigerter Erregung innerhalb des Tanzverhaltens der Honigbiene. *Naturwissenschaften* **47** : 335-336.

---------, 1966. Das Durchwinden, eine Tanz-und Alarmierungsform der Honigbiene. *Beinenzucht* **19** : 14-24.

Wolf, E. 1927. Ueber Das HeimKehrvermogen der Bienen, II *Zeitschr.Vergl.Physiol.* **6** : 227-254.

Wooley, T.A. and C.R. Vossbrinck, 1977. SEM as a tool in anthropod taxonomy. SEM/1977/11. *IIT Res.Inst.Chicago II*, 60616, 645-652.

Yadava, R.P.S. and M.V. Smith, 1971. Aggressive behaviour of Apis mellifera L. workers towards introduced queens. I. Behavioural mechanisms involved in the release of worker aggression. *Behaviour*, 39 : 212-236.

Zander, E. 1951. Der bau der biene stuttgart : Eugen ulmer.

Zdarek, J. and O. Harhgsim. 1974. *Jour.Ins.Physiol.*, 7 : 258.

Zill and Moran, 1981. *Jour.Morphol.Comp.Physiol.*