MATERIALS AND METHODOLOGY

All brood Stages: eggs, young & old larvae & capped cells.
A detailed morphometric and behavioural characters of *Apis cerana indica* Fabricius were undertaken during the study period 2000 - 2004. The different materials and methods employed during this research period are presented hereunder.

3.1 Morphometry of Indian honeybee *Apis cerana indica* Fabricius

Shimoga district is situated between 13° 27' and 14° 39' – north latitude and between 74° 38' and 76° 4' – east longitude, in about the mid-south western part of the Karnataka state.

Worker bee samples were collected during summer and winter seasons from different geographical areas viz., Megaravalli of Apiary – Agumbe, Theerthahalli; Bheemanakone of Apiary - Purappemane, Hosanagar; Hegdekkoppa of Apiary - Agumbe, Theerthahalli; Halasinahalli of Apiary - Hosanagar, Hosanagar; Nittur of Apiary - Hosanagar, Hosanagar; of Shimoga district, and are summarized as follows.
Table 3.1: Study sites, Altitudes and their Apiaries in Shimoga District.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Name of the Place or Village</th>
<th>Altitudes in meters</th>
<th>Name of the Taluk</th>
<th>Name of the Apiary</th>
</tr>
</thead>
<tbody>
<tr>
<td>01.</td>
<td>Megaravalli</td>
<td>642</td>
<td>Theerthahalli</td>
<td>Agumbe</td>
</tr>
<tr>
<td>02.</td>
<td>Bheemanakone</td>
<td>640</td>
<td>Hosanagar</td>
<td>Purappemane</td>
</tr>
<tr>
<td>03.</td>
<td>Hegdekoppa</td>
<td>610</td>
<td>Theerthahalli</td>
<td>Agumbe</td>
</tr>
<tr>
<td>04.</td>
<td>Halasinahalli</td>
<td>608</td>
<td>Hosanagar</td>
<td>Hosanagar</td>
</tr>
<tr>
<td>05.</td>
<td>Nittur</td>
<td>572</td>
<td>Hosanagar</td>
<td>Hosanagar</td>
</tr>
</tbody>
</table>

Figure 1: Map of Shimoga District

Table 3.1: Study sites, Altitudes and their Apiaries in Shimoga District.
Worker bees were collected at the entrance of the beehive. The honeybees were culled in acetone to ensure full extension of morphological parts of the entire body. Hundred bees were collected from each colony. Out of the hundred individuals, twenty-five bees were carefully studied for the different morphometric features.

The various morphological characters used by Kshirsagar (1980) are being referred for this present study about the morphological characters of Indian honeybee *Apis cerana indica* Fabricius. With the help of standardized ocular micrometer placed in the eyepiece of the compound microscope, the measurements of different body parts were made. The several measurements taken are as follows.

3.1.1: **Length of the body**: The entire size of the body of a bee extending between the anterior tip (head region) of the body to that of tip of abdomen (Figure 2).

3.1.2: **Antennal length**: This is the distance between the base of the scape to the tip of the flagellum (Figure 3).

3.1.3: **Length of Proboscis**: The total distance present between the base of the postmentum to that of the tip of the glossae (Figure 4).

3.1.4: **Length of Prementum**: The total distance measured between the tip of the postmentum to that of the base of the glossae (Figure 4).
Fig. 2: Externals of Honeybee (Snodgrass)

Ab = Abdomen;  Ant = Antenna;
E = Compound eye;  H = Head;
L1, L2 and L3 = Legs;
Md = Mandible;
Prb = Proboscis;
Sp = Spiracle;
Th = Thorax;
W2, W3 = Wings;
I = Propodeum;  II – VII = Abdominal segments
**Fig: 3: The Antenna**
- SpL = Length of Scape
- pdL = Length of Pedicel
- FgL = Length of Flagellum

**Fig: 4: Proboscis of Worker bee**

**Fig: 5: Thorax - Ventral surface**
- N1 = Notum – 1
- S2 = Sternum – 2
- CxC = Coxal cavity
- IS = Intersegmental grooves of Thorax
Fig: 6: Forewing of Worker bee
PwL = Length of fore wing
FwB = Breadth of fore wing
RcL = Length of radial cell
RcB = Breadth of radial cell
RcL1 = Length of basal portion of radial cell
RcL2 = Length of apical portion of radial cell
a = Length of 1st abscissa of vein;
b = Length of 2nd of abscissa

Fig: 7: Hindwing of Worker bee
HwL = Length of hindwing
HwB = Breadth of hindwing
RL = Length of basal portion of radial vein
ML = Length of apical portion of radial vein
VL = Length of discoidal vein
IL = Length of indica vein
NH = Number of hamuli
EH = Extent of hamuli
JL = Length of jugal lobe
VnL = Length of vannal lobe
Fig: 8: Fore leg of Worker bee – left anterior

Tb = Tibia;
a = Clasp of Antenna cleaner;
b = Notch of Antenna cleaner;
Btar = Basiatarsus

Fig: 9: Middle leg of Worker bee
Fig: 10: Hind leg of Worker bee

Btar = Basitrasus;
Cx = Coxa;
Fm = Femur;
Pr = Pollen press;
Tb = Tibia;
Tr = Trochanter

Fig: 11: Abdomen of Worker bee

T = Tergum
S = Sternum
Sp = Spiracle
3.1.5: **Length of Postmentum:** The total distance present between the base of the postmentum to the base of prementum (Figure 4).

3.1.6: **Length of Thorax:** The distance measured between the anterior and posterior tips of the thorax i.e., the distance between prothorax and the metathorax (Figure 5).

3.1.7: **Breadth of Thorax:** The distance measured nearer to the posterior angles where the thorax is broadest (Figure 5).

3.1.8: **Length of Forewing:** The distance measured between the articulator point of forewing with its apical tip (Figure 6).

3.1.9: **Breadth of Forewing:** The distance measured between the middle of forewing where it is maximum (Figure 6).
3.1.10: **Length of Hindwing**: The distance measured between the articulatory point of hindwing with its apical tip (Figure 7).

3.1.11: **Breadth of Hindwing**: The distance measured in the middle of hindwing where it is maximum (Figure 7).

3.1.12: **Extent of Hamuli**: The distance measured between the first hook to the last hook (Figure 7).

3.1.13: **Number of hooks**: Number of hooks present on the coastal margin of the hindwing (Figure 7).

3.1.14: **Length of Corbicula**: The distance present between the sides of the concave surface of the corbicula (Figure 10).

3.1.15: **Corbicula breadth**: Distance measured between the anterior tip and posterior tip of the corbicula (Figure 10).

3.1.16: **Length of Abdominal sternum**: Distance measured between the anterior tip to the posterior base of the sterna. All six sterna were measured (Figure 11).

3.1.17: **Length of Sting**: The distance measured from the base to the tip of the sting (Figure 12).
3.2 Behavioural traits of *Apis cerana indica* F.

Fortnightly observations on behavioural traits of Indian honeybee, *Apis cerana indica* of Shimoga district, Karnataka were made on yellow and black strains of normal colonies numbered serially and kept at random for a period of 12 months of each year of study period. The honey and pollen stores and bee population were kept more or less equal in each colony at the beginning of study.

3.2.1 Pollen load carrying capacity of black and yellow strains of *Apis cerana indica* F.

To estimate the amount of pollen carried by individual honeybee colonies were selected and were designated for studying pollen and honey storage. The hive entrance was closed for a short while at the time of observation. Ten foraging bees returning with pollen load were collected from each hive and the pollen pellets were brushed off from the pollen basket and the fresh weight of the pollen pellets was taken. Such observations were recorded at 09.00 hr, 12.00 hr and 15.00 hr, during the day at an interval of 15 days, uniformly for all the observations, as envisaged for the study.
3.2.2 Pollen and honey stores of black and yellow strains of Apis cerana indica F.

Observations on pollen and honey stores were recorded at 15 days interval in all the experimental colonies using the marked transparency sheet, which consisted of number of squares and each square with an area of 1 cm\(^2\). This sheet was placed on the brood and super frames and the number of squares with honey and pollen were recorded. Cells filled with pollen or honey scattered in different parts in a comb were counted separately and converted into square centimeter area. The above parameters were recorded on both faces of all brood and super frames.

3.2.3 Bees’ population of black and yellow strains of Apis cerana indica F.

Bees’ population per colony was estimated at an interval of one month. The hive entrance was closed at late night, next day morning weight of the whole colony (C), empty box (B) and frames along with comb without bees (F) were taken. The weight of frames (F) and empty box (B) were subtracted from weight of whole colony (C), which gives the net weight of bees (E) \[E = C - B - F\]. Three samples of 100 bees were anaesthetised using ether and weighed, the average individual bee weight was calculated; net weight of the bee colony (E) was converted to number of bees to study the bee population of the colony.
3.3 Bee flora and Melissopalynology of Apis cerana indica F.

The studies on bee flora, foraging behaviour and melissopalynology were conducted for a period of one year i.e., from June 2000 – May 2001 in different locations of Shimoga district. Honey samples were collected from Megaravalli of Apiary – Agumbe, Theerthahalli; Bheemanakone of Apiary - Purappemane, Hosanagar; Hegdekoppa of Apiary - Agumbe, Theerthahalli; Halasinahalli of Apiary - Hosanagar, Hosanagar; Nittur of Apiary - Hosanagar, Hosanagar; of Shimoga district (Table 3.1 and Figure 1).

The materials used, techniques adopted and observations recorded during the study course are presented here under.

3.3.1 Bee flora

Bee flora of Apis cerana indica Fabricius was studied regularly once a week from June 2000 to May 2001 by recording different angiospermic plants that were visited by the foragers. The flowering period of each of such bee plant was noted by recording the period of commencement and cessation of flowering. During the full blooming period the foraging activities of bees were recorded. It is noted that the bees visited varieties of plant groups including trees, field crops, fruit crops, plantation crops, ornamental plants, vegetable crops etc. The flowers were collected from those plants, which were frequently visited by the foragers, and the same were used to prepare the pollen photomicrographs (Erdtman, 1952).
3.3.1.1 Preparation of palynographs

Few freshly blossomed flowers of different bee plants were collected in separate polythene bags. In the laboratory, the anthers of these flowers were disturbed gently in order to dust the pollen at the center of a clean glass slide. With the help of a drop of water, the pollen was mounted using a cover slip. The pollen grains were observed under the compound microscope and photographed. Such photographs are referred to as Pollen photomicrographs or Palynographs. These photomicrographs are used as reference pollen slides for identifying the pollen source in the melissopalynological studies.

3.3.2 Melissopalynological studies

Honey samples were collected from each of colonies of the above said study areas at an interval of one month. Palynographs were prepared from honey samples and pollen types in various honey samples were identified with the help of reference palynographs. Then the pollen grains were counted with the aid of compound microscope. Depending upon the frequencies of pollen grains present in various honey samples the absolute pollen count and percentage of pollen types were made and pollen spectrum was constructed on the basis of these percentages (Louveaux et al., 1970).
3.3.2.1 Preparation of Palynographs from honey samples

Pollen photomicrographs were prepared from the honey samples to identify the major pollen source in the study area. About 10 gm of honey samples was diluted with distilled water in a clean glass test tube (1:1). These diluted samples were taken in eppendorf tubes and centrifuged at 2000 rpm for a period of 2 minutes. The supernatants were discarded and the pellet is placed on the center of a glass slide. To this preparation, a drop of water is added and mounted using a cover slip. The palynographs were prepared by photography and pollen grains are observed under the compound microscope (Sheshagiri, 1985). The numbers of pollen grains of each plant type were counted at 3-4 microscopic fields. Later the total number of pollen of each type in all the study centers was counted. The frequency of each type of pollen was expressed as percentage of the total number of pollen.

3.3.2.2 Preparation of pollen spectra from bee visiting flowers

A pollen spectrum was prepared from pollen grains collected from the flowers of different species within a specific locality. The per cent pollen grains of each type is represented in a pie diagram. This pollen spectrum was to act as a reference for the pollen types collected from the pollen loads of foraging bees within the locality. Different pollen types are placed under the four classes of pollen frequency according to the recommendations of the International Commission for Bee Botany (Moore and Webb, 1978), as follows.
<table>
<thead>
<tr>
<th>Pollen frequency class</th>
<th>Percentage of pollen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predominant pollen</td>
<td>Above 45%</td>
</tr>
<tr>
<td>Secondary pollen</td>
<td>16 – 45%</td>
</tr>
<tr>
<td>Important minor pollen</td>
<td>3 – 15%</td>
</tr>
<tr>
<td>Minor pollen</td>
<td>Less than 3%</td>
</tr>
</tbody>
</table>

3.3.2.3 Preparation of pollen spectra from pollen loads of honeybees

Two colonies of almost uniform strength were selected from the study sites. In each colony three foragers returning with pollen loads were captured at 09.00 hr, 12.00 hr and 15.00 hr. From the pollen baskets of each forager, the pollen was taken out with the help of a fine brush. These pollen grains were fixed in 70 per cent alcohol in small glass vials. These samples were used for the preparation of pollen photomicrographs. Reference palynographs were used to identify the sources of pollen. The per cent frequencies of each pollen source was calculated and a palynograph was constructed to identify the predominant, secondary, important minor and minor sources of pollen grains (Sheshagiri, 1985).