PATHOGENICITY OF THAI SACBROOD VIRUS TO THE ECOTYPES OF *Apis cerana indica* Fab. IN KERALA

BY

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THESIS

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1998

DECLARATION

I hereby declare that this thesis entitled **"Pathogenicity of Thai Sacbrood Virus to the Ecotypes of *Apis cerana indica* Fab. in Kerala"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

Vellayani,
31 - 7 - 1998.

S. DEVANESAN 31/7/98

CERTIFICATE

Certified that this thesis entitled **"Pathogenicity of Thai Sacbrood Virus to the Ecotypes of *Apis cerana indica* Fab. in Kerala"**, is a record of research work done independently by **Sri. S. Devanesan** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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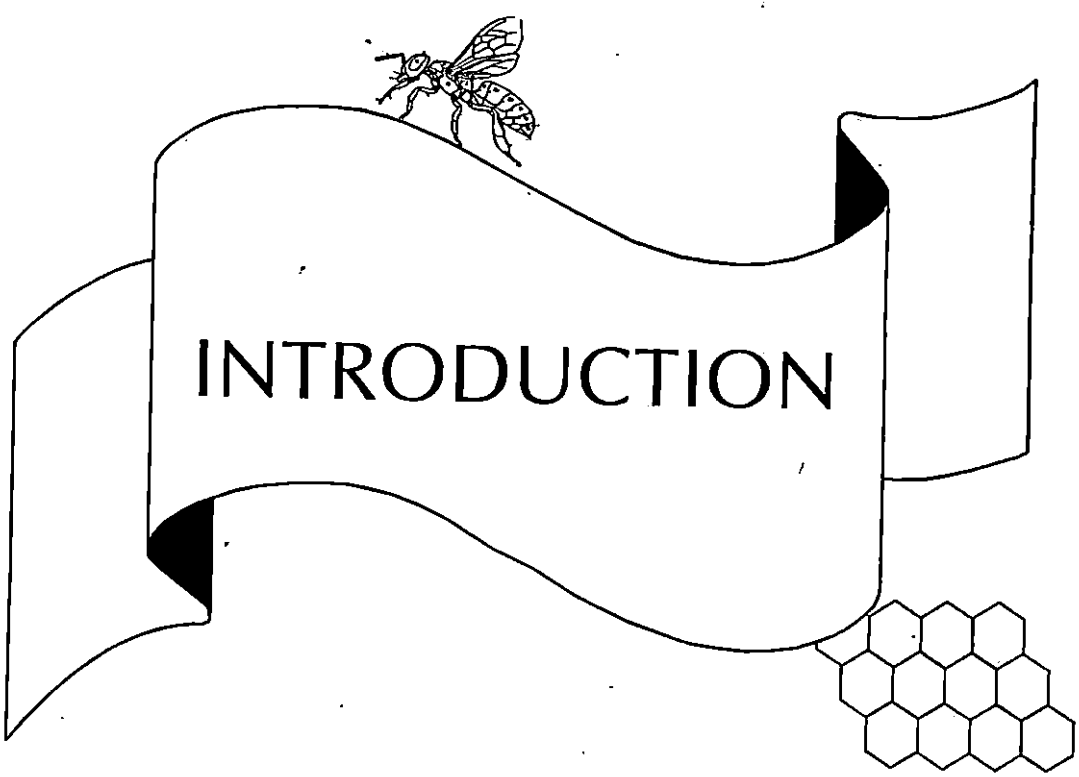

INTRODUCTION

INTRODUCTION

Apiculture has been an important source of additional income for small and marginal farmers, landless labourers and other weaker sections of society all over the world. Hive products such as honey, beeswax, royal jelly and pollen are much in demand in internal and export markets. Another significant, but not widely recognized, role of honeybees is that they enhance productivity of crops through cross pollination.

Beekeeping was practised in the erstwhile Travancore in a primitive way, using different types of crude hives. Later, hives with movable frames were introduced under the pioneership of YMCA, Marthandam in 1924 and they revolutionized bee culture practices in the region. Subsequently many organisations like Khadi and Village Industries Commission, Kerala Khadi and Village Industries Board, Kerala Sarvodaya Sangh, Kerala Gandhi Smaraka Nidhi, Rubber Board and Spices Board took up leading roles in the popularisation of apiculture. Beekeeping made rapid strides in Kerala during the second half of the twentieth century and it got established as a major activity of the cottage industry sector.

Detection of rubber (*Hevea brasiliensis* Muell. Arg.) as a rich source of nectar, during seventies, gave a big boost to the beekeeping

industry in the state. Subsequently, honey production through migratory beekeeping got established and Kerala state became the topmost producer of honey in India.

The Indian bee, *Apis cerana indica* Fab., has been the only domesticated species utilized for commercial beekeeping in Kerala. In 1991-92, the catastrophic outbreak of Thai Sacbrood Virus (TSBV) disease resulted in the destruction of more than 90 per cent of the then existing bee colonies in the state. This resulted also in a drastic reduction in the honey production. Kerala now has to import honey to meet its internal demand.

Thai Sacbrood Virus disease was first observed in 1976 in Thailand on *Apis cerana* causing 100 per cent mortality (Bailey *et al.*, 1982). In India, this disease first appeared in parts of Meghalaya and Assam in 1978 and by 1985 it had spread to Uttar Pradesh, Punjab and neighbouring states and had virtually wiped out the colonies of *A. cerana indica* from these areas (Shah and Shah, 1988).

Inspite of the dreadful nature of the disease, systematic studies on it are limited. Information available is restricted to its occurrence in various localities with brief symptomatology, electron microscopy and serology of the causative agent as well as suggestions for the management of the disease (Kshirsagar *et al.*, 1975; Bailey *et al.*, 1982; Kshirsagar 1983 a, b; Joshi and Verma 1985; Rana *et al.*, 1988, 1991; Shah and Shah 1988; Abrol and Bhat 1990; Jacob *et al.*, 1992).

Being a viral disease, drugs are not very useful to prevent or control the disease. Management practices suggested so far are of little benefit in containing the malady (Phadke, 1983; Abrol and Bhat, 1990). In this context introducing *Apis mellifera*, known to be resistant to TSBV, into the diseased areas if it is ecologically compatible, and selecting out a strain of native Indian bee, *A. cerana indica* showing resistance to the virus from the surviving populations would appear to be the reasonable approaches to tackle the problem.

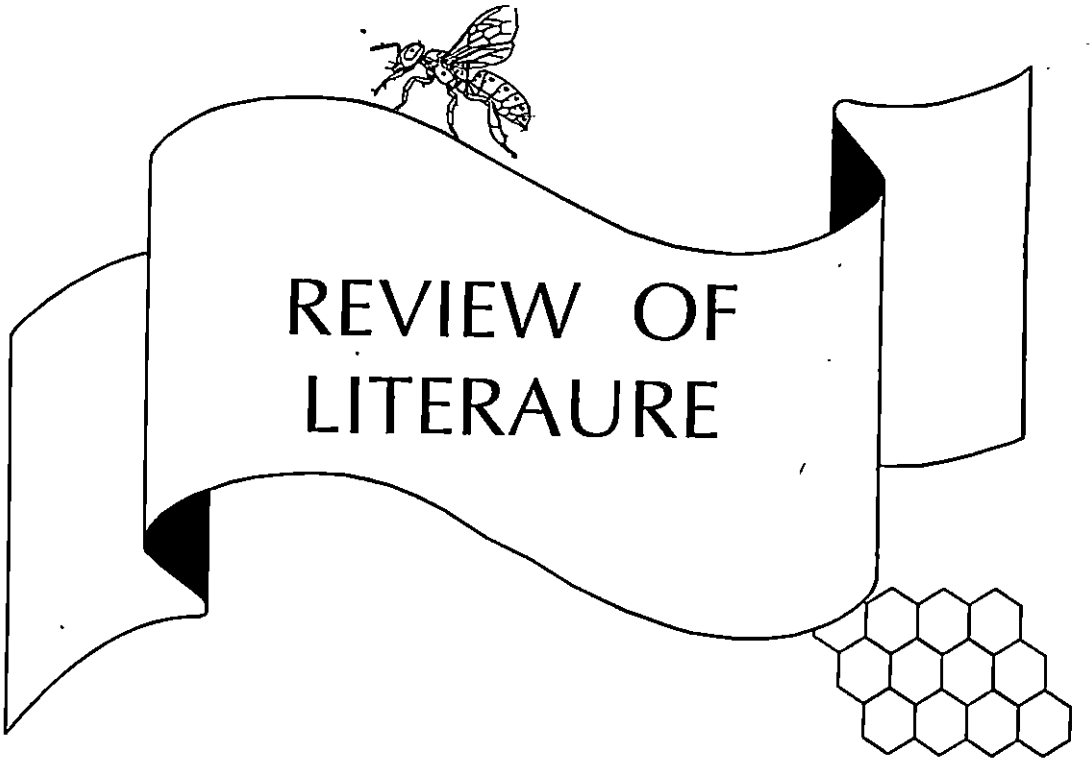
It has been widely reported that a small percentage of bees (*A. cerana indica*) survives in some areas even after severe outbreak of the disease (Verma and Joshi, 1988; Abrol and Bhat, 1990; Abrol, 1993). This has been attributed to the immunity developed by them and it may be associated with the geographic races or ecotypes of bees. By selective breeding of such surviving colonies, it could be possible to evolve a resistant strain of *A. cerana indica.*

As a result of continuous process of natural selection through centuries, different geographic races of a particular species of honeybee have been evolved (Verma, 1992). Geographic races of *Apis mellifera* have been extensively reported from tropical Africa, North America and near east and west Mediterranean region (Ruttner, 1985, 1986). The geographic races have further locally adapted populations called "ecotypes". Biological and economic variations existing in different geographic races and ecotypes of *A. mellifera* have been successfully exploited for their genetic

improvement through selection and breeding. In *A. cerana*, hilly and plain varieties of the species have been reported (Kapil, 1956; Narayanan *et al.*, 1960, 1961a and 1961b; Kshirsagar, 1976). Ruttner (1985, 1986, 1988) has distinguished *A. cerana indica* as the sub-species/race present in South India. Verma (1992), through discriminant analysis, indicated the existence of two biometric sub groups/ecotypes in south India. The first group is distributed in the Kerala region and the second in the Karnataka and Tamil Nadu regions. But these results are based on studies with very few locations and bee samples from Kerala. With its wide range of geographic and ecological conditions, Kerala is likely to have more than one ecotype as reported earlier. Further, little is known about the resistance of the strains of *A. cerana indica* thriving under the different geographic locations to TSBV.

The present studies were hence taken up with the following objectives :

1. Characterisation of the Indian bees (*Apis cerana indica*) collected from different tracts of Kerala by biometric studies to fix up their ecotypes and assessment of their susceptibility to Thai Sacbrood Virus.
2. Assessment of the economic attributes of the resistant ecotypes if any.
3. Collection of detailed information on the susceptibility of different larval instars of *A. cerana indica* to Thai Sacbrood Virus and the symptomatology of the disease.


REVIEW OF
LITERAURE

REVIEW OF LITERATURE

Information on subjects related to the research programme has been briefly reviewed in this chapter.

2.1 Species diversity within *Apis*

The genus *Apis* has recently been recognized as being more diverse than was previously believed to be (Ottis, 1991). Four species of the genus *Apis* are Rockbee, *Apis dorsata* Fab., Little bee, *A. florea* Fab., Asian hivebee/Indian honeybee, *A. cerana indica* Fab. and European hive bee, *A. mellifera* Linn. Of these the former two species are wild and the latter two are domesticated and used for commercial beekeeping. First three species were present in India and *A. mellifera* was introduced from European countries.

Three more species of the genus *Apis* have been suggested to exist. *A. koschevnikovi* (Ruttner *et al.*, 1989) reported from South East Asia and it appears to be similar to *A. florea*. Another closely related species known to exist in Thailand, Malaysia and Southern China is *A. andreniformis* (Wongsiri *et al.*, 1990). *A. laboriosa* from the Himalayas is another new species which looks like *A. dorsata* (Sakagami *et al.*, 1980).

2.2 Races and ecotypes of the Asian hivebee (Indian honeybee) *Apis cerana* Fab.

Apis cerana had been the base of Indian beekeeping and is found throughout India except in the plains of north India. This species is distributed in Pakistan, Sri Lanka, Malaysia, Philippines, China, USSR, Japan, Thailand and Indonesia also. It is native to Southern and Eastern Asia (Verma, 1992) and thrives upto 2500 m mean sea level (MSL) (Mishra, 1995). It is a bee with gentle temperament and it responds to smoking. It is frugal in habit but lack of flora is quickly reflected in the absconding of the hives. It also has a strong tendency for swarming (Mishra, 1995).

2.2.1 Genetic diversity of *Apis cerana*

Extensive geographic variation is exhibited by honeybees (Ottis, 1991; Verma, 1992). Such geographic races of *A. mellifera* existing in tropical Africa, North Africa and near east and west Mediterranean regions have been identified through computer-based biometric analysis (Ruttner, 1985, 1986, 1988). Each geographic race of honeybee species has further locally adapted distinct populations called ecotypes which differ from each other in several biological and economic characters (Verma, 1992).

The biological and economic differences existing in different geographic races and ecotypes of honeybees provide an excellent opportunity

for genetic improvement by selection and breeding. These differences have been extensively exploited in *A. mellifera* with remarkable successes (Freshaye and Lavie, 1976; Nye and Mackenson, 1970).

Very little is known about the geographical variations exhibited by *A. cerana indica* except the arbitrary hilly and plain varieties of this species reported. Kapil (1956) compared the biometric characters of *A. cerana indica* workers of plains (Allahabad 26° N lat.) and hill (Nagrota 32° N lat.) varieties. The differences between the two varieties were significant for many characters except for the number of hamuli and cubital index. Narayanan *et al.* (1960, 1961a) recorded the tongue length and number of hooks on the hindwings of plain and hill races. The tongue length was 4.84 ± 0.05 mm for leather coloured plain race, 5.16 ± 0.01 mm for leaden grey hill race and 4.9629 ± 0.0041 mm for bees from Pusa. Number of wing hooks were 16.91 ± 0.09 and 18.26 ± 0.06 for the plains and hill races respectively. Tongue length increased with altitude. The studies led the authors to conclude that three races (or biological varieties) of *A. cerana indica* i.e., Himalayan, Gangetic plains and South Indian could be distinguished.

Mattu and Verma (1983, 1984 a, b) made exhaustive morphometric studies of tongue, antennae, wings, hindlegs, tergites and sternites of *A. cerana indica* in North-West Himalayas. The characters varied between the bees of Himachal and Kashmir regions and most characters were correlated with altitude. Similar observations were reported earlier by

Kshirsagar and Ranade (1981) and they found geographical gradient in several characteristics.

Mattu and Verma (1980) found that some biometrical characters in *A. cerana indica* varied with altitude and some values were even higher than those of introduced *A. mellifera*.

Kshirsagar (1981) reported the effect of geographical position on morphometric characters. He found that latitude apparently affected morphology more than longitude and that altitude also had an influence. Positive correlations with altitude were found for 23 morphometric characters of bees from Manipur, Mizoram and Nagaland (Singh, 1984).

Many of the morphological characters such as length of flagellum, antenna, forewing, radial cell, wing vein, antennal lobe, femur, tibia, third tergite, wax mirror, 3rd and 6th sternites and breadth of hindwing had significantly higher values in bees collected at Shimla during summer and autumn than those collected in other seasons (Mattu and Verma, 1984 c). Tongue, length of scape, hind wing, radial cell etc. had no seasonal variations. Thus they concluded that summer and autumn were the periods of honey flow and colony strength, and presence of surplus food for larvae might affect the biometry of *Apis cerana*.

In the recent past attempts have been made to identify different races of *A. cerana* by using computer-assisted standard statistical methods.

Ruttner (1985, 1986, 1987) identified four races of *A. cerana* in different parts of Asia.

His findings were based on 34 morphological characters in 68 samples of *A. cerana* collected from different parts of Asia and data were statistically analysed using principal component analysis and discriminant analysis or cluster analysis. Research group in Himachal Pradesh Agricultural University, Shimla had also carried out biometric studies on *A. cerana* worker bees found in north-east, north-west and south India representing different physiographic conditions. In all, 55 morphological characters related to the tongue, antenna, forewing, hindwing, hindleg, tergites and sternites were studied. Computer-based univariate and multivariate analysis were done (Verma, 1992). The results of this analysis and those of Ruttner (1985, 1986, 1987) led to the identification of the following four sub species of *Apis cerana* and some ecotypes under each.

2.2.1.1 *Apis cerana cerana*

Apis cerana cerana showed significant variations in 28 characters of the bees of Himachal region and in 18 characters in the bees of Kashmir region. In bees of Himachal, 40 characters showed a significant positive correlation with altitude, whereas in Kashmir only 22 characters were positively correlated with altitude. Worker bees from the

mountainous zone above 1800 m MSL were significantly bigger in size and darker in colour than those of the sub-mountainous zone (below 1800 m MSL). Bees of the Kashmir region were significantly larger in 39 morphometric characters as compared to the bees of Himachal region. This sub-species was also longer in body size as compared to bees of north east and south India. It was also found that the bees from the Kashmir and Himachal region of north-west India could be clustered biometrically into two separate sub groups. It was concluded that *A. cerana cerana* in north west India may further contain two separate ecotypes or geographic populations.

2.2.1.2 *Apis cerana himalaya*

Ruttner (1985) reported that bees from eastern Himalaya form a separate cluster distinct from the bees of western Himalaya and he named it as *Apis cerana himalaya*. Biometric studies on *A. cerana* from north-east India comprising Nagaland, Manipur, Mizoram, Assam, Meghalaya, Arunachal Pradesh and Sikkim also supported the findings (Verma, 1987). Further three geographic ecotypes of *A. cerana himalaya* were reported among the bees of north-east India viz.,

(a) Naga and Mizo Hill bees

(b) Brahmaputra valley bees and

(c) Bees of the main axis of the Himalaya

2.2.1.3 *Apis cerana japonica*

Ruttner (1985) identified this sub-species from Japan. According to him this sub-species in Japan could further be divided into two ecotypes or sub-groups i.e., Honshu and Tsushima bees.

2.2.1.4 *Apis cerana indica*

The worker bees of *A. cerana* from south India, Sumatra, Malaysia, Sri Lanka, Java and Thailand have been separately grouped through principal component analysis and it was named as *Apis cerana indica* (Ruttner, 1985). Discriminant analysis of morphometric data from 14 localities of south India comprising Kerala, Tamil Nadu and Karnataka indicated the existence of two separate biometric sub-groups/ecotypes in south India. The first group was seen distributed in Kerala region. Verma (1992) suggested that in addition to the seven sub-groups/ecotypes identified in different races of *A. cerana* in India, there could be much greater number of ecotypes existing in varying geographical regions of the country. This may be more relevant to Kerala since Verma (1992) collected samples from only very few locations of the state.

2.3 Diseases of honeybee in India

Honeybees have many attributes which favour quick transmission and spread of disease pathogens. Since bees of a colony have very frequent contact among themselves and trophyllaxis is one of the important aspects

of their social behaviour, any pathogen when present, spreads with great ease. Behavioural specialities such as swarming, absconding and foraging also help in the rapid spread of diseases in contiguous areas. Migratory beekeeping widely adopted by bee-keepers also causes wide spread of bee diseases. Spread of diseases from affected to healthy colonies occurs through manipulative operations in the apiary such as exchange of combs, uniting of colonies and provision of drinking water also (Mishra, 1995).

The problems of diseases and pests are very complex in Asia. Because of the wide range of ecological conditions prevalent, occurrence of different species of native honey bees as well as *A. mellifera*, the bees introduced from abroad, there are greater chances of interspecific transmission of bee diseases in India. Many diseases which affect honeybees in other countries are not yet serious in India. But any known serious disease of the west can appear at any time in epidemic form in India also (Mishra, 1995). The microbial diseases of honeybee include those caused by bacteria, protozoa, fungi and viruses.

2.3.1 Bacterial diseases

Bacterial brood diseases are severe and common in foreign countries. From India, there were only two reports and that too was not in a severe form (Mishra and Shihag, 1987). Singh (1961) reported the occurrence of American Foul Brood (AFB) in *A. indica* colonies from Jeolykote, U.P. However, the occurrence of it had not been reported from

any other part of India subsequently (Kshirsagar, 1983a). A bacterial disease, European Foul Brood (EFB), affecting the brood was reported by Diwan *et al.* (1971) from Maharashtra. The causative organism was identified to be an unusual strain of *Streptococcus* (*Mellissococcus*) *pluton* White (Bailey, 1974). But the incidence of the disease has not been reported subsequently (Kshirsagar, 1983a).

2.3.2 Protozoan diseases

Infection of *A. indica* colonies by *Nosema apis* Zander in two apiaries was reported by Singh (1974, 1975) from Uttar Pradesh. Subsequently it was reported from Himachal Pradesh, Jammu and Kashmir and Punjab (Kshirsagar, 1983a).

2.3.3 Fungal diseases

Though two fungal diseases viz., chalk brood (*Ascophaera apis*) and stone brood (*Aspergillus flavus*) have been reported from abroad, these diseases are unknown to Indian apiculture (Mishra, 1995).

2.3.4 Viral diseases

Many viral diseases of honeybees are known, but the extent and severity of different viruses vary. The bee viruses appear to be species-specific. Three viruses viz., *Apis* iridescent virus, Kashmir bee virus and Thai sacbrood virus (TSBV) have been reported from India (Mishra, 1995).

2.3.4.1 *Apis* iridescent virus

This viral disease was reported from north western states of India on adults of *A. cerana indica* (Kshirsagar, *et al.*, 1975; Bailey *et al.*, 1976; Shah and Shah, 1977, 1979; Bailey and Ball, 1978; Mishra *et al.*, 1980).

Mishra (1995) summarised the symptoms of the disease as follows: Bees leave the comb and form clusters on the walls of the hive or outside the hive, bees become sluggish, queen stops egg laying and crawlers appear around the colony. Large colonies have been said to perish within two months of becoming visibly affected (Bailey and Ball, 1978) although Mishra *et al.* (1980) observed that the symptoms subsided when foraging of bees increased. Mishra *et al.* (1980) fed vitamin B complex, vitamin B complex + antibiotics and yeast as protein source in sugar syrup to overcome the infection. Yeast gave encouraging results in less severely infected colonies and they believed that the virus multiplied in the fat bodies, depleted the stored food and the treatment restored the health status.

According to Mishra (1995) the virus was specific to *A. cerana indica* and even in mixed apiaries the disease did not appear in *A. mellifera.*

2.3.4.2 Kashmir Bee Virus

This virus was isolated from diseased adults of *A. cerana* sent to Rothamsted from Kashmir (Bailey and Woods, 1977) and Mahabaleshwar

(Bhambure and Kshirsagar, 1978; Bailey *et al.*, 1979). Strains of Kashmir bee virus was also reported in adults of *A. mellifera* in Australia (Bailey *et al.*, 1979).

2.3.4.3 Thai Sacbrood Virus

A disease similar to sacbrood infecting *A. mellifera* appeared in an epidemic form in *A. cerana* in Thailand during 1976 causing 100 per cent mortality. Because of certain physiochemical and serological properties this was identified as a new strain of sacbrood virus called as Thai sacbrood virus (TSBV) (Bailey *et al.*, 1982). This viral disease was first observed in India in *A. cerana indica* in Meghalaya in 1978 (Kshirsagar *et al.*, 1981). The disease spread in an epidemic form from north east to Sikkim in 1980 (Kshirsagar *et al.*, 1982), Bihar in 1981 (Kshirsagar *et al.*, 1982), Uttar Pradesh in 1982-83 (Kshirsagar and Phadke, 1984), Himachal Pradesh in 1984, Jammu in 1985, and Kashmir in 1986 (Joshi and Verma, 1985; Verma and Joshi, 1985; Singh, 1985; Abrol and Bhat, 1990).

The disease made its appearance in Kerala in 1991 (Jacob *et al.*, 1992) and during the later part of 1991 or early in 1992 it struck the bee keepers in Dakshina Kannada and Kodagu Districts of Karnataka (Channa Basavanna, 1992).

2.3.4.3.1 Symptoms, severity and spread of TSBV

The common symptoms of the disease as reported by various workers are summarised below :

The disease affected the larvae in the early stages and the symptoms developed slowly causing death in the late larval stage or in the prepupal stage. The dead prepupae turned into sac like structures. The affected larvae could be seen lying on the floor of the cells with the heads directed outwards and turned upwards. The cadavers dried out as thin scales, lying on the floor of the cell. The infection was common in worker brood.

The worker bees could be seen ejecting the diseased larvae from the cells during the early phase of infection. Many diseased and dead larvae could thus be seen strewn on the floor board as well as on the ground below the hives. When the infection became severe and persistent, the diseased and dead larvae were seen left in the cells. Bees of most of the affected colonies were seen deserting the hives, thus causing total loss to the apiary (Phadke, 1983; Joshi and Verma, 1985; Jacob *et al.*, 1992; Channa Basavanna, 1992).

2.3.4.3.2 Causal organism

Bailey *et al.* (1982) made detailed investigations on the TSBV. The virus particles were isometric in shape with a diameter of about 30 nm. TSBV had a single stranded RNA of molecular weight 2.8 x 10^6 dalton determined on agarose-acrylamide gels and sedimented at 160 S in 0.1 m KCl. In these respects it resembled sacbrood virus. However, it sedimented

at 150 S only in 0.01 M phosphate and produced three close but well defined bands of protein on 5% SDS - acrylamide gels with apparent molecular weights 30200, 34000 and 38700, whereas SBV sedimented at 160 S in phosphate and presented one broad band of molecular weight range 29000 to 34000. Further, TSBV did not react with antiserum in immunodiffusion tests using either a borate-buffered saline agar or a phosphate-buffered agar + 2% EDTA. Electron microscopic and serological studies conducted by Rana *et al.* (1991) on TSBV of *A. cerana indica* showed a close relationship with sacbrood virus (SBV) of *A. mellifera.* Isometric particles of 30 nm in size were seen in ultra thin sections as well as in purified preparations of the diseased larvae.

2.3.4.3.3 Mode of transmission

Mode of transmission of this disease has not been ascertained clearly. TSBV had not been found to infect *A. mellifera* (Kshirsagar and Phadke, 1984; Rana *et al.*, 1986b). But whether *A. mellifera* could act as a carrier of the disease is not properly understood. Similarly, whether *A. dorsata* and *A. florea* are infected by TSBV or are only carriers of this disease is also not clearly known. Kshirsagar (1983b) and Abrol and Bhat (1990) stated that in nature TSBV multiplied in adult bees without showing any obvious disease symptoms in them. They further stated that the youngest workers were more susceptible to infection. After infection, the virus accumulated in the hypopharyngeal glands of the insect.

Thus it was assumed that infected nurse bees transmitted the disease when they fed the larvae with secretions from their glands. TSBV was usually associated with certain aerobic bacteria which was found to enhance the intensity of the disease (Abrol and Bhat, 1990). Kshirsagar (1982) suggested that capturing of natural swarms from infected area would accelerate spread of the disease.

Studies conducted at U.P. by Verma and Joshi (1985) revealed that TSBV disease was more prevalent during winter months indicating that low temperature played an important role in the intensity of the disease and it appeared to spread quickly when the brood rearing was at its highest.

2.3.4.3.4 Persistence of the disease

TSBV disease disappeared after a period of over four years in most places of North India as well as in Thailand (Kshirsagar, 1983b; Kshirsagar and Phadke, 1984; Shah and Shah, 1988). A small percentage of *A. cerana indica* survived in some areas even after severe outbreak of the disease. Abrol (1993) had observed that in Kashmir valley about 5.7 per cent of the total bee colonies survived. Verma and Joshi (1988) and Abrol and Bhatt (1990) encountered similar survival of bee colonies. They suggested that the survival of some bee colonies might possibly be due to the immunity developed by them. It was also reported that wild bee colonies of *A. cerana indica* (wall bees) showed higher percentage of survival as against those in the box hives, perhaps due to inbreeding in the latter case (Abrol, 1993).

2.3.4.3.5 Control of the disease

Being a virus disease, no specific chemotherapeutic measures are known for the control of TSBV. Abrol and Bhat (1990) tried various antibiotics and vitamins to suppress the viral infection. But none of these chemicals was effective.

Phadke (1983) and Abrol and Bhat (1990), based on their studies, made certain suggestions to prevent the spread of the disease.

1. Isolate the infected colonies and destroy them to avoid infection

2. Maintain strong and vigorous colonies and ensure surplus honey stores with the colonies

3. Avoid overcrowding of the colonies as well as exchanging brood combs from one colony to another

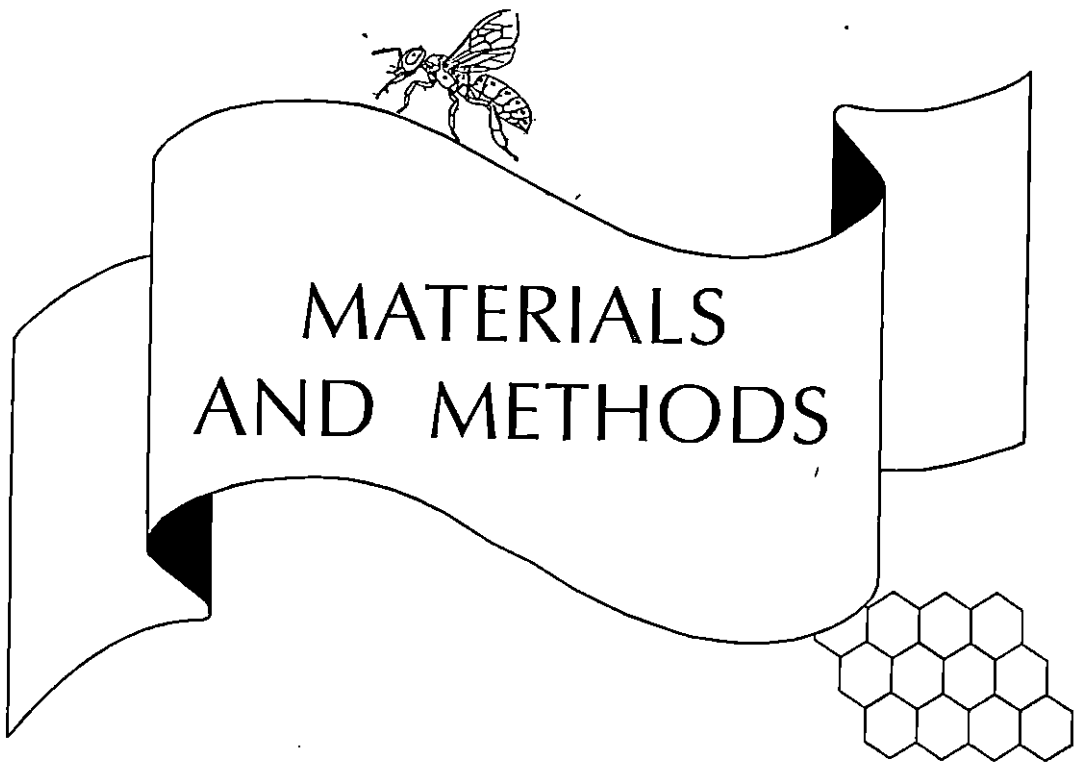
4. Destroy the infected colonies along with brood and comb by burning when not many colonies are involved

5. Avoid migration of infected colonies

6. Avoid procurement of beekeeping materials from the area of infection

7. Inspect brood combs at regular intervals to find disease symptoms.

It had also been suggested that dequeening the infected colonies for a specific period would be a good management practice against Thai sacbrood disease. Rahman (1992) carried out a preliminary experiment in Assam by dequeening an infected bee colony for a period of six weeks and found satisfactory recovery from the disease. Similarly, Shah and Shah (1988) also found that if there was a break in the breeding either by dequeening or caging the queen, the bees in the hive were able to eject out the diseased / dead brood efficiently thereby keeping the infection under check.


MATERIALS
AND METHODS

MATERIALS AND METHODS

The investigations carried out in the present research programme included biometric studies of Indian bee *Apis cerana indica* collected from different locations of Kerala and the pathogenicity studies on Thai Sacbrood Virus of *Apis cerana indica*.

The above studies were carried out in the Department of Entomology, College of Agriculture, Vellayani and the apiaries spread over different parts of the state of Kerala.

3.1 Maintenance of *A. cerana indica* colonies at the college

ISI - A type hives with eight frames were used for rearing bees. Each colony was inspected at two week intervals and the observations on the condition of the bees and brood were recorded in proper colony registers.

The colonies were kept strong and healthy by adopting the following management practices : (i) using only standard hives and other equipment, (ii) cleaning of hives at regular intervals, (iii) timely renewal of old and worn out combs, (iv) renewal of old and weak queens with young and healthy ones, (v) uniting weak colonies with stronger ones, (vi) feeding sugar syrup (1:1) during periods of dearth as and when

required, (vii) removal and destruction of any comb showing signs of infection and (viii) isolation of colonies with suspected infection to far away places and destroyal if necessary.

3.2 Biometric studies on *A. cerana indica*

3.2.1 Selection of locations for collecting bee samples

The following four natural topographic divisions have been recognised in Kerala.

Highrange

The mountainous land with an elevation of 750 m to 2500 m above MSL including Western ghats, with jutting rocks and loamy soils constitute the highrange.

Highland

The hilly tracts on the western side of the Western ghats, comprising about 43 per cent of the land and supporting 14 per cent of the population covered with forests and small streams and having an elevation ranging from 75 to 750 m above MSL constitute highland.

Midland

The midland plains cover about 42 per cent of the land mass having an undulating terrain intersected by numerous rivers, small hills and valleys with an elevation of 7.5 to 75 m above MSL.

Lowland

The lowland bordering the Arabian sea is a strip of land running along the coast.

Only highrange, highland and midland were included for the present investigations since beekeeping is rarely practised in the lowland. For each division, two locations were identified in each of the three agroclimatic zones of Kerala (Table 1; Fig. 1). These 18 locations were identified ensuring the complete coverage of the state in the survey.

3.2.2 Collection of bee samples

Samples of worker honeybees were collected from the above 18 locations. Each sample consisted of 60 field bees taken from five colonies as a composite sample. Each colony was given separate number as an identification mark for future monitoring of disease incidence.

Bees were anaesthetised with chloroform, killed in warm water and preserved in Pampell's fixative (Ruttner *et al.*, 1978) with the following composition:

Glacial acetic acid	4 ml
Distilled water	30 ml
Formaldehyde (40%)	6 ml
Alcohol (95%)	15 ml

Table 1. Details of locations identified for collection of bee samples

Sl. No.	Locality	Altitude in m (MSL)	Agroclimatic zone	Topographic division
1.	L_1 Pampadumpara	1050	Southern	Highrange
2.	L_2 Ponmudi	850		
3.	L_3 Attapady	761	Central	
4.	L_4 Kumbalacode	905		
5.	L_5 Wayanad	764	Northern	
6.	L_6 Mananthavadi	780		
7.	L_1 Kulathupuzha	125	Southern	Highland
8.	L_2 Kanjirappally	105		
9.	L_3 Palakkad	95	Central	
10.	L_4 Malampuzha	100		
11.	L_5 Nilampur	80	Northern	
12.	L_6 Malappuram	80		
13.	L_1 Neyyattinkara	27	Southern	Midland
14.	L_2 Pathanamthitta	30		
15.	L_3 Thrissur	33	Central	
16.	L_4 Vadakkancherry	33		
17.	L_5 Pilicode	30	Northern	
18.	L_6 Aralam Farm	45		

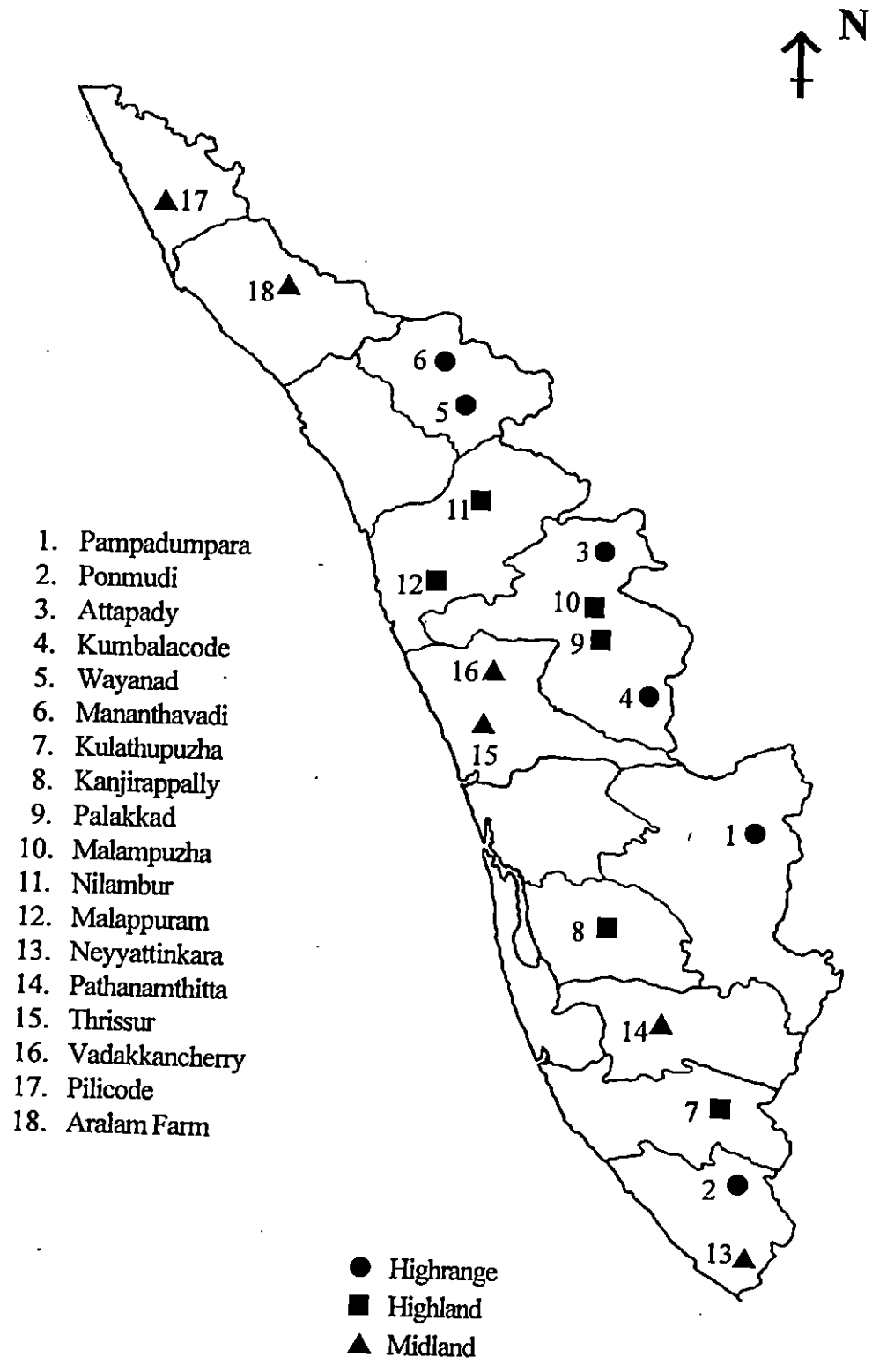


Fig. 1. Map of Kerala state showing the locations selected for collection of bee samples

3.2.3 Biometry

From each sample, 30 worker bees were used for measurements. All measurements were taken with a stereomicroscope equipped with an ocular micrometer. Angles of the wings were measured with the help of a projection microscope.

The different parts to be measured were carefully dissected out and mounted on a slide in glycerine or adjusted on the slide (Alpatov, 1929; Narayanan *et al.*, 1960; Ruttner *et al.*, 1978; Fernando, 1979).

Fifty morphometric characters (vide Table 2) were selected based on the works of Alpatov (1929), Goetze (1964), Du Praw (1965), Ruttner *et al.* (1978), and Mattu and Verma (1983, 1984 a and b).

3.2.4 Statistical analysis

3.2.4.1 Univariate analysis

The data on the morphometric measurements of all the 50 characters of worker bees collected from 18 locations were analysed separately using analysis of variance (univariate analysis) (Rao, 1952; Ruttner *et al.*, 1978; Mattu and Verma, 1983) to find out the significance of variations in the morphometric characters among bees collected from various locations.

Table 2. List of characters selected for morphometric studies

Sl. No.	Character	Author	Figure
Head			
1.	Tongue length	Alpatov	2
2.	Length of scape	Alpatov	3
3.	Length of pedicel	Alpatov	3
4.	Length of flagellum	Alpatov	3
5.	Total length of antenna	Alpatov	3
Forewing			
6.	Length of forewing	Alpatov	4
7.	Breadth of forewing	Alpatov	4
8.	Length of radial cell	Alpatov	4
9.	Breadth of radial cell	Alpatov	4
10.	Length of basal portion of radial cell	Alpatov	4
11.	Length of apical portion of radial cell	Alpatov	4
12.	Length of first abscissa	Alpatov	4
13.	Length of second abscissa	Alpatov	4
14.	Wing angle No. 31	Goetze	5
15.	Wing angle No. 32	Goetze	5
16.	Wing angle No. 33	Goetze	5
17.	Wing angle No. 34	Goetze	5
18.	Wing angle No. 35	Goetze	5
19.	Wing angle No. 36	Goetze	5
20.	Wing angle No. 37	Goetze	5
21.	Wing angle No. 38	Goetze	5
22.	Wing angle No. 39	Goetze	5
23.	Wing angle No. 40	Goetze	5
24.	Wing angle No. 41	Goetze	5
Hindwing			
25.	Length of hindwing	Ruttner	6
26.	Breadth of hindwing	Ruttner	6

Table 2. (Contd....)

Sl. No.	Character	Author	Figure
27.	Length of vein RL	Ruttner	6
28.	Length of vein ML	Ruttner	6
29.	Length of vein VL	Ruttner	6
30.	Length of vein IL	Ruttner	6
31.	Number of hamuli	Du Praw	6
32.	Extent of hamuli	Du Praw	6
33.	Length of jugal lobe	Mattu & Verma	6
34.	Length of vanal lobe	Mattu & Verma	6
Hind leg			
35.	Length of femur	Alpatov	7
36.	Length of tibia	Alpatov	7
37.	Length of metatarsus	Alpatov	7
38.	Breadth of metatarsus	Alpatov	7
Third tergite			
39.	Width of light band	Mattu & Verma	8
40.	Width of dark band	Mattu & Verma	8
41.	Total width of tergite	Mattu & Verma	8
Fourth tergite			
42.	Width of light band	Mattu & Verma	9
43.	Width of dark band	Mattu & Verma	9
44.	Total width of tergite	Mattu & Verma	9
Third sternite			
45.	Length of wax mirror	Ruttner	10
46.	Breadth of wax mirror	Ruttner	10
47.	Distance between wax mirrors	Ruttner	10
48.	Total width of sternite	Ruttner	10
Sixth sternite			
49.	Depth of sternite	Ruttner	11
50.	Breadth of sternite	Ruttner	11

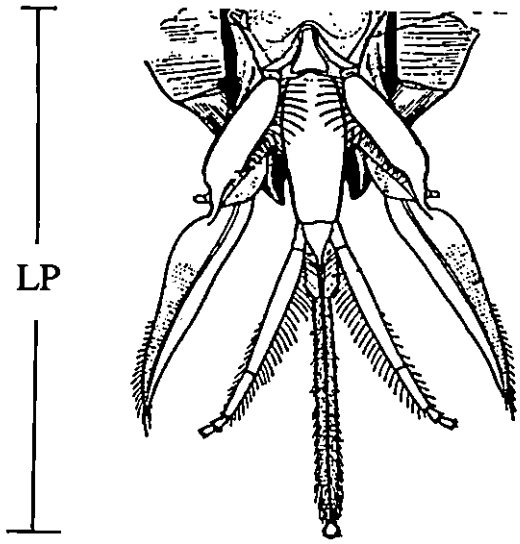


Fig. 2. Length of proboscis (LP)

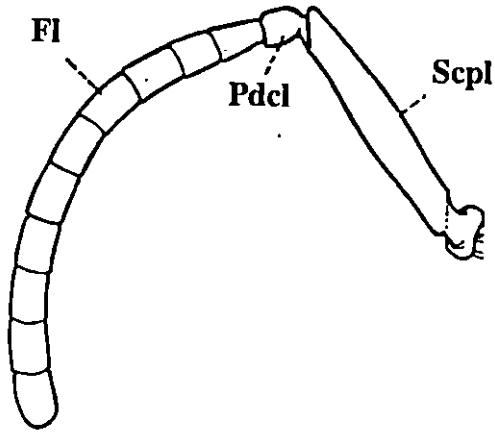


Fig. 3. Characters measured for the antenna

Scpl - length of scape Pdcl - length of pedicel Fl - Length of flagellum

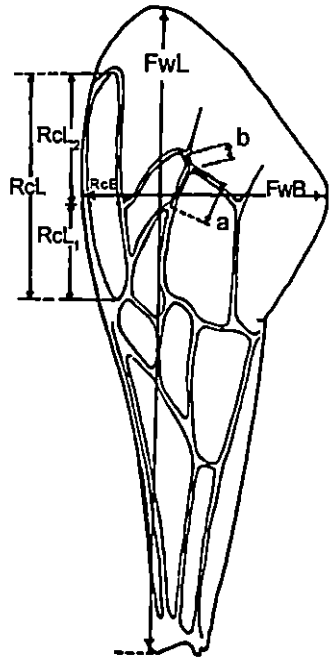


Fig. 4. Characters measured for the forewing

FWL - length of forewing FWB - breadth of forewing RcL - length of radial cell
RcB - breadth of radial cell RcL_1 - length of basal portion of radial cell
RcL_2 - length of apical portion of radial cell
a, b - length of first and second abscissa respectively of vein M3+M4 in second median cell

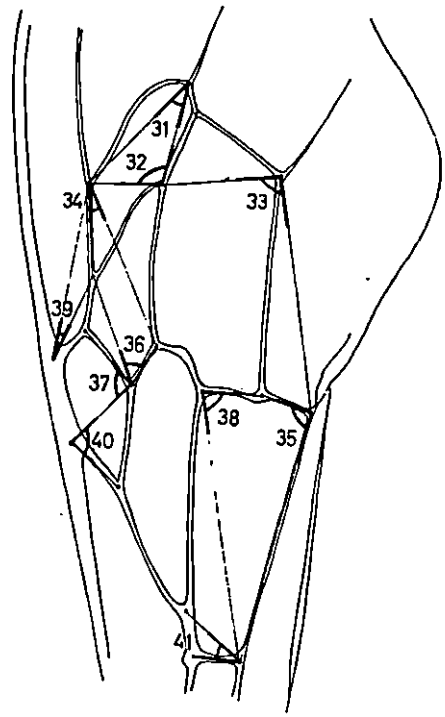


Fig. 5. Numbering of forewing vein angles

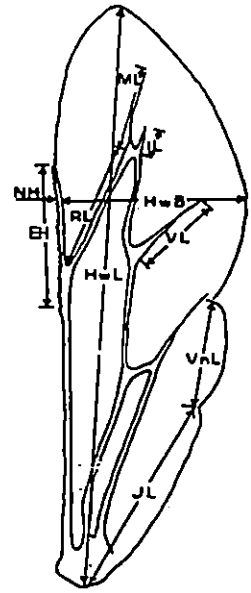


Fig. 6. Characters measured for the hindwing

HWL- length of hindwing
RL - length of basal portion of radial vein
VL - length of discoidal vein
JL - length of jugal lobe
EH - extent of hamuli
HWB - breadth of hindwing
ML - length of apical portion of radial vein
IL - length of indica vein
VnL - length of vanal lobe
NH - number of hamuli

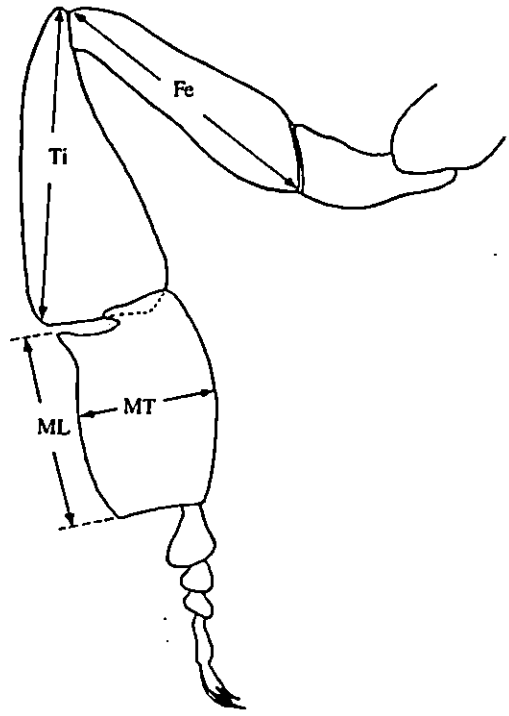


Fig. 7. Characters measured for the hind leg of the worker bee

Fe - length of femur
ML - length of metatarsus
Ti - length of tibia
MT - width of metatarsus

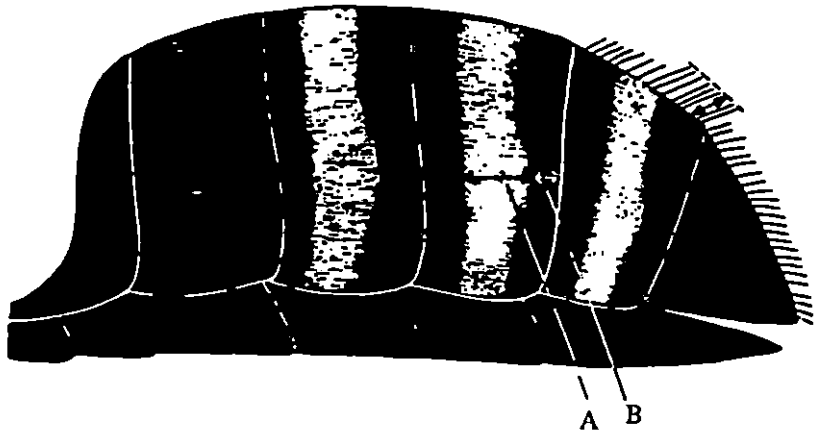


Fig. 8. Characters measured for the abdomen of the worker bee

A - width of tomentum, tergite 4
B - width of the dark stripe between tomentum and posterior rim of the tergite

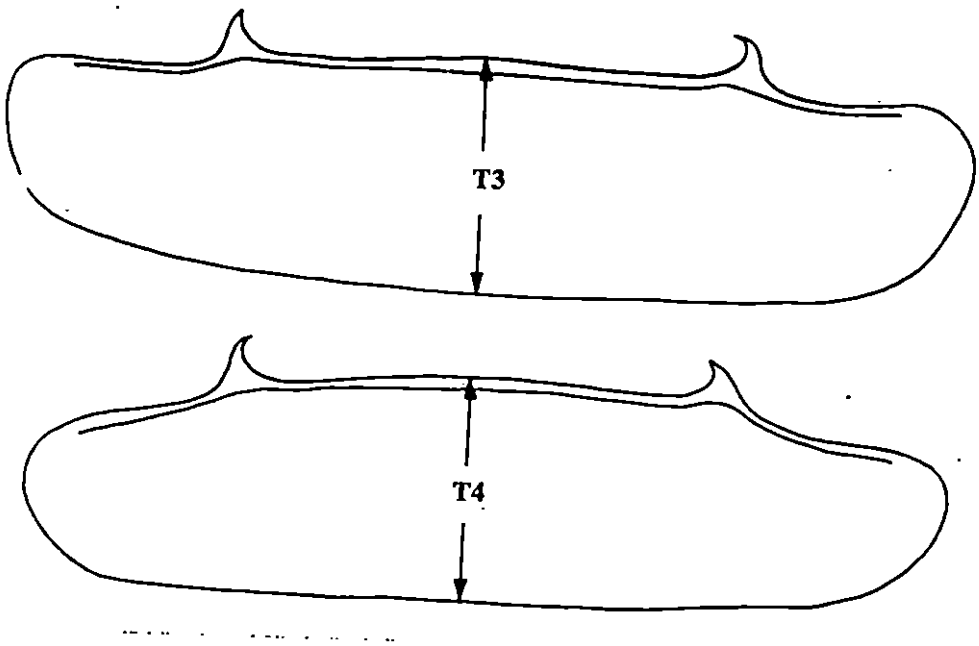


Fig. 9. Longitudinal diameter of tergite 3 and 4

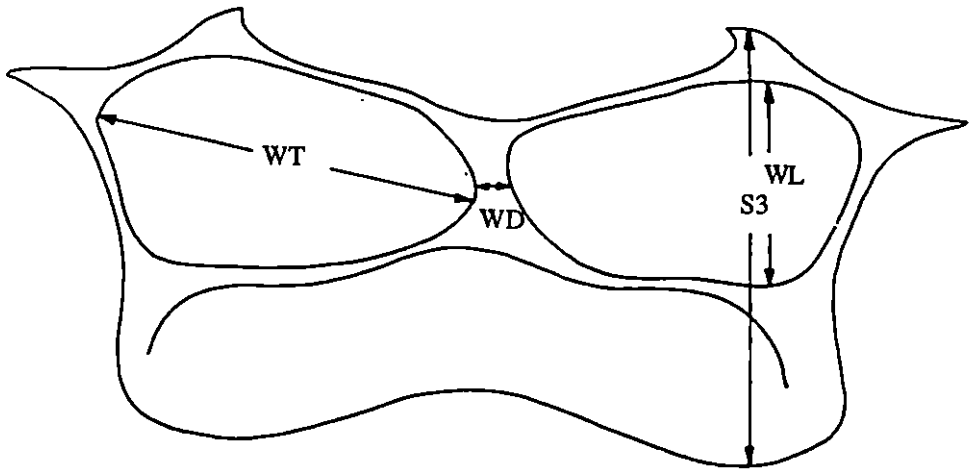


Fig. 10. Characters measured for sternite - 3

S3 - total width of sternite
WT - length of wax mirror
WL - width of wax mirror
WD - distance between wax mirrors

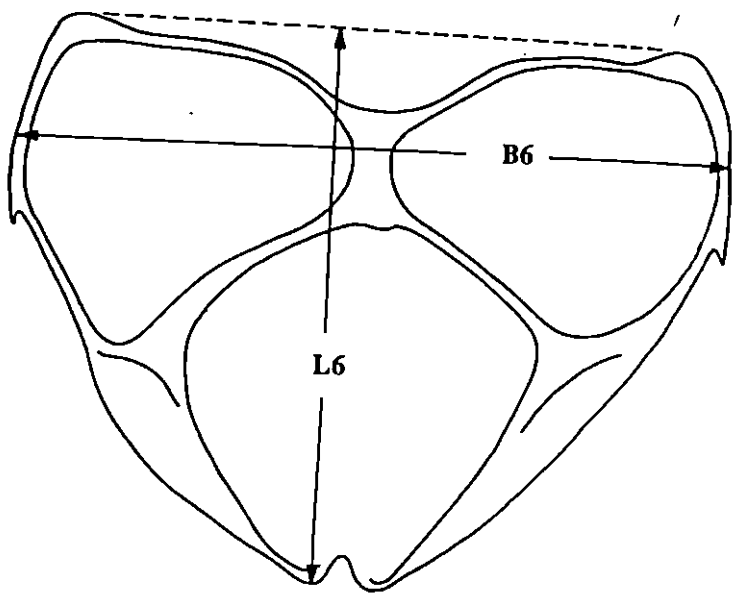


Fig. 11. Characters measured for sternite - 6

B6 - breadth of sternite
L6 - depth of sternite

3.2.4.2 Correlation of morphometric characters with altitude

The coefficients of correlations of altitude with the morphometric characters were estimated following the method of Snedecor and Cochran (1976).

3.2.4.3 Multivariate analysis

Computerised multivariate analysis viz., D^2 analysis (Mahalanobis, 1928) was done with all the 50 characters to identify the divergent groups / clusters of the bees in the localities.

The various steps involved in the estimation of D^2 values are listed below :

1. Measuring all the 50 morphometric characters of worker bees from 18 locations

2. Estimate the variations among the characters with respect to locations and error

3. Test the simultaneous significance of mean differences among the characters in bees of different locations using the method of analysis of dispersion in respect of the pooled effect of the character

4. The morphometric characters of the bees from various locations were then transferred into a set of uncorrelated variables using pivotal condensation method of the error variance co-variance matrix

5. Estimation of D^2 values using the transformed means was done as follows:

   D^2 with respect to bees from k^{th} and l^{th} locations was estimated using the formula

   $D^2 = \Sigma_i \ ((Yi^{(k)} - Yi)^{(l)})^2$ where.

   $Yi^{(k)} - Yi^{(l)}$, is the mean values of character 'i' with respect to bees from 'k^{th}' and 'l^{th}' locations. As such with 'n' locations nc_2 distances were worked out.

6. Bees from different locations were grouped based on their D^2 values using Tocher method (Singh and Chaudhary, 1985).

7. The average inter and intra cluster distances were estimated.

8. A cluster diagram (dendogram) was drawn using the group distance D (not exactly to scale).

3.2.4.4 Contribution of morphometric characters towards the divergence

The contributions of individual character towards divergence was worked out (Singh and Chaudhary, 1985).

In all the combinations [$18C_2 = (18 \times 17)/2 = 9 \times 17 = 153$], each character was ranked on the basis of difference in the pairs of values. Rank one was given to the highest mean difference and rank 50 to the lowest mean difference where 50 was the lowest number of character.

Based on this ranking the contribution of each character towards divergence was found out as follows :

Percentage contribution = No. of times a character appear first in ranking / Total ranks = $18C_2$.

3.2.5 Monitoring of TSBV incidence in bee colonies

The colonies in different locations kept numbered as explained in para 3.2.2 were inspected at two month intervals to check the incidence of TSBV. The conditions of each colony were recorded.

3.3 Pathogenicity studies on TSBV

3.3.1 Preparation of virus suspension

TSBV was extracted from freshly collected diseased larvae which were still alive when removed from brood comb cells. Ten larvae were ground in 10 ml of distilled water (1 larva / ml) using a mortar and pestle kept in an ice bath. Resulting suspension was filtered through muslin cloth and the filtrate was centrifuged at 8000 g for 10 minutes. The clean supernatent fluid was used for inoculation (Bailey *et al.*, 1964; Bailey, 1965).

3.3.2 Selection of larvae for inoculation

Healthy colonies of *A. cerana indica* mentioned in para 3.1, which were found disease free for a minimum period of six months, were selected for the inoculation studies.

Larvae of known ages were obtained by confining a laying queen to a limited area on one side of a brood comb (Hitchcock, 1966). The queen was confined for 18 to 24 hours using a wire mesh cage which would allow free passage of worker bees but not the queen. After this, the queen was released from the cage though the cage was retained in the same place to prevent the queen from laying any more eggs in the test area. The eggs hatched on the third day. From this, 1, 2, 3 and 4 day old larvae were selected for inoculation.

3.3.3 Inoculation of the virus

The virus suspension (vide para 3.3.1.) was mixed with 50 per cent (w/v) sugar syrup in the ratio of 1:10 and was used for inoculation. Using a Hamilton microsyringe 1 µl of the suspension was added to the food in which an young larva lies in each individual brood cell. ,For older larvae, the suspension was placed near the head of the larvae. Fifty larvae in each age group, in alternate rows, were treated with virus inoculum. For control, larvae in the same comb in alternate rows were inoculated with sugar syrup without the virus suspension (Bailey *et al.*, 1964). The position of each treated larva was determined by a cell locating frame of Woodrow (1941) suitably modified (Fig. 12). This device helped to identify the exact location of the individual cells / larvae exposed to a specific treatment and to make repeated examinations of the same at regular intervals.

Modified Woodrow's unit consisted of a four sided wooden frame. A vertical scale was permanently fixed on to the side frame on the left.

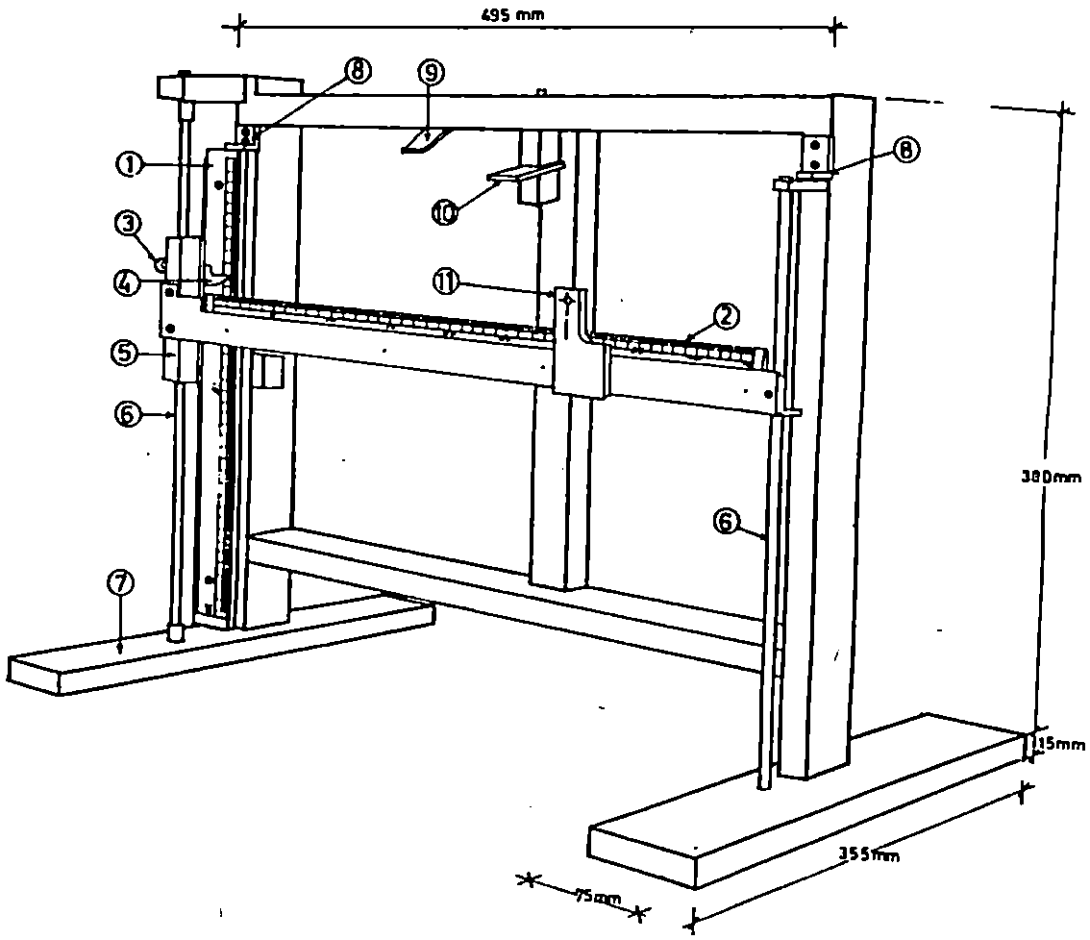


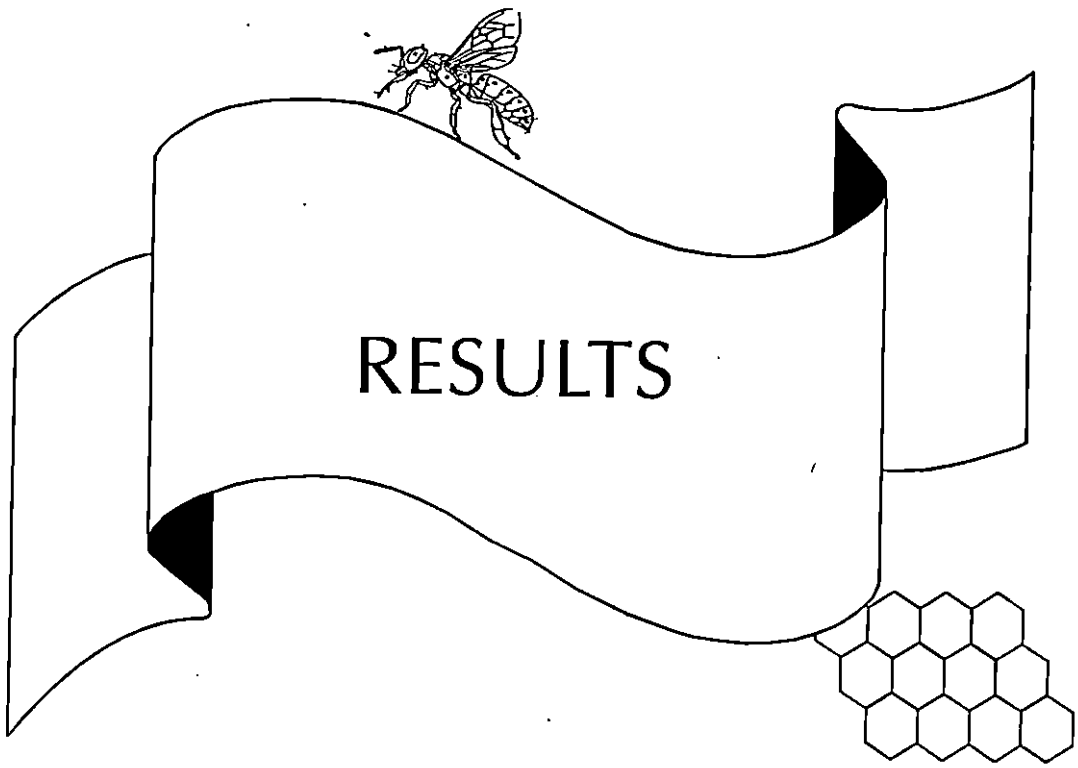
Fig. 12. Cell locating device

1. Vertical scale
2. Horizontal scale
3. Scale holder adjuster
4. Pointer
5. Scale holder
6. Aluminium rod
7. Wooden base
8. Mellifera frame holder
9. Frame tightener
10. Adjustable clamp for ISI frames
11. Cursor

Another metal scale, carried by a scale holder sliding up and down over a vertical aluminium rod, was connected between the wooden base and the top bar. This scale could be fixed at any position by the aid of a scale holder adjuster and it was intended to note the position of cells in the horizontal line using the cursor which by itself was movable on the scale.

The comb of ISI-A type frames used for rearing *A. cerana indica* could be placed correctly with two frame holders and adjustable clamps. A frame tightener provided on the top bar of the device kept the bee frame pressed in position. By noting the position of the horizontal scale with reference to the vertical scale, any cell could be located repeatedly.

The condition of the larvae were observed at 24 hour intervals and recorded till they died / pupated. The pupated larvae were kept under observation till adult emergence / death. The experiment was done thrice during 1994-95, 1995-96 and 1996-97 respectively.


RESULTS

RESULTS

Results obtained from different experiments are presented in this chapter.

4.1 Variations in the morphometric data of worker honeybees collected from different locations of Kerala

4.1.1 Variation in tongue length

The relevant data and results of statistical analysis of the same are presented in Table 3. Tongue length ranged from 4.5156 mm to 5.3399 mm in bees collected from 18 locations and the variations were statistically significant. The bees collected from the three topographic regions viz., highrange, highland and midland also varied significantly, the highest mean measurement being in bees of the highrange (5.139 mm) followed by those of highland (4.831 mm) and midland (4.762 mm)

4.1.2 Mean length of scape

The data and results of statistical analysis of the same are presented in Table 4. The variation in scape length ranging from 1.0753 mm to 1.1901 mm obtained in bees collected from the 18 locations were

Table 3. Mean length of tongue (mm) in workers of *Apis cerana indica* collected from different locations in Kerala

Topographic division	Location						Mean
	1	2	3	4	5	6	
Highrange	5.3399	5.1994	5.0963	5.0944	5.0798	5.0254	5.139
Highland	4.8341	4.6629	4.8050	4.8033	4.9360	4.9439	4.831
Midland	4.6676	4.5156	4.8017	4.9781	4.7627	4.8471	4.762

CD (topographic division) : 0.02284
CD (location) : 0.05595

Table 4. Mean length of scape (mm) in workers of *Apis cerana indica* collected from different locations in Kerala

Topographic division	Location						Mean
	1	2	3	4	5	6	
Highrange	1.1901	1.1673	1.0753	1.0785	1.1255	1.1076	1.124
Highland	1.1078	1.0964	1.1142	1.1020	1.1191	1.1303	1.112
Midland	1.0802	1.0931	1.1351	1.1383	1.0799	1.1045	1.105

CD (topographic division) : 0.00708
CD (location) : 0.01735

statistically significant. The mean scape length was highest in bees collected from highrange (1.124 mm) while those of highland (1.112 mm) and midland (1.105 mm) were on par and significantly lower than that of the highrange bees.

4.1.3 Mean length of pedicel

The data and results of statistical analysis are presented in Table 5. The length of pedicel ranged from 0.1788 mm to 0.2169 mm and the variations were statistically significant. The highest mean length of pedicel was recorded in bees collected from highrange (0.192 mm) and this was significantly higher than that from highland (0.187 mm) but on par with midland (0.189 mm).

4.1.4 Mean length of flagellum

The data on the length of flagellum and results of statistical analysis of the same are presented in Table 6. It was found that the variations in length of flagellum ranging from 2.2498 mm to 2.4818 mm and were statistically significant. The mean length of flagellum of bees collected from highrange (2.440 mm) varied significantly from those of highland (2.315 mm) and midland (2.307 mm), while the latter two were on par. Bees from highrange recorded the highest mean.

4.1.5 Mean length of antenna

The data presented in Table 7 showed that the length of antenna varied significantly among the bees collected from different locations

Table 5. Mean length of pedicel (mm) in workers of *Apis cerana indica* collected from different locations in Kerala

Topographic division	Location						Mean
	1	2	3	4	5	6	
Highrange	0.2169	0.1788	0.1908	0.1860	0.1892	0.1884	0.192
Highland	0.1940	0.1965	0.1828	0.1820	0.1812	0.1860	0.187
Midland	0.1965	0.1828	0.1909	0.1876	0.1828	0.1924	0.189

CD (topographic division) : 0.00320
CD (location) : 0.00785

Table 6. Mean length of flagellum (mm) in workers of *Apis cerana indica* collected from different locations in Kerala

Topographic division	Location						Mean
	1	2	3	4	5	6	
Highrange	2.4737	2.4818	2.4359	2.4057	2.4219	2.4187	2.440
Highland	2.2498	2.2604	2.3347	2.3315	2.3186	2.3929	2.315
Midland	2.2991	2.2664	2.3526	2.3429	2.2814	2.2992	2.307

CD (topographic division) : 0.01311
CD (location) : 0.03210

Table 7. Mean length of antenna (mm) in workers of *Apis cerana indica* collected from different locations in Kerala

Topographic division	Location						Mean
	1	2	3	4	5	6	
Highrange	3.8786	3.8099	3.6604	3.6960	3.7687	3.7331	3.758
Highland	3.5496	3.5528	3.5993	3.6151	3.6198	3.7087	3.608
Midland	3.5819	3.5408	3.6928	3.6683	3.5374	3.5859	3.601

CD (topographic division) : 0.01556
CD (location) : 0.03810

Table 8. Mean length of forewing (mm) in workers of *Apis cerana indica* collected from different locations in Kerala

Topographic division	Location						Mean
	1	2	3	4	5	6	
Highrange	7.9204	8.1191	7.7926	7.7880	7.8605	7.8636	7.891
Highland	7.8022	7.5920	7.8524	7.8540	7.8690	7.7991	7.795
Midland	7.6793	7.6051	7.9089	7.7781	7.6665	7.7375	7.729

CD (topographic division) : 0.02573
CD (location) : 0.06303

and ranged from 3.5374 mm to 3.8786 mm. The highest mean length (3.8786 mm) of antenna was noticed in bees from Pampadumpara. The bees collected from highrange recorded the highest mean length of antenna (3.758 mm) which varied significantly from that of highland (3.608 mm) and midland (3.601 mm) and the latter two were on par.

4.1.6 Mean length of forewing

The data on the length of forewing and the results of statistical analysis of the same are presented in Table 8. It was observed that the forewing length ranged from 7.5920 mm to 8.1191 mm and that the variations were statistically significant. The mean length of forewing of bees collected from the three topographic regions also varied significantly the highest mean measurement being in bees of the highrange (7.891 mm) followed by those of highland (7.795 mm) and midland (7.729 mm).

4.1.7 Mean breadth of forewing

The data pertaining to forewing breadth and results of statistical analysis are presented in Table 9. It showed that variations in breadth of forewing ranging from 2.7227 mm to 2.9153 mm obtained in bees collected from various locations were statistically significant. The mean breadth of forewing of bees collected from highrange (2.794 mm), highland (2.754 mm) and midland (2.809 mm) did not differ significantly.

Table 9. Mean breadth of forewing (mm) in workers of *Apis cerana indica* collected from different locations in Kerala

Topographic division	Location						Mean
	1	2	3	4	5	6	
Highrange	2.8959	2.8546	2.7437	2.7405	2.7614	2.7663	2.794
Highland	2.7583	2.7615	2.7534	2.7297	2.8003	2.7227	2.754
Midland	2.7987	2.7453	2.8229	2.8035	2.9153	2.7655	2.809

CD (topographic division) : 0.04669
CD (location) : 0.11438

Table 10. Mean length of radial cell (mm) of forewing in workers of *Apis cerana indica* collected from different locations in Kerala

Topographic division	Location						Mean
	1	2	3	4	5	6	
Highrange	3.0218	2.9862	2.8763	2.8747	2.9765	2.9700	2.951
Highland	2.7778	2.8196	2.8245	2.8213	2.9231	2.8295	2.833
Midland	2.8326	2.8392	2.8683	2.8585	2.7971	2.7776	2.829

CD (topographic division) : 0.01085
CD (location) : 0.02658

4.1.8 Mean length of radial cell

The data presented in Table 10 showed that the mean length of radial cell ranged from 2.7776 mm to 3:0218 mm in bees collected from 18 locations and the variations were statistically significant. Bees collected from highrange varied significantly in the mean length of radial cell (2.951 mm) from that in bees of the highland (2.833 mm) and midland (2.829 mm). The latter two values were on par.

4.1.9 Mean breadth of radial cell

Data and results of statistical analysis relating to the breadth of radial cell are presented in Table 11. The mean breadth of radial cell ranged from 0.4328 mm to 0.4858 mm and the variations were statistically significant. The mean breadth was highest in bees collected from midland (0.464 mm) followed by those of highland (0.458 mm) and highrange (0.444 mm) and the variations among them were significant.

4.1.10 Mean length of basal portion of radial cell

Table 12 presents the data on mean length of basal portion of radial cell of bees collected from the different locations. It was observed that the mean length ranged from 1.0042 mm to 1.1643 mm. The mean length in bees collected from highrange (1.129 mm) was significantly higher than

Table 11. Mean breadth of radial cell (mm) of forewing in workers of *Apis cerana indica* collected from different locations in Kerala

Topographic division	Location						Mean
	1	2	3	4	5	6	
Highrange	0.4370	0.4586	0.4422	0.4328	0.4433	0.4481	0.444
Highland	0.4530	0.4675	0.4706	0.4594	0.4642	0.4337	0.458
Midland	0.4530	0.4393	0.4754	0.4858	0.4674	0.4642	0.464

CD (topographic division) : 0.00447
CD (location) : 0.01095

Table 12. Mean length of basal portion of radial cell (mm) in workers of *Apis cerana indica* collected from different locations in Kerala

Topographic division	Location						Mean
	1	2	3	4	5	6	
Highrange	1.1643	1.1642	1.1367	1.1157	1.1102	1.0800	1.129
Highland	1.0042	1.1028	1.0397	1.0341	1.0802	1.0591	1.053
Midland	1.0771	1.0738	1.0778	1.0510	1.0397	1.0542	1.062

CD (topographic division) : 0.01140
CD (location) : 0.02793

those from highland (1.053 mm) and midland (1.062 mm) while the latter two were on par.

4.1.11 Mean length of apical portion of radial cell

The data on the mean length of apical portion of radial cell and results of statistical analysis are presented in Table 13. Data showed that the variations ranging from 1.7123 mm to 1.8674 mm were statistically significant. The mean length in bees collected from highrange (1.804 mm), highland (1.777 mm) and midland (1.765 mm) showed significant variations among them.

4.1.12 Mean length of first abscissa

The data presented in Table 14 showed that the mean length of first abscissa varied significantly among the bees collected from 18 locations. It ranged from 0.4625 mm to 0.5384 mm and the variations were statistically significant. The mean length in bees collected from highrange (0.517 mm) was significantly higher than those of highland (0.482 mm) and midland (0.479 mm), the difference between the latter two being insignificant.

4.1.13 Mean length of second abscissa

The data relating to the mean length of second abscissa and results of statistical analysis of the same are presented in Table 15. It was observed

Table 13. Mean length of apical portion of radial cell (mm) in workers of *Apis cerana indica* collected from different locations in Kerala

Topographic division	Location						Mean
	1	2	3	4	5	6	
Highrange	1.8674	1.8320	1.7398	1.7592	1.8157	1.8125	1.804
Highland	1.7607	1.7123	1.7931	1.7891	1.8368	1.7705	1.777
Midland	1.7507	1.7607	1.7907	1.8109	1.7584	1.7204	1.765

CD (topographic division) : 0.01052
CD (location) : 0.02577

Table 14. Mean length of first abscissa (mm) in workers of *Apis cerana indica* collected from different locations in Kerala

Topographic division	Location						Mean
	1	2	3	4	5	6	
Highrange	0.5160	0.5103	0.5384	0.5296	0.5076	0.5012	0.517
Highland	0.4762	0.4805	0.4786	0.4714	0.4997	0.4859	0.482
Midland	0.4691	0.4755	0.4891	0.4947	0.4625	0.4851	0.479

CD (topographic division) : 0.00587
CD (location) : 0.01437

that the mean length of second abscissa ranging from 0.0986 mm to 0.1884 mm varied significantly. The bees collected from the three topographic regions also varied significantly. Highest mean measurement was in bees of the midland (0.169 mm) followed by those of highland (0.156 mm) and highrange (0.131 mm).

4.1.14 Mean wing vein angle 31

The data on mean dimension of wing vein angle 31 and results of statistical analysis are presented in Table 16. The variation in the data was statistically significant. The bees collected from highrange, highland and midland also varied significantly. The highest mean was recorded in bees from highrange (31.711°) followed by those of highland (30.883°) and midland (31.278°).

4.1.15 Mean wing vein angle 32

Table 17 presents the data on mean dimension of wing vein angle No. 32. Data ranged from 102.0333° to 109.9667° and the variations were statistically significant. Bees collected from highrange (107.550°) and highland (107.550°) did not differ significantly while bees from midland (104.822°) showed the lowest vein angle and it differed significantly from the angles of the former two regions.

4.1.16 Mean wing vein angle 33

The data on mean dimension of wing vein angle 33 are presented in Table 18. The data varied significantly among the bees collected from

Table 15. Mean length of second abscissa (mm) in workers of *Apis cerana indica* collected from different locations in Kerala

Topographic division	Location						Mean
	1	2	3	4	5	6	
Highrange	0.1444	0.1231	0.1076	0.1117	0.1636	0.1369	0.131
Highland	0.1652	0.1884	0.1619	0.1595	0.1652	0.0986	0.156
Midland	0.1748	0.1724	0.1877	0.1731	0.1539	0.1539	0.169

CD (topographic division) : 0.01099
CD (location) : 0.02693

Table 16. Mean dimension of wing vein angle No.31(degrees) in workers of *Apis cerana indica* collected from different locations in Kerala

Topographic division	Location						Mean
	1	2	3	4	5	6	
Highrange	30.5667	32.3333	31.5000	31.7000	32.1333	32.0333	31.711
Highland	30.6667	31.7333	30.4667	30.4333	30.0000	32.0000	30.883
Midland	32.6000	30.6667	31.3333	31.4667	31.0000	30.6000	31.278

CD (topographic division) : 0.32312
CD (location) : 0.79147

Table 17. Mean dimension of wing vein angle No.32 (degrees) in workers of *Apis cerana indica* collected from different locations in Kerala

Topographic division	Location						Mean
	1	2	3	4	5	6	
Highrange	109.9667	109.6667	105.2333	105.5667	107.5000	107.3667	107.550
Highland	107.7667	104.6000	107.6667	107.4667	108.4667	109.3333	107.550
Midland	102.0333	105.4333	103.9333	104.0667	109.4667	104.0000	104.822

CD (topographic division) : 0.74667
CD (location) : 1.82895

Table 18. Mean dimension of wing vein angle No.33 (degrees) in workers of *Apis cerana indica* collected from different locations in Kerala

Topographic division	Location						Mean
	1	2	3	4	5	6	
Highrange	99.1333	94.6000	96.3667	97.0667	96.2000	96.4333	96.633
Highland	96.8333	97.1333	96.9333	97.0333	96.8667	94.0000	96.467
Midland	100.5000	100.9000	96.4333	95.5333	97.5667	95.0667	97.667

CD (topographic division) : 0.77317
CD (location) : 1.89386

the 18 locations and it ranged from 94.000° to 100.900°. In the bees collected from the different topographic divisions, the mean angle 33 was highest in midland (97.667°) which was significantly different from highrange (96.633°) and highland (96.467°) there being no significant difference between the latter two regions.

4.1.17 Mean wing vein angle 34

Table 19 presents the data on mean dimension of wing vein angle 34 and the variations ranging from 18.9333° to 22.1333° which were statistically significant. The mean angles in bees of highrange (20.306°) was the lowest and it differed significantly from those of highland (20.961°) and midland (20.694°) which were on par.

4.1.18 Mean wing vein angle 35

Table 20 summarises the data on mean dimensions of wing vein angle 35 in bees collected from the different locations. The data ranged from 85.3333° to 99.0333° and the variations were statistically significant. The bees collected from the three topographic divisions also showed significant variations. Highland bees recorded the largest angle (95.494°) followed by midland (93.756°) and highrange (91.417°).

4.1.19 Mean wing vein angle 36

The data relating to mean dimension of wing vein angle 36 are presented in Table 21. It ranged from 42.8000° to 48.4000°. Statistical

Table 19. Mean dimension of wing vein angle No.34 (degrees) in workers of *Apis cerana indica* collected from different locations in Kerala

Topographic division	Location						Mean
	1	2	3	4	5	6	
Highrange	18.9333	20.5667	20.3000	20.4000	20.6667	20.9667	20.306
Highland	21.4000	20.6000	20.6000	20.6333	21.1333	21.4000	20.961
Midland	21.4667	22.1333	19.9667	20.5333	20.1333	19.9333	20.694

CD (topographic division) : 0.27222
CD (location) : 0.66682

Table 20. Mean dimension of wing vein angle No.35 (degrees) in workers of *Apis cerana indica* collected from different locations in Kerala

Topographic division	Location						Mean
	1	2	3	4	5	6	
Highrange	85.3333	93.7333	91.5000	91.5000	92.8667	93.5667	91.417
Highland	95.0333	99.0333	93.2000	93.1667	94.7333	97.8000	95.494
Midland	91.0667	94.7000	92.9000	94.8000	95.3333	93.7333	93.756

CD (topographic division) : 0.61656
CD (location) : 1.51027

Table 21. Mean dimension of wing vein angle No.36 (degrees) in workers of *Apis cerana indica* collected from different locations in Kerala

Topographic division	Location						Mean
	1	2	3	4	5	6	
Highrange	44.2667	45.6000	44.7333	44.5333	42.8000	43.5333	44.244
Highland	48.4000	46.7000	47.5333	47.5333	46.0000	47.8667	47.339
Midland	44.1667	46.5333	45.5333	45.3333	46.4667	46.4000	45.739

CD (topographic division) : 0.60937
CD (location) : 1.49264

Table 22. Mean dimension of wing vein angle No.37 (degrees) in workers of *Apis cerana indica* collected from different locations in Kerala

Topographic division	Location						Mean
	1	2	3	4	5	6	
Highrange	95.5333	99.6000	102.4667	102.7333	100.2667	100.3333	100.156
Highland	101.9333	103.2333	104.2667	103.9333	100.1333	100.6000	102.350
Midland	101.6000	100.9000	101.6667	103.4000	104.8000	102.6000	102.494

CD (topographic division) : 0.68534
CD (location) : 1.67874

analysis revealed that the variations were significant. Wing vein angle 36 in bees of highrange (44.244°) was the least and in highland (47.339°) it was highest and midland (45.739°) came in between.

4.1.20 Mean wing vein angle 37

The relevant data and results of statistical analysis are presented in Table 22. Variations in wing vein angles ranging from 95.5333° to 104.8000° were statistically significant. Bees collected from highrange showed the lowest mean angle (100.156°) which differed significantly from those of highland (102.350°) and midland (102.494°) while the latter two were on par.

4.1.21 Mean wing vein angle 38

The data presented in Table 23 showed that the mean measurements of angle 38 ranged from 69.8667° to 81.1000° and the data varied significantly. Wing vein angle 38 in bees of highrange (78.928°) was the highest and significantly different from highland (75.661) and midland (77.283°).

4.1.22 Mean wing vein angle 39

Relevant data and results of analysis are presented in Table 24. Mean dimensions ranging from 12.8000° to 15.6333° varied significantly. The three topographic regions also showed significant variations. The highest mean dimension was in bees of the highland (14.533°) followed by those of highrange (14.261°) and midland (13.811°).

Table 23. Mean dimension of wing vein angle No.38 (degrees) in workers of *Apis cerana indica* collected from different locations in Kerala

Topographic division	Location						Mean
	1	2	3	4	5	6	
Highrange	74.6667	79.2667	79.1000	78.9000	80.5333	81.1000	78.928
Highland	72.8667	69.8667	78.0000	77.8333	78.8667	76.5333	75.661
Midland	76.5000	77.6667	77.8667	75.4000	77.9333	78.3333	77.283

CD (topographic division) : 0.62544
CD (location) : 1.53200

Table 24. Mean dimension of wing vein angle No.39 (degrees) in workers of *Apis cerana indica* collected from different locations in Kerala

Topographic division	Location						Mean
	1	2	3	4	5	6	
Highrange	14.1333	13.6667	14.6333	14.4333	14.5333	14.1667	14.261
Highland	15.6333	14.5000	14.5333	14.4000	13.4000	14.7333	14.533
Midland	13.7000	12.8000	13.6333	14.9333	14.2667	13.5333	13.811

CD (topographic division) : 0.25634
CD (location) : 0.62789

4.1.23 Mean wing vein angle 40

Table 25 presents the data and results of statistical analysis. It was observed that the mean angles ranged from 74.3667° to 87.0667° and that the variations were statistically significant. The bees collected from highland recorded the highest mean wing vein angle 40 (84.072°) followed by those of midland (83.489°) and highrange (80.039°), the latter being the lowest. The mean values for highland and midland bees did not differ significantly.

4.1.24 Mean wing vein angle 41

Results presented in Table 26 showed that there was significant variation in the mean dimensions of wing vein angle 41 of bees collected from different locations. It ranged from 30.6667° to 36.3333°. There was no significant variations in the means of vein angle 41 in bees collected from highrange (33.489°), highland (33.506°) and midland (33.228°).

4.1.25 Mean length of hindwing

Data relating to the mean length of hindwing are presented in Table 27. The mean wing length ranging from 5.2447 mm to 5.6424 mm varied significantly. Highrange (5.525 mm), highland (5.482 mm) and midland (5.424 mm) also varied significantly with reference to this criterion.

Table 25. Mean dimension of wing vein angle No.40 (degrees) in workers of *Apis cerana indica* collected from different locations in Kerala

Topographic division	Location						Mean
	1	2	3	4	5	6	
Highrange	74.3667	82.5333	83.1000	82.6000	78.4000	79.2333	80.039
Highland	81.5000	82.8000	87.0667	86.8000	85.4000	80.8667	84.072
Midland	79.7333	82.9667	82.5667	86.6000	84.8000	84.2667	83.489

CD (topographic division) : 0.74801
CD (location) : 1.83224

Table 26. Mean dimension of wing vein angle No.41 (degrees) in workers of *Apis cerana indica* collected from different locations in Kerala

Topographic division	Location						Mean
	1	2	3	4	5	6	
Highrange	33.2333	33.2333	34.6000	34.3333	33.0000	32.5333	33.489
Highland	33.9000	33.5000	33.0667	32.9333	34.6333	33.0000	33.506
Midland	36.3333	32.9667	33.4000	30.6667	33.4667	32.5333	33.228

CD (topographic division) : 0.57770
CD (location) : 1.41507

Table 27. Mean length (mm) of hindwing in workers of *Apis cerana indica* collected from different locations in Kerala

Topographic division	Location						Mean
	1	2	3	4	5	6	
Highrange	5.5649	5.6424	5.5146	5.5163	5.4743	5.4403	5.525
Highland	5.5065	5.3806	5.4936	5.4807	5.5519	5.4760	5.482
Midland	5.2770	5.2447	5.5616	5.4743	5.5227	5.4612	5.424

CD (topographic division) : 0.02105
CD (location) : 0.05156

Table 28. Mean breadth (mm) of hindwing in workers of *Apis cerana indica* collected from different locations in Kerala

Topographic division	Location						Mean
	1	2	3	4	5	6	
Highrange	1.6073	1.6217	1.5281	1.5362	1.5587	1.5587	1.568
Highland	1.6218	1.6234	1.6269	1.6008	1.6363	1.6135	1.620
Midland	1.6639	1.5700	1.6283	1.6316	1.6170	1.6201	1.622

CD (topographic division) : 0.01363
CD (location) : 0.03337

4.1.26 Mean breadth of hindwing

The data on the breadth of hindwing of bees collected from different locations are presented in Table 28. Breadth of hindwing ranged from 1.5281 mm to 1.6639 mm and the variations were statistically significant. The mean breadth was highest in bees collected from midland (1.622 mm) which was on par with those of highland (1.620 mm). Highrange bees (1.568 mm) recorded the lowest mean.

4.1.27 Mean length of vein RL

The data presented in Table 29 showed that the mean length of vein RL varied significantly among the bees collected from different locations. It ranged from 1.2145 mm to 1.3858 mm. Vein RL was significantly longer in bees of highrange (1.364 mm) and it was followed by those of midland (1.337 mm) and highland (1.336 mm), latter two being on par.

4.1.28 Mean length of vein ML

The data relating to the length of vein ML and results of statistical analysis of the same are presented in Table 30. It was observed that the mean length of vein ML ranged from 1.0996 mm to 1.2695 mm and that the variations were statistically significant. The bees collected from highrange, highland and midland also varied significantly, the highest mean length being in bees of the highland (1.189 mm) followed by highrange (1.176 mm) and midland (1.151 mm).

Table 29. Mean length (mm) of vein RL in workers of *Apis cerana indica* collected from different locations in Kerala

Topographic division	Location						Mean
	1	2	3	4	5	6	
Highrange	1.3729	1.3405	1.3858	1.3777	1.3549	1.3518	1.364
Highland	1.3210	1.3066	1.3557	1.3348	1.3791	1.3163	1.336
Midland	1.3665	1.2145	1.3645	1.3491	1.3550	1.3712	1.337

CD (topographic division) : 0.00919
CD (location) : 0.02253

Table 30. Mean length (mm) of vein ML in workers of *Apis cerana indica* collected from different locations in Kerala

Topographic division	Location						Mean
	1	2	3	4	5	6	
Highrange	1.1789	1.2695	1.1255	1.1304	1.1740	1.1788	1.176
Highland	1.1950	1.2352	1.1740	1.1691	1.2160	1.1449	1.189
Midland	1.0996	1.1141	1.1800	1.1868	1.1707	1.1578	1.151

CD (topographic division) : 0.01295
CD (location) : 0.03171

4.1.29 Mean length of vein VL

The data on the mean length of vein VL and results of statistical analysis of the same presented in Table 31 showed that the variations ranging from 1.0688 mm to 2.5104 mm were statistically significant. Bees collected from different topographic regions did not vary significantly.

4.1.30 Mean length of vein IL

The data and results of statistical analysis are presented in Table 32. Data ranging from 0.3333 mm to 0.4432 mm varied significantly. The mean length in bees of highrange (0.405 mm) and midland (0.403 mm) did not differ significantly. The highland bees (0.370 mm) recorded the lowest mean length and it was significantly lower than those of the other two regions.

4.1.31 Mean number of hamuli

Table 33 presents the data on mean number of hamuli and results of statistical analysis. Mean number (17.0667 to 19.5333) in samples varied significantly. The mean number of hamuli in bees of highrange (18.022), highland (17.961) and midland (17.911) did not differ significantly.

Table 31. Mean length (mm) of vein VL in workers of *Apis cerana indica* collected from different locations in Kerala

Topographic division	Location						Mean
	1	2	3	4	5	6	
Highrange	1.3001	1.2145	1.1724	2.5104	1.1967	1.1853	1.430
Highland	1.1982	1.2209	1.1967	1.1918	1.2354	1.1804	1.204
Midland	1.1262	1.0688	1.2073	1.2471	1.2063	1.2323	1.181

CD (topographic division) : 0.35612
CD (location) : 0.87231

Table 32. Mean length (mm) of vein IL in workers of *Apis cerana indica* collected from different locations in Kerala

Topographic division	Location						Mean
	1	2	3	4	5	6	
Highrange	0.4385	0.4416	0.3430	0.3333	0.4335	0.4415	0.405
Highland	0.3752	0.4271	0.3527	0.3527	0.3657	0.3495	0.370
Midland	0.4255	0.3738	0.3333	0.4432	0.4173	0.4239	0.403

CD (topographic division) : 0.01464
CD (location) : 0.03585

Table 33. Mean number of hamuli in workers of *Apis cerana indica* collected from different locations in Kerala

Topographic division	Location						Mean
	1	2	3	4	5	6	
Highrange	19.2667	17.6000	17.8333	18.1000	17.5333	17.8000	18.022
Highland	17.6667	19.5333	18.2000	17.9667	17.2000	17.2000	17.961
Midland	18.9667	18.2000	17.7333	18.1000	17.0667	17.4000	17.911

CD (topographic division) : 0.27024
CD (location) : 0.66195

Table 34. Mean extent (mm) of hamuli in workers of *Apis cerana indica* collected from different locations in Kerala

Topographic division	Location						Mean
	1	2	3	4	5	6	
Highrange	1.0882	1.1334	1.1221	1.1237	1.1318	1.0802	1.113
Highland	1.0884	1.0818	1.1480	1.1206	1.1077	1.1157	1.110
Midland	1.1011	1.0915	1.0964	1.1448	1.1464	1.1222	1.117

CD (topographic division) : 0.00973
CD (location) : 0.02383

4.1.32 Extent of hamuli

The data on extent of hamuli and results of statistical analysis of the same are presented in Table 34. The extent ranging from 1.0802 mm to 1.1480 mm varied significantly in samples. The three topographic regions did not show significant variations.

4.1.33 Length of jugal lobe

The data presented in Table 35 showed that the mean length of jugal lobe of bees collected from various locations differed significantly. It ranged from 1.4742 mm to 1.7689 mm. The mean length of jugal lobe of bees collected from highland (1.642 mm) was significantly higher than those of highrange (1.614 mm) and midland (1.627 mm) while the latter two were on par.

4.1.34 Length of vanal lobe

The data and results of statistical analysis of the mean length of vanal lobe of bees collected from various locations are presented in Table 36. Variation in length of vanal lobe ranging from 1.0140 mm to 1.1900 mm were statistically significant. Bees collected from the three topographic regions viz., highrange, highland and midland also varied significantly, the highest mean length being in bees of highrange (1.107 mm) followed by those of highland (1.084 mm) and midland (1.063 mm).

Table 35. Mean length (mm) of jugal lobe in workers of *Apis cerana indica* collected from different locations in Kerala

Topographic division	Location						Mean
	1	2	3	4	5	6	
Highrange	1.4742	1.7042	1.7156	1.6719	1.5619	1.5555	1.614
Highland	1.7365	1.6574	1.6331	1.6298	1.6298	1.5652	1.642
Midland	1.5652	1.5523	1.7689	1.6233	1.6039	1.6493	1.627

CD (topographic division) : 0.01560
CD (location) : 0.03822

Table 36. Mean length (mm) of vanal lobe in workers of *Apis cerana indica* collected from different locations in Kerala

Topographic division	Location						Mean
	1	2	3	4	5	6	
Highrange	1.1900	1.1172	1.0674	1.0609	1.0898	1.1157	1.107
Highland	1.0980	1.0156	1.0867	1.1287	1.1318	1.0445	1.084
Midland	1.0543	1.0140	1.0883	1.0817	1.0801	1.0608	1.063

CD (topographic division) : 0.01116
CD (location) : 0.02731

4.1.35 Mean length of hind femur

Table 37 presents the data on the length of hind femur and the results of statistical analysis. The femur length ranged from 2.0773 mm to 2.3055 mm and the variation was found to be statistically significant. The mean lengths of hind femur in bees collected from highrange (2.213 mm), highland (2.184 mm) and midland (2.153 mm) were significantly different from one another.

4.1.36 Mean length of hind tibia

The data pertaining to the length of hind tibia and the results of the statistical analysis of the same are presented in Table 38. It was observed that the mean length ranged from 2.4656 mm to 2.8051 mm and that the variations were statistically significant. There was no significant difference in the mean length of hind femur of bees collected from highrange (2.670 mm) and highland (2.689 mm). But bees from midland recorded the lowest mean of 2.616 mm which was significantly lower than those of the other two regions.

4.1.37 Mean length of metatarsus

The data relating to the length of metatarsus and the results of statistical analysis are presented in Table 39. Data showed that the variations among the different locations were statistically significant and it ranged from 1.6202 mm to 1.8325 mm. The variation in the mean

Table 37. Mean length (mm) of hind femur in workers of *Apis cerana indica* collected from different locations in Kerala

Topographic division	Location						Mean
	1	2	3	4	5	6	
Highrange	2.2247	2.3055	2.1862	2.1650	2.1989	2.1973	2.213
Highland	2.2085	2.1246	2.1957	2.1778	2.2119	2.1860	2.184
Midland	2.1617	2.0773	2.1779	2.1923	2.1407	2.1665	2.153

CD (topographic division) : 0.01271
CD (location) : 0.03113

Table 38. Mean length (mm) of hind tibia in workers of *Apis cerana indica* collected from different locations in Kerala

Topographic division	Location						Mean
	1	2	3	4	5	6	
Highrange	2.5837	2.8051	2.6742	2.6661	2.6451	2.6451	2.670
Highland	2.6419	2.5918	2.6937	2.7920	2.7389	2.6775	2.689
Midland	2.5916	2.4656	2.6645	2.6969	2.6127	2.6646	2.616

CD (topographic division) : 0.03172
CD (location) : 0.07769

Table 39. Mean length (mm) of metatarsus in workers of *Apis cerana indica* collected from different locations in Kerala

Topographic division	Location						Mean
	1	2	3	4	5	6	
Highrange	1.7802	1.8325	1.7140	1.7075	1.7770	1.7737	1.764
Highland	1.7204	1.6882	1.7688	1.7543	1.7947	1.7721	1.750
Midland	1.6848	1.6202	1.7689	1.7883	1.7852	1.7625	1.735

CD (topographic division) : 0.01181
CD (location) : 0.02892

Table 40. Mean breadth (mm) of metatarsus in workers of *Apis cerana indica* collected from different locations in Kerala

Topographic division	Location						Mean
	1	2	3	4	5	6	
Highrange	0.7972	0.9865	0.9168	0.9120	0.9020	0.9249	0.907
Highland	0.8992	0.8847	0.9491	0.9419	0.9799	0.9379	0.932
Midland	0.9217	0.9185	0.9411	0.9362	0.9606	0.9249	0.934

CD (topographic division) : 0.01271
CD (location) : 0.03112

length of metatarsus of bees collected from highrange recorded the highest mean length (1.764 mm) followed by highland (1.750 mm) and midland (1.735 mm), the differences among the three regions being statistically significant.

4.1.38 Mean breadth of metatarsus

The data on mean breadth of metatarsus and the results of statistical analysis are presented in Table 40. The variations (0.7972 mm to 0.9865 mm) were found to be statistically significant. The mean breadth of metatarus of bees collected from highrange was the lowest (0.907 mm) which was significantly different from those of highland (0.932 mm) and midland (0.934 mm), the latter two being on par.

4.1.39 Mean width of light band of third tergite

Table 41 summarises the data on width of light band of third tergite and the results of statistical analysis of the same. The mean width of tergite varied significantly in bees collected from different locations and the width ranged from 0.5920 mm to 1.1772 mm. Highrange bees recorded the highest mean width of light band of third tergite (0.880 mm) and it was followed by those of highland (0.766 mm) and midland (0.724 mm), the differences among the three regions being statistically significant.

Table 41. Mean width (mm) of lightband of 3rd tergite in workers of *Apis cerana indica* collected from different locations in Kerala

Topographic division	Location						Mean
	1	2	3	4	5	6	
Highrange	0.9589	0.8022	1.1433	1.0850	0.6457	0.6469	0.880
Highland	1.0017	1.1772	0.6049	0.5920	0.6179	0.6049	0.766
Midland	0.7393	0.9847	0.7342	0.6840	0.5950	0.6081	0.724

CD (topographic division) : 0.01722
CD (location) : 0.04218

Table 42. Mean width (mm) of darkband of 3rd tergite in workers of *Apis cerana indica* collected from different locations in Kerala

Topographic division	Location						Mean
	1	2	3	4	5	6	
Highrange	0.7924	0.9491	0.6906	0.8135	1.0058	1.0009	0.875
Highland	0.6686	0.6616	0.9669	0.9588	1.0025	1.0510	0.885
Midland	0.6404	0.5320	0.9023	0.8991	0.9604	0.9896	0.821

CD (topographic division) : 0.01784
CD (location) : 0.04371

4.1.40 Mean width of dark band of third tergite

The data relating to the width of dark band of third tergite and results of statistical analysis of the same are presented in Table 42. It revealed that the mean width of dark band in bees collected from different locations varied significantly. It ranged from 0.5320 mm to 1.0510 mm. The mean width was least in bees collected from midland (0.821 mm) and it was significantly lower than those of highland (0.885 mm) and highrange (0.875 mm) the latter two being on par.

4.1.41 Mean total width of third tergite

The data on mean width of third tergite and results of statistical analysis are presented in Table 43. The mean total width of third tergite of bees collected from various locations differed significantly and it ranged from 1.5878 mm to 1.8982 mm. The mean width of third tergite in bees collected from highrange (1.755 mm), highland (1.679 mm) and midland (1.658 mm) differed significantly.

4.1.42 Mean width of light band of fourth tergite

The relevant data and results of statistical analysis of the same are presented in Table 44. The mean width ranging from 0.5371 mm to 1.1578 mm varied significantly. Mean width of light band in bees collected from highrange (0.758) and highland (0.750) were on par and significantly higher than that of midland (0.729).

Table 43. Mean total width (mm) of 3rd tergite in workers of *Apis cerana indica* collected from different locations in Kerala

Topographic division	Location						Mean
	1	2	3	4	5	6	
Highrange	1.7510	1.7446	1.8401	1.8982	1.6492	1.6476	1.755
Highland	1.6963	1.7597	1.6460	1.6428	1.6606	1.6687	1.679
Midland	1.6622	1.8174	1.6540	1.6297	1.5878	1.5975	1.658

CD (topographic division) : 0.01705
CD (location) : 0.04178

Table 44. Mean width (mm) of lightband of 4th tergite in workers of *Apis cerana indica* collected from different locations in Kerala

Topographic division	Location						Mean
	1	2	3	4	5	6	
Highrange	0.8362	0.6065	0.9442	0.9281	0.6016	0.6307	0.758
Highland	1.0203	1.1578	0.5887	0.5774	0.6113	0.5468	0.750
Midland	0.9717	1.0300	0.6760	0.6080	0.5531	0.5371	0.729

CD (topographic division) : 0.02269
CD (location) : 0.05559

4.1.43 Mean width of darkband of fourth tergite

The data relating to the width of darkband of fourth tergite of bees collected from various locations and results of statistical analysis of the same are presented in Table 45. It was observed that the variations in mean width (from 0.7278 mm to 1.1466 mm) were statistically significant. The mean width in bees collected from highrange (1.016 mm) was significantly higher than those of highland (0.939 mm) and midland (0.935 mm), the latter two being on par.

4.1.44 Mean total width of fourth tergite

The data and results of statistical analysis of the same are presented in Table 46. Bees collected from various locations varied significantly in the width of fourth tergite (from 1.5684 mm to 1.8900 mm). The mean width in bees collected from highrange (1.770 mm) was significantly higher than those of highland (1.690 mm) and midland (1.670 mm) which were on par.

4.1.45 Mean length of wax mirror in third sternite

Table 47 presents the data on mean length of wax mirror in third sternite of bees collected from different locations. The mean length of wax mirror ranging from 1.5717 mm to 1.9031 mm varied significantly.

Table 45. Mean width (mm) of darkband of 4th tergite in workers of *Apis cerana indica* collected from different locations in Kerala

Topographic division	Location						Mean
	1	2	3	4	5	6	
Highrange	0.9913	1.1466	0.9345	0.9409	1.0511	1.0300	1.016
Highland	0.7343	0.7278	1.0316	1.0252	1.0122	1.1027	0.939
Midland	0.7826	0.7957	0.9799	0.9865	1.0187	1.0478	0.935

CD (topographic division) : 0.01988
CD (location) : 0.04869

Table 46. Mean total width (mm) of 4th tergite in workers of *Apis cerana indica* collected from different locations in Kerala

Topographic division	Location						Mean
	1	2	3	4	5	6	
Highrange	1.8270	1.7527	1.8900	1.8359	1.6525	1.6606	1.770
Highland	1.7544	1.8886	1.6201	1.6024	1.6250	1.6493	1.690
Midland	1.7912	1.8287	1.6557	1.5942	1.5684	1.5847	1.670

CD (topographic division) : 0.02089
CD (location) : 0.05119

Table 47. Mean length (mm) of wax mirror in 3rd sternite in workers of *Apis cerana indica* collected from different locations in Kerala

Topographic division	Location						Mean
	1	2	3	4	5	6	
Highrange	1.6299	1.9031	1.8545	1.8657	1.6073	1.5959	1.743
Highland	1.7108	1.6023	1.6235	1.6153	1.6072	1.6429	1.634
Midland	1.6541	1.5976	1.6654	1.5749	1.5717	1.5781	1.607

CD (topographic division) : 0.02256
CD (location) : 0.05526

Table 48. Mean breadth (mm) of wax mirror in 3rd sternite in workers of *Apis cerana indica* collected from different locations in Kerala

Topographic division	Location						Mean
	1	2	3	4	5	6	
Highrange	1.1270	1.0640	1.0979	1.0710	0.9443	0.9340	1.040
Highland	1.1368	1.1466	1.0187	1.0122	0.9120	0.9508	1.030
Midland	1.1869	1.1142	1.0220	1.0284	0.9767	0.9477	1.046

CD (topographic division) : 0.02310
CD (location) : 0.05659

The mean length in bees collected from the three topographic regions also differed significantly with the highest mean measurement in bees of the highrange (1.743 mm) followed by those of highland (1.634 mm) and midland (1.607 mm).

4.1.46 Mean breadth of wax mirror in third sternite

The data pertaining to the breadth of wax mirror in third sternite and results of statistical analysis are presented in Table 48. The mean breadth ranged from 0.9120 mm to 1.1869 mm and the variations were statistically significant. But the mean breadth of wax mirror of bees collected from highrange (1.040 mm), highland (1.030 mm) and midland (1.046 mm) did not differ significantly.

4.1.47 Mean distance between wax mirrors in third sternite

The data on the mean distance between wax mirrors in third sternite and results of statistical analysis are presented in Table 49. It was observed that the data ranged from 0.2914 mm to 0.5611 mm and that the variations were statistically significant. The mean distance in bees collected from highland (0.390 mm) was significantly lower than that of highrange (0.449 mm) and midland (0.438 mm) while the latter two were on par.

Table 49. Mean distance (mm) between wax mirrors in 3rd sternite in workers of *Apis cerana indica* collected from different locations in Kerala

Topographic division	Location						Mean
	1	2	3	4	5	6	
Highrange	0.4998	0.3980	0.5305	0.4837	0.3688	0.4102	0.449
Highland	0.3544	0.5466	0.4237	0.4205	0.3041	0.2914	0.390
Midland	0.5611	0.5112	0.3679	0.4366	0.4239	0.3301	0.438

CD (topographic division) : 0.01756
CD (location) : 0.04300

Table 50. Mean total width (mm) of 3rd sternite in workers of *Apis cerana indica* collected from different locations in Kerala

Topographic division	Location						Mean
	1	2	3	4	5	6	
Highrange	1.9937	2.2134	2.0582	2.0324	2.0599	2.0340	2.065
Highland	2.1826	1.9548	2.0534	2.0469	2.0211	2.0986	2.060
Midland	1.9466	1.9338	2.0309	2.0825	2.0179	2.0533	2.011

CD (topographic division) : 0.01858
CD (location) : 0.04551

4.1.48 Mean width of third sternite

The data on width of third sternite and results of statistical analysis of the same are presented in Table 50. The mean width ranging from 1.9338 mm to 2.2134 mm varied significantly. The mean width in bees collected from midland (2.011 mm) was significantly lower than those of highrange (2.065 mm) and highland (2.060 mm) the latter two being on par.

4.1.49 Mean depth of sixth sternite

Table 51 summarises the data on mean depth of sixth sternite in bees collected from the different locations. There was significant variations in the data ranging from 2.0695 mm to 2.2976 mm. The mean depth in bees of highland (2.205 mm) was significantly higher than those of midland (2.149 mm) and highrange (2.142 mm) the latter two being on par.

4.1.50 Mean breadth of sixth sternite

The relevant data and results of statistical analysis of the same are presented in Table 52. The mean breadth ranged from 2.2183 mm to 2.5949 mm and the variations were statistically significant. The mean breadth of sixth sternite of bees collected from midland (2.397 mm) was significantly lower than those of highrange (2.462 mm) and highland (2.446 mm) while the latter two were on par.

Table 51. Mean depth (mm) of 6th sternite in workers of *Apis cerana indica* collected from different locations in Kerala

Topographic division	Location						Mean
	1	2	3	4	5	6	
Highrange	2.1294	2.2394	2.1504	2.1294	2.1084	2.0971	2.142
Highland	2.2976	2.1197	2.2118	2.1989	2.2361	2.1633	2.205
Midland	2.0695	2.1489	2.1763	2.2182	2.1779	2.1053	2.149

CD (topographic division) : 0.01894
CD (location) : 0.04638

Table 52. Mean breadth (mm) of 6th sternite in workers of *Apis cerana indica* collected from different locations in Kerala

Topographic division	Location						Mean
	1	2	3	4	5	6	
Highrange	2.3720	2.5949	2.5045	2.5117	2.3994	2.3897	2.462
Highland	2.5061	2.4051	2.4705	2.4591	2.4317	2.4059	2.446
Midland	2.2183	2.3493	2.4801	2.5565	2.4091	2.3703	2.397

CD (topographic division) : 0.02806
CD (location) : 0.06874

4.2 Correlation of different morphometric characters of *A. cerana indica* with varying altitudes of different locations

Correlations between various morphometric characters of Indian honeybees collected from different locations in Kerala and altitudes of the locations are presented in Table 53. The tongue length showed a highly significant positive association with altitudes of the eighteen locations (r = 0.8427). Total length of antenna (r = 0.7924) and length of flagellum (r = 0.8567) also showed a positive correlation significant at 1 per cent level while the length of scape and pedicel did not show significant association with altitude.

Length of radial cell (r = 0.7926), length of basal portion of radial cell (r = 0.7850) and Ist abscissa (r = 0.8291) showed a highly significant positive association with varying altitudes at 1% level. Length of forewing also showed a positive correlation (r = 0.5291) with altitude at 5 per cent level. But the wing vein angles 35 (r = -0.6001), 36 (r = -0.6530) and 40 (r = -0.5957) showed a highly significant negative correlation with varying altitudes.

The breadth of radial cell (r = -0.5750), length of IInd abscissa (r = -0.5746) and vein angle 37 (r = -0.5701) showed a negative correlation at 5 per cent level. The forewing breadth, length of apical portion of radial cell and wing vein angles 31, 32, 33, 34, 38, 39 and 41 did not show any significant association with altitude.

Table 53. Morphometric characters of Indian honeybee *Apis cerana indica*, collected from different locations in Kerala, and coefficients of correlation with altitude

Sl. No.	Characters	Correlation coefficient (r)
Head		
1.	Tongue length	0.8427**
2.	Length of scape	0.3341
3.	Length of pedicel	0.2937
4.	Length of flagellum	0.8567**
5.	Length of antenna	0.7924**
Forewing		
6.	Length of forewing	0.5291*
7.	Breadth of forewing	0.1499
8.	Length of radial cell	0.7926**
9.	Breadth of radial cell	-0.5750*
10.	Length of basal portion of radial cell	0.7850**
11.	Length of apical portion of radial cell	0.4399
12.	Length of Ist abscissa	0.8291**
13.	Length of IInd abscissa	-0.5746*
14.	Vein angle no. 31	0.3180,
15.	Vein angle no. 32	0.3480
16.	Vein angle no. 33	-0.0852
17.	Vein angle no. 34	-0.4127
18.	Vein angle no. 35	-0.6001**
19.	Vein angle no. 36	-0.6530**
20.	Vein angle no. 37	-0.5701*
21.	Vein angle no. 38	0.3497
22.	Vein angle no. 39	0.0895
23.	Vein angle no. 40	-0.5957**
24.	Vein angle no. 41	0.0543
Hindwing		
25.	Length of hindwing	0.6827**
26.	Breadth of hindwing	-0.6320**
27.	Length of vein RL	0.3440
28.	Length of vein ML	0.1004

Contd...

Table 53 (Contd...)

Sl. No.	Characters	Correlation coefficient (r)
29.	Length of vein VL	0.4099
30.	Length of vein IL	0.1974
31.	Number of hamuli	0.1272
32.	Extent of hamuli	-0.0477
33.	Length of jugal lobe	-0.1678
34.	Length of vanal lobe	0.4545
Hind leg		
35.	Length of femur	0.4984*
36.	Length of tibia	0.1070
37.	Length of metatarsus	0.4814*
38.	Breadth of metatarsus	-0.3961
Third tergite		
39.	Width of light band	0.3443
40.	Width of dark band	0.0546
41.	Total width of 3rd tergite	0.5113
Fourth tergite		
42.	Width of light band	0.0671
43.	Width of dark band	0.2957
44.	Total width of 4th tergite	0.4195
Third sternite		
45.	Length of wax mirror	0.5661*
46.	Breadth of wax mirror	0.0624
47.	Distance between wax mirrors	0.2031
48.	Total width of 3rd sternite	0.2069
Sixth sternite		
49.	Depth of 6th sternite	-0.2362
50.	Breadth of 6th sternite	0.2291

Items 14 to 24 are in degree and the rest are in mm.

* Significant at 5% level

** Significant at 1% level

In hindwing, the length showed a significant positive correlation (r = 0.6827) while the breadth (r = –0.6320) was negatively correlated at 1 per cent level with varying altitudes. Length of vein RL, length of vein ML, length of vein VL, length of vein IL, number of hamuli, extent of hamuli, length of jugal lobe and length of vanal lobe did not exhibit significant association with altitude.

The length of femur (r = 0.4984) and length of metatarsus (r = 0.4814) showed a positive correlation with altitude at 5 per cent level, while there were no significant associations with altitude in length of tibia, and breadth of metatarsus.

In third and fourth tergites, width of light bands, dark bands and their total width did not show any correlation with altitude. Among the characters of third sternite, length of wax mirror (r = 0.5661) was positively correlated with altitudes at 5% level while the breadth of wax mirror, distance between wax mirrors, width of 3rd sternite, depth of 6th sternite and its breadth did not show significant correlation with altitude.

4.3 Multivariate analysis of the morphometric data of the bee populations collected from different locations in Kerala

Since all the 50 characters showed significant variations in the morphometric data in univariate analysis a multivariate analysis (Mahalanobis D^2 analysis) was done.

4.3.1 Clustering of bee population

The multivariate analysis showed that the samples could be clustered biometrically into four clusters (ecotypes).

Cluster I included ten locations, six from midland (altitude 27 to 45m above MSL) and four from highland (altitude 80-100m MSL) viz., Palakkad, Malampuzha, Nilampur, Malappuram, Neyyattinkara, Pathanamthitta, Thrissur, Vadakkancherry, Pilicode and Aralam Farm.

Cluster II included two locations of highland only viz., Kulathupuzha and Kanjirappally (105 and 125 m above MSL).

Cluster III included five locations (altitude 761 to 905 m above MSL), Ponmudi, Attapady, Kumbalacode, Wayanad and Mananthavadi and cluster IV was with a single location viz., Pampadumpara (1050 m above MSL).

The inter cluster distances among the identified clusters based on D^2 analysis are shown in Table 54 and illustrated in Fig.13.

Table 54. Inter- and intra-cluster distances of various subgroups / ecotypes of *Apis cerana indica* in Kerala

Cluster I	Cluster II	Cluster III	Cluster IV
*8.73	12.56	13.92	17.23
	*4.58	15.61	13.86
		*6.21	12.11
			*0.00

* Intra cluster distances

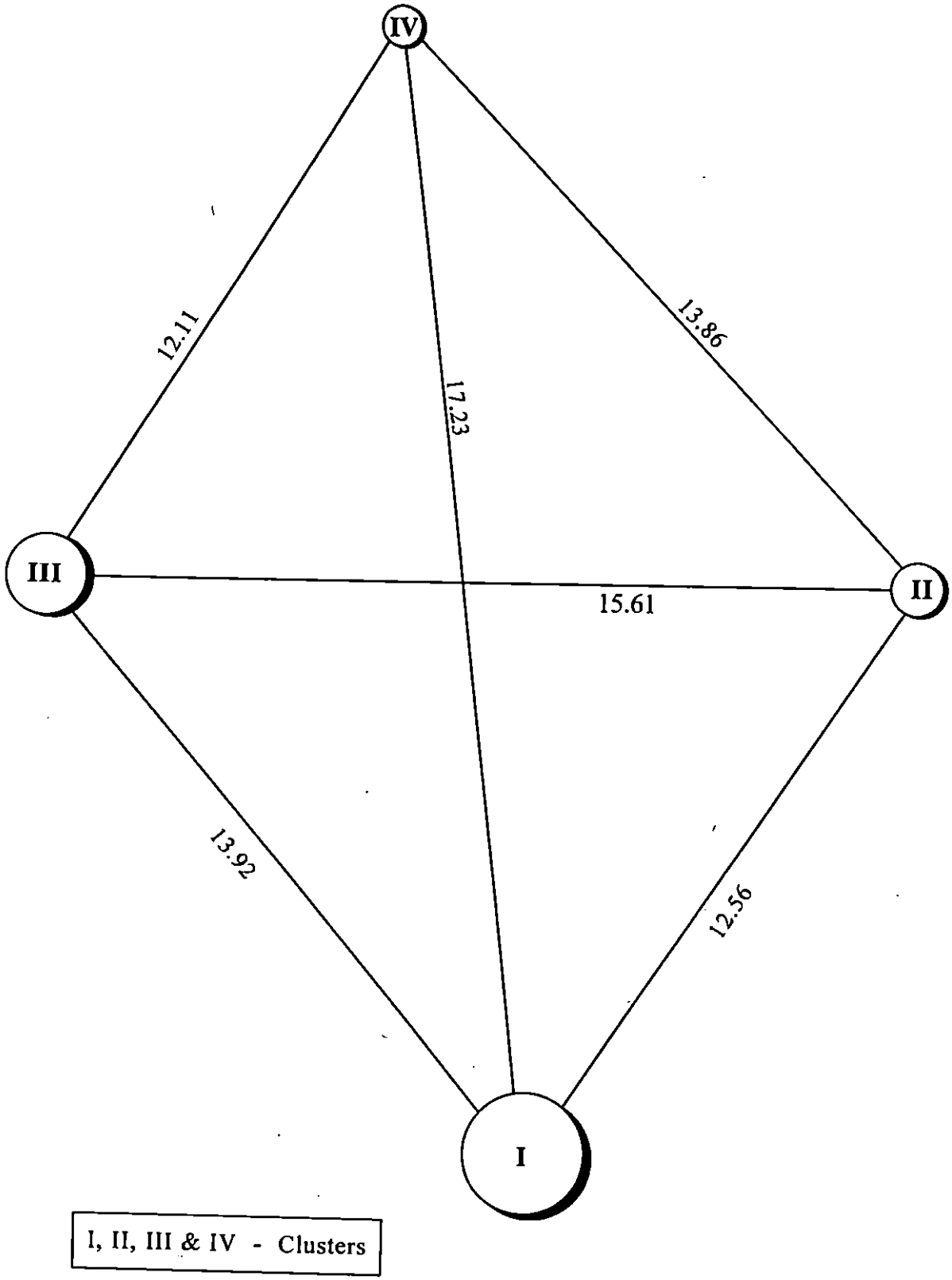


Fig. 13. Dendogram showing inter cluster distances of ecotypes of *Apis cerana indica* in Kerala

Cluster I contained bees from 10 locations. Among the clusters cluster IV was seen far away from cluster I having an inter cluster distance of 17.23. This indicated that with reference to morphometric data, bees belonging to cluster IV had the highest deviation from cluster I and it was followed by cluster III (distance 13.92) and cluster II (12.56).

Cluster II contained bees from two locations of highland and it remained closer to cluster I (12.56) than with cluster IV (13.86) and most distant from cluster III (15.61). Cluster III appeared to have evolved more towards Cluster IV, the distance between the two being 12.11 compared to the distance with cluster I ie., 13.92. Intra cluster distances of clusters I, II, III and IV were 8.73, 4.58, 6.21 and 0.00 respectively.

4.3.2 Means of different morphometric characters used for the identification of clusters in the population

The data along with the ranges of values for different means are presented in Table 55. The lowest mean tongue length (4.7485 mm) was observed in cluster II and it was closely followed by that in cluster I (4.8061 mm). The range in the measurements indicated a lot of overlapping in the data of the two clusters. The highest mean measurement (5.3999 mm) was observed in cluster IV and it was followed by the mean in cluster III (5.0991 mm) there being significant overlapping of the measurements observed in the two clusters.

Table 55. Cluster means of different characters of worker bees of *Apis cerana indica* from different locations of Kerala

Sl. No.	Characters	Cluster I	Cluster II	Cluster III	Cluster IV
Head					
1.	Tongue length	4.8061 (4.516-4.979)	4.7485 (4.663-4.834)	5.0991 (5.025-5.199)	5.3999 (5.00-5.578)
2.	Length of scape	1.1097 (1.080-1.138)	1.1021 (1.096-1.108)	1.1108 (1.075-1.167)	1.1901 (1.164-1.261)
3.	Length of pedicel	0.1865 (0.178-0.196)	0.1953 (0.194-0.196)	0.1866 (0.179-0.191)	0.2169 (0.199-0.238)
4.	Length of flagellum	2.3219 (2.266-2.393)	2.2551 (2.250-2.260)	2.4328 (2.419-2.482)	2.4737 (2.377-2.571)
5.	Length of antenna	3.6150 (3.531-3.709)	3.5512 (3.550-3.553)	3.7336 (3.660-3.809)	3.8786 (3.735-4.026)
Forewing					
6.	Length of forewing	7.7749 (7.605-7.909)	7.6971 (7.592-7.802)	7.8848 (7.788-8.119)	7.9204 (7.760-8.003)
7.	Breadth of forewing	2.7857 (2.279-2.915)	2.7599 (2.758-2.762)	2.7733 (2.741-2.855)	2.8959 (2.765-3.007)

Items 14 to 24 are in degrees and the rest are in mm. The values in parantheses show the range.

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Sl. No.	Characters	Cluster I	Cluster II	Cluster III	Cluster IV
8.	Length of radial cell	2.8372 (2.778-2.923)	2.7987 (2.820-2.978)	2.9367 (2.875-2.986)	3.0218 (2.910-3.153)
9.	Breadth of radial cell	0.4613 (0.433-0.486)	0.4603 (0.453-0.467)	0.4450 (0.433-0.458)	0.4370 (0.232-0.453)
10.	Length of basal portion of radial cell	1.0587 (1.034-1.080)	1.0535 (1.004-1.103)	1.1214 (1.080-1.164)	1.1643 (1.066-1.213)
11.	Length of apical portion of radial cell	1.7781 (1.720-1.837)	1.7365 (1.712-1.761)	1.7918 (1.739-1.832)	1.8674 (1.746-2.086)
12.	Length of Ist abscissa	0.4812 (0.463-0.499)	0.4784 (0.467-0.480)	0.5174 (0.501-0.538)	0.5160 (0.521-0.526)
13.	Length of IInd abscissa	0.1601 (0.154-0.188)	0.1768 (0.165-0.188)	0.1286 (0.108-0.164)	0.1444 (0.125-0.252)
14.	Vein angle no. 31	31.0567 (30.00-32.60)	31.2000 (30.667-31.733)	31.9399 (31.500-32.333)	30.5667 (29.00-32.00)
15.	Vein angle no. 32	106.1867 (102.033-109.467)	106.1834 (104.60-107.767)	107.0667 (105.237-109.667)	109.9667 (108.00-112.00)
16.	Vein angle no. 33	97.0833 (94.00-100.90)	96.9833 (96.833-97.133)	96.1333 (94.600-97.067)	99.1333 (90.00-107.00)
17.	Vein angle no. 34	20.7933 (19.933-22.133)	21.0000 (20.600-21.400)	20.5800 (20.300-20.967)	18.9333 (15.00-23.00)

Items 14 to 24 are in degrees and the rest are in mm. The values in parantheses show the range.

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Sl. No.	Characters	Cluster I	Cluster II	Cluster III	Cluster IV
18.	Vein angle no. 35	94.1433 (91.067-97.800)	97.0333 (95.033-99.033)	92.6333 (91.500-93.733)	85.3333 (82.00-89.00)
19.	Vein angle no. 36	46.3367 (44.167-47.867)	47.5500 (46.700-48.400)	44.2399 (42.800-45.600)	44.2667 (40.00-46.00)
20.	Vein angle no. 37	102.3900 (100.133-104.80)	102.5833 (101.933-103.233)	101.0800 (99.600-102.733)	95.5333 (92.00-98.00)
21.	Vein angle no. 38	77.4933 (75.40-78.867)	71.3667 (69.867-72.867)	79.7800 (78.90-81.100)	74.6667 (72.00-76.00)
22.	Vein angle no. 39	13.9933 (12.800-14.933)	15.0667 (14.500-15.633)	14.2867 (13.667-14.633)	14.1333 (12.00-16.00)
23.	Vein angle no. 40	84.1067 (79.733-87.070)	82.1500 (81.500-82.800)	81.1733 (78.40-83.100)	74.3667 (73.00-75.00)
24.	Vein angle no. 41	33.3000 (30.667-36.333)	33.7000 (33.500-33.900)	33.5399 (32.533-34.600)	33.2333 (30.00-44.00)
Hindwing					
25.	Length of hindwing (mm)	5.4544 (5.245-5.562)	5.4436 (5.381-5.506)	5.5176 (5.440-5.642)	5.5649 (5.432-5.723)
26.	Breadth of hindwing	1.6208 (1.570-1.664)	1.6226 (1.622-1.623)	1.5607 (1.528-1.622)	1.6073 (1.552-1.649)
27.	Length of vein RL	1.3407 (1.214-1.379)	1.3138 (1.307-1.321)	1.3621 (1.341-1.386)	1.3729 (1.310-1.407)

Items 14 to 24 are in degrees and the rest are in mm. The values in parantheses show the range.

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Sl. No.	Characters	Cluster I	Cluster II	Cluster III	Cluster IV
28.	Length of vein ML	1.1613 (1.099-1.415)	1.2151 (1.195-1.235)	1.1756 (1.125-1.179)	1.1789 (1.067-1.261)
29.	Length of vein VL	1.1892 (1.126-1.247)	1.2096 (1.198-1.221)	1.4559 (1.172-1.510)	1.3001 (1.164-1.407)
30.	Length of vein IL	0.3837 (0.333-0.443)	0.3401 (0.375-0.427)	0.3986 (0.333-0.442)	0.4385 (0.2318-0.4831)
31.	Number of hamuli	17.8033 (17.20-18.967)	18.6000 (17.667-19.533)	17.7733 (17.563-18.100)	19.2667 (17.00-21.00)
32.	Extent of hamuli	1.1194 (1.091-1.220)	1.0851 (1.082-1.088)	1.1182 (1.080-1.133)	1.0882 (1.067-1.116)
33.	Length of jugal lobe	1.6221 (1.522-1.770)	1.6969 (1.657-1.737)	1.6418 (1.556-1.716)	1.4742 (1.358-1.649)
34.	Length of vanal lobe	1.0771 (1.014-1.132)	1.0568 (1.016-1.098)	1.0902 (1.061-1.117)	1.1900 (1.116-1.3100)
Hind leg					
35.	Length of femur	2.1688 (2.077-2.212)	2.1666 (2.125-2.209)	2.2106 (2.165-2.306)	2.2247 (1.940-2.571)
36.	Length of tibia	2.6598 (2.465-2.792)	2.6168 (2.592-2.642)	2.6871 (2.645-2.805)	2.5837 (2.328-2.959)

Items 14 to 24 are in degrees and the rest are in mm. The values in parantheses show the range.

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Sl. No.	Characters	Cluster I	Cluster II	Cluster III	Cluster IV
37.	Length of metatarsus	1.7499 (1.620-1.795)	1.7043 (1.688-1.720)	1.7609 (1.707-1.832)	1.7802 (1.698-1.940)
38.	Breadth of metatarsus	0.9412 (0.919-0.979)	0.8919 (0.885-0.899)	0.9284 (0.902-0.986)	0.7972 (0.776-0.983)
Third tergite					
39.	Width of light band	0.6838 (0.605-0.985)	1.0895 (1.033-1.119)	0.8646 (0.646-1.143)	0.9589 (0.728-1.261)
40.	Width of dark band	0.8903 (0.532-1.640)	0.6651 (0.662-0.669)	0.8919 (0.691-1.006)	0.7924 (0.728-1.064)
41.	Total width of 3rd tergite	1.6567 (1.588-1.817)	1.7280 (1.696-1.760)	1.7559 (1.648-1.899)	1.7510 (1.698-1.843)
Fourth tergite					
42.	Width of light band	0.6700 (0.537-1.030)	1.0891 (1.020-1.158)	0.7431 (0.602-0.944)	0.8362 (0.728-1.213)
43.	Width of dark band	0.9784 (0.783-1.103)	0.7311 (0.728-0.734)	1.0206 (0.934-1.147)	0.9913 (0.728-1.358)
44.	Total width of 4th tergite	1.6519 (1.568-1.828)	1.8215 (1.754-1.889)	1.7583 (1.653-1.890)	1.8270 (1.698-2.134)

Items 14 to 24 are in degrees and the rest are in mm. The values in parantheses show the range.

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Sl. No.	Characters	Cluster I	Cluster II	Cluster III	Cluster IV
Third sternite					
45.	Length of wax mirror	1.6141 (1.572-1.665)	1.6566 (1.602-1.711)	1.7653 (1.596-1.903)	1.6299 (1.455-1.698)
46.	Breadth of wax mirror	1.0169 (0.912-1.187)	1.1417 (1.137-1.147)	1.0222 (0.934-1.098)	1.1270 (0.970-1.358)
47.	Distance between wax mirrors	0.4071 (0.291-0.561)	0.4505 (0.350-0.547)	0.4382 (0.369-0.531)	0.4998 (0.291-0.534)
48.	Total width of sternite	2.0285 (1.934-2.099)	2.0687 (1.955-2.183)	2.0796 (2.032-2.213)	1.9937 (1.892-2.086)
Sixth sternite					
49.	Depth of sternite	2.1706 (2.069-2.370)	2.2087 (2.120-2.298)	2.1429 (2.097-2.239)	2.1294 (1.940-2.377)
50.	Breadth of sternite	2.4151 (2.105-2.556)	2.4556 (2.405-2.506)	2.4800 (2.389-2.595)	2.3720 (2.134-2.571)

Items 14 to 24 are in degrees and the rest are in mm. The values in parantheses show the range.

The lowest mean length of scape (1.1021 mm) was observed in cluster II and it was closely followed by the mean measurements in cluster I (1.1097 mm) and cluster III (1.1108 mm). The range in the measurements in above three clusters indicated a lot of overlapping in the data. The highest mean measurement (1.1901 mm) was observed in cluster IV and the range in the measurement in this cluster did not overlap with those of cluster I and II while there was a minor overlapping with that of cluster III.

The lowest mean length of pedicel (0.1865 mm) was observed in cluster I and it was closely followed by the mean measurements in cluster III (0.1866 mm) and II (0.1953 mm). The range in these clusters indicated overlapping in the data. The highest mean measurement (0.2169 mm) was observed in cluster IV and its range did not overlap with any of the other three clusters.

The lowest mean length of flagellum (2.2551 mm) was observed in cluster II and it was closely followed by the mean measurements in cluster I (2.3219 mm). The highest mean length of flagellum was observed in cluster IV (2.4737 mm) closely followed by that in cluster III (2.4328 mm). The ranges in the measurements in the clusters I, III and IV showed overlapping to varying degree while cluster II stood low and separate without overlapping.

The lowest mean length of antenna (3.5512 mm) was observed in cluster II and it was closely followed by that in cluster I (3.6150 mm). The highest mean measurement (3.8786 mm) was observed in cluster IV, closely followed by the mean measurements in cluster III (3.7336 mm). The ranges in the measurements in cluster I and II showed overlapping while there was no overlapping of measurements in cluster II with III and IV. But cluster I showed slight overlapping with cluster III.

The lowest mean length of forewing (7.6971 mm) was observed in cluster II and it was closely followed by the mean measurement in cluster I (7.7749 mm). The highest mean measurement (7.9204 mm) was observed in cluster IV closely followed by that of cluster III (7.8848 mm). The ranges in the measurements in clusters I and II as well as III and IV overlapped.

The lowest mean breadth of forewing (2.7599 mm) was observed in cluster II and it was closely followed by cluster III (2.7733 mm) and cluster I (2.7857 mm). The highest mean measurement (2.8959 mm) was observed in cluster IV. The ranges of measurements in various clusters had high overlapping.

The lowest mean length of radial cell (2.7987 mm) was observed in cluster II and it was closely followed by cluster I (2.8372 mm). The highest mean measurement (3.0218 mm) was observed in cluster IV closely followed by that in cluster III (2.9367 mm). The ranges in the measurements in the four clusters indicated overlapping of the data.

The lowest mean breadth of radial cell (0.4370 mm) was observed in cluster IV closely followed by that in cluster III (0.4450 mm). The ranges in the measurements in the four clusters showed a lot of overlapping. The highest mean breadth of radial cell (0.4613 mm) was observed in cluster I and it was closely followed by that in cluster II (0.4603 mm).

The lowest mean length of basal portion of radial cell (1.0535 mm) was observed in cluster II and it was closely followed by cluster I (1.0587 mm) and cluster III (1.1214 mm). The highest mean was observed in cluster IV (1.1643 mm) followed by that in cluster III. The ranges of clusters I and II as well as III and IV showed considerable overlapping.

The lowest mean length of apical portion of radial cell (1.7365 mm) was observed in cluster II and it was closely followed by the mean measurement in cluster I (1.7781 mm) and cluster III (1.7918 mm) while cluster IV showed the highest mean measurement (1.8674 mm). The ranges in the clusters showed overlapping of the data.

The lowest mean length of Ist abscissa (0.4784 mm) was observed in cluster II and it was closely followed by that in cluster I (0.4812 mm). Cluster III (0.5174 mm) recorded the highest measurement which was almost equal to cluster IV (0.5160 mm). The ranges in the measurements in cluster I and II showed overlapping as also in cluster III and IV.

The lowest mean length of second abscissa (0.1286 mm) was observed in cluster III followed by that in cluster IV (0.1444 mm) and cluster II (0.1768 mm) showed the highest value which was closely followed by that in cluster I (0.1601 mm). The ranges in the measurements in cluster I, II and IV overlapped but those in cluster III overlapped with cluster I and IV only.

The lowest mean of angle No. 31 (30.5667°) was observed in cluster IV and it was closely followed by cluster I (31.0567°) and cluster II (31.2000°). Cluster III (31.9399°) showed the highest mean measurement. The ranges in measurements in the above four clusters indicated overlapping of the data.

The lowest mean of angle No. 32 (106.1834°) in cluster II was closely followed by those of cluster I (106.1867°) and cluster III (107.0667°). Cluster IV (109.9667°) showed highest mean. The ranges in the measurements of the above four clusters overlapped.

The lowest mean of angle No. 33 (96.1333°) was observed in cluster III and it was followed by cluster II (96.9833°) and cluster I (97.0833°). Cluster IV (99.1333°) showed the highest mean. The ranges in measurements of all clusters overlapped.

The lowest mean of angle No. 34 (18.9333°) was observed in cluster IV. Cluster II (21.0000°) showed the highest mean which was followed by those in cluster I (20.7933°) and cluster III (20.5800°) in

descending order. The ranges in measurements in the four clusters indicated high overlapping of the data.

Wing vein angle No. 35 showed widely varying cluster means for the four clusters. The lowest mean of angle No. 35 (85.3333°) was observed in cluster IV and was followed by cluster III (92.6333°), cluster I (94.1433°) and cluster II (97.0333°). The ranges in the measurements in cluster I, II and III overlapped each other but cluster IV did not.

The lowest mean of angle No. 36 (44.2399°) was observed in cluster III and it was followed by that in cluster IV (44.2667°). The highest mean value was recorded in cluster II (47.5500°) which was followed by that of cluster I (46.3367°). The ranges in the measurements in clusters overlapped.

The lowest mean of angle No. 37 (95.5333°) was observed in cluster IV. The highest mean was recorded in cluster II (102.5833°) followed by those in cluster I (102.3900°) and cluster III (101.0800°). The ranges in measurements in different clusters did not show much overlapping.

Cluster II showed the lowest mean of angle No. 38 (71.3667°) while the highest mean measurement was noticed in cluster III (79.780°) and the means of the cluster I (77.4933°) and cluster IV (74.6667°) were of intermediate values. The ranges in measurements in different clusters did not overlap significantly.

The lowest mean of angle No. 39 (13.9933°) was observed in cluster I and it was closely followed by the mean measurements in cluster IV (14.1333°) and cluster III (14.2867°). The highest mean vein angle was found in cluster II (15.0667°). The ranges in the measurements in various clusters overlapped considerably.

The lowest mean of angle No. 40 (74.3667°) was observed in cluster IV while cluster I (84.1067°) showed the highest mean vein angle. Cluster III (81.1733°) and cluster II (82.1500°) had values which were intermediate. The ranges in the measurements in cluster IV did not overlap while all the other three clusters overlapped each other.

The mean values for angle No. 41 in all the clusters did not show wide variations and it was lowest (33.2333°) in cluster IV and highest in (33.7000°) in cluster II. Clusters I (33.3000°) and III (33.5399°) came in between.

In the case of the hindwing length also, the mean values for the different clusters did not show wide variations. The lowest mean of hindwing length (5.4436 mm) was observed in cluster II and was closely followed by the mean measurement in cluster I (5.4544 mm). Cluster IV (5.5649 mm) recorded the highest mean closely followed by that of cluster III (5.5176 mm). The ranges in measurements in all the four clusters overlapped considerably.

The lowest mean hindwing breadth was observed in cluster III (1.5607 mm) followed by cluster IV (1.6073 mm) and cluster I (1.6208 mm).

Cluster II (1.6226 mm) recorded the highest mean. The ranges in the measurements of the four clusters showed overlapping.

Cluster II (1.3138 mm) recorded the lowest mean length of vein RL and it was closely followed by cluster I (1.3407 mm) and cluster III (1.3621 mm). Cluster IV showed the highest mean (1.3729 mm). It was observed that the ranges in the measurements in various clusters overlapped except those in cluster II and cluster III.

The lowest mean length of vein ML was recorded in cluster I (1.1613 mm) followed by cluster III (1.1756 mm) and cluster IV (1.1789 mm). The highest mean was recorded in cluster II (1.2151 mm). The ranges in the measurements in various clusters showed overlapping except those in cluster II and III.

Cluster I (1.1892 mm) recorded the lowest mean length of vein VL followed by cluster II (1.2096 mm) and cluster IV (1.3001 mm). Cluster III (1.4559 mm) recorded the highest mean. It was observed that the ranges in measurements of various clusters overlapped.

The lowest mean length of vein IL was observed in cluster II (0.3401 mm) and it was followed by that in cluster I (0.3837 mm) and cluster III (0.3986 mm). Cluster IV recorded the highest mean of 0.4385 mm. There were significant overlapping of the ranges in the measurements of the four clusters.

The lowest mean number of hamuli was observed in cluster III (17.7733) closely followed by that in cluster I (17.8033) and cluster II (18.6000). But cluster IV indicated the highest mean number of hamuli (19.2667). The ranges in the measurements in the four clusters showed lot of overlapping.

The lowest mean extent of hamuli (1.0851 mm) was noted in cluster II and it was followed by cluster IV (1.0882 mm). Cluster I (1.1194 mm) recorded the highest mean extent of hamuli which was closely followed by that in cluster III with 1.1182 mm. The ranges in the measurements in various clusters indicated a lot of overlapping in the data except those in cluster I and II.

The lowest mean length of jugal lobe was observed in cluster IV (1.4742 mm). Cluster II recorded the highest mean length (1.6969 mm) followed by cluster III (1.6418 mm) and cluster I (1.6221 mm) with very near values. There were significant overlapping in the ranges in the measurements of different clusters.

Cluster II was observed to have the lowest mean length of vanal lobe (1.0568 mm) closely followed by cluster I with 1.0771 mm and cluster III with 1.0902 mm while cluster IV recorded the highest mean length of 1.1900 mm. The ranges in the measurements in the four clusters overlapped.

The lowest mean length of femur (2.1666 mm) was observed in cluster II which was closely followed by the mean measurement in cluster I (2.1688 mm). Cluster IV with 2.2247 mm recorded the highest mean length of femur followed by cluster III with mean value of 2.2106 mm. Ranges in measurements of different clusters overlapped.

The lowest mean length of tibia was observed in cluster IV (2.5837 mm) which was closely followed by that in cluster II (2.6168 mm). It was highest in cluster III (2.6871 mm) and it was closely followed by that in cluster I with 2.6598 mm. The ranges in measurements in the four clusters overlapped.

Cluster II recorded the lowest mean length of metatarsus (1.7043 mm) followed by cluster I (1.7499 mm). Cluster IV recorded the highest mean length of 1.7802 mm while that in cluster III (1.7609 mm) was in between. The ranges in measurements of different clusters overlapped.

The lowest mean breadth of metatarsus was observed in cluster IV (0.7972 mm) followed by that in cluster II (0.8919 mm). Cluster I recorded the highest mean breadth of metatarsus (0.9412 mm) closely followed by that of cluster III with 0.9284 mm. The ranges in the measurements in the cluster I, III and IV overlapped while cluster II did not overlap with clusters I and III.

The lowest mean width of light band of 3rd tergite (0.6838 mm) was observed in cluster I and it was followed by that in cluster III

(0.8646 mm). Cluster II was observed to have the highest width of light band (1.0895 mm) and cluster IV was very near with the mean value of 0.9589 mm. The ranges in the measurements in the cluster I and II did not show overlapping while cluster IV overlapped with all other cluster measurements.

The lowest mean width of dark band of 3rd tergite was noted in cluster II (0.6651 mm) followed by that in cluster IV (0.7924 mm). Cluster III (0.8919 mm) recorded the highest mean which was almost equal with the mean width of cluster I (0.8903 mm). The ranges in the measurements in cluster I and II were distinct while measurements in clusters I, III and IV were overlapping.

Total width of the third tergite was least in Cluster I (1.6567 mm). Cluster III (1.7559 mm) recorded the maximum width of third tergite which was closely followed by those in cluster IV (1.7510 mm) and cluster II (1.7280 mm). There were significant overlapping in the ranges in measurements in different clusters.

Cluster I showed the lowest mean light band width of 4th tergite (0.6700 mm) followed by that of cluster III (0.7431 mm) and cluster IV (0.8362 mm). Cluster II was observed to have highest mean width in light band (1.0891 mm). The ranges in the measurements of the above four clusters showed significant overlapping.

The lowest mean width of dark band of 4th tergite (0.7311 mm) was observed in cluster II followed by cluster I (0.9784 mm). While cluster III (1.0206 mm) showed maximum width, cluster IV with 0.9913 mm and cluster I with 0.9784 mm closely followed it. The ranges in the measurements in clusters overlapped considerably.

Cluster I showed the lowest mean total width of 4th tergite (1.6519 mm) followed by that in cluster III (1.7583 mm). Cluster IV (1.8270 mm) showed the highest mean width while cluster II was close to it with mean width of 1.8215 mm. The ranges in the measurements in four clusters were overlapping.

The lowest mean length of wax mirror 1.6141 mm was observed in cluster I and it was followed by cluster IV with 1.6299 mm and cluster II with 1.6566 mm. Cluster III with 1.7653 mm showed the highest mean length. The ranges in the measurements in the various clusters exhibited considerable overlapping.

The lowest mean breadth of wax mirror was observed in cluster I (1.0169 mm) followed by 1.0222 mm in cluster III and 1.1270 mm in cluster IV. The highest mean breadth was observed in cluster II (1.1417 mm). The ranges in the measurements in the various clusters showed significant overlapping.

The lowest mean distance between wax mirrors (0.4071 mm) was observed in cluster I and it was followed by the mean measurements in

cluster III (0.4382 mm) and in cluster II (0.4505 mm) while cluster IV (0.4998 mm) measured the highest. The range in the measurements indicated a lot of overlapping in the data of the four clusters.

Cluster IV recorded the lowest total width of third sternite (1.9937 mm) and it was closely followed by cluster I with a mean value of 2.0285 mm. Cluster III (2.0796 mm) showed the highest mean width closely followed by cluster II with mean width of 2.0687 mm. The ranges in the measurements indicated overlapping in the data of the different clusters.

Cluster IV showed the lowest mean depth of sixth sternite (2.1294 mm) followed by the cluster III (2.1429 mm), cluster I (2.1706 mm) and cluster II (2.2087 mm), the last being the highest. The range in the measurements indicated overlapping in the data of the four clusters.

The lowest mean breadth of sixth sternite (2.3720 mm) was observed in cluster IV and it was followed by cluster I (2.4151 mm) and cluster II (2.4556 mm). Cluster III recorded the maximum mean breadth (2.4800 mm). The ranges in the measurements in the four clusters showed significant overlapping.

4.4 Contribution of different characters towards the divergence

The percentage contribution of each morphometric character towards divergence of the clusters of *A. cerana indica*, identified in

discriminant analysis, are presented in Table 56. The highest contribution was by wing vein angle 32 and length of vein VL (each appeared 10 times as first in ranking), the per cent contribution towards divergence being 6.4 each. These were followed by length of antenna, length of basal portion of radial cell and number of hamuli (each appeared 7 times in ranking as first) with 4.5 per cent contribution to divergence.

The morphometric characters viz., wing vein angles 33, 39 and length of vein RL contributed 3.8 per cent each while breadth of metatarsus contributed 3.2 per cent towards divergence.

Length of pedicel, length of apical portion of radial cell, length of first abscissa, wing vein angle 35, 37, 41, extent of hamuli, length of femur, width of dark band of fourth tergite appeared four times each as first in ranking, contributing 2.5 per cent towards divergence.

The contribution towards divergence by length of radial cell, wing vein angle 31, length of vein IL, length of metatarsus, total width of third sternite and breadth of third sternite was 1.9 per cent each and each appeared three times as first in the ranking.

Tongue length, length of scape, length of forewing, breadth of forewing, length of second abscissa, wing vein angle 40, length of hindwing, breadth of hindwing, length of jugal lobe, length of vanal lobe, length of tibia, total width of fourth tergite and length of wax mirrors

Table 56. Contribution of different characters towards the divergence of the clusters of *Apis cerana indica* identified in discriminant analysis

Sl. No.	Characters	No. of times appearing first in ranking	Percentage contribution
Head			
1.	Tongue length	2	1.3
2.	Length of scape	2	1.3
3.	Length of pedicel	4	2.5
4.	Length of flagellum	0	0
5.	Length of antenna	7	4.5
Forewing			
6.	Length of forewing	2	1.3
7.	Breadth of forewing	2	1.3
8.	Length of radial cell	3	1.9
9.	Breadth of radial cell	0	0
10.	Length of basal portion of radial cell	7	4.5
11.	Length of apical portion of radial cell	4	2.5
12.	Length of Ist abscissa	4	2.5
13.	Length of IInd abscissa	2	1.3
14.	Vein angle no. 31	3	1.9
15.	Vein angle no. 32	10	6.4
16.	Vein angle no. 33	6	3.8
17.	Vein angle no. 34	1	0.6
18.	Vein angle no. 35	4	2.5
19.	Vein angle no. 36	1	0.6
20.	Vein angle no. 37	4	2.5
21.	Vein angle no. 38	1	0.6
22.	Vein angle no. 39	6	3.8
23.	Vein angle no. 40	2	1.3
24.	Vein angle no. 41	4	2.5
Hindwing			
25.	Length of hindwing	2	1.3
26.	Breadth of hindwing	2	1.3
27.	Length of vein RL	6	3.8
28.	Length of vein ML	0	0

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Table 56 (Contd...)

Sl. No.	Characters	No. of times appearing first in ranking	Percentage contribution
29.	Length of vein VL	10	6.4
30.	Length of vein IL	3	1.9
31.	Number of hamuli	7	4.5
32.	Extent of hamuli	4	2.5
33.	Length of jugal lobe	2	1.3
34.	Length of vanal lobe	2	1.3
Hind leg			
35.	Length of femur	4	2.5
36.	Length of tibia	2	1.3
37.	Length of metatarsus	3	1.9
38.	Breadth of metatarsus	5	3.2
Third tergite			
39.	Width of light band	1	0.6
40.	Width of dark band	1	0.6
41.	Total width of 3rd tergite	1	0.6
Fourth tergite			
42.	Width of light band	1	0.6
43.	Width of dark band	4	2.5
44.	Total width of 4th tergite	2	1.3
Third sternite			
45.	Length of wax mirror	2	1.3
46.	Breadth of wax mirror	0	0
47.	Distance between wax mirrors	1	0.6
48.	Total width of 3rd sternite	3	1.9
Sixth sternite			
49.	Depth of 6th sternite	1	0.6
50.	Breadth of 6th sternite	3	1.9

Items 14 to 24 are in degree and the rest are in mm.

appeared two times each as first rank and contributed 1.3 per cent towards divergence.

Wing vein angle 34, 36, 38, width of light band of third tergite, width of dark band of third tergite, total width of third tergite, width of light band in fourth tergite, distance between wax mirrors and depth of sixth sternite contributed 0.6 per cent each appearing one time each as first in ranking.

The morphometric characters viz., length of flagellum, breadth of radial cell, length of vein ML in hindwing and breadth of wax mirrors in third sternite have not made any contribution towards divergence.

4.5 Relative susceptibility of ecotypes of *Apis cerana indica* in Kerala to TSBV

The colonies selected for collecting bee samples for morphometric studies were kept numbered in the concerned locations and bimonthly observations were recorded on the incidence of TSBV disease in them. The cumulative percentage mortality / desertion of colonies due to TSBV infection are presented in Table 57. It was found that all the bee colonies maintained in different locations got infected with TSBV and got lost in the course of two to twelve months indicating the lack of resistance of the different ecotypes to TSBV.

Table 57. Fate of *Apis cerana indica* colonies sampled for morphometric studies from various locations

Sl. No.	Name of location	Cumulative percentage mortality of colonies due to TSBV in					
		2 months	4 months	6 months	8 months	10 months	12 months
1.	Pampadumpara	—	—	40	80	100	—
2.	Ponmudi	—	20	40	80	100	
3.	Attapady	20	40	60	100	—	—
4.	Kumbalacode	—	—	20	100	—	—
5.	Wynad	—	20	80	100	—	—
6.	Mananthavadi	—	20	80	100	—	—
7.	Kulathupuzha	20	20	40	80	100	—
8.	Kanjirapally	—	—	—	20	100	—
9.	Palakkad	—	—	40	80	100	—
10.	Malampuzha	—	—	—	20	60	100
11.	Nilampur	20	40	60	100	—	—
12.	Malappuram	—	20	40	80	100	—
13.	Neyyattinkara	20	20	40	60	100	—
14.	Pathanamthitta	—	—	—	60	60	100
15.	Thrissur	—	—	40	60	100	—
16.	Vadakkancherry	—	—	20	40	100	—
17.	Pilicode	—	—	—	60	100	—
18.	Aralam Farm	20	20	40	40	80	100

Note: Bees from five different colonies were sampled in each location.

4.6 Susceptibility of different larval instars of *Apis cerana indica* to TSBV and symptomatology

4.6.1 Larval susceptibility

The larvae were inoculated with TSBV when they were 1, 2, 3 and 4 days old. The results are presented in Table 58.

When the larvae were inoculated on the first day, nearly 50 per cent died within two days after inoculation and all the larvae were found dead on the 3rd day after inoculation. Mean incubation period was 2.52 to 2.54 days. Thus 100 per cent of larvae died before reaching the prepupal stage and hence the cells were not capped by the nurse bees. The cadavers were being promptly removed by the worker bees and the cells were seen mostly emptied during the observation.

When the larvae were inoculated on the second day, the incubation period was found slightly longer (3.45 to 3.48 days) than that of the first instar. Here also about 50 per cent of larvae died on the third day of inoculation and 38 to 44 per cent, died by the fourth day leaving eight to sixteen per cent to undergo normal development. They emerged as disease free adults. All the dead larvae were seen in unsealed cells and their size was small.

When three day old larvae were inoculated, the incubation period was observed to be comparable to that of two day old larvae (3.41 to

Table 58. Mortality of larvae of *Apis cerana indica* inoculated with TSBV

Age of larvae at inoculation	Year of experiment	Treatments	No. of larvae died due to TSBV infection within					No. of larvae pupated	Per cent mortality	Incubation period (days)	
			24 hr	48 hr	72 hr	96 hr	120 hr			Range	Mean
	1994-95	TSBV treated	—	23	—	27	—	Nil	100	2-3	2.54
		Control	—	—	—	—	—	50	Nil	—	—
1 day	1995-96	TSBV treated	—	24	26	—	—	Nil	100	2-3	2.52
		Control	—	—	—	—	—	50	Nil	—	—
	1996-97	TSBV treated	—	24	26	—	—	Nil	100	2-3	2.52
		Control	—	—	—	—	—	50	Nil	—	—
	1994-95	TSBV treated	—	—	23	19	—	8	84	3-4	3.45
		Control	—	—	—	—	—	50	—	—	—
2 day	1995-96	TSBV treated	—	—	24	22	—	4	92	3-4	3.48
		Control	—	—	—	—	—	50	Nil	—	—
	1996-97	TSBV treated	—	—	24	22	—	4	92	3-4	3.48
		Control	—	—	—	—	—	50	Nil	—	—

Note : 50 larvae were used in each test.

Contd...

Age of larvae at inoculation	Year of experiment	Treatments	No. of larvae died due to TSBV infection within					No. of larvae pupated	Per cent mortality	Incubation period (days)	
			24 hr	48 hr	72 hr	96 hr	120 hr			Range	Mean
3 day	1994-95	TSBV treated	—	—	24	24	—	2-4	96	3-4	3.50
		Control	—	—	—	—	—	50	Nil	—	—
	1995-96	TSBV treated	—	—	23	19	—	8-16	84	3-4	3.71
		Control	—	—	—	—	—	50	—	—	—
	1996-97	TSBV treated	—	—	24	17	—	9-18	82	3-4	3.41
		Control	—	—	—	—	—	50	Nil	—	—
4 day	1994-95	TSBV treated	—	—	11	15	10	14	72	3-5	3.97
		Control	—	—	—	—	—	50	Nil	—	—
	1995-96	TSBV treated	—	—	12	18	7	13	74	3-5	3.86
		Control	—	—	—	—	—	50	Nil	—	—
	1996-97	TSBV treated	—	—	12	16	9	13	74	3-4	3.92
		Control	—	—	—	—	—	50	Nil	—	—

Note : 50 larvae were used in each test.

3.71 days). Here also nearly 50 per cent of the larvae died within three days and the rest within four days of inoculation. A low percentage (4-18) pupated and adults emerged normally. It was observed that the larvae which died on the third day after inoculation were in uncapped cells. But those dying on the fourth day after inoculation were found capped by the worker bees in the normal pattern. The condition of larvae and mortality had to be recorded after removing the cappings.

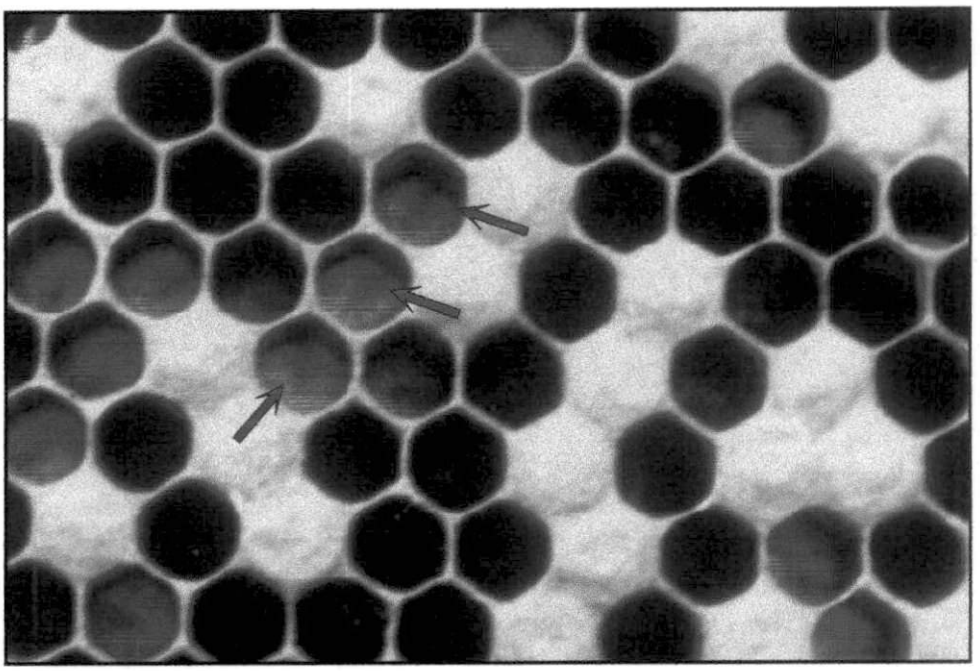
In the case of four day old larvae, the mean incubation period ranged from 3.86 to 3.97 days. It did not significantly differ from those of two and three day old larvae. Though the mortality commenced from third day after inoculation, the number of dead larvae were comparatively lower (11-12 per cent only) on the third day after inoculation. On fourth day the mortality percentages ranged from 30-36 and death continued upto the fifth day when there was 14 to 20 per cent mortality. Further, in experiments repeated during the three years more than 25 per cent of four day old larvae inoculated with TSBV pupated normally leading to adult emergence and while disease affected ones did not reach the pupal stage. Since the mortality commenced only on the third day of inoculation ie., seven days after hatching, the cells of diseased larvae were found normally capped by the worker bees. Later, those cells accomodating dying larvae were seen bitten by the workers and opened up.

4.6.2 Symptomatology

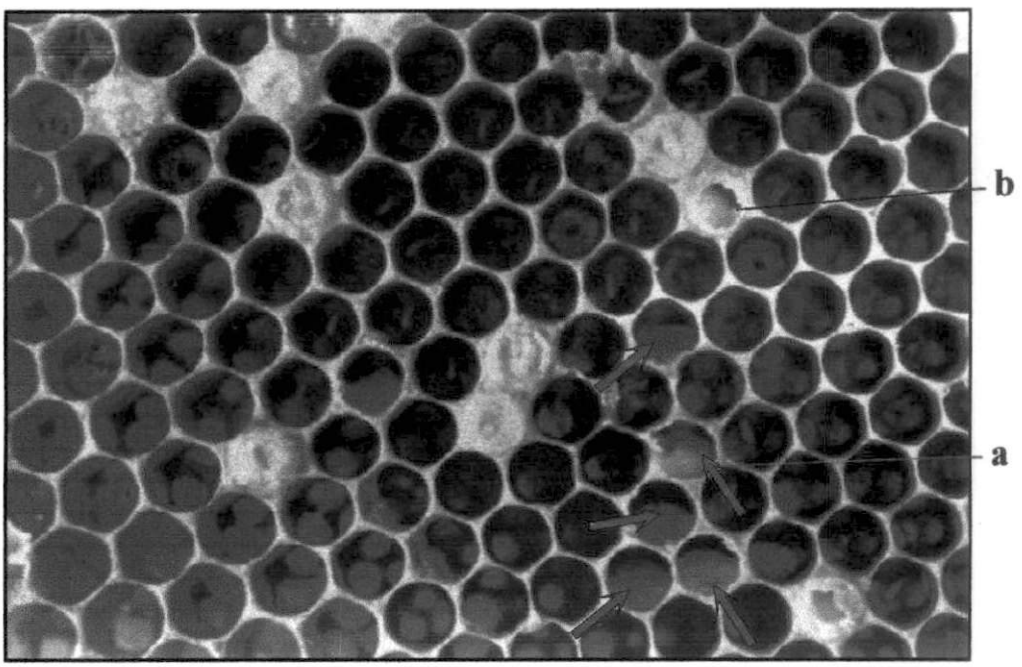
Symptomatology was studied on larvae artificially inoculated with Thai Sacbrood Virus. No perceptible symptoms could be observed on larvae inoculated in the first and second instars except that they did not show the normal increase in size. Larvae inoculated in the third and subsequent instars appeared slightly plumpy on the second day of inoculation (four day or more old) compared to healthy larvae of the same age. Soon the diseased larvae were seen lying on the lower side of the cells with the head directed outwards and turned upwards somewhat like the prow of a boat. Dead larvae were mostly seen in uncapped cells (Fig. 14), though some larvae which got infected late could be found in capped cells which often got perforated by the worker bees. In later stages the body of the larvae appeared as sacs and it was seen filled with a milky fluid (Fig. 15). The larval cuticle was thin and got ruptured even with mild pressure. The body colour of the dead larvae changed from pearly white to pale yellow. The cadavers dried up in 10-15 days and became thin brownish black boat-shaped scales lying at the floor of the cells which could be easily removed (Fig. 16). The dead larvae did not show any ropiness and had no putrid odour.

Infected larvae failed to pupate. Those larvae which survived infection, pupated normally and adults emerged. No malformation or death of such pupae and adults was noticed.

Fig. 14. Healthy and dead larvae of *Apis cerana indica* in the comb cells



A. Healthy larvae within comb cells



B. Dead larvae lying at the floor of comb cells

(a) dead larvae in uncapped cells
(b) dead larvae in capped cells which are perferated by worker bees

Fig. 15. Healthy and TSBV infected larvae of *Apis cerana indica*

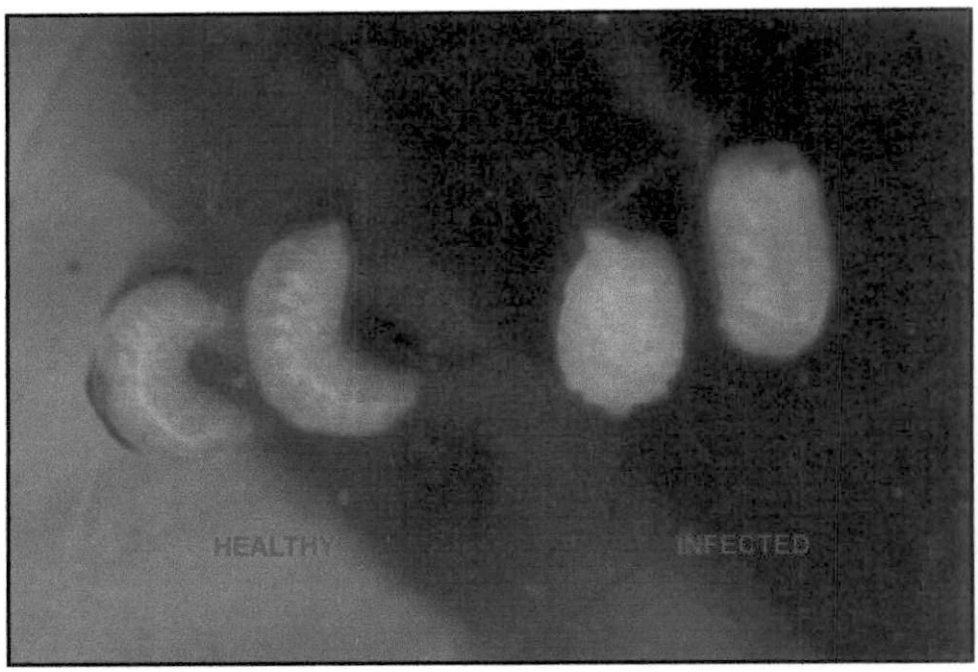
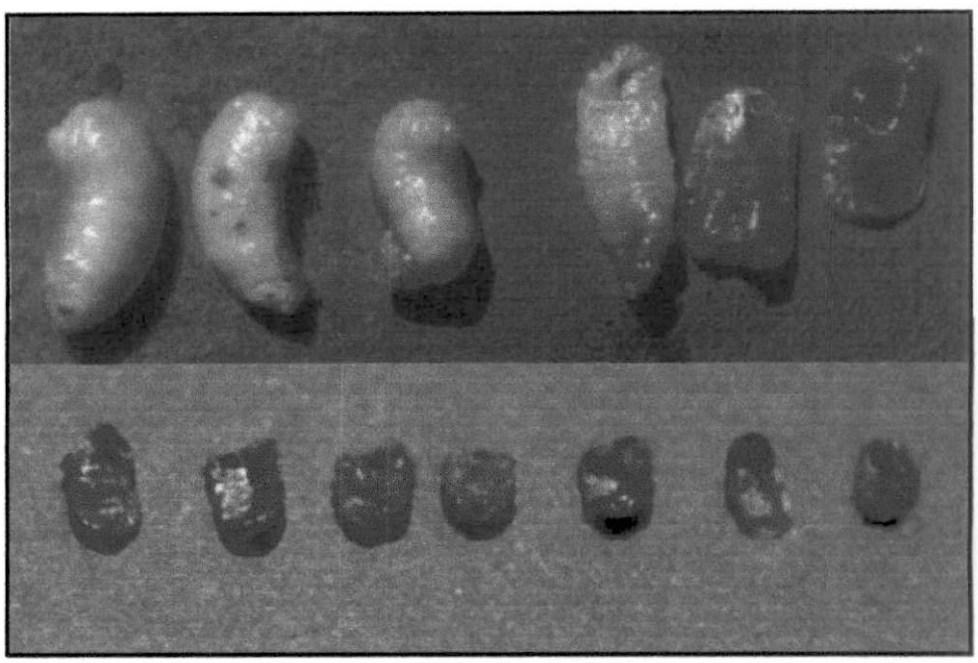


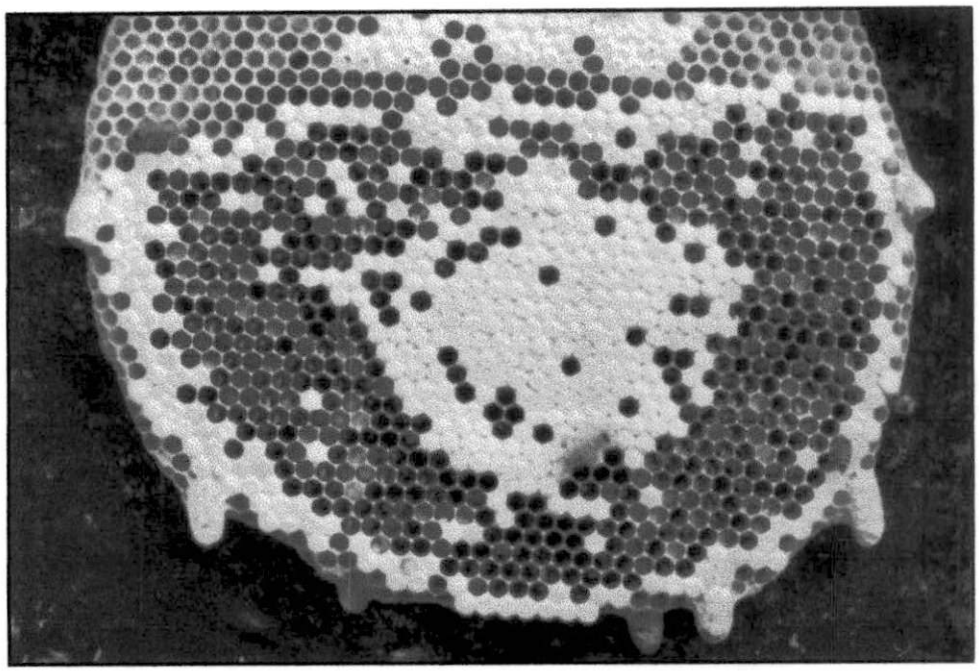
Fig. 16. Cadavers of the larvae of *Apis cerana indica* showing formation of scales



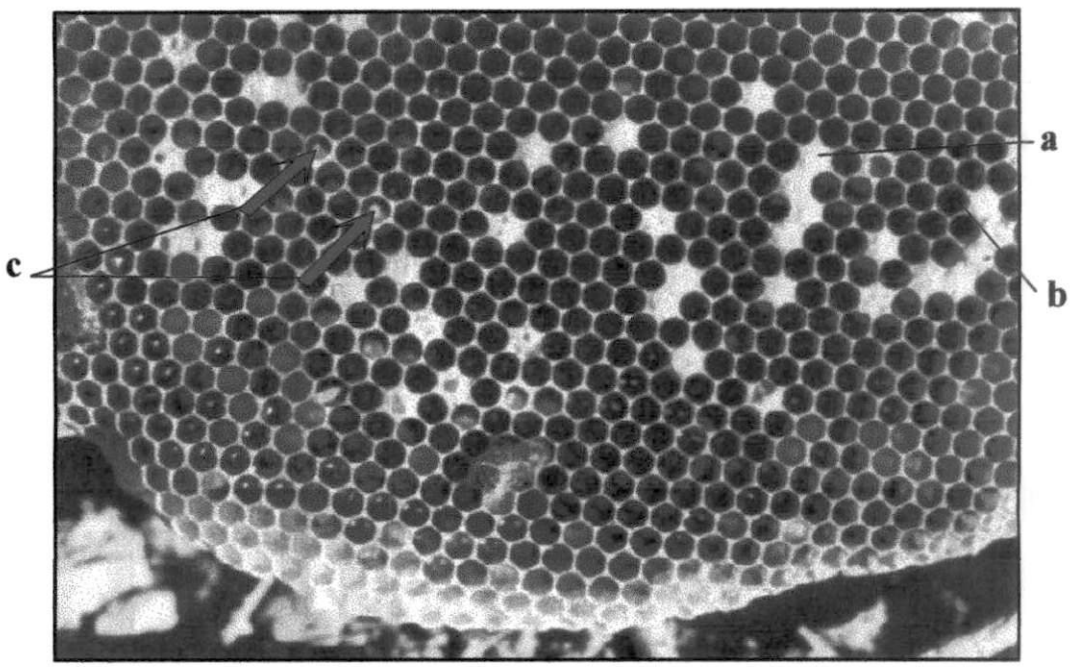
The symptoms of infected colonies in the apiary were also observed. In the beginning, diseased larvae could be seen in the cells lying with the head directed outwards and turned upwards like the prow of a boat (Fig. 17B). Soon dead larvae lying on the floor of the cells as described earlier also were noticed. Usually only a few dead larvae were thus seen in the beginning. Further, the workers were seen ejecting the dead larvae from the cells. They were seen scattered on the floor board, alighting board as well as on the ground below the hive (Fig.18). The infection occurred in worker brood only. No first and second instar larvae could be found lying dead in the cells.

With the progress of the disease, infected combs showed a mottled appearance with a large number of uncapped empty cells, with a few normally sealed cells scattered among them and a few cells with perforated cappings (Fig. 17B) and some cells having dead larvae in it. In course of time the disease spread in the brood fast and more and more dead larvae were seen left within the cells without being ejected by the worker bees. Nurse bees became inactive and failed to tend the brood properly. The foraging activity of the worker bees also decreased as the disease advanced and the workers were seen remaining idle in the colony. Further, the bees showed increased agressiveness compared to those of healthy colonies. The infection noticed in some colonies progressed rapidly causing 95 to 100 per cent brood mortality in the course of 8-10 days. During this time the hives had an unpleasant

Fig. 17. Healthy and TSBV infected combs of *Apis cerana indica*



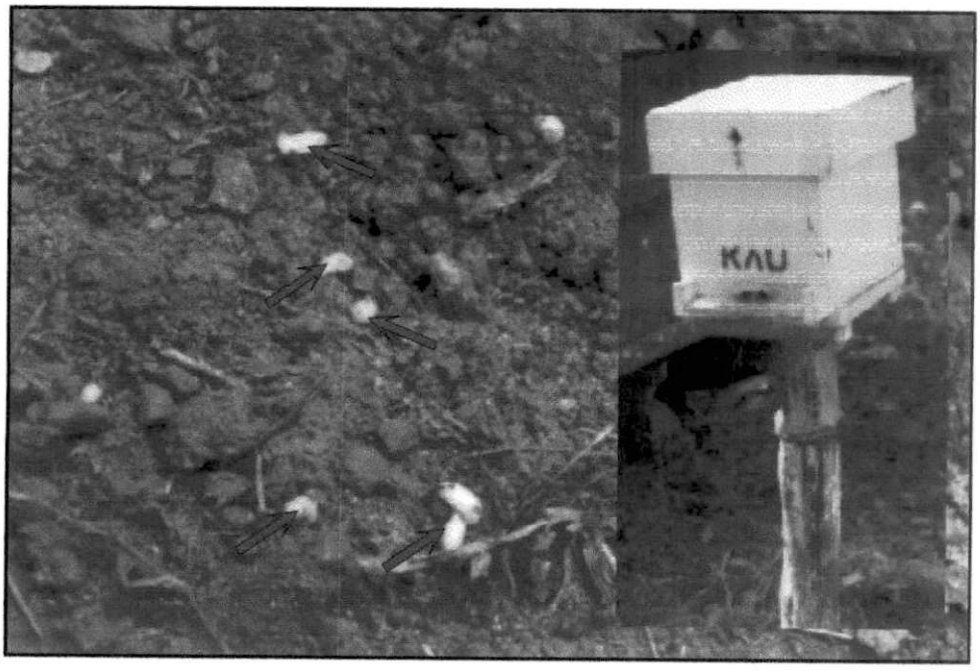
A. Healthy comb showing the normal pattern of brood rearing and storage of honey and pollen



B. Infected comb showing capped cells (a) which are few in number interspersed with uncapped cells (b) and prow-like appearance of the head of diseased larvae (c)

Fig. 18. Cadavers of *Apis cerana indica* larvae infected with TSBV strewn on the floor board and on the ground

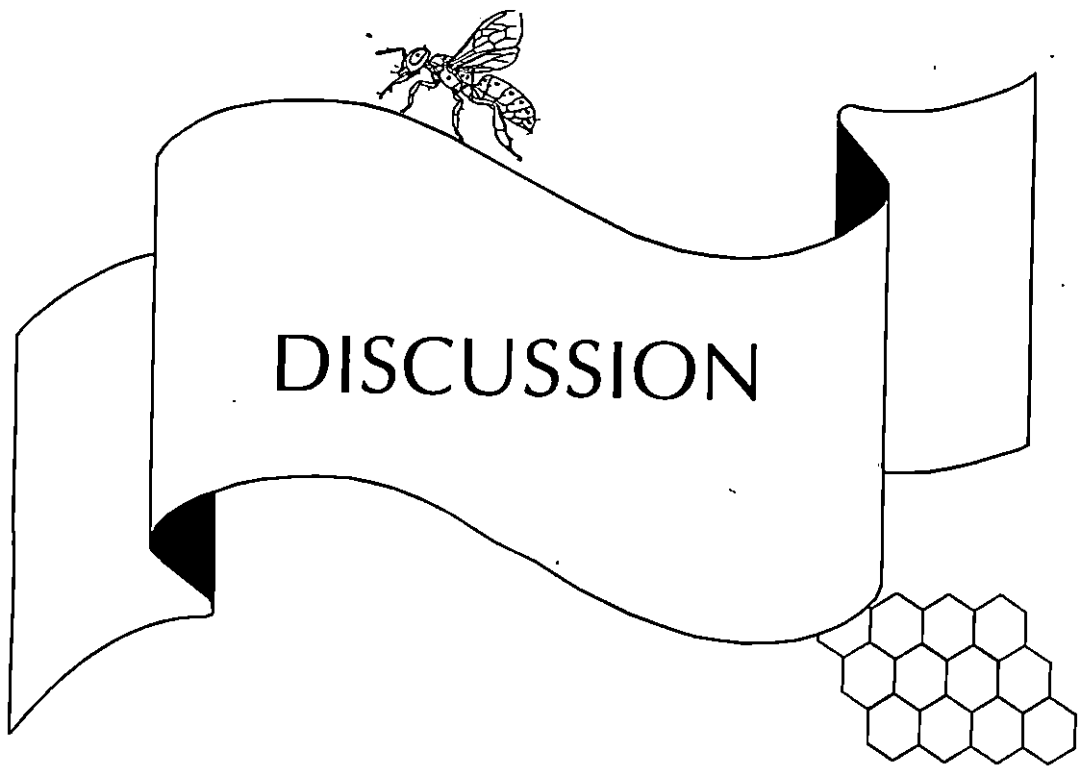
A. Cadavers on the floor board



B. Cadavers on the ground

odour. The behaviour changes in workers affected the activity of the queen also. The eggs became sparse and irregularly placed in the cells. The emerging larvae died due to TSBV infection. Consequently emergence of new bees got reduced or even ceased completely and the population of the colony dwindled down. The remaining bees in such colonies often deserted the hives causing total loss to the apiary.

It was observed in the field that some colonies exhibited intermittent infection and recovery and some colonies which had recovered from infection continued to be healthy for varying periods extending upto twelve months though they succumbed finally to the virus infection.


DISCUSSION

DISCUSSION

The present investigations were undertaken with a view to identifying ecotypes of *Apis cerana indica* Fab. in Kerala and to assess their susceptibility / resistance to Thai Sacbrood Virus (TSBV). It was also intended to make detailed investigations on the pathogenicity of TSBV on *A. cerana indica.*

Extensive geographic variations have been earlier recognised in honeybees (Ottis, 1991). Detailed taxonomic studies on the basis of morphometric analysis of these variations have been done on the European bee *Apis mellifera* Linn. (Ruttner, 1985, 1986). But the information on these aspects is limited in *A. cerana indica.*

Kerala with its varied topograhical, environmental and floral diversity is likely to harbour a number of ecotypes of *A. cerana indica.* Hence, the present studies were conducted with 540 bee samples collected from 18 locations covering the whole length, breadth and altitudes of Kerala. Since the precision of the classification is highly influenced by the coverage of different components of morphometrics, 50 characters covering various aspects of bee morphology were chosen for the investigation. The results obtained from the studies are discussed below:

5.1 Ecotypes of *A. cerana indica* in Kerala

5.1.1 Univariate analysis

The results of univariate analysis of the data on morphometric characters of worker honeybees of *A. cerana indica* collected from different locations of Kerala are presented under 4.1.1 to 4.1.50. Data showed significant variations indicating the feasibility of identifying the ecotypes, if any, through a discriminant analysis.

Since samples were collected with reference to the three topographic divisions of the state viz., highrange, highland and midland, the honeybee populations of the three regions were also compared with reference to each morphometric character and the results have been summarised and presented in Table 59.

It was observed that the bees collected from these divisions did not show any significant variation with reference to six characters viz., breadth of forewing, wing vein angle 41, length of vein VL, number of hamuli, extent of hamuli and breadth of wax mirror.

Fernando (1979) did not observe any significant variation in the number of hamuli in *A. cerana* collected from various locations in lowland and mountainous districts of Sri Lanka. Narayanan *et al.* (1961 b) also reported that there was no difference in the number of hamuli between hill bees and plain bees.

Table 59. Ranking of the bee populations in highrange, highland and midland based on morphometric characters

Sl. No.	Characters	Rank I	Rank II	Rank III
Head				
1.	Tongue length	HR*	HL*	ML*
2.	Length of scape	HR*	HL	ML
3.	Length of pedicel	HR	ML	HL
4.	Length of flagellum	HR*	HL	ML
5.	Length of antenna	HR*	HL	ML
Forewing				
6.	Length of forewing	HR*	HL*	ML*
7.	Breadth of forewing	NS	NS	NS
8.	Length of radial cell	HR*	HL	ML
9.	Breadth of radial cell	ML*	HL*	HR*
10.	Length of basal portion of radial cell	HR*	ML	HL
11.	Length of apical portion of radial cell	HR*	HL*	ML*
12.	Length of Ist abscissa	HR*	HL	ML
13.	Length of IInd abscissa	ML*	HL*	HR*
14.	Wing vein angle no. 31	HR*	ML*	HL*
15.	Wing vein angle no. 32	HR	HL	ML*
16.	Wing vein angle no. 33	ML*	HL	HR
17.	Wing vein angle no. 34	HL	ML	HR*
18.	Wing vein angle no. 35	HL*	ML*	HR*
19.	Wing vein angle no. 36	HL*	ML*	HR*
20.	Wing vein angle no. 37	ML	HL	HR*
21.	Wing vein angle no. 38	HR*	ML*	HL*
22.	Wing vein angle no. 39	HL*	HR*	ML*
23.	Wing vein angle no. 40	HL	ML	HR*
24.	Wing vein angle no. 41	NS	NS	NS
Hindwing				
25.	Length of hindwing	HR*	HL*	ML*
26.	Breadth of hindwing	ML	HL	HR*
27.	Length of vein RL	HR*	ML	HL
28.	Length of vein ML	HL*	HR*	ML*
29.	Length of vein VL	NS	NS	NS
30.	Length of vein IL	HR	ML	HL*
31.	Number of hamuli	NS	NS	NS
32.	Extent of hamuli	NS	NS	NS
33.	Length of jugal lobe	HL*	ML	HR
34.	Length of vanal lobe	HR*	HL*	ML*

Contd...

Sl. No.	Characters	Rank I	Rank II	Rank III
Hind leg				
35.	Length of femur	HR*	HL*	ML*
36.	Length of tibia	HL	HR	ML*
37.	Length of metatarsus	HR*	HL*	ML*
38.	Breadth of metatarsus	ML	HL	HR*
Third tergite				
39.	Width of light band	HR*	HL*	ML*
40.	Width of dark band	HL	HR	ML*
41.	Total width of 3rd tergite	HR*	HL*	ML*
Fourth tergite				
42.	Width of light band	HR	HL	ML*
43.	Width of dark band	HR*	HL	ML
44.	Total width of 4th tergite	HR*	ML	HL
Third sternite				
45.	Length of wax mirror	HR*	HL*	ML*
46.	Breadth of wax mirror	NS	NS	NS
47.	Distance between wax mirrors	HR	ML	HL*
48.	Total width of 3rd sternite	HR	HL	ML*
Sixth sternite				
49.	Depth of sternite	HL*	ML	HR
50.	Breadth of sternite	HR	HL	ML*

NS - Not significant * Significantly distinct

Morphometric characters in which *Apis cerana indica* populations showed variations in three topographic divisions of Kerala

Topographic divisions	No. of characters in which the variations were					Total number of characters studied
	HR	on par HL	ML	Significantly different	Non significant	
HR	—	7	5	32	6	50
HL	7	—	14	23	6	50
ML	5	14	—	25	6	50

HR - Highrange HL - Highland ML - Midland

With references to 32 characters, highrange bees were significantly different from highland and midland bees. Of these, in 21 characters they showed highest values viz., tongue length, length of scape, length of flagellum, length of antenna, length of forewing, length of radial cell, length of basal portion of radial cell, length of apical portion of radial cell, length of first abscissa, wing vein angles 31 and 38, length of hindwing, length of vein RL, length of vanal lobe, length of femur, length of metatarsus, width of light band in third tergite, width of third tergite, width of dark band in fourth tergite, width of fourth tergite and length of wax mirror and in nine characters they had the lowest values viz., breadth of radial cell, length of second abscissa, wing vein angles 34, 35, 36, 37 and 40, breadth of hindwing and breadth of metatarsus. Wing vein angle 39 and length of vein ML showed middle values. Highrange bees were on par with highland bees in seven characters and with midland bees in five characters. Similarly bees collected from highland and midland were distinct in 23 and 25 characters respectively and they were on par in 14 characters.

An overview of the above results showed that the bee population of the three topographic divisions of Kerala are morphometrically distinct from one another. The trend in the evolutionary / adaptive changes were in the lengthening of proboscis, antennae, wings (consequent to which some of the wing veins and angles got reduced and showed a negative trend in data) and legs. The highrange bees ranked high in this evolutionary trends and highland bees came in between highrange and midland bees. Highland bees appeared to be nearer to midland bees (the two being on par in 14

characters) than with highrange bees (on par in five characters only). The results thus showed that highland and highrange bees might have evolved from the midland bee stock through a continuous process of natural selection from the variants showing greater morphological changes adapted to the changed ecological situations of the highland and highrange.

Altitude- and latitude-linked morphometric variations have been reported by earlier workers. Kapil (1956) found that hill varieties of *A. cerana* had a longer proboscis than those of plain varieties. Narayanan *et al.* (1961 a and b) and Fernando (1979) found longer tongues in *A. cerana indica* at higher elevations. Bouchner (1977) found a statistically significant difference in proboscis length among different races of *A. mellifera.* Tongue length was reported to influence nectar gathering (Rahman and Singh, 1948; Hassanein and El Banby, 1956; Morimoto, 1968). Shafikov (1976) also found a positive correlation between the length of proboscis and honey production. However, Mattu and Verma (1983) did not observe any positive association between proboscis length and altitude and suggested that the tongue length may be related more to flower morphology. Probably the morphological changes in the flora of different ecological situations of the topographic regions would have exerted a selection pressure on the bee population and resulted in the lengthening of the proboscis.

Kshirsagar (1976) reported greater antennal length at higher elevations. Mattu and Verma (1983) also found significant higher mean

value for length of pedicel, flagellum and antennae in the bees of the mountainous locality than that in submountainous bees of the Himalayan region.

Morphological characteristics of wings are important in classifying different races and strains of bees. Wing size influences the flight ability of bees and hence the observed variations might be an adaptive modification in evolution (Horowitz, 1953; Akahira and Sakagami, 1959b; Abdellatif *et al.*, 1977; Ruttner *et al.*, 1978). Several investigators have studied the relationship between the morphometric characters of the wings and geographical position such as altitude, latitude, etc., for the European honeybee, *A. mellifera* (Alpatov, 1929; Carlisle, 1955; Ruttner *et al.*, 1978; Dutton *et al.*, 1981). But comparatively little is known about Indian honeybee (Kapil, 1956; Kshirsagar, 1976, 1981; Kshirsagar and Ranade, 1981). In the present investigation, most of the parameters of the forewing and hindwing, which determine their size, were seen significantly higher in highrange bees than in midland and highland bees.

It was observed that lengths of femur and metatarsus were highest in highrange bees while breadth of metatarsus was lowest. Kapil (1956) also reported that hill variety of *A. cerana* had longer leg length than that of the plain variety. However Akahira and Sakagami (1959a) did not find any relationship between altitude and various characters of hind leg for *A. cerana* in Japan.

The width of third and fourth tergite were highest in highrange bees compared to those of bees from other regions. This indicated larger size of the abdomen and possibly the capacity to produce more honey in bees of the highrange region. The results are consistent with those of Kshirsagar (1976) and Kshirsagar and Ranadae (1981) for the Srinagar area of Kashmir and the Kangra area of Himachal.

The larger size of wax mirrors observed in highrange bees indicated their ability to produce more wax. However, Mattu and Verma (1984b) did not find any significant difference in the size of wax mirror between Kashmiri and Himachali bees.

5.2 Correlation of different morphometric characters of *Apis cerana indica* with varying altitudes of different locations

Results presented in para 4.2 showed that the tongue length of *A. cerana indica* had a highly positive correlation with altitude. Similar results were reported by Narayanan *et al.* (1961a and b) and Fernando (1979). But Kshirsagar (1976) and Mattu and Verma (1983) found that the character was not related to the altitude and suggested that tongue length might be related more to floral morphology than to the altitude. Carlisle (1955) did not find relationship between tongue length and altitudes of the habitats of *A. mellifera.*

A significant positive correlation was established between altitude and length of flagellum as well as total length of antennae. No such

significant correlation was reported for the Japanese bee *A. cerana cerana.* But Mattu and Verma (1983) found significant positive correlation between altitude and all antennal measurements ie., length of scape, pedicel, flagellum and overall length for bees of the Himachal region, while in Kashmiri bees only total length was positively correlated.

Among the characters of forewing, length of radial cell, length of basal portion of radial cell and length of first abscissa showed a highly significant positive correlation with altitude at 1% level. Length of forewing also showed a positive correlation with altitude at 5% level. Mattu and Verma (1984a) also found significant positive correlations between altitude, length of forewing, length of radial cell, apical portion of radial cell, length of first abscissa and breadth of forewing of *A. cerana indica* collected from Himachal region. They also found significant positive correlation between altitude and angle No. 31, 37 and 38 though the present results did not agree with that.

It was observed in the present investigations that among the forewing characters, wing vein angle 35, 36 and 40 showed a highly significant (at 1% level) negative correlation with altitude while breadth of radial cell, length of second abscissa and wing vein angle 37 also showed a negative correlation at 5% level. In contradiction to this Mattu and Verma (1984a) found significant positive correlations between altitude and length of radial cell, length of first and second abscissa, breadth of radial cells, size of wing vein angles 34, 35, 39 and 40, length of vein VL, number of hamuli and breadth of forewing in the case of bees of Kashmir region.

At the same time bees of Himachal region showed negative correlation between altitude and wing vein angle 40.

In the hindwing characters, only length of hindwing was positively correlated at 1% level while the breadth was negatively correlated. Mattu and Verma (1984a) found a positive correlation for altitude with length of hindwings, length of veins RL, ML, IL, length of jugal and vanal lobe and number and extent of hamuli for Himachali bees. It was observed in the present investigation that the number of wing hooks had no correlation with altitude. Fernando (1979), in his studies on *A. cerana* in different localities of Sri Lanka, also reported lack of significant differences in the number of wing hooks among bees collected from these localities.

The characters of hind leg, length of femur and length of metatarsus showed a positive correlation with altitude at 5% level. Among the characters of third sternite, length of wax mirror was positively correlated with altitude at 5% level. These correlations of the different morphometric characters and altitudes broadly agree with the characters differentiating the bees of highrange, highland and midland in Kerala.

Among the 50 characters subjected to univariate analysis 18 characters showed positive or negative association with varying altitudes. These were found as important ones among the characters which led to the identification of three distinct sub groups of honeybee population in the different regions also. It is hence indicated that the evolutionary changes in the morphometric characters of honeybee is an altitude-linked phenomenon.

5.3 Multivariate analysis

5.3.1 Clustering of biometric data

Multivariate analysis of the data on the morphometric characters of bees collected from different locations presented in para 4.3.1 showed the existence of four different clusters (ecotypes) in the Indian bee population of Kerala.

Cluster I contained bees from all the six locations of midland and four locations in highland of comparatively lower elevations. Since midland and highland are regions where beekeeping is being widely practised from very early times and as all the locations in midland fall under cluster I, it may be considered as the basic ecotype from which other clusters would have evolved. Cluster IV showed highest divergence from cluster I in morphometric data. It could be further seen that cluster III underwent higher morphometric changes compared to cluster II. Cluster III contained locations in highrange with altitudes varying from 761 m to 905 m above MSL, while in cluster II, the altitude of the locations varied from 105 to 125 m MSL only. Above findings in multivariate analysis broadly agree with the findings from the univariate analysis of the data in which highrange, highland and midland bee population were identified as distinct groups significantly different from one another in morphometric characters. Highrange includes cluster IV and III and two locations of highland fall in cluster II and rest of highland and entire midland fall in cluster I. Since two locations of cluster II and four locations of cluster I came in highland,

that region stood significantly different from the midland region, which came fully in cluster I. The results further showed the necessity for detailed analysis of the data of different topographic regions for identifying all the ecotypes present in each region. The identification of four clusters / ecotypes in the population of *A. cerana indica* in the state is seen linked with the topographic divisions of Kerala based on varying agroecological conditions and altitudes.

This is the first report of the existence of different ecotypes of *A. cerana indica* in Kerala. Verma (1992) reported the existence of only one ecotype of *A. cerana indica* in the state. This might be due to the very limited number of samples and locations included in the survey. Studies by Mattu and Verma (1983, 1984 a and b) showed that *A. cerana cerana* in north west India contained two separate ecotypes / geographical populations. Verma (1992) also reported that *Apis cerana himalaya* in north east India could be grouped under three ecotypes viz., Naga and Mizoram bees, Brahmaputra valley bees and bees of the main axis of Himalaya.

5.3.2 Means of morphometric characters of different clusters / ecotypes of *A. cerana indica*

Results presented in para 4.3.2 revealed high variability of the morphometric characters contributing the divergence of clusters. Most of the characters showed a positive or negative trend among the clusters I, II, III and IV. However, the ranges in the measurements in different clusters were generally seen overlapping. Thus the relative importance of the characters in recognizing subgroups of *A. cerana indica* could not be

decided from the data. Hence the data were statistically analysed for estimating the percentage contribution of different characters to the divergence, following the methods of Singh and Chaudhary (1985).

5.4 Contribution of morphometric characters towards divergence

Results presented in para 4.4 showed that wing vein angle 32, length of vein VL, length of antenna, length of basal portion of radial cell, number of hamuli, wing vein angle 33, 39 and length of vein RL which contributed 3.8 to 6.4 per cent for the separation of clusters may be treated as more important characters for the identification of the ecotypes. Length of pedicel, length of apical portion of radial cell, length of first abscissa, wing vein angles 35, 37, 41, extent of hamuli, length of femur and width of dark band of fourth tergite which contributed up to 2.5 per cent of the divergence also may be included for the identification of ecotypes probably from within a single topographic region where greater precision is needed.

As mentioned earlier information available on the taxonomy of *A. cerana indica* below the rank of subspecies is very meagre. From India seven ecotypes have been reported so far (Verma *et al.*, 1984; Verma, 1987, 1992).

This is the first study in India for a biometric classification of *A. cerana indica* with representative sampling of the population covering the entire beekeeping area of any State. The identification of more ecotypes, when followed up with biological, behavioural and economical

components, will open up high possibilities for breeding bee stocks with desirable traits in yield, behaviour and pest / disease resistance. It will be particularly significant and relevant in the context of TSBV incidence and consequent destruction of bee colonies in the State.

5.5 Relative susceptibility of different ecotypes of *A. cerana indica* in Kerala to TSBV infection

Results presented in para 4.5 showed that all the four ecotypes of Indian bee identified in Kerala contracted TSBV disease under field situations and the intensity reached the peak in 2 to 12 months time resulting in the absconding of bees from the affected hives. Even the varying rate of development of disease symptoms in the colony was not seen associated with the different ecotypes of the bee race. It was concluded that none of the four ecotypes had any tolerance / resistance to TSBV infection. Hence further studies in disease resistance of the ecotypes envisaged, as part of the objectives of this study, could not be pursued.

5.6 Pathogenicity of TSBV on *A. cerana indica*

5.6.1 Larval susceptibility to TSBV and the symptoms caused

Thai Sacbrood Virus disease was first observed in India on *A. cerana indica* in Meghalaya in 1978 which soon spread to other parts of North India (Kshirsagar *et al.*, 1981, 1982; Kshirsagar and Phadke, 1984; Joshi and Verma, 1985; Singh, 1985; Verma and Joshi,

1985; Abrol and Bhat, 1990). The disease made its first appearance in Kerala in 1991 and subsequently spread to Tamil Nadu and Karnataka also (Jacob *et al.*, 1992).

Earlier studies on TSBV in India relate mainly to its occurrence and spread, electron microscopy of the causative agent and observations on symptomatology. No attempt has been made so far to study the stage susceptibility of the insect to the virus, detailed symptomatology and detection of infection in the colony. Emphasis was hence given to the above aspects in the present investigations.

Lack of detailed pathogenicity studies on brood diseases might be due to the special nature of bee development. From egg to adult stage, the development takes place inside the comb cells attended regularly by the nurse bees. Food is provided by the nurse bees at different stages of development as royal jelly and bee bread and brood development require a homeostatic condition manipulated by the bee population inside the hive. Hence the development cannot be observed outside the hive by maintaining the larvae in open rearing devices. Under these conditions inoculation of the pathogen, continued observation of individual larva at intervals to record the signs of infection and symptoms is difficult. Most of these difficulties were overcome by suitable rearing methods evolved and use of cell locating frame, devised by Woodrow (1941), with modification to suit the requirements of *A. cerana indica* colonies.

Observations recorded in para 4.6.1 showed that all the four larval instars of *A. cerana indica* were susceptible to TSBV. One day old larvae were highly susceptible, recording 100 per cent mortality in two days and had the shortest incubation period of 2 to 3 days. The 2 and 3 day old larvae were also susceptible resulting in 84 to 92 per cent mortality with an incubation period of 3 to 4 days. The 4 day old larvae were comparatively less susceptible since 26 to 28 per cent of inoculated larvae escaped the infection and developed into healthy adult bees. The results indicated an inverse relationship between mortality and larval age and a positive relationship between larval age and incubation period. Pathogenicity of the TSBV on different larval stages of *A. cerana indica* was being studied for the first time. Further the susceptibility of first and second instars to TSBV has not been elucidated in any of the previous studies. Hitchcock (1966) in his studies on Sacbrood Virus of *A. mellifera* found that larvae inoculated at 1 to 2 or 2 to 3 days of age appeared more susceptible. Bailey and Ball (1991) reported that 2 day old larvae of *A. mellifera* were the most susceptible to SBV. Differential susceptibility to virus infection of Lepidopteran larvae of different ages had been observed by Bergold (1943), Steinhaus (1949), Smith *et al.* (1953), Tanada (1953), Morris (1962) and Jacob and Subramanian (1972). This increase in resistance associated with the growth of larvae was regarded by some authors as a 'maturation immunity'. Ignoffo (1966) attributed this partly to the normal increase in body weight which might serve to 'dilute' a constant virus dose.

The present observation on sac formation of infected larvae did not agree with the earlier reports. Abrol and Bhat (1990) reported that the sac-like appearance of dead larvae was due to the filling up of greenish yellow ecdysial fluid in between the hard unshed last larval skin and newly formed pupal skin. It was observed in the present studies that the internal body tissues of the infected larvae got liquefied as a milky white fluid and collected within the cuticle which gave the characteristic sac like appearance of the larva. Further, the cuticle was thin and fragile and it broke open even with mild pressure releasing the liquefied body contents.

Viruses generally require a minimum incubation period for their development and multiplication within the hosts and for the resultant exhibition of symptoms (Steinhaus, 1949). The finding that the minimum period required to initiate mortality in *A. cerana indica* was 2 to 3 days explains the earlier observations that late larval stages of the bee alone get affected by TSBV. Even if the larvae were infected early in development, mortality could occur only in advanced larval phase due to the required incubation period.

The infected larvae failed to grow and pupate. Some of the four day old larvae remained live in sealed cells upto 8 days when inoculated with TSBV and they continued in larval stage failing to enter the prepupal and pupal phases. Moulting and metamorphosis in insects are under hormonal control. It is possible that the infection of various tissues affected the hormonal balance. Morris (1963) had observed that virus infections affected the hormonal balance and time of hormone activity in the infected Lepidopteran larvae.

Adult bees in an infected colony normally detected and removed dead larvae promptly (Bailey *et al.*, 1964). Thus in the initial stages, the dead larvae which were few in number, would have been removed and thrown out by the worker bees and hence the disease incidence went unnoticed. However, as the disease became severe, it will be difficult to the worker bees to clean up the diseased and dead brood and they will be left in the brood cells making the disease condition more evident.

In the case of TSBV, the infection progressed rapidly causing 95 to 100 per cent mortality in 8 to 10 days. The high susceptibility of all the four larval instars and the short incubation period as observed in the present studies, would clearly explain why in *A. cerana indica* TSBV incidence caused total loss while in *A. mellifera* the incidence of Sacbrood disease never became severe and often went unnoticed by beekeepers (Gochnauer *et al.*, 1975).

Infection of the brood affected the behaviour of the workers and queen in the colony. There is a normal rhythm in bee activities inside a hive. Anything which upsets the normal routine will make the bees nervous and irritable. Infection of TSBV was found to cause disturbance in the normal activities like keeping cleanliness in the hives by the worker bees, egg laying by the queen, development of brood and population build-up and foraging activity.

It is the usual habit of honey bees to prefer a congenial abode which is neat and tidy and free from natural enemies. Besides, the behavioural changes in the worker bees due to TSBV infection, emergence of new brood gets inhibited resulting in a decrease of worker bee population, especially the house bees and this also affects the timely cleaning and other routine activities within the hives. The accumulation of dead and decaying brood within the cells further aggravate the condition. Finally, finding the hive quite unsuitable, the remaining bees fly off. Mass absconding of *A. cerana* colonies infected with TSBV has been reported earlier also. Such desertion seldom occurs in SBV infected colonies of *A. mellifera* (Suchwantsingh and Koul, 1985).

The occurrence of intermittent infection and recovery of bee colonies was observed for the first time in the case of TSBV infection. Similar observations had been reported by Woodrow (1941) on *A. mellifera* infected with American foul brood (AFB). This indicates the possibility of behavioural immunity possibly related to the efficiency in cleaning the diseased larvae from the combs by the worker bees. This also indicates the necessity for detailed information on the cleaning behaviour of bees in diseased colonies and its effect in reducing infection. That may help in evolving suitable management practices to reduce the rate of spread of infection in bee colonies.

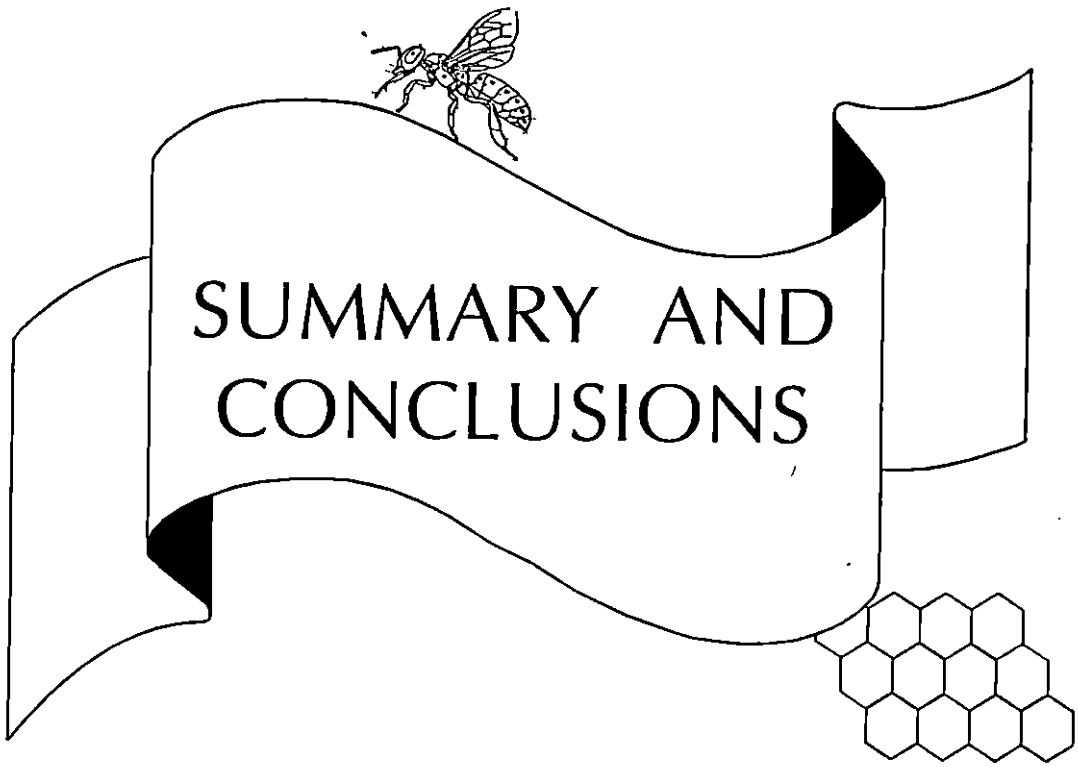
Symptomatology of TSBV infection in *A. cerana indica* as observed in the present studies, can be summarised as follows :

- ❑ All the larval instars of *A. cerana indica* are susceptible to the disease, earlier instars being affected faster and more severely.
- ❑ Affected larvae appear slightly plumpy compared to healthy ones when examined after taking out of the comb cells.
- ❑ The infected larvae lie stretched out on their back in the cells with the head of larvae directed outwards and turned upwards like the prow of a boat.
- ❑ The dead larvae look like a sac when lifted and it ruptures easily releasing a milky white fluid probably formed by the hystolysis of the larval tissues.
- ❑ The cadavers change their colour from white to pale yellow and sink down on the floor of the cells and gradually dry up in 10 to 15 days as brownish black boat like scales which are easily removable from the cell.

Following sequence in the visible symptoms of infected colonies also could be deduced from the present investigations:

- Presence of unsealed cells in brood area containing infected larvae with the head directed outwards like a prow of a boat.
- Dead larvae seen lying stretched out on their back on the floor of brood cells.
- Appearance of dead larvae strewn on the floor board, hive entrance or on the floor near the hive.

- Mottled appearance of brood combs with uncapped cells interspersed with capped cells or cells with perforated cappings.
- Appearance of more and more dead larvae within the cells without being ejected by the worker bees.
- Appearance of scale-like remnants of dead larvae within the cells.
- Lack of cleaning activity within the hive.
- Decrease in egg laying rate and irregular placement of eggs.
- Decrease in foraging activity and presence of idling workers inside the hive.
- Dwindling of bee population of the colony.
- Desertion of the infected hive by the bees.



SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

This investigation entitled "pathogenicity of Thai Sacbrood virus to the ecotypes of *Apis cerana indica* Fab. in Kerala" was carried out in the Department of Entomology, College of Agriculture, Vellayani and in the apiaries spread over the different parts of Kerala.

The main objectives of the investigations were identifying ecotypes of *A. cerana indica* in Kerala and to assess their susceptibility / resistance to Thai Sacbrood Virus (TSBV) and to gather detailed information on the pathogenicity of TSBV to *A. cerana indica.*

1. Identification of ecotypes of *A. cerana indica*

Statistical analysis of morphometric data was adopted to identify the ecotypes. Samples of worker honeybees were collected from 18 different locations covering the whole length, breadth as well as different altitudes of Kerala. In highrange collections were made at Attappady (761 m above MSL), Wayanadu (764 m), Mananthavadi (780 m), Ponmudi (850 m), Kumbalacode (905 m) and Pampadumpara (1050 m). In highland, collections were made at Malappuram (80 m), Nilampur (80 m), Palakkad (95 m), Malampuzha (100 m), Kanjirappally (105 m) and Kulathupuzha

(125 m) while in midland region Neyyattinkara (27 m), Pathanamthitta (30 m), Pilicode (30 m), Thrissur (33 m), Vadakkancherry (33 m) and Aralam Farm (45 m) were included. In each location 60 worker bees were collected from five different colonies.

Fifty morphometric characters in 540 worker bees (30 each from 18 locations) viz., tongue length, length of scape, length of pedicel, length of flagellum, length of antenna, length of forewing, breadth of forewing, length of radial cell, breadth of radial cell, length of basal portion of radial cell, length of first abscissa, length of second abscissa, wing vein angle 31 to 41, length of hindwing, breadth of hindwing, length of vein VL, length of vein IL, number of hamuli, extent of hamuli, length of jugal lobe, length of vanal lobe, length of femur, length of tibia, length of metatarsus, breadth of metatarsus, width of light band in third tergite, width of dark band, width of third tergite, width of light band in fourth tergite, width of dark band, width of fourth tergite, length of wax mirrors, breadth of wax mirrors, distance between wax mirrors, total width of third sternite, depth of sixth sternite and breadth of sixth sternite were measured.

Univariate analysis of the data on morphometric characters of worker honeybees showed significant variations indicating the feasibility of identifying the ecotypes through discriminant analysis.

Since samples were collected with reference to the three topographic divisions of the State viz., highrange, highland and midland

the honeybee populations of the three regions were also compared with reference to each morphometric character. There was no significant variation with reference to six characters viz., breadth of forewing, wing vein angle 41, length of vein VL, number of humuli, extent of hamuli and breadth of wax mirror in bees collected from the three division. With reference to 32 characters highrange bees were significantly different from highland and midland bees. Of these 21 characters showed highest values in highrange bees. These characters were tongue length, length of scape, length of flagellum, length of antenna, length of forewing, length of radial cell, length of basal portion of radial cell, length of apical portion of radial cell, length of first abscissa, wing vein angle 31 and 38, length of hindwing, length of vein RL, length of vanal lobe, length of femur, length of metatarsus, width of light band in third tergite, total width of third tergite, width of dark band in fourth tergite, width of fourth tergite and length of wax mirrors. Nine characters had lowest values. These characters were breadth of radial cell, length of second abscissa wing vein angles 34, 35, 36, 37 and 40, breadth of hindwing and breadth of metatarsus. Wing vein angle 39 and length of vein ML showed middle values.

Bees collected from highland and midland were distinct from highrange bees in 23 and 25 characters respectively and they were on par in 14 characters. Highland bees were on par with highrange bees in seven characters while midland bees were on par with highrange bees in five characters.

Comparison of the morphometry of bees from the three divisions would suggest that bees from highrange, highland and midland were

distinct, there being higher parity between the bees of the latter two topographical divisions. It was also indicated that the trend in the evolutionary changes in the morpometrics of the subgroups was in having longer proboscis, antennae, wings, legs and larger abdominal size.

Correlation of different morphometric characters of *A. cerana indica* with various altitudes of the locations was worked out. Tongue length, length of flagellum, length of antenna, length of radial cell, length of basal portion of radial cell, length of first abscissa, length of hindwing, length of forewing, length of femur, length of metatarsus and length of wax mirror showed significant positive correlations. Wing vein angles 35, 36, 40, breadth of hindwing, breadth of radial cell, length of second abscissa and wing vein angle 37 showed significant negative correlation.

Multivariate (discriminant) analysis of the morphometric data showed the existence of the following four different clusters (ecotypes) in the Indian honeybee *A. cerana indica* population of Kerala:

Cluster I included bees from ten locations, six from midland (altitude 27-45 m MSL) and four from highland (80-95 m MSL) viz., Neyyattinkara, Pathanamthitta, Thrissur, Vadakkancherry, Pilicode, Aralam Farm, Palakkad, Malampuzha, Nilampur and Malappuram.

Cluster II included two locations of highland only (105-125 m MSL) viz., Kulathupuzha and Kanjirappally.

Cluster III included bees from five locations of highrange (altitude 761 to 905m above MSL) viz., Ponmudi, Attappady, Kumbalacode, Wayanadu and Mananthavadi and cluster IV with a single location of highrange viz., Pampadumpara (1050 m above MSL). Clustering also was found related to the altitudes of different locations and the results were in over all agreement with the findings of univariate analysis.

Contribution of different morphometric characters towards divergence was also worked out. It was found that wing vein angle 32, length of vein VL, length of antenna, length of basal portion of radial cell, number of hamuli, wing vein angle 33, 39 and length of vein RL made higher contribution towards the divergence of clusters. These characters may be used for identifying the ecotypes of unclassified specimens.

2. Relative susceptibility of different ecotypes of Kerala to Thai Sacbrood Virus

The susceptibility or resistance of the bee colonies selected for sampling was assessed by monitoring the incidence and intensity of TSBV, if any, at bimonthly intervals. Results showed that all the bee colonies got infected with TSBV and got lost in the course of two to twelve months indicating the lack of resistance of the different ecotypes to TSBV.

Existence of four different clusters based on morphometric characters may be confirmed with detailed studies on their biological and

economic characters also. This will facilitate the breeding of improved stocks of *A. cerana indica* having desirable characters as is now being done extensively in the case *A. mellifera* all over the world.

3. Pathogenicity studies on *Apis cerana indica*

Pathogenicity studies were made in the apiary attached to the College of Agriculture, Vellayani. Inoculations were done on 1, 2, 3 and 4 day old healthy larvae using virus suspension extracted from freshly collected diseased larvae. The development of the disease symptoms on individual larvae inoculated with TSBV was followed up using a 'cell locating device' designed in the laboratory. Experiment was done three times during 1994-95, 1995-96 and 1996-97.

Results revealed that all the four instars of *A. cerana indica* were susceptible to TSBV. One day old larvae were highly susceptible recording 100 per cent mortality and had the shortest incubation period of 2-3 days. Two and three day old larvae were also susceptible recording 84 to 92 and 82 to 96 per cent mortality respectively with an incubation period of 3 to 4 days. Four day old larvae were less susceptible showing 72 to 74 per cent mortality with an incubation period of 3-5 days and more than 25 per cent of the inoculated larvae escaped infection and developed as unaffected adult bees.

The symptomatology can be summarised as follows. All the larval instars were susceptible to the disease, earlier instars showing faster and

severe infection. Affected larvae appeared slightly plumby compared to healthy ones when examined by taking out of the comb cells. The infected larvae were seen stretched on their back in the cells with the head directed outwards and turned upwards like the prow of a boat. The dead larvae looked like a sac filled with milky white fluid when lifted up and it ruptured even with the slight pressure releasing the milky fluid. The cadavers changed their colour from white to pale yellow and sank down to the floor of the cell and dried up in 10-15 days as brownish black boat like scales which were easily removable from the cell.

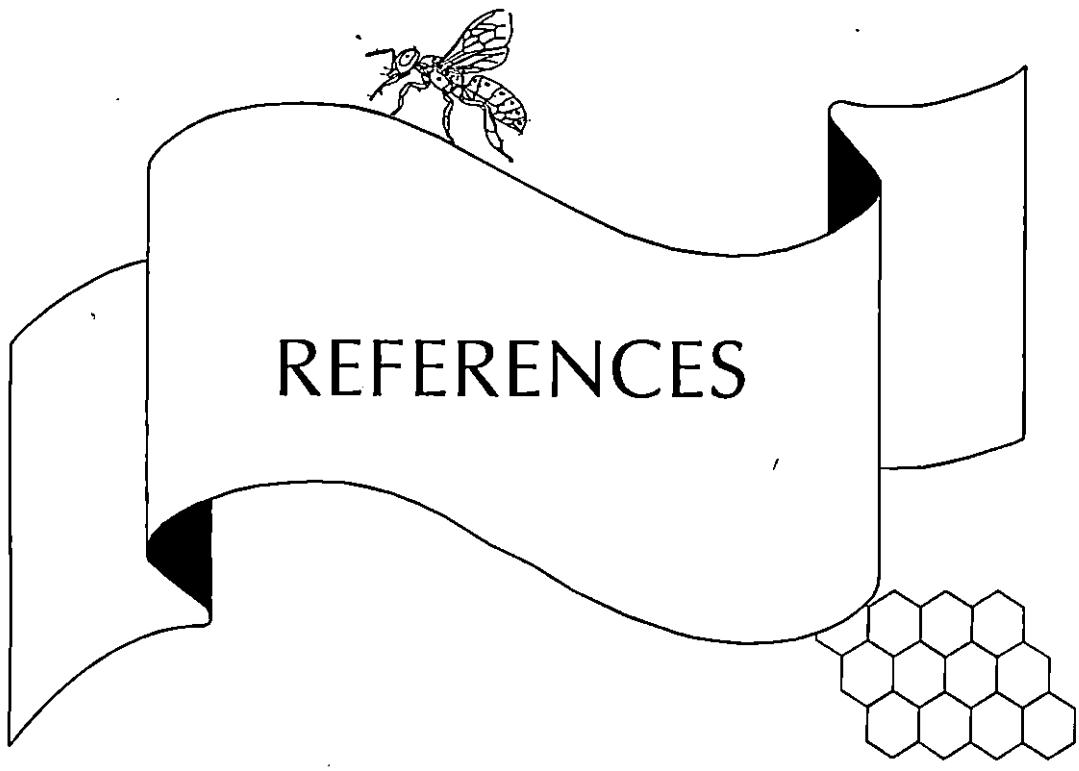
Following sequence was observed as the visible symptoms in infected colonies of *A. cerana indica* :

- Presence of unsealed cells in brood area containing diseased larvae with their head directed outwards like the prow of a boat.
- Dead larvae seen lying stretched out on their back on the floor of brood cells and looked like a sac filled with milky white fluid when lifted up.
- Appearance of dead larvae strewn on the floor board, hive entrance or on the floor near the hive.
- Mottled appearance of brood combs with uncapped cells interspersed with capped cells or cells with perforated cappings.

- Appearance of more and more dead larvae left within the cells without being ejected by the worker bees.
- Appearance of scale like remnants of dead larvae within the cells.
- Lack of cleaning activity within the hive
- Decrease in egg laying rate and irregular placement of eggs.
- Decrease in foraging activity and presence of idling workers inside the hive.
- Dwindlig of bee population of the colony
- Desertion of the infected hive by the bees.

Future line of work

The results of the present investigations have shown the existence of four clusters / ecotypes of *Apis cerana indica* Fab. in Kerala. Biological and economical traits of these ecotypes should be worked out which will help in breeding bee stocks with desirable attributes.



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* Original not seen

PATHOGENICITY OF THAI SACBROOD VIRUS TO THE ECOTYPES OF *Apis cerana indica* Fab. IN KERALA

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ABSTRACT OF THE THESIS
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ABSTRACT

Identification of ecotypes of the Indian bee *A. cerana indica* Fab. in different ecological niche in Kerala adopting statistical analysis of the morphometric data was attempted in the investigation. Sixty worker bees each were collected from 18 locations distributed throughout Kerala and data on 50 selected characters were collected. ***Univariate analysis*** showed significant variations in the data with reference to all the fifty characters indicating the desirability of a multivariate analysis for identifying sub groups of *A. cerana indica* available in the state.

A comparison of the honeybee population of the three topographic divisions of the state viz., highrange, highland and midland, with reference to each morphometric character revealed that the bees from highrange were distinct from those of highland and midland. There was less distinction between the bees of the latter two divisions. It also indicated that the highrange bees possessed longer proboscis, antennae, wings and legs. The abdominal size also showed an increasing trend in highland and highrange bees.

Eleven morphometric characters were positively correlated with altitude while seven characters showed negative correlation.

Multivariate (discriminant) analysis of the morphometric data revealed the existence of four different clusters / ecotypes in *A. cerana indica* populations of Kerala. Cluster I included all the six locations of midland and four locations of highland. Two locations of highland at higher altitude formed cluster II. Pampadumpara of the highest altitude in highrange came in cluster IV and remaining locations of the highrange constituted cluster III.

Contribution of each morphometric character towards divergence of the clusters was also assessed. Seventeen characters contributing 2.5 to 6.4 per cent of divergence were thus identified.

Bees from all the four clusters / ecotypes showed susceptibility to Thai Sacbrood Virus (TSBV) infection.

Studies on the pathogenicity of TSBV showed that all four larval instars of *A. cerana indica* were susceptible to TSBV. One day old larvae were highly susceptible recording 100 per cent mortality closely followed by 2 and 3 day old larvae showing 84 to 92 and 82 to 96 per cent mortality respectively, with an incubation period of 3-4 days. Four day old larvae were comparatively less susceptible recording 72 to 74 per cent mortality with an incubation period of 3 to 5 days.

The infected larvae were seen lying on the floor of the brood cells on their back with the head directed outwards and turned upwards like the

prow of a boat. In later stages they became plumbier than healthy larvae. After death each larva showed a sac like appearance when lifted up and it was filled with a milky fluid formed probably by the histolysis of the tissues. In 10 to 15 days the sac got shrunk into a small browinish black scale loosely lying at the floor of the cell. The presence of diseased larvae was found to upset the behaviour of workers and queen. These resulted in the fast dwindling of the population and cessation of cleaning activities in the hive. The hive lost the desired qualities of a bee abode and hence the surviving bees deserted the same causing total loss to apiary.