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Biodiversity in honey bees: Scanning electron microscopic analysis of antenna of plain and hill populations of *Apis cerana* F. (Hymenoptera: Apidae)

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Abstract
Antenna of honey bees is geniculate and has been known to possess a wide variety of sensilla. The sensilla are sensitive to odor, temperature, humidity, air pressure and gustatory stimuli. The antennal form as well as the sensilla arrangement has been suggested to be adapted to the pheromone perception need of a particular species. Electron microscopy is today considered an important tool in elucidating the external morphological details of diverse biological material. In the present investigations, ultrastructural study on the antenna of plains and hill populations of *Apis cerana* F. was taken into account. Studies revealed differences at electron microscopic level in the antennal morphology and types and distribution of sensilla on the antenna in different populations of honey bees.

Keywords: *Apis cerana*, antenna, plains, hills, sensilla, SEM

Introduction
South-east Asia is the centre of honey bee diversity and the evolutionary homeland of honey bees. *Apis cerana* are cavity-nesting bees which build several parallel combs. The head appendages constitute an important morphological feature to study honey bee systematics. The antenna of honey bees is important for understanding behavior of honey bees. It consists of a basal scape, pivoted pedicel and a ten-segmented flagellum. Antennae are important for sensory perception. Various types of sensilla are observed on the segments of flagellum. According to [1], the antennae are the main sites of olfactory reception in most insects. Six types of antennal sensilla have been observed on the flagellum of the European honey bee *A. mellifera* drone and worker. They have been referred to as a thick basiconic sensillum, a tapered basiconic sensillum, a trichoid sensillum, a placoid sensillum, a coeloconic or ampullaceal sensillum and a coelocapitular sensillum, formerly known as campaniform sensillum [2, 3]. SEM studies on antennal sensilla of honey bee workers have been performed by [4] from Saudi Arabia. [5] described occurrence of sensilla trichodea types A, B, C and D on different segments of flagellum of honey bees from Thailand. The aim of the present study was to document the diversity in *Apis cerana* plains and hill populations on the basis of scanning electron microscopic investigations of an important morphological character *i.e.* the antenna.

Material and Methods
Study Material
Worker bees of *A. cerana* from plains (apiaries in Chandigarh) and hills (high hills of Distt. Kinnaur) were collected from the hive entrance.

Preservation
The collected material of *A. cerana* was preserved in 70% alcohol and the protocol of [6] was followed.

Preparation of material for scanning electron microscopy
The antennae were carefully excised from the freshly collected worker bees of *A. cerana*. These were then washed with phosphate buffer. The samples were fixed in 5% glutaraldehyde for 2 hrs. Subsequently these were washed with phosphate buffer 2 to 3 times and then

dehydrated through graded series of acetone and dried in a critical point drier. Dehydrated samples were mounted on slides in the desired orientation with the help of double side adhesive tape under binocular microscope. The samples were attached in such a way that they became visible from all sides. The stubs were placed inside the sputter for gold coating to overcome the problem of "charging" and "beam damage". The sputtered specimens were examined in Jeol JS-6100 scanning electron microscope operated at an acceleration voltage of 10KV at Regional sophisticated instrumentation centre, Panjab University, Chandigarh.
The results of scanning were preserved as photographs used in this presentation.

Results

Apis cerana (plains population)

The antenna of *A. cerana* showed 3 distinct regions under the scanning electron microscope, the scape attached to pedicel which bears the flagellum.

Scape: Two types of hair like sensilla, one longer and another smaller could be identified. The longer sensilla were scattered among the smaller one and did not possess any serration (Fig. 1).

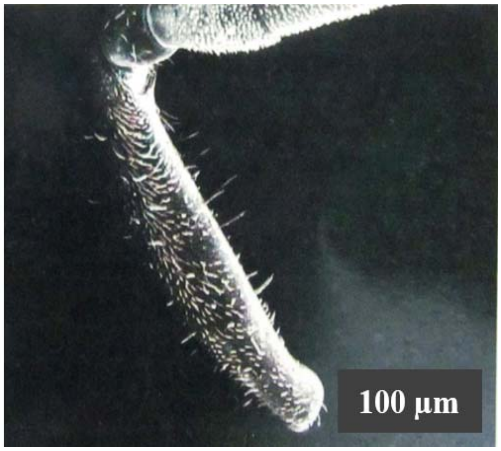

Fig 1: SEM of scape of antenna of *A. cerana* (plains population) (Bar= 100 µm)

Pedicel: It was short and distinctly cup-shaped. The distal end of pedicel showed distinct fimbriated margin. Only one type of sensilla were sparsely scattered over the pedicel (Fig. 2).

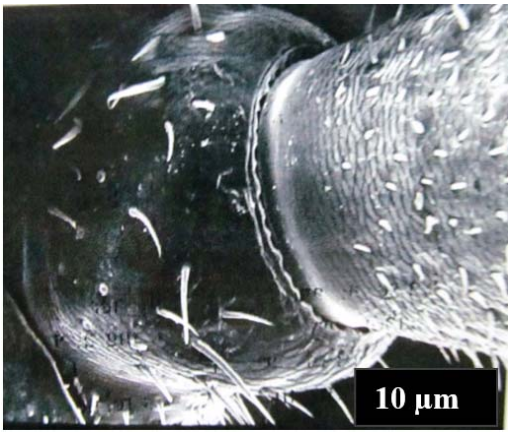

Fig 2: SEM of pedicel showing fringed margin and one type of sensilla (Bar= 10 µm)

Segment 1st and 2nd: These segments showed the presence of sensilla trichodea of type I in abundance and few of type IV (Fig. 3 and 4).

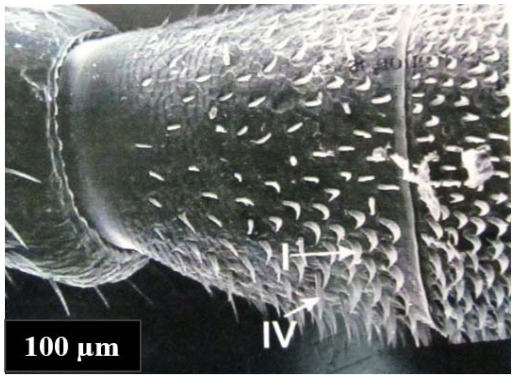

Fig 3: SEM of first segment showing sensilla trichodea of type I and few of type IV (Bar= 100 µm)

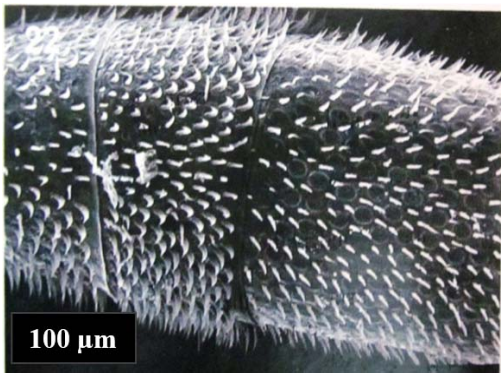

Fig 4: SEM of second segment (Bar= 100 µm)

Segments 3rd, 4th and 5th: These segments showed the presence of sensilla trichodea of type I, type III, IV and few sensilla placodea and sensilla ampullacea (Fig.5).

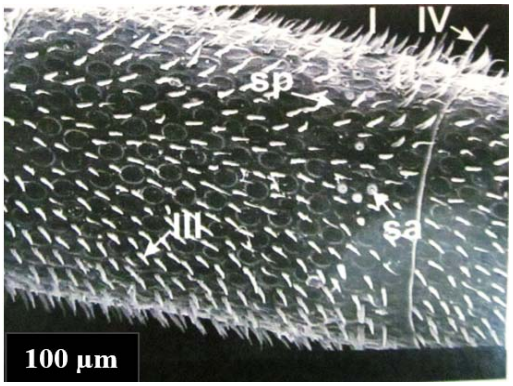

Fig 5: SEM of third segment showing sensilla placodea, ampullacea and trichodea of type I, III and IV (Bar= 100 µm)

Segments 6th and 7th: These showed the sensilla trichodea of type III and IV along with sensilla placodea and sensilla ampullacea.

Segment 8th, 9th and 10th: These showed the sensilla trichodea of type II and sensilla basiconica in addition to type III, IV and sensilla placodea and sensilla ampullacea. 8th and 9th segments also showed the presence of sensilla campaniformia which was made up of depressed structure bearing mushroom-like body in the centre (Fig. 6, 7, 8).

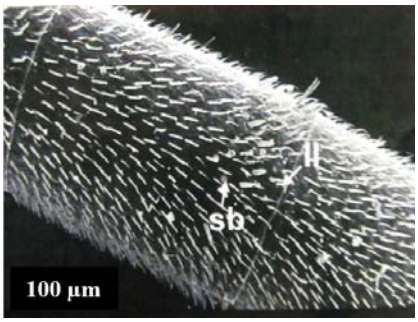

Fig 6: SEM of eighth segment showing sensilla trichodea of type II, III and IV, sensilla campaniformia, placodea, ampullacea and basiconica (Bar= 100 µm).

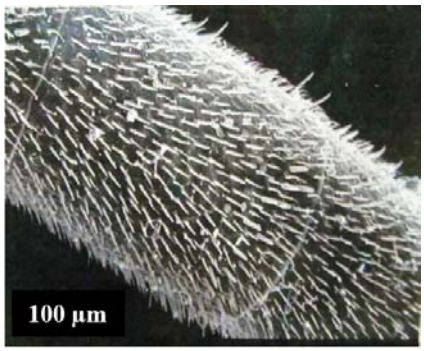

Fig 7: SEM of ninth segment showing sensilla trichodea of type II, III and IV, sensilla campaniformia, placodea, ampullacea and basiconica (Bar=100 µm).

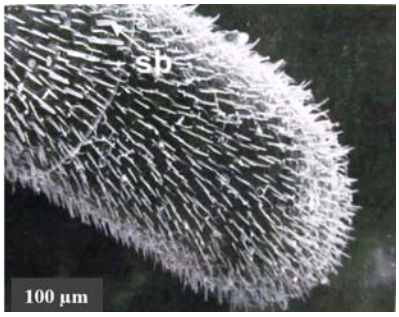

Fig 8: SEM of tenth segment (Bar= 100 µm)

***Apis cerana* (hills population)**

Scape: Scape possessed two types of hair-like sensilla. The longer ones were without serration and were more numerous on one side of scape.

Pedicel: It was cup-shaped, bearing only one type of sensilla and having fimbriated margins. (Fig. 9).

Fig 9: SEM of antenna of *A. cerana* (hills population) showing two types of sensilla and pedicel with one type of sensilla (Bar= 100 µm).

Segment 1st and 2nd: Sensilla of type I could be identified in these segments and very few of type IV (Fig. 10).

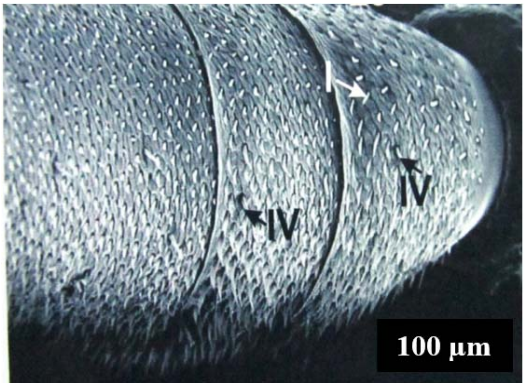

Fig 10: SEM of first and second segment showing trichodea of type I and few of type IV (Bar= 100 µm).

Segment 3rd and 4th and 5th: Sensilla placodea were present in addition to sensilla trichodea type I and type IV. The sensilla placodea were restricted to sides only (Fig. 11).

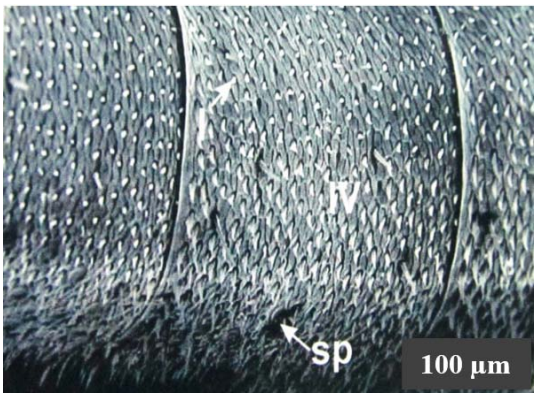

Fig 11: SEM of third segment showing sensilla trichodea of type I and IV along with sensilla placodea (Bar= 100 µm).

Segment 6th and 7th: These segments showed the presence of 4 types of sensilla, sensilla trichodea of type III, IV, sensilla placodea and sensilla ampullacea.

Segments 8th, 9th and 10th: Sensilla of type II, were found in addition to others. Sensilla basiconica were present near the margins of the segments. The 8th and 9th segment showed the presence of sensilla campaniformia (Fig. 12-15).

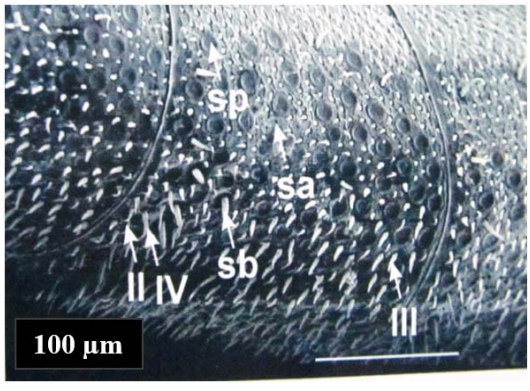

Fig 12: SEM of eighth segment showing sensilla trichodea of type II, III and type IV, along with sensilla placodea, basiconica and ampullacea (Bar= 100 µm).

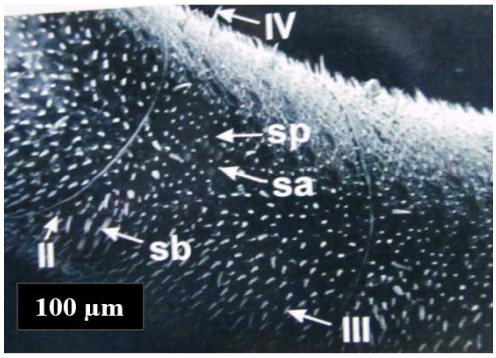

Fig 13: SEM of ninth segment ((Bar= 100 µm).

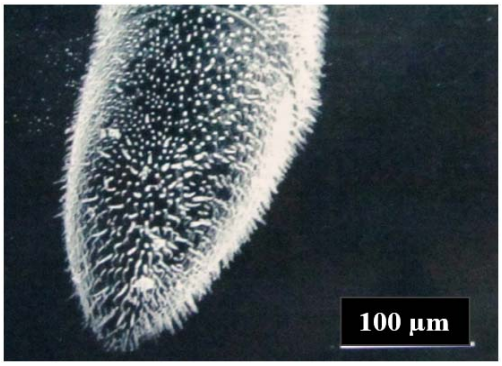

Fig 14: SEM of tenth segment ((Bar= 100 µm). sensilla trichodea of type II, III and type IV, along with sensilla placodea, basiconica and ampullacea (Bar= 100 µm).

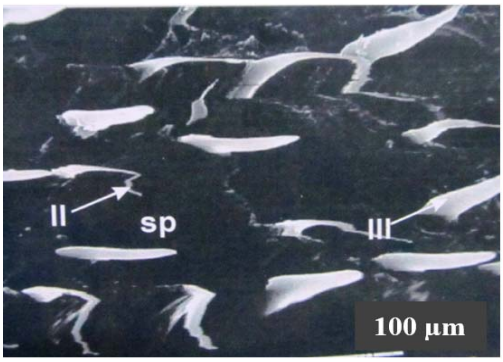

Fig 15: Higher magnification of tenth segment showing sensilla placodea, trichodea of type II, III (Bar= 100 µm).

Discussion

Several variants of the Asian honey bee *A. cerana* have been reported from time to time. Each geographic race has been shown to have locally adapted populations called ecotypes which differ from each other in several biological and economic traits [7-15]. [11, 16, 12, 17] have been able to distinguish 2 ecotypes in the *A. cerana* of North-West India. [18] Collected much scattered information and reported the presence of 4 distinct sub-species of *A. cerana*. [19, 15, 20] identified characteristic ultra-structural variations in different parts of tongue of 2 cavity-nesting species- *A. mellifera* and *A. cerana*. [21] Observed different types of antennal sensilla of *A. dorsata* from Chandigarh and Jalandhar and observed significant differences. [22] performed SEM studies in antenna of *A. florea* and *A. dorsata* and observed the presence of sensilla ampullacea and sensilla basiconica only in *A. florea* and not in *A. dorsata.* [23] observed ultrastructural differences in proximal region, middle region and flabellum of tongue in *A. cerana* plains and hill populations.

During the present observations, regarding the 3rd, 4th and 5th segments, the distribution of sensilla differed in the 2 species, sensilla trichodea type III and sensilla ampullacea being absent in the hill populations. Similarly, in 8th, 9th and 10th segments, sensilla trichodea type III, IV, sensilla placodea and sensilla ampullacea were lacking in hill populations. This can be regarded as an adaptation of the antenna with regard to the hygroreception and olfaction which varies according to the *A. cerana* plains and hill populations.

Conclusion

The findings during the present investigations are interesting and helped to identify characteristic ultrastructural variations in different parts of the antenna of the plains and hill populations of *A. cerana*. The distribution of sensilla 3rd, 4th, 5th, 8th, 9th and 10th flagellar segments differed in the two populations reflecting variation under the influence of native flora. The present findings are illuminatory and emphasize advantage of applying and incorporating SEM data for separating the two populations of *A. cerana*.

Acknowledgements

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