



Scanning electron microscopic comparison of endophallus of *Apis cerana* and *Apis mellifera*

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Abstract

During the present investigations, the detailed morphoarchitecture of the male genitalia viz., the endophallus of the drone of *A. cerana* F. and *A. mellifera* L. was scanned under the electron microscope. Endophallus of each species was studied for its unique characteristics. Significant interspecific differences were observed in the 2 different species. These differences highlight the reproductive isolation in the genus. These species specific differences in the endophallus are highlighted in the paper.

Keywords: *Apis cerana*; *Apis mellifera*; drone; scanning electron microscopy; endophallus.

INTRODUCTION

The drones of all *Apis* species have a copulatory structure consisting of a large complicated tube- the endophallus, present inside the abdomen. The everted endophallus comprised vestibule, bursal cornua (dorsal and ventral), cervix and endophallic bulb. Due to high level of variation within species, the systematic of honey bees is not clearly understood. The endophallus is a reliable character for the separation of species. The eversion process of the drone's endophallus of *Apis mellifera* was described by [1], [2] and [3] and of *Apis cerana* by [4]. According to [3] and [5] in *Apis mellifera* a "comet" of drones follows the female and the winner surrenders a portion of his phallus at the end of coitus and dies later on. Everted endophalli of *Apis dorsata* drones were described by [6] and [7] and of *A. laboriosa* by [8]. [9] demonstrated that although *A. cerana* and *A. mellifera* queens and drones attempted to mate with each other under experimental conditions, the matings were not successful. Even instrumental insemination of heterospecific semen did not produce any offspring. [7] published detailed descriptions and drawings of male genitalia in honey bees and suggested endophallus variations among bees could be employed for generic or subgeneric distinction within Apidae. [10] examined the substances present in the mating sign and observed that three substances were ejected during endophallus eversion and natural mating with queen bees viz., semen, mucus and epithelial membranes.

MATERIAL AND METHODS

The study material of drones of *Apis cerana* was collected from the hive entrance of a natural wall hive while drones of *A. mellifera* were collected from the comb frame in Langstroth hives

kept Panjab University, Chandigarh. The drones were caused to evert their genitalia which were carefully removed. The material was preserved in 5% gluteraldehyde. It was dehydrated in graded series of acetone for 15 minutes each followed by acetone - amyl acetate in the ratio of 50:50 for 15 minutes and then transferred to amyl acetate for 15 minutes and dried upto critical point. Dehydrated samples were mounted on stubs in the desired orientation with the help of double side adhesive tape under binocular microscope. Stubs were then placed inside the sputter for gold coating. The sputtered specimens were examined in Jeol JSM-6100 scanning electron microscope operating at an accelerating voltage of 10 KV at Regional Sophisticated Instrumentation Centre, Panjab University, Chandigarh and the ultra structural differences were studied.

RESULTS

Endophallus of *Apis cerana*- The everted genitalia of *Apis cerana* comprised vestibule, hairy dorsal plate, bursal cornua (dorsal and ventral), cervix and endophallic bulb. The dorsal cornua were short and stout. The ventral cornua was somewhat triangular. The region between the horns of dorsal cornua was short and revealed few bristly projections (Fig.1).

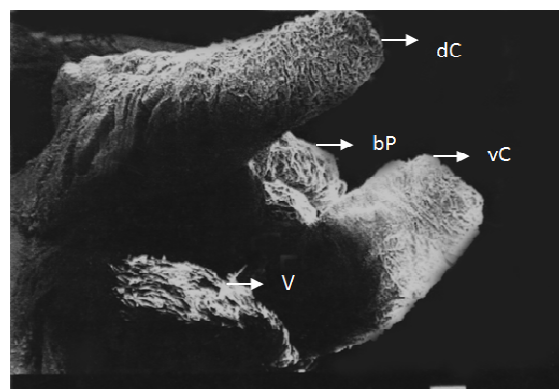


Fig 1.tiff Higher magnification of dorsal cornua with bristly projections and hairy dorsal plate of *Apis cerana* (Bar= 100 micrometer)
 dC- dorsal cornua, VC- ventral cornua, V- vestibulum, bP- bristly projections

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The cervix showed characteristic hairy folds. The cervical lobe was prominent and possessed unique fimbriae which were large lobes arranged in a rosette (Fig.2).

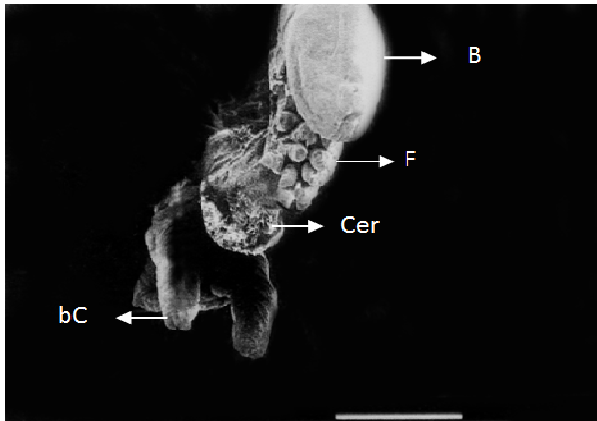


Fig 2.tiff Scanning Electron Micrograph (SEM) of endophallus of *A. cerana* (Bar= 1 mm) F- fimbriae, Cer- cervix, bC- bursal cornua

The bulb was globular with characteristic chitinous plate at the tip (Fig.3).

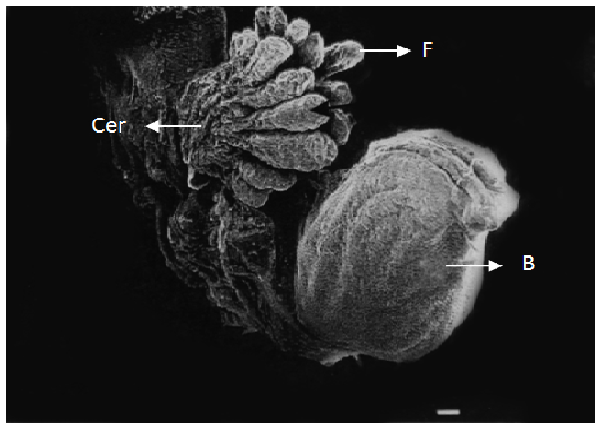


Fig 3.tiff Higher magnification of bulb showing rosette shaped fimbria of *Apis cerana* (Bar= 100 micrometer)

Endophallus of *Apis mellifera* revealed specific variations. The dorsal and ventral cornua were longer and more pointed (Fig.1a).

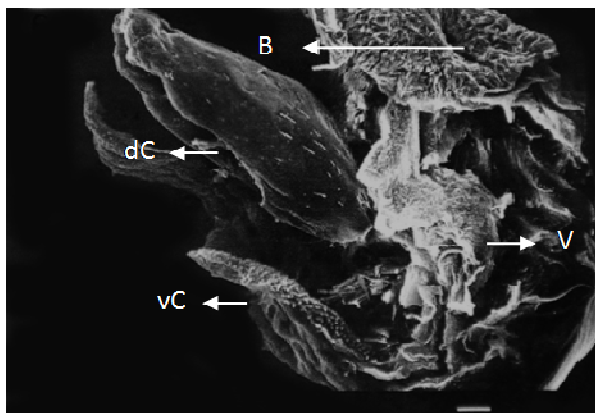


Fig 1a.tiff Higher magnification of vestibule showing dorsal and ventral cornua of *Apis mellifera* (Bar= 100 micrometer) B- endophallic bulb

The hairy dorsal triangular plate was present. The hair were numerous and larger than those found in *Apis cerana* (Fig. 2a).

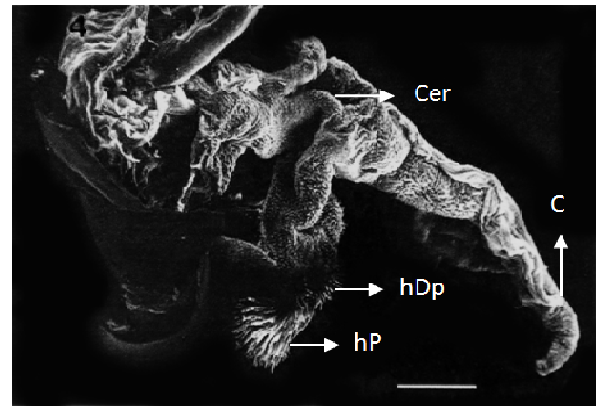


Fig 2a.tiff SEM of endophallus of *Apis mellifera* (Bar= 1 mm) C- cornua, hDP - hairy dorsal plate, hP - hairy projections

The cervix was characterized by the presence of chitinous plate not found in *Apis cerana*. The cervical lobe showed longer basal part on which numerous cylindrical hair like fimbriae were present. These were arranged in a cluster that did not extend beyond the endophallus bulb, which was smaller than that of *A. cerana* (Fig.3a).

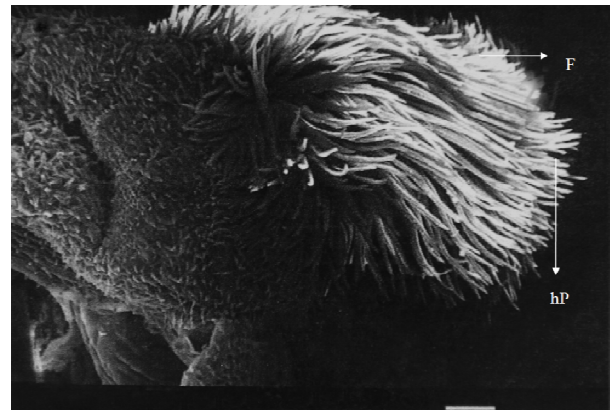


Fig 3a.tiff Higher magnification of cervical lobe showing fimbria of *Apis mellifera* (Bar= 100 micrometer)

DISCUSSION

According to [11] the differences in the endophallus along with studies on mating behavior, congregation areas can serve as key factors in separating different honey bee species. [12] and [13] distinguished 4 honey bee species on the basis of gross morphology of the male genitalia while [14] on the basis of SEM studies on the sting (ovipositor) concluded that genus *Apis* could be separated into 5 species viz. *Apis andreniformis*, *Apis florea*, *Apis cerana*, *Apis mellifera* and *Apis dorsata*. For the separation of species, the endophallus is a reliable character. Any diagnostic difference could not be observed in the endophallus of *A. laboriosa* and *A. dorsata* by [8]. However, the published drawings of the endophallus of *Apis dorsata* [6] and *Apis laboriosa* [8] show distinct differences. The present SEM study clearly brings out specific differences between *A. cerana* and *A. mellifera* which as reported by [5] are otherwise so similar in morphology and behavior that they have been considered

as distant races of the same species.

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REFERENCES

- [1] Woyke J 1955. Multiple mating of the honey bee queen (*Apis mellifera* L.) in one nuptial flight. *Bull. Acad. pol. Sci. II* 3 (5): 175-180
- [2] Woyke J 1958. The histological structure of the reproductive organs of the drone, *Poznan Soc. Friends of Sci., Publ. Sect. Agric. Sylvic.* 19, 38-50
- [3] Woyke J, Ruttner H 1958. An anatomical study on the mating process in the honey bee. *Bee wld* 39 (1): 3-18
- [4] Ruttner F., Woyke J, Koeniger N. 1972. Reproduction in *Apis cerana* 1. Mating behavior. *J. apic. Res.* 11 (3): 141-146
- [5] Winston ML 1987. *The Biology of the honey bee.* Harvard University Press, Cambridge. London
- [6] Simpson H 1970. The male genitalia of *Apis dorsata* F (Hym: Apidae). *Proc. R Entomol Soc Lond A* 45, 169-171
- [7] Koeniger G, Wissel M, Herth W 1990. Cornual secretion on the endophallus of the honey bee drone (*Apis mellifera* L.). *Apidologie* 21, 185-191
- [8] McEvoy M, Underwood BA 1988. The drone and species status of the Himalayan honey bee, *Apis laboriosa* (Hymenoptera: Apidae). *J Kans entomol Soc* 61, 246-249
- [9] Ruttner F., Maul V. 1983. Experimental analysis of reproductive interspecies isolation of *Apis mellifera* L. and *Apis cerana* L. *Apidologie* 14 : 309-327
- [10] Woyke, J. 2010. Three substances ejected by *Apis mellifera* drones from everted endophallus and during natural matings with queen bees. *Apidologie* 41, 613-621.
- [11] Yoshida T, Yamazaki, M 1993. Difference in drone congregation areas of *Apis mellifera* and *A. cerana japonica* as a reproductive isolation mechanism. In: *Asian Apiculture* (Lawrence J. Conner, Thomas Rinderer, H.A. Sylvester and Siriwat Wongsiri eds.). pp: 99-103
- [12] Simpson J (1960). Male genitalia of *Apis* species. *Nature*, 185,56
- [13] Patinawin S, Wongsiri S. 1994. Scanning electron microscopy of cervical lobes of *Apis* drones. *J. Apic. Res.* 33 (2) pp. 65-68
- [14] Jayasvati S. 1990. Scanning electron microscopy and analysis of honey bee (*A. florea* F., *A. dorsata* F., *A. cerana* F., *A. mellifera* L., *A. andreniformis* S.) stings in Thailand. *Proc. 1st Asia-Pacific Conf. Entomol.* Pp: 89-98