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Original article

Morphology of the cervical lobe of the endophallus in *Apis mellifera* drones*

Lien MOORS, Johan BILLEN

Zoological Institute, University of Leuven, Naamsestraat 59, 3000 Leuven, Belgium

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Abstract – We investigated the morphology and ultrastructure of the cervical lobes of *Apis mellifera* drones with respect to age. The lobe epithelium decreases while the cuticle thickness increases as the drone gets older. Ultrastructural study revealed that the lobe has no secretory function but possibly acts as an inflatable bag during copulation.

morphology / lobe / *Apis mellifera* / drones

1. INTRODUCTION

In *Apis* species, mating occurs high in the air on the wing (Woyke, 1958; Koeniger and Koeniger, 1991). During copulation, the drone everts his endophallus into the sting chamber of the queen (Woyke and Rutter, 1958). Three major parts of the membranous endophallus can be distinguished: the vestibulum with the paired cornua, the cervix with the fimbriated lobe, and the bulbus (Snodgrass, 1956). Koeniger et al. (1991) showed that the lobe has a different appearance among the various species of *Apis*. It is similar in *Apis koschevnikovi* v. Buttel-Reepen, *Apis mellifera* L., and *Apis cerana* Fabricius. In contrast to these cavity-nesting species, the lobe of free-nesting species has no fringes or fimbria. However, Woyke et al. (2001) recognized fimbria on the cervical lobes of *Apis dorsata* Fabricius, but only when completely everted. The everted lobes have the same characteristic number of fimbria and subdivisions as they have in situ, however, they are extremely extended by pressure (Koeniger et al., 1991). A scanning electron microscopic study of the cervical lobes of the endophallus in the males of five *Apis* species provided evidence of reproductive isolation. Structural similarities suggest three evolutionary groups, namely *Apis mellifera* and *Apis cerana*, the dwarf honeybees *Apis florea* Fabricius and *Apis andreniformis* Smith, and *Apis dorsata* (Patinawin and Wongsiri, 1994). The same authors made a comparison between uneverted and everted cervical lobes. However, they do not give a well defined function for the lobe.

The present study documents a morphological and ultrastructural approach of the lobe, which is the first ultrastructural study of this organ in honeybees.

2. MATERIALS AND METHODS

Drones of *Apis mellifera* were obtained from hives in Oberursel (Germany). The lobe was studied in 5 age categories of adult drones (0–3–6–9–12 days after emergence). After day 12, the drone is sexually mature and ready to copulate (Mindt, 1962). The lobes were dissected and fixed in cold 2% glutaraldehyde, buffered at pH 7.3 with 50 mM Na-cacodylate and 150 mM saccharose. After postfixation in 2% osmium tetroxide in

Corresponding author: L. Moors,
Lien.Moors@bio.kuleuven.be
* Manuscript editor: Klaus Hartfelder

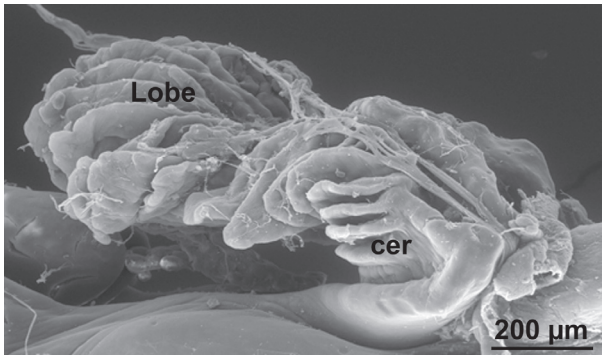

Figure 1. Scanning microscopic image of the cervical lobe of a 6-days-old *Apis mellifera* drone. cer, cervix; Lobe, cervical lobe. Anterior side to the left.

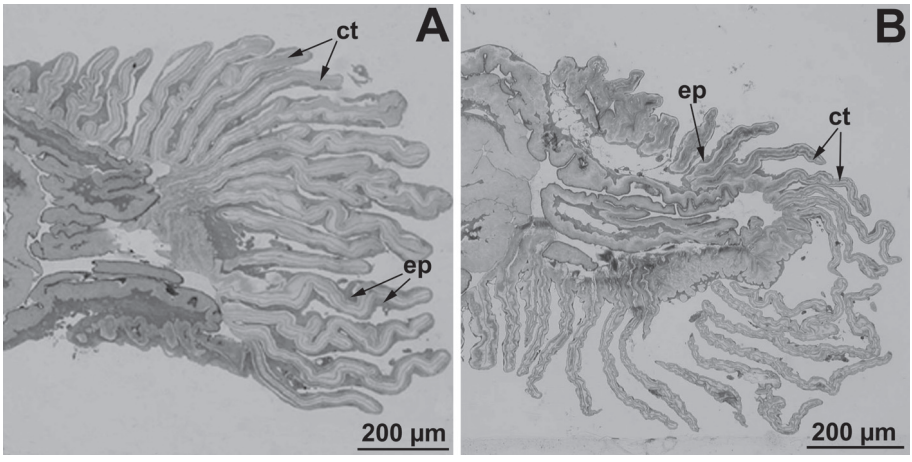

Figure 2. Semithin cross sections of the lobe, (A) a just emerged drone, (B) a 12-day-old drone. ct, cuticle; ep, epithelium.

the same buffer, a dehydratation in a graded acetone series (50%–70%–90%–100%) was carried out. Afterwards, the samples were embedded in Araldite.

The samples were sectioned with a Reichert OmU2 microtome and stained with methylene blue-thionin (1%–1%). These semithin sections (1 μm) for light microscopy of both everted and non-everted endophalli were observed with an Olympus BX-51 microscope. Ultrathin sections (70 nm) of endophalli in situ were made with a Reichert Ultracut E microtome and manually double stained with uranyl acetate and lead citrate. The sections were studied with a Zeiss EM900 electron microscope. Uneverted lobes used for scanning microscopy were first dehydrated in a graded ethanol series (30%–50%–70%) after postfixation and were critical point dried. They were coated with gold and viewed in a Philips SEM 515 microscope.

3. RESULTS

The lobe is unpaired and situated in the posteroventral region of the drone abdomen. The appearance can be compared with a glove. The fingers of the lobe are orientated to the anterior side (Fig. 1). The lobe has approximately 15–20 fingers (Figs. 2A, B).

Histological sections showed considerable differences with respect to age. Immediately after emergence, the epithelial lining has a thickness of about 7 μm. During sexual maturation, the epithelial thickness decreases to

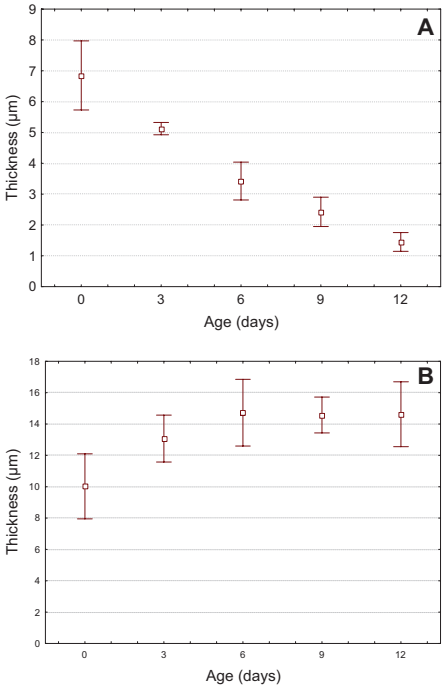

Figure 3. Measurements of the epithelial thickness (A) and cuticular thickness (B) of the cervical lobe in relation to age (n = 4 individuals; 6 measurements/structure).

less than 2 μm (Fig. 3A). The maximum thickness of the cuticle is approximately 15 μm and is more or less reached at an age of day 6 (Fig. 3B).

During eversion, the lobe turns inside out. The cuticle becomes the exterior lining and attains a thickness of approximately 20 μm (Fig. 4).

As the lumen in the lobe of both young and sexually mature drones is empty, the opposite epithelial walls touch each other. The epithelium and the cuticle are folded. The cuticle is divided into an outer relatively dense layer and a fibrillar layer (Figs. 5A–C). The cytoplasm of the lobe contains numerous mitochondria, while the intercellular borders are very much interdigitated. The apical plasmalemma has a simple topography without microvilli and other interdigitations. At the basal side of the epithelium, the basement membrane is very thin (Fig. 5D).

4. DISCUSSION

Our measurements show that when drones emerge, the epithelium of the cervical lobe of the endophallus has a thickness of about 7 μm. At that moment, the epithelium is involved in the formation of the cuticle, which has a thickness of 10 μm. The older the drone gets, the more the epithelium thickness decreases to less than 2 μm and the cuticular thickness increases to approximately 15 μm. It is probable that the reduction in epithelial thickness in an indication for the non-glandular nature of the epithelium. This is supported by the ultrastructural features of the epithelial cells, such as the absence of secretory vesicles within the cytoplasm, the simple apical topography and the empty lumen of the lobe.

The cuticular lining of the lobe, on the other hand, displays an increasing thickness until the age of 6 days and then stays constant. This observation is in accordance with the functional aspects described by Koeniger et al. (1991). These authors mention that the lobe extends during mating. As the queen and a drone are connected together as a cork in a bottle during copulation, the lobe could function as an inflatable bag. Hence, it should possess a strong cuticle in order to resist pressure during extension without rupturing. As the thickness of the cuticle in the everted lobe is similar to that of the lobe in situ, the cuticle does not appear to be stretched during the eversion process. Moreover, we could not recognize special constructions of the everted cuticle which could indicate a dilatable character. When extended, the cuticular lining of the everted lobe is its outermost border. The lobe therefore seems to provide an additional reinforcement to the mechanical connection between the queen and the drone.

As far as we know, non-*Apis* bees do not possess an endophallus and hence also lack a lobe. Its presence in *Apis* only therefore may reflect the very peculiar mating system of the genus, in which a tight anchoring between both partners during copulation is necessary. Males of non-*Apis* bees have e.g. enlarged and modified legs that help in holding females for mating (Michener, 2000).

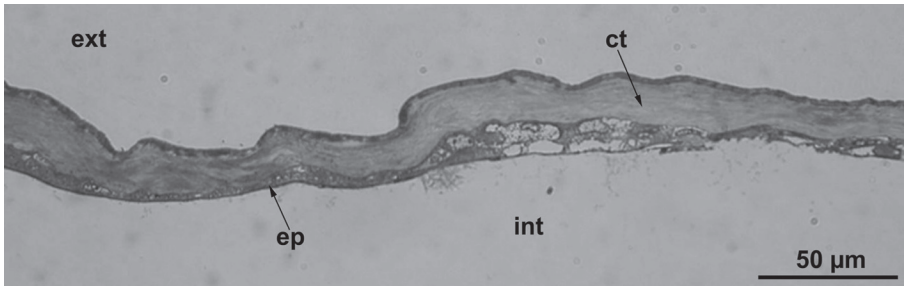

Figure 4. Semithin section of the everted lobe. ct, cuticle; ep, epithelium; ext, exterior; int, interior.

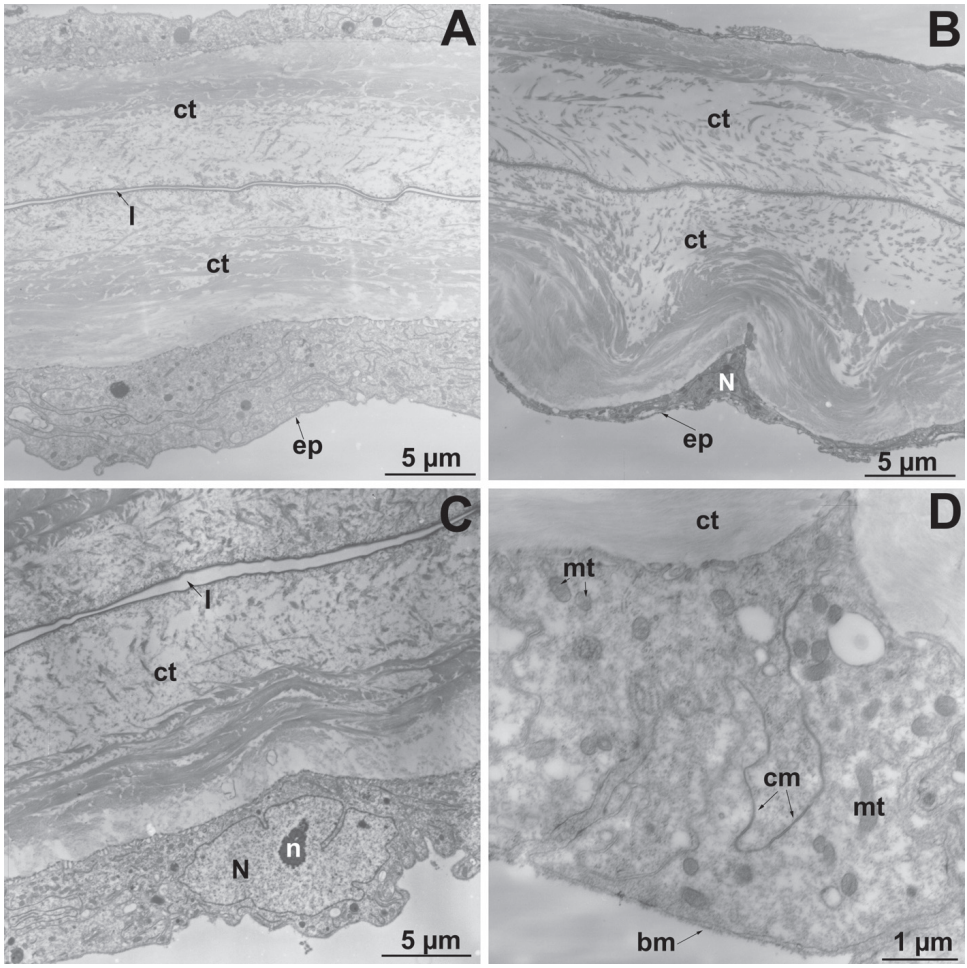

Figure 5. Transmission electron micrographs of the cervical lobe of adult drones, (A, B) overview of respectively 0 and 12 days, (C) detail of the epithelium and cuticle at 0 days, (D) detail of cytoplasm at 6 days. ct, cuticle; ep, epithelium; l, lumen; N, nucleus; n, nucleolus; mt, mitochondria; cm, cell membrane; bm, basement membrane.

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Morphologie du lobe cervical de l'endophallus des mâles d'*Apis mellifera*.

***Apis mellifera* / mâle / lobe / morphologie**

Zusammenfassung – Bau des Federanhangs am Begattungsorgan von *Apis mellifera* Drohnen. Auch wenn Drohnen meist nur eine geringe Beachtung bekommen, so ist ihre Rolle in der Bienengemeinschaft doch von essentieller Bedeutung, denn die Weitergabe ihres Spermas ist die Voraussetzung für Königinnen, als Nachkommen Arbeiterinnen zu erzeugen.
Während der Paarung stülpt der Drohn sein Begattungsorgan aus und führt es gleichzeitig in die Stachelkammer und in den medianen Eileiter der Königin ein. Das Begattungsorgan unterteilt sich in drei Hauptabschnitte, das Vestibulum mit den beiden Hörnchen, den Bulbus und die Cervix mit dem Federanhang.
Unter Anwendung von Licht-, Elektronen- und Rasterelektronenmikroskopie wurde der Federanhang in allen Einzelheiten untersucht. Die Abhängigkeit der Dicke des Epithels und der Kutikula vom Alter wurde gemessen. Das Epithel des Anhangs ist beim Schlupf sehr dünn. Während der sexuellen Reifung wird das Epithel noch dünner, während die Kutikula an Stärke zunimmt (Abb. 3). Das Lumen bleibt leer (Abb. 5C)
In der Ultrastruktur fanden wir Mitochondrien, aber keine sekretorischen Vesikel (Abb. 5D). Das extrem dünne Epithel, fehlende Sekretvesikel und das leere Lumen weist darauf hin, dass es sich hier nicht um ein Drüsenepithel handelt. Da Königin und Drohn während der Kopulation so fest wie „ein Korken mit der Flasche" verbunden sind, könnte der Anhang die Funktion eines aufblasbaren Sackes haben. Diese Deutung ist durch das graduelle Anwachsen der Kutikula bei der sexuellen Reifung unterstützt.

Morphologie / Federanhang / *Apis mellifera* / Drohn

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