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Comparative anatomy of male genital organs in the genus *Apis*

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Summary — Comparative morphological studies of male genitalia from 6 honey bee species revealed distinct qualitative differences in each species in both everted endophalli and endophalli in situ. The endophalli in situ can be studied in drones preserved in ethanol. The following characters are most suitable for classification in situ: 1) pattern of the ventral hairy field of the vestibulum; 2) number of folds and hairy patches of the ventral cervix; 3) form of the dorsal hairy field of the cervix; and 4) the form of its appendage (lobe). Three types of endophalli can be classified. One type is present in the cavity-nesting honeybees *A mellifera*, *A cerana* and *A koschevnikovi*, the second in the free-nesting dwarf honeybees *A andreniformis* and *A florea* and the third in the *A dorsata* complex.

*Apis* / morphology / reproduction / endophallus / systematics

INTRODUCTION

In *Apis* species, especially in *A mellifera*, there is a broad geographic variation in both morphometric and behavioral characters which when quantified allows the characterization of many subspecies. Maa (1953) classified many of these geographic variants as true species. However, the "well documented quantitative intraspecific variability of *A mellifera* has to be regarded as exemplary for selecting species-specific characters within the genus" (Ruttner, 1988). Male genitalia, for example, show qualitative differences in morphology, which are absolutely specific in *Apis mellifera*, *A cerana*, *A dorsata* and *A florea* (Simpson, 1960, 1970; Ruttner, 1988). This also proved to be true for the recently rediscovered species *Apis koschevnikovi* (Tingek et al, 1988) and *Apis andreniformis* (Wongsiri et al, 1990).
Thus the anatomy of the male genitalia becomes an important tool for the classification into true species or subspecies. In *Apis* the male copulatory organ is a membranous endophallus. The drone everts it during mating by contracting the abdominal muscles and pumping hemolymph into it. During its insertion into the queen it is encountered by pressure from the small sting chamber and bursa copulatrix. Eversion can be induced artificially by pressing the abdomen, but it is different from the natural eversion in the presence of the queen. The counter-pressure of the queen is missing (Woyke and Ruttner, 1958; Koeniger, 1985, 1986). Further, only with sexually mature and well-fed drones can eversion be induced easily.

The anatomy of endophalli has been studied mostly from everted endophalli. The results are sometimes contradictory, because artificial eversion is often incomplete. As a result the shapes of endophalli of the same species look quite different. For example, there are 3 different drawings of the endophallus of *A. dorsata*: by Simpson (1970), McEvoy and Underwood (1988) and Koeniger *et al* (1990a). (The drawing by McEvoy and Underwood (1988) is actually of the *A. laboriosa* penis. They found “no diagnostic differences between the male genitalia of *A. laboriosa* and *A. dorsata*”). The drawings of the endophalli of *A. koschevnikovi* and *A. andreniformis* by Mathew and Mathew (1990) again show differences from the previous ones (Tingek *et al*, 1988; Wongsiri *et al*, 1990).

Further, it is not possible to obtain everted endophalli from fixed or dried drones. Thus many drone samples cannot be used for studies if only everted endophalli are examined.

To avoid these difficulties and to find an additional method to recognize the characteristic structures of the endophalli, a detailed description of the uneverted endophallus *in situ* could be helpful.

Snodgrass (1956) gives the anatomy of uneverted and everted endophalli in *A. melillera*. Bährmann (1961) examined the uneverted endophalli of *A. mellifera, A. cerana, A. florea* and *A. dorsata* from dried museum specimens. He found several diagnostic differences in the morphology of the organ in the 4 species.

In this paper uneverted and everted endophalli of 6 different species are described and compared.

**MATERIALS AND METHODS**

Samples of drones were collected in Malaysia: *A. koschevnikovi* (*n* = 5) in Sabah, *A. andreniformis* (*n* = 5) in Johore, *A. cerana* and *A. dorsata* (*n* = 50) in Selangor. Drones of *A. florea* (*n* = 7) were collected in Bangkok, Thailand and drones of *A. mellifera* in Oberursel, Germany.

To study everted endophalli drones were collected during the time of drone flight for each species. By pressing the abdomen between finger and thumb the endophallus was everted (Moritz, 1989). A photo was taken immediately following which the drone was preserved in 70% ethanol. Fine structures were studied under the microscope (magnification 60–250X).

Uneverted endophalli were dissected and isolated either from freshly killed drones or from drones preserved in 70% ethanol. After removal of the tergites and digestive tract, the mucus glands were easy to recognize. Then the genital apparatus was isolated by seizing the ductus ejaculatorius between both mucus glands and pulling all the genital apparatus up. The last sternite (sternum 9), penis valves and lamina parameralis were separated from the drone and remained attached to the endophallus. The isolated apparatus was transferred into a new dissections dish and fixed with a needle through the last sternite. Very carefully the cornua were straightened with a small soft brush. The epithelia were removed with the brush until the chitinized structures were easy to recognize. Some samples were stained with methylene blue. The
isolated genital apparatus was preserved in 70% ethanol.

RESULTS

In all Apis species 3 main parts of the membranous endophallus can be distinguished: 1), the vestibulum with cornua and hairy patches; 2), the cervix with hairy patches and a special lobe ("fimbriate lobe"); 3) the bulbus.

Uneverted endophalli

Differences in morphology between the species occurred in all parts (figs 2 and 3).

Vestibulum (V)

The cornua

All cornua in situ are extremely wrinkled and folded. They can be unfolded and stretched easily when pulled from the tip. The cornua are different in form, length and subdivisions. All species have 1 pair of ventral cornua (vC). Usually one ventral cornus is a simple tube, but in A dorsata each ventral cornus branches into 2 long tubes. The dorsal cornua (dC) are also paired. They are much shorter and have several bulges and notches. In A mellifera, A florea and A andreniformis the bulges are small and at different positions. In A koschevnikovi 3 bulges are elongated into tubes; this elongation is even more pro-

![Fig 1. Scheme of an uneverted endophallus: a) dorsal view; b) ventral view. Symbols for all figures: B: Bulbus; Cer: cervix; chP: chitinous plates of bulbus; dC: dorsal cornua; Dej: ductus ejaculatorius; F: folds of cervix; L: lobe; V: vestibulum; vC: ventral cornua; \( \text{hairy fields} \).]
nounced in *A. cerana*. In *A. dorsata* each ventral cornus is elongated into 2 long tubes, 1 of them with an additional bulge.

In all species sexually mature drones (undertaking mating flights) have an orange-colored secretion inside the cornua (Koeniger *et al.*, 1990b).

The hairy fields on the ventral side of the vestibulum in most species are pointed at the border with the cervix. Only in *A. cerana* the border is flat. The density of the hair varies within the field, thus forming different patterns. The field ends in *A. cerana* with a densely haired stripe.

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Fig 2. Drawing of uneverted endophalli of 6 *Apis* species (scale 7:1): a) dorsal view; b) ventral view.
Cervix (Cer)

The cervix starts behind the origin of the cornua and the ventral hairy field of the vestibulum and ends after the lobe. Often it is twisted into a spiral. Only in A florea and A andreniformis is it straight.

Hairy patches

In all species there are hairy patches on the ventral and dorsal side of the cervix.

In A koschevikovi, A cerana and A mellifera the ventral side of the cervix has folds (FCer) : 6 in A cerana, 10–12 in A...
koschevnikovi and = 9 in A mellifera. Some of the folds carry hairs. In the other 3 species there are no folds, but different numbers of small hairy patches: 9–11 in A dorsata, 4 in A andreniformis and very thin stripes in A florea. The dorsal hairs form one big patch with different shapes.

The lobe (L)
The lobe is similar in A koschevnikovi, A mellifera and A cerana. It has a notch at the end, and each side has 13–15 fringes (fimbria). In A cerana the notch is much deeper than in the other 2 species. The lobe of the free-nesting types has no fringes (fimbria). In A dorsata it has 4 deep notches that divide the lobe into 4 un-scored appendages. The lobes of A florea is subdivided into 3 different bulges, and A andreniformis has one lobe with one pair of long thin appendages and 3 pairs of short ones.

Bulbus (B)
The bulbus of A mellifera is large and has chitinous plates (chP), which are not present in the other species. The bulbus in A dorsata is relatively small compared to that of A cerana, A koschevnikovi and A mellifera. The bulbus opens into the ductus ejaculatorius (Dej). This junction forms the gonoporus in the everted endophallus. In A andreniformis and A florea the bulbus is a thin tube and its apex cannot be distinguished from the beginning of the ductus ejaculatorius.

Everted endophalli
During eversion the endophallus is filled by hemolymph and air to increase its stability by inner pressure. The former “inside” then becomes the “outside”. All wrinkles and folds are extended and as long as the high pressure persists all endophalli have typical curves and shapes (fig 4).

Vestibulum (V)
The ventral cornua (vC) of A mellifera, A koschevnikovi and A cerana first extend posteriorly but the tips are recurved ventrally to different degrees. This is opposite in A andreniformis and A florea, where the cornua curve back dorsally. The 2 long tubes of the ventral cornua of A dorsata are coiled to a spiral.

The dorsal cornua (dC) are smaller and have 2 or 3 appendages. In A koschevnikovi they look like little balls; in A cerana they form small tubes. A dorsata drones have the longest dorsal cornua (3 tubes); A mellifera drones have the shortest dorsal cornua (one bump). The orange secretion of the everted cornua covers their outer surface.

The hairy patches on the ventral side correspond to the shapes in situ, but because of the extension of the vestibulum the hairs appear less dense.

Cervix (Cer)
The cervix, which is a thin tube at the beginning of the eversion, is soon widened to a thick duct in A mellifera and A cerana. The spiral character has vanished. The duct is a little elongated in A koschevnikovi and long and thin in A dorsata. The ventral hairy patches are placed on little humps, but it is difficult to recognize the same number of humps as folds in the uneverted endophallus. Presumably the extension of the cervix eliminates some of them.

The relative length of the extended cervix – measured from the end of the ventral hairy field of the bulbus to the orifice of the lobe – is different. It is one quarter of the total length of the endophallus in A mellife-
ra, one third in *A. cerana*, two fifths in *A. koschevnikovi*, and one half in *A. dorsata*. In *A. andreniformis* and *A. florea* it is less than one quarter. In the unevorted stage the cervix has about the same length in 5 species. *A. dorsata* is the only exception, because the cervix is not folded.

The dorsal hairy patches are broadened but show similar shapes as in the unevorted endophallus.

The lobes have the same characteristic number of fringes (fimbria) and subdivisions as they have in situ. However, they are extremely extended by pressure, thus changing the shape considerably.

**Bulbus**

The bulbi are bent dorsally in *A. mellifera*, *A. cerana* and *A. koschevnikovi*. They often contain mucus and sometimes the mucus is ejaculated but remains on the outer surface of the bulb. The bulb of *A. mellifera* contains chitinous plates (chP).

The bulb of *A. dorsata* is bent ventrally and is smaller than in the former species. It also contains some mucus. In *A. andreniformis* and *A. florea* the bulb forms a thin tube and is rather pointed apically. Mucus was not recognized.

**DISCUSSION**

In all species examined, distinct qualitative differences in the morphology of the endophallus could be recognized. Comparing endophalli in situ gives a reliable method to classify the *Apis* species, using fresh or preserved drones. Even dry specimens can be used for examination (Bährmann, 1961). This method may help to clarify whether there are differences between the endophalli of *A. dorsata* and *A. laboriosa*, a question still under debate (Koeniger et al., 1990a).

The following characters are clearly different in the unevorted endophalli of the 6 examined species: 1) pattern of the ventral hairy field of the vestibulum; 2) number of folds and hairy patches of the ventral cervix; 3), form of the dorsal hairy field of the cervix; and 4), the form of its lobe.

On the other hand, during preparation of endophalli in situ the length and form, particularly of the cornua and cervix, may be changed. These parts are easily stretched because they are extremely wrinkled and folded.

Everted endophalli give additional information which cannot be obtained with endophalli in situ. For example, the bend of the cornua either dorsally or ventrally cannot be recognized in the unevorted organ. The same is true for the bending of the bulbus, which also differs between the species. While the bulb turns dorsally in the cavity-nesting species, it turns ventrally in *A. dorsata*. Also in *A. andreniformis* and *A. florea* the thin bulb turns ventrally directly after the lobe, then forming a "dorsal hook".

The different length of the cervix can also only be easily recognized in the everted stage. Its length steadily increases from *A. mellifera*, *A. cerana* to *A. koschevnikovi*. The longest and thinnest cervix is in *A. dorsata*. The dwarf honeybees *A. andreniformis* and *A. florea* have extremely short cervixes.

Evaluating the endophalli, both in situ and in the everted state, one can distinguish 3 main types: 1), the bulb is thick and bent dorsally; the ventral cornua is bent ventrally; production of mucus and lobe with fimbria are observed. This type is found in the cavity-nesting species: *A. mel-
Fig 3. Photos of uneverted endophallii of 6 Apis species: a) dorsal view; b) ventral view.
lifera, A cerana and A koschevnikovi; 2), The bulbus is thin and elongated; the cervix short, the ventral cornua bent dorsally; there is little or no mucus; the lobe is without fimbria. This type is found in free-nesting dwarf honeybees A andreniformis and A florea. 3) The bulbus is small and bent ventrally; there are ventral cornua with 2 long tubes; the lobe is divided into 4 parts and is without fimbria; the cervix is extremely long. This type is found in the dorsata group.

Fig 4. Drawings of everted endophalli of 6 Apis species (lateral view). For abbreviations, see figs 1 and 2.
The differences in morphology may provide additional information for evaluating the relation and the phylogeny of the honey bee species. Form, course and length of the bulb and the cervices seem to be suitable for this evaluation, especially in combination with sperm number and the mechanism of sperm transfer (Koeniger and Koeniger, 1990a,b; Koeniger et al, 1990c).

Little is known about intraspecific variation of morphological characters. For example, the number of hairy patches of the cervix varies between 9–11 in *A dorsata* drones from 1 colony. Further, the type of hairs on the endophallus might be useful for both inter- and intraspecific variations. In *A mellifera*, Woyke (1958) describes 7 different types of hairs on different parts of the endophallus. Thorough studies of intraspecific variation of the endophalli are still lacking.


Le plan d'organisation de l'endophallus membraneux est identique chez toutes les espèces *Apis*. Trois parties principales peuvent être distinguées (fig 1):

1) Le vestibule (V) avec des cornules ventrale et dorsale (vC et dC), et une aire pileuse.

2) Le cervix (Cer), qui comporte également des surfaces pileuses et un lobe spécial (le lobe feuilleté).

3) Le bulbe (B).

Dans ces différentes parties, il y a des différences nettes entre les espèces d'abeilles (figs 2, 3 et 4):

1) Les aires pileuses du vestibule ont une forme différente dans chaque espèce.

2) Les cornules (C) sont subdivisées en une partie ventrale (vC) et une partie dorsale (dC). La forme et la taille sont différentes, mais une sécrétion de couleur orange est présente dans toutes les espèces.

3) La longueur du cervix (Cer) est différente : *A dorsata* a un très long cervix; il est très court chez *A florea* et *A andreniformis*, ce qui rend sa reconnaissance aisé dans l'endophallus après éversion. Les différences suivantes sont plus faciles à mettre en évidence dans des endophalli *in situ* : nombre des petites taches poilues et des plis du côté ventral, forme des surfaces pileuses dorsales.

4) Le lobe feuilleté (L) a des poils seulement chez les espèces nichant à l'intérieur de cavités; chez *A dorsata*, le lobe est divisé en 4 prolongements. Le lobe d'*A florea* est subdivisé en 3 renflements différents, et *A andreniformis* a un lobe comportant une paire d'appendices longs et minces et 3 paires d'appendices courts. Les différences dans ce lobe sont plus faciles à reconnaître dans l'endophallus *in situ*. 
5) Le bulbe est hypertrophié uniquement chez les espèces nichant dans des cavités, et chez *A dorsata*, il est rempli de mucus au cours de l'éversion. Il comporte des plaques chitineuses spéciales (chP) uniquement chez *A mellifera*. Chez *A florea* et *A andreniformis*, le bulbe forme un tube long et fin et se termine en pointe.

Les endophallus *in situ* montrent suffisamment de différences qualitatives entre les espèces pour être utilisés pour la classification de ces espèces. Les endophallus *in situ* et après éversion, permettent de séparer 3 types différents:

1) Les espèces *d'Apis* nidifiant dans des cavités ont un bulbe bien développé, qui se recourbe dorsalement après éversion, et qui contient du mucus; les cornules sont courbées ventralement après l'éversion; le lobe du cervix est feuilleté.

2) Les abeilles naines *A florea* et *A andreniformis* ont un bulbe long et fin, qui contient peu ou pas de mucus; les cornules sont courbées dorsalement après l'éversion; le cervix est très court et le lobe n'est pas feuilleté.

3) Le groupe *dorsata* a un bulbe relativement plus petit, qui se recourbe dorsalement après l'éversion. Il contient du mucus; le cervix est très long, son lobe a 4 appendices. Les cornules ventrales sont branchées dans 2 longs tubes enroulés. Les cornules dorsales se ramifient en 3 parties.

**Apis / endophallus / morphologie / reproduction / systématique**

**Zusammenfassung — Vergleichende Anatomie der männlichen Begattungsorgane innerhalb der Gattung *Apis*.** Vergleichende morphologische Studien von evertierten Endophalli der Drohnen haben gezeigt, daß bei 6 Honigbienenarten deuts-
Dorsalseite. Das wiederum ist leichter in situ zu sehen.

4) Der “Federanhang” (L) ist nur bei den höhlenbrütenden Arten, _A mellifera_, _A cerana_ und _A koschevnikovi_ federförmig. Beim Endophallus in situ ist in der Mitte eine Kerbe zu erkennen, die bei _A cerana_ tief eingeschnitten ist. Der Anhang bei _A dorsata_ ist grob vierlappig, bei _A andreniformis_ ist der Anhang zu verschiedenen langen dünnen Schläuchen ausgezogen, bei _A florea_ sind nur kurze Ausläufer vorhanden. Die Unterscheidung des “Federanhangs” ist am deutlichsten am nicht evertierten Endophallus zu erkennen.

5) Der Bulbus ist bei den höhlenbrütenden Arten und _A dorsata_ zwiebelförmig erweitert und ist im evertierten Zustand mit weißem Mucus gefüllt, bei _A mellifera_ enthält er zusätzlich paarige Chitinspangen. Bei _A andreniformis_ und _A florea_ ist der Bulbus lang ausgezogen und endet in einer Spitze.

Endophalli in situ lassen ausreichend qualitative Unterschiede zwischen den Arten erkennen, um sie zur Klassifizierung zu benutzen. Sowohl Endophalli in situ als auch im evertierten Zustand zeigen, daß es 3 Typen gibt.

Die höhlenbrütenden Honigbienenarten haben einen dicken, dorsal gebogenen Bulus, der im evertierten Zustand Mucus enthält, die Cornua sind ventral gebogen, der Anhang an der Cervix ist federförmig.

Beiden Zwerghonigbienen _A andreniformis_ und _A florea_ ist der Bulbus dünn und lang und enthält wenig oder keinen Mucus, die Cornua sind dorsal gebogen, die Cervix ist sehr kurz und ihr Anhang ist nicht federförmig.

Beide _A dorsata_ ist der Bulbus relativ klein und dorsal gebogen und enthält Mucus. Die Cervix ist extrem lang, ihr Anhang besteht aus 4 ungekerbten Lappen, die ventralen Cornua sind zweigeteilt und sehr lang ausgezogen. Auch die dorsalen Cornua sind relativ lang und sind dreigeteilt.

**Apis / Morphologie / Reproduktion / Endophallus / Systematik**

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