

Mating and sperm transfer in *Apis florea*

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Summary — The mating flights of 3 virgin queens of *Apis florea* have been observed. The queens carried no mating sign when they returned to their colonies. In 2 of the queens, dissected several minutes following their return, spermatozoa were found in the spermatheca. The filling of the spermatheca during mating and the anatomical structure of the *Apis florea* drone's endophallus suggested direct sperm transfer into the spermatheca. The 3rd queen was dissected 16 h after mating. At that time, more than 200 eggs were present in the previously empty comb. The queens had 0.48, 0.72 and 1.35 million spermatozoa in the spermatheca. On average, drones of *A. florea* were found to produce about 0.44 million spermatozoa. So, the queens were presumably mated by 1, 2 and 3 drones, respectively.

***Apis florea* — mating — sperm transfer**

INTRODUCTION

The mating behaviour and sperm transfer of the Western honeybee *Apis mellifera* and the Eastern hive bee *A. cerana* seem to follow a similar pattern. For mating, the young virgin queen leaves the hive and flies to a drone congregation area. There the queen mates high in the air with, on average, 6 to 12 drones. In *A. cerana*, polyandry seems to occur to a similar extent. Woyke (1975) calculated that *A. cerana* queens are mated by an average of 10 drones.

During mating, the first drone inserts his endophallus into the bursa copulatrix

of the queen and ejaculates his spermatozoa into the oviducts of the queen. Then the queen separates from the drone, which falls paralyzed to the ground. The drone's mating sign (mucus plus some orange pigment) remains in the queen's sting chamber. Each consecutive drone inserts his endophallus beneath the mating sign of his predecessor and removes it by a special organ on the endophallus (Koeniger, 1986). In the course of copulation, each drone deposits his spermatozoa into the oviducts and leaves the mating sign in the queen. After the last mating, the queen returns to the hive carrying the mating sign of the last drone in her sting chamber. The mating sign is clearly visible to the naked eye.

After mating, the spermatozoa are transferred from the oviduct into the spermatheca. More than 90% of the spermatozoa are lost during this transportation process. In *A. mellifera* about 6–8 million spermatozoa are found in a fully mated young queen, while the *A. cerana* queen has only 1.3–3.5 million spermatozoa in the spermatheca (Ruttner *et al.*, 1973).

The number of spermatozoa of drones differ between both species. *A. mellifera* drones have 10–11 million spermatozoa (*A. mellifera carnica*) in their seminal vesicles, while *A. cerana* drones contain only 1 million spermatozoa (Woyke, 1975)

In *A. florea*, the only previously reported details are spermatozoa counts from the spermathecae of 2 laying queens, where 0.38 and 0.922 million spermatozoa were found (Ruttner, 1988).

MATERIAL AND METHODS

Colony management and queen production

Colonies of *A. florea* were collected from the campus of Chulalongkorn University in Bangkok and from Mae Klong village (70 km south east of Bangkok). The colonies were installed at 2 balconies in the first story of the biology building. After an initial orientation period of 2 days, the queens were removed from the colonies and dissected. Ten days after removal of the queens, the colonies were inspected and all sealed queen cells were cut out from the comb together with some surrounding comb cells. Each queen cell was kept in its natural orientation in a small plastic cage and placed into an incubator at 34.5 °C and 60% relative humidity. Sixteen days after the removal of the queen, the first queen cells hatched. Each young queen was marked by a numbered plastic tag on the

thorax and introduced into a queenless colony, where the young queen was observed daily from 12.00 to 16.00 (local time). Our previous observations showed that drone flights of *A. florea* in Bangkok occurred between 13.45 and 15.30. Surplus queens were kept in small cages with an escort of 20 workers and fed with candy for several days. The workers were changed after 3 days.

Dissection and counting of spermatozoa

Drones were caught at the colony when they returned from their mating flights. They were kept in a cage (without workers) for about 12 to 16 h until their movements were slow. This treatment prevented the drones from emptying the vesicles when being killed. Then the vesiculae seminales were carefully isolated and transferred into 1 ml of 0.9 NaCl solution. The vesicles were opened and the sperm evenly dispersed. For counting, a Fuchs-Rosenthal counting chamber (haemocytometer) was used.

The original queens were dissected immediately after removal from the colony. Each spermatheca was isolated and its tracheal net removed. Then the intact spermatheca was transferred into 1 ml of 0.9% NaCl solution, where the spermatozoa were evenly distributed. Again, a Fuchs-Rosenthal chamber was used for counting. In the freshly mated queens the oviducts were examined for spermatozoa under a microscope.

RESULTS

Number of spermatozoa in drones and queens

We counted the vesiculae seminales of 8 drones (Table I). The average number of spermatozoa was 0.44 million per *A. florea*

Table I. Number of spermatozoa (in millions) and body mass of drones.

| <i>Drone No.</i> | <i>Weight (mg)</i> | <i>Spermatozoa (millions)</i> |
|------------------|--------------------|-------------------------------|
| 1 | 90.7 | 0.45 |
| 2 | 82.7 | 0.46 |
| 3 | 86.9 | 0.4 |
| 4 | 80.9 | 0.39 |
| 5 | 87.9 | 0.49 |
| 6 | — | 0.48 |
| 7 | — | 0.45 |
| 8 | — | 0.41 |
| Average | 85.8 | 0.44 ± 0.037 |

drone. The results of sperm counting in laying queens of unknown age are shown in Table II. The maximum number found was 1.35 million.

Premating behaviour

The first queen emerged on Feb. 5th and was labelled and introduced into a queenless colony that day. During the first days the queen patrolled (Lindauer, 1952) main-

ly on the comb beneath the curtain of bees which covered the colony. On days 3 and 4, the queen crossed the platform (upper portion of the comb) several times. The movements of the queen by this time were much faster and the workers which came into contact with the queen on the platform reacted with dorso-ventral vibrations, as described for *A. mellifera scutellata* (Fletcher, 1977). On the 6th day the queen entered the platform. She ran fast with several sudden breaks during which she displayed intensive cleaning behaviour. At the

Table II. Number of spermatozoa (in millions) found in the spermatheca of laying queens collected in Thailand.

| <i>Queen no.</i> | <i>Spermatheca (without tracheas) diameter (mm)</i> | <i>Spermatozoa (million)</i> |
|------------------|---|------------------------------|
| 1 | 0.825 | 0.85 |
| 2 | 0.75 | 0.25 |
| 3 | 0.81 | 1.2 |
| 4 | 0.8 | 1.2 |
| 5 | 0.75 | 0.62 |
| 6 | 0.7 | 1.35 |
| 7 | 0.8 | 0.8 |

same moment, cleansing and orientation flights of the workers took place. The queen started flying at 13.44 and returned, after some circling around, to the colony at 13.46.

On the 7th day, the queen frequently crossed the platform and the dorso-ventral movements of the workers near her became intensive. No drone flight occurred during this day.

Mating flight and sperm transfer

On the 8th day, the queen started flying at 14.16 and flew away in a straight line high up in the sky. She returned at 14.34 and was caught at once. No mating sign was found but nevertheless, we dissected her without delay. Her spermatheca was examined 10 min after her return; it was filled with fast moving spermatozoa. We counted 0.48 million spermatozoa from the spermatheca. The oviduct was rinsed in NaCl solution and the fluid was carefully inspected under a microscope. We detected 15 moving spermatozoa.

The second queen had a short flight (2 min) on her 5th day. On the 6th day she started flying at 14.04 and returned at 14.25. Again, no mating sign was observed and the examination of the queen's spermatheca only 5 min afterwards showed there to be 0.72 million spermatozoa in her spermatheca. No spermatozoa were detected in the oviducts.

Start of oviposition

The third queen flew on the 7th day of her life from 15.02 until 15.32 and returned without a mating sign. This queen was al-

lowed to stay in the colony for 16 h, after which we found 200 freshly laid eggs in the comb. The comb did not contain eggs 1 day before. The queen had 1.17 million spermatozoa in her spermatheca. None was found in the oviducts.

The fourth queen flew 8 days after emergence and left from 14.00 until 14.15. Her spermatheca and oviducts were empty.

DISCUSSION

These first observations of the mating flight of *A. florea* were carried out with a limited number of virgin queens and at one location. Nevertheless, data from the 3 queens allow some conclusions to be drawn on the mating of *A. florea*. Apparently, no mating sign is left in the queen's sting chamber after mating and the sperm is transferred directly into the spermatheca. Anatomical 'evidence' for a direct sperm transfer into the spermatheca can be concluded from the morphology of the genital organs. The *A. florea* drone has rudimentary mucus glands and no mucus for a mating sign is produced. In contrast to *A. mellifera*, where the drone is attached during the copulation to the queen by the endophallus filled with mucus (Koeniger, 1986), the *A. florea* drone has clasper organs at the basitarsus of its hind leg which are suitable and may function to hold the queen's leg (Ruttner, 1975). The endophallus of *A. florea* is "an isolated, highly specialized type within the genus *Apis*" (Ruttner, 1988). The shape of this endophallus, especially the tip at the distal end, seems to fit the hypothesis of direct sperm transfer (Fig. 1). Anatomical studies of the queen's genitalia and the ductus spermaticus are in preparation.

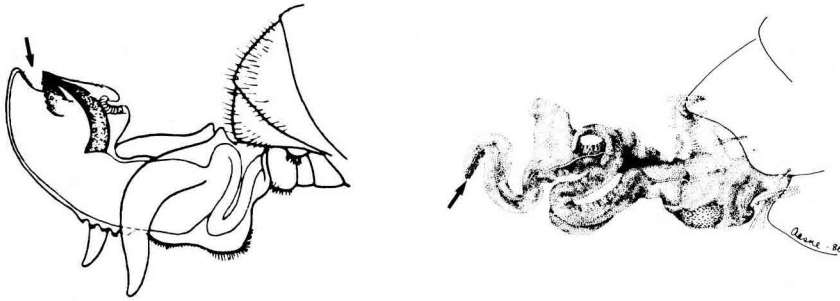


Fig. 1. Everted endophallus: **a.** *Apis mellifera* (stage of ejaculation); bulb short and broad; **b.** *Apis florea*; bulb elongated with thin tip (arrow = opening of ejaculatory duct).

Furthermore the number of spermatozoa found in the spermathecae of the freshly mated queens seems to be related to the number of matings. One *A. florea* drone has an average of 0.44 million spermatozoa (Table I). So we can assume that

- i) the first queen (0.48 mio) was inseminated by 1 drone;
- ii) the second queen (0.72 mio) was inseminated by 2 drones;
- iii) the third queen (1.17 mio) was inseminated by 3 drones.

This idea again is based on direct sperm transfer into the spermatheca, which can function without major losses of spermatozoa. Under these assumptions, the number of spermatozoa found in laying *A. florea* queens (Table II) indicates that *A. florea* queens copulate with a maximum of 4 drones. The largest number found was 1.35 million. This could be the result of 3 to 4 matings. So, the degree of female polygamy in *A. florea* is much lower than in *A. mellifera* or *A. cerana*.

In *A. mellifera* and *A. cerana* about 90% of the spermatozoa deposited by the drones in the oviducts gets lost, with only 10% reaching the spermatheca. This transport of spermatozoa within the queen is in-

efficient compared to the direct transfer of spermatozoa into the spermatheca during mating by drones of *A. florea*.

Résumé — Accouplement et transfert de sperme chez *Apis florea*. On a observé le vol nuptial de 3 reines vierges d'*Apis florea*. Elles ne portaient pas de signes d'accouplement lorsqu'elles sont rentrées à la ruche. Deux des reines, disséquées quelques minutes après leur retour, avaient leur spermathèque remplie de spermatozoïdes. On n'a relevé aucune quantité notable de sperme dans les oviductes. Cette observation, ainsi que la structure anatomique de l'endophallus du mâle, suggèrent le transfert direct du sperme dans la spermathèque lors de l'accouplement chez cette espèce. La troisième reine a été disséquée 16 h après l'accouplement. A ce moment là plus de 200 œufs étaient présents dans les cellules du rayon, vides à l'origine.

Les reines renfermaient dans leur spermathèque 0,48, 0,72 et 1,35 million de spermatozoïdes. L'examen des vésicules séminales des mâles a montré qu'elle contenaient environ 0,44 million de sper-

matozoïdes. On peut donc penser que les reines se sont accouplées avec 1, 2 ou 3 mâles, respectivement.

Zusammenfassung — Paarungsverhalten und Spermatransfer bei der Zwerg-honigbiene *Apis florea*. Der Hochzeitsflug von 3 jungfräulichen Königinnen wurde am Volk beobachtet. Die Königinnen kehrten vom Paarungsflug ohne Begattungszeichen zurück. Zwei Königinnen, die wenige Minuten nach ihrer Rückkehr präpariert wurden, hatten bereits eine mit Spermien gefüllte Spermatheka. In den Ovidukten waren keine nennenswerten Spermamengen vorhanden. Diese Beobachtung sowie der anatomische Bau des Endophallus des Drohns sprechen für eine direkte Spermaübertragung in die Spermatheka bei der Paarung von *Apis florea*. Die dritte Königin wurde 16 Stunden nach Rückkehr vom Paarungsflug präpariert. Zu diesem Zeitpunkt wurden bereits mehr als 200 Eier in den vorher leeren Wabenzellen gefunden.

Die Königinnen hatten 0,48 Millionen, 0,72 Millionen und 1,35 Millionen Spermien in den Spermatheken. Eine Überprüfung der Vesiculæ seminales der

Drohnen ergab eine durchschnittliche Spermazahl von 0,44 Millionen. So kann vermutet werden, daß die Königinnen von jeweils 1, 2 und 3 Drohnen begattet worden waren.

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