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Original article

Mating flights and sperm transfer in the dwarf honeybee *Apis andreniformis* (Smith, 1858)

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(Invited paper)

Abstract – Mating flights of 3 virgin queens of *Apis andreniformis* were observed at their natural nesting site. They initiated mating flights between 12.33 and 12.50 h. The flight duration was between 19 and 23 minutes. The sting chamber of the returning queens contained the orange-colored secretion from the cornual gland of the drone's endophallus. Immediately after the mating flights, the queens were dissected. No sperm was detected in the oviducts, but spermatozoa were found in the spermathecae. In 2 queens, the spermathecae contained 0.09 million spermatozoa, which corresponds to about 75% of the sperm of 1 drone. The third queen had 0.31 million spermatozoa. The spermatozoa in the spermatheca were observed to be moving, and formed an undulating thread. These results suggest that sperm is transferred not into the oviducts but directly into the spermatheca (via the spermaduct). Seven egg-laying queens of unknown age had between 0.33 and 1.26 million spermatozoa in their spermathecae. The mode of sperm transfer is discussed in relation to the number and sequence of the spermatozoa received from each drone in the spermatheca.

***Apis andreniformis* / queen / mating flight / sperm transfer/ spermatozoa count**

1. INTRODUCTION

At the beginning of this century, more than 100 honeybee species were described based solely on morphological characters. When the high level of intraspecific polymorphy within *Apis mellifera* became evident during the years 1950 to 1960 [21], the number of *Apis* species was reduced to 4, and *Apis florea* was regarded as the only dwarf honeybee species. With the report by Wu and Kuang [28], however, the species *Apis andreniformis* described in 1858 by

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Smith [22] was recognized as the second dwarf honeybee species. Research on the distribution and biology of this bee and its status as a separate species has been confirmed by several authors [6, 13, 17–19, 24, 25].

Reproductive isolation between honeybee species has attracted much attention. It has been shown for several sympatric species that the different daily mating flight times function as a mating barrier [8, 12]. The period of drone flight activity has been observed in several locations (peninsular Malaysia, south-eastern Thailand [17], and Sabah, Malaysia [12]). At all locations recorded so far, all *A. andreniformis* drones fly around noon (when the sun passes its zenith), a time at which drones of other sympatric honeybee species stay in the nest.

In addition to studies on flight times, the occurrence of polyandry in *A. andreniformis* queens has been investigated. In 2 ovipositing queens, sperm numbers in the spermathecae were counted and compared with the sperm number from the vesiculae seminales of drones [5]. According to these sperm counts, it was suggested that queens mate a minimum of 8 times. The offspring of 4 *A. andreniformis* queens were analyzed by Oldroyd et al. [14] using 4 polymorphic microsatellite loci with sufficient allelic variation. They found that each of these 4 queens mated between 10 and 21 times. Thus, polyandry is as common as in other *Apis* species.

The aim of this study was to investigate queen mating flights and sperm transfer in *A. andreniformis*. The observations and experiments were carried out in February and March 1998 at the Agricultural Research Station Tenom, Sabah, Malaysia.

2. MATERIALS AND METHODS

Between the middle of February and the middle of March 1998, 6 colonies of *A. andreniformis* were found, located in cocoa fields at the Agricultural Research Station in Tenom, Sabah, Malaysia. The colonies were hanging beneath small branches, which were not higher than 2 m. Thus we were able to make the observations at the natural nesting site.

2.1. Rearing queens

In colony 2, we detected 1 queen and 7 sealed queen cells. Using a bee smoker, the bees and the queen were chased from the comb. They settled nearby in the branches. We carefully removed all 7 queen cells. The bees returned to the comb within 30 min. The queen was caught, marked (Edding 750 paint marker) and immediately put back into the colony. The egg-laying queens were removed from 5 other colonies. One of the colonies remained on the comb after the successful introduction of a young queen. Another colony produced 5 queen cells. All queen cells were transferred after sealing into an incubator, and the queens emerged without any losses. Seven of them were marked with different colors and used for replacement of queens. Four of these replacement queens were accepted.

2.2. Observation of mating flights

The colonies were observed from 9.30 to 16.00 h. Drone mating flights were recorded from colony 2, and queen mating flights from colonies 2 and 6. As previously described, drones departed from the upper part of the colony (dance floor) [17].

2.3. Dissection of queens and spermatozoa counts

Three queens were examined 5–10 min after they had returned from mating flights that lasted more than 15 min. The sting chambers were inspected for a mating sign, and the oviducts and spermathecae were

examined for the presence of spermatozoa. Further, spermatozoa were counted. The spermathecae were dissected and transferred separately into a small dish containing 0.5 mL of Hyes' solution. The sperm was suspended evenly by stirring and blowing air into the droplet with a pipette, and the spermatozoa were counted in a hemocytometer (Rosenthal counting chamber).

In addition, the spermatozoa from egg-laying queens were counted. Seven queens of unknown age were collected from wild colonies in different years and from different locations (Tab. III). Two queens were dissected 1989, 1 queen in 1993 and 1997 respectively, and 3 queens in 1998.

3. RESULTS

3.1. Status of colonies

The combs of 4 colonies were of normal size (ca. 10 × 15 cm) and had egg-laying queens. They were totally covered by bees. Eggs and all stages of worker brood were present, but no drone cells. While the comb of one colony was smaller (5 × 6 cm), another (colony 2) had a larger comb (about 15 × 21 cm) with 460 drone cells. The latter did not have sufficient bees to cover the whole comb, so that sometimes queen cups were visible at the lower edge. The worker and drone brood cells were sealed, and there were no eggs or larvae. A young queen was observed, moving steadily on the comb. We saw her regularly on the surface of the comb (at locations which were not covered by the curtain of bees). During the observation period, several drones matured. The number of drones present on the dance floor between 12.00 and 13.30 h increased from 14 to about 50 over a 1-week period. The number of drones that initiated mating flights also increased. The peak of drone flights was between 12.33 and 13.10 h (GMT +8). Two hundred flights were recorded.

3.2. Reaction to removal of queens

After dequeening of 4 colonies, only one of these colonies produced queen cells; the other 3 colonies left the comb and dispersed in small groups on the branches nearby. Fighting and biting were observed among the worker bees in all groups. Bees were balled and then often fell to the ground. We tried to introduce newly-emerged queens from the incubator, but they were not accepted and subsequently were no longer seen in the colony. A fifth colony, however, accepted a newly-emerged foreign queen a few minutes after removal of the old queen.

3.3. Mating flights of queens

The orange-marked queen in colony 2 could be seen moving on the surface of the curtain on 24.2.1998 for several hours. She was attacked by worker bees several times. Every few minutes she stopped moving, and displayed dorso-ventral shaking behavior. Around noon, while drones were flying, the queen's activity increased and then calmed down again after 13.30 h. The next day the queen appeared on the dancing area on the top of the comb at 12.35 h. She was very seldom chased, and most of the time moved calmly. At 12.50 h she departed on her mating flight, and returned after 21 min at 13.10 h (Tab. I). This queen was caught and dissected within 10 min of her return.

Her sister queen (marked in yellow) had emerged on 24.2.1998 in the incubator. She was introduced immediately after removing the orange-marked queen. She was accepted by the colony, and went on a mating flight at the age of 3 days (26.2.1998). She was dissected shortly after her return, and replaced by her sister queen who emerged on 25.2.1998. This queen showed the same behavior as the previous ones, but did not take a mating flight. Instead, on March 1st, the colony absconded with the virgin queen and left the comb behind without any brood.

Table I. Mating flights of queens.

Colony/queen	Date of emergence	Date of mating flight	Time of mating flight
2 / orange	Unknown	25.2	12.50–13.10 h
2 / yellow	24.2	27.2	12.40–13.03 h
6 / white	8.3	11.3	12.33–12.52 h

After dequeening, colony 6 readily accepted a newly-emerged queen from the incubator, originating from another colony on March 7th. At the age of 4 days, she took a mating flight (Tab. I), and was also dissected upon return.

3.4. A mating sign

All 3 queens examined after their mating flight had the orange-colored cornual secretion of the drone's endophallus adhering to the ventral side within the sting chamber. The thread-like ends had merged and protruded 0.5 to 1 mm from the last sternite (Fig. 1). No mucus was added to the mating sign, as has been observed for the hive-nesting *Apis* species.

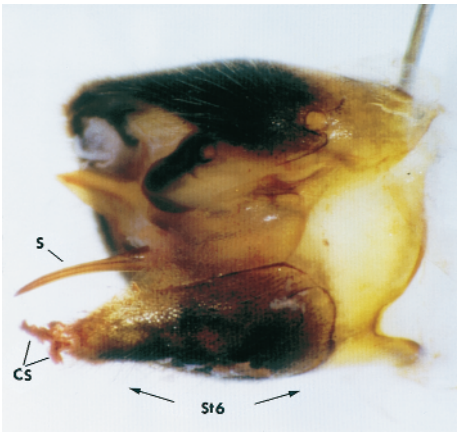

Figure 1. The open sting chamber of a queen which had just returned from a mating flight. Part of the orange-colored cornual secretion could be seen protruding from the last sternite. S: sting; CS: cornual secretion; St 6: 6th sternite.

3.5. Distribution and number of spermatozoa in the spermatheca

3.5.1. Queens after one mating flight

After one mating flight, no sperm could be detected in the lateral oviducts. The spermatozoa in the spermathecae were moving, and formed a ring-shaped undulating thread. The sperm tails moved in a coordinated manner. While in 2 queens only 1 thread could be seen, the third had 2 different threads which seemed to cross twice (Fig. 2). Both of the spermathecae with 1 ring contained 90 000 spermatozoa; the other with 2 rings had 310 000 spermatozoa.

3.5.2. Egg-laying queens of unknown age

In the spermathecae of ovipositing queens, the spermatozoa were evenly distributed and no fast movement could be observed. The spermathecae were either opalescent or marbled in appearance. The two opalescent spermathecae contained 330 000 spermatazoa, and the marbled spermathecae contained between 980 000 and 1 260 000 spermatozoa (Tab. III).

4. DISCUSSION

4.1. Mating flights of queens

Queens initiated mating flights at the age of 3 days. This is the youngest age reported for *Apis*; in all other species queens were at least 5 days old. All queens departed between 12.30 and 13.00 h, during the peak period of drone flights. They returned after 19–23 min. This is about the same queen

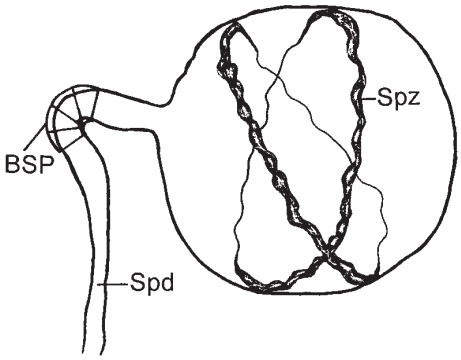

Figure 2. Spermatheca of a queen which had just returned from a mating flight. Two undulating rings of spermatozoa could be seen in the spermatheca. BSP: Bresslau's sperm pump; Spz: spermatozoa; Spd: spermaduct.

flight duration as described for other *Apis* species: *A. koschevnikovi*, 19 min [11]; *A. mellifera*, 15–26 min; *A. cerana*, 10 min [27] and 29 min [16]; *A. florea*, 18–30 min [10]; and *A. dorsata*, 15 min [23]. None of the 3 queens performed a separate orientation flight during the period of drone flight. The first flights of most queens of the other species are orientation flights, also in the free-nesting *A. florea* [11] and *A dorsata* [23]. Probably queens of *A. andreniformis* can learn about their surroundings while still on the outer curtain of the comb. This should be possible in the other free-nesting species, but these queens have short flights. There are too few observations for any final conclusions to be made.

4.2. Mating sign?

The sting chamber of newly-mated *A. florea* queens was empty [10], whereas queens of *A. andreniformis* returned with the orange-colored cornual secretion of the drone's endophallus. It adhered within the sting chamber, and also protruded between 0.5 and 1 mm from the last sternite. It was clearly a sign of mating, as it was the secretion from the cornual glands of drones, as described for *A. mellifera* [7]. Its function, i.e., to strengthen the connection between the queen and the drone during copulation, seems unlikely because this connection is mainly guaranteed by the thumbs of the hind tibia of drones, as described for *A. florea* [9, 21].

In *A. andreniformis* no mucus is added to the cornual secretion, as the mucus glands are tiny and do not appear to produce much mucus. In the hive-nesting bees and in *A. dorsata*, the mucus glands of drones are well developed [9]. During the mating process, mucus fills the endophallus and thus strengthens the connection between the queen and the copulating paralyzed drone until sperm transfer has occurred [9] After mating, mucus remains together with the cornual secretion as a mating sign which fills the sting chamber. The mating sign keeps the sting chamber open and can be clearly seen. In *A. mellifera*, it has been shown that drones prefer to mate queens that already have a mating sign [3]. Because the orange-colored secretion protrudes from the last sternite in *A. andreniformis*, drones might be able to recognize it as well.

4.3. Sperm transfer and number of spermatozoa

In *A. andreniformis*, no spermatozoa were found in the oviducts. Drones seemed to inject the sperm into the spermaduct, as described for *A. florea* [10], from where the spermatozoa obviously reached the spermatheca within a few minutes. In both species the morphology of the endophallus is similar, ending in a fine tip which fits into the orifice of the spermaduct. The tip is not long enough to reach the spermatheca [6]. The undulating threads of the spermatozoa looked as if they had just left the spermaduct and did not disperse immediately, as they had in ovipositing queens. They were ring-shaped, possibly because spermatozoa tend to adhere to surfaces as they move along the

Table II. Number of spermatozoa in the spermatheca of queens after 1 mating flight in Tenom in 1998.

Colony/queen	Flight duration (min)	Sperm No. (million)
2 / orange	20	0.09
2 / yellow	23	0.31
6 / white	19	0.09

spermathecal membrane [1]. The second ring in 1 queen (Fig. 2) may have resulted from a situation where 1 ring was shifted from the entrance before the second thread left the spermaduct. Ruttner [20] studied a number of queens immediately, and then 2 to 25 h after mating. The spermatozoa were dispersed, even when only a few had entered the spermatheca.

In 2 queens the spermathecae contained 0.09, and in the third 0.31 million spermatozoa after 1 flight (Tab. II). These sperm counts were very low compared to the ovipositing queens with 0.33–1.26 million spermatozoa. It is possible that there was a low concentration of drones in the surrounding plantations, as we found drones only in 1 of the 6 discovered colonies. The duration of mating flight time, i.e., between 19 and 23 minutes, was in the normal range of other *Apis* species. It is also possible that queens generally perform several flights to obtain more spermatozoa.

As 2 queens had fewer spermatozoa in the spermatheca than a drone in the vesiculae seminales [4], we assumed that the sperm came from mating with 1 drone. In this case, about 70% of the spermatozoa from 1 drone reached the spermatheca. The third queen, which had 310 000 spermatozoa in the spermatheca, may have mated with 3 drones on average, which corresponds to 79% of each drone's sperm. There are several possible explanations for this: a) dissecting the queen 10 min after her return from mating did not allow enough time for the spermaduct to be emptied; b) *A. andreniformis* drones may not succeed in transferring their total amount of spermatozoa; c) not all spermatozoa may be able to reach the spermatheca, even if it is still empty (as demonstrated in this study); and d) queens eject the spermatozoa, as suggested by Oldroyd et al. [15]. It does not seem likely that the *A. andreniformis* queen can expel much of the semen that has reached the lumen of the spermaduct or of the spermatheca. Spermatozoa have the tendency to adhere onto surfaces and also to unite in a string with coordinated swinging tails [1], corresponding to the observation that the spermatozoa form an undulating thread immediately after mating and are still active within the spermatheca. Furthermore, it does not seem feasible that sperm is moved with the Bresslau's sperm pump into the spermatheca and in the opposite direction towards the oviducts at the same time.

The transfer of sperm into the spermaduct in *A. florea* and *A. andreniformis* and the filling process in the spermatheca is almost finished by the time the queen returns from her flight. Thus the queen has less than 1/2 h to influence the composition of the spermatheca. In the hive-dwelling species of *Apis* in which the endophallus of the drones ends in a bulb, spermatozoa are injected into the oviducts [2, 4, 26]. In this case, the filling of the spermatheca takes more than 25 h and only between 5 and 10% of the spermatozoa reach the spermatheca. The results of this study suggest that in *A. andreniformis* between 70 and 80% of the spermatozoa from the first drones reach the spermatheca. Comparing the average number of matings (13.5; [14]) and the average number of spermatozoa in the vesiculae seminales (0.13 million; [5]) and in the spermatheca (0.78 million; Tab. III), each drone could transfer an average of 0.05 million spermatozoa, which corresponds to about 40% of their spermatozoa. Considering 9 effective matings [14], most drones inject 0.086 million spermatozoa – on average 66%. The same calculation for *A. mellifera* (drones: 10 million; queens: 5 million; and about 13 matings [14]) shows that 3.5% of their spermatozoa reach the spermatheca.

Table III. Number of spermatozoa in the spermatheca of egg-laying queens of unknown age.

Location	Colony	Year	Sperm No. (million)
Tenom	1	1998	0.33
Tenom	4	1998	0.33
Tenom	6	1998	1.26
Tenom	–	1997	1.10
Tenom	–	1993	0.37
Johore*	1	1989	0.98
Johore*	2	1989	1.09

* Koeniger et al. [5].

4.4. First male advantage?

The mode of sperm transfer seems to affect the number and sequence of the spermatozoa of each drone that reach the spermatheca. When sperm is injected into the oviducts, as is normal in all cavity-dwelling honeybees, the queen may be able to directly influence how much sperm, and which sperm is stored. By muscular activity of the abdomen she can control the speed at which the sperm passes the entrance to the spermaduct, thus influencing the chances of the spermatozoa of being able to enter. The rate of expelled spermatozoa can be regulated additionally by the valvula vaginalis. The queen expels between 90 and 95% of the sperm received during mating [1, 20].

Thus, the type of sperm transfer may have an influence on the first or last male's advantage. In the case when sperm is injected into the oviducts, most sperm from the first drone possibly lies near the ovaries in the lateral oviducts, while sperm from the last drone may remain in the median oviducts closer to the spermaduct and may have the opportunity of being able to enter the spermatheca first. In addition, the queen can regulate how much sperm is stored. The muscular activity of the oviducts may mix the sperm, and also may control the speed at which the sperm passes the entrance to the spermaduct. Thus, the queen can influence the chances of spermatozoa being able to enter the spermaduct.

In the case when sperm is injected into the spermaduct, the queen's influence on the amount of sperm per drone that can migrate during the mating process is restricted to less than 1/2 h. We hypothesise that the first drones have a mating advantage until the spermatheca contains a fair amount of spermatozoa. As already discussed above, it does not seem likely that a queen can expel spermatozoa from the spermatheca.

4.5. Number of spermatozoa in the spermatheca

Spermatozoa of ovipositing queens of all species were dispersed in the spermathecae, and only slow movements could be recognized. The concentration of spermatozoa (million/mm^3) in the spermatheca is similar in all studied species: concentrations of fully mated, ovipositing queens normally range between 3 and 5 million/mm^3 [5]. In *A. andreniformis* up to 1.26 million spermatozoa were counted in the spermatheca with volumes of about 0.28 mm^3, which corresponds to 4.63 million spermatozoa per 1 mm^3 [5]. There are no reported sperm numbers of queens that have successfully initiated oviposition after mating flights.

The above results and discussion are based solely on observations of 3 queens returning from mating flights. It is likely that during other seasonal conditions the results may differ. For example, a higher number of matings may occur during one flight. Though these observations are still incomplete, some interesting results have been obtained. We hope that these observations and discussion may stimulate researchers to conduct further studies to complete our knowledge on the different mating systems in *Apis* species.

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Résumé – Vols de fécondation et transfert de sperme chez l'abeille naine, *Apis andreniformis* (Smith, 1858). L'abeille naine *Apis andreniformis* n'a été redécouverte comme espèce propre qu'en 1987 [28]. Depuis les études de biologie comparée avec les autres espèces d'*Apis* ont été nombreuses. Nous décrivons ici les vols de fécondation des reines et le mode de transfert du sperme.

Six colonies ont été trouvées dans les plantations de cacaoyers de la Station Expérimentale d'Agriculture de Tenom, Sabah, en Malaisie. Une colonie possédait sept cellules royales ; elles ont été prélevées et placées en étuve. Toutes les reines ont éclos. Les colonies avec les jeunes reines ont été observées de 9.30 à 16.00 h. Lorsque la reine rentrait d'un vol de fécondation, elle était immédiatement capturée et disséquée. Dans les dix minutes qui suivaient, l'une des reines écloses en étuve était introduite dans la colonie.

Nous avons pu observer les vols de fécondation de trois reines. Les reines se sont envolées pendant la principale période de vol des mâles, entre 12.33 et 12.50 h. Le vol durait entre 19 et 24 min (Tab. I). Toutes les reines avaient dans la chambre de l'aiguillon la secrétion de couleur orange des cornules de l'endophallus du mâle (Fig. 1), qui peut donc être considérée comme un signe de fécondation.

Les reines ont été disséquées entre cinq et dix minutes après leur retour dans la colonie. Aucun sperme n'a été trouvé dans les oviductes, mais les spermathèques renfermaient entre 0,09 et 0,31 millions de spermatozoïdes (Tab. II). Ils bougeaient et formaient un ruban ondulant en forme d'anneau. Dans la spermathèque qui renfermait 0,31 million de spermatozoïdes, il y avait deux anneaux (Fig. 2). D'après ces résultats le transfert de sperme a eu lieu, comme chez *A. florea*, directement dans le canal de la spermathèque et non pas dans les oviductes, comme c'est le cas chez les espèces d'*Apis* qui nidifient dans des cavités.

Les spermathèques de sept reines pondeuses d'âge inconnu renfermaient entre 0,33 et 1,26 million de spermatozoïdes qui étaient toujours répartis régulièrement. Le faible nombre de spermatozoïdes chez les reines qui ne s'étaient accouplées qu'une fois pourrait s'expliquer par le fait qu'habituellement elles effectuent plusieurs vols de fécondation. Il est aussi possible que le nombre de mâles présents ait été trop faible.

Deux des reines fraichement fécondées possédaient 0,09 million de spermatozoïdes, ce qui ne représente que 70 % des spermatozoïdes produits dans les vésicules séminales d'un mâle. La spermathèque de la troisième reine renfermait 0,31 million de spermatozoïdes, soit 79 % des spermatozoïdes produits par trois mâles. Si l'on prend pour base le nombre moyen des accouplements [14], le nombre moyen de spermatozoïdes dans les vésicules séminales [5] et celui dans les spermathèques des reines pondeuses (Tab. III), 40 % environ des spermatozoïdes d'un mâle atteignent la spermathèque de la reine chez *A. andreniformis*. Si l'on considère les accouplement effectifs, c'est 66 %. D'après les résultats de notre étude nous estimons que 70 à 80 % des spermatozoïdes des trois premiers mâles qui s'accouplent avec une reine atteignent sa spermathèque.

Lors des accouplements chez les espèces d'*Apis* qui nidifient dans des cavités, les spermatozoïdes parviennent d'abord dans les oviductes. Puis, durant plus de 24 h, a lieu le transfert dans la spermathèque, au cours duquel seuls 3 à 10 % des spermatozoïdes reçus atteignent la spermathèque.

Dans le cas du transfert du sperme dans le canal de la spermathèque (*A. florea*, *A. andreniformis*), le remplissage de la spermathèque est achevé dès que la reine rentre de son vol de fécondation. La reine dispose alors moins d'une demi-heure pour influencer le remplissage de sa spermathèque. Le mode de transfert du sperme pourrait avoir une influence sur le nombre de spermatozoïdes et sur l'ordre dans lequel les spermatozoïdes des divers mâles atteignent la spermathèque.

***Apis andreniformis* / vol de fécondation / transfert de sperme / nombre de spermatozoïdes / reine**

Zusammenfassung – Hochzeitsflüge und Spermaübertragung bei der Zwerghonigbiene *Apis andreniformis* (Smith, 1858). Die Zwerghonigbiene *Apis andreniformis* wurde erst 1987 als eigene Art wieder entdeckt. Seit dieser Zeit wurden viele Untersuchungen zu einer vergleichenden Biologie mit den anderen Arten der Honigbienen durchgeführt. Hier beschreiben wir Hochzeitsflüge der Königinnen und den Modus der Spermaübertragung.

In den Kakoaplantagen der landwirtschaftlichen Versuchsstation in Tenom (Sabah, Malaysia) wurden sechs Völker gefunden. Ein Volk hatte sieben Weiselzellen, die ausgeschnitten und in einem Brutschrank gehalten wurden. Alle Königinnen schlüpften. Die Völker mit jungen Königinnen wurden von 9.30 bis 16.00 Uhr beobachtet. Nach Rückkehr von einem Hochzeitsflug wurden die Königinnen sofort abgefangen und seziert. Innerhalb von 10 Minuten wurde dem Volk jeweils eine der im Brutschrank geschlüpften Königinnen zugesetzt.

Die Hochzeitsflüge von insgesamt drei Königinnen konnten beobachtet werden. Die Königinnen starteten während der Hauptflugzeit der Drohnen zwischen 12.33 und 12.50. Die Flugdauer betrug 19 bis 23 Minuten (Tab. I). In den Stachelkammern aller Königinnen befand sich das orangefarbene Sekret der Cornua des Begattungsorgans der Drohnen (Abb. 1). Es kann somit als Zeichen der Begattung gewertet werden.

Die Königinnen wurden 5 bis 10 Minuten nach ihrer Landung auf der Wabe seziert. In den Ovidukten wurden keine Spermien entdeckt, aber die Spermatheken enthielten 0,09 und 0,31 Millionen Spermatozoen (Tab. II). Die Spermien bewegten sich und bildeten ein wellenförmiges Band. In der Spermatheka mit 0,31 Millionen gab es sogar zwei Bänder (Abb. 2). Die Spermaübertragung erfolgt nach diesen Ergebnissen wie bei der *A. florea* direkt in den Spermadukt und nicht in die Ovidukte, wie es bei den höhlenbrütenden Honigbienen geschieht.

Bei sieben eierlegenden Königinnen unbekannten Alters enthielten die Spermatheken zwischen 0,33 und 1,26 Millionen Spermatozoen, die immer gleichmäβig verteilt waren. Die geringe Anzahl an Spermatozoen in den Königinnen nach einem einzigen Hochzeitsflug könnte zum einen damit erklärt werden, dass sie normalerweise mehrere Hochzeitsflüge durchführen. Zum anderen ist es auch möglich, dass zu wenig Drohnen vorhanden waren.

Zwei der frisch begatteten Königinnen hatten mit 0,09 Millionen jeweils nur 70 % der Spermatozoen aufgenommen, die ein Drohn in seinen Vesiculae seminales produziert. Die Spermatheka der dritten Königin enthielt mit 0,31 Millionen Spermien 79 % der Anzahl, die von drei Drohnen produziert werden. Legt man die Durchschnittszahlen von Paarungen [14] und der mittleren Anzahl der Spermatozoen in den Vesiculae seminales [5] und in den Spermatheken eierlegender Königinnen (Tab. llI) zugrunde, erreichen etwa 40 % der Spermatozoen eines Drohns bei *Apis andreniformis* die Spermatheka. Bei der Berücksichtigung der effektiven Paarungen sind es 66 %. Nach den Ergebnissen unserer Versuche halten wir es für wahrscheinlich, dass von den ersten drei Drohnen, die eine Königin paaren, meist 70 bis 80 % ihrer Spermatozoen in die Spermatheka gelangen.

Bei den höhlenbrütenden Arten der Honigbiene gelangen die Spermien bei den Paarungen zunächst in die Ovidukte. Danach dauert die Übertragung in die Spermatheka mehr als 24 Stunden, wobei nur 3 bis 10 % der aufgenommenen Spermatozoen in die Spermatheka gelangen. Im Falle der Übertragung des Spermas in den Spermadukt (*A. florea* und *A. andreniformis*) ist die Füllung der Spermatheka bereits mit der Rückkehr der Königin vom Hochzeitsflug abgeschlossen. Damit bleibt der Königin weniger als eine halbe Stunde Zeit, die Füllung der Spermatheka zu beeinflussen. Die Art der Spermaübertragung könnte damit Einfluβ darauf haben, wie viele und in welcher Reihenfolge die Spermien der einzelnen Drohnen in die Spermatheka gelangen.

***Apis andreniformis* / Königin / Hochzeitsflug / Spermaübertragung / Anzahl der Spermatozoen**

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