Apidologie 32 (2001) 407–416

Original article

Apis dorsata drone flights, collection of semen from everted endophalli and instrumental insemination of queens

Jerzy WOYKE[a]*, Jerzy WILDE[b], Maria WILDE[c]

[a] Agricultural University – SGGW, Bee Division, 166 Nowoursynowska 02-787 Warsaw, Poland
[b] WM University, Apiculture Division, Olsztyn, Poland
[c] Dabur Apicultural Center, Jugedi, Chitwan, Nepal

(Received 30 November 2000; revised 28 April 2001; accepted 18 May 2001)

Abstract – We observed drone flights of 16 colonies of *Apis dorsata* in Chitwan, Nepal. At the end of February drone flights occurred between 18:15 and 18:35 h. By April, as day length increased, drones flew gradually later. Within 2 months the start of drone flights was delayed by 42 min. High correlation ($r = 0.99$) was found between the sunset time and the start of drone flights. After the thorax or abdomen of drones were squeezed, seven stages of endophallus eversion occurred. Semen appeared as a small drop at the ventral side of completely everted cervix. We collected 8 mm^3 of semen from 41 drones. Thus, one drone produced on average 0.2 mm^3 of semen. Three *Apis mellifera* queens were inseminated with 2–3 mm^3 of *A. dorsata* semen. All queens started to lay eggs. Larvae hatched from 3% of eggs. After sealing of the larvae, only drone pupae were found. We speculate that embryos in rest of the eggs did not develop due to genetic incompatibility.

drones / drone flight time / eversion of endophallus / semen / instrumental insemination / *Apis dorsata*

1. INTRODUCTION

Until now, no one has collected semen from everted *Apis dorsata* Fabricius drone endophalli. The volume of semen produced by *A. dorsata* drones also is not known, probably due to difficulties in collecting semen from everted endophalli of *A. dorsata* drones. In preliminary attempts, we did not find any semen in everted *A. dorsat*a endophalli. The semen also was not presented in published figures and descriptions

* Correspondence and reprints
E-mail: woyke@alpha.sggw.waw.pl

of everted *A. dorsata* endophalli (Simpson, 1970; Koeniger et al., 1990). We think that the difficulties in collecting *A. dorsata* semen are due to the lack of the knowledge of the eversion process of *A. dorsata* endophallus, and the phenomena occurring during that process.

The eversion process of *A. mellifera* L. endophalli was described by Woyke (1955, 1958) and Woyke and Ruttner (1958) and of *A. cerana* by Ruttner et al. (1973).

If the appearance of semen during the eversion of *A. dorsata* endophalli was known, the semen could be collected and measured. It could be used in homo- or heterospecific instrumental inseminations. Heterospecific instrumental inseminations of *Apis* species were initiated by Woyke (1973). He collected the semen from everted drone endophalli and inseminated both *A. cerana indica* and *A. mellifera* with heterospecific semen. Ruttner and Maul (1983) also inseminated both *A. mellifera* and *A. cerana* with heterospecific semen. Woyke (1993) inseminated *A. florea* queens with *A. mellifera* semen. Koeniger et al. (1996) did not collect the semen from drone endophalli but from the *vesiculae seminale*s and inseminated both *A. cerana* and *A. koschevnikovi* with heterospecific semen and *A. koschevnikovi* queens with *A. dorsata* semen. In all those crosses, the semen of one species entered the queen spermatheca of the other species. Ruttner and Maul (1983) showed that the spermatozoa were able to fertilize the eggs. However, the embryo did not develop. Koeniger et al. (1998) reported on hybrids obtained from crosses between the closely related *A. cerana* and *A. koschevnikovi*.

We observed drone flights and investigated the eversion process of *A. dorsata* endophallus, to find out how to collect the semen, and to measure the volume of semen ejaculated. We also inseminated *A. mellifera* queens with the semen of *A. dorsata* drones.

2. MATERIALS AND METHODS

The investigation on the eversion of *A. dorsata* endophalli was started by Woyke in the Central Bee Research Institute in Poona, India in 1973/1974, continued in the Bee Biology Research Unit, Chulalongkorn University in Bangkok, Thaïland in 1992 and followed in the Bee Division of the Agricultural University, Warsaw, Poland. We conducted investigations in Dabur Apicultural Center, Jugedi, Chitwan, Nepal in 1999. The investigations on drone flights and semen collection were conducted in the Institute of Agriculture and Animal Science of Tribhouven University in Rampur (latitude 28°46' North, longitude 84°21' East).

2.1. Time of drone flights

Drone flights of 16 colonies were observed. The nests were 4–5 m above the ground in windows and under roof of a building. One person observed drone flights from the ground through binoculars (12 × 50 Zenith). This person walked along the building from one end to the other, which took 30 s to 1 min. A second person made close up observations of one nest through a window, from a distance of 25–50 cm. The local time was + 6:15 h GMT. The sunset time was determined by data presented by CNN for Pokhara (www.cnn.com/WEATHER/as/Nepal/PokharaVNPK.html) (lat. 28°55' N., long. 84°00' E.). Since Rampur is 0°09' South and 0°21' East of Pokhara, the sunset occurs here 1:24 min earlier.

2.2. Eversion of the endophallus and collection of semen

A. dorsata drones of various ages were examined. Endophallus eversion of hundreds of drones was investigated. To investigate the eversion process, the drone thorax or abdomen was gradually squeezed. Endophalli under full internal pressure were

examined under a stereomicroscope, and were drawn with the aid of a Nikon drawing apparatus. The drawings were scanned into a computer and were elaborated with CorelDRAW© 9.0 software.

Semen was collected from drones caught during their flights at dusk. The drone thorax or abdomen was squeezed and the pressure was gradually increased. The stage at which the semen appeared outside the everted endophallus was recorded, as well as the number of drones ejaculating the semen. The semen was collected in calibrated tip of the syringe for instrumental insemination. Results of two trials, one consisting of 70 drones and the other of 90 drones are presented here.

2.3. Instrumental insemination of queens

Three *A. mellifera* queens were inseminated with the semen of *A. dorsata* drones. The queens were maintained in populous mating nuclei with three trapezoid combs $13 \times 9 \times 8$ cm. The nuclei were periodically reinforced with sealed brood from other nuclei. Queen excluders were attached to the entrances to prevent natural matings of queens. Egg laying of the queens was noted and the presence or absence of larvae were checked in weekly intervals. The few larvae that hatched were allowed to develop into pupae.

3. RESULTS

3.1. Time of drone flights

Drones flew from all 16 colonies. The characteristic "wuum" sound was audible. However, due to distance and lack of sunlight, it was difficult to determine the exact time of the beginning and especially the end of flights of particular colonies. Drones flew from all colonies at dusk, but those from some colonies started or ended the flights a few (2–3) minutes earlier or later. Table I shows the results of the colony observed at close range. At the end of February drone flights occurred between 18:15 and 18:35 h. and lasted 20 min. As the time passed and day length increased, drones flew gradually later. At the end of April, drones flew between 18:57 and 19:17 h. Thus, within 2 months, the start of drone flights was delayed by 42 min. The duration of flights oscillated between 20 to 21 min.

Table I shows that the sunset in Rampur on 24 February occurred at 18:19 h and on 27 April at 18:55 h, which is 36 min later. A high correlation was found between sunset time and the start of drone flights ($r^2 = 0.989$, $p = 0.0002$). At the time of

Table I. Flights of *Apis dorsata* drones from one colony observed at close range in Rampur, Chitwan, Nepal, 1999.

Date	Flight time h	Duration min	Sunset h	Diff.* min	Sunrise h	Day length h
Feb. 24	18:15–18:35	20	18:19	4	6:52	11:27
March 27	18:37–18:58	21	18:38	1	6:19	12:25
April 2	18:40–19:00	20	18:41	1	6:12	12:29
April 12	18:42–19:03	21	18:47	5	6:01	12:46
April 20	18:50–19:10	20	18:51	1	5:54	12:57
April 27	18:57–19:17	20	18:55	–2	5:47	13:08
Diff.**	0:42–0:42		0:36		1:05	1:41

* Difference between sunset time and start of drone flights.
** Difference between data concerning February 24 and April 27.

investigation, a delay of sunset by 1 min was related with a delay of drone flights by 1:06 min. On average, the drones started to fly 1:40 min before the sunset time. However, variation was found from 5 min before to 2 min after the sunset time.

24 February was 11:27 h long, and 27 April was 13:08 h, a difference of 1:41 h. High correlation was found between the length of the day and the start time of drone flights (r^2 = 0.988, p = 0.0002). In addition, there was a high negative correlation between sunrise time and the start of drone flight (r^2 = – 0.987, p = 0,0003). Acceleration of sunrise by 1 m was related with 36 s delay of the start of drone flights.

3.2. Eversion of the endophallus and collection of semen

After the thorax of a drone was squeezed, one of the three phenomena occurred:

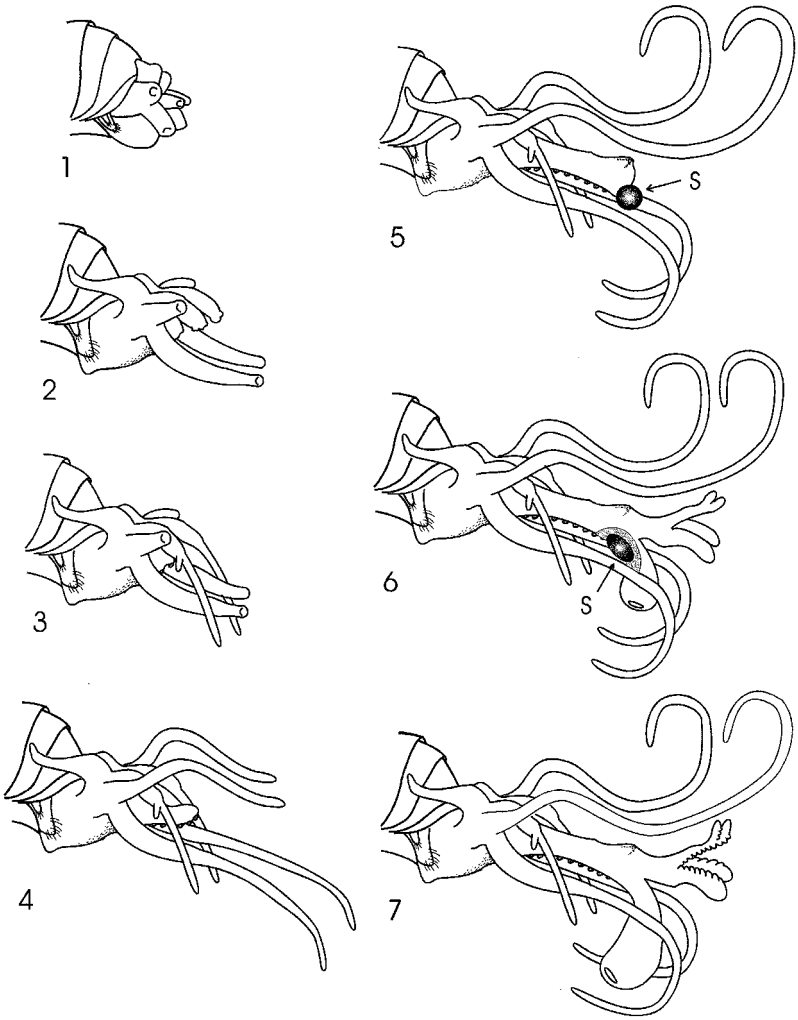

Figure 1. Lateral view of seven stages (1–7) of eversion of the drone endophallus of *Apis dorsata*. S – semen.

(1) no eversion of endophalli, (2) partial eversion, or (3) complete or almost complete eversion of endophalli.

When the abdomen of a drone, which did not evert the endophallus, was squeezed from both sides, the vestibulum with rudiments of all cornua appeared at first (Fig. 1.1). Next the eversion continued through two stages (no. 2 and 3) in which the eversion of the cornua proceeded. The eversion stopped at the stage of partial eversion (Fig. 1.4) At this stage the dorsal and medial cornua had everted completely, the ventral cornua everted almost completely, and the lateral ones everted to about half their length. Quite high pressure was required to induce eversion of the cervix (Fig. 1.5). At this stage all the cornua had everted completely. This stage was followed by almost complete eversion of the endophallus (Fig. 1.6). Again, increased pressure was required to induce completely eversion of the endophallus with the bulbus everted completely and fimbria visible on the fimbriate lobe (Fig. 1.7). No semen appeared during the process of eversion in this group of drones.

Semen appeared in drones, which, after the thorax was squeezed, suddenly everted the endophallus to the partially everted stage. (Fig. 1.4). Out of 70 drones whose thorax was squeezed, this phenomenon occurred only in 8 (11%). A very careful increase of pressure was required to evoke complete eversion of the endophallus cervix. A small drop of creamy semen appeared at the anal-ventral edge of the cervix (Fig. 1.5 S). If the increase of pressure was not controlled adequately, the next stage of almost completely eversion occurred (Fig. 1.6). In this case, the semen was found on a layer of white mucous located at the ventral side of the endophallus, in an area between the end of the cervix and the beginning of the bulbus (Fig. 1.6 S).

Semen was found also in 3 (4%) out of 70 drones which everted the endophallus almost completely or completely after their thorax was squeezed. The semen was located in the same place as described above (Fig. 1.6 S). Thus out of 70 drones whose thoraces were squeezed, only 11 (15.7%) everted the endophallus partly or almost completely, and, it was possible to collect the semen from them. Together, 2 mm^3 of semen was collected from those 11 drones. Thus, one drone supplied on average 0.2 mm^3 of semen.

A very high proportion (84%) of drones failed to ejaculate semen during lateral squeezing of the abdomen. We undertook different efforts to increase the proportion of drones ejaculating the semen. We found that after the abdomen was pushed in the anal direction, instead of being squeezed sideways, some drones everted the endophallus suddenly up to the partly everted stage. Further increase of abdominal pressure resulted in complete eversion of the cervix with a drop of semen on its end (Fig. 1.5). This way, out of the next series of 90 drones, we were able to collect the semen from a total number of 30 (33%) drones.

In the case when individual drones produced a small amount of semen, relatively large amounts adhered to the inside syringe wall on either end of the semen cylinder. This amount could not be measured, and consequently the measured volume of semen from individual drones is inaccurate. When semen from several drones was collected together, the adhering amount outside the semen cylinder was small relative to the larger volume of semen. Consequently, the mean volume collected from multiple drones was more accurate. Therefore, we measured the volume "step by step" during the whole process of semen collection from all those drones. We collected 2 mm^3 of semen from the first 10 drones. Continuing the collection into the same syringe tip, we accumulated 4 mm^3 from 20 drones and further collection resulted in 6 mm^3 of semen from the total number of the 30 drones.

Together in both trials, we collected $2 + 6 = 8$ mm^3 of semen from $11 + 30 = 41$ drones. Thus, one drone produced on average 0.2 mm^3 of semen.

3.3. Instrumental insemination of queens

Three *A. mellifera* queens were inseminated with semen of *A. dorsata* drones. One queen was inseminated with 2 mm^3 of semen and two with 3 mm^3. All queens started to lay eggs several days after insemination. Weekly examinations revealed eggs present in the central comb of the nucleus within an elliptical area of 8×5 cm. About 250 eggs were present on both sides of the comb. One queen survived 35 days, the second 100 days, and the third one 130 days. Together 750, 3000 and 4000 of eggs of the three respective queens were examined. About 3% of eggs hatched. Thorough examinations revealed 3 to 5 larvae within the 125 eggs present on each comb side. After the brood cells were sealed, only protruding cappings were present, and examination of the contents revealed drone pupae. Five combs (1, 2 and 2 from the respective queens) containing about 250 eggs each, were put in an incubator (34 °C, 90% RH) and again about 3% of eggs hatched.

4. DISCUSSION

A. dorsata drones fly at dusk between 18:00 and 18:45 h. The flight period of 7 colonies ranged over 45 min counted on 9 days. 90% of flights occurred within 30 min (Koeniger and Wijayagunasekera, 1976; Koeniger et al., 1988). Flight duration of drones from individual colonies lasted on avg. 24.0 or 25.6 min (Tan et al., 1999). This duration is similar to the 20–21 min period presented in this paper.

Drone flights were observed over 9 days (Koeniger and Wijayagunasekera, 1976); 2 days (Koeniger et al., 1988), 4 days (Rinderer et al., 1993); 5 days (Tan et al., 1999) or 10 days (Koeniger et al., 1994). We observed drone flights over 2 months, which enabled us to detect changes in the start of drone flights relative to the progress of the season and increase in daylength.

Previous investigations on *A. dorsata* drone flights were conducted in lower latitudes: Sri Lanka 8°, Sabah 5°, Thailand 12°, and Vietnam 14°. In those places the day length is about 12 h, with only slight changes during the year. We observed drone flights at high latitude of 28°46', at the northern border of *A. dorsata* distribution where the changes of day length are much larger.

For the first time, we compared the beginning of drone flight with exactly determined time of sunset. Thus, we were able established high correlations between the beginning of drone flights and sunset time, sunrise time, and length of day. The flight period was delayed 42 min over two months as daylength increased. This finding has not been reported previously. Although Koeniger et al. (1994) did not specifically mention this phenomenon, the same conclusion can be drawn from their data, according to which the drones flew on 1 January between 18:27–18:40, and on 14 January between 18:30–18:52.

When semen was collected from *A. dorsata* it appeared on the ventral edge of the completely everted cervix of the endophallus. In contrast, semen appears at the anal dorsal edge in *A. mellifera* (Woyke, 1955; Woyke, 1958) and *A. cerana* (Ruttner et al., 1973). The volume of semen produced by one *A. dorsata* drone has not been measured previously. It is interesting to note that the big *A. dorsata* drone produced only 0.2 mm^3 of semen. This amount is much smaller than the 1.2 mm^3 of semen produced by *A. mellifera* drones (Woyke, 1960). This amount is similar to the 0.16 mm^3 (Woyke, 1973) or 0.21 mm^3 (Woyke, 1975) of semen produced by the much smaller *A. cerana* drone. This small volume of *A. dorsata* semen corresponds to the low number of spermatozoa found previously in the *vesiculae seminales* of *A. dorsata* drones: 2.46×10^6 ($n = 5$) (Koeniger et al., 1990) or 1.24×10^6 ($n = 31$) (Tan et al., 1999). The number of spermatozoa in *A. dorsata* is higher than the 0.93×10^6 spermatozoa

produce by *A. cerana* drones (Ruttner et al., 1973), but much lower than the 11×10^6 produced by *A. mellifera* drones (Woyke, 1960).

The concentration of spermatozoa in 1 µl of *A. dorsata* semen from everted endophalli (but not from *vesiculae seminales*) would be $2.46 \times 10^6 / 0.2 = 12.3 \times 10^6$ for data presented by Koeniger et al. (1990) and $1.24 \times 10^6 / 0.2 = 6.2 \times 10^6$ for presented by Tan et al. (1999). The latter figure is closer to the 7.7×10^6 spermatozoa concentration in *A. mellifera* semen (Woyke, 1960).

It is well known that semen of one *Apis* species enters into the spermathecae of other species (Woyke, 1973; Ruttner and Maul, 1983; Woyke, 1993; Koeniger et al., 1996). According to Ruttner and Maul (1983, p. 317), "Heterospecific insemination is as efficient as homospecific". According to Koeniger et al. (1996, p. 304), "the two queens inseminated heterospecifically with *A. dorsata* semen had spermatozoa in their spermatheca. In all experiments heterospecific spermatozoa were stored in the spermathecae. It seems that the physiology of sperm storage is similar for all *Apis* species even within the less related open nesting *A. dorsata*". Thus, there is no reason to doubt that *A. dorsata* spermatozoa entered the spermathecae of queens inseminated by us. In our investigations most eggs (97%) did not hatch. This percentage is similar to the 92% of fertilized eggs deposited by heterospecifically inseminated queens (Ruttner and Maul, 1983). Thus the explanation may be similar. Our results suggest that the eggs were fertilized, but due to genetic incompatibility the embryos did not develop and consequently the eggs did not hatch.

ACKNOWLEDGEMENTS

The senior author would like to express sincere thanks to Dr. R.P. Phadke and Dr. K.K. Kshirsagar, Central Bee Research Institute, Pune, India, and Dr. S. Wongsiri, Chulalongkorn University, Bangkok, Thailand. Our special thanks are due to the chairman of the Dabur Company Mr. A.C. Burman for providing us the facilities to conduct these investigations in Nepal. We thank also M. Sci Robert Kolasiński for his initiative to conduct research investigations in Nepal, and for his interest and help, and Mr. K.M. Neupane for his help in the access to *A. dorsata* colonies.

Résumé – Les vols des mâles d'*Apis dorsata*, prélèvement de sperme dans l'endophallus en éversion et insémination artificielle des reines. Jusqu'à présent personne n'a prélevé de sperme dans l'endophallus en éversion des mâles d'*Apis dorsata*. Le volume de sperme produit est également inconnu, vraisemblablement par méconnaissance du processus d'éversion de l'endophallus. À Rampur (Népal), nous avons observé les vols de mâles de 16 colonies d'*A. dorsata*, étudié le processus d'éversion de l'endophallus et trouvé le moyen de prélever du sperme et de mesurer le volume de sperme injecté lors de l'accouplement. Nous avons également inséminé des reines d'*A. mellifera* avec du sperme de mâles d'*A. dorsata* afin de déterminer l'intercompatibilité entre ces deux espèces. Le tableau I montre qu'à la fin de février les vols des mâles avaient lieu entre 18 h 15 et 18 h 35. Au fur-et-à-mesure que le printemps avançait et que la longueur du jour augmentait, les mâles s'envolaient plus tard. Une corrélation élevée ($r = 0,99$) a été trouvée entre l'heure du coucher du soleil et le début de l'envol des mâles. En deux mois, le début de l'envol des mâles a reculé de 42 min. L'éversion de l'endophallus de centaines de mâles a été étudiée. Les mâles ont été capturés à la tombée de la nuit. Une pression croissante a été appliquée sur le thorax et l'abdomen des mâles. Le sperme a été récolté dans l'extrémité calibrée d'une seringue pour insémination artificielle. Après avoir pressé le thorax des mâles, nous avons observé l'un des trois phénomènes suivants : (i) pas d'éversion de l'endophallus, (ii) éversion partielle, (iii) éversion presque complète. Lorsque l'abdomen des mâles, dont l'endophallus ne s'était pas retourné, était pressé des deux

côtés, le vestibulum apparaissait d'abord avec des rudiments des cornules (Fig. 1.1). Puis l'éversion se poursuivait jusqu'au stade d'éversion partielle (Fig. 1.4). Une augmentation de la pression provoquait l'éversion brutale du cervix (le « cou » de l'endophallus) (Fig. 1.5). Ce stade était suivi par l'éversion presque complète de l'endophallus (Fig. 1.6). Une pression encore plus forte était nécessaire pour obtenir l'éversion complète (Fig. 1.7). Le sperme apparaissait chez les mâles qui, suite à la pression exercée sur leur thorax, renversaient soudainement l'endophallus jusqu'au stade d'éversion partielle (Fig. 1.4). Le phénomène n'est survenu que chez huit (11 %) des 70 mâles dont le thorax avait été pressé. Une augmentation très prudente de la pression était nécessaire pour obtenir l'éversion complète du cervix de l'endophallus. Une gouttelette de sperme crémeux apparaissait alors à son extrémité (Fig. 1.5 S). Le sperme est également apparu chez 3 (4 %) des 70 mâles qui avaient presque complètement ou complètement retourné leur endophallus après pression sur le thorax. Dans ce cas le sperme a été trouvé sur une couche de mucus blanc située sur la face ventrale de l'extrémité du cervix (Fig. 1.6 S). Nous n'avons donc pu prélever du sperme que chez 11 (15,7 %) des 70 mâles étudiés. Au total 2 mm^3 de sperme ont été récoltés. Donc un mâle a fourni en moyenne 0,2 mm^3 de sperme. Lorsque l'abdomen était pressé en direction de l'anus au lieu de l'être sur les côtés, certains mâles retournaient brusquement leur endophallus jusqu'au stade d'éversion complète. Une pression plus forte provoquait l'éversion complète du cervix avec une goutte de sperme à son extrémité (Fig. 1.5 S). De cette façon, sur une nouvelle série de 90 mâles, nous avons récolté 6 mm^3 de sperme provenant de 30 (33 %) mâles. Sur l'ensemble des deux expériences, nous avons récolté 8 mm^3 de sperme provenant de 41 mâles. Ainsi un mâle a produit en moyenne 0,2 mm^3 de sperme. Trois reines d'*A. mellifera* ont été inséminées avec 2–3 mm^3 de sperme d'*A. dorsata*. Toutes les reines se sont mises à pondre quelques jours après l'insémination. Nous avons examiné un total de 7 750 œufs. Trois pour cent des œufs ont éclos et donné des larves. L'operculation du couvain a montré qu'il n'y avait que des nymphes de mâles (cellules à l'opercule bombé). Les données suggèrent que le sperme a probablement pénétré dans la spermathèque et que les œufs ont été fécondés de façon hétérospécifique (par des mâles d'une espèce différente). Mais nous pensons que pour des raisons d'incompatibilité génétique les embryons ne se sont pas développés et que les œufs n'ont donc pas éclos.

***Apis dorsata* / comportement de vol / mâle / éversion de l'endophallus / sperme / insémination artificielle hétérospécifique**

Zusammenfassung – Flüge von *Apis dorsata* Drohnen, Gewinnung von Sperma von ausgestülpten Endophalli und instrumentelle Besamung von Königinnen. Bisher hat noch niemand Sperma von ausgestülpten Endophallen von *Apis dorsata* Drohnen gesammelt. Die Spermamenge, die von einem *A. dorsata* Drohn produziert wird, ist ebenfalls nicht bekannt, da der Eversionsprozess des Endophallus nicht bekannt ist. Wir beobachteten den Flug der *A. dorsata* Drohnen und untersuchten den Eversionsprozess, um eine Möglichkeit der Spermagewinnung zu finden und die Menge des injizierten Spermas zu messen. Auβerdem inseminierten wir *A. mellifera* Königinnen mit Sperma von *A. dorsata* Drohnen, um die Interkompatibilität beider Arten zu bestimmen.

Drohnenflüge wurden an 16 *A. dorsata* Völkern beobachtet. Tabelle I zeigt, dass die Flüge Ende Februar zwischen 18.15 und 18.35 Uhr erfolgten. Mit fortschreitender Jahreszeit nahm die Tageslänge zu und die Drohnen flogen immer später. Es ergab sich eine hohe Korrelation (r = 0,99) zwischen dem Zeitpunkt des Sonnenuntergang und dem Beginn des Drohnenflugs. Im Laufe

von 2 Monaten verschob sich der Beginn der Flugzeit um 42 Minuten.

Die Eversion des Endophallus wurde bei mehreren Hundert Drohnen untersucht. Drohnen wurden während des Drohnenflugs in der Abenddämmerung gefangen. Der Thorax oder der Hinterleib wurden gedrückt, wobei der Druck allmählich verstärkt wurde. Das Sperma wurde mit einer Spritze für instrumentelle Besamung mit kalibrierter Spitze aufgenommen. Nach Druck auf den Thorax erfolgte eines von 3 Phänomenen: (1) keine Eversion des Endophallus, (2) partielle Eversion, oder (3) eine fast vollständige Eversion. Wenn der Hinterleib von den Drohnen, die ihren Endophallus nicht evertiert hatten, von beiden Seiten gedrückt wurde, erschien zunächst das Vestibulum mit den Anfängen von allen 4 Cornua (Hörnchen) (Abb. 1.1). Danach ging die Eversion weiter, bis sie in einem bestimmten Stadium einer partiellen Eversion stoppte (Abb. 1.4). Bei einer weiteren Erhöhung des Druckes erfolgte eine plötzliche Eversion der Cervix (Halsstück) (Abb. 1.5). Auf dieses Stadium erfolgte meist eine fast vollständige Eversion des Endophallus (Abb. 1.6). Noch stärkerer Druck war nötig, um die vollständige Eversion zu erreichen (Abb. 1.7). Sperma wurde von Drohnen ejakuliert, die nach Druck auf den Thorax plötzlich ihren Endophallus bis zum partiell evertierten Stadium evertierten (Abb. 1.4). Von 70 Drohnen, deren Thorax gedrückt wurde, erfolgte dieses Phänomen nur bei 8 (11 %). Sehr vorsichtige Erhöhung des Druckes war nötig, um die komplette Eversion der Cervix zu erreichen. Ein kleiner Tropfen von cremigen Sperma erschien an deren Ende (Abb. 1.5 S). Sperma wurde auch bei 3 (4 %) von 70 Drohnen erhalten, die ihren Endophallus fast vollständig oder vollständig evertierten, nachdem ihre Thoraces gedrückt wurden. In diesem Fall wurde das Sperma auf einer Schicht aus weiβem Mucus gefunden, der auf der ventralen Seite am Ende der Cervix lag (Abb. 1.6 S). Entsprechend erhielten wir von den 70 Drohnen nur Sperma von insgesamt 11 (15,7 %). Insgesamt wurden 2 mm^3 Sperma aufgenommen. Demnach trug ein Drohn im Mittel 0,2 mm^3 Sperma bei. Wir entdeckten, dass einige Drohnen beim Druck auf das Abdomen in der Analregion statt von den Seiten den Endophallus plötzlich bis zur partiellen Eversion austülpten. Weitere Erhöhung des Drucks führte zu einer vollständigen Eversion mit einem Spermatropfen an der Spitze (Abb. 1.5 S). Auf diese Weise sammelten wir von der nächsten Serie mit 90 Drohnen 6 mm^3 Sperma von 30 Insekten (33 %). Insgesamt sammelten wir in beiden Versuchen 8 mm^3 von 41 Drohnen. Demnach produziert ein Drohn im Mittel 0,2 mm^3 Sperma. Drei *Apis mellifera* Königinnen wurden mit 2–3 mm^3 *A. dorsata* Sperma besamt. Alle Königinnen begannen einige Tage nach der Besamung mit der Eilage. Insgesamt wurden 7 750 Eier untersucht. Larven schlüpften bei 3 % der Eier. Nachdem die Zellen verdeckelt waren, wurden nur Drohnenpuppen gefunden. Diese Daten lassen vermuten, dass das Sperma vermutlich in die Spermatheka eindrang und die Eier heterospezifisch befruchtet wurden. Wir ziehen jedoch in Betracht, dass auf Grund genetischer Incompatibilität die Embryonen sich wahrscheinlich nicht entwickelten.

Drohnen / Drohnenflugzeit / Eversion des Endophallus / Sperma / instrumentelle Besamung / *Apis dorsata*

REFERENCES

Koeniger G., Koeniger N., Tingek S. (1996) Interspezifische instrumentelle Besamung von *Apis cerana* Fabricius, 1793, *Apis koschevnikovi* Buttel-Reepen, 1906 und *Apis dorsata* Fabricius, 1793, Apidologie 27, 302–304.

Koeniger G., Koeniger N., Tingek S., Kalitu A. (1998) Vorläufiger Bericht über mit instrumentaller Insemination erzeugte Hybriden zwischen *Apis cerana* Fabricius, 1793 und *Apis koschevnikovi* v. Buttel-Reepen 1906, Apidologie 29, 462–463.

Koeniger G., Mardan M., Ruttner F. (1990) Male reproductive organs of *Apis dorsata*, Apidologie 214, 161–164.

Koeniger N., Koeniger G., Tingek S., Mardan M., Rinderer T.E. (1988) Reproductive isolation by different time of drone flight between *Apis cerana* Fabricius, 1793 and *Apis vechti* (Maa, 1953), Apidologie 19, 103–106.

Koeniger N., Koeniger G., Tingek S., Kalitu A., Mardan M. (1994) Drones of *Apis dorsata* (Fabricius 1793) congregate under the canopy of tall emergent trees in Borneo, Apidologie 25, 249–264.

Koeniger N.,Wijayagunasekera H.N.P. (1976) Time of drone flights in the three Asiatic honeybee species (*Apis cerana, Apis florea, Apis dorsata*), J. Apic. Res. 15, 67–71.

Rinderer T., Oldroyd B.P., Wongsiri S., Sylvester H.A., Guzman L.I. De, Potichot S., Sheppard W.S, Buchmann S.L. (1993) Time of drone flight in four honey bee species in south-eastern Thailand, J. Apic. Res. 32, 27–33.

Ruttner F., Woyke J., Koeniger N. (1973) Reproduction in *Apis cerana* 2. Reproductive organs and natural insemination, J. Apic. Res. 12, 21–34.

Ruttner F., Maul V. (1983) Experimental analysis of reproductive interspecific isolation of *Apis mellifera* L. and *Apis cerana* Fabr., Apidologie 14, 309–327.

Simpson J. (1970) The male genitalia of *Apis dorsata* (F.) (Hymenoptera: Apidae), Proc. R. Entomol. Soc., London 45, 169–171.

Tan Q.N., Mardan M., Yhai P.H. Ch. (1999) Observations on multiple mating flights of *Apis dorsata* queens, Apidologie 30, 339–346.

Woyke J. (1955) Multiple mating of the honeybee queen (*Apis mellifica* L.) in one nuptial flight, Bull. Acad. Pol. Sci. Cl. II 3, 175–180 (available: http://www.sggw.waw.pl/~woyke/MultMat.pdf/).

Woyke J. (1958) The process of mating in the honeybee, Pszcz. Zesz. Nauk. 2, 1–42 (in Polish, English summary).

Woyke J. (1960) Natural and artificial insemination of queen honeybees, Pszcz. Zesz. Nauk. 4, 183–275 (in Polish, summarized Bee World (1962) 43, 21–25).

Woyke J. (1973) Instrumental insemination of *Apis cerana indica* queens, J. Apic. Res. 12, 151–158.

Woyke J. (1975) Natural and instrumental insemination of *Apis cerana indica* in India, J. Apic. Res. 14, 153–159.

Woyke J. (1993) Rearing and instrumental insemination of *Apis florea* queens, in: Asian Apiculture, Proc. 1st Int. Conf. on Asian Honey Bees and Bee Mites, Bangkok, Thailand, Wicwas Press, Cheshire, Ct., USA, pp. 206–210.

Woyke J., Ruttner F. (1958) An anatomical study of the mating process in the honeybee, Bee World 39, 3–18.