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Original article

Mating flight duration of *Apis mellifera* queens: As short as possible, as long as necessary*

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Abstract – Polyandry in queen honey bees (*Apis mellifera*) prolongs the duration of nuptial flights which increases costs and risks. Under conditions of limited drone numbers the hypothesis was tested whether or not there is a threshold for successful mating during mating flight. In 29 queens we found a significant negative correlation between mating flight duration and number of spermatozoa in the spermatheca (Pearson r = −0.38, $P = 0.04$). This negative correlation supports the idea that queens continuously get information on her mating success during flight and return to the colony as soon as they have met a sufficient number of drones. In case of normal availability of drones queens fly from 10 to 30 minutes, so we compared 2 groups of queens (flight duration less than 30 versus more than 30 minutes). Sperm numbers differed significantly between the two groups (3.0 ± 0.77 and 1.1 ± 1.04 million, Wilcoxon, $P < 0.001$). These results further indicate that queens monitor mating success during flight.

mating success / nuptial flights / flight duration / sperm numbers / behavioral response

1. INTRODUCTION

To raise and maintain a sound and reproductive successful colony, honeybee (*Apis mellifera* L.) queens require a large storage of sperm in the spermatheca ranging from 2 to 6 million. About 5 to 10 days after emergence, young queens leave their hives for mating and, after one or two short orientation flights (from 1 to 2 min), they visit drone congregation areas (DCA, as rendez vous places in honeybees are named). There they copulate with several drones, which deposit their sperm in the queen's oviducts. Back in the colony less than 5% of the received sperm is transferred from the oviducts into the queen's spermatheca. This filling process takes more than 24 hours (Woyke, 1960, 1988). Woyke (1960) measured the sperm volume in the oviducts from 129 returning queens. On average the volume was equivalent to that of 10 to 12 drones. Other authors analyzed patrilines of the offspring and estimated between 5 and 10 copulations during one flight (Franck et al., 2002; Schlüns et al., 2005a). Queens regularly fly 1 to 2 km to reach a DCA (Ruttner and Ruttner, 1972). The time to reach the DCA and the highly polyandric mating system in the honeybee involves costs and risks for the queen. Since queens are not able to mate again and replenish their sperm storage once oviposition has started, a behavioral control of mating success has a high evolutionary significance.

Mating flights in general last from 10 to 30 min (Ruttner, 1954). About 60% of the queens perform two mating flights and 10% fly out more often (Woyke, 1964). Similar results were published by Schlüns et al. (2005a). In experiments of Tarpy and Page (2000), however, only 27% of the queens (8 queens out of 30) had more than one mating flight. Due to predation, weather conditions, mistakes in

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relocating the colony and several other causes, 10% to 20% of queens are lost during these flights (Ruttner, 1980; Ratnieks, 1990; Schlüns et al., 2005a). Queen loss during mating is disastrous for a bee colony. The old queen has already left the nest together with a swarm before emergence of young queens and the sexual maturation of a young queen takes at least 5 days. Thus, at the time of the queen's mating flight the female larvae in the nest are too old to be transformed into new queens. Queen loss during mating flights therefore results in the inevitable death of the colony. Limitation of number and duration of mating flights by the queen to an optimum would minimize the mating risks and increase her fitness.

Woyke (1964) suggested that queens adjust their nuptial flight frequency to the amount of sperm from the previous mating flight. He proposed that the queen's behavior is influenced either by the amount of sperm in the oviducts or by the concentration of spermatozoa in the spermatheca. Schlüns et al. (2005a) analyzed the number of patrilines after one flight. Queens which tried to perform additional mating flights (but were hindered from doing so) had significantly less copulations and tended to have fewer spermatozoa in the spermatheca than queens that started oviposition without further flights. They suggest that the queen "counts" the number of copulations "to decide" whether or not an additional mating flight is required. Both findings support the hypothesis that queens are able to adjust their behavior according to the mating success of a previous nuptial flight. Tarpy and Page (2000), however, did not find a correlation between mating flight duration and frequencies of copulation or mating flights, respectively. They concluded that there is no behavioral control by queens, neither by adjusting flight duration nor by taking extra mating flights.

Our experiment was designed to extend the queen's flight time required to contact a sufficient number of drones. In an isolated Alpine valley, we adjusted the number of drones to 2.500 drones which was about 25% of the previous years (Koeniger and Koeniger, 2005) and we monitored the duration of one mating flight. Later, we counted the number of spermatozoa in the queen's spermatheca and compared this to her flight duration. In case queens tend to copulate with as many drones as possible, sperm numbers in the spermatheca should increase with the duration of mating flight (positive correlation). A "no correlation" between flight duration and sperm numbers may have several causes. A negative correlation between mating flight duration and sperm numbers would support the idea that queens continuously get information on her mating success during the nuptial flight and return to the colony as soon as they have mated with a sufficient number of drones.

2. MATERIALS AND METHODS

2.1. Queens

All queens were sisters. They were kept in three-frame nucleus hives with about one thousand bees. All queens were individually marked by number tags. Also the nucleus hives were numbered, so we knew which queen belonged to which hive.

2.2. Design of experiment

The experimental site is situated in the Austrian Alps in the Salza valley near Gschöder (Koeniger et al., 2005). This steep valley is surrounded by high mountains which prevent the intrusion of drones from outside. From July 15th to August 4th we placed 35 virgin sister queens in nucleus hives at 2 locations which were 1.2 km apart. In between both locations 15 drone colonies were placed which were without queens and drone brood. We counted the number of drones in the early morning on the combs and adjusted the number of flying drones to about 2 500 by blocking the drone flight of some colonies. This was achieved by inserting queen excluder screens. There are 2 DCAs in this area, known for the last 15 years. The presence of drones at both DCAs was checked by using pheromone baited drone traps (Williams, 1987).

Eight-to-nine nucleus hives were placed on one stand side by side. Altogether we had 4 stands with 35 queens. The flight entrances of the nucleus colonies were equipped with transparent plastic tubes (diameter 3 cm, length 5 cm). They were closed by a queen excluder to prevent unobserved mating flights. Four observers patrolled the flight entrances between 13.00 and 17.30 h, which is the mating period in this region (Koeniger et al., 1989). As soon as a queen appeared in the tube, the queen excluder was removed, flight was permitted and the take off time was recorded. After the queen's start the excluder was re-installed to prevent uncontrolled returns of the queen into the colony. As soon as a returning queen was sighted near the entrance of a nucleus hive the queen's number and the time of return were noted. Further, the queens were examined for mating signs (indication of successful mating flight). After entering the tube of her hive's entrance the time was noted a third time. If a queen did not find the colony for more than 10 minutes we caught the queen by an insect net and put her into her nucleus colony. Queens which had returned and were carrying a mating sign were confined to the hive and no second mating flight was allowed.

2.3. Sperm counts

After the queens had started oviposition the spermathecae were extirpated and placed in 1 mL of Hyes solution, torn apart and the spermatozoa were dispersed. Subsequently they were killed by adding 9 mL distilled water. They were then counted in a Fuchs-Rosenthal hemocytometer (Koeniger et al., 2005). We counted 3 samples from the sperm dispersion of each queen. For each sample the spermatozoa were re-dispersed by blowing air through the sample with a pasteur pipette. For each sample we counted 100 units of the counting chamber (1 unit = 0.0125 mm^3).

2.4. Statistical analyses

We used Pearson's coefficient of correlation to analyze associations between mating flight duration and number of spermatozoa in the spermatheca. To compare the differences in sperm numbers between two groups of queens with mating flight durations below or above 30 minutes we applied the Wilcoxon Test.

3. RESULTS

All queens performed 1 or 2 orientation flights (flight duration less than 5 min, no mating sign). Twenty nine queens returned to their hives with mating signs and could be included

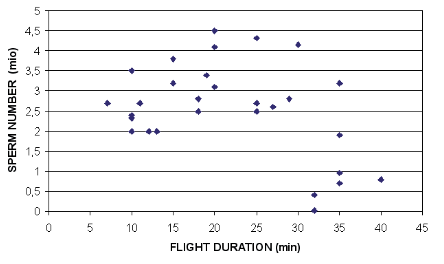

Figure 1. Relation between number of spermatozoa in the spermatheca and duration of nuptial flight.

in the analyses. These queens mated on 3 successive days (July 22–25, 2002). The mean flight duration was 21.8 ± 9.67 min. There was a significant negative correlation between mating flight duration and number of spermatozoa in the spermathecae (Pearson r = –0.38, P = 0.04). So, queens with longer flight duration had received less sperm. For a further analysis we referred to the mating flight data of Ruttner (1954) and Koeniger et al. (1989), which were done in the same region but with "normal" drone numbers and which demonstrated that all queens returned within 30 min from the mating flight. So we compared the number of spermatozoa between queens flying shorter and queens flying longer than 30 min ("a priori reason") and found a significant difference (Wilcoxon, $P < 0.001$) (Fig. 1). Queens with flight duration below 30 min had an average of 3.0 ± 0.77 million spermatozoa in their spermathecae. Seven queens (24%) stayed away longer than 30 minutes, and had 1.1 ± 1.04 million spermatozoa. Neither within the group with flight durations from 10 to 30 minutes nor within the group longer than 30 minutes a correlation between duration of flight and number of spermatozoa in the spermatheca is significant (Pearson's r = 0.403, P = 0.06 and respectively Pearson's r = 0.225, P = 0.6).

4. DISCUSSION

Drone congregation areas in regions with intensive bee keeping are visited by 12 to 15 thousand drones (Koeniger and Koeniger, 2005). There mating flights of queens last from

10 to 30 min (Ruttner, 1956). In our set up the 29 experimental queens could encounter only about 20% of drones available at DCAs in regions with beekeeping. Under these conditions we found nuptial flight duration was 21.8 ± 9.67 min while in earlier experiments in the same region with plenty (>10 000) drones it was 13.7 ± 6.1 min (Koeniger et al., 1989). This supports our presumption that the number of available drones influences nuptial flight duration of queens and might be an initial indication that queens monitor the mating success.

There was a negative correlation between flight duration and the number of spermatozoa in the spermatheca. This result suggests that queens may have an ability to assess mating success and consequently return to their hives as soon as this is achieved. At the same time, other queens will still continue to search for additional drones. Considering only the group of queens returning with mating signs within 30 minutes no significant correlation could be detected between mating flight time and number of spermatozoa. Queens seem not to copulate with as many drones as possible but return to the hive after meeting the threshold for mating success.

Generally, the flight duration of bees (and queens) is limited by the honey content of the crop. After a long flight the honey content might be exhausted and queens are immediately forced to return to the colony; regardless of the number of copulations obtained so far. The group of 7 queens with a flight duration of 30 min and longer had significantly less spermatozoa. Therefore we conclude, that queens access the progressing multiple copulations already completed during the mating flight. Further, the significantly higher standard deviation of sperm numbers within the group of queens flying 30 min and longer compared to that of queens with shorter flight durations is consistent with the hypothesis of a timed limitation of nuptial flights.

When a queen returns after mating flight the sperm is still in the oviducts. Thus queens cannot rely on signals which are related to the sperm transfer into the spermatheca, which happens later, after her return in the colony. Schlüns et al. (2005a) looked at the queen's onset of oviposition. They reported that the number of copulations rather than sperm volume seems to serve as a signal for undertaking a second flight or start of oviposition. On the other hand these authors also reported a positive, (non linear) correlation between the number of spermatozoa in the spermatheca and the number of copulations. This is a further confirmation that the number of spermatozoa found in the spermatheca is correlated to a distinct behavioral response. The reported number of spermatozoa in the spermatheca, however, differ among authors from 1.88 and 1.01 (Schlüns et al., 2005a) to 3.0 and 1.1 million in our results and 5.1 and 3.4 million (Woyke, 1964) respectively. These differences might reflect a dependency of the threshold of mating success on local environmental conditions.

In contrast to the results discussed above Tarpy and Page (2000) could not detect a relationship between flight frequencies and number of copulations nor between number of nuptial flights and number of copulations. Queens in our study and in the experiments of Schlüns et al. (2005a) were of the same age and mated within 3 successive days, while Tarpy and Page (2000) tested their 32 queens from April to August. Differences in seasonal conditions may have masked behavioral differences. The low number of copulations per queen compared to results of other authors (review Schlüns et al., 2005b) and the high variance militate in favor of differences in environmental conditions.

There are also several other facts which point to a strong selective force to optimize the mating flight time of a queen. In drone congregation areas drones of more than 200 colonies aggregate, which contributes to the extreme outbreeding typical for honey bees in a short time (Baudry et al., 1998). Further, in many insects species male use secretions of the accessory glands as mating plugs to block off competitors (Thornhill and Alcock, 1983). In contrast the *A. mellifera* drone's secretions are used to mark the queen to facilitate the identification of a queen by other drones and facilitate additional copulations (Koeniger, 1990). This phenomenon was termed "post mortem cooperation" and also serves to ensure multiple mating within a mating flight time reduced to a minimum.

Our results support the hypothesis that there is a threshold for mating success which correlates with the number of copulations (Schlüns et al., 2005a) and number of spermatozoa in the spermatheca (Woyke, 1964). In addition our results suggest that queens may acquire information about mating success during the mating flight. The actual stimuli for the perception are still under debate (Colonello and Hartfelder, 2005).

La durée du vol nuptial chez *Apis mellifera* : aussi court que possible, aussi long que nécessaire.

succès reproducteur / vol d'accouplement / durée de vol / nombre de spermatozoïdes / réponse comportementale

Zusammenfassung – Die Dauer des Hochzeitsfluges der Bienenkönigin (*Apis mellifera*): So kurz wie möglich und so lange wie nötig. Die Mehrfachpaarung der Bienenkönigin führt zu einer Verlängerung des Paarungsfluges. Dadurch steigen die „Kosten“ und vor allem auch die Risiken des Paarungsfluges erheblich und einer Optimierung der Flugdauer im Sinne eines Selektionsvorteils kommt zweifelsfrei eine wesentliche evolutive Bedeutung zu.

Wir haben in einem Gebiet, in dem die Anzahl der vorhandenen Drohnen auf 2500 experimentell vermindert war, die Frage untersucht, ob Königinnen den Paarungserfolg überwachen und heimkehren, sowie die Schwelle für erfolgreiche Paarungen erreicht ist. Bei einer Drohnenanzahl von 2500 dauerten die Paarungsflüge von 29 Königinnen im Durchschnitt $21,8 \pm 9,67$ Minuten, während wir früher bei mehr als 10 000 Drohnen eine signifikant kürzere Flugdauer von $13,7 \pm 6,1$ Minuten (Koeniger et al., 1989) gemessen hatten. Das war eine Bestätigung für unsere Annahme, dass die Anzahl der Drohnen die Dauer des Paarungsfluges der Königin beeinflusst und zugleich ein erster Hinweis darauf, dass Königinnen ihren Paarungserfolg während des Fluges „überwachen“.

Bei 29 Königinnen ergab sich eine negative Korrelation zwischen der Flugdauer und der Anzahl der in der Spermatheka gefundenen Spermien (Pearson $r = -0,38$, $P = 0,04$). Diese negative Korrelation spricht für die Idee, dass die Königin während des Fluges kontinuierlich Information über den Paarungserfolg erhält und sofort zurück fliegt, wenn sie genügend Drohnen getroffen hat.

Sind genügend Drohnen vorhanden so streut die Dauer der Hochzeitsflüge, wie wir am gleichen Ort in den Vorjahren feststellten, zwischen 10 und 30 Minuten. Auf dieser Grundlage (Apriori – Annahme!) wurden die Daten in 2 Gruppen unterteilt: Zum ersten in die Gruppe der Königinnen, die weniger als 30 Minuten ausgeflogen waren und zum zweiten in die Gruppe von Königinnen, die länger als 30 Minuten gebraucht hatten. Die Spermienzahlen zwischen den beiden Gruppen unterschieden sich erheblich. Die kürzer als 30 Minuten geflogenen Königinnen hatten im Durchschnitt $3,0 \pm 0,77$ Millionen Spermien in der Spermatheka während die länger als 30 Minuten geflogenen Königinn mit nur $1,1 \pm 1,04$ Millionen signifikant weniger Spermien hatten (Wilcoxon, $P < 0{,}001$). Bei längerem Flug der Königin könnte der Honigvorrat im Kropf zu Ende gegangen sein. Die betroffene Königin wäre dann gezwungen sofort den Heimflug anzutreten und könnte nicht weiterfliegen, bis sie eine ausreichende Zahl von Paarungen erzielt hätte.

Unsere Ergebnisse sprechen dafür, dass die Königin während des Hochzeitsfluges den Paarungserfolg kontrolliert, der mit der Anzahl der Kopulationen (Schlüns et al., 2005) sowie auch mit der Anzahl der Spermien in der Spermatheka (Woyke, 1964) zusammenhängt und zurück fliegt sobald die Reizschwelle erreicht ist.

Paarungsflug / Flugdauer / Paarungserfolg / Spermienzahl / Verhaltensreaktion

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