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Original article

Observations on gynes and drones around nuptial flights in the stingless bees *Tetragonisca angustula* and *Melipona beecheii* (Hymenoptera, Apidae, Meliponinae)

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Abstract – The nuptial flight of gynes of *Tetragonisca angustula* and *Melipona beecheii* was studied. The moment of nuptial flight was found to be related to the ambient temperature, and the duration of the nuptial flight for *M. beecheii* was longer in November (rainy season) than in March (dry season). A repeated mating flight was recorded for two gynes of *T. angustula*. Three of five *T. angustula* queens and all six *M. beecheii* queens were mated successfully. Behavioural data of drones and gynes shortly before and after the nuptial flight are presented. Drones of *T. angustula* participated in a congregation for up to three days. The importance of pheromones for the attraction of drones and gynes is discussed. An hypothesis explaining the observed seasonal occurrence of male congregations near nests of *T. angustula* is presented.

mating / drone congregation / *Melipona beecheii* / *Tetragonisca angustula* / stingless bees

1. INTRODUCTION

The complete mating flight of any stingless bee has not been observed so far. The mating flight is undertaken from the mother colony in case of supersedure and from the filial nest in case of swarming. For *Meliponini* it is known that gynes are between 3 and 8 days old when they undertake the nuptial flight [12, 16], although in one case a 17 hours old gyne flew out on a nuptial flight and mated successfully [16]. In confinement, gynes of *M. quadrifasciata* were able to mate on the day of emergence [2]. Based on sperm counts of open mated queens of *M. quadrifasciata* compared with

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controlled mated queens, insemination by a single drone was concluded [2, 7]. That single mating seems to be the rule in stingless bees was confirmed through genetic relatedness studies between queen and daughters for thirteen species [10]. Multiple mating was however concluded in one case for *T. angustula* [6], and for several cases in *Scaptotrigona postica* [9]. The nuptial flight may last as short as six minutes in *M. beecheii* [16], but up to 102 minutes in *M. quadrifasciata* [12]. In *Melipona*, drone congregations are not nest associated [14], which may explain the generally large duration of the nuptial flight if compared with those of gynes of *Trigonini*, in which nest-associated drone congregations occur [5, 11]. It was observed that drones and gynes of *M. favosa* flew off after arrival of the gynes at a drone congregation area and did not mate at the site [14].

Data are presented on how environmental cues and behaviour of gynes and drones influence the nuptial flight in *M. beecheii* and *T. angustula*, and discussed in relation with mating strategies of stingless bees.

2. MATERIALS AND METHODS

Between November 1991 and May 1996, during six observation periods, each lasting 1 to 7 months, totaling 23 months, a total of 58 hived colonies of *T. angustula* and 19 experimentally established queen deprived colonies of *M. beecheii* were studied, at four different locations in Costa Rica [16, 17]. Only the experimental set up of the colonies from which mating flights were undertaken will be described here.

2.1. Bee colonies

In the case of *T. angustula*, two of the seven observed mating flights were undertaken from naturally founded colonies recently established in hive boxes. The other five nuptial flights were undertaken from queenless colonies, established by putting 200 to 400 workers, several combs with emerging brood, one royal cell and some food stores in a small observation box. To study the mating flight of gynes of *M. beecheii*, small nuclei were formed by putting 50 to 180 workers and some 100 to 400 brood cells in small observation boxes [16].

2.2. Measurements

The presence of gynes in the colonies of *M. beecheii* was checked every two hours between 6 and 18 h. Once a gyne was accepted [17], observations were intensified. The moment and duration of the nuptial flight and related behaviour of the gyne were recorded. The age of the gyne, outside temperature and humidity were measured. Special attention was paid to drone presence, and to the presence of a mating sign upon returning of the gyne.

The boxes, located to attract swarms of *T. angustula*, were hung up at 2 to 20 m distance from the occupied hives, and had a capacity of 3 to 5 l. These boxes were checked daily for the presence of entering workers and new structures such as an entrance tube [5]. Once evidence of nest foundation was seen, observations were carried out every hour during daylight and records made as described above for *M. beecheii*. Drone presence was counted every fifteen minutes for two colonies on the day of the nuptial flight and the day after. All recordings of behaviour were through direct observations. An analysis of variance was performed to calculate the correlation between the moment nuptial flights took place and several environmental cues.

3. RESULTS

3.1. Nuptial flight and gyne behaviour

3.1.1. Tetragonisca angustula

The nuptial flight of five gynes of *T. angustula* was observed (Tab. I). For

Table I. Mating flights, weather conditions and subsequent oviposition in *T. angustula* (n.k. = not known).

Gyne number	1	2	3		4	5		Average
Date	17/11	12/11	7/11	8/11	25/3	29/3	1/4	
Age (days)	n.k.	n.k.	9-12	10-13	7	12	15	11
Time	11:01	10:41	14:30	14:00	12:12	15:00	13:30	12:59
Duration (s)	400	146	270	589	1224	180	692	500
Temp. (°C)	–	–	28.4	31.2	28.4	28.1	31.9	29.6
Rel. humidity	–	–	78	61	72	86	65	72
Air press. (mbar)	–	–	989	988	1001	979	997	991
Male presence	300	20	No	No	No	No	No	
Start egg-laying	19/11	> 15/11		13/11	No		No	3.3

gynes number three and five two successive nuptial flights were recorded for the first time in stingless bees. Queen number 5 did not return inside the hive immediately after the mating flight, but flew on both days respectively for 27’00 and 11’58 minutes outside in front of the hive, apparently having problems in finding the entrance. Once she landed on the wall close to the entrance she was helped in by us after a few minutes.

The data clearly show nuptial flights of a short duration, especially when a male aggregation was present close to the hive. This was the case for gynes one and two. None of the gynes had any mating sign upon return.

All gynes performed a short orientation flight when leaving the hive, flying backwards in circles, with the head directed towards the entrance. Gyne number three performed this orientation flight only the first day, and flew straight out the second day. Gynes number four and five probably did not mate successfully, because no egg-laying was observed within six weeks after the nuptial flight(s). All other gynes started egg-laying within two to five days after their nuptial flight and samples showed worker production, which is a clear indication of successful mating. Seven to 15 days after her nuptial flight, gyne number four was observed 32 times in the entrance tube for five to ten minutes, between 9:53 and 15:05, but did not leave for a second nuptial flight.

3.1.1. *Melipona beecheii*

The mating flight of gynes of *M. beecheii* was recorded (Tab. II). All gynes left the hive flying very fast, without performing any noticeable orientation flight. When gyne number five flew out, she landed on a tree branch about 30 cm from the entrance, where she sat for 17 minutes, auto grooming, before she left the site in a straight line. She returned 37 minutes later. Gyne number four entered a neighbouring hive when she returned from her nuptial flight, where she was received by worker aggression before she was put in the correct box by us. Two times a gyne was observed with a mating sign upon her return in the hive. No workers were seen to help the gyne to get rid of the mating sign. The gynes rubbed their abdomen over the brood comb surface and hive walls, and were not observed with the male genitalia after about one hour.

The durations of the nuptial flights for *M. beecheii* were found to be quite different between those observed in March and November (limited data do not allow for statistical testing).

For four more gynes, the age when the nuptial flight was undertaken, was 4, 16, 17

Table II. Mating flights and subsequent oviposition in *M. beecheii* (* duration in days after nuptial flight before egg-laying started).

Gyne number	1	2	3	4	5	6	Average
Date	March	March	March	18/11	16/11	28/11	
Age	6	4	4	8	7	10	6.5
Time	9:23	10:00	10:43	11:05	12:01	11:30	10:47
Duration	7’00	7’00	6’00	15-40	37”00	5-35	17’30
Mating sign	No	No	No	Yes	No	Yes	
Temp. °C	34.0	36.5	37.5	33.0	34.0	34.0	34.8
Start egg-laying*	9	11	8	14	17	4	10.5

and 18 days respectively. No drones were seen near the hives during the observation period.

3.2. Comparison of species

Based on the assumption that the environmental cues that determine the conditions under which the nuptial flight takes place are the same for both species, all data of temperature and the starting time of the nuptial flight were used to calculate the correlation ($F = 15.7$, $P = 0.003$). Neither date nor duration of the nuptial flight were related to mating success ($F = 2.1$, $P = 0.15$ and $F = 1.3$, $P = 0.18$ respectively).

3.3. Male aggregations near the nest of *Tetragonisca angustula*

Drones were present in aggregations near two of the hives of *T. angustula* at the moment a virgin queen was present (Tab. I), and in three more aggregations observed near colonies of which the internal condition was not known.

From about 9 h onwards, between 150 and 200 drones were observed sitting on a palm leaf 40 cm from the entrance of the mother colony of gyne number 1, the morning this gyne flew to the newly founded nest nearby. No drones were observed at this spot the day before. Around 12 h some fifty of these drones were observed flying in a dense “cloud” with some fifty more workers, close to the entrance of the hive. At 12:48 h the gyne flew out, after which the drones took off immediately. After seven minutes the gyne entered the “daughter” nest, located 5 m distance. Since the gyne flew out of sight it was not possible to observe her behaviour during these seven minutes. Ten minutes after that, the drones started arriving at the filial nest, and landed on leaves of plants nearby (40 to 70 cm distance). Returning drones, that joined a “cloud” now estimated to consist of more than 1500 workers in front of the mother nest, were attacked by the workers. Between 16:30 and 17:00 h, all drones left. The following morning about 300 drones were observed on the same location as the day before close to the daughter nest. Around 11:00 h all drones started flying in front of the entrance, and left suddenly at 11:01 h, following the gyne on her nuptial flight. The gyne returned alone after 400 s (Tab. I). The first drones returned shortly after her and at 11:11 h about 120 drones had returned. During the afternoon the drones vanished and by 15:45 all drones had left. For the following four days between 80 and 100 drones were still present between 7:30 h and 16:45 h. On day five after the nuptial flight, only four drones were left, and after that no more drones were observed near the hive.

The presence of drones in two aggregations, in front of two colonies on the day of the nuptial flight of a gyne and the day after, was measured (Fig. 1). On the first day a maximum number of 88 drones was seen near one colony and 14 near the other. The drones started to arrive around 9 h, and 50% of the aggregation was established within the first half hour. The maximum number of drones was observed just before the gyne left. After the return of the mated queen, the number of drones in the aggregation increased gradually until reaching a number of about half the maximum presence observed. During the nuptial flight no drones were present near the hive. On the day after the nuptial flight fewer drones (Fig. 1, gray bars) participated in the aggregation, at most 34% of the maximum of the day before, i.e. some thirty (only present for the first colony). Although drones started coming at the same time, the last male left before 11:15 h.

In another aggregation that was discovered near a hive on 18 January 1993, about 100 drones participated. After two days their number diminished to about 80, followed by four days with an average presence of some 60 drones, interrupted by one rainy day on which no drones were observed. On the last day the aggregation was seen (26 January), a maximum of thirty drones was counted. Individual marking revealed that on successive days 20 to 22.5% of the drones returned to the aggregation, and that drones may participate for up to three days. The drones spent about 80% of their time "standing still", occasionally rubbing their abdomen with their hind tibiae, and the other 20% on flying around near the aggregation site. It was observed 49 times within 5 minutes, that a sitting drone flew away when approached by a flying male, the latter occupying its place. On January 18, between 10:00 and 13:20 h, eight drones entered the nest box and stayed inside for an average 174 s (S.D. = 90 s).

4. DISCUSSION

In stingless bees, drones are produced in varying quantities all year round, depending on species, colony conditions and season [1, 3, 13, 15]. The variation found in this study in duration of the nuptial flight for

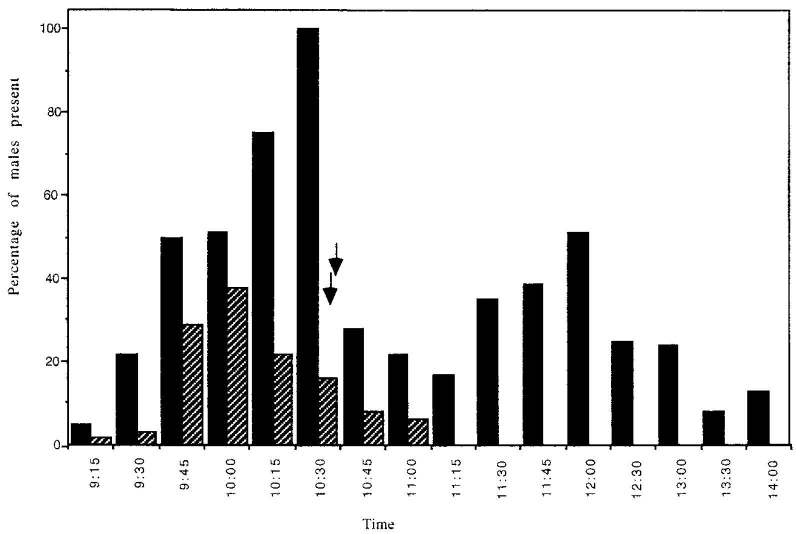

Figure 1. Presence of drones of *T. angustula* in two aggregations near the entrance of two hives the day of the nuptial flight (black bars) and the day after (gray bars), expressed as percentages of the maximum presence, which was 88 drones on the first day at 10:30 h and 14 drones on the second day at 10:00 h. The nuptial flight was at 10:32 h for one colony and at 10:35 h for the other (arrows).

both species, the two cases for gynes of *T. angustula* in which a second nuptial flight was undertaken, and a mating success of only 60% for this species, may well be caused by the inconstant production and therefore varying availability of drones [15]. The fact that the two mating flights undertaken by gynes of *T. angustula* from a hive where a male aggregation was present lasted shorter than the others observed, is consistent with this explanation. Also, for *M. beecheii*, a considerable difference was found in duration between the mating flights performed in November and March. A 5.5% higher production of drones was found for *M. beecheii* in the period March to July than in November [15]. If on one hand the duration of the nuptial flight and the seasonal availability of drones is related, and on the other hand, as was found for *T. angustula*, the mating success depends on the season, then this has important implications for the reproductive strategy of these species. Supersedure and social reproduction (swarming) should take place in a period with sufficient male production, because the mating success of the gyne, and therewith the individual reproduction of the (newly founded) colony, depends on it. Obviously the season for social reproduction must be favourable for the development of the new colony.

A negative correlation was found for the moment at which the nuptial flight is undertaken and the temperature. This is the first evidence that environmental cues influence the mating behaviour of stingless bees.

Drones of *T. angustula* were attracted to nests that contained virgin queens. At the moment the gyne left the nest, either for her nuptial flight or go to the site of a new nest, drones almost immediately perceived this movement and followed the gyne. Returning drones were attacked at the mother nest shortly after the gyne had left, whereas they were allowed to enter the daughter nest. In the days following the nuptial flight, the presence of drones near the nests gradually diminished, indicating a reducing attractiveness of the newly mated queen. All these observations indicate a pheromonal mechanism, which is responsible for attracting drones to the gyne and for having them become tolerated in its direct vicinity.

The nest associated male congregations occurred in only two of the five hives with gynes. The nuptial flights were less successful and lasted longer in these cases. We hypothesize that many drones are produced in a "reproductive season". These drones, instead of forming large congregations, distribute between nests with virgin queens, so increasing their mating change. In this way the gynes are assured of a short and thus less risky mating flight. Outside this reproductive season, the chance of drones for mating is reduced to incidental superseding gynes only. We hypothesize that these gynes are attracted by drones to a congregation area, so increasing mating probability for both.

Gynes of *T. angustula* performed an orientation flight before leaving for the nuptial flight, whereas gynes of *M. beecheii* fly out in a straight line, without performing a characteristically orientation flight. Gynes of *T. angustula* were also seen flying from the mother nest to the new nest unaccompanied. These observations suggest that scent released by workers is important for the orientation of the gyne.

Based on the comparison of sperm counts in open-mated *Melipona quadrifasciata* queens and queens mated under controlled conditions [2], it is assumed that gynes of stingless bees mate with only one drone. The remains of the torn off male genitalia ("mating sign"), which are removed by the queen herself [12] would probably prevent a second copulation [5]. There are however indications that the mating sign facilitates a following mating in *Apis mellifera* [8]. In *Scaptotrigona postica* multiple mating was found in several cases [4, 9]. Especially if the long lasting mating flights of *Melipona* are taken into consideration, multiple mating can not be excluded. For a good understanding of the reproductive biology of stingless bees it is very important to have this issue resolved.

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Résumé – Observations sur les vols de fécondation des femelles et des mâles des abeilles sans aiguillon *Tetragonisca angustula* et *Melipona beecheii* (Meliponinae). Le vol de fécondation des femelles et des mâles de *Tetragonisca angustula* et *Melipona beecheii* a été étudié au Costa Rica entre novembre 1991 et mai 1996. Le moment où a lieu le vol de fécondation dépendait de la température ambiante et la durée était plus longue en novembre (saison des pluies) qu'en mars (saison sèche) pour *M. beecheii*. Lorsque les congrégations de mâles de *T. angustula* étaient près de la ruche, les vols de fécondation étaient de courte durée. Nous avons pour la première fois enregistré des vols de fécondation répétés pour deux reines de *T. angustula*. Trois des cinq reines de *T. angustula* et les six reines de *M. beecheii* se sont accouplées avec succès.

Deux reines de *M. beecheii* ont été vues avec un signe de fécondation lorsqu'elles rentraient à la ruche après le vol de fécondation. Elles s'en sont débarrassées elles-mêmes en frottant leur abdomen sur les rayons de couvain et les parois de la ruche. Nous n'avons observé de signe de fécondation chez aucune des reines de *T. angustula*. Les mâles de *T. angustula* ont participé à un rassemblement jusqu'à trois jours d'affilée durant lesquels ils passaient le plus clair de leur temps à rester tranquilles ou à voler autour du nid.

Nous émettons l'hypothèse selon laquelle les mâles sont produits en grande quantité pendant la « saison de reproduction » et attirés par des phéromones vers les nids possédant des reines vierges. Ces rassemblements de mâles près des nids donnent lieu à des vols de fécondation couronnés de succès et de courte durée. Nos données indiquent que les vols de fécondation connaissent plus d'échecs et durent plus longtemps lorsque les rassemblements de mâles ne sont pas à proximité directe des nids. C'est ce qui se passe en dehors de la saison de reproduction, lorsque les possibilités d'accouplement des mâles se réduisent aux seules reines de supersédure rencontrées fortuitement. Nous supposons que dans ces cas-là les reines sont attirées par le rassemblement de mâles. L'importance des phéromones dans l'attraction des reines et des mâles est discutée.

accouplement / rassemblement de mâles / *Melipona beecheii* / *Tetragonisca angustula* / abeille sans dard

Zusammenfassung – Beobachtungen von Hochzeitsflügen von Weibchen und Drohnen der stachellosen Bienen *Tetragonisca angustula* und *Melipona beecheii* (Meliponinae). Wir untersuchten die Paarungflüge der Königinnen von *Tetragonisca angustula* und *Melipona beecheii* in Costa Rica von November 1991 bis Mai 1996. Der Zeitpunkt der Hochzeitsflüge war von der Umgebungstemperatur abhängig, und die Flüge von *M.beechii* dauerten im November (Regenzeit) länger als im Mai (Trockenzeit). Wenn es Drohnenansammlungen von *T. angustula* in Nestnähe gab, waren die Hochzeitsflüge nur von kurzer Dauer. Zum ersten Mal konnten bei 2 Königinnen von *T. angustula* wiederholte Paarungsflüge beobachtet werden. Drei von 5 *T angustula* Königinnen und alle 6 *M. beechii* paarten sich erfolgreich.

Bei 2 *M. beechii* Königinnen wurden nach dem Hochzeitsflug bei ihrer Rückkehr zum Nest Begattungszeichen beobachtet, die sie selbst durch Reiben ihres Hinterleibs über den Brutzellen entfernten. Bei *T. angustula* wurden keine Begattungszeichen entdeckt. Drohnen von *T. agnustula* blieben bis zu 3 Tagen in den Ansammlungen, wo sie ihre Zeit vor allem mit Stillsitzen oder Flügen in der Nähe des Nestes verbrachten.

Es wird eine Hypothese vorgestellt, nach der Drohnen während der Reproduktionsphase in groβen Mengen erzeugt werden und durch Pheromone zu den Nestern mit unbegatteten Königinnen gelockt werden. Diese Drohnenansammlungen in der Nähe des Nests ermöglichen kurze erfolgreiche Hochzeitsflüge. Unsere Daten zeigen, dass die Hochzeitsflüge weniger erfolgreich sind und länger dauern, wenn es keine Ansammlungen von Drohnen in der näheren Umgebung des Nests gibt. Letzteres kommt ausserhalb der Reproduktionszeit vor, wenn die Möglichkeiten der Paarung für Drohnen auf die zufälligen Nachschaffungsköniginnen reduziert sind. Wir nehmen an, dass in diesem Fall die Königinnen zu den Drohnenansammlungen angelockt werden. Die Bedeutung von Pheromonen für die gegenseitige Anlockung von Drohnen und Königinnen wird diskutiert.

Paarung / Drohnenansammlungen / *Melipona beecheii / Tetragonisca angustula* stachellosen Bienen

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