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Research article

Behavioural development and abdomen inflation of gynes and newly mated queens of *Melipona beecheii* (Apidae, Meliponinae)

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Summary. The behaviour of gynes of *Melipona beecheii* in queen-deprived colonies was studied. The period after emergence until acceptance is characterized by agonistic behaviour of workers towards the gynes. The gynes escaped from this worker aggression by hiding in the periphery of the nest, by performing rapid turn-arounds once grabbed by a worker, and „feigning death". Between acceptance and nuptial flight, gynes spent most of their time in pushing, hiding, and antennal contact with workers, and self-grooming or food solicitation. After the nuptial flight the queen's behavioural repertoire shifted to less pushing and food solicitation, to an increase in standing, tapping and antennal contact.

Accepted gynes had a significantly more prolonged abdomen inflation than gynes that were eliminated.

An hypothesis is presented to explain how abdomen enlargement and behavioural development influences the acceptance of gynes and the establishment of a dominance relation with workers under queenless conditions.

Key words: Pre-mating behaviour, gyne, abdomen enlargement, *Melipona beecheii*, stingless bee.

Introduction

As emergency queen rearing is unknown in stingless bees because of the mass-provisioning system (Sakagami, 1982), gynes are produced regularly throughout the year (Engels and Imperatriz-Fonseca, 1990; Imperatriz-Fonseca, 1977, 1978; Van Veen et al., 1992). Surplus queens of *Melipona* are usually eliminated by workers after a short period, although occasionally they are tolerated, causing an oligogynous colony state (Engels and Imperatriz-Fonseca, 1990; Sommeijer, 1994). In some Trigonini special chambers are constructed, egg-shaped enclosures made of cerumen, where the gynes live temporarily (Imperatriz-Fonseca and Zucchi, 1995; Kerr et al., 1962). In some *Melipona* gynes may also live in the nest for some time, alone, or in small groups (Kleinert and Imperatriz-Fonseca, 1994) without a specially built refuge, using empty pots or nest crevices. In *M. beecheii* no special refuges are constructed and the gynes can move freely throughout the nest.

Koedam et al. (1995a) hypothesize that reproductive competition between the physogastric queen and the unmated new gynes is a mechanism by which the quality of the laying queen is continuously tested. These authors present a detailed study on behaviour of gynes in *Melipona favosa*. The competitive behaviour between gynes leads to either elimination of excess queens under queen-right conditions, or acceptance of the gyne in case of supersedure (Koedam et al., 1995b). They assume that pheromones released by abdominal glands which are exposed when the abdomen of gynes becomes enlarged, may play an important role in the acceptance of the virgin queen by workers, as was also found for *M. marginata* and *M. quadrifasciata* (Cruz-Landim and Mota, 1990; Imperatriz-Fonseca and Zucchi, 1995) and for *Paratrigona subnuda* (Cruz-Landim et al., 1980; Imperatriz-Fonseca, 1977). Acceptance of a gyne may occur from a few hours up to until one week after emergence, depending on the condition of the colony and its queen (Kerr et al., 1962; Koedam et al., 1995a; Silva et al., 1972; Imperatriz-Fonseca, 1977, 1978; Imperatriz-Fonseca and Zucchi, 1995).

Only general information is available about the behavioural development of a gyne after being accepted by the colony, before she leaves the nest for her nuptial flight (Engels and Imperatriz-Fonseca, 1990; Kerr et al., 1962; Imperatriz-Fonseca and Zucchi, 1995; Sakagami, 1982;

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Sommeijer, 1994). The most detailed study is by Koedam et al. (1995b), who found that the acceptance process of gynes of *M. favosa* under queenless conditions started about 15 hours after emergence and was characterized by an abrupt behavioural change and simultaneous abdomen enlargement. Imperatriz-Fonseca (1977, 1978) and Da Silva et al. (1972) give the following description of behaviours performed by gynes of *P. subnuda* and *M. quadrifasciata*: auto-grooming and walking for recently emerged (non attractive) gynes, followed after acceptance by a period of intensified activity characterized by moving rapidly through the hive and soliciting for food by workers. After this phase the gyne became attractive, inflated her abdomen, walked excitedly through the hive, vibrating her wings, and had more trophallactic and antennal contact with courting workers. In this phase workers became aggressive towards gynes more frequently. Finally the gyne left with a swarm, replaced the physogastric queen or was eliminated by the workers.

Here data are presented about the process of acceptance of gynes in queenless groups of workers of *M. beecheii*, focusing on how their behavioural development from the moment of acceptance to the nuptial flight, and thereafter until the first egg-laying, contributes in establishing their dominance relation over workers. Data about the elimination of gynes and their specific defense behaviour are presented.

Material and methods

Colonies of *Melipona beecheii* housed in wooden boxes in Pozo Azul (Costa Rica, Guanacaste Province), where our experiments took place between November, 1991 and April, 1992, provided 30 experimental colonies with brood and worker bees. One group consisted of eleven colonies with 94 to 120 worker bees and 48 to 100 cells of combs with emerging brood, a second group consisted of ten colonies with about 60 workers and 90 to 145 brood cells, and a third group consisted of nine colonies with 120 to 180 workers and 250 to 400 brood cells. All colonies were established in glass-covered wooden observation hives of 16 (length) × 11 (width) × 7 (height) cm (inside dimensions), with a plastic entrance tube (10 to 20 cm long, diameter of 12 mm). If more than 50% of the brood had emerged, additional brood from the same mother colony was added. Occasionally colonies were fed with honey of *Apis* on the brood comb. All recordings on the behaviour of gynes were made through direct observation under red light. In all artificial colonies the number of gynes that were eliminated before a gyne was accepted was scored.

The colonies of group one were inspected daily at 6 a.m., 12 a.m. and 6 p.m. for the presence of an emerged gyne. Once a gyne emerged, her behaviour was observed (focal animal method; Lehner, 1979) every hour for 10 min, from 8 a.m. until 5 p.m., throughout the pre-mating period and after her nuptial flight, until she started egg-laying. An ethogram was made and the total time spent per ten minutes was scored. Special attention was paid to the behaviour of workers and gynes towards emerging new gynes, and to gyne behaviour immediately before and after the nuptial flight.

The experimental colonies of groups two and three were used only to score emergence, acceptance, nuptial flight and the start of egg-laying by gynes and newly mated queens. Every two hours these colonies were checked for the presence of emerged gynes, which were marked immediately with a small two-letter-code paper tag, glued on their thorax.

Results

1. Emergence of gynes

The moment of emergence was observed for three gynes. These gynes cut off the capping of the cell around the border with their mandibles, leaving an almost perfectly round cover. Immediately after leaving the cell, the gyne ran over the comb, through the nest to the periphery, away from the two to four workers that were around her cell. The gynes were seen hiding in or under empty storage pots.

Eleven of 38 gynes were attacked by workers while trying to leave their cell. The workers grabbed a gyne by the legs or antennae and started pulling her. The gyne escaped by turning rapidly around, while vibrating her wings. She pushed firmly with her hind legs and tried to rub her abdomen over the head of the attacking workers, and finally ran to the food storage area, thereby sometimes even passing over a worker. The attacked gynes were always eliminated by the workers within the next 20 h.

A very peculiar behaviour performed by some (not all) gynes, as a reaction to aggression by workers, was feigning death. The gyne kept completely immobile, with her legs and antennae folded under her thorax and abdomen. The workers dragged her to the waste dump, where the gyne stayed for several minutes in this state, until she started moving again. There upon agonistic interactions with workers followed until the gyne was killed.

During the contacts between gynes, lasting 5.4 s (SD = 4.1, n = 39) on average, one gyne functioned as aggressor, trying to grab the other gyne with its mandibles. The other gyne pushed the aggressor away with its hind legs, making oscillating movements with its abdomen.

2. Gyne longevity in queenless colonies

Between zero and 13 gynes were killed in the colonies before one was accepted, the average being four (SD = 5, n = 16). Between the acceptance of a gyne by the colony and her nuptial flight two (SD = 3, n = 11) more gynes emerged, all of which were eliminated. The gynes that were eliminated before acceptance of the new virgin queen, lived an average 46 h (SD = 30, n = 5), whereas gynes that emerged after acceptance of a new virgin queen lived only for an average 14 h (SD = 18, n = 24), which is significantly different (T-test, df = 27, t = 3.3, p = 0.003). In one case a gyne coexisted for 64 h in a nest with an accepted virgin queen.

3. Abdominal inflation in gynes

In seven artificial nests the morphological development of gynes was followed with respect to abdomen enlargement. Gynes showed no noticeable enlargement the first 12 h after emergence. During the following phase, 56 h (SD = 60, n = 15) lasting periods in which the abdomen enlarged by approximately 30%, were alternated with significantly

shorter periods of 11 h (SD = 5, n = 16; T-test, df = 29, t = 3.0, p = 0.006) during which this phenomenon was absent. Virgin queens that were eliminated without an accepted gyne being present, enlarged their abdomen for only 11 h (SD = 1) at intervals of 12 h (SD = 4, N = 12). Of all gynes (n = 24) that were eliminated after a virgin queen was accepted, only one had an enlarged abdomen once for about 12 h. Without exception all gynes (n = 36) were eliminated when they did not have an enlarged abdomen. When an accepted gyne was also present, she always had an enlarged abdomen (n = 24).

4. Pre- and post-mating behaviour

In four experimental nests, accepted gynes were scan sampled or followed as long as possible (focal animal method) in order to register the behavioural repertoire. Observed were pushing, food soliciting, getting fed (Kolmes and Sommeijer, 1992), auto-grooming, standing, tapping (Koedam et al., 1995b), hiding in or under storage food pots and antennal contact with workers. Walking through the nest box was not scored as a separate behaviour.

The gynes were observed for 15, 14, 12 and 13 days respectively, and realized their nuptial flight on day six, three, three and four. In order to compare the behavioural development of the four gynes, the data were standarized related to the day of their nuptial flight, using only the data obtained during the three days preceding the nuptial flight and nine days afterwards.

Daily average values are presented for the frequency of different behaviours of the four gynes (Fig. 1). The behaviours hiding and tapping showed a significant increase in the time the gynes spent on these behaviours for the observed period, and pushing a significant decrease (Multiple Regression ANOVA, $F = 38.2$, $P < 0.001$; $F = 10.9$, $p = 0.004$ and $F = 3.9$, $p = 0.049$, resp.). Tapping was especially low on the first three days (Fig. 1). Although no overall effect could be noted for the other behaviours, a strong tendency of intensified antennal contact between the newly mated queens and

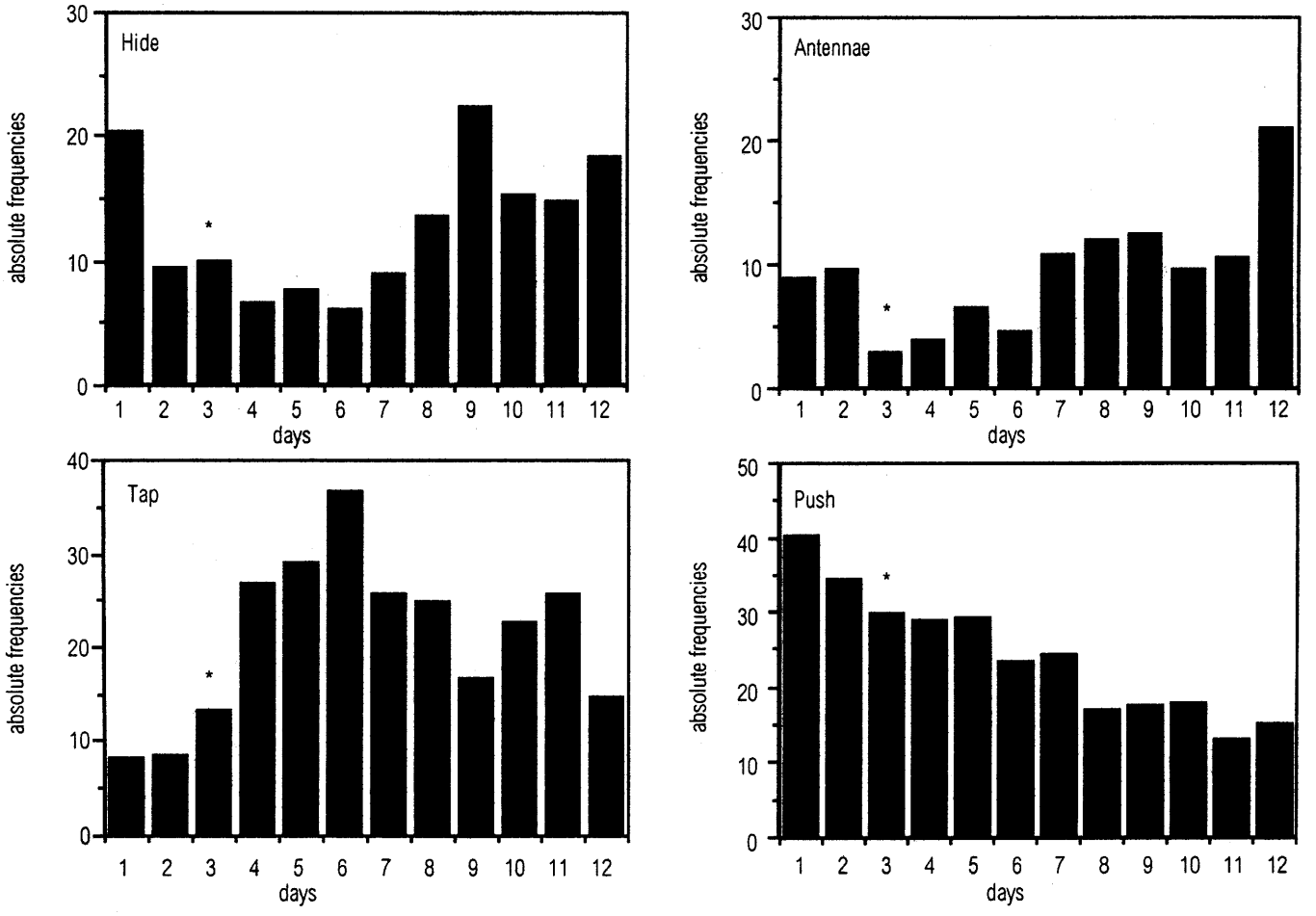

Figure 1. The activity of accepted gynes starting three days before their nuptial flight (indicated with *) and afterwards, until the moment of first egg-laying. The values presented are daily average frequencies of behaviours for four gynes, during nine observation periods of ten minutes each. It should be noted for a correct interpretation of the data that the scan sampling method used for this study provides no information about the duration of the behaviours.

the workers could be observed during the days preceding their first egg-laying. The time spent on standing followed a similar pattern.

The behaviour „auto groom“ was performed more or less constantly during the observation period. Soliciting for food was especially high on the first day after acceptance and was relatively low awarded. The ratio soliciting – getting fed increased from 0.45 on the first day to 1.37 on day twelve ($F = 3.502$, $p = 0.091$), which means that occasionally gynes were offered food by workers without having to solicitate for it (after day 9).

Discussion

The presence of gynes in nests of *Melipona beecheii* is a situation characterized by agonistic interactions between a gyne and workers or other gynes. Gynes were found to open the cell from which they emerged. Immediately afterwards they were active and seemed to avoid worker aggression by running to the periphery where they hid. Pushing was found to be especially high during the first three days (Fig.1) for accepted gynes. If grabbed by a worker the gynes rubbed their abdomen over the head and the thorax of this worker and tried to run away. Often gynes feigned death to escape from worker aggression. This behaviour was very effective at the moment of performance, but apparently performed by “weaker” gynes, since none of the gynes was later accepted. Interactions between gynes were always of an agonistic character. Since all colonies were hived in the same size nest-boxes, aggressive encounters between gynes and workers might have been influenced by the population density. However no relation was found with the size of the experimental colonies and queen longevity nor with the number of gynes killed.

The 19.6 % gynes that were not eliminated within 12 h after emergence developed a temporarily inflated abdomen. In gynes that subsequently became accepted this enlargement lasted significantly longer than for gynes that were eliminated. This indicates that gynes with a more prolonged abdomen inflation were more attractive to workers. Gynes of *M. favosa* with abdomen inflation had an increased number of workers in the court around them (Koedam et al., 1995b). Inflation is a display that suggests development, similar to that found in mated physogastric queens. It is therefore likely that gynes in queenright colonies of *M. beecheii* are usually eliminated before the phase of abdomen distension. This assumption is supported by the fact that gynes in our study were eliminated within 14 h after emergence if an accepted gyne was present in the nest boxes, and until after 46 h if no accepted gyne was present. In this respect it is important to note that the presence of an accepted gyne in the nest reduced the life expectations of newly emerging gynes significantly. It is not clear whether the working of some pheromone in the process of acceptance of the dominant gyne also stimulate the workers to eliminate the newly emerged gynes. Many remarks are made in the literature about gyne attractiveness and pheromone production based on increased glandular activity (Cruz-Landim et al., 1980; Cruz-Landim and Mota, 1990; Engels and Imperatriz-Fonseca, 1990; Imperatriz-Fonseca et al., 1998; Kleinert and Imperatriz-Fonseca, 1994; Koedam et al., 1995 a and 1995 b), but so far the exact functioning is not known. Our data seem to support an hypothesis (Koedam et al., 1995 b) that abdominal glandular development and resulting pheromone release play a role in the acceptance of a gyne by workers and the establishment of her dominance.

The behavioural repertoire of gynes and newly mated queens of *M. beecheii* was rather limited. Koedam et al. (1995 a) obtained similar results for *M. favosa*. No cerumen-working activities, as for *Plebeia* and *F. varia* gynes which participated in the construction of refuges (Imperatriz-Fonseca and Zucchi, 1995), or direct uptake of food from storage pots (Silva et al., 1972) were observed. In the process by which gynes obtained a gradual dominance once accepted, two phases could be distinguished: before and after the nuptial flight. Before the nuptial flight took place gynes were frequently involved in agonistic interactions. Pushing, which can be explained as an agonistic behaviour (Sakagami, 1982) was frequent, and diminished gradually. Hiding, which may be explained as a behaviour to avoid aggression was especially high on the first day. Another indication of a gradually increasing dominance by the gyne over the workers is the way in which food soliciting by the gyne was awarded more frequently with time. Koedam et al. (1995a) obtained similar results for *M. favosa*. They assumed that food deprivation could be a major reason for death of gynes in queenright colonies of *M. favosa*. After mating, the behavioural repertoire of the queens was characterized by a sudden increase in tapping workers on head or thorax with forelegs and antennae, demonstrating the dominance of the queen and the submissive attitude of the workers, less agonistic interactions occurred, pushing diminished and the workers started offering food to the queen.

Our data suggest that in *M. beecheii* under queenless conditions behavioural interactions between workers and gyne(s) and her abdomen inflation are of importance for initial acceptance of a gyne. Gynes that cannot avoid worker aggression will be eliminated before they display an inflated abdomen. In our study this was 80.4 % of the gynes. Final acceptance was characterized by abdomen distension of the gyne and presumably accompanying pheromone production. If multiple gynes are in the phase of abdomen enlargement, the pheromonally most attractive gyne is likely accepted. In our study these were the gynes that had significantly more prolonged abdomen enlargement.

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