

A NOTE ON THE RITUALISED BEHAVIOUR OF *MELIPONA* BEES FROM XAPURI

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ABSTRACT

The behaviour during the provisioning and oviposition process (POP) of three species of Amazonian *Melipona* bees, native in Acre, are compared: *M. crinita*, *M. eburnea fuscopilosa* and *M. grandis*. The POP of the stingless bees is considered to contain a number of ritualized behavioural elements. This means that during evolution a functional shift has taken place from an act serving the individual into a communicative signal, thus serving the interaction among individuals. Because stingless bees live in the dark, communication can be expected to be mainly of a pheromonal nature. Yet the descriptions of POP behaviour concern what is visible to the human eye: postures and movements. In this paper an attempt is made to connect some of such postures and movements with odourous signals.

Key words: Chemical communication, *Melipona*, ritualisations

INTRODUCTION

The inducement for this study was the variety in some, and the conformity in other POP behavioural elements I observed among the stingless bees. These bees are reknown for their complex provisioning and oviposition process (POP), in which workers and the queen of a colony closely cooperate. Although there are considerable differences in several aspects of this process among the many genera of the stingless bees, it always concerns the assembling of the queen and a number of workers around a completely constructed cell. In response to a signal from the queen the workers, one by one, deposit the contents of their stomach into this cell. In some species food deposition may occur in a number of cells synchronously, once it has been started in the first one. If there is enough food in the cell, the queen places an egg on top of this food and departs, leaving the sealing of the cell to one of the workers. In a number of genera, before the queen's oviposition, a worker may lay a trophic egg, which is eaten by the queen and which constitutes an important element in her acquisition of proteins.

Leaving aside the variations in the POPs of the many species, it is obvious that such an efficient

process can only exist if there is a system of communication among the participants. Sakagami and co-workers described these variations in a serial study. In his review [6] these behaviour patterns are called ritualized, which means that they are considered to be communicative and, being the product of an evolution process, derived from a more original element of behaviour.

It is remarkable that the descriptions of POP behaviour concern the postures and movements of the individuals involved, though we know that stingless bees have their nests in a dark environment. Those characteristics that we can see probably are only accompanying the real communications, which might be of a chemical nature. Postures enable the emanation of a pheromone, movements bring them to the required place or are needed to evade the signal produced by another. In addition to such specific signals, there may be odours that are not produced in order to elicit a specific response in a partner in a communication, but that are just a more or less permanent characteristic of an individual. For instance, differences in body odour among individuals provide clues that regulate behaviours such as aggression, and victims would profit from not disclosing their identity.

With this kind of problem in mind I studied the POP behaviour of some *Melipona* species, hoping to better understand the ritualized behaviour. Such

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understanding is supported by the many studies by C. da Cruz-Landim and co-workers [2,3] on the glandular systems of the bees. Bees possess a number of glands which, together, secrete a great variety of substances used in communication. The products of a given gland often differs according to caste, age or function, and therefore, provide an excellent background for the necessary differentiation of signals. However, neither the human nose, nor the technology for capturing and characterizing the minute amounts of substances used in a moment of communication, allow a direct correlation between the presentation of a chemical signal by a messenger individual and the response of the receiver. Therefore, this analysis concerns a mixture of objective data and subjective interpretation, or of what is visible in the behaviour and what I consider a plausible explanation of the nature of the interaction.

MATERIALS AND METHODS

The three species of *Melipona* reported on in this study were kept in the Experimental Station maintained by Dr. Paulo Nogueira-Neto in Xapuri, Acre, Brazil. The species are *M. crinita*, *M. eburnea fuscopilosa* and *M. grandis*. The observations were made in October-November of the years 1999, 2000 and 2004. Three colonies of *M. crinita* were used, and one each of the other species.

Colonies were kept in hives, as designed by Dr. Nogueira-Neto [5]. After the removal of the hive lid, one has access to the involucre surrounding the brood area of the nest. By removing the very top of this involucre it was possible to see whether cell construction was taking place on the uppermost comb. Only colonies constructing brood cells at this comb were used, because taking apart the combs often disturbs the normal division of labour. In such a case many bees become involved in repair activities. Once cell production on the upper comb was ascertained, the opening in the involucre was enlarged and a glass plate, with a circular opening of 12 cm diameter was put on the hive. The inner margin of the opening was wetted with paraffin oil, a material that inhibits the bees to cross. The chinks between the glass and the hive wall were sealed with tape. The opening in the glass was covered by a separate smaller glass plate, which was at times replaced by a clean one when necessary. When the colony was not under observation, this glass plate was covered with black plastic foil and the wooden hive lid.

Direct observations were made through the glass. Events were also observed and registered using a Sony video walkman and a digital camera (Watec 902H, black/white, and Watec 217HS colour camera) equipped with a Watec Rainbow manual zoom lens, enabling macro

registrations at rather low light intensities. Observations had to be made at a low light intensity, to prevent bees from running or even flying around within the hive space, thereby disturbing the normal activities in the nest. This meant that a tent-like construction above the glass plate was necessary, made of wire and black plastic foil. This plastic was semitransparent for light; eventually, two layers were used. A cold light source equipped with a red filter, was sometimes used for illumination.

Video registrations were aimed at the recording of interactions prior to and during the POPs. Therefore, the camera was focused at one or some nearby new cells in a progressed stage of construction, that is that they already had a collar. While this area could be watched on the screen of the recorder, the events near these cells were recorded on tape as soon as they became too complex to write down. Analyses of these tapes was done later.

For the description of the temporal subdivision and behavioural elements of the POP the definitions given by Zucchi *et al.* [10] were used. In the stingless bees, distinct periods of ovipositions are separated in time by interoviposition periods. During the oviposition period, several cells may each receive food and an egg, either independently of each other (Unit Oviposition Period) or in an more or less integrated manner. In the latter case the UOPs may come in a quick succession, as a batch. The more integrated form, IOP, is characterized by a common signal releasing the synchronous start of a number of POPs. Such IOP, however, does not occur in *Melipona*. A POP is subdivided into a provisioning stage, an oviposition stage and an operculation stage. The provisioning stage is subdivided into three substages: a preprovisioning, a provisioning, and a postprovisioning substage. In this study I concentrate on the observations concerning the provisioning stage.

RESULTS

Melipona crinita

The POPs of this species occurred in batches of 2-7 cells, which were often located on three consecutive combs, the largest being the lowest of the three, the smallest on top. A batch could either start slowly, in which case the queen cruised the comb for a relatively long time, followed by long preprovisioning and provisioning substages of the POPs. In such a case, there were few POPs in a batch. More often, the queen did not cruise at all or only for a brief period of time. In these cases the different substages were of a brief duration and the number of POPs in the batch more numerous. Such a batch commenced with a number of workers surrounding the cells ready to be provisioned, in the absence of

the queen. Workers showed what in earlier papers, on other species, has been called “agitation”, which in this case means that they predominantly pointed with their heads towards the cell while they were in constant motion. In these motions, each bee moved alternately forward and backward as well as sideways. Characteristically, the forward movement was combined with frequent touching the comb surface with the mouthparts, while the backward movement was more rapid, lacking such touching. The sideways movement was combined with going forward and was more prominent at the beginning of the forward movement. The surface area for one cycle of movements of a single bee equalled about one cell of the comb surface. Bees on the side wall of the empty cell performed similarly, as did those clinging on the neighbouring cells. While performing this kind of dance, usually the bee was slowly moving forward. When it ultimately reached the cell, the bee went deep into it, then came out again and usually left the area. It was remarkable to see that in such situations the queen, who usually approached from lower combs, climbed onto the higher comb at the place where bees were dancing. It may mean that she didn't need to cruise the comb in order to find a cell ready to be provisioned. Usually, within a minute after her arrival the first food deposition into the cell took place. When, after oviposition, the queen moved on to the next cell of the batch, she usually moved to a cell surrounded by dancing bees, ignoring other cells that appeared to me to be just as suitable. Around the subsequent cells there could be fewer bees, especially for the last cell of the batch.

At other times the queen arrived at the comb without such dancing bees, in which case she walked around and found cells, but she usually moved on and eventually disappeared from the comb. If she stayed at a cell, and in case this was followed by the start of a POP, the duration of the preprovisioning substage could be as long as 7 min 30 sec.

The provisioning substage could be rather brief and compact, but sometimes took much more time. If compact, there was a quick succession of workers depositing food into the cell; if it took more time, not only was there an increase in the intervals between subsequent food depositions, but there were also workers that merely inserted their heads into the cell. While food depositing workers left the area around

the cell after they had provisioned, workers that merely inserted often stayed nearby.

After the provisioning substage there usually was a distinct postprovisioning substage. During this period a sequence of workers came to the cell, inserted, and continued with stretching their bodies over the cell. This behaviour was observed previously in *M. rufiventris flavolineata* [4] and, in a somewhat different form, in *M. seminigra merrillae* [1]. This behaviour was called “cobrir a célula” (covering the cell) and “crouching”. I suggest to use the term “Standing Over the Cell”, abbreviated as STOC, which expresses the aspect covering the cell as well as the absence of movement. During the postprovisioning substage it often occurred that workers laid a trophic egg, executed in the same manner as in other *Melipona*'s.

The different phases of the POP could also be characterized by the behaviour of the queen. In all cases she stood oriented towards the cell. In the preprovisioning substage she usually had the mandibles very close to the cell margin; during the provisioning substage the front of her head was at 0.5-1.0 cell diameter distance, allowing her to position the tip of the antennae at the cell margin, while during the postprovisioning substage she usually stood at a distance of 1.0-2.5 cell diameter. The queen occasionally inspected the cell by directing the antennae towards and above the cell, and eventually she came forward. She could briefly insert her head into the cell. As in the other *Melipona*'s, she stimulated the first worker to regurgitate food by drumming on the abdomen once the worker was deeply inserted into the cell. For this drumming she used mainly her antennae, but sometimes her front legs as well. Often the second and third depositing worker received a similar drumming on their abdomen, but frequently only when this deposition had already started. After the queen had inserted into the cell herself, she no longer performed the drumming behaviour.

Egg laying by the queen, in a direct sequence of trophic egg consumption and food uptake from the cell, frequently concluded her participation in the POP. Often a period with STOCs was terminated by the queen in that she moved to the cell, ingested some of the larval food and oviposited. She then departed, found another cell of the batch or disappeared from sight. Only a few times did she continue cruising the comb after the last POP of the batch.

Melipona eburnea fuscopilosa

Several colonies of this species were available. In one of them cell construction occurred at the upper comb and therefore, this colony was chosen for observations. Three combs were visible with many cells in all stages of construction at their margins. The uppermost comb had a diameter of 12 cells, the second one of 22 cells and the third was of an oval shape and measured approximately 30 x 25 cell diameters. The next day, the first cell of a new comb was already constructed on top of the youngest comb. This shows that in this species cell construction may take place at several combs at the same time, at places wide apart.

Interactions prior to the preprovisioning substage

The queen, cruising over the comb, occasionally found a just completed cell and positioned herself at its margin. But there appeared to be something missing: bees that could deposit food. For some minutes the queen would persist, and it could happen that a worker arrived and went deep into the cell. This worker was beaten by the queen with the antennae and frontlegs. The worker then responded by coming upward until only her head was still within the collar of the cell, and she started working the inside of the cell. Such a response usually evoked in the queen an immediate departure. She would resume walking, and could find another cell where a similar sequence of events would take place. This kind of activity of the queen often continued for 30-40 min. At other times she remained hidden somewhere in the nest. At such times there was little activity of workers on the combs, except for cell builders.

No difference was found in the average duration of the visits of the queen at the cell during the cruising stage compared to her last presence, that is during the preprovisioning substage (s.s.) of the POP (Table 1). A difference, however, was discovered in the behaviour of the workers. During the major part of this presence the queen was awaiting a worker to

go deep into the cell; if in response to the queen's beatings this worker immediately and persistently shifted to working the inside of the collar of the cell, the queen moved away within a few seconds. If, however, the worker remained with the head deep inside the cell, or returned quickly to the bottom of the cell after an ephemeral upward movement, the queen stayed and continued her beatings. Instead of working the collar, workers could also quickly depart from the cell. In that case the queen usually remained at the cell.

A further inquiry was possible into the question of what determined the transition from mere presence at the cell and the start of the provisioning. During the time interval of 40 min in between two POPs, it occurred 6 times that the queen positioned herself near the same cell and waited for a worker to arrive and to go deep into the cell. The queen interacted with that worker, and once the worker started to manipulate the inner side of the collar, the queen left. For the intervals when the queen was absent, the duration of worker activities at the cell was determined. It was found that a gradual shift occurred from mainly working at the collar region of the cell towards working at the bottom (Table 2). Furthermore, the frequency of workers coming to this cell had increased dramatically in the last period, prior to the arrival of the queen and the start of the preprovisioning substage of the resulting POP.

Workers selecting cells to be provisioned

While the queen was cruising, or absent from the combs with new cells, there usually were some workers standing on the comb and mandibulating its surface. They had mainly jerky head movements. In addition, in anticipation of the batch of POPs there appeared workers that showed "agitation", that is, while standing more or less at the same place, they made rocking movements and hit the surface below with their mandibles. They stood on the comb as well as on the side walls of the empty cell, and on the

Table 1. The duration of the presence of the *M. eburnea fuscopilosa* queen at a cell during the cruising phase compared to the preprovisioning substage of the POP.

	number of observations	duration (sec)			
		mean	min	max	median
Cruising phase	42	60	5	124	29
Preprovisioning substage	27	60	7	224	50

side walls of already closed cells next to the cell to be provisioned. Sometimes these workers appeared to stand in a random direction, but subsequently they became oriented towards the cell. Especially when the bees were positioned at the side walls they could be seen to touch the cell surface with their mandibles in a rhythmic and frequent beating. A similar behaviour was shown by workers in the court surrounding the queen. Such groups of “dancing” bees usually occurred simultaneously around some of the completed cells. It appeared as if a few workers started the behaviour and then were joined by others, thereby enlarging any local effect. When the queen arrived near such a group she usually went straight to the cell and, standing on the comb, initiated the preprovisioning by positioning herself with the mandibles practically touching the cell margin. In only a few cases she positioned herself to the side wall of the cell, in which case it was clearly visible that she too extended her mouthparts. However, in distinction to the workers, she was not beating the cell surface.

After the first discharge the queen inserted the head into the cell and moved backward, to maintain a distance of about 0.5-1 cell diameter during the provisioning substage. No further beatings of discharging workers occurred. Most POPs were of a compact nature, with only a few workers inserting in between the dischargers. STOCs did occur, and interestingly, the first STOCs did occur before the last food depositions had taken place. It also happened that some of the last discharging workers continued with a STOC. Depending on the duration of the postprovisioning stage, the queen could come to the cell and eat from the provisionings. A few times it was ascertained that a worker, after

performing a STOC, returned to the cell and laid a trophic egg; others laid their egg immediately after having performed a minute STOC.

Melipona grandis

The *M. grandis* colony available for observation had an involucre of a single layer, heavier than the lamellae of the composite involucre of the other species. Due to its thickness it was less flexible, almost leatherlike. It was greyish in colour. After the top of this involucre was removed, the two combs under construction were already visible. The lower one had a diameter half that of the first completed comb below it, while the upper one consisted of only 12 completed cells. The completed comb that was visible was oval in shape, with a long axis of 15 cells and 10 at its widest cross section. From the comb below it an additional number of 30 cells were visible. From this it was estimated that a completed comb contained 120-150 cells.

In comparison to other species of *Melipona*, the workers of *M. grandis* were rather sluggish in their movements on the comb. However, in the preparation of the POP this sluggishness disappeared. While in the interoviposition periods only few workers, mainly cell builders, were on the comb, in the preparation of POPs more and more workers showed up. Here too POPs are of the batched type. The 35 ovipositions recorded were in 8 batches, varying from 3-7 POPs each. Batches appeared to be separated in time by a minimum of 4-5 hours. The construction of an individual cell was estimated to take at least 12 hours, after which there could be a delay before they were included in a POP. During this period workers frequently came to the cell and worked on the inside as well as at its outside.

Table 2. Temporal aspects of the cell polishing activity of *M. eburnea fuscopilosa* workers in the absence of the queen. Worker behaviour during five time slots in the cruising phase of the queen was analysed. The duration of the element “worker staying deep into the cell” is compared to the time spent on “working the inside of the collar”.

Time slot preceding the POP	worker deep in the cell		worker in the collar	
	number of observations	median duration	number of observations	median duration
- 40 till - 38 min	8	0.82 sec	7	4.41 sec
- 35 till - 34	7	0.88	7	2.72
- 30 till - 25	4	1.90	0	
- 20 till - 18	5	2.52	4	1.97
- 4 till - 1	29	2.69	25	0.91

Outside the periods with POPs the queen usually was hidden deep in the nest, but when she appeared she cruised the comb and occasionally requested a trophallaxis from workers. However, she hardly had any success. Instead, workers were seen to dart at her. When, infrequently, she stood still, a court of workers formed around her. The queen requested trophallaxis from these workers as well, but again without success. The queen clung to the outer wall of a fully constructed but empty cell for long periods of time, apparently “at rest”.

Preparations for a batch to start

Near the start of the first POP of a batch more and more workers arrived at the comb. Initially they just stood motionless or worked the comb surface with the mandibles, but then progressively they became more active and walked. In this phase trophallactic contacts became more frequent and included both worker-worker as well as worker-queen interactions. The often rapid increase in the mobility of the workers indicated that the first POP was to occur within minutes. Workers formed groups around collared cells and quickly moved forward and backward over short ranges. The forward movement predominated. While doing so, their mandibles, which were kept ajar, touched the comb surface. In this way they approached the cell, and one after the other they entered the cell up to the front legs, which were held perpendicular to the body. After such a deep insertion the bee withdrew from the cell and moved a few cells backward, where she continued with the forward and backward motions. If the queen came near such a group, some bees turned towards her, but continued with their actions. The queen usually moved in a straight line towards the cell and positioned herself in a vertical position along the side wall of the cell.

The first POP of a batch was often characterized by having a distinct preprovisioning substage. The duration of this substage depended on the number of workers present and the vehemence of their movements before the arrival of the queen. If there were already many workers present, there was no preprovisioning proper. If there was, now and then a worker went deep into the cell, with the queen beating the worker laterally with antennae and front legs, mainly on the wings. Often, the worker then departed. By definition, the transition to the provisioning substage occurred when such a worker,

in response to the queen’s drumming, regurgitated food into the cell. If there was no preprovisioning, the circle of workers around the cell was found by the queen, and while she was descending to the side wall, in passing she could beat a worker that was going into the cell. This worker responded immediately and regurgitated. The first POP of the batch was on its way.

In the meantime, the “dancing” bees present around other collared cells, on this and the other comb, continued their activities. After her oviposition in the cell, the queen usually found one of these groups of bees within a couple of seconds, positioned herself on the side wall of the cell and beat the first worker that went deep into the cell. Regurgitation occurred immediately, followed by more regurgitations by other workers. Again, the provisioning of the cell was a compact and efficient process.

The batch ended either by lack of empty collared cells or by lack of workers prepared to regurgitate. In the latter case the queen would come to a cell, but the few workers around that cell only had slow motions and hardly ever inserted into the cell, and if they did, they responded to the drumming by the queen with a quick retreat. The batch, therefore, has a distinct beginning and a well recognisable end.

DISCUSSION

In anticipation of a batch of POPs there is, at the individual level as well as at colony level, the necessary preparation. Synchronisation is needed in the preparation of the queen and of the workers. Within the context of this synchronisation I will suggest the explanation for some of the features of and the variation in the POP behaviour. But let us first consider the individual level.

For the queen preparation concerns the ripening of the eggs in her ovarioles. Given that in *Melipona* each ovary has only four ovarioles, there might be only a limited number of eggs ready at the beginning of the batch, perhaps only a maximum of eight eggs. This physiological aspect might be the reason why there exists a period of ovipositions, in our species concentrated in the batch, alternating with long resting periods for the queen, the interoviposition period.

The workers have to accumulate food in their stomachs in order to deposit it during the provisioning of the cell. This food should be of the proper composition, that is a well-defined mixture of pollen

and honey. The components of this larval food are available in the food storage pots, however, the two components of the food are stored separately. It is supposed that, like in *M. favosa* [8], some workers collect these constituents from the pots, and that other workers then sample these in a mixed form during trophallaxis. The exchange of food among workers is most prominently seen during the relatively brief period prior to the POP, and during the provisioning of the (series) of cell(s). Once deposited into the brood cell, for a given species the composition of the mixture is remarkably constant [9]. Mixing and distributing the food items is a process that needs time. I suppose the mixing takes place well before the start of the batch, and that the collection of it by the provisioners occurs in a shorter period of time, mostly before the start of the first POP, but also during the progress of the entire batch.

Synchronisation in these developments in the queen and the workers is needed to arrive at the POP. However, often either the queen or the workers are ready prior to the partner(s) in this process, and such variability is expressed by the variable presence of some behaviours: cruising by the queen and what I called “dancing”, or that, in other papers on the subject, were described as “agitation” or “excitement”.

Apart from queen and workers, a third factor needs to be mentioned, namely that there should be cells ready to be involved in the POPs. This not only concerns their erection, but also the perfection of the walls. Such perfection is perhaps not just the smoothening, but might imply the application of a substance that provides protection: against infestation of the food by micro-organisms as well as preventing leakage and exchange of food among neighboring cells. Such lining of cells is known to occur in many solitary bees. Table 2 indicates that the completion of the cell includes, as a last treatment, the careful manipulation of the inner side. Such worker behaviour is known from other *Melipona* species as well, however the change over time in the relative attention given to cell collar and cell bottom has not been reported before.

Let us suppose that *Melipona* workers, as a last action in the preparation of the cell, apply a substance when working at the inside of the cell. If, during the application of this lining, the queen comes to the cell, she will beat any worker inside. In response to this beatings the worker starts to work on the inside

of the collar. The queen of *M. eburnea flavolineata* responded immediately, she departed. This suggests that the worker is secreting a substance. Apart from a possible function as a kind of lining, this secretion also indicates to the queen that the cell is not ready yet. The same kind of communication is also suggested by the data reported in Table 1. The average duration of the queen’s stay during the cruising phase and the preprovisioning subphase was found to be the same. This suggests that this duration was determined by the probability of the worker being present. Once the worker goes deep into the cell she receives the queen’s beatings, and she either starts applying a secretion at the inside of the collar, or she signals her preparedness to deposit food. Her choice between these two alternatives depends on the condition of the collar as well as on the amount of food she carries.

A negative signal produced by the worker, communicating that the cell is not ready yet, is only functional when the queen is prepared to engage in POPs before there are sufficient workers to deposit food. In such a case we may find a cruising phase, during which the queen probes the preparedness of the workers by means of requests for trophallaxis. Such cruising includes attempts to start a POP. In the opposite situation, the workers are ready but the queen is still absent. All three species of this study provide indications of what happens if the workers are waiting for the queen to become active. They assemble around those brood cells that are ready to receive food and show some kind of special movements. In the existing literature, such movements were considered to express agitation and excitement, terms that are probably too much anthropomorphic. I prefer to use “dance”. Though the species differ in the details of this dance, in all cases the workers touch the substrate with their mouthparts. It is suggestive of the deposition of odour marks. In solitary bees we find an equivalent in the marking of nest entrances, which serve as recognition cue for the owner of the nest and as a warning signal for other females: upon entering such a nest aggression by the owner should be expected.

The supposed presence of such odour marks around cells would explain why the *M. crinita* queen so often mounted the comb exactly at the site where these dancing bees had gathered. An odour gradient, with the brood cell as its center, would also explain observations of bees that crossed the

entire comb in a straight line and arrived at the cell, where they immediately deposited a portion of food. In this context, it should be remembered that depositing workers are able to make more than a single contribution to a given provisioning process. In a short time they were able to collect this food for a second time, at a place distant from the cell. For such an efficient orientation, an odour field appears to be more likely than a return based entirely on memory or a random search. The attraction of a sufficient number of provisioners within a short time appears to be warranted by the presence of a specific pheromonal signal around the cell.

After the arrival of the queen at the cell, she inserts her head into the cell ("inspects"), upon which she positions herself with the mandibles above or just before the cell collar. In cases where the queen stands on the comb, we do not see what she is doing by observing her from above. However, when she positions herself onto the side wall of the cell, it is possible to see that she has the mandibles spread out, and that the other mouthparts are extended and kept in motion. This suggests that in the preprovisioning subphase also the queen produces a chemical signal. For *M. grandis*, clinging to the wall of the cell is the normal position. However, it also occurs in all other species, when it concerns the initial cells of a new comb. In that situation it can be seen that here too the queen operates with her mouthparts, just in the same way. It could be that the queen exposes larval food (which she has obtained a few minutes before during trophallactic contacts), or it may concern exocrine secretions.

In addition to such a chemical stimulus, the queen stimulates workers by beating them with antennae and front legs. In *M. eburnea fuscopilosa*, the queen stimulated only the first worker by beatings to deposit food. In *M. crinita* the queen stimulated the first one before this worker deposited, while in case of the second or third she only started her beatings while the worker was already regurgitating. In case of *M. grandis* also several workers received beatings. In all three species, however, once the queen had inserted the head into the cell, she would retreat to a greater distance from the cell margin, would no longer beat the workers and would stop the movements of the mouthparts. This suggests that for the continuation of the provisioning the presence of the queen is no longer indispensable. During the provisioning as well as the postprovisioning subphase, it may even

occur that the queen temporarily moves away from the cell, without causing a change in the behaviours of the workers.

The colonies of the three species differed in the duration of a POP and in the compactness of the batch. On average, a POP of *M. crinita* took 518 sec ($n = 58$), in case of *M. eburnea fuscopilosa* this value was 237 sec ($n = 27$) while for *M. grandis* a value of 134 sec ($n = 35$) was calculated. In *M. grandis* this even included the time the queen needed to go from one cell to the next. Such a rapid servicing of a batch of cells, that are scattered along the periphery of several combs, is only possible when the queen does not need to search for the next cell. She probably was guided by the groups of workers that applied odour marks near such a cell. As soon as there was no longer such a circle, there was no additional cell incorporated in the batch and the queen would no longer search. In the other two species, and especially in *M. eburnea fuscopilosa*, these groups of workers were less prominent, the workers being also more dispersed, and the queen needed more time to find a next cell of the batch.

That the number of eggs the queen can lay in a single batch is limited, is suggested by the durations of her successive ovipositions. The oviposition by the queen took in *M. crinita* on average 20 sec (range 12-185 sec), in *M. eburnea fuscopilosa* it took her on average 14 sec (range 10-80 sec) and in *M. grandis* 25 sec (range 18-40 sec). The extremely long values found for the first two species concerned the last cells of the batches. Also in this aspect *M. grandis* appeared to be the most efficiently organised species.

STOCs occurred in *M. crinita* and in *M. eburnea fuscopilosa*. These two species belong to the sub-genus *Michmelia* [7]. Also the other two species known to have STOCs, *M. rufiventris flavolineata* and *M. seminigra merrillae* belong to this subgenus. However, there is variation in the moment this behaviour can be seen. In *M. s. merrillae* it is performed by the provisioning workers, directly following the discharge, as well as during the postprovisioning subphase [1]. In *M. eburnea fuscopilosa*, at the end of the provisioning subphase, discharging as well as non-discharging workers were seen to have the STOC, while in *M. crinita*, like in *M. r. flavolineata* [4], STOCs only occur in the postprovisioning subphase. In *M. compressipes*, also a member of this subgenus, STOCs were not

observed [1]. The movement pattern suggests it is a ritualised derivative of egg laying: first inserting into the cell, then coming out of the cell followed by a movement forward, then a period (of variable duration) of remaining motionless. However, the communicative function of this ritualisation needs further investigation.

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