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STUDIES ON PARATRIGONA SUBNUDA (MOURE) HYMENOPTERA, APIDAE, MELIPONINAE III: QUEEN SUPERSEDURE

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ABSTRACT

In this paper the process of queen supersedure is described for *Paratrigona subnuda* (Moure), and some considerations on the conditions under which that process was observed are reported. The virgin queen plays a definite part in the natural replacement of the fertilized queen of the colony, because the process of substitution is started by the virgin queen when she drops an amount of her mandibular gland secretion onto the body of the physogastric queen of the colony, who then begins to be intensily licked by the workers. Generally the laying queen presents a particular defense behaviour; she only grooms herself, probably spreading in this way her own pheromones over her body.

The workers of *P. subnuda* court the physogastric queen by moving to and fro, but normally they do not lick her as it occurs in *Apis*. Sometimes the queen was observed accompanied by an unusual court, and ten or more workers licked her body strongly. At the beginning of the substitution process, the court of the queen increases with the participation of workers belonging to the virgin queen's court. They lick the virgin queen's secretion delivered on the physogastric queen's body. At this time the virgin queen is very active: she moves throughout the colony and when she stops many workers encircle her, lick the tip of her abdomen and begin trophallaxis with her. When the number of workers that lick the physogastric queen decreases, the virgin queen goes toward the laying queen's body and delivers some secretion over it again.

In the first phase the frequency of visits of the virgin queen to the physogastric queen increases gradually. Later the virgin queen's visits occur less often, until the workers that lick the physogastric queen's body begin to attack her. Generally her body is cut into pieces, starting with the abdominal glands on the terga, and she is thus eliminated from the colony.

Although the attempts to replace the physogastric queen are part of the normal life cycle of the virgin queens, supersedure is not often observed. It is important to consider that when the laying queen is more attractive than the virgin queen, she is not disturbed by the latter's secretions. Then the workers that encircle her kill the virgin queen.

The queen supersedure occurs frequently when the colony is swarming. At this time many virgin queens are together in the colony, and the relationships between several virgin queens and queen supersedure has already been explained. Queens that have suffered injury are also superseded, but the mechanism of the substitution is a different one: the virgin queen shows no active role in it.

RESUMO

Nesse trabalho a substituição natural de rainha em *Paratrigona subnuda* (Moure) e as condições em que esse processo ocorre, são descritas.

A rainha virgem (V_q) atrativa tem uma participação ativa na substituição natural da rainha fecundada (Q) da colônia, pois o processo é iniciado quando a V_q coloca uma gota de sua substância mandibular sobre o corpo da Q da colônia, que passa a ser intensamente lambida pelas operárias. Geralmente a Q conseque distribuir os seus próprios feromônios massageando somente o seu próprio corpo.

As operárias de *Paratrigona subnuda* têm um corte de avanço e recuo para a Q parada, a qual normalmente não é lambida, como acontece em *Apis mellifera*. Algumas vezes as Q de *Paratrigona* são lambidas intensamente por mais de 10 operárias. Esse comportamento está relacionado com a tentativa de substituição da rainha fecundada. No início do processo de substituição, a corte da Q aumenta com a participação de operárias pertencendo à corte da V_q: elas lambem a secreção da V_q depositada sobre o corpo da Q. Enquanto isso, a V_q anda ativamente pela colônia; quando para, infla o abdomen, cuja ponta é lambida por operárias que lhe fazem a corte, e às vezes entram em trofalaxis com ela. Quando o número de operárias que lambe a Q diminui, a V_q aproxima-se novamente e excreta sobre o seu corpo.

Na primeira fase do processo de substituição a frequência das visitas da V_q ao corpo da Q aumenta gradualmente. Mais tarde essas visitas tornam-se raras, até que as operárias que lambem o corpo da Q começam a atacá-la. O corpo da rainha é cortado em pedaços e eliminado da colônia (as glândulas abdominais dos tergitos são as primeiras a serem cortadas).

Embora a tentativa de substituição natural da rainha da colônia faça parte do ciclo biológico das V_{qs}, a substituição não é frequentemente observada. Quando a Q é mais atrativa que a V_q, não é substituida, e a V_q é morta por operárias.

A substituição natural de rainha ocorre frequentemente quando a colônia está enxameando. Nessa ocasião há muitas V_q na colônia, e as relações entre enxameagem e presença de várias V_q foi explicada. As rainhas injuriadas também são substituídas, mas o mecanismo de substituição é diferente: a V_q não tem papel ativo nesse tipo de substituição.

INTRODUCTION

The Meliponinae are eusocial bees presenting perennial colonies. In fact, there are nests that have been seen for at least 20 years at the same nest-site. However, the queen of the colony does not live for such a long time: in *Paratrigona subnuda* the physogastric queen lives during about 3 years. The constant natural queen replacement allows the colony a long existence.

The queen supersedure, which is the process of queen replacement without swarming, occurs frequently in *Apis mellifera*. Butler (1957) explained that, "sometimes in a case of supersedure the old queen and her newly mated daughter remain together in the colony for some time without apparent animosity, but, more often, the old queen desappears shortly before the young virgin queen mates, if not earlier.

According to Root (1945), it is not known whether she dies a natural death or whether she is killed by the young queen or by the worker bees."

In Meliponinae the queen supersedure was observed by Silva (1972) in *Plebeia droryana;* by Silva et al. (1972) in *Melipona quadrifasciata;* by Terada (1974) in *Leurotrigona muelleri* and *Frieseomelitta varia;* by Simões (1974) in *Scaptotrigona postica.*

In the present paper, the process of queen supersedure is described in *Paratrigona subnuda* (Moure), and some considerations on the conditions under which that process occurs are reported. The virgin queen acts directly in the natural queen replacement, being selected by the workers before the queen's supersedure (Imperatriz-Fonseca, 1977).

MATERIAL AND METHOD

The material used consisted of colonies of *Paratrigona subnuda* (Moure) in experimental hives. Films were taken with a Cannon 814-E super-8 movie camera.

The method was mainly the direct observation.

In this paper the physogastric queen is called Q, the virgin queen V_q, and the workers W.

RESULTS

In Meliponinae a corting of the queen by workers is not observed as it is in *Apis.* Sakagami (1971) compared the different kind of court in Apinae and Meliponinae. The relationships between queen and workers in the oviposition process, as well as the kind of court presented by the workers to the queen of *Paratrigona lineata,* were reported by Zucchi (1977).

The workers of *Paratrigona subnuda* court the physogastric queen mainly by moving to and fro, but normally they do not lick her as is observed in *Apis.* However, sometimes the queen of *P. subnuda* was observed with an unusual court, and ten or more workers licked her body strongly. After the elucidation of the biological cycle virgin queens (I-Fonseca, 1975), this fact was related with the queen supersedure: the workers lick the Vq's secretions delivered over the Q's body. Generally the Q presents a particular defense behaviour; she only grooms herself, probably spreading in this way her own pheromones over her body.

1 – *Description of a process queen's supersedure.*

The colony where the process of queen's supersedure was observed was very weak. The physogastric queen did not lay eggs. An attractive virgin queen was introduced into this colony, before her settlement phase.

After some days the process of queen's supersedure began. At 10 a.m., the Vq settled down in the colony. The Vq was on a structure of cerumen with a court

of 20 workers, all of them moving very actively; this situation is a characteristic of the settlement process. The physogastric queen had a court of two or three workers on the floor of the colony. The Vq was very active in the colony: she moved throughout it, and when she stopped, she showed movements of establishment, with inflation of the abdomen and strong trophalaxis.

At 11:00 a.m., the Q was near the lamelae of the involucrum, with a court of four or five workers that moved to and fro. Another active worker appeared near the Q, and also fourteen workers surrounded the Q, trying to touch her body (mainly her abdomen). Then the Vq was seen near the Q moving very rapidly and sometimes with circular movements of the inflated abdomen. The Vq was followed by several workers, and attacked by one of them. The court to the Q increased, and twenty or more workers came around. Some W did not leave the Q.

At 11:40 a.m. the Vq came near the Q and delivered a drop of buccal substance over her body. When the Vq arrived, there was a court of 15 workers to the Q; when the Vq touched the Q's body, about 30 workers surrounded the two queens actively. The Vq moved very actively. and was followed and attacked in the abdomen by some W of Q's court. Some workers tried to push the Q near the region of comb involucrum, others licked her mesosoma and metasoma; others bit the rear tergites of the Q, the same that are licked during the Vq's establishment. After the excretion over the Q's body, the Vq went away, and the Q had a court of several workers that licked her body, mainly the abdomen. Some W moved the wings actively, as an alarm attitude. The W that licked the Q were not always the same: some of them licked the Q and went away afterwards.

At 0:25 p.m., when the Q's court began to decrease, (it consisted of 6 or 7 workers) the Vq came again, releasing some substance over the Q's body. In that way, the court to the Q increased again. The activity of the Vq increased when she delivered a drop of substance over the Q's body. The Q tried to move, but the workers over her body or those that surrounded her did not allow her to move freely About 24 workers surrounded the Q, while the Vq moved in the colony searching for trophallaxis. In 3 minutes, the Vq came 14 times over Q's body, releasing substance over her body. Sometimes it seemed that there was some a delivery of fecal substances too.

At 0:36 p.m. the Q moved slowly along the wall of the hive. About 10 workers licked her and paid court. Some of them climbed over her body to lick it. For 3 minutes, the Vq visited the Q's body only twice. The Q moved her antennae, and some workers bit her rear tergites.

At 1:03 p.m., the Q's abdomen was very damaged, mainly in the region of rear tergites. The were 7 W near the Q when the Vq came to excrete over the Q's body; so, 15 W licked the Q, in the abdomen and legs.

At 1:10 p.m., two W cut the rear segments of the Q's abdomen so strongly that the queen lost her equilibrium. At 1:25 p.m. the central part of the 5th tergite of the Q's body was cut, and the W went away carrying her in their mandibles. The regions of the abdominal glands of the queen were damaged. At 1:43 p.m. the final part of the Q's abdomen was very damaged; a W pulled the Q by the leg, another licked her. The Vq came near the Q; the W came with their open mandibles to attack the queen.

At 1:48 p.m. the Q's tergites had no cuticle, but the Q walked very slowly on the floor, where she was licked and cut.

At 2:25 p.m., the Q was dead without wings and antennae. The Vq was very attractive, in her "settlement territory", and adopted attitudes of establishment.

2 – *Circumstances under which the supersedure was not observed*

The queen of *Paratrigona subnuda* spends most of her life on the comb, which is considered her main territory (I.•Fonseca,1975). Therefore sometimes the Q moves through the colony, and does not go to the new comb even if there are new cells being construted or being filled with larval food. This behavior of the Q is related to the activities of the active V qs in the colony. The Q can be found: a) resting on some involucrum surface, where she vibrates her wings and is paid court to by retreating and advancing workers; b) walking around the region of Vq's pots, where she searches for contacts with W; c) walking on the floor of the hive. Sometimes there are regions the Q is seen more often; at this time she is in the quiescent phase (Q, cf. Sakagami et Zucchi, 1974).

When many Vqs live in the colony, any one of them may finish her biological cycle. This coincides with a decrease in the number of cells constructed in each batch (the construction of cells is Be Sy (exclusively batched and synchronic), cf Sakagami et Zucchi, op. cit.). The Q can have different kinds of answers to one attempt at supersedure, according to the health of the colony and the relationships between Q and W It is very common, at this time, for the Q to change her normal attitudes towards the construction of cells: the phases of Cruizing, Waiting, Arousal e Patroling disappear, and the Q searches for more and more contact with W Sometimes the W put larval food in the cells, but the cells and W's eggs do not attract the Q. For some time, the Q only eats the W's eggs, but does not lay eggs. In the next IOP there are one or two brood cells, with a different kind of construction: 8 to 10 workers construct each brood cell. Only very attractive queens lay eggs shortly after the attempt at supersedure.

After the Vq begins to deliver drops of mandibular substances over the Q's body. the Q generally goes to the involucrum only to return to the new comb after the Vq's death.

Sometimes, when there are a few cells being cosntructed in each batch, one Vq completes her cycle. In two experiments, col. *Q* (1972), col. *2* (1977), examples of this could be seen. The Q seemed to be very well, with a very large abdomen. In both cases the Vq were taken out of the colony when they were attacked by the W mainly at the tip of the abdomen. After a few hours the rate of cell construction was normal again, and so was the activity of the Q. This shows that some Vqs try replace the Q of the colony even if she is in good condition.

Twice very attractive Qs were seen laying eggs even when their bodies were licked by the W: in col. *A* (3/2/70) the Q and all the places where she walked were licked strongly by the workers, and in col. P (5/3/1974) the Q was licked even when laying eggs in 10 cells. The two colonies (A and P) were in conditions of over-population, and the Vqs moved actively through the comb region and near the involucrum.

Generally while the Vq's excretions are rare, the Q stays on the new comb, sear-

ching for different kinds of contacts with W, depending on the phase of the supere-dure. When the colonies are weak, the Q seldom stays the comb region, and walks freely in the colony. mainly on the floor of the hive. The supersedure occurs where the queen is, and the attractive Vq can have the comb as her main territory. If we move one attractive Vq from a colony A to a colony B, where the Q is in bad condition, the supersedure will occur only after some time: the Vq must have a kind of relation-ship with the workers of the new colony.

3 – *Circumstances in which the supersedure was observed.*

Supersedure is mainly observed when the colony is about to swarm, because in this phase there are many virgin queens living together in the colony. Sometimes there is supersedure when the queen is not productive, and each batch always has few cells, generally from 2 to 6. Natural queen replacement also occurs when the Q is injured, but in this case the Vq does not act in the process. Queen maintained in artificial prisons, out of the comb, are also supersedured; this particular case will be discussed later, in another paper.

TABLE 1

Circumstances in which queen supersedure was observed.

colony	date	general health of the colony	swarming	cell construction
J	17/01/73	++++	yes	reduced
J	27/01/73	++	yes	absent
J	26/02/73	+	yes	reduced
F	16/11/72	++++	yes	reduced
L	03/01/73	+	not	absent
F	04/02/73	++	yes	reduced
J	12/11/73	++	yes	reduced
U	20/05/77	++	not	reduced
U	30/08/77	++	not	reduced
Ay	25/07/77	++	not	reduced
A_2	01/10/77	++	yes	reduced

+ weak colony ++ good colony +++ strong colony ++++ very strong colony

Comparing queen supersedures, the following observations can be pointed out:

a) the time spent for the queen's elimination varies with the situation. Once a Q that had been attacked for an hour and 35 min. was put in a glass box with pores (W and Vq could pass through them). The Q was almost dead, and the box was kept at 10 cm from the territory of the Vq. The Vq did not feel immediately the Q's change and had strong trophalaxis with W of her court. Some W were in the box near the Q, but did not touch her. Only after 20 hours did the Q die when W and Vq were inside the box; the Vq came over the Q's body to deliver her substances, and after this movement some W attacked the Q.

b) at the moment of the substitution, it seems that the Vq is more attractive than the Q.

c) the court of the Vq has more workers than the Q's one.

d) at the beginning of the substitution process, the court of the Q increases with W belonging to the Vq court. These W are aggressive to the Q, the Q's workers attack the Vq, when she approaches.

e) the rear terga are licked in the Vq when she is establishing herself, and the terga are also destroyed first when the W kill the Q. These facts seem to show the probability of the existence of special glands which are important to social regulation.

DISCUSSION

Simpson (1968) said that, in *Apis mellifera,* the beginning of the new queen's rearing in the presence of the physogastric queen can be followed by swarming, queen supersedure or nothing. It is difficult to know that will happen. In efficient supersedure the new queen hatches, is fertilized and begins to lay eggs while the old queen is alive and laying. In successful supersedure, the Vqs were not observed to produce sound or remain confined in their cells, but this can happen when the old queen is dead (Simpson, 1961). The cause of the absence of fighting between Vq and Q during supersedure is not yet explained. Simpson saw a fertilized queen cut and killed by young queen, three weeks after she began to lay eggs.

In Meliponinae some observations were made on queen supersedure. Silva (1972) observed, in *Plebeia droryana* that the Vq can attack the fertilized one even at the moment of hatching. The Vq bites the Q, and there is a fight between them. She also found in one colony two physogastric queens, a young one and an older one. Both laid eggs, in the same process, but the younger one was able to lay more eggs. The older queen, transferred to another colony of the same species, was also accepted and laid a few eggs (the younger queen also laid more eggs). The old Q could be accepted by another colony; this reinforces the idea that the amount of pheromones produced by the old queen decreases, because queens introduced into normal colonies are killed by the queen or workers of the colony.

Simões (1974), working with *Scaptotrigona postica,* saw the physogastric queen being attacked by the workers and pushed slowly over the comb, and the Ws tried to put cerumen over her body to recover it. After three days the new Vq had a court of Ws and seemed fertilized; after four days she began to lay eggs. Simões (1974) also observed that thrirteen days before the death of the Q a Vq ran about in the colony; many workers ran backwards, and often licked her body. This Vq sometimes entered into the comb region and was not attacked by the workers. Probably in *Scaptotrigona postica* the Vq that is to substitute the Q is chosen before the Q's death, but the mechanism of the queen's supersedure and the attitude of the Vq related with the Q was not observed.

Terada (1974) observed natural queen replacement in *Leurotrigona muelleri* and *Frieseomelitta varia;* at the time of the substitution, there was a high production of males and Vq, and the Q was and old one. The colony was not well. The newly hatched Vq attracted W and had a court, which was sometimes aggressive. The substituted queen was not in her main territory (the comb), but at the entrance tube. The Q had few workers in her court. This Q was altached by ws that did not paid court to her; others climbed over her body, stimulated by the presence of the Vq. Silva et al. (1972), observing natural queen replacement in *Melipona quadrifasciata,* verified that the Vq was selected and tolerated by workers prior to her mother's death. On the eve of the nuptial flight, in one of the cases of natural queen replacement, the accepted Vq actively participated in chasing the younger queens away. She excitedly ran all over the hive, mainly to the younger queens away. She excitedly ran all over the hive, mainly to the corners and cavities, chasing away other virgins so that, after leaving their hiding places, they could be caught by the workers. In these instances she stood in the fighting area and roughly attacked the virgins with mandibles and frontal legs, without being disturbed by the workers. These authors also think that "if there is a "queen substance" in stingless bees, the first queen to be accepted might be just after the disappearance of a given kind of queen pheromone released by the old queen"

Queen supersedure observed several times in *Paratrigona subnuda* shows the importance of substance produced and eliminated by the Vq (certainly pheromones), ando also how active the role of the Vq is in supersedure.

It was verified that many process of queen supersedure are observed when the colony is swarming out. It has already been explained (I. Fonseca, 1977) that the attempt of the Vq to substitute the Q is one phase of the Vq's biological cycle. The substitution process is the result of many interactions with the workers of the colony, which begins when the Vq is found hidden in her pot. The success of the Vq's role depends on her relationship with the workers of her court. In swarming time there are many Vq in the colony, so the probability of a queen supersedure is greater: this problem will be discussed at greater lenght in our considerations about swarming activity.

At the moment of the Q substitution, the Vq may produce a great quantity of pheromones (Vq S). The Q also produces pheromones (QS).

Vq S > Qs	the Q is eliminated
Q S < Vqs	the Vq is eliminated

At the moment of the Q substitution generally the Q grooms herself, with a normal court. The Q does not react to the excretions of the Vq over her body, she stays quiet, passing her front legs over her head and body, spreading her own substances over her body. If her substances are more attractive to the workers than the substances delivered by the Vq, the Q will kill the Vq.

In the same way, a change of dominance may occur through of a Vq_1 by another Vq_2 of the colony. When a Vq_1 substitutes the Q of a colony, she spends a great quantity of energy and also of glandular substances. As the existence of several Vq in the colony at the same time is possible, and as the physiological state of one Vq does not interfere directly in the physiology of other Vqs, any Vq can be completing her cycle at the same time as V_{q2} may settle down in the colony while V_{q1} is restoring her own substances. Just once it was observed that an attractive substitute Vq, which settled on the comb surface, was repelling another one with strong abdominal movements. This behavior is common in some Meliponine, as in *Plebeia remota* and *Plebeia droryana*, even when the Vq is inside the prison cell (I. Fonseca et al, 1975). Nevertheless it occurs mainly when the Vq is completing her cycle, as Zucchi (1977) also observed in several *Plebeia, Friesella schrottkyi, Frieseomelitta varia* and *Melipona quinquefasciata.* In *Paratrigona* the Vq is killed by the workers; a fight between Vqs was never seen, nor was a Vq seen attacking a Q with her mandibles, as Silva (op.cit:) described for *Plebeia droryana.*

Queen supersedure is also observed when the queen is not productive. When it occurs, the Q's elimination is carried out by the process described in this work.

Just once an injured physogastric queen (without one antenna) was observed. After she had lost her antennae, this queen did not come to the comb region, but walked on the floor of the colony in circles. This queen was killed by the workers 1 month later, but there was no Vq involved in the process of killing.

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