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PRODUCTION OF MALES IN COLONIES OF MELIPONA BEECHEII, COSTA RICA

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SUMMARY

Because of the great importance for an evolutionary approach of social behaviour in eusocial insects, we studied the presence of egg-laying workers, related to the production of males in the stingless bee *Melipona beecheii.*

In the most favourable season we found a large number of male pupae in the brood, while a relatively small number of adult males was present. A colony kept in captivity in Utrecht showed an absolute absence of both adult males and male pupae in the nest. The results support the hypothesis of a season bound production of males by workers and/or the queen, formulated in 1983 by Sommeijer. The results are discussed in relation with the size of the colony, the age of the queen and the high mortality found for young males. Results on the presence of queen pupae are presented.

Key words: stingless bee, Melipona beecheii, social regulation, egg-laying workers, male-production.

RESUME

Production de mâles dans une colonie de *Melipona beecheii*, Costa Rica

Cette étude porte sur la présence d'ouvrières pondeuses, liée à la production de mâles, chez *Melipona beecheii.* La présence de telles ouvrières est d'une grande importance: elle permet une approche de l'évolution du comportement social chez les insectes eusociaux.

Les mâles, issus d'oeufs haploïdes, peuvent provenir soit des reines, soit, en totalité ou en partie, des ouvrières pondeuses. Les ouvrières de Melipona favosa, qui produisent des mâles en abondance dans les colonies sans reine, sont aussi capables de pondre des oeufs reproducteurs en présence d'une reine (Van Buren et Sommeijer, 1987). Ces oeufs donnent également des mâles.

En 1983, Sommeijer (et al., 1984) a formulé l'hypothèse d'une production saisonnière des mâles par les ouvrières pondeuses. Ce phénomène serait lié à l'affaiblissement de la dominance et du contrôle physiologique des reines dans des colonies qui ne cessent de s'accroître en saison favorable.

Mots clés: abeille sans dard, Melipona beecheii, production de mâles, régulation sociale, ouvrière pondeuse.

INTRODUCTION

The presence of egg-laying workers in eusocial insects is of great importance for an evolutionary approach of social behaviour in insects. Because this feature is clearly developped in *Melipona beecheii*, we made researches into the egg-laying behaviour of workers in this stingless bee.

Because males are born from unfertilized (haploid) eggs, it is possible that they are sons of queens or exclusively (or mainly) sons of egg-laying workers. After it was found that they produce plenty of males in queenless colonies, workers of *Melipona favosa* also appeared to be able to produce male-born reproductive eggs in presence of the queen (Van Buren & Sommeijer, 1987).

In1983 Sommeijer (et al., 1984) has formulated a hypothesis that supposes a production of males by egg-laying workers depending on the season. According to this hypothesis this would be connected with the diminishing reproductive dominance and physiological control of queens in nests that increase in size when the season is favourable. Workers of suchs nests would be able to produce reproductive eggs because of incomplete "suppression" of ovary activation in these workers, e.g. by pheromones, by the queen.

To test this hypothesis we investigated the production of males in *Melipona beecheii*, both in Costa Rica and in Utrecht (The Netherlands) on one hand, and a possibly seasonal presence of males in natural nests on the other hand.

MATERIAL AND METHODS

In the period from March until May 1989, observations were made in Costa Rica on four colonies of *Melipona beecheii*. Shortly after sampling, the colonies were put in small wooden observation hives (plywood 30(l)*15(w)*10(h)cm) covered with a glass plate, in a dark room. The hives were connected with the outside through the wall, by a plastic transparent tube (ø 20mm, length~25 cm). With the use of red light, observations in the nests could be made without disturbance. During a period of 21 days spread over 6 weeks, the egg-laying was studied. The Provisioning and Oviposition Process (POP) was observed 54 times in three of the four nests. All colonies studied in Costa Rica were regularly checked on the presence of adult males. Because of the slight morphological differences with workers, the males were marked individually. Two large colonies, A and B, contained each more than 1500 individuals. The two small colonies, C and D, contained less than 500 individuals. In two ways the presence of males in the nests was measured. Firstly by sampling the broodcombs on male pupae, and secondly by estimating the number of adult males in the nests.

The results found in Costa Rica were compared with those measured in July in Utrecht on a colony of *Melipona beecheii* kept there since January 1989. These bees were kept in similar observation hives as used in Costa Rica, in a constantly darkened room, without any possibility to fly out. They

were fed daily with a pollen mixture (prepared after Camargo, 1976) and syrup. Brood samples were taken from combs with pupae, from eggs layed in July.

RESULTS

Before we give the results of our observations concerning the production and presence of males, we shall mention some general aspects of the colony size and the provisioning and oviposition process.

1. Colony size and oviposition rate

The size and the daily oviposition are important indicators for the condition of a colony and are therefore important for the interpretation of the results. These data are presented in *table 1.* The table shows that the large colonies that were used contained on the average equal numbers of adult bees.

Colony	Size	Average daily oviposition	Observation period (days)
A	>1500	15.8	30
B	>1500	14.6	23
C	< 500	7.8	36
D	< 250	8.4	27
Utrecht	>1500	13.8	23

Table 1*: - Size and oviposition rate, for the free-flight colonies in Costa Rica and the colony kept in captivity in Utrecht.*
Tableau 1: - *Taille et taux de ponte dans les colonies en liberté de Costa Rica et la colonie en captivité d'Utrecht.*

2. Observations on the presence of males

At the end of the dry season in Costa Rica, in the period March-May, observations on the egg-laying behaviour were made. Special attention was given to the laying of eggs by workers during the POP. The average duration of the POP, measured from the first food discharge by a worker untill the egg-laying of the queen was 12.6 $\pm$ 4.9 min. During the POP the appearance of egg-laying by workers in the cell before the oviposition of the queen is very common (Michener, 1974; Sakagami, 1982). During the 54 times a POP was observed, the queen always produced the reproductive eggs. Immediately after the oviposition of the queen a worker closed the cell. We were able to follow the POP 43 times from the beginning. For 31 of the 43 occasions in which measurement was possible, workers laid one or more of these trofic eggs.In all cases the queen devoured these trofic eggs (Sommeijer, 1984). The laying of reproductive eggs by workers has not been observed in this particular period.

Figure 1 shows the results of the worker-laid eggs, for three different colonies (A, B and C).

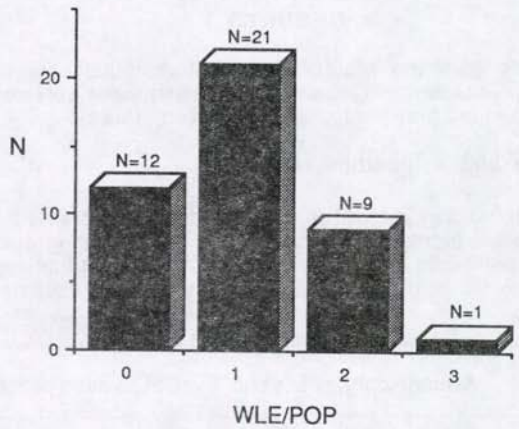

Fig. 1: - *Number (N) of worker-laid eggs (WLE) per POP.*
Fig. 1: - *Nombre (N) d' oeufs issus d' ouvrières pondeuses (WLE) par POP.*

To estimate the presence of males in the nests, samples of broodcombs with pupae were taken from two nests. Concerning the stage of development of the pupae, the combs must have been produced in January and February 1989. Because of the fact that the development of brood takes about 45 days (unpublished information Sommeijer), and the short period in which we observed the egg laying, this was 42 days, it was not possible to check brood of the period March-May for the presence of male pupae. For this reason it is not known whether the queen or the workers produced this brood. The percentage of male pupae in the brood appeared to be relatively high, about 33%. In Utrecht we compared these results with a broodsample collected in September. So this brood was produced in July 1989 *(table 2)*. We also looked for the presence of young queens in the brood. In three occasions it was not possible to determine the sex of a pupa.

Colony	Workers	Males	Queens	?	Sample size
A Costa Rica	35(56.5)	20 (32.3)	5 (8.1)	2	62
B Costa Rica	41(59.4)	23 (33.3)	5 (7.2)	0	69
Utrecht	72(92.3)	0	5 (6.4)	1	78

Table 2: -*Sampling for the presence of pupae of workers, males and queens: absolute and relative ( ) numbers.*

Tableau 2: *-Présence de nymphes de mâles, d' ouvrières et de reines: nombre et pourcentage ( ).*

All colonies in Costa Rica were regulary examined on the presence of adult males. Males found were marked individually to facilitate recognition in the nest. In two small colonies (<500 bees) no adult males were found. In the two large colonies A and B few males were found *(table 3).*

Colony	Workers	Males	Sample size
A Costa Rica	92 (94.8)	5 (5.2)	97
B Costa Rica	55 (92.7)	4 (7.3)	59
Utrecht	50 (100)	0	50

Table 3: *-Sampling for the presence of adult males: absolute and relative ( ) numbers.*

Tableau 3: *-Présence de mâles adultes: nombre et pourcentage ( ).*

The Utrecht colony, examined on males in September, did not contain any male. The young born males, observed in Costa Rica during this period, were mostly killed by workers shortly after their emergence from the broodcells. From the nine marked males, three were killed on the first day after their emergence, four the second day, one the third day and the last one on the fifth day. They were seen on the centrally constructed broodcombs only occasionally. The males mostly stayed in or between the surrounding food storage pots, showing a similar behaviour as queens of some stingless bee species (Sakagami, 1982). They hide between or in empty food storage pots to escape from worker aggression.

DISCUSSION

At the end of the dry season the average daily oviposition is high, the colonies are large, and the food storage pots are filled with pollen and honey. According to the theory that males might be produced by workers in the most favourable season, because of the expected queen's decrease in dominance due to the size of the colony, this was to be expected during this observation period. Indeed adult males were found in the two large colonies during this period, and even a much higher percentage of males in the brood. Small colonies did not contain any males. Van Buren and Sommeijer (1988) assume that in *Melipona trinitatis* the age of a queen can be of importance for her reproductive success. So the size of a colony as well as the age of the queen can also be factors influencing the production of males. Of the two small colonies at least one had a young queen. This colony was split off in January 1989.

The large colony in Utrecht that did not contain males in the months July and August, might be an indication that the production of males is not constant during the whole year. This means that beside the size of a colony and the age of a queen, seasonal factors can also be of importance for the production of males.The results show that the number of male pupae in the brood is not a reflection of the number of adult males present in the nest. We estimated a high mortality of males in the first few days after emergence from the brood.

On account of these results it is not yet possible to establish who exactly produced the males. Apparently a great number of males are already being produced before the ending of the dry season. More research, concerning a longer period, will be necessary to test the hypothesis of a season bound production of males by either workers or the queen.

If we compare the production of young queens in the two observed periods, January and February in Costa Rica, and July and August in Utrecht, it is strikingly constant.

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