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Repartition of reproduction among queens in the polygynous stingless bee *Melipona bicolor*

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The mechanisms leading to reproductive skew among queens in a polygynous colony of *Melipona bicolor* were studied. Queens differ in the probability of remaining the sole queen at a cell during the provisioning and oviposition process. Being alone enhances the probability of receiving a trophic egg. When together with another queen egg-laying is random. Therefore, reproductive dominance is the consequence of differential food uptake, not of a dominance hierarchy.

Keywords: polygyny, reproductive skew, trophic eggs, Melipona bicolor

If environmental conditions are unfavourable for solitary breeding, animals may cooperate in their reproductive effort. Sometimes this cooperation allows all the participants to reproduce, be it that some dominant members of the group might have a bigger share than others, sometimes one or a few individuals dominate the other individuals to such an extent that the helpers only obtain indirect fitness or have to postpone reproduction until they have become sufficiently dominant. Such relationships have been described for various birds and mammals (see Stacey & Koenig, 1990; Emlen, 1991), and among the Hymenoptera this early stage of their evolution of sociality is well represented by a large number of wasp and the bee species. In addition, among the ants, the presence of several queens within a eusocial colony is widespread (Hölldobler & Wilson, 1990). The investigations to understand such social forms concern two types of questions: what kind of mechanisms govern the dominance relationships among the reproductives, and what could be the adaptive value of this system, compared to the supposedly original solitary way of reproduction? In this contribution we will concentrate on the first type of question.

Among eusocial bees polygyny is very rare. It only occurs among a few species of stingless bees, a group representing an ancient lineage of Apidae and which is characterized by a highly social colony structure with complex communication systems. We studied the polygyny in Melipona bicolor, a species occurring in the Atlantic Rain Forest along the Brazilian coast. In this species polygyny is facultative (Bego, 1983), meaning that some colonies are headed by just a single queen, whereas others have more than one queen. We observed up till five physogastric queens being present in a colony and laying eggs.

In the stingless bees egg-laying is part of a complex social process (the Provisioning and Oviposition Process, POP, reviewed by Sakagami, 1982 and Zucchi, 1993). Brood cells are generally arranged in horizontal combs, and new cells are constructed by workers along the margin of the newest combs. Once the construction of a particular cell is completed, cell provisioning by workers is triggered by the appearance of the queen on the comb. She positions herself in front of the cell, probably emitting a pheromone that attracts workers, and when a worker enters that cell she beats her with antennae and front legs on the very tip of the abdomen. This stimulates food regurgitation, and this first food is a strong stimulus for other workers to regurgitate in the cell too. When the food inside the cell reaches a certain level, further food deposition is inhibited. The queen eats some of this provision and lays an egg on top of the food. Often this food consumption and oviposition is preceded by the production of a trophic egg by a worker, an egg that is consumed by the queen prior to her own egg laying. Then the cell is sealed by a worker.

Trophic eggs probably constitute a valuable food component for the queen. While the digestion of pollen, the common source of protein in bees, is a time consuming process, the digestion of eggs may be much more rapid, and a higher proportion of trophic eggs in the diet of a queen could mean an accelerated production of eggs in her ovaries. In this respect it is interesting that queens solicit trophic eggs: once the provisioning of a cell comes to an end, the queen may withdraw from the cell and wait several minutes before she will oviposit, thus providing an

opportunity for laying workers to oviposit during this interval. Occasionally, even two or more trophic eggs are consumed before the queen oviposits. In a strong *M. bicolor* colony 20-30 cells are produced per day, each well separated in time from the others.

In the polygynous colonies of *M. bicolor* often more than one laying queen is attending the provisioning of a cell. Here we report on the repartition of egg laying opportunities among these queens, based on direct observations and on videotaped registrations of the provisioning and oviposition processes.

MATERIAL AND METHODS

The colonies used in this study were collected in the vicinity of Cunha, São Paulo State, and transferred to observation hives (Sakagami, 1966) in the Bee Laboratory at the University of São Paulo. The queen(s) present in these colonies were marked with paint. Observations and filming were done using low light intensities and additional red light. During the initial phase of the study, POP's from monogynous and polygynous colonies were randomly videotaped, while later a colony headed by three queens was studied during a period of three weeks. All POP's were registered.

RESULTS

Random videotaping of egg laying in polygynous colonies, for each colony during a period ranging from several months till over a year, revealed that generally more than one queen was producing the eggs. However, though several queens were laying, the queens differed in the proportions of eggs produced, and when more than two physogastric queens were present, these additional queens were almost excluded from reproduction. Fig. 1 shows these skewed distributions for 6 different colonies.

Continuous observation of a single colony during a three week period (over 500 POP's were recorded) showed that most of the time queens stay away from the comb where cell construction takes place, but when construction of a cell is (nearly) completed, a queen may arrive on the comb. Sometimes the same cell is found by another queen, and then both queens stay at the cell and stimulate workers to regurgitate. Occasionally, there is a ritualised form of aggression among the queens, during which each queen attempts to touch the expanded abdomen of the other, which causes the pair of queens' temporarily departure from the cell, but usually both return soon. In cases where two or three queens are present when egg laying in the cell becomes possible, each of them has the same probability of laying the egg (Table 1). Apparently, differences in strength leading to behavioural dominance, or in responsiveness to stimuli that might come from the cell or from the attending workers are not at stake here. Queens never remove the egg laid by another queen, though they eagerly consume trophic eggs laid by workers.

Table 1. The frequency of ovipositions for three queens, marked blue, green and white resp., in situations where they were singly, in pairs or all three together at a cell. Given that the queens differ in their frequencies of appearance on the comb (as reflected in the frequencies of being alone at the cell), the frequencies of the combinations are random ($\chi^2 = 2.4$), and the queens have equal chances to oviposit

Situation:	Number of cells	Ovipositions by queen		
		blue	green	white
One queen	248	128	71	49
Two queens:				
Bl + Gr	109	54	55	-
BI + W	56	31	-	25
Gr + W	41	-	22	19
Three queens	62	25	21	16

If we compare POP's attended by a single queen with those having more queens it becomes clear that the probability of receiving one or more trophic eggs is lower in the second group of POP's (Fig. 2). For the period of observation the three queens of the colony in question did not differ significantly in the ratio between the number of trophic eggs consumed and eggs laid; this ratio was 0.35. However, they differed in the proportion of POP's which they attended alone (Table 1, $\chi^2 = 13.6$).

Once the provisioning of a cell is completed the duration of the following post-provisioning period (lasting till the egg laying by a queen) varied. It was on average of shorter duration when there was no trophic worker egg in comparison to those POP's in which one or more trophic eggs were laid, and there were no post-provisioning periods of long duration that remained without a trophic egg (Fig. 3).



Figure 1. The relative frequencies of ovipositions of the queens in six polygynous colonies as revealed by random videotaping over periods of 3-14 months



Figure 2. The probability of receiving one or more trophic eggs depends on whether the queen is alone at the cell or together with another queen



Figure 3. The frequency distributions of the duration of the post-provisioning phase in POP's with and without trophic eggs

DISCUSSION

Our results indicate that the differences in the reproductive output of queens in a polygynous colony result from differences in the frequencies with which the queens arrive at the comb at the time cell construction is completed. This frequency probably is related to the egg maturation rate in their ovaries.

If a queen remains singly at such a cell, she may solicit worker oviposition by postponing her own oviposition. This enhances the probability of receiving a trophic egg. However, not always does the queen wait for the egg, she may oviposit shortly after the last food deposition has taken place. Her appetite, therefore, plays a role as well. If she waits long enough she will get the egg (Fig. 3). If two or more queens attend the same cell, they are competing for the opportunity to lay the egg. This means that their tendency to solicit worker egg-laying should be reduced, and indeed the laying queen obtains relatively less worker eggs (Fig. 2). Taking food from the cell prior to oviposition, however, is not impeded. These provisions are mainly composed of diluted honey and pollen. Depending on the diet a queen gets from a cell, the rate of developing a new egg is regulated.

The proportion of cells with multiple queens, relative to single queen attendance, therefore, is part of the mechanism that regulates egg production at colony level. During periods in which queen eggs mature more rapidly than workers provide new cells, queens will frequently meet each other at a cell, thereby reducing their food intake, while at times when their egg maturation rates lag behind cell production rate, they will frequently attend the POP singly, will be able to solicit worker oviposition and, if receiving such eggs, will increase their egg maturation rate. The mechanism functions equally well in monogynous and polygynous colonies.

However, such a mechanism does not explain why over a longer time period the repartition of egg-laying among the queens in a polygynous colony remains stable. This probably depends on differences in the attractiveness of the queens. Some queens incite workers sooner to start food deposition in the cell than other queens, and as a consequence, for the supposedly more attractive queen the probability to be joined by another queen is less than for less attractive queens. Indeed, preliminary observations indicate important differences in the duration of the pre-provisioning phase (the time interval between arrival at the cell and the first food deposition) of POP's attended singly by these queens. From this it would follow that the most attractive queen is able to satisfy her desired proportion of trophic eggs received, leading to a certain reproductive capacity, after which the second queen may use the remaining egg laying opportunities. Slight differences in attractiveness, therefore, are supposed to be at the base of long-lasting differences in reproductive output, as illustrated in Fig. 1.

Various theoretical studies investigate the kind of mechanisms that could produce the unequal reproduction of the partners in a cooperative reproduction effort. Such theories are reviewed and synthesised by Johnstone (2000). Though our empirical data are still insufficient to select the

proper model, the "incomplete control" model proposed by Reeve, Emlen & Keller (1998) fits our results best.

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