

Egg laying and oophagy by reproductive workers in the polygynous stingless bee *Melipona bicolor* (Hymenoptera, Meliponini)*

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Abstract – We studied queen-worker conflict over male production in a *Melipona bicolor* colony, having three physogastric queens and individually marked workers, by means of observations of the processes of cell oviposition. The gender that developed from these cells showed that queens produced mainly female offspring. The overall percentage of the males that were workers' sons was estimated between 27 and 82%. Forty-two times workers were seen to deposit a male egg, normally following the queen's oviposition, in the same cell and in sixteen cases, the reproductive worker ate the egg already present in the cell before ovipositing in it. Workers not only were more likely to lay their egg next to that of another worker than next to a queen's egg, they also were more likely to replace the egg already present when it was worker-derived. Their conduct agrees with predictions from kin-selection theory because workers are better served when rearing sons at the cost of other workers' sons than at the cost of queens' daughters.

stingless bees / *Melipona bicolor* / kin conflict / worker reproduction / worker oophagy

1. INTRODUCTION

Colonies of stingless bees (Apidae: Meliponini) have a high level of social organisation. In almost all species the colony contains a single queen, a morphologically differentiated female specialised in egg laying, and many workers, these constituting the worker caste (Michener, 1974). The queen lays each egg in a separate cell made out of wax, but not before it has been supplied with food for the future larva. Within minutes workers regurgitate this liquid food into the cell before the queen lays her egg on top of it and a worker closes the cell rapidly. This process has been extensively described for several

species of stingless bees by Sakagami (1982) and Zucchi (1993), and is also known as the 'provisioning and ovipositioning process' or POP. Because the development from the egg till the adult stage takes place inside a closed cell, decisions are made during the POP that affect sex and caste of this new individual.

In Hymenoptera, to which the stingless bees belong, females can lay fertilized eggs giving rise to female offspring, or unfertilized eggs giving rise to male offspring. Only the queen is fecundated. Workers, therefore, are only able to lay haploid eggs, that develop into males or serve as food for the queen, the so called 'trophic' eggs that occur in many species (Sakagami and Zucchi, 1966; Zucchi, 1993). The proportion of males coming from worker eggs seems to vary considerably among and within species (Beig, 1972; Beig et al., 1985;

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Drummond et al., 2000; Grosso and Bego, 2000; Machado et al., 1984; Paxton et al., 2003; Sommeijer et al., 1999; Tóth et al., 2002a,b, 2004). In *M. subnitida* about a third of the males are workers' sons (Contel and Kerr, 1976), whereas in *M. favosa* workers produce most males (Sommeijer et al., 1999). Reproductive egg laying by workers can occur either before or after the queen's oviposition. In the latter situation, the cell contains two eggs. Beig (1972) found that in *Scaptotrigona postica* cells having received an egg by the queen and an egg by a reproductive worker almost invariably gave rise to a male. He described how the larva eclosing from this worker-derived egg actively injured the queen-derived egg or larva in a lethal way. For other species of bees, the occurrence of larvae killing other larvae or eggs present in the same cell has not yet been studied.

Kin-selection theory assumes that in social Hymenoptera the asymmetric relatedness causes a queen and workers to be in conflict over the production and investment in females and males (Hamilton, 1964). Because the mating system in stingless bees typically leads to monandrous colonies (Peters et al., 1999; Strassmann, 2001), workers would prefer sons ($r = 0.5$) and nephews ($r = 0.375$) over brothers ($r = 0.25$), while queens would prefer sons ($r = 0.5$) over grandsons ($r = 0.25$) (Ratnieks and Reeve, 1992). The male production by workers recorded for some stingless bee species shows that the expected dispute over male parentage in these cases is real (Tóth et al., 2004).

We studied male-production conflict in *Melipona bicolor*, a species occurring in the southern part of the Atlantic Rain Forest and the adjacent inland area of Brazil. Whereas most species of stingless bees have monogynous colonies, this species is facultatively polygynous, with several queens laying eggs (Bego, 1983, 1989; Velthuis et al., 2001). The queens are singly mated and worker relatedness was estimated to be 0.62 (Peters et al., 1999). This high relatedness implies that workers, as in monogynous species, should value their sons more than sons of queens. In this paper, we confirm this conflict by the occurrence of reproduction by workers in a colony having three queens active in egg lay-

ing. From cells for which we monitored ovipositions, the gender was determined at emergence. We also give a detailed description of the way reproductive workers laid eggs up to the moment the cells are sealed. We discuss the typical procedure of their egg laying behavior from a kin selection perspective.

2. MATERIALS AND METHODS

2.1. The colony and queens used

In a colony of *M. bicolor*, having individually marked workers and three queens active in egg laying, observations on egg laying were executed 24 hours per day from October 14th till November 3rd, 2000. Originally, this colony had only one unmarked queen. Since Alonso et al. (1998) showed that physogastric queens can be exchanged and are readily accepted by their new colony, on June 28th, 2000, two physogastric queens were collected from other nests and introduced into the colony having the unmarked queen (Velthuis et al., 2006). One of them was marked with a white dot of paint on the thorax, the other with a pink dot. When the observations started, the three queens had already been together for three and a half months. The three queens of the colony were unrelated. Although reproduction was skewed, all of the queens participated in egg laying. After about more than a week of observations, the participation of the "white" queen in egg laying diminished significantly and ceased completely shortly thereafter.

The colony was housed in a wooden observation hive, placed in an outer incubator box, with a glass cover on top. The incubator temperature was kept at about 27 °C. The colony was connected to the outside via a plastic tube, thereby allowing workers to forage freely.

2.2. Colony feeding

On October 15th, 17th and 19th, pollen from other *Melipona* colonies and a small quantity of *Apis* honey was placed on the inner layers of involucre surrounding the brood nest and in empty food pots. From October 19 onwards, this food was supplied daily, until the end of the observations on November 3rd.

2.3. Marking individuals and doing observations

From October 4th till October 28th emerging workers were individually marked. The combs, originating from the colony on which the observations were made and from which bees were to emerge, were placed in a small wooden box connected via a plastic tube with the main hive, the small box being placed inside the incubator box as well. Through this connection workers could reach the combs in the small box and could assist young bees emerging. At a fixed hour of the day these freshly emerged bees were collected and a paper tag was glued onto their thorax. The tags held unique combinations of letters, numbers and symbols. After marking, the workers were placed in the main hive. Altogether, 437 individuals were marked, of which 409 were workers, 23 were gynes and 5 were males.

During 16 h of the day and part of the night, the behaviour of the queens and the workers at the combs was observed by means of direct observations, and simultaneously recorded on video. During the remaining 8 hours, behaviour was only recorded on video. While the direct observations concerned the whole nest, video recording concentrated on the cells where a POP was expected. The camera was centred on these cells, and was adjusted in such a way that the tags could be read from the monitor. In order to mark and introduce newly emerged bees, the recording of some cells was stopped shortly after their closure. Some minutes later recordings were resumed. In addition, the high rate of ovipositions occasionally caused recording at one cell to be stopped and to proceed to another cell that was being prepared for oviposition. Thus, the data on the closure of some cells are incomplete.

During the night hours, especially when the combs had grown large, the field of view of the camera had to be enlarged to cover the whole comb for monitoring. As a consequence the tags could not always be read, and in this case the information about the identity of egg-laying bees was lost. Observations started at 0000 h on October 14th and ended at 1600 h on November 3rd.

Workers laid the two types of eggs known from other species, trophic and reproductive (Koedam et al., 2001). Notwithstanding the absence of a complete correlation between egg morphology and worker behaviour, in this paper we will use only worker behaviour to distinguish between trophic and reproductive workers' eggs.

2.4. Data analyses

On the day that observations were terminated, all bees were captured from the colony. It was shown that 348 marked workers, including seven individuals that had lost their tag, were still alive. Worker survival was thus 85.1 per cent. Mortality was not age-related, but condition-related to the day of their emergence. Young queens are normally killed some days after emerging, while males leave the nest after a few days (Imperatriz-Fonseca and Zucchi, 1995; Koedam et al., 1995; Wenseleers et al., 2004), so, therefore, marked gynes and males were no longer found.

When brood was near to emergence and therefore could easily be identified as being a queen, a worker, or a male, we opened the cells from which the POPs were recorded, and we checked for the sex and caste of the pupae inside (Koedam, 2003). Like for other *Melipona* species studied, the opening of cells provided the most reliable data when it was done not later than 36 days after they had received an egg (Koedam, 1999; Koedam et al., 1999).

A chi-square test for Poisson distributed frequencies was executed according to Zar (1999). A Change-point test was applied according to Siegel and Castellan (1988).

3. RESULTS

3.1. General occurrence of worker egg laying

During the 496 hours of observations, the bees completed 501 new cells. Of these, the information whether or not worker egg laying had occurred was lacking for eight cells. In one of the cells, however, a worker laid at least one egg, but further information was lost. The unmarked queen oviposited in 261 of the cells, whereas queen "pink" oviposited in 190 cells and queen "white" in only 50 cells.

When a worker abandoned the cell immediately after egg laying, this egg was considered trophic (Koedam et al., 2001). When instead the worker commenced closing the cell following her egg laying, this egg was considered reproductive. In total, workers laid 256 trophic and 42 reproductive eggs. Trophic eggs were laid regularly all through the observation period. The deposition of reproductive eggs by

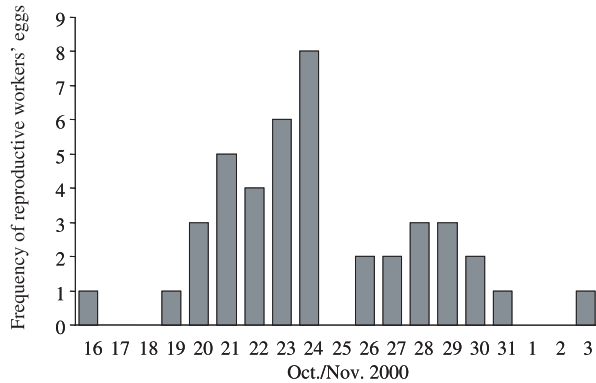


Figure 1. The laying of reproductive eggs by workers on subsequent days in October and November 2000, in a colony of *M. bicolor*.

workers occurred over a period of 19 days. It commenced on October 16th and slowly increased in number until it reached a peak on October 24th (Fig. 1). Thereafter, production rates were lower but worker reproductive egg laying continued till observations were stopped.

3.2. Egg laying by reproductive workers

In total, 14 workers (13 marked and one unmarked) laid reproductive eggs. They were seen to lay 50 eggs, of which 42 were reproductive as indicated by the worker's closing the cell directly after her oviposition. As reproduction among the workers was unequal, the estimated effective number of egg-laying workers was 16. Except for two cases, the reproductive eggs were all laid after a queen's oviposition in that cell and consequently, these cells received an egg two times, sometimes even more.

Three times it was observed that a reproductive worker laid in a cell that had not yet received a queen's egg. Once the queen left the cell after her oviposition, but the egg stuck to the tip of her abdomen, and became adhered to the upper side of the inner cell wall of the cell. Her egg was eaten by a non-reproductive worker. After more than a minute a reproductive worker oviposited in the abandoned cell and sealed it. In a second case, the queen suddenly left an already provisioned cell without

ovipositing. After the cell had remained abandoned by queens for more than five minutes, a worker appeared and laid a trophic egg. One minute later this was consumed by a passing worker, which then proceeded to lay her own reproductive egg and seal the cell. In a third case, after workers had provisioned the cell, the queen assumed a position on top of it but failed to lay an egg. Subsequently, a non-reproductive worker started to seal this cell in a normal way and when this process was halfway through, a reproductive worker interrupted it and laid her egg in that cell.

To lay their eggs, workers showed no preference for cells which had shortly before been oviposited in by either one or the other two queens (Chi-square = 4.17, NS, $df = 2$). Most of the cells used by reproductive workers received only one of their eggs each ($n = 29$). However, five cells were more than once oviposited in by reproductive workers, receiving up to four subsequent ovipositions. These multiply oviposited cells occurred more often than expected based on a random Poisson-distribution (467 cells receiving no reproductive worker egg; 29 cells receiving one egg; three cells two eggs; one cell three eggs and one cell four eggs: Chi-square = 9.16, $P = 0.01$, $df = 2$). The probability that a worker would lay an egg in a cell already containing a queen's egg was significantly lower than the probability that workers would lay an extra egg in a cell already containing a workers' egg

Table I. The frequency of egg eating by reproductive workers preceding their egg laying in *M. bicolor*. Workers oviposited 1. directly following oviposition by the queen, 2. while the cell was being closed by a non-reproductive worker, 3. while the cell had already been closed by a non-reproductive worker or 4. after oviposition by another reproductive worker.

| Reproductive worker oviposition following queen oviposition in the same cell | Egg eating by worker preceding her oviposition | |
|--|--|-----|
| | No | Yes |
| Immediately after queen oviposition | 11 | 0 |
| While non-reproductive worker seals | 8 | 4 |
| After cell has been sealed by non-reproductive worker | 3 | 5 |
| After oviposition by another reproductive worker | 1 | 7 |

(26/493 (5.3%) versus 4/12 (33.3%), Fischer's exact test, $P = 0.003$).

In five out of eight cases of subsequent worker oviposition in the same cell, the reproductive worker, when sealing the cell, could be substituted by a next worker. The latter positioned herself very close to the sealing worker, which, in order to continue cell sealing, had to make certain movements. The new worker took advantage of these movements to slowly take position on top of the cell. No aggression was noticed in such replacements. In other cases the cell, after being closed, was abandoned for only about two minutes when a new worker arrived (126.7 s, SD = 58.8, $n = 3$). In all eight cases the replacing worker opened the cell again by rough, jerky movements, tearing the cell lid with her mandibles, and bending the parts to the side, before she could position herself on it and oviposit. She then closed the cell again.

3.3. The moment the reproductive worker egg is laid, and its consequences

Of 42 reproductive egg layings by workers, 39 occurred following the reproductive egg laying of a queen. Subsequent to a queen's oviposition, a worker could lay a reproductive egg in the cell with or without delay, and with or without first eating the egg(s) already present in that cell. By sticking her head into the cell for some seconds, a worker could eat the formerly laid egg, its absence being visually confirmed just before the worker would

lay her own egg and close the cell. As a consequence, after closing, the cell would contain only the worker's egg. If oophagy was lacking, the cell would contain more than one egg. Table I summarizes the moment of reproductive worker egg layings and whether or not they were preceded by oophagy conducted by the egg layer. A more extensive description of these egg layings is presented below. The three remaining cases of reproductive worker egg laying occurred when the cell had not yet received a queen's egg and are outlined in the previous section.

As can be seen in Table I, the 39 reproductive worker egg layings were split into four categories according to the delay with which the worker laid her egg. In only eleven cases a reproductive worker laid an egg immediately following the queen's oviposition, proceeding with closure of the cell (category one). In these instances, the queen's egg was never devoured, resulting in the cell containing two eggs. It happened that twenty times a reproductive worker laid an egg after a non-reproductive worker had already been active in closing that cell (category two and three). In 12 of these cases, closure had not yet been completed (category two). In some of these instances the reproductive worker had to widen the cell opening, before being able to sit. In four of these 12 cases, the worker ate the queen's egg prior to her oviposition. In eight cases, when a reproductive worker arrived at the cell it had just been completely closed after having received a queen's egg and so she had to re-open the cell first before oviposition

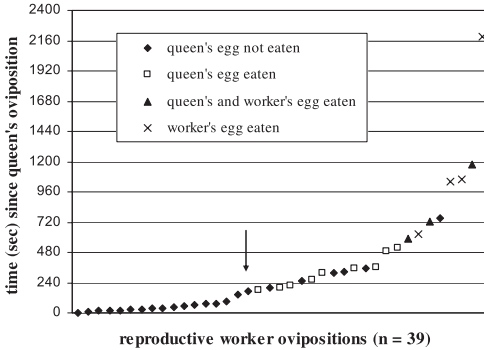


Figure 2. The occurrence of egg eating prior to egg laying by a reproductive worker and the time that passed since the queen oviposition had taken place ($n = 39$). Because workers could oviposit in the same cell several times, it happened that workers ate the eggs of sister workers as well. A Change-point test reveals that the shift from not eating to eating occurs at worker oviposition No. 17 (see arrow) which is about three minutes after a queen's oviposition (Change-point test: $m = 16$, $n = 23$, $D_{\max} = 0.72$, $P < 0$).

(category three). The cell lid was torn in a rather rough way in these cases and in five of these eight cases the worker ate the queen's egg prior to her oviposition. As another variant, a reproductive worker re-opened a cell that had just been closed by another reproductive worker ($n = 8$, category four). Such a cell either contained two eggs (one from the queen, the other from the worker, $n = 3$), three eggs (one from the queen and two from workers, $n = 1$), or the single egg of the previous worker ($n = 4$). In seven of the eight cases observed, the eggs were eaten, including the eggs of the queen. The probability that a worker ate a worker's egg already present in the cell was significantly higher than the probability that a queen's egg was eaten (4/4 (100%) versus 9/31 (29.0%), Fischer's exact test, $P = 0.014$).

For the 39 reproductive worker ovipositions we determined the time that had passed since the queen had oviposited in a particular cell until the moment that a worker oviposited in it (Fig. 2). The resulting order of incidents of oophagy by reproductive workers preceding their egg layings shows that the probability of replacement rose over time. A shift from non-

replacement to replacement behaviour was statistically determined to occur about three minutes after a queen's oviposition (Fig. 2).

Ten out of twelve queens' eggs devoured by workers were laid by the unmarked queen. However, this proportion was not different from what might be expected on the basis of the frequencies of egg laying by workers in cells oviposited in by the different queens (Chi-square = 0.004, NS, $df = 1$). Whereas only reproductive workers ($n = 7$) ate reproductive eggs, both queen and worker-derived, and the queens ate trophic eggs, egg eating by non-reproductive workers hardly occurred. Only once did such a worker eat a trophic egg because the queen abandoned the cell and left the comb altogether and only once did we observe a reproductive worker consuming a trophic egg (see above). In one instance we observed that a reproductive worker had laid an egg and started to close the cell when it was disturbed by the queen's approach. The queen ate this worker's egg and oviposited anew, the cell then being closed in a regular manner by a non-reproductive worker.

Altogether, reproductive workers' actions resulted in 19 sealed cells containing the egg of a queen as well as that of a worker and 14 sealed cells containing only a worker's egg.

3.4. The production of females and males

Due to an unknown cause, the workers demolished a part of the cells from which the details on egg laying were recorded. Of the 468 cells that had received only a single reproductive egg, laid by a queen, 190 had been emptied by the workers; of the 33 cells oviposited in by reproductive workers, 19 were found emptied (Chi-square = 3.65, $P = 0.055$, $df = 1$). From the remaining 278 cells in which only a queen oviposited, 230 workers, 44 queens and four males developed. However, in the case of two of these male bees, there exists uncertainty. By coincidence, recording of these cells was stopped shortly after the closure of these cells, in order to mark and introduce newly born bees, and it could have occurred that these cells were re-opened by a

reproductive worker. When, after a few minutes, recording was resumed, a worker was seen mandibulating the top of these two cells in the manner characteristic of reproductive workers. In the other two cases recording was complete. This means that 2–4 males were sons of the queen.

Of the 19 cells that contained two eggs, one laid by the queen, the other by a worker, 11 cells remained, producing seven females and four males. Of the 14 cells containing only a worker egg three remained, producing three males. The overall percentage of the males that were workers' sons was estimated to be at least 27% but no more than 82%.

4. DISCUSSION

4.1. Male production in *M. bicolor*

In the *M. bicolor* colony we studied, both the queen and the worker produced males, although the queens seemed to participate by producing male eggs in very low numbers (4 males; 274 females). The sporadic production of males by *M. bicolor* queens suggests that the four males that developed from cells containing a queen's egg besides a worker's egg may have been of worker origin.

Queen-worker male production ratios are different among the different species and even among nests of the same species (Tóth et al., 2002b, 2004). For stingless bees, kin-selection theory predicts all males to be produced by workers but its lack in uniformity points out the fact that workers lack complete control, that their male production entails costs to the colony or that their reproduction is limited by phylogenetic constraints (Tóth et al., 2004). Despite *M. bicolor*'s facultative polygyny, relatedness between workers in this species is still high (Peters et al., 1999) which suggests that male production by workers is consistent with predictions that derive from kin-selection theory.

The presence of several unrelated queens in a single colony, all active in egg laying, makes the mean relatedness between colony members lower, although reproductive skew among the queen caste will have a moderating effect. Whatever the number of unrelated

queens, workers should always value the son of a randomly chosen worker nearly 1.5 times as much as the son of a randomly chosen queen. In a similar way, workers should value a randomly chosen female offspring twice as much as the son of a randomly chosen worker.

In contrast to a situation in which queens are highly related, lower intra-colony relatedness should make workers value their own sons relatively more. In such a case, reproductive worker behaviour may therefore appear more selfish. However, as far as egg-laying by reproductive workers is concerned, no particular selfish conduct was documented for the colony studied. The total number of eggs produced by individual workers and the sequence in which they laid these eggs revealed patterns comparable to what was found for individual workers of the monogynic species *M. subnitida* (Koedam et al., 1999; see Koedam and Imperatriz-Fonseca, 2004). Furthermore, the eating of formerly laid eggs by reproductive *M. bicolor* workers, as was observed in this study, seems to reflect a species-specific behaviour since the same egg-eating behaviour was observed in a *M. bicolor* colony with highly related queens (Velthuis et al., 2002).

Our use of multiple, non-related queens also did not affect queen behaviour or their interactions with workers in any noticeable way. For example, reproductive skew among the queens from this study is very similar to what Velthuis et al. (2001) previously reported for this species in colonies with highly related queens. In addition, worker behaviour never seemed biased towards one of the three queen. For instance, a possible preference of reproductive workers to lay their egg only after the oviposition of a particular queen was statistically discarded (this study, see also Cepeda, 2006). Only the occurrence of a considerable number of closed cells emptied by workers remains puzzling. However, workers behaved in a similar fashion in another monogynic *Melipona* species (Koedam et al., 1999, 2005) which makes this fact unlikely to be related to the presence of unrelated queens. Clearly, the data presented here on reproductive caste behaviour and male production have to be substantiated by more behavioural observations

and molecular genetic work (Velthuis et al., 2005).

4.2. General occurrence of reproductive workers

For various species it has been documented that workers can lay their reproductive eggs following a series of regurgitations, thereby excluding the queen from ovipositing in that cell (Chinh et al., 2003; Koedam et al., 1999; Sommeijer et al., 2003). This behaviour was occasionally observed in *M. bicolor* as well (Koedam et al., 2001) but in the current study nearly all reproductive worker ovipositions occurred in cells which had shortly before been oviposited in by a queen. As a matter of fact, the constant presence of the queens on the combs did not seem to stop reproductive workers from egg laying, neither were these workers halted during egg-laying when, in a few cases, a queen physically tried to impede them.

Despite the multiple ovipositions in cells, which not only occur in *M. bicolor* but happen in other stingless bee species as well (Bego, 1990; Beig, 1972; Beig et al., 1985; Chinh et al., 2003; Koedam et al., 1999, 2005; Sommeijer et al., 1984; Tóth et al., 2002a), only a single individual will always develop from each cell. The laying of more than one reproductive egg per cell is therefore an expression of conflict. The eating of the formerly laid egg prior to oviposition in the same cell is even a stronger indication of conflict. However, in *M. bicolor* only reproductive workers were seen replacing a reproductive egg for one of their own whereas queens were rarely seen to do this.

In many eusocial bees, wasps and ants, cannibalism of reproductive eggs is evident (Crespi, 1992). However, in stingless bees this phenomenon has only been observed in *Melipona scutellaris trinitatis* (Sommeijer et al., 1984) and in *Scaptotrigona postica* (Bego, 1990) in which it were the queens that ate functional worker-laid eggs. Egg cannibalism by workers is common under queenless colony conditions (Sakagami, 1982) and in queenright colonies it has so far only been documented for colonies of *S. postica* where

workers infrequently ate a functional egg deposited by another worker (4 out of 73 eggs) but ate a considerable amount of trophic eggs (23 out of 118 eggs) (Bego, 1990). The eating of reproductive eggs by workers, which we report here for *M. bicolor*, seems to be exceptional among stingless bees.

4.3. Gender from multiply oviposited cells

When it is assumed that all eggs or larvae have similar probabilities in reaching adulthood, the chance that a next deposited egg will hatch will be, on average, equal to one divided by the total number of eggs that are present when the cell is closed. In that case, therefore, it is in the interest of the ovipositing female to eliminate the egg, laid by the foregoing individual, because it gives her more certainty that it is her egg that will develop into an adult. Moreover, the highly nutritive value of the egg, which this worker may eat, could be an important prerequisite for her to produce rapidly some more eggs (Velthuis, 1993). On the other hand, if a worker does not eliminate an egg prior to her egg laying in that cell, her offspring might benefit from the extra nutrients left by the other egg or larva that, for some reason, will not develop. Reproductive *S. postica* workers may pursue the latter strategy

Our study lacks the genetic confirmation of male maternity but the numbers of female offspring developing from cells in which the egg of the queen and that of a worker co-occurred, show that a queen's egg can hatch at the cost of a worker's egg. When we consider the males from these cells are worker-derived, the survival chances for queen- and worker-derived eggs were not significantly different (Binomial test, $N = 11$, $k = 4$, NS). The possibility of queen-derived eggs having a probability to develop into full-grown offspring means that reproductive workers should invariably replace such an egg. Yet, our data show that in only nearly half of the cases of reproductive worker egg laying, a worker was seen to eat the egg previously deposited prior to ovipositing in that cell.

4.4. Selective worker egg laying and egg replacement

Egg replacement was not biased towards one of the queens, nor was it executed by a particular worker; as much as seven different workers of a total of fourteen did it. The only bias we found was that workers tended to avoid eating the queen's eggs. This is probably because most of those, as we assumed earlier, are female, to which the workers are related twice as much as to other workers' sons, and therefore workers always benefit from replacing those by sons. So, from a kin selection perspective the workers always benefit from replacing other workers' sons with their own. Of course, the same logic is true for simply the deposition of eggs by workers in cells already oviposited in; workers would be better off by placing their egg next to that of a worker than that of a queen and this is what we actually witnessed. The reproductive conduct of workers in *M. bicolor* would therefore be an example of policing by reproducing workers, also named selfish policing (Wenseleers et al., 2005; Ratnieks et al., 2006).

4.5. Possible egg marking by *M. bicolor* queens

Egg marking by queens has been confirmed for honeybees and ants (Ratnieks, 1995; Monnin and Peeters, 1997). In some cases hydrocarbons and esters seem to play a significant role in recognition (Endler et al., 2004; Martin et al., 2004a), in others the chemical cues responsible are still largely unknown (Martin et al., 2004b). Our data on the selective egg laying and replacement by reproductive workers indicate that queens' eggs in *M. bicolor* are likely to receive some kind of cue during ovipositing which allows workers to recognize them as such (Fig. 2). We suggest that the queens cover their eggs or its nearby surroundings with a pheromone which causes reproductive worker bees at first to refrain from eating these eggs. Successively, this active substance loses its inhibitory power over a time track of a few minutes, allowing reproductive workers to cannibalize on these eggs.

Whether in *M. bicolor* the active queen substance on the egg loses its power due to evaporation and diffusion, or that workers get rapidly adapted to this substance needs as yet to be determined. However, in the majority of cases after the oviposition of a reproductive egg by a queen, the cell is quickly sealed by a worker, a behaviour which is both unique to and universal among stingless bees (Sakagami, 1982). So, most of the time, eggs are protected. It is only up till the moment a cell is completely sealed that a deposited queen's egg is really vulnerable because a queen is never seen to look after it. This could indicate that an egg-marking pheromone to frustrate reproductive workers to cannibalise a queen's egg is short-lived; it would suffice to let the deposited egg be chemically protected for no more than several minutes which is the time usually necessary to close a cell (unpubl. data).

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Ponte et oophagie chez les ouvrières reproductrices de *Melipona bicolor* (Hymenoptera, Meliponini), l'abeille sans aiguillon polygyne.

***Melipona bicolor* / abeille sans aiguillon / conflit de parenté / reproduction des ouvrières / oophagie**

Zusammenfassung – Eierlegen und Eierfressen bei reproduktiven Arbeiterinnen der polygynen Stachellosen Biene *Melipona bicolor* (Hymenoptera, Meliponini). Da Stachellose Bienenköniginnen nur einmal begattet werden, sagt die Theorie der Verwandtschaftsselection voraus, dass die Arbeiterinnen ihre Söhne höher werten als ihre Brüder und Neffen. In neuerer Zeit wurden in mehreren Studien zum Königinnen-Arbeiterinnenkonflikt die mütterliche Herkunft der Männchen bestimmt. Diese zeigen, dass die Männchenproduktion durch die Arbeiterinnen nicht nur zwischen den Arten, sondern auch zwischen verschiedenen Nestern der

gleichen Art stark differiert. Die meisten Stachellosen Bienen haben nur eine Königin, in Nestern von *Melipona bicolor* kommen manchmal aber auch mehrere Königinnen vor. Der mittlere Verwandtschaftsgrad zwischen Arbeiterinnen betrug dennoch 0,62.

In diesem Beitrag untersuchten wir durch Beobachtung des Eiablageprozesses den Konflikt über die Produktion von Männchen in einem Volk von *M. bicolor* mit drei physogastrischen Königinnen. Zwei der Königinnen entstammten anderen Nestern. Um ihre Teilnahme am Eiablageprozess zu ermitteln, wurden die Arbeiterinnen mit kleinen Markierungen auf dem Thorax individuell gekennzeichnet. An 21 aufeinanderfolgenden Tagen wurde die Eiablage in die Zellen über alle 24 Stunden des Tages ununterbrochen registriert, entweder durch direkte Beobachtung oder durch Videoaufzeichnung. Das Geschlecht der sich in diesen Zellen entwickelnden Tiere wurde bestimmt, dies zeigte dass die Königinnen nur gelegentlich Männchen produzierten. Der Gesamtanteil an Männchen betrug schätzungsweise zwischen 27 und 82 %. Der in unserem Untersuchungsvolk von den Arbeiterinnen erzeugte Anteil an Männchen steht daher mit den Vorhersagen der Verwandtschaftsselection im Einklang.

Die Arbeiterinnen wurden 42 mal dabei beobachtet, dass sie ein männliches Ei ablegten, hierbei verschlossen sie nach der Eiablage die Zellen. Der Verlauf der Anzahl täglicher Eiablagen ist in Abbildung 1 dargestellt. Die Arbeiterinnen legten hierbei ausnahmslos ihr Ei in Zellen, nachdem die Königin in die gleiche Zelle bereits ein Ei abgelegt hatte. In fünf Zellen wurden wiederholt von verschiedenen Arbeiterinnen Eier abgelegt. Die Wahrscheinlichkeit, dass eine Arbeiterin ihr Ei benachbart zu einem anderen Arbeiterinnenei ablegte, war größer als die, dass die Arbeiterin ihr Ei benachbart zu einem Königinnenei ablegte. Von elf Zellen, die ein Ei von der Königin und einer Arbeiterin gemeinsam enthielten, stammten mindestens sieben der schlüpfenden Tiere von der Königin ab. Wir nehmen daher an, dass es im Interesse der Arbeiterinnen ist die vorhandene Eier zu entfernen. Allerdings wurde nur in 16 Fällen ein Ei von einer reproduzierenden Arbeiterin aufgefressen, bevor sie ein eigenes Ei in die Zelle legte (Tab. I). Darüber hinaus war die Wahrscheinlichkeit, hierbei das Ei einer anderen Arbeiterin zu fressen höher als die, ein Königinnenei zu fressen.

Dass die Königinnen nicht verwandt waren, sollte keinen Einfluss darauf haben, in welcher Weise Arbeiterinnen die Nachkommen der anderen Arbeiterinnen im Vergleich zu denen der Königinnen werten. Vom Gesichtspunkt der Verwandtenselektion ist daher die hier dargestellte selektive Eiablage der Arbeiterinnen zusammen mit dem Ersatz der Eier für sie vorteilhaft, da der Austausch eines eigenen Sohns für einen Nachkommen der Königin, der höchstwahrscheinlich eine Schwester ist, doppelt so kostspielig ist als der Austausch gegen den Sohn

einer Schwester. Um diesen Mechanismus von eigennützigem „Policing“ (Entfernen von Arbeiterinneneiern) möglich zu machen, sollten die Königinneneier eine schützende Markierung haben. Unsere Verhaltensdaten unterstützen diese Ansicht, da mit einem Anstieg des Zeitabstandes zwischen der Eiablage der Königin und der Eiablage einer reproduktiven Arbeiterin in die gleiche Zelle die Wahrscheinlichkeit zunahm, dass das zuerst abgelegte Ei der Königin von der Eierlegenden Arbeiterin gefressen wurde (Abb. 2).

Stachellose Bienen / *Melipona bicolor* / Verwandtschaftskonflikt / Arbeiterinnenreproduktion / Arbeiterinnen - Eifraß

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