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Cell-sealing efficiency and reproductive workers in the species *Melipona bicolor* (Hymenoptera, Meliponini): double standard and possible rogue conduct

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Abstract – In many hymenopteran insect societies, selfish workers are policed, as selfishness can negatively affect the average inclusive fitness of one or both castes by reducing either the degree of average relatedness to the colony's male offspring or colony efficiency. In stingless bees, the rapid capping of brood cells could aid in controlling selfishness; to this end, we studied cell-sealing efficacy in *Melipona bicolor*. Execution of cell sealing was found to be both rapid and almost continuous. Comparing the performance of reproductive and non-reproductive workers, the former sealed the cells more efficiently when they contained their own eggs, but less so when the queens' eggs were involved. We argue that the occurrence of disruptions in cell sealing through self-serving reproductive workers is capable of undermining sealing efficacy as a policing instrument, thus making reproductive workers potential rogue individuals.

stingless bees / *Melipona bicolor* / cell sealing / efficiency / reproductive workers / rogue conduct

1. INTRODUCTION

In insect societies, the queens produce the bulk of the progeny, although individual reproduction by nest-helpers occasionally occurs as a manifestation of self-interest. Morphological and physiological caste differentiation has set certain limits to worker reproduction (Wilson 1971). In addition, policing behavior, such as egg eating or aggression against workers with developed ovaries could reduce this ongoing social conflict (see Ratnieks 1988). Hypotheses about relatedness and efficiency are used to

explain this social-repression (Trivers and Hare 1976; Hammond and Keller 2004).

In the Hymenoptera, a female is symmetrically related to her offspring but asymmetrically related to her siblings, due to the haplo-diploid determination of sex (see Kerr 1962). Furthermore, queens have a spermatheca to store sperm, whereas workers, while lacking this organ, have retained functional ovaries, thereby having the faculty to produce offspring albeit only males (Wilson 1971). Thus, both queens and workers can produce males. Therefore, conflict over rearing males can be expected, though its intensity may depend on the number of queens heading the colony and on the number of males with which the queen has mated (Ratnieks and Reeve 1992). For instance, in *Apis* (Apini), workers are more related to the sons of a

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multiply-mated queen ($r=0.25$) than to the sons of sisters ($r<0.25$). In addition, they should always prefer their own sons ($r=0.5$) over brothers or nephews. Possibly, the mutual eating of each other's eggs, also called worker policing, has led to a situation in which worker reproduction seldom occurs (Ratnieks 1988). It is believed that worker policing is the reason why an *Apis* queen produces the bulk of the colony's males.

In contrast to *Apis*, stingless bee (*Meliponini*) colonies are normally headed by one singly-mated queen (Peters et al. 1999). In this case, kin-selection theory predicts that males should be predominantly produced by the workers, through their higher degree of relatedness to their sisters' sons ($r=0.375$) than to their brothers ($r=0.25$). The overall picture, based on behavioural observations of reproductive-egg laying and on analysis of emerging brood and genetic finger-printing studies, fits this prediction. Even so, male-maternity ratios are quite variable, even within a single species (Tóth et al. 2004), especially since in a few species, workers completely lack activated ovaries (Sakagami and Zucchi 1968; Terada 1974). This observation, plus the patterns of worker male production in several other eusocial Hymenoptera, like in a wasp (Foster et al. 2002) and various ant species (Evans 1998; Hammond et al. 2003; see Hammond and Keller 2004), demonstrates that factors other than genetic forces are at work to reduce selfishness in helper individuals.

The harmful effect that the behaviour of selfish workers may have on colony efficiency is believed to be another justification for policing (Hammond and Keller 2004). For instance, in stingless bees, the production of males, including that by worker reproduction (see Tóth et al. 2004), may be at the expense of a future worker-force (Koedam 1999; Santos-Filho et al. 2006). Furthermore, reproductive or dominant hymenopteran workers were found to execute certain nest-tasks in a mediocre or inefficient way (Ross 1985; Cole 1986; Hillesheim et al. 1989). Even so, reproductive workers of the stingless bee *Melipona bicolor* were recorded as being much more active in mass-provisioning brood cells than non-reproductive workers (Cepeda 2006; but also

see Tóth et al. 2002). Finally, an occasional attack on the physogastric queen in *Melipona subnitida* (Koedam et al. 2005) reveals that reproductive workers may be overtly aggressive.

In stingless bees, each individual develops within a single, sealed cell. This development starts after several different workers deposit food into vertically oriented cells, whereupon the queen lays an egg on top of this food mass (Sakagami 1982). Shortly after egg deposition, the cells are sealed with a wax cap. Generally, soon after food provisioning ceases, or immediately after queen oviposition, workers have an opportunity to deposit male-destined eggs (see Velthuis et al. 2005). Neither queens nor non-reproductive workers noticeably interfere with workers depositing these eggs (see Velthuis et al. 2005). The real threat of intra-colonial cell usurpation, which occurs in many stingless bee species, is curtailed by the rapid capping of cells with a protective cover. Rapid capping is thus an important mechanical defense against nest mates seeking egg-laying opportunities. Sealed cells may also safeguard the brood against hetero-specific parasitism, though this type of parasitism seems to be uncommon (Kistner 1982).

Together with the presumed use of chemical substances as a defense against replacement of queen-derived eggs (Koedam et al. 2007), workers also block the cell opening with their bodies most of the time, while they are sealing the cell (Sakagami and Zucchi 1963). This "body-plugging" can be more or less continuous, since these workers do not need to interrupt their work to procure building materials for producing the cell caps, because the collars of recently constructed cells typically provide sufficient wax for this purpose (Sakagami 1982). Hence, based on cell-anatomy alone, the actual closing of cell openings is a continuous procedure within a narrow time frame, as would be expected if it is to be an effective control of cell usurpation. On the other hand, the participation of selfish workers in cell sealing could be prejudicial to the quality of this task.

For this study, we examined both speed and efficiency in cell-sealing performance by mon-

itoring individually tagged workers of the facultatively polygynous species *M. bicolor*. We document how reproductive workers, as compared to non-reproductive workers, performed this task. We then provide explanations why they might do so and discuss how their typical participation in the sealing of cells into which queens oviposited may augment worker parasitism.

2. METHODS

2.1. Study organism and behavioural observations

We used one queenright, polygynous colony of *M. bicolor* to study cell-sealing performance, which was observed during October and November of 2000. The egg-laying career of 14 reproductive workers was monitored and has been described elsewhere (Koedam et al. 2007). When a worker abandoned the cell immediately after egg laying, her egg was considered trophic. When instead the worker commenced closing the cell following laying an egg, her egg was considered reproductive.

The colony was maintained inside the bee laboratory at the University of São Paulo (USP) and had a population of about 350 bees when observations were terminated. During 3 weeks (496 consecutive hours of observation), the colony produced 501 capped brood cells. Virgin queens and males emerged from several of these cells. The colony's physogastric queens and the workers were individually marked on the dorsal part of the thorax. Queens were marked with minute dots of paint, while workers were marked at emergence with small paper tags, each having a unique number and letter code. Behavioural observations were undertaken with the help of video recordings and direct observations. Using a non-invasive method of brood-comb sampling (Koedam 2003), the contribution of both reproductive workers and queens to male production was determined.

For this study, formerly recorded but as yet unused video material on cell sealing was analyzed. Recordings, as well as analyses of cell sealing were made with a Philips VR857 video-cassette recorder. Time was read from the recorder's display, accurate to the nearest second.

2.2. Cell sealing and its sub-phases

Cell sealing follows a typical course and has already been documented for various stingless-bee species (Sakagami and Zucchi 1974; Zucchi 1993). Sakagami and Zucchi (1974) recognized four sub-phases of cell sealing: pre-operculation, rotation, side-working, and post-operculation. Here, we stress that the sub-phase pre-operculation, in contrast to post-operculation, is not an element of cell sealing. It is simply a time interval during which a cell already oviposited into by a queen remains untouched until a worker begins sealing.

The actual closing or operculation of the cell in *M. bicolor* consists of two, sequential behavioral sub-phases, namely rotation and side-working, with no transient sub-phase. Rotation starts from the moment a worker takes a position on top of the cell and inserts the posterior half of her abdomen inside the cell opening itself. Then, she begins reducing the cell opening by bending the cell rim inwards with her mandibles, at the same time revolving on her body axis in small steps (the rotation sub-phase). This sub-phase ends when the central gap becomes so small that the worker has to remove the tip of her abdomen from the inner part of the cell. She then immediately repositions herself on the top, now more laterally, to effectively close the rest of the opening by gnawing with her mandibles (the side-working sub-phase). The behavioral transition between rotation and side-working occurs as a straightforward event and is therefore easy to determine.

After the original opening has already been fully closed, a worker often continues gnawing the waxen cell-top. This part of cell sealing was previously termed the post-operculation sub-phase (Sakagami et al. 1964) and has received some attention mainly in early studies on in-nest stingless bee behaviour (Sakagami and Oniki 1963; Sakagami and Zucchi 1963, 1967, 1968; Beig and Sakagami 1964). The moment of transition from side-working to post-operculation is marked by the disappearance of the central hole. However, in many cases, this exact moment can only be estimated, such as when a gnawing worker obscures a clear view of the cell apex with her body. In contrast to side-working, the end of the post-operculation sub-phase is not characterized by a visible, and therefore measurable,

morphological condition of the cell apex but is defined by the final departure of the worker involved. To emphasize the behavioral and integrated aspect of this sub-phase in the process of cell sealing, in this paper, the term “post-oviposition” is referred to as “cell-cap mandibulation”.

2.3. Measuring and comparing cell-sealing performance

Cell-sealing performance was studied by determining how often individuals participated in this task and how fast and efficiently they did so. Efficiency was estimated by measuring parameters, such as delays in starting and whether execution was continuous. These aspects were first studied in a number of randomly selected provisioning and oviposition processes (POPs), in which cells had been oviposited into by only the queen and subsequently sealed by a non-reproductive worker. This outcome was then used for developing a reference point for evaluating the performance of reproductive workers. The possible consequences of differences in cell sealing (e.g., final cap structure or hatching success) were not investigated.

The process of cell sealing was considered fully concluded only when the worker involved finally departed, either during mandibulation of the cell-cap or when the worker had completely closed the cell opening. If a worker interrupted cell-cap mandibulation, she sometimes returned to resume with a few more short bouts of the same procedure. This additional activity was considered as part of this sub-phase. The time taken by a single worker to complete the whole cell-sealing process defined the cell-sealing tempo.

Continuity in execution was determined by calculating the proportion of processes completed by single workers and by measuring the rate and duration of each temporary interruption. The possible cause for discontinuity was recorded. Both the change of body-position when a worker stopped rotating and started side-working and the moment a worker abandoned cell-sealing during cell-cap mandibulation were considered acts inherent to cell sealing. Therefore, these interruptions and what induced them were excluded from analysis. When a worker abandoned sealing prematurely, another

worker would always resume the task. The performance of subsequent workers, other than reproductive workers, also was not included for analysis.

To examine a possible bias in favor of their own eggs when sealing cells, the behavior of reproductive workers on cells oviposited into only by queens was analyzed separately from their behavior on cells into which they also deposited eggs. As a result, the analyses of cell-sealing performance often involved three groups: non-reproductive workers, reproductive workers that sealed queen-oviposited cells, and reproductive workers that sealed subsequent to their own egg laying.

2.4. Data and statistical analysis

To be able to read the small letter and number codes on worker identification tags when analyzing video tapes, it was necessary to focus the camera on the cell that was most likely to be provisioned and oviposited into. As soon as the process with this particular cell was complete, the position of the camera was changed to the next potential cell. Especially during these changes of camera positioning, but also during the change of video tapes, the recordings of certain cells might have been interrupted for a certain time. For this reason, small amounts of information on the behavior at these cells occasionally were not available for analysis.

Possible differences in duration of behavior among the three groups were determined by using Kruskal–Wallis tests. Only if significant, testing was continued with multiple Mann–Whitney *U* tests between the different pairs of groups. Possible independence of characteristics was determined by contingency table chi-square analysis. In the cases of multiple comparisons, a Bonferroni correction was applied.

3. RESULTS

3.1. Reproductive workers and cell sealing

The sealing of brood cells in *M. bicolor* takes place in a manner that has already been described for most other stingless bee species (Sakagami 1982, see also “Methods”). After a

queen has laid her egg on top of the liquid food, she leaves the cell. Shortly after, a worker begins sealing taking up a position on the top of the cell with the tip of her abdomen projecting into the cell opening. During the 3-week observation period, reproductive workers would seal cells into which a queen had oviposited without laying their own egg in the same cell. Within the same time frame, the reproductive workers would then oviposit in other recently oviposited cells, sealing all of them consecutively. Some workers exhibited such dual participation within a time frame of less than 24 h.

In the case of cell re-oviposition, a reproductive worker would arrive at the cell, either subsequent to queen-oviposition, or following partial or full cell sealing by a non-reproductive or reproductive worker. The laying of a functional egg by a worker became apparent because she subsequently closed the cell by herself. Fourteen workers laid functional eggs a total of 42 times, and in 40 of these, the same pattern was adhered to. In the two remaining cases, a queen ate the worker's egg immediately after deposition in one case, and in the other, sealing was halted by the observer's interference to check cell contents. Five cells were each repeatedly oviposited into by different reproductive workers.

Prior to putting the tip of the abdomen inside a cell and ovipositing, a reproductive worker sometimes had to partly or completely re-open it. This happened as a consequence of sealing activity by a prior, non-reproductive worker. In all cases of serially repeated oviposition by several reproductive workers in the same cell, the next worker was obliged to open the cell capping before egg-laying became possible.

Thirty-seven POPs were randomly selected to study the performance of non-reproductive workers in cell sealing. In 32 of these, the cell-sealing individuals' tag-codes were undeniably identifiable and revealed that nearly every capping procedure was executed by a different worker. As it turned out, only one of 31 unique individuals participated in two separate capping procedures. The 13 individually tagged repro-

ductive workers, however, were seen to participate 97 times in the sealing processes of 94 separate cells, each of which had been solely oviposited into by one of the queens. In 84 of these 97 instances, the workers began cell sealing, following the same procedure as non-reproductive individuals. Thus, the participation of an individual reproductive worker, compared to a non-reproductive, was at least six times more frequent on average.

In 13 of the 97 occasions, reproductive workers became involved in the sealing of only queen-oviposited cells when sealing was already in progress or had terminated. In one instance, a single reproductive worker consecutively went through cell opening and sealing of a recently capped cell containing a queen's egg. In this case, the reproductive worker opened the cell slightly, and then closed it again, all within 95 s, without perceptible oviposition. On another occasion, a reproductive worker attempted to open a queen-oviposited cell, while a non-reproductive worker in turn tried to close it. This combined activity lasted nearly 4 min, until the cell was finally re-opened and re-oviposited into by the reproductive worker. In two other cases, a reproductive worker visibly continued gnawing the cap of a cell previously closed by a non-reproductive worker, for several seconds prior to finally re-opening the cell and laying her own egg.

There was a significant relationship between the reproductive state of a sealing worker and the length of the interval following the moment an egg was deposited until this worker initiated the sealing process. The mean length of this interval (or pre-oviposition sub-phase) for the arrival of a non-reproductive worker to begin closing the cell mouth was 22.9 s (SD=20.1, $n=37$). Subsequent to queen oviposition, a reproductive worker arrived about 1.5 times more quickly at a cell to begin sealing compared to a non-reproductive worker (mean=15.2 s, SD=17.6, $n=84$). After laying her own egg, a reproductive worker always went ahead with sealing without delay (mean=0 s, $n=41$, Kruskal–Wallis, $DF=2$, $H=96.8$, $p<0.001$, Mann–Whitney, $n=121$, $Z=-2.63$, $p=0.026$, $n=78$, $Z=-8.21$, $p<0.001$, $n=125$, $Z=-9.22$, $p<0.001$).

3.2. Cell-sealing tempo

To complete a cell-sealing process, a single non-reproductive worker would work for about 3 min on average (182.0 s, SD=63.9 s, $n=29$, Figure 1), with rotation taking about four times as long as either side-working or cell-cap mandibulation (rotation, 116.1 s, SD=39.2 s; side-working 34.5 s, SD=27.8 s; cell-cap mandibulation 31.4 s, SD=34.9 s).

Individual reproductive workers were observed closing a cell from the beginning to the end in 65 cases. Only 55 of these could be used for measuring the duration of each behavioral phase, as the remaining 10 video recordings were incomplete (see “Methods”). Reproductive workers sealed cells that had been oviposited into by only the queen, in the same way as non-reproductive workers. On average, sealing took nearly three minutes (167.5 s, SD=54.1 s, $n=24$, Figure 1). Rotation (106 s) took about 2.5 times longer than side-working, whereas cell-cap mandibulation took about half as long as side-working (rotation 106.3 s, SD=21.2 s; side-

working 39.6 s, SD=31.6 s; cell-cap mandibulation 21.6 s, SD=20.5 s). In 31 cases, a reproductive worker went through a complete sealing sequence after laying her own egg. On average, she worked for nearly 20 min (total cell operculum, mean=1,110.6 s, SD=950.0 s; rotation, mean=68.4, SD=30.2 s; side-working, mean=43.3 s, SD=36.5 s; cell-cap mandibulation, mean=998.8 s, SD=944.7 s, $n=31$, Figure 1), this being significantly longer than in the other two groups of cell sealing. This overall difference in time-spans came from the differences in the rotation and cell-cap mandibulation tasks, not from differences in side-working (Figure 1). After laying their own eggs, reproductive workers rotated for a shorter time, with mandibulation taking much longer.

In one out of the 31 complete cell-sealing procedures involving reproductive workers, the worker laid her own egg in a cell that had already received larval food but had been left unattended. In another case, the worker ate a trophic egg, deposited by another worker, prior to ovipositing. In six more cases, a reproductive worker laid an egg following queen-oviposition

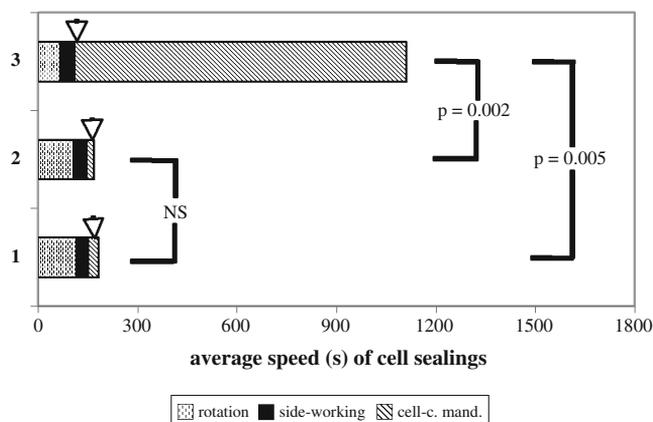


Figure 1. The average speed (s) of complete cell-sealing processes, as performed by individual workers in a *Melipona bicolor* colony, and of the corresponding subsequent sub-phases; i.e., rotation, side-working and cell-cap mandibulation. Cells were sealed by: (1) non-reproductive workers—cell containing a queen's egg ($n=29$), (2) reproductive workers—cell containing a queen's egg ($n=24$) and (3) reproductive workers—after their own oviposition ($n=31$). Cells were considered fully capped when workers switched from side-working to cell-cap mandibulation, indicated with an arrow. See the results section for standard deviations. Kruskal–Wallis, $DF=2$, $H=16.83$, $p<0.001$; Mann–Whitney, $n=53$, $Z=-0.88$, NS; $n=60$, $Z=-3.42$, $p=0.005$, $n=55$, $Z=-3.49$, $p=0.002$; side-working, $n=60$, $Z=-1.14$, NS, $n=55$, $Z=-0.65$, NS; rotation, $n=60$, $Z=-4.81$, $p<0.001$, $n=55$, $Z=-4.47$, $P<0.001$; cell-cap mandibulation, $n=60$, $Z=-4.94$, $p<0.001$, $n=55$, $Z=-5.13$, $p<0.001$.

and started cell sealing; in five of these cases, she did so after having rotated the cell rim several seconds before her egg laying (mean=19.5 s, SD=19.9). In the other 23 cases of worker egg-laying and complete cell sealing, a worker laid her egg following partial or complete sealing by a previous worker. We examined whether the activity of the previous worker was responsible for the shorter duration of rotation done by a subsequent egg-laying worker. We found that the total time taken for rotation (118.8 s, SD=32.6, $n=6$), after worker oviposition following queen-oviposition, was not significantly different from the time taken by a non-reproductive worker (Mann–Whitney, $n=35$, $Z=-0.74$, NS). As a whole, this outcome did not change when the time involved in a bout of rotation of the cell opening prior to her egg-laying was subtracted from the total rotation time (Mann–Whitney, $n=35$, $Z=-0.94$, NS). However, partial or complete sealing by a previous worker caused the succeeding one to rotate for significantly less time, the difference being more than 50% (56.7 s, SD=22.8, $n=23$, Mann–Whitney, $n=52$, $Z=-5.6$, $p<0.001$). This reduction in rotation time is the reason that worker-oviposited cells generally reached the capped phase about 25% faster than those that were exclusively queen-oviposited.

3.3. Cell-sealing continuity

During the sealing of 146 separate cells, both non-reproductive and reproductive workers were seen to halt activities 223 times. In some cases, a worker would continue sealing after a short break of only some seconds (12.6 s, SD=25.1 s, $n=154$), whereas in other cases, she would not resume sealing, but would leave the cell altogether. In general, breaks occurred at a low rate of about once every 4 min of sealing. In comparison to a non-reproductive worker, a reproductive one halted sealing twice as frequently when sealing purely queen-oviposited cells (Figure 2); when sealing cells containing her own egg, stoppages were seven times less frequent.

When a worker temporarily interrupted rotation, she would withdraw the tip of her

abdomen from the cell aperture. If she withdrew her abdomen during side-working, she would merely change the position of her body in relation to the cell aperture or step a little aside. During both types of interruption, the cell contents could briefly be physically uncovered.

During rotation, a worker was capable of stopping and abandoning the cell. With non-reproductive workers, this occurred during two of 37 occasions (Figure 3). On the whole, just over one-fifth of all non-reproductive workers left the process incomplete. In contrast, in 65.8% of the cases involving queen-oviposition alone, reproductive workers left the process incomplete ($n=79$, Figure 3). Nearly 50% ceased cell sealing early on, before starting side-working. In five more cases, reproductive workers took up position on the cell to start sealing, and then immediately withdrew and left.

Reproductive workers finished the sealing of cells, after laying their own eggs, more often than in all other cases (Figure 3, $DF=4$, $\chi^2=54.5$, $p<0.001$). In 38 out of 40 cell closings (95%), the reproductive workers reached the final stage of cell sealing; not a single individual was seen to abandon this activity during the rotation sub-phase.

3.4. Causes of breaks

More than half of the 223 interruptions occurred on the queen's arrival at the cell, the remainder occurred without any visible external cause. An immediate effect of the queen's approach was that the cell-sealing worker crawled down from the cell in an opposite direction to that of the arriving queen, which may have been an avoidance response. The ratio of breaks in cell sealing caused by queen approach as opposed to some undetected cause was significantly different between non-reproductive and reproductive workers (Figure 2, $DF=2$, $\chi^2=13.5$, $p=0.001$). A non-reproductive worker was as likely to stop sealing due to queen approach as she was to some undetected cause. On the other hand, a reproductive worker was somewhat more likely to stop sealing in response to an undetected cause when the cell contained a queen's egg. In

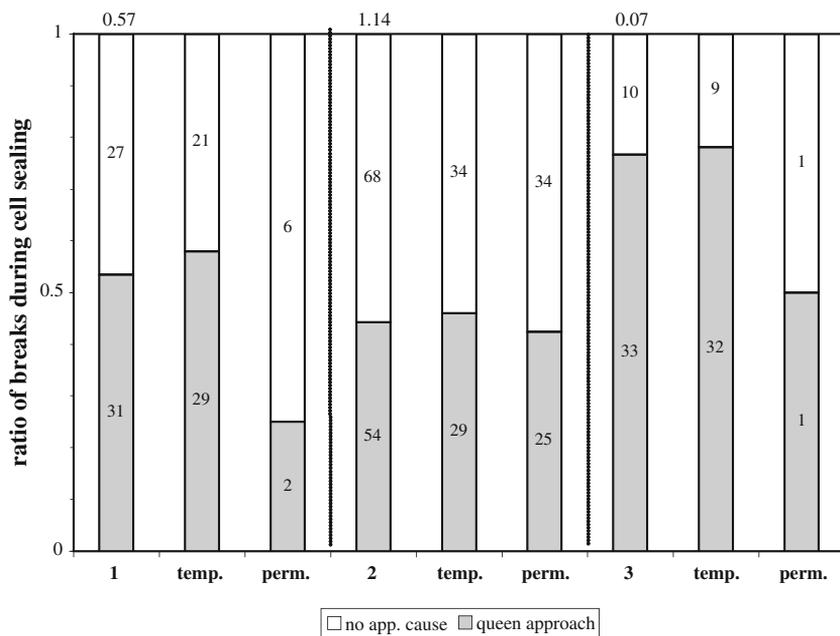


Figure 2. The breaks in cell sealing caused by queen approach and those that occurred without any visible external cause, during cell sealing by (1) non-reproductive workers—cell containing a queen's egg ($n=37$), (2) reproductive workers—cell containing a queen's egg ($n=76$), and (3) reproductive workers—cell containing their own egg ($n=33$). The rate of interruptions (no./min sealing) appears above the bars, whereas the absolute number of interruptions is within each bar segment. For all cases of cell sealing, the number of breaks was split into temporary (temp) and permanent (perm).

addition, when the cell contained her own egg, a reproductive worker was much more likely to be influenced by queen approach. The ratios of the number of times workers temporarily interrupted sealing due to queen approach versus an undetected cause were significantly different for the three sets of cell-sealing ($DF=2$, $\chi^2=10.5$, $p=0.005$), although this was not so for permanent breaks in cell sealing ($DF=2$, $\chi^2=0.96$, NS).

Temporary interruptions that were set off by an undetected cause lasted as long as those caused by queen approach (Figure 4). The length of interruption differed significantly among the three sets of cell sealing (Figure 4). Interruptions during the sealing of cells containing a worker's egg lasted significantly longer, around five times longer, than those occurring during the sealing of cells with only a queen's egg (Figure 4).

In the five cells that were multi-oviposited by different reproductive workers, we investigated whether the substitution of a reproductive worker by another at the same cell caused a shortening in the total time of cell-cap mandibulation by the first reproductive worker. A comparison was made between the duration of cell-cap mandibulation by workers that were displaced and substituted by a second worker at the same cell (cell-cap mandibulation=434.5 s, $SD=332.0$, $n=8$) relative to those that were the last to have oviposited therein, including those cases in which cells received a worker's egg only once (cell-cap mandibulation=1,274.0 s, $SD=997.7$, $n=21$). It was found that the duration of cell-cap mandibulation in the first group was, on average, nearly two thirds shorter than that of the second group; the difference was almost significant (Mann-Whitney, $n=29$, $Z=-1.76$, $p=0.079$).

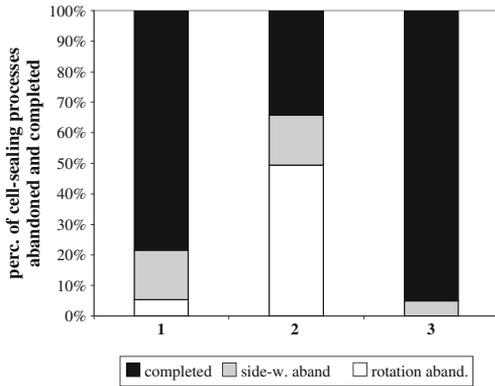


Figure 3. The percentages of cell-sealing processes abandoned prematurely and completed by single workers, in a *Melipona bicolor* colony. The sealing of cells was started by (1) non-reproductive workers—cell containing a queen's egg ($n=37$), (2) reproductive workers—cell containing a queen's egg ($n=79$), and (3) reproductive workers—after their own oviposition ($n=40$). Abandonment could occur during either the rotation or side-working sub-phases. In cases of abandonment, other workers would resume until completion. The participation of succeeding workers was not considered here.

4. DISCUSSION

4.1. Cell-sealing speed, efficiency, and reproductive worker participation

On average, *M. bicolor* workers sealed cells rapidly. In the taxon *Melipona*, worker bees seal cells from about 2 ½ min (*Melipona puncticollis*; Venturieri 1991) to 8 min (*M. beecheii*; Avila et al. 2005). We found that individual, non-reproductive *M. bicolor* workers took only about 2 ½ min to cap an open cell. Thereafter, they spent around half a minute gnawing the cell lid. On average, workers delayed less than half a minute to initiate sealing.

On average, *M. bicolor* workers sealed cells efficiently with few disruptions. Task partitioning is an important and widespread feature of work organization in social insects (Ratnieks and Anderson 1999) and in *Melipona favosa* and other species, cell building and provisioning is done in this way (Sakagami 1982; Sommeijer et al. 1982). In contrast, we found that cell sealing in *M. bicolor* was task

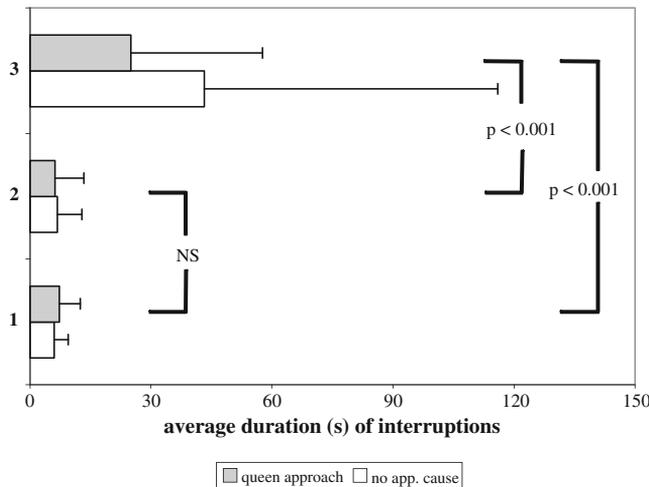


Figure 4. The average duration (s) of breaks occurring during cell-sealing processes involving single workers, caused by queen approach ($n=64$) or for no apparent reason ($n=90$, Mann–Whitney; $n=154$, $Z=-1.33$, NS). Non-reproductive workers interrupted sealing 1—of cells containing a queen's egg 21 and 29 times ($N=50$), respectively. Reproductive workers interrupted sealing 2—of cells containing a queens' egg 34 and 29 times ($N=63$), respectively, and 3—of cells after own oviposition 9 and 32 times ($N=41$), respectively. Lines above bars indicate standard deviations, Kruskal–Wallis; $DF=2$, $H=50.5$, $p<0.001$, Mann–Whitney; $n=113$, $Z=-1.41$, NS, $n=104$, $Z=-6.49$, $p<0.001$, $n=91$, $Z=-5.89$, $p<0.001$.

partitioned only on some occasions. A non-reproductive worker prematurely abandoned sealing, thereby causing more than one worker to be involved in the closing of a single cell, only once out of five times.

The participation of reproductive workers in sealing cells containing an egg other than their own was previously demonstrated in *M. subnitida*, but without details (Koedam et al. 1999). Here, we found that reproductive workers repeatedly participated in the sealing of exclusively queen-oviposited cells, capping the cell as rapidly as non-reproductive workers. They did so sometimes even after having laid their own egg therein. Rotation time was shorter only when reproductive workers first re-opened a closed or nearly closed cell prior to egg laying, thus less work was involved. This is logical, because workers make an opening just large enough to be able to insert the tip of their abdomen into the cell to oviposit. As a result, openings for re-ovipositing were typically smaller than holes made immediately after cell construction.

Importantly, by predominantly using cells that had just been sealed or were halfway through the process, reproductive workers were able to reduce the time required for capping cells by about 25% after depositing their own eggs. Instead of laying eggs right after provisioning or immediately after queen-oviposition, as has been recorded for several other species (see Velthuis et al. 2005), the strategy of sneaking eggs into cells, as done mostly by reproductive *M. bicolor* workers, could avail workers fitness benefits through the economy in valuable time.

4.2. Double standard performance and selfishness

In contrast to speed, other aspects of the performance of reproductive workers during cell sealing revealed two distinct patterns that were related to the caste origin of laid eggs. When dealing with cells only oviposited into by a queen, reproductive workers mostly underperformed in comparison with non-reproductive

workers. Once busy, they interrupted sealing more often, slightly more so for apparently no other reason than queen approach. More than half the time, they abandoned sealing at an early stage. Finally, after completing actual cell sealing, they spent on average of only 20 s gnawing the cell cap. Nevertheless, reproductive workers participated at significantly higher rates and started sealing significantly sooner following queen oviposition compared to non-reproductive workers.

A completely different pattern became obvious when reproductive workers sealed cells containing their own eggs. Using the performance of non-reproductive workers as a benchmark, the overall performance of reproductive workers was superior. First, there was no delay in initiating sealing, with hardly any premature abandonment. Second, they would rarely temporarily interrupt cell sealing, and if they did, it occurred much more frequently at queen approach than for no apparent cause. Also, cell-cap mandibulation lasted an exceptionally long time.

Selfish workers are rarely seen to be directly restrained from reproducing, other than by reproductive non-queen sisters (Koedam et al. 2007); this seems to be a general trend among stingless bees (Koedam et al. 1999, 2005; Tóth et al. 2002). Here, it was seen that as with non-reproductive workers, the *M. bicolor* physogastric queens sometimes caused reproductive workers to interrupt sealing. Whether or not these types of interruptions were of higher impact when workers were sealing cells containing their own eggs is unclear, but in these cases, the queens hardly ever managed to scare them away. The conclusion must be that during sealing, reproductive workers experience little effective nest-mate control. Thus, a reproductive worker uses the origin of an egg as a major criterion in her decision to adapt her cell-sealing performance. She steps up her mechanical defense scheme by carrying out cell sealing in a highly persistent manner when cells contain her own egg. As a result, she creates a constant physical barrier with her body until the cell capping is fully in place. Subsequently, she continues her presence on top of the cell for

quite some time, only gnawing the capping. The main function of this behavior may be to offer further protection to her enclosed egg, as pressure from potential usurpers continues strong after capping (this study; see also Koedam et al. 2007). When cells contain a queens' egg, a reproductive worker acts to the contrary and directs care away from this egg.

We consider the double standard in reproductive worker cell-sealing performance, as with their egg-laying strategy, to be part of their pursuit of optimizing personal output. Even the rather unexpectedly quick initiation and relatively frequent involvement in the sealing processes of only queen-oviposited cells can be considered as a manifestation of this selfish behavior. One should realize that worker egg-laying opportunities are strongly limited by their physiology, since each, at best, can produce only a small clutch of eggs under queenright conditions, and this at a rate of only one egg a day (Koedam, unpubl. data). Thus, they need to closely follow the progress of brood-cell preparation, in order to increase their own egg-laying opportunities. The best way to achieve this is to actively participate in the various stages. The above-average participation of this group of workers in cell provisioning (Cepeda 2006) underscores this idea and may also explain why some even emerged as specialist first-dischargers (Pioker et al. 2003). Furthermore, the exercising of non-risky or non-energetically costly tasks could improve their future reproduction, as reproductive workers can have a higher life expectancy than non-reproductive ones (Alves et al. 2009). The conclusion is reached that selfishness caused reproductive *M. bicolor* workers to occupy a threshold lower than that of other workers for engaging in brood tasks. This serves primarily to boost personal reproduction. A similar involvement by these workers in cell construction is supposed, but is devoid of data.

4.3. Selfishness effects: colony benefits and possible rogue conduct

Our point is that self-serving behavior in sealing and other brood-preparing tasks can have different consequences for the average

fitness of colony members, some of which are in opposition. First, the effects may be positive, such as the possible benefits to colony productivity derived from the highly frequent involvement of reproductive workers in provisioning and cell sealing. As in several ant species (Robson and Traniello 1999), this type of task specialization may lead to increased colony efficiency. The impact from other effects may be more neutral, as when sealing processes were left unfinished, to subsequently be picked up by succeeding workers. A potentially more far-reaching, negative outcome may be the increase in cell-sealing disruptions; reproductive workers displayed mediocre sealing performance by leaving not-yet-sealed, queen-oviposited cells physically unblocked about twice as often as normal.

There are indications that temporarily uncovered cells that are in the process of sealing are easy targets for usurpation. For instance, a closer examination of the way the 14 reproductive workers took control of cells already occupied by an egg shows that usurpation occurred in four out of five cases at not-yet-capped cells that had been left visibly unattended for various seconds ($n=19$), rather than by pushing away the workers occupied in sealing ($n=5$). It is therefore inferred that the typical way these workers participated in the sealing of cells other than those containing their own eggs, probably resulted in less rather than more protection against usurpation. This element of a reproductive helper's repertoire may therefore turn these workers into rogue individuals within a policed context. When the efficacy of normal sealing as a mechanical defense tactic against usurpation is undermined, it should be in favor of the genetic interests of the worker caste. On the other hand, a structural incapacity of controlling worker parasitism by means of cell sealing could inflict significant costs on colony productivity. In cases in which reproductive workers are not the offspring of the resident queen but the offspring of a previous, superseded queen, one would expect this contrast between worker benefits and colony costs to become more accentuated (see Alves et al. 2009).

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Efficacité dans l'operculation des cellules et ouvrières pondueuses chez *Melipona bicolor* (Hymenoptera, Meliponini)

Abeilles sans aiguillon / *Melipona bicolor* / operculation des cellules / efficacité / ouvrières pondueuses

Zellverdeckungseffizienz bei reproduktiven Arbeiterinnen der Stachellosen Biene *Melipona bicolor*: Doppelte Masstäbe und schurkenhaftes Verhalten

Stachellose bienen / *Melipona bicolor* / Zellverdeckung / Effizienz / reproduktive Arbeiterinnen / Schurkisches Verhalten

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