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Genetic and behavioral conflict over male production between workers and queens in the stingless bee *Paratrigona subnuda*

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Abstract Though social-insect colonies are highly cooperative, conflicts of interest can sometimes occur. In this study, we examined conflicts over male production in the stingless bee, *Paratrigona subnuda*. Microsatellite genotyping of workers confirmed that the queen was always singly mated, as in other stingless bees. As a consequence, workers are more related to the sons of other workers than they are to the queen's sons, and conflict is expected with the queen over who produces the males. A likelihood analysis shows that both the queen and the workers contribute substantially to male production, with workers typically contributing more, an average of 64%. The likelihood curves are sharp enough to show that the worker fraction varies among colonies and over time, consistent with a shifting balance of power between queen and workers. Workers laid eggs in 31% of cells recently oviposited in by the queen, and in some other cells as much as 1–2 days old. Queens sometimes forcefully pushed a laying worker from the cells, but the worker returned to finish laying. There was no evidence that queens were effective in preventing workers from laying eggs, yet queens produce some of the males. Worker behavior during oviposition suggests that they do not discriminate between cells destined to produce queen males versus workers, and thus the cost of losing too many future workers may limit worker laying.

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Introduction

The evolution and maintenance of social groups depends on how much conflict of interest there is and on how well the groups are able to resolve such conflicts (Maynard Smith and Szathmáry 1995; Queller and Strassmann 1998). Genetic relatedness among group members facilitates cooperation while differences in relatedness can cause conflicts of interest within a group (Trivers and Hare 1976; Ratnieks 1988; Bourke and Franks 1995; Reeve and Keller 1999). The haplodiploid genetic structure of social Hymenoptera makes them particularly valuable for the study of cooperation and conflict. As a consequence of haplodiploidy, certain family members are highly related, which facilitates altruistic behavior (Hamilton 1964, 1972). But the asymmetrical relatednesses of colony members also generate a variety of potential conflicts. Whether potential conflict becomes real conflict depends on how good colony members are at resolving their disharmony (Ratnieks and Reeve 1992).

One important conflict is over the parentage of males. In multiply mated honeybees, workers are most related to their own sons, but least related to the sons of other workers, so they police each others' reproduction, with the result that males are produced by the queen (Ratnieks 1988; Ratnieks and Visscher 1989). Stingless bees are an intriguing group for the study of this conflict because they are a highly eusocial group in the same family as honeybees, but unlike honeybees, their colonies are typically headed by a singly mated queen (Camargo 1972; Contel and Kerr 1976; Machado et al. 1984; Oliveira Campos 1990; Peters et al. 1999; Palmer et al. 2002). Workers are therefore full sisters (r=0.75) and this high relatedness has consequences for male production. Workers that produce unfertilized haploid eggs that give rise to males are more related to their own sons (r=0.5) than to the sons of others, just as in honeybees. However, stingless bee workers are more closely related to their nephews (sisters' sons *r*=0.375) than to their brothers (mother's sons *r*=0.25). In stingless bees, therefore, workers should prefer their own and their sisters' sons over queen-produced sons while the queen, of course, favors her own sons. This situation can lead to conflict between workers and their queen (Ratnieks 1988; Crespi 1992; Peters et al. 1999).

Workers in many stingless bee species can produce haploid, male-destined eggs (Sakagami et al. 1963; Sakagami 1982; Engels and Imperatriz-Fonseca 1990; Zucchi 1993). Often these are eaten by the queen (Sakagami 1982; Crespi 1992; Zucchi 1993), but worker production of males has been confirmed for a number of species (Sakagami et al. 1965; Beig 1972; Contel and Kerr 1976; Machado et al. 1984; Sommeijer et al. 1984; da Cunha et al. 1986; Inoue and Roubik 1990; Sommeijer and van Buren 1992; van Benthem et al. 1995; Inoue et al. 1999; Koedam et al. 1999; Drumond et al. 2000; Palmer et al. 2002; Tóth et al. 2002a). Clearly the list is far from complete since few of the several hundred species of stingless bees have been carefully studied. The use of molecular markers such as microsatellites for analyzing relatedness and parentage (Queller and Goodnight 1989; Queller et al. 1993; Peters et al. 1999) greatly enhances the prospects for more information on this point, and should also allow for a better delimitation of how queens and workers divide reproduction.

The conflict of interest over male production may lead to overt behavioral conflict, or it may be settled peacefully (Ratnieks and Reeve 1992). Egg laying involves a complex, ritualized, and species-specific set of interactions between workers and their queen, wherein the workers build and provision a cell with food before the queen lays an egg in it and leaves it to the workers to close it (Sakagami 1982; Engels and Imperatriz-Fonseca 1990; Zucchi 1993). Some interactions of this provisioning and oviposition process (POP) appear agonistic, leading to the suggestion that they function in queen-worker conflict over reproduction, or at least originally evolved in that context (Crespi 1992; Zucchi 1993; Drumond et al. 1999; Peters et al. 1999). Worker laying can occur during POP before the queen oviposits (Sommeijer et al. 1984; Koedam et al. 1999), before cell closure after the queen's oviposition (Beig 1972; van Benthem et al. 1995; Imperatriz-Fonseca and Kleinert 1998), or after reopening of recently closed cells (Imperatriz-Fonseca and Kleinert 1998; Koedam et al. 1999).

We studied the outcome of conflict over male production in *Paratrigona subnuda*, a species known to have singly mated queens (Peters et al. 1999). This species has multi-cell provisioning and an oviposition process called an integrated oviposition process (IOP), in which workers build 8–33 cells at a time for queen oviposition (Zucchi et al. 1997). Workers have ovarian development and have been seen laying trophic eggs (Zucchi et al. 1997), but actual worker reproduction has not been verified. Therefore, our first goal was to determine if workers or the queen produced the males, or in what proportions they divided the male production. We also sought to assess the degree to which this potential conflict led to overt behavioral conflict, and what determines the relative power of workers and queens.

Methods

Species and study area

P. subnuda is a common stingless bee in the Brazilian Atlantic rain forest. It nests 25–120 cm under ground (Schwarz 1948; Imperatriz-Fonseca et al. 1972). The nests are spherical and are protected by several sheets of involucrum. Inside these sheets is a helicoidal brood comb with small, egg-shaped honey and pollen pots around it (Imperatriz-Fonseca et al. 1972; Imperatriz-Fonseca 1976). This species is very suitable for behavioral study because new combs are built on top of older combs, facilitating behavioral observation of the oviposition process. The size difference between workers and queens is pronounced. Not only does the queen have a larger head and thorax (Imperatriz-Fonseca 1976), but physogastric queens (1.2–1.8 cm) are at least twice as big as workers (0.5–0.8 cm) and can push workers away relatively easily.

The behavioral observations were carried out at the University of São Paulo (Brazil) between March and December 1999. We used six colonies of *P. subnuda*, which we dug up in the Atlantic rain forest near Cotia (46°56′ W, 23°39′ S), Brazil. After excavation, we transported the colonies to the bee laboratory in São Paulo, removed the involucrum layers on the upper side of the colonies, and transferred the nests into observation hives. The observation hives consisted of wooden boxes covered with a glass lid. We placed these hives into a thermo box with a glass lid that kept the colonies at a constant temperature of 28°C (Sakagami 1966). When the colonies were not used, the thermo boxes were covered with thick, black fabric and styrofoam plates. All colonies had an exit tube leading outside, so that the bees could forage normally.

All the colonies we studied were similarly sized and were producing males. Colonies contained 1 laying queen, 3,000–4,500 workers, 150–400 males, a few virgin queens, and 6–8 layers of brood comb.

Genetic data

In all we genotyped 107 workers (10–34 per colony) and 418 males (from 27 to 108 per colony). We collected two or three batches of males at 3-week intervals over a period of 2 months. We genotyped workers and males at five moderately polymorphic microsatellite loci: Mbi232AAG, Mbi233AAG, Mbi254AAG, Mbi259AAG, Mbi278AAG (Peters et al. 1998). These loci had from two to eight alleles in our population. We used workers and males too young to have left their natal colony. We identified these individuals by their lighter pigmentation and their often bent-down wing tips. We extracted DNA, set up polymerase chain reactions, and visualized PCR products on polyacrymalide gels following the protocols of Strassmann et al. (1996). To estimate relatedness within colonies for our microsatellite data, we used the computer program Relatedness 5.07 for Macintosh (Goodnight and Queller 2000). Standard errors were based on jackknifing over loci for within-colony estimates and over colonies for population estimates (Queller and Goodnight 1989). To calculate population allele frequencies, we weighted colonies equally in all analyses. We calculated 95% confidence intervals (CI), assuming that the jackknifed pseudovalues followed a *t*-distribution.

To estimate the proportion of males that were produced by the queen or the workers, we used a modified version of the maximum likelihood method from Arévalo et al. (1998). The test is based on a set of worker and male genotypes, with the queen genotypes inferred from the workers. The likelihood, L, for any value of the parameter Q, the fraction of males drawn from the queens, is calculated as $L = K \prod_{males} \left(Q \prod_{loci} f_{qi} + (1-Q) \prod_{loci} f_{wi} \right)$. K is a multinomial constant that never has to be calculated because it multiplies all Ls by a constant. For each male allele considered in turn, f_{qi} and f_{wi} are the frequencies of that allele in the queen and in the workers. We calculate L for all values of Q between 0 and 1 at increments of 0.01 (further details in Tóth et al. 2002a, b).

Behavioral data

We videotaped a total of 37 integrated oviposition processes, which included provisioning and laying in 536 cells. Our video-recordings showed workers building and the queen ovipositing in, on average, 14.5 cells per IOP (SD=4.9, range: 7–27, N=37 IOPs). The actual number of cells must have been somewhat higher though, because sometimes the involucrum covered a part of the comb and sometimes the comb was too big to get analyzable video-footage of the whole. The number of cells/IOP in our colonies was similar to that observed in other studies on *P. subnuda* (Zucchi et al. 1997, range 10–35). Colonies did not differ in the number of cells built per IOP (Kruskal-Wallis, P=0.91, df=5), so we pooled colony values for further analyses.

Our behavioral observations focus on when and how workers oviposit, their behavioral interactions with queens, and specifically on whether queens attempt to, and succeed at, limiting worker oviposition. We focused our videotaping on the IOP period because that is when worker egg laying has been observed in stingless bees. We started the analysis of the videotapes 2 min before general arousal of the workers that precedes IOP, and we stopped the analysis 3–22 min after the closure of the last cell that was laid in by the queen. In order to determine the effectiveness of queen disturbance, for every IOP we counted the total number of cells built and determined the frequency at which queens displaced workers that were involved in the IOP. To estimate worker reproduction behaviorally, we calculated the proportion of cells reopened later by reproducing workers. We included only 32 IOPs (435 cells) in the analysis, and excluded IOPs where we stopped recording less than 5 min after the last cell was laid in, because cell reopening might have been missed if fewer than 5 min after laying was watched.

Results

Genetic data

As expected, workers in the colonies were full sisters (pooled R=0.74, 95% CI±0.052, N=107). In each colony, all worker genotypes were consistent with a full sister group. Workers contributed substantially to male production. Depending on the colony, the maximum likelihood estimate of worker male production ranged from 20% to 85% (Fig. 1). The average of these estimates was 63.7% (±17.1 SD). One advantage of the likelihood method is that the steepness of the curves indicates how much less likely other hypotheses are (Tóth et al. 2002a). The likelihood curves were steep enough to conclude that male production was shared. In no colony was there a significant likelihood that the queen produced all the males. In only one colony was there a reasonable chance that workers produced all the males, but even in this colony this hypothesis was only one-quarter as likely as shared male production.

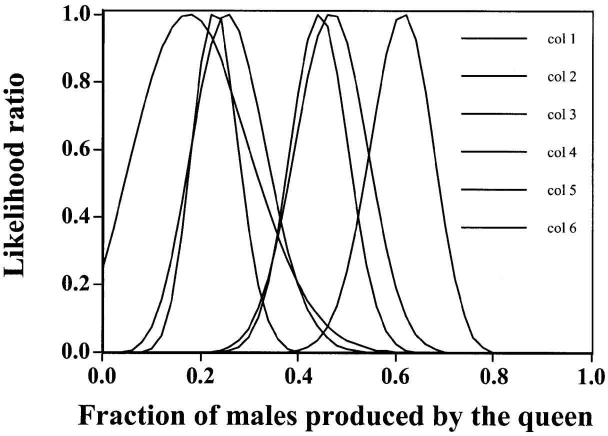

Fig. 1 The likelihood for the fraction of males produced by the queen for six colonies of *Paratrigona subnuda*. In order to standardize the maximum likelhood to unity, the likelihood is presented as a ratio, with the maximum likelihood in the denominator. The number of males analyzed for colonies 1–6, respectively, are 85, 27, 43, 89, 66 and 108

When we looked at the samples separated in time by at least 3 weeks, the range of worker-produced males was broader, from almost none to all males produced by workers (Fig. 2). In four colonies, the proportion of males produced by workers did not substantially differ over time, but in two colonies they did, as indicated by little overlap of the likelihood curves from different time samples (Fig. 2). We do not see any consistent effect of the absolute or relative time period on the fraction of males produced by workers at different times (Fig. 2).

Behavioral data

Worker oviposition

Worker reproduction happened in two ways. Some reproducing workers laid their eggs by reopening closed cells that were a part of the most recent IOP (we call those workers immediate layers), a behavior previously noted for *Friesella schrottkyi* (Imperatriz-Fonseca and Kleinert 1998). In addition, some workers adopted the previously unreported strategy of opening cells that had been provisioned 1–2 days earlier (we call those workers delayed layers). We observed 134 immediate ovipositions and 60 delayed ones, but the proportion of delayed ones may be higher because our videotaping was focused on the IOP period when immediate laying was expected. Delayed laying was seen when it happened to occur close in time and near the same place as a filmed IOP.

In both types of oviposition, reproducing workers made a very small hole in the cell cap (approximate diameter=0.5 mm). Neither workers nor the queen could put their head or abdomen in. Laying workers just dropped an egg through the hole, but queens apparently could not do this; in three cases we observed the queen apparently trying to lay an egg in the opened cells, but

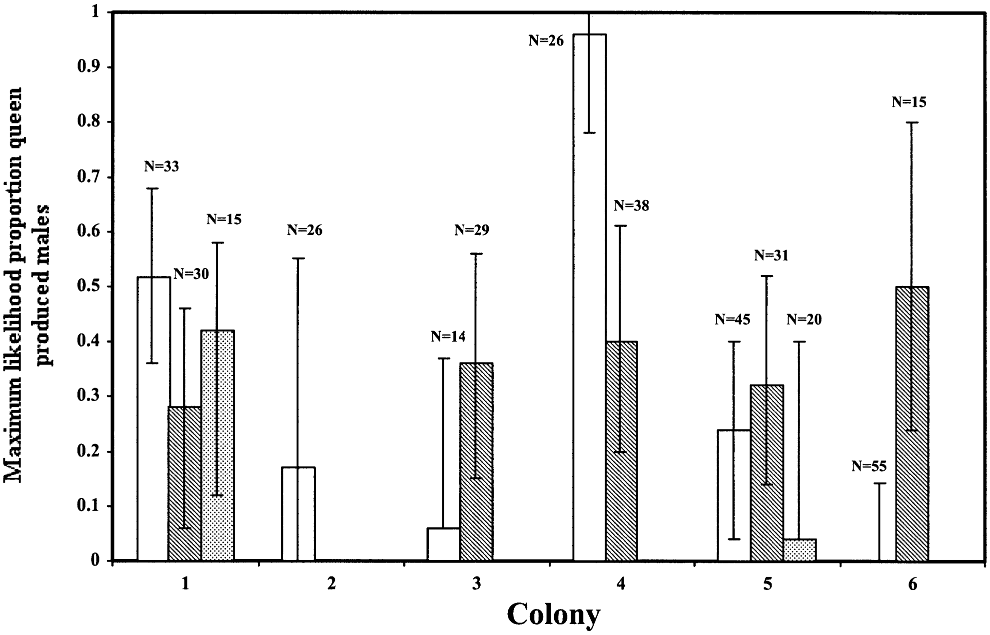

Fig. 2 The maximum likelihood for the fraction of queen-produced males for the individual colonies, at sampling periods 3 weeks apart. The *error bars* represent likelihood values that are 10% as likely as the maximum. Samples shown with *unfilled*, *shaded*, and *striped columns* were taken with 3-week intervals. Samples for colony 1 were taken at a different time of the year, but all the samples from other colonies were taken in the same time period

they seemed not to be able to find the opening with their abdomens (laying workers also sometimes had difficulty finding the cell opening, but eventually succeeded).

We never observed other workers reopening cells that had been oviposited in by worker layers (observation periods 3–22 min after oviposition). We do not know if worker-oviposited cells were opened later; in particular, we do not know if any immediate worker oviposition cells were subsequently reopened for delayed worker oviposition.

Workers should prefer to replace the queen's sons rather than her daughters, but we observed no behaviors suggesting that workers assess the sex of brood before laying. They did not antennate numerous cells before choosing one to open. Nor did they probe through the tiny cell opening. Finally, there was apparently no assessment of opened cells because all were oviposited in, instead of some being reclosed without oviposition.

Although we did not mark workers in the colonies, it seemed that immediate worker layers had not been involved in the IOP because they came from underneath the comb right before they started to open the cell. Immediate worker layers acted individually; other workers did not orient towards their activity. They reopened one-third of the cells that were provisioned in the most recent IOP (31.2%, SD 22.3, *N*=32 IOPs, Fig. 3), shortly after they were closed (average=80.3 s after cell closure, SD=74.3, *N*=134 cells). They needed, on average, just 45 s (SD=26) to open the cell, lay an egg and reclose the cell.

Delayed-worker oviposition took place in older cells away from the comb's edge. These had been filled 7–15 IOPs previously, which we estimate to be a period of about 1 or 2 days. The time required for a delayed oviposition was 76.8 s (SD=37.4, *N*=60), significantly longer than immediate ovipositions (*t*=6.26, *P*<0.0001, *df*=19).

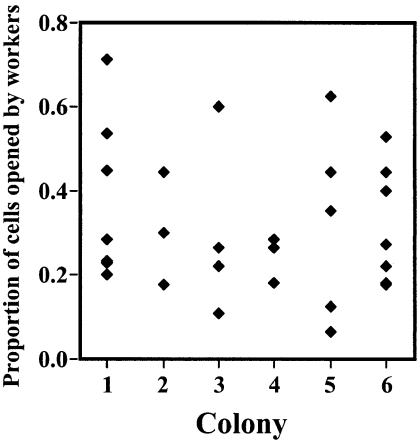

Fig. 3 Proportion of cells opened by workers during different IOPs pooled in six colonies

Unlike immediate layers, delayed layers were surrounded by a court of workers consisting of 6–12 individuals for the period the cell was open. The attending workers did not hinder the egg layers in their work. The queen was less likely to walk by a reopened cell laid by delayed layers outside the IOP than by reopened cells laid by immediate layers that were a part of the IOP (*G*=10.3, *P*=0.0013, *df*=192). Often, when the queen came close to the laying worker, the workers that were around the reproductive worker darted towards the queen, but this did not appear to alter the queen's path, and in fact whenever the queen approached more closely, the attending workers dispersed.

Worker oviposition also occurred during IOP, prior to queen oviposition. On average, workers laid 0.24 (SD=0.19, range 0–2) eggs at this time. These functioned as trophic eggs, as they were always eaten by the queen, who then laid her own egg in the cell. It is unknown whether these eggs could have developed.

We found no evidence for oviposition by workers closing cells after queen oviposition, a time when they have their abdomens in cells and might sneak in eggs. Cell-closing workers did not assume the position observed in laying workers, nor did they pause long enough to lay eggs in the manner observed for other workers. Unlike laying workers (see below), cell-closing workers were easily displaced by body contact of the patrolling queen, or by another worker. Queens interrupted closing workers 2.8 times on average (SD= 2.1, range=0–7). Also unlike laying workers, who return after displacement (see below), displaced cell closers were often replaced by a different worker. On average, 2.1 workers were necessary to close each cell (SD=1.7, range=1–5). Finally, on perhaps 10% of the occasions when closing workers were displaced, the videotape gave a clear view of the cell contents, and they never included an extra egg.

Queen response to worker oviposition

As is typical for species that have integrated ovipositions, the queen kept patrolling over the comb after she oviposited. While patrolling over the comb, the queen walked over the reopened cells that were part of the most recent IOP in 46% of cases. On only 28% of these occasions did she pause to antennate the opened cell. When the queen paused at an opened cell, she pushed the worker who had opened the cell with her body, and the worker actively resisted displacement, a behavior not seen in any other context; 54.8% of the pushed workers gave way, but as soon as the queen left they always came back to the cell to finish laying and closing the cell. Similarly, of the 60 delayed worker layers, 12 were approached by the queen, 4 were pushed by her, and 2 were displaced, but again only temporarily.

We found no evidence for effective queen control of worker reproduction. Of the cells provisioned in the most recent IOP, the queen did not frequent cells that would be reopened by workers more often than cells that would not (*t*-test, P=0.96, t=–0.05, df=433), suggesting that she could not predict which would be reopened. There was no correlation between the proportion of workers backing off the queen while she was patrolling over the comb during an IOP and the proportion of cells reopened by workers right after the IOP (Spearman rank correlation, rho=0.01, P=0.97, N=32 IOP), suggesting that patrolling did not elevate queen control. Likewise, there was no correlation between the proportion of time the queen spent on the comb's edge where the new cells were, and the proportion of cells reopened by workers (rho=–0.16, P=0.38), indicating that queen presence did not increase her control. We also did not find any correlation between the number of cells per IOP and the proportion of cells reopened (rho=–0.10, P=0.59). This indicates that the queen was not able to control worker reproduction more when there were fewer cells to monitor than when there were more. When we looked at each colony separately, no significant correlations were found for the above effects.

Discussion

P. subnuda queens are singly mated, like queens of other stingless bees (Camargo 1972; Contel and Kerr 1976; Machado et al. 1984; Oliveira Campos 1990; Peters et al. 1999). This makes workers more closely related to each others' sons than to the queen's sons. Each party – workers and queens – should therefore prefer to produce the males, other things being equal (Ratnieks 1988). We confirmed that workers laid eggs that developed into males, as has been reported for some other stingless bees (Beig 1972; Machado et al. 1984; Koedam et al. 1999; Drumond et al. 2000; Tóth et al. 2002a). In *P. subnuda*, workers produced, on average, 64% of total male output, towards the high end of the spectrum for stingless bees. The general prediction that singly mated stingless bees should show more worker male production than multiply mated honey bees holds. However, the pattern is not perfect; workers in some other stingless bee species do not produce males (Sakagami and Zucchi 1974; Inoue et al. 1999; Grosso et al. 2000; Palmer et al. 2002; Tóth et al. 2002a, b). A similar conclusion holds for vespine wasps, with a strong trend for singly mated species to have more worker male production, but with some exceptions (Foster and Ratnieks 2001).

The microsatellite markers were fairly powerful, leading to likelihood curves that were steep enough to show that the worker-laid fraction of males sometimes differed between colonies and between time periods within a colony. Such variation is expected if there is a variable and shifting balance of power between workers and queens. However, other explanations are certainly possible, and our behavioral studies provide more insight into the balance of power.

Workers lay eggs in three time periods. Some eggs are laid during the IOP, before queen oviposition. While it is possible that this egg laying initially evolved as reproductive competition (Crespi 1992), in *P. subnuda* these function as trophic eggs. The queen eats these eggs before depositing her own. This pattern is common in stingless bees (Sakagami 1982; Crespi 1992; Zucchi 1993), but in some species, workers can close the cells before the queen oviposits (Koedam et al. 1999, 2001). *P. subnuba* workers make no effort to close cells before the queen arrives.

Workers also lay eggs shortly after the cells are sealed, after reopening a tiny hole in a cell cap. This is an effective competitive strategy. The tiny hole seems to prevent the queen from sticking her head into the cells and eating reproductive worker eggs, and perhaps also prevents the queen from laying another egg in the opened cell, judging from the observed attempts that failed. The tiny hole also precluded removal of the queen's egg, and the success of worker-produced males likely results from them developing more rapidly and killing the queen's offspring, as has been documented in *Scaptotrigona postica* (Beig 1972).

Conflict occurred when a queen encountered a worker engaged in reopening and ovipositing in a cell. The queen actively pushes the worker and the worker strongly resists the queen's efforts to displace her. This behavior was not observed in any other context. Although this interaction was apparently agonistic, it was limited to mutual pushing and never escalated to overt fighting. Although the queen often succeeded in displacing the worker, she failed to prevent the worker from returning and laying an egg. Other evidence also supports the lack of queen control of worker oviposition. One might expect greater control when queens spend more time on the comb edge, when they force more backing down from workers, and when they need to monitor fewer cells at a time, but none of these measures was correlated with the fraction of cells reopened by workers.

Workers laid their eggs, not only in freshly closed cells, but also in cells 1–2 days old. Why they delay is not clear. Though there was less queen interference during delayed oviposition, this seems an unlikely explanation because such interference is ineffective anyway. However, laying in older cells might be less effective because it gives the queen's eggs a developmental head start (though perhaps 1–2 days is not too much in the context of the 35- to 50-day developmental time of stingless bees (Salmah et al. 1996). An alternative hypothesis is that older cells opened by workers contained dead or inviable eggs, though if this were true one might expect workers to remove those eggs for hygienic reasons.

The court of workers surrounding the delayed ovipositors suggests a more coordinated activity than normally surrounds worker oviposition, with workers cooperating against the queen. Court members occasionally darted at the queen if she approached, suggesting they were trying to protect the laying worker. However, this behavior did not appear to deter the queen (the same darting occurs during IOP when the queen approaches provisioned cells; see Zucchi et al. 1997) and her further approach would disperse the court. Moreover, the laying worker did not appear to need protection because with the immediate layers, a delayed layer resisted queen pushing, and even if the queen succeeded in displacing her, the worker simply returned and oviposited. One possibility is that the court is simply workers attracted to the smell of an open, older cell, a stimulus that might normally recruit workers to make repairs.

Whatever the reason for delayed worker oviposition, it has a wider significance for understanding conflict over male production. The provisioning and oviposition process of stingless bees involves elaborate ritualized interactions between queens and workers, including elements that appear to be agonistic. This has led to the suggestion that these interactions may either function in the context of queen-worker conflict over oviposition, or be relics of past queen-worker conflict (Crespi 1992; Zucchi 1993; Drumond et al. 1999; Peters et al. 1999). This is supported by the comparison with honey bees, because honey-bee workers are predicted to agree that the queen should produce the males, and honey bees lack elaborate interactions between the queen and workers at oviposition. However, an alternative explanation is that the POP behaviors constitute the communication required to coordinate provisioning and oviposition, something that does not need to be done in honey bees who do not mass provision (Alonso and Schuck-Paim 2002). In *P. subnuda*, workers do not successfully reproduce during the POP, so their behaviors there are either communicative or relics of conflict (or both). However, the delayed oviposition in *P. subnuda* weakens the communication explanation in general for stingless bees. It shows that there is no constraint that provisioning and oviposition must be closely coordinated.

Given that the queen did not have control over worker reproduction and that other workers agreed with their reproducing sisters, it seems strange that workers did not dominate male production completely. Nonacs and Carlin (1990) suggested that workers are unlikely to be able to distinguish between male and female eggs laid by the queen. Workers may therefore restrict their reproduction in order not to destroy too many worker-destined eggs. Our observations strongly suggest that *P. subnuda* workers cannot, or at least do not, distinguish male and female eggs. First, reproducing workers that were not involved in the IOP could not know the sex of queen-laid eggs because the cells were closed when laying workers arrived. Second, workers usually opened a cell very close to the place they entered the comb and they never walked around on the comb antennating cells before they started their opening activity. Finally, we never observed a worker opening a cell and closing it without laying an egg in it.

In summary, *P. subnuda* supports the prediction of queen-worker conflict in species with one singly mated queen. Male production is divided between queens and workers, each of which uses different levers of power. Though queens are larger and can often push workers out of the way, the workers win these individual contests, in the sense that they ultimately succeed at laying eggs. The queen's primary advantage appears to be that she is the sole source of worker-destined eggs, and workers cannot replace too many of her eggs without damaging their own inclusive fitness interests.

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