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**Review article**

# Conflict over male parentage in stingless bees

**E. Tóth[1, 2], D.C. Queller[1], A. Dollin[3] and J.E. Strassmann[1]**

[1] *Ecology and Evolutionary Biology, Rice University, P.O. Box 1892, Houston, Texas 77251, USA, e-mail: queller@rice.edu; strassm@rice.edu*
[2] *Current address: Evolutionary Ecology, Virginia Institute of Marine Science, Route 1208 Greate Rd, Gloucester Point, VA 23062-1346, USA, e-mail:etoth@vims.edu*
[3] *Australian Native Bee Research Centre, PO Box 74, North Richmond NSW 2754, Australia, e-mail: anbrc@zeta.org.au*

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**Summary.** Stingless bees usually have one, singly-mated queen. This can lead to a genetic conflict of interest between the queen and the non-laying workers over who should produce the males. In many stingless bee species workers have developed ovaries and can produce male-destined eggs. In this study we compile the available data on who produces the males in stingless bees. Worker reproduction is common but less frequent than expected from predictions built on relatedness-based preferences of non-laying workers. We tested whether the pattern in worker reproduction can be explained best by queen control, by an arms race between workers and their queen, by the costs of losing workers to reproductive competition, or by phylogenetic constraints. The data are consistent with the view that there is ongoing conflict over male production that is resolved differently depending on the specific dynamics of costs and benefits of worker reproduction. There was also a role for phylogeny; *Melipona* workers often reproduced while *Plebeia* and Australian stingless bee workers seldom or never did. The high worker reproduction in *Melipona* may reflect low costs, because many of the replaced queen-laid eggs would become excess queens.

*Key words*: Meliponinae, conflict, male parentage, relatedness, worker reproduction.

## Introduction: conflicts of interests in insect colonies depending on queen mating frequency

Sociality in Hymenoptera has evolved and persisted independently perhaps a dozen times (Wilson, 1971). Insect societies are very successful, since their high degree of cooperation allows them to ecologically dominate many terrestrial ecosystems (Wilson, 1971). Social groups, however, are predisposed to internal conflicts and these conflicts potentially weaken the forces that hold colonies together (Trivers and Hare, 1976; Bourke and Franks, 1995; Queller and Strassmann, 1998). How contests are settled and how insect societies remain functional despite the destructive effects of conflict are important questions (Reeve and Keller, 1999).

In social insects, conflicts arise because genetic differences within the colony give rise to relatedness differences (Ratnieks and Reeve, 1992; Queller and Strassmann, 1998). For example, haplodiploid females are always more related to their own sons than they are to the sons of the any other colony member. Other things being equal, each female therefore favors rearing her own sons. But if each worker is selected to compete to produce males, what keeps anarchy at bay?

Sometimes collective interests can suppress this conflict. In haplodiploid insects that have a multiply mated queen, such as honeybees, workers are on average less related to the sons of other workers (r close to 1/8) than to sons of their mother, the queen (r = 1/4) (Starr, 1984; Woyciechowski and Lomnicki, 1987; Ratnieks, 1988). This has favored the evolution of worker policing. Honeybee workers eat each others' eggs (Ratnieks and Visscher, 1989) and are aggressive towards the few nestmates with developed ovaries (Sakagami, 1954; Evers and Seeley, 1986; Visscher and Dukas, 1995). In honeybees the queen thus produces virtually all the adult males in all species studied (Visscher, 1989; Ratnieks and Visscher, 1989; Ratnieks, 1993; Barron et al., 2001, Halling et al., 2001; Oldroyd et al., 2001; Wattanachaiyingcharoen et al., 2001).

However, in species where the queen mates singly, workers are more related to their sisters' sons (r = 3/8) than they are to their mother's sons (r = 1/4). Hence, on relatedness grounds these workers should collectively oppose queen production of males and allow each other to reproduce (Ratnieks, 1988; 1990). For example, in stingless bees, that have one, singly mated queens (Silva et al., 1972; Contel and Kerr, 1976; Peters et al., 1999; Paxton et al., 2001; Palmer et al., 2002; Green and Oldroyd, 2002; Tóth et al., 2002a, b; 2003) we would therefore expect worker reproduction to be quite prevalent (Ratnieks, 1988; 1990; Peters et al., 1999). The only exception is *Melipona bicolor* where colonies can have 1 to 5 reproductive queens (Velthuis et al., 2002). In fact, in contrast to honeybees, workers with developed ovaries are very common in stingless bees (Sakagami et al., 1963, Engels and Imperatriz Fonseca, 1990). Moreover, behavioral conflict is suggested by the often intricate interactions between workers and their queen during cell provisioning and subsequent oviposition (the provisioning and oviposition process or POP; Sakagami, 1982; Peters et al., 1999). Stingless bees therefore seem an important test group for theories of worker male production.

It has long been known from behavioral studies that workers lay eggs in many species (reviewed below), though queen production of males appears to be the rule in some species. While behavioral studies are essential to understand how the workers are able to reproduce, when quantifying worker reproduction these studies should be interpreted with some caution for the following reasons. First, many worker-laid eggs are infertile trophic eggs destined to be eaten by the queen (Sakagami, 1982; Sommeijer and van Buren, 1992; Sommeijer et al., 1984b; Koedam et al., 2001), and queens might also eat some fertile eggs (e.g. Sommeijer et al., 1984a). Second, some worker laying may be cryptic, occurring outside the observers range of view or at times when it is not expected, such as when workers reopen and oviposit in cells hours or days after their initial closure (Tóth et al., 2002b). Third, behavioral observation is time intensive, and the difficulty of intensively observing many colonies could lead to missing variation in worker laying due to the season (e.g. Machado et al., 1984; van Veen et al., 1990), the stage of the male production period (Koedam et al., 1999), or the strength of the colonies (Cortopassi-Laurino, 1979; Machado et al., 1984; van Veen et al., 1990). Finally, even if the relative amounts of worker and queen laying are well characterized, the proportion of worker-produced males would remain unknown because the queen-laid eggs usually include an unknown fraction of females (but see Koedam et al., 1999; Sommeijer et al., 1999). Genetic studies get around many of these problems.

Genetic methods can distinguish worker males, queen males and queen females. They usually test maternity at a later stage (older larvae, pupae, young adults), and it is easy to scale up to multiple colonies. There are now a number of genetic studies of male production in stingless bees (e.g. Contel and Kerr, 1976; Machado et al., 1984; Drumond et al., 2000; Paxton et al., 2001, 2003; Green and Olroyd, 2002; Palmer et al., 2002; Tóth et al., 2002 a, b; 2003). However, they have not simplified the picture. Like the behavioral studies, they show considerable variation in the proportion of males produced by workers. Relatedness, though it is clearly important in selection, does not explain this variation because all stingless bees appear to be singly mated. Other factors must therefore account for this variation, and it seems useful to start considering other hypotheses.

The aim of this paper is thus to review data available so far on worker reproduction in stingless bees and to test what factors could cause the observed variation.

## Possible factors explaining variation in worker reproduction

There are several hypotheses that could explain why workers in some species produce males while in other species they do not. One is that the queen sometimes has the power to go against worker interests. The relative power of workers and queens may evolve in arms races. Another hypothesis is that workers refrain from reproducing because of costs to the colony. Moreover, current male production patterns in stingless bees may not reflect ongoing conflict, but could be a relic of prior conflict. Here we review aspects of these hypotheses that can lead to predictions that can be tested with available comparative data (Table 1).

**Table 1.** Predictions for worker production of males in stingless bees

| Worker reproduction if: | Why | Kind of reason |
|---|---|---|
| 1) Colonies have a single and once-mated queen | Worker relatedness is higher to nephews than to brothers | Relatedness |
| 2) Little queen/worker dimorphism | Queens cannot force workers behaviorally | Control |
| 3) Many cells prepared for eggs at once | Queens fail to control oviposition situation | Control |
| 4) Arms race won by workers | Workers escape queen control temporarily | Control |
| 5) Large colonies | Many workers harder to control and make worker reproduction less costly | Control;<br>Cost/benefit |
| 6) Unproductive or inexperienced queen | Queen cannot force workers behaviorally, best to focus her little reproduction on females | Control;<br>Cost/benefit |
| 7) Seasonal male production | Workers can tell when to produce males without hurting worker production | Cost/benefit |
| 8) Abundant food | Worker reproduction will not greatly reduce colony productivity | Cost/benefit |
| 9) Many excess queens | Replacing female eggs has less cost when they often become excess queens killed shortly after emergence | Cost/benefit |
| 10) Other species in genus have it too | Pattern in worker reproduction determined in the past | Phylogeny |

### *Queen policing hypotheses*

*Queen power through size*
As it is in the queen's interest to produce males, she may be more likely to do so when she is more capable of forcefully preventing the workers from reproducing. This might be the case when she is larger than the workers (Oster and Wilson, 1978; Bourke and Ratnieks, 1999). Her large-size advantage need not be fixed at emergence but could also be gained through physogastry, when the abdomen is swollen with many eggs (Engels and Imperatriz Fonseca, 1990), at least if something like shoving is what determines the winner. Shoving could be effective in displacing workers from cells to prevent them from ovipositing, or in gaining access to worker-laid eggs in order to eat them. Of course the workers may be selected to resist this control. In this hypothesis, the bigger the difference between worker and queen size the better the queen should be able to manipulate workers (Oster and Wilson, 1978).

*Oviposition process*
Variation in the nature of the cell provisioning and oviposition process could also affect whether the queen can prevent workers from producing males. All stingless bees are mass provisioners, meaning that workers have to provision a cell with larval food before eggs can be laid in it, after which the cell will be closed until the callow bee hatches from it (Sakagami, 1982). Species differ in the number of cells they build and oviposit at the same time. In some species cells are prepared for oviposition one at a time (Provisioning and oviposition process or POP, Sakagami, 1982). In other species the workers finish a whole row of cells at once (Integrated oviposition process or IOP). When many cells are provisioned at once the queen may be less able to monitor them and therefore may lose control of who lays eggs in them (Zucchi, 1993; Drumond et al., 2000). Also, when more cells have to be oviposited in at the same time, more workers are involved in the oviposition process, and the queen would need to suppress more workers from reproducing. As a consequence, we would predict that if queen power is important then IOP would be more favorable for worker-laying than POP.

*Arms races*
If workers evolve to escape queen control and the queen in turn can evolve new ways to suppress the workers, this can lead to an arms race between the castes. Queen manipulation could happen through aggressive behavior backed by increased mass, or through pheromones (Fletcher and Ross, 1985; Ratnieks and Reeve, 1992). Although pheromonal manipulation of workers by the queen should not be evolutionarily stable since workers should be selected to ignore such signals (Keller and Nonacs, 1993), it could function in arms races, allowing the queens to stay a step ahead of the workers, particularly if changing the chemical composition of pheromones is not too costly (West Eberhard, 1981; Foster et al., 2000). Queens could also evolve strategies to detect and disturb ovipositing workers and workers could avoid queen control by laying their eggs when the queen is not present. In this sort of arms race we expect considerable variation in who wins across species. There could even be variation among closely related species (Crespi, 1992). If variation in worker reproduction occurs for this reason, it may not be correlated with any easily measured colony or individual parameters.

*Colony size*
We also predict that larger colonies with more workers may have a higher level of worker reproduction (Oster and Wilson, 1978; Ratnieks and Reeve, 1992). The main reason is that in larger colonies the queen is likely to be less able to control a larger number of workers. The same prediction may follow if the cost of worker male production is less for large colonies because they can spare the loss of the work when workers focus on reproducing (Oster and Wilson, 1978). This assumes that small colonies are more in need of workers' effort while the workforce in larger colonies is more often idle. In small colonies workers need to be able to perform many tasks at the same time while in big colonies there is a tendency to have stronger caste differentiation and specialization (Bourke, 1999).

### *Cost of worker reproduction*

We consider three predictions exclusively based on costs and benefits of worker reproduction. First, when food is abundant, worker male production will not have as large of a cost to the productivity of the colony as it would if food were scarce. When food is abundant the cost of a reduced worker force is ameliorated relative to when food is scarce and more workers are needed.

Second, if male production is all concentrated in a brief period, then a worker laying in that period has a better chance of replacing queen-produced males than queen-produced workers, because the fraction of males will be high. This assumes that workers cannot distinguish between a male and a female egg of the queen (Nonacs and Carlin, 1990). Therefore we predict more worker male production in species with short male-production periods because of the lower cost. These two factors may operate together; seasonal male production may occur because of greater food abundance at certain times of year.

The argument above focuses on replacing workers rather than new queens because the latter are normally produced in identifiable types of cells. However, in *Melipona*, workers and queens develop in identical cells and up to 25% of all females become excess queens (Kerr, 1950), which are executed by the workers (Engels and Imperatriz Fonseca, 1999). This is thought to be the result of female brood being able to choose to become queens rather than workers given that the food supplied is the same (Bourke and Ratnieks, 1999; Ratnieks, 2001; Wenseleers et al., 2003). We predict more worker reproduction in such species because a significant fraction of worker-produced males will not exact the cost of replacing a useful worker, but instead will replace a useless queen.

### Phylogenetics

Adaptive change may be limited by phylogenetic constraints. For example, a settlement of conflict reached long ago may have involved a suite of characters that make subsequent changes unlikely or impossible. In this case we would expect that who produces the males is strongly influenced by phylogenetic relationships, with closely related taxa being similar for this trait. Unfortunately, despite several attempts, no single clearly resolved phylogenetic tree is available for stingless bee species (Wille, 1979; Michener, 1990; Camargo and Pedro, 1992; Costa et al., 2003). Trees based on morphological and behavioral characters do not agree with each other (Wille, 1979; Michener, 1990; Camargo and Pedro, 1992; Zucchi, 1993) and the only study based on genetic data separates broad geographical groups, but does not generally provide much resolution for taxa within those geographical groups (Costa et al., 2003).

## Data on worker oviposition in stingless bees

To address the different predictions on the pattern of worker reproduction we surveyed the literature for studies on the amount of worker male production, and gathered available data on other variables (e.g. worker ovarian development, type of oviposition etc.) relevant to the predictions in Table 1.

Many published studies that dealt with worker reproduction failed to include colony size so we collected colony size from a limited number of other studies (Table 2). Only three studies give colony sizes that are based on exact counts (von Ihering (1903), Darchen and Delage-Darchen (1975) and Wenseleers unpublished); the others are estimates carried out in diverse ways. Though we tested for an effect of colony size using these literature data, we also conducted the test a second time using, wherever possible, newly collected data from our personal observations and personal communications on the particular species (see acknowledgements). This had the advantage of increasing the number of usable species and making the estimates in a uniform manner whenever possible. We did colony estimations the same way scientists at Utrecht University do. We counted 50 bees in the colonies in one area, then extrapolated that amount over other areas in the nest where bees were present. We estimated the number of bees that were covered by colony structures, such as involucrum (insulating sheaths built by the bees) or brood combs, depending on how big the covering surface was. This method usually slightly underestimates the number of bees. We chose this relatively crude estimation method because it was rapid and non-destructive. The upper limits for colony sizes came from our estimates of wild colonies that were transferred into man-made boxes, the lower limit from colonies kept in laboratories, where colonies often do less well than in the field.

**Table 2.** Colony estimation data from the literature.

| Species | Colony size | Average | Reference |
|---|---|---|---|
| *Melipona beecheii* | 532 | | Darchen and Delage-Darchen, 1975 |
| | 612 | | |
| | 1273 | | |
| | <500 | | van Veen et al., 1990 |
| | >1500 | | |
| | 500–600 | | van Veen et al., 1997 |
| | 1000 | | |
| | 450 | | Biesmeijer and Tóth, 1998 |
| | 350 | | |
| | 2500–3000 | | |
| | 2500–3000 | | Biesmeijer et al., 1999 |
| | 800 | | Hart and Ratnieks, 2002 |
| | 1200 | | |
| | 1800 | | |
| | 1131 | | Wenseleers unpubl. |
| | 1843 | | |
| | 1226 | | |
| | | 1192 | |
| *M. favosa* | 100–200 | | van Veen et al., 1997 |
| | 200–300 | | Sommeijer et al., 1983 |
| | 285–345 | | Sommeijer and van Buren., 1992 |
| | 100–400 | | Sommeijer et al., 2003 |
| | | 241 | |
| *M. fasciata** | 500 | | Biesmeijer et al., 1998 |
| | 800–1000 | | |
| | 2000–2500 | | Biesmeijer and Ermers, 1999 |
| *M. panamica** | 500–600 | | Nieh and Roubik, 1998 |
| | | 1128 | |
| *M. marginata* | 160–243 | | von Ihering, 1903 |
| | | 202 | |
| *M. quadrifasciata* | 300–400 | | Lindauer and Kerr, 1958 |
| | 300–400 | | Lindauer and Kerr, 1960 |
| | | 350 | |
| *M. scutellaris* | 400–500 | | Sommeijer et al., 1983 |
| | 400–600 | | Lindauer and Kerr, 1958 |
| | 400–600 | | Lindauer and Kerr, 1960 |
| | | 483 | |
| *M. subnitida* | 400–500 | | Koedam et al., 1999 |
| | | 450 | |
| *Friesella schrottkyi* | 300–2500 | | von Ihering, 1903 |
| | | 1400 | |
| *Frieseomelitta nigra* | 1500 | | Hart and Ratnieks, 2002 |
| | 400–500 | | Sommeijer et al., 1983 |
| | 400–700 | | Sommeijer et al., 1984a |
| | | 720 | |
| *Geotrigona mombuca* | 2000–3000 | | Lindauer and Kerr, 1958 |
| | 2000–3000 | | Lindauer and Kerr, 1960 |
| | | 2500 | |
| *Plebeia droryana* | 8000–10000 | | Lindauer and Kerr, 1958 |
| | 2000–3000 | | Lindauer and Kerr, 1960 |
| *Strong* | 1500–2000 | | Cortopassi-Laurino, 1979 |
| *Medium* | 700–1500 | | |
| *Weak* | 200?–700 | | |
| | | 2960 | |
| *P. remota* | 2000–5000 | | van Benthem et al., 1999 |
| | | 3500 | |
| *Paratrigona subnuda* | 3000–4500 | | Tóth et al., 2002b |
| | | 3750 | |

Queen/worker body size ratios were not given in the literature, so we calculated them by averaging the length from the tip of the head to the tip of the abdomen of three physogastric queens (fully developed functional queens) and three

**Table 2** (continued)

| Species | Colony size | Average | Reference |
|---|---|---|---|
| *Scaptotrigona postica* | 3000–5000<br>2000–50000 | 15000 | Lindauer and Kerr, 1958<br>Lindauer and Kerr, 1960 |
| *Schwarziana quadripunctata* | 800–2500 | 1650 | Tóth et al., 2003 |
| *Tetragona dorsalis* | 24423–75000<br>1800 | 27774 | von Ihering, 1903<br>Slaa et al., 2003 |
| *H. carbonaria* | 2500–3000 | 2750 | Nieh et al., 1999 |
| *Heterotrigona minangkabau* | 700–800 | 750 | Suka and Inoue, 1993 |

* Some researchers classify *M. panamica* to include: *M. fasciata*, *M. rufiventris*, and *M. burnea* (Inoue et al., 1999).

workers per species using enlarged pictures (for some species we had only one or two queens available). For a few species no pictures were available. We did one analysis without these species and a second with an estimated value. In all these species queens are produced in bigger cells than workers and thus the physogastric queens are expected to fall in the range common to such species of 1.5 to 1.8 times the size of a worker. For these species' queen/worker body size we used the average of those two numbers, a ratio of 1.65.

When no numerical estimate of the proportion of worker reproduction was reported in behavioral studies, we labeled them as "much" or "little" if the investigator reported it to be quite common or occurring at a low frequency, respectively. In order to include these species in our correlations, albeit roughly, we used a value of 45% of males produced by workers for much, and 3% for little. When we found several citations for the same species we averaged those values for the analysis.

We categorized species into three groups depending on their oviposition process. Some species build and oviposit cells mainly one at the time (provisioning and oviposition process, POP). Other species provision and oviposit their cells in groups (integrated oviposition process, IOP). Some other species usually oviposit several cells shortly after each other (facultative oviposition process, FAC). For analyses we treated FAC species and IOP species together because FAC species usually have more than one cell ready for oviposition and those cells are usually scattered over the comb making it impossible for the queen to be present at all of them at the same time.

Comparative tests should be corrected for shared phylogenetic history (Felsenstein, 1985) but unfortunately there is no well resolved tree for stingless bees. As a partial solution we averaged characters within genera, using only one entry per genus in the analyses. Because we have seven data points for the genus *Melipona*, we also conducted separate tests within that genus. Spearman rank correlations are corrected for ties.

## Results: variable worker reproduction in stingless bees

We found published studies of male production for 31 species and 14 genera (Table 3). Twenty-four of those species are Neotropical, six are Australian, and one is Asian. Twelve of these species had cells prepared one at a time for oviposition (POP), and fifteen had a group of cells prepared together for oviposition (IOP). In four species workers could build one to a few cells at the same time (FAC). Average colony size varied from 400 to 10,000 individuals per species. Functional physogastric queens varied from almost as small as workers to more than three times the size of workers.

Table 3 shows that there is a great amount of variation in whether the workers or the queens produce the males. Male production by workers is common in stingless bees, but not universal; half of the studied species had no worker reproduction. Lack of worker reproduction has been confirmed for a few species. In some species workers never have developed ovaries (*Frieseomelitta varia, Duckeola ghilianii*) or workers do not develop their ovaries in the presence of a functional queen (*Friseomelitta (Trigona) nigra, Hetreotrigona (Trigona) minangkabau, Leurotrigona mulleri*) (Sakagami and Zucchi, 1968; 1974; Sakagami et al., 1973; Terada, 1974; Sommeijer et al., 1984a). However workers fail to produce any males even in a number of species where workers have developed ovaries in the presence of their queen (Table 3).

The proportion of males produced by workers varies across species (Table 3). Moreover, some of the genetic studies show that the amount of worker reproduction varies between colonies of the same species as well (*M. subnitida* (Contel and Kerr, 1976) *M. scuttellaris* (Tóth et al., 2002a) *M. marginata* (Tóth et al., 2002a) *P. droryana* (Machado et al., 1984), *Paratrigona subnuda* (Tóth et al., 2002b), *S. postica* (Tóth et al., 2002a; Paxton et al., 2001, 2003). In some species this might be just sampling variation, but in others the likelihood that all colonies have the same percentage of worker laying appears to be very small. In some species workers appear not to reproduce at all in some colonies, while in other colonies they appear to produce 100% of the males (e.g. *M. scutellaris* and *S. postica*: Tóth et al., 2002a).

The differences between genera in queen and worker body size do not correlate with the degree of worker reproduction. This was true when we excluded the species with no body size ratio data (Spearman, rho = 0.125, p = 0.80, N = 8 genera) or included them with estimates of 1.65 (Spearman, rho = 0.031, p = 0.88, N = 14 genera). The same correlation for the seven *Melipona* species was closer to being significant (Spearman rho = 0.680, p = 0.0956, N = 7 species).

Consistent with our prediction, there was a trend towards greater worker male production in genera where multiple cells are simultaneously provisioned (IOP or FAC mean = 0.23 and POP mean = 0.10) but this difference was not significant (POP versus IOP and FAC; Mann-Whitney Test, p = 0.18, N = 14 genera).

Mean worker reproduction does also not correlate across genera with colony size, measured as numbers of workers, either using literature data only (Spearman, rho = 0.03, p = 0.99, N = 10 genera) or when using our new data whenever possible (Spearman, rho = 0.11, p = 0.86, N = 14 genera). The same correlation within *Melipona*, using literature data, was significant, but in the opposite direction of our prediction (Spearman rho = –90.857, p = 0.036, N = 7 species).

*Melipona,* the genus with excess queen production, has high average levels of worker male production, although *M. beecheii* and maybe *M. fasciata* seem to be exceptions to this (Table 3). This general pattern supports the prediction that colonies will have more worker reproduction when worker-produced male eggs would be replacing excess queens, instead of workers.

Worker reproduction levels tend to be similar within genera (Table 3). *Melipona*, except for *M. beecheii* and *M. fasciata*, has high worker reproduction on average. Similarly, in both studied *Scaptotrigona* species workers contribute to male production. By contrast, *Plebeia* species generally have little or no worker male production. The *Austroplebeia* species have little worker reproduction and the *Heterotrigona* species little or none. Although the above mentioned genera have similar worker reproduction values, the two studied *Tetragona* species appear to differ markedly. However, the data on *T. dorsalis* come from a behavioral study and probably from a single colony, while the genetic study on *T. clavipes* suffers from poor resolution that does not exclude the possibility of very low worker production.

**Table 3.** Stingless bee species for which it is known from the literature whether workers reproduce or not. The different columns indicate (from left to right) worker ovary development, the mean % of worker produced males (L = little, M = much), the range of worker produced males, the type of study (B = behavioral, G = genetic), range of colony size, average colony size, queen-to-worker body size ratio (+ indicates unknown but estimated at 1.65), the type of oviposition (POP = one cell is built and oviposited at the same time, IOP = few to many cells are oviposited at the same time, FAC = one or several cells are oviposited at the same time) and the source cited. The first group of bees up to *Duckeola* are from America, the second group up to *H. mellipes* are Australian and *H. minangkabau* is Asian. Colony sizes in italics are our own estimations.

| Species | W ovaries | Avg % W males | Range W males | Type study | Range colony size | Avg. col. size | # col studied | Q/W size | Qvip. type | References |
|---|---|---|---|---|---|---|---|---|---|---|
| *Melipona beecheii* | + | 0 | | G | 300–3000 | 1192 | 13 | 1.2 | POP | Paxton et al., 2001 |
| *M. bicolor** | + | M | | B | *150–800* | *425* | 3 | 1.3 | POP | Koedam et al., 2001 |
| *M. fasciata* | + | 1 | | B | 500–2500 | 1128 | ? | 1.3 | POP | Inoue et al., 1999 |
| *M. favosa* | + | 95 | | B | 100–*700* | *400* | 4 | 1.5 | POP | Sommeijer et al., 1999 |
| *M. quadrifasciata* | + | 40 | | B | 300–*1500* | *900* | 1? | 1.2 | POP | Tambasco, 1971 |
| *M. quadrifasciata anth.* | + | 64 | 55– 70 | G | | | 2 | | POP | Tóth et al., 2002a |
| *M. subnitida* | + | 39 | 0–100 | G | *200–1200* | *700* | 14 | 1.2 | POP | Contel and Kerr, 1976 |
| *M. subnitida* | + | M | | B | | | 4 | | POP | Koedam et al., 1999 |
| *M. scutellaris* | + | 49 | 0–100 | G | 1000–2000 | *1500* | 5 | 1.3 | POP | Tóth et al., 2002a |
| *M. marginata* | + | 46 | 15– 85 | G | *160–2500* | *1330* | 3 | 1.5 | POP | Tóth et al., 2002a |
| *Friesella schrottkyi* | + | 100 | | B | 300–2500 | 1150 | 1 | 1.5 | IOP | Fonseca and Kleinert, 1982 |
| *Frieseomelitta varia* | – | 0 | | B | *800–1600* | *1200* | ? | 1.3 | IOP | Zucchi, 1993 |
| *F. nigra* | – | 0 | | B | 400–1500 | 950 | 1 | + | IOP | Sommeijer et al., 1984a |
| *Geotrigona mombuca* | + | 0 | | B | 2000–3000 | 1500 | 3 | + | IOP | Lacerda and Zucchi, 1999 |
| *Plebeia droryana* | + | 16 | 0– 57 | G | 200–10000 | 2960 | 14 | 1.9 | IOP | Machado et al., 1984 |
| *P. doryana* | + | 0 | | G | | | 1 | | IOP | Tóth et al., 2002a |
| *P. remota* | + | L | | B | *800*–5000 | *2900* | 6 | 2.2 | IOP | van Benthem et al., 1995 |
| *P. remota* | + | 3 | 0– 25 | G | | | 5 | | IOP | Tóth et al., 2002a |
| *P. saiqui* | + | 0 | | G | *1000–2000* | *1500* | 3 | 1.8 | IOP | Tóth et al., 2002a |
| *Paratrigona subnuda* | + | 64 | 28– 80 | G, B | *2000–5000* | *3500* | 6 | 1.7 | IOP | Tóth et al., 2002b |
| *Scaptotrigona postica* | + | 42 | 0–100 | G | 2000–50000 | 15000 | 3 | 1.5 | IOP | Tóth et al., 2002a |
| *S. postica* | + | M | | B | | | 4 | | IOP | Beig, 1972 |
| *S. postica* | + | 13 | | G | | | 10 | | IOP | Paxton et al., 2003 |
| *S. barrocoloredensis* | + | 18 | 10– 25 | B | *3000–7000* | *5000* | ? | 1.6 | IOP | Inoue et al., 1999 |
| *Schw. quadripunctata* | + | 0 | | G, B | *500*–2500 | 1500 | 16 | 1.5 | POP | Tóth et al., 2003 |
| *Tetragona dorsalis* | + | 0 | | B | 1800–75000 | *10000* | ? | + | FAC | Inoue et al., 1999 |
| *T. clavipes* | + | 66 | 0–100 | G | *4000–10000* | *7000* | 5 | + | FAC | Tóth et al., 2002a |
| *Tetragonisca angustula* | + | 0 | | B | *2000–8000* | *5000* | 2 | 3.3 | IOP | Grosso et al., 2000 |
| *Leurotrigona mulleri* | – | 0 | | B | 500–1000 | 750 | 1 | + | POP | Sakagami and Zucchi, 1974 |
| *Duckeola ghilianii* | – | 0 | | B | very big | 10000 | ? | 1 + | POP | Sakagami and Zucchi, 1968 |
| *Austroplebeia australis* | + | L | 0–L | G, B | *1000–5000* | *3000* | 2 | 1.6 | FAC | Drumond et al., 2000 |
| *A. symei* | + | 3 | 0–13 | G | *1000–5000* | *3000* | 4 | 1.9 | FAC | Palmer et al., 2002 |
| *Hetreotrig. carbonaria* | ? | 0 | | G | *2500–8000* | *5250* | ? | 1.6 | IOP | Drumond et al., 2000 |
| *H. carbonaria* | ? | L | 0–L | G | | | | | IOP | Green and Olroyd, 2002 |
| *H. clypearis* | ? | 0 | | G | *200–600* | *400* | 4 | 1.7 | IOP | Palmer et al., 2002 |
| *H. hockingsi* | ? | 0 | | G | *3000–10000* | *7000* | 4 | 1.6 | IOP | Palmer et al., 2002 |
| *H. mellipes* | ? | 0 | | G | *1000–5000* | *2000* | 4 | 1.6 | IOP | Palmer et al., 2002 |
| *H. minangkabau* | – | 0 | | B | 700–800 | 750 | 1 | + | POP | Suka and Inoue, 1993 |

* *M. bicolor* is an exception of the single queen rule in stingless bees.

## Which hypothesis fits stingless bees?

### *Relatedness predictions*

Although queens in stingless bees are singly mated and thus workers are more related to their own and other worker-produced males than to queen produced ones, males are mainly produced by workers in some stingless bees, but not in others. Relatedness does explain the general differences between honeybees and stingless bees, but does not explain the wide variation in worker reproduction in stingless bees. The one species that does have lower relatedness, *Melipona bicolor,* seems to go against the predicted trend because it has worker male production. On the other hand, as expected, it also shows evidence of policing; during male production reproductive workers guard the cell they lay in while others try to open and cannibalize the contents of worker laid cells (Velthuis et al., 2002; Cepeda, pers. comm.).

### *Queen policing hypotheses*

We did not find compelling evidence supporting the hypothesis that variation in abilities of queens to control workers explains variation in who produces males. An observation consistent with the hypothesis that large queens have more control is that the lowest queen/worker dimorphism is found in *Melipona,* which also has high worker reproduction. *Melipona* produces queens from the same size cells as workers and queens are actually smaller than workers until the queens become physogastric. Even with physogastry, *Melipona* queens are smaller on average relative to their workers than is the case for other species (Table 3). However, comparing across all genera, and also within *Melipona*, there was no support for the hypothesis that larger queen/worker size ratios lead to less worker male production. For this reason we favor an alternative explanation for the generally high worker male production in *Melipona* (see below).

The hypothesis that queens would be less able to maintain control of worker reproduction when many cells are prepared for oviposition simultaneously (IOP species) was not supported (Table 3). Although the trend was in the right direction, some results are counter to our queen control prediction; *Melipona* with POP has high worker oviposition, while *Plebeia* and *Heterotrigona* with IOP and *Austroplebeia* with FAC have low worker reproduction.

We hypothesized that queens would be less able to monitor and control workers when colonies are large. However, there was no support for this hypothesis from correlations of colony size and worker male production, across genera. Within *Melipona* based on colony sizes gathered from the literature the results are opposite to the expectations. While colony size might not explain worker reproduction across taxa, there is a suggestion from the literature that colony size might explain when workers start reproducing in species that have worker reproduction (e.g. Cortopassi Laurino, 1979; van Veen et al., 1990; Sommeijer et al., 2003).

Behavioral observations in the literature give a mixed picture on the possibility of queen control. We could not find extensive information on special interactions between the two castes when males are produced, but queen pushing and/or vigorous tapping of reproducing workers is common, for example in *M. scutellaris trinitatis* (Sommeijer et al., 1984a) *M. subnitida* (Koedam et al., 1999), *M. fasciata* (Inoue et al., 1999) and *P. subnuda* (Tóth et al., 2002b). This seems to suggest a conflict over male production. Yet, even when the queen was able to drive the worker away from the cell, in most cases she was not able to prevent worker reproduction. In two exceptional species, *S. barrocoloradensis* (Inoue et al., 1999) and *M. favosa* (Chinh et al., 2003), queens do not attempt to intervene with laying workers. Generally, queen eating of worker eggs is common, a possible indicator of conflict (Crespi, 1992). Many of these consumed eggs are inviable trophic eggs, but queens may also eat viable eggs (Koedam et al., 2001).

Some role for queen control may also be suggested by sporadic data in the literature that workers lay when the queen is inexperienced, inattentive or too old (e.g. van Buren and Sommeijer, 1988). Recently mated young queens need to 'learn' how to interact with workers and when to lay their eggs (Beig, 1972). In *Melipona scutellaris trinitatis* and *Scaptotrigona postica* worker reproduction occurred when the queen apparently did not notice when the workers were ready with cell provisioning (Camilo-Atique, 1977; van Buren and Sommeijer, 1988; Sommeijer and van Buren, 1992). It was confirmed that an older queen of *M. trinitatis* did not react rapidly enough when a cell was ready to be oviposited (Sommeijer and van Buren, 1992). However, these examples could be consistent with simple queen signaling as well as queen control (Keller and Nonacs, 1993).

Even though we do not find much direct evidence of queen control, the high variability in worker reproduction is consistent with the expectation of an evolutionary arms race over control. *Melipona* species however, have worker reproduction, two species (*M. fasciata* and *M. beecheii*) are an exception (Table 3). Among the *Plebeia, P. droryana* has considerably more worker reproduction than *P. remota* and *P. saiqui* do. Also, among the *Heterotrigona, H. carbonaria* shows some worker reproduction while in the other species the queen produces the males. The two specific castes where queens do not intervene with reproducing workers also seem consistent with the arms-race hypothesis. *S. barrocoloradensis* (Inoue et al., 1999) and *M. favosa* (Chinh et al., 2003) do not have similar oviposition type, colony size, or queen/worker size ratio, but they share the trait that workers and queens do not compete with each other over male progeny. Finally, an arms race is consistent with the fact that reproducing workers use varying methods to reproduce, even within the same species. Workers have been observed to lay reproductive eggs before the queen would lay her egg in *M. fasciata*, *M. favosa*, *M. subnitida*, *M. trinitatis*, *S. postica* and *S. barrocoloradensis* (Beig et al., 1982; van Buren and Sommeijer, 1992; Inoue et al., 1999, Koedam et al., 1999; Chinh et al., 2003). Workers also laid right after queen oviposition while closing a cell in *M. subnitida, S. postica and F. schrottkyi* (Beig, 1972, Beig et al., 1982; Imperatriz Fonseca and Kleinert, 1998; Koedam et al., 1999). Sometimes workers reopen already closed cells, as in *F. schrottkyi* and *P. subnuda* (Imperatriz Fonseca and Kleinert, 1998; Tóth et al., 2002 b). Sometimes cells receive more than one worker egg (e. g. *M. favosa, M. quarifasciata, S. postica*) suggesting competition among laying workers as well (Beig, 1972; Sommeijer et al.,1999).

### *Cost/benefit hypotheses*

We did not find sufficient, precise data on seasonality of male production to test the prediction that seasonality favors worker male production because the workers would be more likely to be replacing queen-produced males. Three studies mention that extensive worker reproduction occurred in bouts of short male-production period (Koedam et al., 1999; Velthuis et al., 2002; Chinh et al., 2003) consistent with this cost/benefit hypothesis. Many other studies mention that workers produce males in the season of maximal male production (Beig, 1972; Sakagami, 1982; Bego, 1982 a; van Veen et al., 1990; Grosso et al., 2000). However, this could either be because of the advantage of replacing queen-produced males, or simply because this is the optimal time to produce males. A few studies mention that the amount of resources might have a positive effect on male production (Beig, 1972; Cortopassi-Laurino, 1979; Bego, 1990; van Veen et al., 1997; Moo-Valle et al., 2001) or that workers might reproduce in the same period (Beig, 1972; Koedam et al., 1999; Tóth et al., 2002 b; Chinh et al., 2003 indirect evidence).

A related cost/benefit hypothesis successfully predicts the high worker reproduction in the genus *Melipona.* Because a large fraction of *Melipona* cells contain surplus queens (Wenseleers et al., 2003) who will be killed anyway, the cost of replacing a queen-produced female egg with a worker-produced egg is correspondingly reduced.

Costs might also vary with colony size, with larger colonies being more able to sustain the effects of worker reproductive competition. However, as noted above, colony size was uncorrelated with worker reproduction across genera and correlated within *Melipona* in the opposite direction to that predicted.

### *Phylogenetic constraints hypothesis*

Although we lack an accurate phylogenetic tree for stingless bees, phylogenetic history appears to have an impact on worker male production. For example, species within the genera *Melipona*, *Scaptotrigona, Plebeia*, *Austroplebeia* and *Heterotrigona* show similar degrees of worker reproduction. Although these data are consistent with phylogenetic-constraints the pattern could also arise from related species having similar ecology, resulting in similar degrees of worker reproduction. However, the range of worker reproduction sometimes differs greatly between colonies of even the same species. Whether workers reproduce at all may be partially phylogenetically constrained, while species where the workers do reproduce may be more influenced by variation in factors like costs and benefits.

## Conclusions

The extensive occurrence of worker male production in stingless bees is exactly what we predict if the genetic interests of workers control variation in who produces the males. Across half of the genera, male production by workers is reported and sometimes predominates. This is in marked contrast to the situation for honeybees where most worker-laid eggs are eaten by other workers if the queen is alive. However, there are also stingless bee species in which the queen produces all or nearly all of the males. Policing of worker eggs seems an unlikely explanation because after cells are filled they are sealed and are not generally reopened to eat or kill eggs or larvae. Queen control or self-restraint due to high costs might explain the failure of workers to produce males but our results did not generally support these hypotheses. Of course, we could only test a limited number of possible correlates of control and costs, and others might be more important. Variation in costs did receive some weak support by the high frequency of worker produced males in *Melipona*, where the cost is lowered whenever these males replace supernumerary queen larvae. A promising approach for future studies is to test between colony patterns within species.

The pattern of variation among species was suggestive in two ways. First, the breadth of variation seems partially consistent with an arms race in which worker and queen control change with time. Second, the similarity within genera supports some role for phylogenetic constraints. A resolved phylogenetic tree will be needed to allow further progress towards understanding these patterns.

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## References

Barron, A.B., B.P. Oldroyd and F.L.W. Ratnieks, 2001. Worker reproduction in honey-bees (*Apis*) and the anarchic syndrome: A review. *Behav. Ecol. Sociobiol. 50*: 199–208.

Beig, D., 1972. The production of males in queenright colonies of *Trigona (Scaptotrigona) postica*. *J. Apic. Res. 11*: 33–39.

Beig, D., O. Correa Bueno, R. Alves de Cunha and H. Jorge de Morales, 1982. Differences in quantity of brood in worker and male brood cells of *Scaptotrigona postica* (Latr. 1807) (Hymenoptera, Apidae). *Insect. Soc. 29*: 189–194.

Bego, L.R., 1982a. The production of males in queenright colonies of *Trigona (Scaptotrigona) postica*. *J. Apic. Res*. *11*: 33–39.

Biesmeijer, J.C. and E. Tóth, 1998. Individual forgaing, activity level and longevity in the stingless bee *Melipona beecheii* in Costa Rica (Hymenoptera, Apidae, Meliponinae). *Insect. Soc. 45*: 427–443.

Biesmeijer, J.C., M.G.L. van Nieuwstadt, S. Lukacs and M.J. Sommeijer, 1998. The role of internal and external information in foraging decisions of the *Melipona* workers (Hymenoptera: Meliponinae). *Behav. Ecol. Sociobiol. 42*: 107–116.

Biesmeijer, J.C. and M.C.W. Ermers, 1999. Social foraging in stingless bees: how colonies of *Melipona fasciata* choose among nectar sources. *Behav. Ecol. Sociobiol. 46*: 129–140.

Bourke, A.F.G., 1999. Colony size, complexity and reproductive conflict in social insects. *J. Evol Biol. 12*: 245–247.

Bourke, A.F.G. and N.R Franks, 1995. *Social Evolution in Ants*. Princeton University Press, Princeton, NJ. 529 pp.

Bourke, A.F.G. and F.L.W. Ratnieks, 1999. Kin conflict over caste determination in social Hymenoptera. *Behav. Ecol. Sociobiol. 46*: 287–297.

Camargo, J.M.F. and S.R. M. Pedro, 1992. Systematics, phylogeny and biogeography of the Meliponinae (Hymenoptera, Apidae): a mini review. *Apidologie 23*: 509–522.

Camillo-Attique, C., 1977. Estudo de variabilidade etológica de *Friesella schrottkyii* incluindo a caracterizaçao de especies cripticas (Hymenoptera, Meliponinae) 203 pp. PhD thesis Fac. Med. Ribeirao Preto.

Chinh, T.X., G.B.J. Grob, F.J.A.J. Meeuwsen and M.J. Sommeijer, 2003. Patterns of male production in the stingless bee *Melipona favosa* (Apidae, Meliponini). *Apidologie 34*: 161–170.

Contel, E.P.B. and W.E Kerr, 1976. Origin of males in *Melipona subnitida* estimated from data of an isozymic polymorphic system. *Genetica 46*: 271–277.

Cortopassi-Laurino, M., 1979. Observaçoes sobre atividades de machos de *Plebeia droryana* Friese (Apidae, Meliponinae), *Rev. Bras. Ent. 23*: 177–191.

Costa, M.A., M.A. del Lama, G.A.R. Melo and W.S. Sheppard, 2003. Molecular phylogeny of the stingless bees (Apidae, Apinae, Meliponini) inferred from mitochondrial 16S rDNA sequences. *Apidologie 34*: 73–84.

Crespi, B.J., 1992. Cannibalism and trophic eggs in subsocial and eusocial insects. In: *Cannibalism: Ecology and Evolution Among Diverse Taxa* (M.A. Elgar and B.J. Crespi, Eds.), Oxford: Oxford University Press. pp. 176–213.

Darchen, R., and B. Delage-Darchen, 1975. Contribution à l'étude d'une abeille du Mexique *Melipona beecheii* B. (Hymenoptère : Apide). *Apidologie 6*: 295–339.

Drumond, P.M., B.P Oldroyd and K. Osborne, 2000. Worker reproduction in *Austroplebeia australis* Friese (Hymenoptera, Apidae, Meliponini). *Insect. Soc. 47*: 333–36.

Engels, W. and V.L. Imperatriz Fonseca, 1990. Caste development, reproductive strategies and control of fertility in honey bees and stingless bees. In: *Social Insects: An Evolutionary Approach to Castes and Reproduction* (W. Engels, Ed.) Berlin: Springer-Verlag. pp. 166–230.

Evers, C.A. and T.D. Seeley, 1986. Kin discrimination and aggression in honey bee colonies with laying workers. *Anim. Behav. 34*: 924–925.

Felsenstein, J., 1985. Phylogenies and the comparative method. *Am. Nat. 125*: 1–15.

Fletcher, D.J.C. and K.G. Ross, 1985. Regulation of reproduction in eusocial Hymenoptera. *Annu. Rev. Entomol. 30*: 319–343.

Foster, K.R., F.L.W. Ratnieks and A.F. Raybould, 2000. Do hornets have zombie workers? *Mol. Ecol. 9*: 735–742.

Green, C.L. and B.P. Oldroyd, 2002. Queen mating frequency and maternity of males in the stingless bee *Trigona carbonaria* Smith. *Insect Soc. 49*: 196–202.

Grosso, A.F., L.R. Bego and A.S. Martinez, 2000. The production of males in queenright colonies of *Tetragonisca angustula angustula* (Hymenoptera, Meliponinae) *Sociobiology 35*: 475–485.

Halling, L., B.P. Oldroyd, B. Patimus, W. Wattanachaiyingcharoen, A.B. Barron, P. Nanork and S. Wongsiri, 2001. Worker policing in the bee, *Apis florae*. *Behav. Ecol. Sociobiol. 49*: 509–513.

Hart, A.G. and F.L.W. Ratnieks, 2002. Task-partitioned nectar transfer in stingless bees: work organization in a phylogenetic context. *Ecol. Entomol*. *27*: 163–168.

Imperatriz Fonseca V.L. and A. Matos de Peixoto Kleinert, 1998. Worker reproduction in the stingless bee *Friesella schrottkyi* (Hymenoptera: Apidae: Meliponinae). *Entomol. Gener. 23*: 169–175.

Inoue, T., D.W. Roubik and T. Suka, 1999. Nestmate recognition in the stingless bee *Melipona panamica* (Apidae, Meliponini). *Insect. Soc. 46*: 208–218.

Keller, L. and P. Nonacs, 1993. The role of queen pheromones in social insects: queen control or queen signal? *Anim. Behav*. *45*: 787–794.

Kerr, W.E. 1950. Genetic determination of castes in the genus *Melipona. Genetics 35*:143–152.

Koedam, D., F.A.L. Contrera and V.L. Imperatriz Fonseca, 1999. Clustered male production by workers in the stingless bee *Melipona subnitida* Ducke (Apidae, Meliponinae). *Insect. Soc. 46*: 387–391.

Koedam, D., H.H.W. Velthuis, M.R. Dohmen and V.L. Imperatriz Fonseca, 2001. The behaviour of laying workers and the morphology and viability of their eggs in *Melipona bicolor bicolor*. *Physiol. Entomol. 26*: 254–259.

Lacerda, L. M. and R. Zucchi, 1999. Behavioral alterations and related aspects in queenless colonies of *Geotrigona mombuca* (Hymenoptera, Apidae, Meliponinae). *Sociobiology 33*: 277–288.

Lindauer, M. and W.E. Kerr, 1958. Die gegenseitige Verständigung bei den stachellosen Bienen. *Z. Vergl. Phys. 41*: 405–434.

Lindauer, M. and W. E. Kerr, 1960. Communication between the workers of stingless bees. *Bee World 41*: 29–71.

Machado, M.F.P.S., E.P.B. Contel and W.E. Kerr, 1984. Proportions of males sons-of-the-queen and sons-of-workers in *Plebeia droryana* (Hymenoptera, Apidae) estimated from data of an MDH isozymic polymorphic system. *Genetica 65*: 193–198.

Michener C.D., 1990. Classification of the Apidae (Hymenoptera). *Univ. Kansas Sci. Bull. 54*: 75–164.

Moo-Valle, H., J.J.G. Quezada-Euán and T. Wenseleers, 2001. The effect of food reserves on the production of sexual offspring in the stingless bee *Melipona beecheii* (Apidae, Meliponini). *Insect. Soc*. *48*: 398–403.

Nieh, J.C. and D.W. Roubik, 1998. Potential mechanisms for the communication of height and distance by a stingless bee, *Melipona panamica*. *Behav. Ecol. Sociobiol*. *43*: 387–399.

Nieh, J.C., J. Tautz, J. Spaethe and T. Bartareau, 1999. The communication of food location by a primitive stingless bee, *Trigona carbonaria*. *Zoology-Analysis of Complex Systems. 102*: 238–246.

Nonacs, P. and N.F. Carlin, 1990. When can ants discriminate the sex of brood? A new aspect of queen-worker conflict. *Proc. Natl. Acad. Sci. USA. 87*: 9670–9673.

Oldroyd, B.P., L.A. Halling, G. Good, W. Wattanachaiyingcharoen, A.B. Barron, P. Nanork, S. Wongsiri and F.L.W. Ratnieks, 2001. Worker policing and worker reproduction in *Apis cerana. Behav. Ecol. Sociobiol. 50*: 371–377.

Oster, G.F. and E.O. Wilson, 1978. *Caste and Ecology in the Social Insects*. Princeton University Press, Princeton, NJ. 352 pp.

Palmer K.A., B.P. Oldroyd, J.J.G. Quezada-Euán, R.J. Paxton and J.W. de May-Itza, 2002. Paternity frequency and maternity of males in some stingless bee species. *Mol. Ecol. 11*: 2107–2113.

Paxton, R.J., N. Weisschuh, W. Engels, K. Hartfelder and J.J.G. Quezada-Euán, 1999. Not only single mating in stingless bees. *Naturwissenschaften 86*: 143–146.

Paxton, R.J., L.R. Bego, H. Ruhnke, F.L. W. Ratnieks and J.J.G. Quezada-Euán. 2001. Social evolution in stingless bees: Are the workers or is the queen in control of male production? *Adv. Ethol.* 236–237.

Paxton, R.J, L.R. Bego, M.M. Shah and S. Mateus, 2003. Low mating frequency of queens in the stingless bee *Scaptotrigona postica* and worker maternity of males. *Behav. Ecol. Sociobiol. 53*: 174–181.

Peters, J.M., D.C. Queller, V.L. Imperatriz-Fonseca, D.W. Roubik and J.E. Strassmann, 1999. Mate number, kin selection, and social conflicts in stingless bees and honey bees, *Proc. R. Soc. Lond. B. 266*: 379–384.

Queller, D.C. and J.E. Strassmann, 1998. Kin selection and social insects, *Bioscience 48*: 165–175.

Ratnieks, F.L.W., 1988. Reproductive harmony via mutual policing by workers in eusocial hymenoptera. *Am. Nat. 132*: 217–236.

Ratnieks, F.L.W., 1990. Worker policing in social insects. In: *Social Insects and the Environment: Proc. 11th Int. Congr. IUSSI* (G.K. Veeresh, B. Mallik and C.A. Viraktamath, Eds), Oxford & IBH, New Delhi. pp. 365–366.

Ratnieks, F.L.W., 1993. Egg-laying, egg-removal, and ovary development by workers in queenright honey-bee colonies. *Behav. Ecol. Sociobiol. 32*: 191–198.

Ratnieks, F.L.W., 2001. Heirs and spares: caste conflict and excess queen production in *Melipona* bees. *Behav. Ecol. Sociobiol. 50*: 467–473.

Ratnieks, F.L.W. and H.K. Reeve, 1992. Conflict in single-queen hymenopteran societies: the structure of conflict and processes that reduce conflict in advanced eusocial species. *J. Theor. Biol. 158*: 33–65.

Ratnieks F.L.W and P.K. Visscher, 1989. Worker policing in the honeybee. *Nature 342*: 796–797.

Reeve, H.K. and L. Keller (Eds), 1999. *Levels of Selection in Evolution.* Princeton University Press, Princeton, NJ. pp 153–175.

Sakagami, S.F., 1954. Occurrence of an aggressive behaviour in queenless hives, with considerations on the social organization of honeybee. *Insect. Soc. 1*: 331–343.

Sakagami, S.F., 1982. Stingless bees. In: *Social Insects*, Vol. III (H.R. Hermann. Ed.) Academic Press, New York. pp. 361–423.

Sakagami, S.F., D. Beig, R. Zucchi and Y. Akahira,1963. Occurrence of ovary-developed workers in queenright colonies of stingless bees. *Rev. Bras. Biol. 23*: 115–129.

Sakagami, S.F. and Zucchi R., 1968. Oviposition behavior of an Amazonic stingless bee *Trigona (Duckeola) ghilianii. J. Fac. Sci. Hokkaido Univ. Zool. 16*: 564–581.

Sakagami S.F. and R. Zucchi, 1974. Oviposition behavior of two dwarf stingless bees *Hypotrigona (Leurotrigona) muelleri and H. (Trigonisca) duckeii* with notes on the temporal articulation of oviposition process in stingless bees*. J. Fac. Sci. Hokkaido Univ. Zool. 19*: 361–421.

Sakagami, S.F., C. Camilo and R. Zucchi, 1973. Oviposition behavior of the Brazilian stingless bee, *Plebeia (Friesella) schrottkyi*, with some remarks on the behavioral evolution in stingless bees. *J. Fac. Sci. Hokkaido Univ. Zool. 19*: 163–189.

Silva D.L.N., R. Zucchi and W.E. Kerr, 1972. Biological and behavioral aspects of the reproduction in some species of *Melipona* (Hymenoptera, Apidae, Meliponinae). *Anim. Behav. 20*: 123–132.

Slaa, E.J., Tack, A.J.M. and M.J. Sommeijer, 2003. The effect of intrinsic and extrinsic factors on flower constancy in stingless bees. *Apidologie 34*: 457–468.

Sommeijer, M.J., G.A De Rooy, W.A. Punt and L.L.M de Bruijn, 1983. Comparative study of foraging behavior and pollen resources of various stingless bees (Hym., Meliponinae) and honeybees (Hym., Apinae) in Trinidad, West-Indies. *Apidologie 14*: 205–224.

Sommeijer M.J., J.l. Houtekamer and W. Bos, 1984 a. Cell construction and egg laying in *Trigona nigra var. paupera* Prov. with notes on the adaptive significance of the typical behavior of stingless bees. *Insect. Soc. 31*: 199–217.

Sommeijer M.J., M. van Zeijl and M.R. Dohmen, 1984b. Morphological differences between worker-laid eggs from a queenright colony and a queenless colony of *Melipona rufiventris paraensis* (Hymenoptera Apidae) *Ent. Ber. 44*: 91–94.

Sommeijer M.J. and N.W.M. van Buren, 1992. Male production by laying workers in queenright colonies of *Melipona favosa* (Apidae, Meliponinae). In: *Biology and Evolution of Social Insects* (J. Billen, Ed.) Leuven University Press, Leuven. pp. 89–97.

Sommeijer M.J., T.X. Chinh and F.J.A.J. Meeuwsen, 1999. Behavioral data on the production of males by workers in the stingless bee *Melipona favosa* (Apidae, Meliponinae). *Insect. Soc. 46*: 92–93.

Sommeijer M.J., L.L.M. de Bruijn, F.J.A.J. Meeuwsen and E.P. Martens, 2003. Natural patterns of caste and sex allocation in the stingless bees *Melipona favosa* and *M. trinitatis* related to worker behaviour. *Insect. Soc. 50*: 38–44.

Starr, C.K., 1984. Sperm competition, kinship and sociality in the aculeate Hymenoptera. In: *Sperm Competition and the Evolution of Animal Mating Systems,* Smith R.L. (Ed). Academic Press, Orlando, FL. pp. 428–459.

Suka, T. and T. Inoue, 1993. Nestmate recognition of the stingless bee *Trigona (Tetragonula) minangkabau* (Apidae: Meliponinae). *J. Ethol. 11*: 141–147.

Tambasco, A.J., 1971. Processo reproductivo em *Melipona quadrifasciata* e seu impacto na população geneticamente ativa. *Ciência e Cultura 23*: 104–105.

Terada, Y., 1974. Contribuição ao estudo da regulação social em *Leurotrigona muelleri* e *Frieseomlitta varia* (Hymenoptera, Apidae), 96 pp. Masters Thesis, University of Sao Paulo.

Tóth, E., J.E. Strassmann, P. Nogueira Neto, V.L. Imperatriz Fonseca and D.C. Queller, 2002a. Male production in stingless bees: variable outcomes of queen-worker conflict. *Mol. Ecol. 11*: 2661–2667.

Tóth, E., D.C. Queller, V.L. Imperatriz Fonseca and J.E. Strassmann 2002 b. Genetic and behavioral conflict over male production in the stingless bee *Paratrigona subnitida*. *Behav. Ecol. Sociobiol. 53*: 1–8.

Tóth, E., J.E. Strassmann, V.L. Imperatriz Fonseca and D.C. Queller, 2003. Queens, not workers, produce the males in the stingless bee *Schwarziana quadripunctata quadripunctata Anim. Behav. 66*: 359–368.

Trivers, R.L. and H. Hare, 1976. Haplodiploidy and the evolution of the social insects. *Science. 11*: 2661–2667.

van Benthem, F.D.J., V.L. Imperatriz Fonseca and H.H.W. Velthuis, 1995. Biology of the stingless bee *Plebeia remota* (Holmberg): observations and evolutionary implications. *Insect. Soc. 42*: 71–87.

van Buren, N.W.M. and M.J. Sommeijer, 1988. Etude des facteurs determinants pour la dominacion reproductive de la reine de *Melipona trinitatis*. *Actes. Coll. Insect.. Soc. 4*: 285–290.

van Veen, J.W., H. Arce and M.J. Sommeijer, 1990. Tropical beekeeping: The production of males in stingless bees (*Melipona*). *Proc. Exp. Appl. Entomol., NEV, Amsterdam. 1*: 171–176.

van Veen, J.W., M.J. Sommeijer and F.J.A.J. Meeuwsen, 1997. Behavior of drones in *Melipona* (Apidae, Meliponinae) *Insect. Soc. 44*: 435–447.

Velthuis, H.H.W., D. de Araujo Alves, V.L. Imperatriz Fonseca and M.J. Duchateau, 2002. Worker bees and fate of their eggs. *Proc. Exp. Appl. Entomol., NEV Amsterdam. 13*: 97–102.

Visscher, P.K., 1989. A quantitative study of worker reproduction in honey bee colonies. *Behav. Ecol. Sociobiol. 25*: 247–254

Visscher, P.K. and R. Dukas, 1995. Honey bees recognize development of nestmates' ovaries. *Anim. Behav. 49*: 542–544.

von Ihering, H., 1903. Biologie der stachellosen Honigbienen Brasiliens. *Zool. Jb 19*: 179–287.

Wattanachaiyingcharoen W., B.P. Oldroyd, G. Good, L.A. Halling, F.L.W. Ratnieks and S. Wongsiri, 2001. Lack of worker reproduction in *Apis dorsata*. *Insect. Soc. 49*: 80–85

Wenseleers, T., F.L.W. Ratnieks and J. Billen. 2003. Caste fate conflict in swarm-founding social Hymenoptera: an inclusive fitness analysis. *J. Evol. Biol. 16*: 647–658.

West Eberhard, M.J., 1981. Intragroup selection and the evolution of insect societies. In: *Natural Selection and Social Behavior* (R.D. Alexander and D.W. Tinkle, Eds.) Chiron Press, New York. pp. 3–17.

Wille, A., 1979. Phylogeny and relationships among the genera and subgenera of stingless bees (Meliponinae) of the world. *Rev. Biol. Trop. 27*: 241–277.

Wilson, E.O., 1971. *The Insect Societies*. Harvard Univ. Press, Cambridge, MA. 549 pp.

Woyciechowski, M. and A. Lomnicki, 1987. Multiple mating of queens and the sterility of workers among eusocial Hymenoptera. *J. Theor. Biol. 128*: 317–327.

Zucchi, R., 1993. Ritualized dominance, evolution of queen worker interactions and related aspects in stingless bees (Hymenoptera: Apidae) In: *Evolution of Insect Societies* (Inoue T. and S. Yamahe, Eds.), Hakuhinsha, Tokyo, Japan. pp. 207–249.