ANIMAL BEHAVIOUR, 2003, **66**, 359–368
doi:10.1006/anbe.2003.2218

Queens, not workers, produce the males in the stingless bee *Schwarziana quadripunctata quadripunctata*

EVA TÓTH*, JOAN E. STRASSMANN*, VERA L. IMPERATRIZ-FONSECA† & DAVID C. QUELLER*
*Department of Ecology and Evolutionary Biology, Rice University
†Departmento de Ecologia Geral, Universidade de São Paulo

(Received 20 November 2001; initial acceptance 20 February 2002;
final acceptance 21 October 2002; MS. number: A9216R)

Most stingless bee colonies have one singly mated queen, resulting in a potential conflict between workers and queen over male production, because workers are more closely related to the sons of other workers than they are to the queen's sons. Furthermore, workers in the majority of stingless bee species have ovarian development, can produce haploid eggs, and show apparently agonistic behaviour towards their queen, suggesting a real conflict. We investigated whether genetic conflict over male production resulted in reproductive and behavioural conflict in *Schwarziana q. quadripunctata*. DNA microsatellite loci showed that, even though workers are more related to other workers' sons than to queen's sons, it is the queen who produces the males. Behavioural interactions between workers and their queen were not uniformly more aggressive during male production than at times with only worker production, although some differences might have been obscured by the fact that food supply was greater during male production. The potential conflict over male production in *S. q. quadripunctata* seems not to lead to an observable conflict between the workers and their queen. Workers might refrain from reproduction because of the costs involved for the colony or because of queen control.

Highly social Hymenoptera live in colonies with most females serving as nonreproducing helpers (Wilson 1971; Michener 1974). Genetic relatedness helps to explain the evolution of cooperation in social insects (Hamilton 1964, 1972), but unequal relatedness between colony members can also be a source of conflict (Trivers & Hare 1976; Queller & Strassmann 1998; Bourke & Ratnieks 1999).

In Hymenoptera, where males arise from unfertilized, haploid eggs, and females are produced by fertilized, diploid eggs, an important issue of conflict is who produces the males. Queens and workers are most related to their own sons and less related to each other's sons. Who gets to produce the males in a colony may be resolved in favour of collective worker interests (Starr 1984; Woyciechowsky & Lomnicki 1987; Ratnieks 1988). Where the mother queen mates more than twice, as in the honeybee, workers will be more related to their queen's sons (r=1/4) than to their sisters' sons (average r near 1/8). Thus, in honeybees, collective worker interests will favour queen's sons above worker sons and workers should police each other (Woyciechowsky & Lomnicki 1987; Ratnieks 1988; Oldroyd et al. 2001). In queenright situations (when the colony has a functional queen), most honeybee workers do not reproduce and have nonfunctional ovaries (Ratnieks 1988; Ratnieks & Visscher 1989). Workers with developed ovaries are attacked (Sakagami 1954; Visscher & Dukas 1995) and most of the eggs they lay are removed from the cells by other workers (Ratnieks & Visscher 1989). In contrast, in species where the queen typically mates only once, as in stingless bees (Camargo 1972; Contel & Kerr 1976; Machado et al. 1984; Oliveira Campos de Melo 1990; Peters et al. 1999), the workers are more related to their own sons (r=1/2) and to sister-produced nephews (r=3/8) than to queen-produced brothers (r=1/4). Based on relatedness alone, workers should thus collectively oppose queen production of males and allow each other to reproduce (Ratnieks 1988; Bourke & Franks 1995; Peters et al. 1999). A potential conflict (Ratnieks & Reeve 1992) between the workers and the queen over male production is thus expected in stingless bees.

Differences in genetic makeup in honeybee and stingless bee colonies could account for many differences

Correspondence and present address: E. Tóth, Virginia Institute of Marine Science, Route 1208 Greate Road, Gloucester Point, VA 23062, U.S.A. (email: etoth@vims.edu). J. E. Strassmann and D. C. Queller are at the Department of Ecology and Evolutionary Biology, Rice University, P.O. Box 1892, Houston, TX 77251, U.S.A. V. L. Imperatriz-Fonseca is at the Departmento de Ecologia Geral, Universidade de São Paulo, P.O. Box 11461, CEP: 05508-900, São Paulo, Brazil.

0003–3472/03/$30.00/0

between the two groups (Peters et al. 1999). Theory predicts that stingless bee workers will be more likely to lay eggs than will honeybee workers and will therefore be in conflict with the queen. In most stingless bees, workers have ovarian development and retain the ability to produce haploid eggs (Sakagami et al. 1963). A fraction of those eggs may be trophic, inviable eggs that are usually laid before queen oviposition and probably are meant to feed the queen (Crespi 1992). However, workers can produce viable eggs that develop into males (Beig 1972). The few studies that have focused on both types of worker egg production showed that workers that produce reproductive eggs are younger than the ones that generate trophic eggs (Bego 1982, 1990; Koedam et al. 1999).

The best way to find out whether genetic conflict becomes a real conflict and thus manifests itself in behaviour is to study the provisioning and oviposition process (POP) in stingless bees. A POP is a complex and ritualized set of behaviours between workers and the queen. It begins with an empty cell and results in a provisioned cell filled with larval food provided by the workers, and an egg that is in most cases produced by the queen (Sakagami 1982; Engels & Imperatriz-Fonseca 1990; Zucchi 1993). Many behaviours during the POP seem to be agonistic between the workers and the queens and are often described in terms of conflict (Engels & Imperatriz-Fonseca 1990), and it has been hypothesized that more intense worker–queen interactions reflect conflict between the castes (Drummond et al. 1999). For example, in *Scaptotrigona postica*, where both the workers and the queen produce males, workers dart and lunge at the queen in a very aggressive way and often barricade the cell with their bodies so that the queen cannot get to it (Sakagami & Zucchi 1963). However, apparently aggressive behaviours might have some other function. One way to test their agonistic nature is to see whether they are more intense at times of higher predicted conflict (e.g. during male production).

In the present study, to predict collective worker interests on male production, we confirmed that the queen in *Schwarziana q. quadripuncata* is singly mated, as found by Peters et al. (1999). We predicted that under single mating, all else being equal, workers would try to monopolize or at least contribute to male production. We tested the accuracy of this prediction using microsatellite genotyping. Finally, we tested the prediction that, if behaviour during POP represents queen–worker conflict, it should be more intense during periods of male production.

METHODS

Study Animals and Data Collection

Schwarziana q. quadripunctata (Lepeletier) nests exclusively underground, 0.3–3.0 m below the surface (Schwarz 1948; Imperatriz-Fonseca 1973; Camargo 1974). The bees enter their nest through a narrow, winding tube leading to the main nesting chamber. This chamber contains a brood pile of five to eight horizontal combs, with the youngest combs on top, surrounded by 20–50 storage pots. A colony consists of one functional queen, up to 800–2500 workers, and sometimes males and a few virgin queens. The nesting chamber is surrounded by several protective sheaths, giving the nest the appearance of a ball. This makes nest collection relatively easy. A nest can be harvested in one piece, avoiding mechanical damage of brood piles and pots or the loss of many bees. We dug up the nests near Cotia, Brazil (23°39′S, 46°56′W), a settlement in the Atlantic Rainforest. The nests were transported to the bee laboratory at the University of São Paulo, São Paulo City, Brazil, and transferred into wooden observation hives. These hives were inside the building and consisted of a heated wooden outer thermo box (28°C) and a smaller inner box that contained the colonies, and both boxes were covered with glass on top (Sakagami 1966). When the nests were not being observed, we covered the thermo box with a black cloth and Styrofoam plates. The colonies were connected to the outside by a plastic tube so that the bees could forage normally.

For the genetic analysis, we sampled workers from 18 *S. q. quadripunctata* nests. Sixteen of these nests produced males, so we collected males from those nests, from February 1997 to May 1999. The collected bees were chilled and then transferred into tubes with 100% ethanol and kept at 4°C until further analysis.

We videotaped POPs from six nests between March and May 1999. We used red or cold lights (to avoid heating colonies) to illuminate the colonies and minimize further disturbance during videotaping. We videotaped POP in four colonies when they were not producing males. After this, since males are normally produced when colonies have plenty of food supplies (Bego 1990; Grosso et al. 2000; Moo-Valle et al. 2001), we attempted to induce male production by regularly supplying the colonies with extra pollen for 8 weeks. Two of these four colonies did produce males (first observed 39 days and 42 days after pollen supplementation started). We also added two more colonies, which were in their male production phase, from the field. Thus, we had behavioural data from two colonies in their male production phase, two colonies outside of male production and two colonies in both phases.

Genetic Data, Relatedness and Male Parentage Estimation

We genotyped samples of workers and males at seven moderately polymorphic microsatellite loci: Mbi201AAG, Mbi215AAT, Mbi219AAT, Mbi232AAG, Mbi254AAG, Mbi259AAG and Mbi278AAG (Peters et al. 1998). In *S. q. quadripunctata*, these loci had three to seven alleles. We genotyped 7–15 workers and 11–39 males per colony, for a total of 189 workers and 314 males. Whenever it was possible, we tried to collect young bees, since they were unlikely to have been drifters from other colonies. We followed protocols of Strassmann et al. (1996) for DNA extraction, polymerase chain reactions and visualization of PCR products of the polyacrymalide gels on autorads. Two people independently scored the autorads and their scores were compared. Discrepancies were rechecked and if necessary the sample was rerun. We used the computer

Table 1. *f* values for the likelihood function, *L*

Worker genotypes	Inferred queen genotype	f_{qi} if male allele matches queen	f_{wi} if male allele matches worker
AA	AA	1	1
AA, AB	AB	1/2	A 3/4; B 1/4
AB, AC	BC	1/2	A 1/2; B 1/4; C 1/4
AB	AA or BB	1 or 0*	A 1/2; B 1/2

*See text for combining these alternatives.

program Relatedness 5.07 for Macintosh (Goodnight & Queller 2000; http://Gsoft.smu.edu/GSoft.html) for relatedness estimations within colonies, based on the microsatellite data. Standard errors were based on jackknifing over loci for within-colony estimates and over colonies for population estimates (Queller & 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 what proportions of males were produced by the queen and the workers we used a maximum likelihood method (modified from Arévalo et al. 1998; Hastings et al. 1998). Under the assumption that we knew the queen, worker and male genotypes at a locus, we sought the maximum likelihood, *L*, of the parameter *Q*, the fraction of males drawn from the queens. For each male-producing colony, we calculated

$$L = K \prod_{\text{males}} \left(Q \prod_{\text{loci}} f_{qi} + (1-Q) \prod_{\text{loci}} f_{wi} \right)$$

for values of *Q* between 0 and 1, which we sampled at intervals of 0.01. *K* is a multinomial constant that never has to be calculated because it multiplies all *L*s by a constant, leaving comparisons between them unaffected. 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. Table 1 shows the values taken by f_{qi} and f_{wi} for the four possible configurations of alleles at a locus when the queen is singly mated. In contrast to earlier papers (Arévalo et al. 1998; Hastings et al. 1998), we used the expected frequencies in workers rather than the observed frequencies in sampled workers. This is because our colonies had a relatively simple structure, with one singly mated queen. Thus, if we sampled 15 AB workers and 5 AC workers, we still assumed that the laying worker pool consisted of 50% of each type, because they must have come from a BC queen mated to an A male.

To avoid damaging the colonies, we did not collect and genotype the queen. Instead, because the colonies are headed by one singly mated queen, we could infer the genotype of the queen and her mate from the worker genotypes. For the second and third cases in Table 1, this is straightforward. For case 1, in which all workers were homozygous AA, we assumed that the queen was AA and her mate A, and we set $f_{qi}=1$ (and also $f_{wi}=1$) whenever a male had the A allele. Because we genotyped 7–11 workers per colony, we neglected the chance that the queen was AB and transmitted no B alleles to the genotyped workers, because the probability of missing an allele of a heterozygote queen when seven daughters are genotyped is 0.008, low enough to be improbable. The more difficult case is the last one in Table 1, in which workers were all heterozygotes and we could not distinguish between AA × B colonies and BB × A colonies. Whenever this happened, we calculated the colony likelihood, *L*, separately for each case, and then combined them (if two loci were ambiguous, four separate cases had to be combined). We combined them by weighting the separate *L* estimates by their relative probabilities. If the frequencies of the two alleles are p_A and p_B, then the probability of an AA × B mating is $p_A^2 p_B$ and of a BB × A is $p_A p_B^2$. The probability that it is AA × B over the summed probabilities of both alternatives is $p_A/(p_A+p_B)$, and the corresponding value for BB × A is $p_B/(p_A+p_B)$. These were the weights used to combine the separate likelihoods. We calculated the *L* values separately across the whole colony before combining them, because to treat each male separately would allow combinations that are not possible; if the males are produced by the queen, they must all be A or B; they cannot be a mixture of A and B.

Behavioural Data

The provisioning and oviposition process in *S. q. quadripunctata* is characterized by interactions with many body contacts between workers and the queen (Nogeira-Ferreira et al. 1998). Below, we describe the typical sequence of behaviours. We then present definitions of each of the behaviours we scored.

When the queen finds an empty cell, ready for oviposition, on the edge of the horizontal comb, she ‘fixes’ it (Sakagami et al. 1965) by staying very close to it and putting her front legs around the cell. Some workers move into a ‘hanging’ vertical position on the outer edge of the comb, on the side of the empty cell, with their heads at the cell opening, facing the queen. Other workers collect on the horizontal comb around the queen. The queen’s head often covers part or all of the cell. Workers hanging from the cell may dart and lunge at the queen. When a worker closely approaches the queen, the queen attempts to tap the worker with her antennae and front legs. Hanging workers sometimes also put their head in the cell or dive partially into it during this preprovisioning period. Over time, their movements become shorter and more frequent. One of the workers then dives into the cell and discharges larval food into it, while the queen taps her side and back. Other workers then follow and also discharge into the cell. After several discharges, a trophic egg may be deposited on the inner rim of the cell. The queen inspects the cell, eats the worker egg if there is one, and often drinks some of the larval food. She subsequently deposits an egg herself on the top of the larval fluid, leaving the cell afterwards. One of the workers, often standing behind the queen before the queen lays her egg, will close the cell by putting her abdomen into the cell and performing rotational movements, pressing the cell rim inward with her mandibles while counterbalancing it with her abdomen inside the cell. During the last phase of cell closure, the worker removes her abdomen from the cell and uses her mandibles to close the tiny hole; others often assist her.

We watched and analysed 92 POPs (N=30 POPs before and N=62 POPs during the period of male production). Definitions of the measured parameters are as follows.

(1) Darting: a hanging worker pushes her body forward towards the queen, then moves away from her again. The heads of the queen and the workers come very close to each other, but do not necessarily touch. As soon as the antennae of the worker and queen touch, the queen moves back a little. Movements in this category vary in velocity from slow to rapid. We often observed that the tongue of the darting worker was stretched out and inserted between the mandibles of the queen. However, we could not always confirm this because the dark tongues of the workers were hard to see against the dark empty cell. For this reason, every time we observed a worker with or without an outstretched tongue approaching the queen at a slower speed than lunging, we identified it as darting.

(2) Lunging: a hanging worker pushes her body forward quickly and hits the head of the queen, pushing the queen away from the cell. This forward movement is followed by a rapid retreat. The worker often pushes the queen's head from underneath at an angle. Lunging is noticeably quicker and more energetic than darting, and the worker often opens her wings in the forward movement. Also during lunging, some workers extrude their tongues.

(3) Inspection: a worker darts forward, pushes the queen away if she blocks the cell and puts her head or a part of it into a cell. The queen taps the worker's back and the worker rapidly retreats.

(4) Diving: same as inspection, except that the worker inserts not just her head but also her thorax into the empty cell.

(5) Discharging: a diving worker contracts her abdomen so that her abdomen visibly shortens, regurgitating larval food into the cell.

(6) Fixation interval: the interval (s) between initial cell fixation by the queen and the first discharge by a worker into the cell.

(7) Operculation interval: the interval (s) during cell closure when the closing worker has her abdomen in the cell.

We also measured (8) the average number of workers hanging from the focal cell and (9) the number of workers standing within a 1.5-cm radius, forming a 'rosette' around the queen during POP.

Tapping of workers by the queen was excluded, because it happened predictably every time a worker came close enough to the queen. Complex queen–worker interactions, such as hypnotic turning and hypnotic cell barricading (Drumond et al. 1996; Nogeira-Ferreira et al. 1998) were also excluded from the analysis, because they occurred in very low frequencies during both periods and nearly always before the queen really fixed the cell, which was before we started recording the elements of the POP. To be able to compare different frequencies in the statistical analysis, we calculated the adjusted rates of darts, lunges, inspections and dives by dividing their number in a POP by the fixation interval.

Although we did not measure ovarian development of workers that were involved in the POP, we know that some had developed ovaries because workers laid trophic eggs. We did not observe worker laying of nontrophic eggs (laid on the fluid rather than on the side of the cell). However, because cryptic worker reproduction often happens in stingless bees while workers close the cell (Beig 1972; Bego 1982; Koedam et al. 1999), we assumed that reproducing *S. q. quadripunctata* workers might apply that strategy as well. Workers might also return and lay eggs after the POP.

Our measured variables did not significantly differ from a normal distribution (Kolmogorov–Smirnov test: NS). For each behavioural variable, we used a one-factor, two-level, incomplete block design within the procedure GLM (SAS Institute 1990). The different parameters were not correlated (r^2=0.08, NS). The two levels in the analysis corresponded to the periods when males and no males were produced. The design was incomplete because some colonies were not observed both with and without males. Because the distribution of trophic worker eggs did not allow us to include them in the GLM calculations, we applied a G test for those. For every test we used a significance level of P<0.05.

Assuming that POP involves conflict, we predicted that POP would take longer and involve more conflict-like behaviour during the period when males are produced compared with periods with just female production. Interactions that seemed agonistic, like darting and lunging between workers and queens, were expected to be more frequent during the male-producing period compared to the period when no males were produced. Although less obviously agonistic, we predicted that workers would inspect and dive into cells more frequently before discharging into a cell in preparation for egg laying. We also predicted that the worker interest in laying male eggs would mean that more of them would be involved in or near the POP to have a hand in the outcome. Above all, we expected the operculation interval to be longer when workers had a chance to lay an egg in the cell they closed.

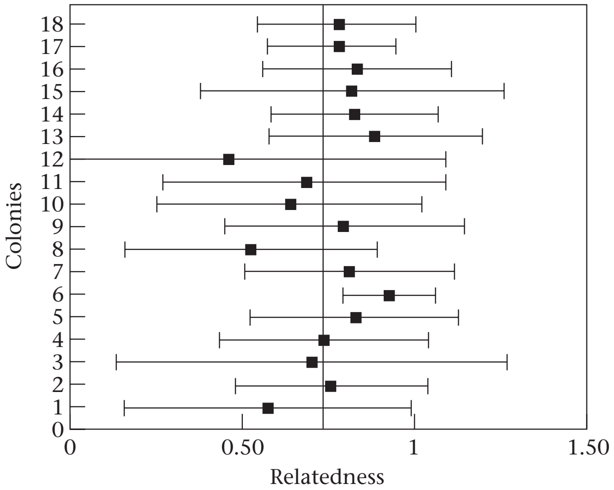

Figure 1. Relatedness values (±95% CI) between workers for 18 colonies of *Schwarziana q. quadripunctata*.

RESULTS

Genetic Data

Single mating

The workers within colonies were highly related to each other (Fig. 1). On average, relatedness for all colonies was 0.75 ± 0.04 (± 95% CI). The 95% confidence interval in all colonies overlapped with full sister relatedness, indicating that the queens were singly mated. Workers generally share one allele (paternal) and no more than two other alleles (maternal). There were six nonmatching genotypes of older workers. We checked and concluded that those individuals could not have been progeny of the queen, even if she was multiply mated, nor could they have been daughters of an older, deceased queen who was the mother of the current queen. Those nonmatching workers must have been drifters from other colonies that were not used for the study. Therefore, we excluded them from further analysis.

Worker preference

The relatedness between workers was higher than the relatedness of workers to queens (Fig. 2). The paired difference test (Queller 1994) showed a significant difference between the two estimates of relatedness (0.28, 95% CI=0.11, $P<0.001$). These results indicate that, on genetic grounds, workers should prefer to produce the males.

Who produces the males

The relatedness of workers to males was 0.51 (95% CI=0.16), which is almost the same as the relatedness of workers to queens (0.48, 95% CI=0.20; Fig. 2). This is what we would expect if the queen produced the males. If workers produced the males, the worker-to-male relatedness of 0.51 ought to equal worker-to-worker relatedness of 0.75, and it did not (paired difference 0.24, 95% CI=0.11, $P<0.002$). This result indicates that the workers were not producing the males.

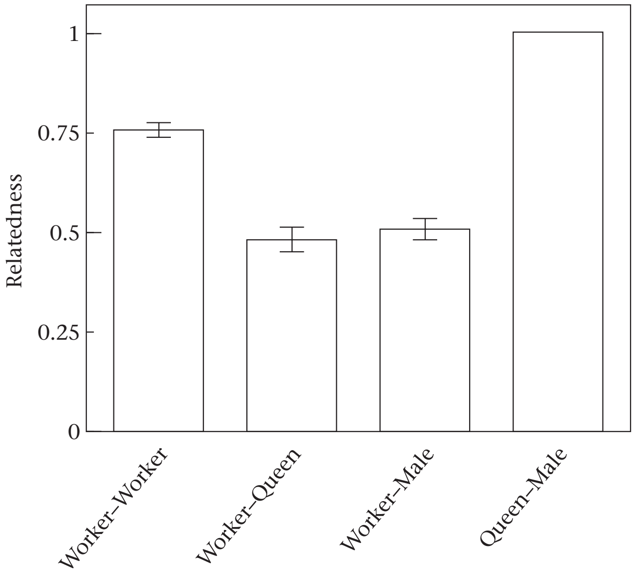

Figure 2. Relatedness of workers to workers, workers to queens, workers to males and queens to males for 16 colonies of *Schwarziana q. quadripunctata*. For the queen–male comparison, the standard error is zero, because males are haploid and they inherit half of the queen's genome when they are all produced by the queen.

Similarly, the results for the maximum likelihood method did not support male production by workers. The maximum of the likelihood estimate was 100% queen production of males for 15 colonies. Furthermore, the likelihood curves dropped off sharply, indicating that anything besides queen production of most males was much less likely (Fig. 3). In one colony, the maximum

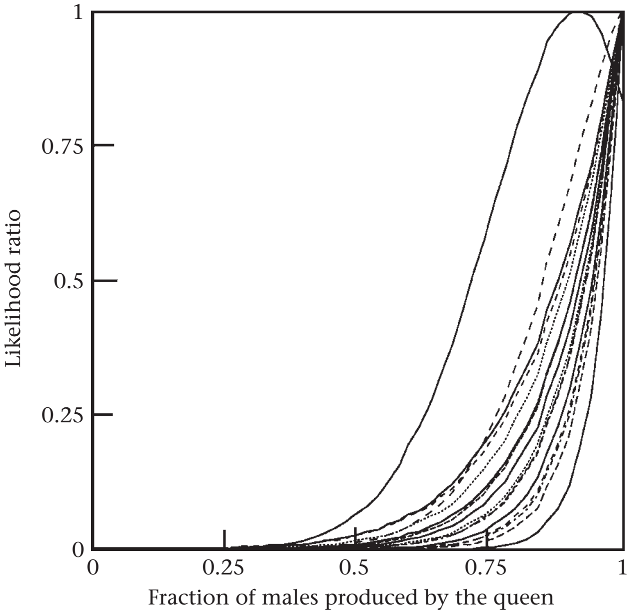

Figure 3. The relative likelihoods for the fraction of males produced by the queen of *Schwarziana q. quadripunctata* (N=16). Each curve represents one colony. For each curve, all values were divided by the maximum likelihood for that colony, thus scaling all values to a maximum of one.

was 90% queen production, but even here this was only about 20% more likely than 100% queen production. Thus, the queens produced all or nearly all of the males.

Behavioural Data

Individual colonies differed from each other and colony effect was highly significant (GLM: $4.47<F_{1,6}<21.01$, $P<0.0005$) for all parameters except diving frequency and operculation interval, which were not significant. Only two of the measured parameters, inspections and fixation interval, differed significantly between the male-producing and the female-producing period (ANOVA: inspections: $P<0.05$; fixation interval: $P<0.001$; Fig. 4a, d). Both of these significant differences during male production (shorter fixation interval, fewer inspections) were in the opposite direction to the one predicted by the conflict theory. Also, against expectations, there were not significantly more workers around the queen or hanging from the cell during male production than when no males were produced (Fig. 4b). Nor were the rates of darts, lunges, or dives significantly different (Fig. 4c, d). Finally, the operculation interval was not significantly different between the two periods, providing no evidence of workers delaying closure in order to lay eggs (Fig. 4a).

We observed fewer trophic eggs laid by workers in the period with no male production (0.10 per cell) than in the period when males were produced (0.42 per cell; G test: $G_1=10.8$ $P=0.001$). Therefore, in the male-producing period, there were probably more workers with developed ovaries present that could have produced males than in the period when only females were produced. Furthermore, observations revealed that hanging workers during the POP consisted of a pool of individuals replacing each other. This was especially obvious in the period of male production; hanging workers were pushing each other for space. The maximum number of hanging workers could have thus been determined by available space. We could often observe the queen fixed at a cell with open mandibles, and also often saw hanging workers with open mandibles.

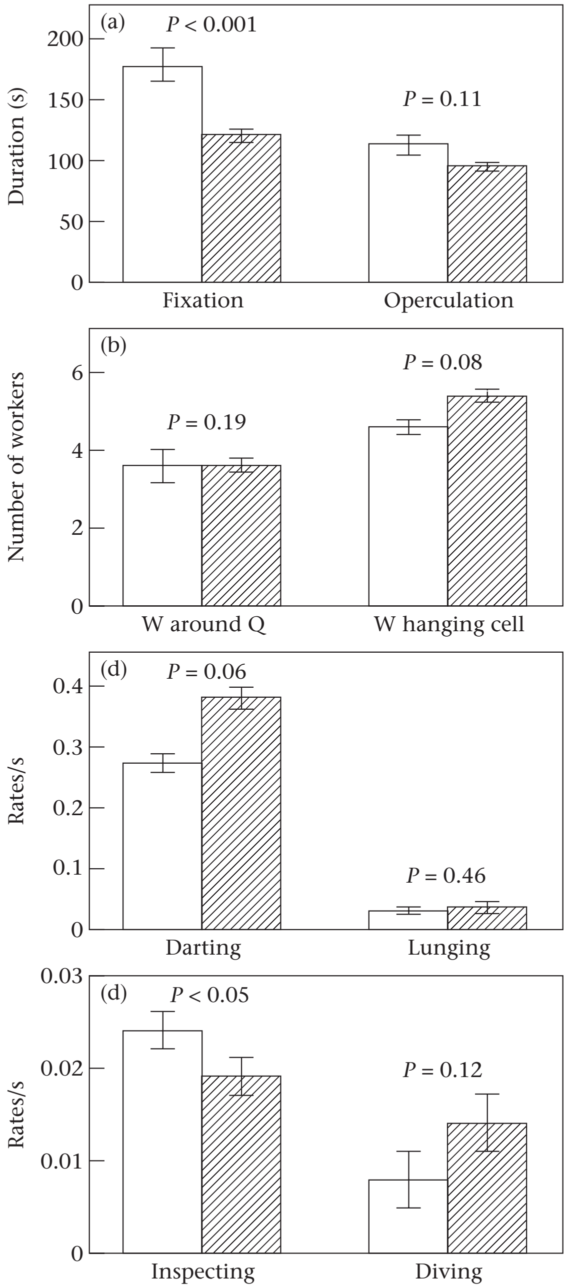

Figure 4. Mean±SE behavioural parameters in the period when no males were produced (□) versus the time of male production (▨). (a) Fixation interval ($F_{1,85}=9.97$) and operculation interval ($F_{1,85}=2.67$); (b) the number of workers around the queen ($F_{1,85}=3.10$) and the number of workers hanging from a cell ($F_{1,85}=1.76$). The values in (c) and (d) indicate the the rate/s during the fixation interval. (c) The rate of darts ($F_{1,85}=4.64$) and lunges ($F_{1,85}=2.53$). (d) The rate of inspection ($F_{1,85}=3.36$) and dives ($F_{1,85}=0.54$) in the cells.

DISCUSSION

Despite the genetic conflict over male production between workers and their queen, in *S. q. quadripunctata*, the queen produces the males. In spite of developed worker ovaries, the genetic conflict does not manifest itself in an increased measurable behavioural conflict between the two castes. We found no real indication that the POP during male production was more aggressive than in the period when no males were produced. The failure of the simple relatedness predictions about collective worker interests raises two related questions. First, why do workers allow the queen to lay the male eggs? Second, why is there little evidence of increased conflict during male production?

Why Do Workers Not Produce the Males?

Worker policing theory predicts that, other factors being equal, a collective worker preference is expected to favour the class of males to which the average worker is more highly related (Woyciechowski & Lomnicki 1987; Ratnieks 1988). In *S. q. quadripunctata* colonies, workers are more related to worker-produced males (sons and nephews) than to queen-produced males (brothers). Based on these results, we expected, assuming equal costs and benefits of worker and queen reproduction, that workers would monopolize male production or at least produce a smaller fraction of the males. The lack of worker reproduction is not a result of undeveloped ovaries, because workers in this species do lay trophic eggs that are eaten by the queen. Why do workers forego male production even though the theory predicts that they should compete with the queen over parentage of male progeny? The two main possibilities are that queens suppress worker reproduction or that workers do not reproduce because reproduction incurs high costs to the colony, reducing everyone's fitness.

Despotic queens could conceivably control the workers either by physical force or by manipulation through signals. A *S. q. quadripunctata* colony would appear to contain too many workers for the queen to suppress (Ratnieks 1988), but relatively few workers (9–30) participate in a given POP. The queen is larger, approximately twice the size of workers. However, her mouth parts are weak (Schwarz 1948; Camargo 1974), so queen control by biting of workers seems unlikely. Even if the queen could control workers during POP, one might expect workers to come back and lay eggs when the queen has gone elsewhere.

The other way that queens might be able to suppress their workers is by pheromones (Fletcher & Ross 1985). However, such manipulation is thought to be difficult to sustain over evolutionary time because the workers would evolve to not respond (Keller & Nonacs 1993). Still, the queen might gain temporary control if she is able to repeatedly evolve new chemical manipulations at low cost (Foster et al. 2000). Queen pheromones may also perform multiple functions, some of which are not connected to queen suppression, so that workers need to remain sensitive to them to perform optimally (Winston 1987). For example, it may be difficult for the workers to evolve to ignore queen pheromones when males are produced, if they convey honest information during the rest of the year (Foster et al. 2000).

If worker reproduction entails high costs to the colony, worker policing could be favoured even when queens are singly mated (Kikuta & Kazuki 1999; Foster et al. 2000). However, in most stingless bee species, workers do not eat each other's eggs (Zucchi 1993), except when the queen fails to eat a trophic egg or when cells are broken down because of damage (E. Tóth & V. Imperatriz-Fonseca, personal observations). Even without policing, high costs could lead workers to individually refrain from attempting to reproduce (Cole 1986; Bourke 1988; Ratnieks & Reeve 1992; Pollock 1996).

Costs could occur in at least three different ways. First, colony performance might drop if reproducing workers consume too much food or neglect other colony tasks. Second, in a contest involving physical fighting, workers might injure or kill their queen (workers in stingless bees regularly kill virgin queens; Imperatriz-Fonseca & Zucchi 1995). Worker production would suffer during the delay before a replacement queen becomes reproductive. A replacement queen also means that workers would suffer a large relatedness loss for new queen production (r=0.75 to 0.375). Thus, physically punishing the queen may not be a very good option, but this still does not explain why workers do not lay eggs when the queen is not present. A third cost, the cost of replacing female with male eggs, may help to explain this (Ratnieks & Reeve 1992; Foster & Ratnieks 2001). If workers replacing queen-laid eggs do not distinguish between female and male eggs (which is the case for the stingless bee *Paratrigona subnuda*, Tóth et al. 2002a), they would be replacing many eggs destined to be workers and depleting the future work force.

Why Is POP Not More Aggressive During Male Production?

The results of the behavioural data do not support the theoretical expectation that conflict between workers and queen would be higher during male production. One possibility is that such an effect might have been counteracted by an opposing effect of increased food during male production. If more workers have larval food in their stomachs, the oviposition process may become more efficient and thus shorter. If so, this suggests that the effects of conflict over male production are not very strong.

Another possible explanation is that aggressive conflict might be adaptive in both periods. Workers with developed ovaries may be a threat all the time. Some workers also have ovarian development during the period when no males are produced, so their egg laying should be prevented by the queen.

However, we now know one important fact that was unknown at the time the prediction was made: workers in this species produce no males. The lack of elevated agonistic interactions during male production could most simply be explained by the fact that workers have lost or given up the contest for male progeny in this species. It would be interesting to repeat this study in a species where workers do produce males.

If there is no conflict in *S. q. quadripunctata*, why do worker–queen interactions appear to be aggressive? Perhaps the interactions between workers and the queen during the POP are a holdover from past evolutionary conflict (Crespi 1992; Zucchi 1993). The aggressive POP behaviours could have been retained as vestigial or nonfunctional traits. However, the behaviours might also have acquired new functions. For example, the queen's eating of nonviable trophic worker eggs (Sakagami 1982) could have derived from eating viable worker eggs (Crespi 1992). Similarly, the darting and lunging behaviours that precede egg laying might now serve primarily for coordination between the workers who provision and the queen who lays the eggs (Sakagami at al. 1965; Sommeijer et al. 1984; Zucchi 1993), but they may have originally evolved in the context of conflict. It is unlikely but not impossible that queen–worker interactions are not aggressive, even if they seem so to humans.

Male Production in Other Species

Although, on relatedness grounds, stingless bee workers should produce at least part of the males, in some species (*Frieseomelitta varia*, *Trigona minangkabau*), workers have no ovarian development and are thus not able to produce eggs (Terada 1974; Cunha et al. 1986; Suka & Inoue 1993). Behavioural studies show that in some other species (*Tetragonisca angustula*, *Trigona pauperea pauperea*, *Tetragona dorsalis*, *Leurotrigona muelleri*), workers have developed ovaries but still do not produce males in queenright colonies (Sakagami & Zucchi 1974; Sommeijer et al. 1984; Inoue et al. 1999; Grosso et al. 2000). However, because oviposition might be very cryptic and overlooked by observers, or because workers might police each other and eat any eggs workers lay, the addition of genetic data is desirable. Our study of *S. q. quadripunctata* is the first one where genetic markers confirm behavioural observations that workers do not produce males.

Although there is worker male production in some species of stingless bees (Beig 1972; Contel & Kerr 1976; Machado et al. 1984; Sommeijer et al. 1984; Inoue & Roubik 1990; van Benthem et al. 1995; Inoue et al. 1999; Koedam et al. 1999; Drumond et al. 2000; Tóth et al. 2002a, b), our finding for *S. q. quadripunctata* is relatively typical for social insects in general. Most social insects appear to have singly mated queens (Owen & Plowright 1982; Bourke 1988; Boomsma & Ratnieks 1996; Arévalo et al. 1998; Walin et al. 1998; Foster et al. 2000, 2001; Strassmann 2001), yet most social insects appear to have queen-produced males (Bourke 1988; Choe 1988; Arévalo et al. 1998; Walin et al. 1998; Foster et al. 2000, 2001). Thus, the prediction that singly mated species should have worker-produced males may fail quite broadly. It therefore becomes an important question to determine the cause and whether it involves fitness costs or queen suppression of workers. Comparative studies are needed between species where workers do and do not contribute to male production. Stingless bees provide species of both types and are thus are an excellent group for further study.

Acknowledgments

We are especially thankful to Eduardo Tadeu Mattos and a friendly anonymous man for helping us to find and dig up the colonies and to Denise de Araujo Alves for collecting some of the samples. This research was funded by NSF grant IBN-9808809 (to J.E.S. and D.C.Q.) and the Brazilian FAPESP grant 96/11093-4 (to V.L.I.-F.) and by a Wray–Todd fellowship (to E.T.). Work in Brazil was conducted under permit 237 from the Brazilian Ministry of Science and Technology.

References

Arévalo, E., Strassmann, J. E. & Queller, D. C. 1998. Conflicts of interests in social insects: male production in two species of *Polistes*. *Evolution*, **53**, 797–805.

Bego, L. R. 1982. On social regulation in *Nannotrigona* (*Scaptotrigona*) *postica* Latreille, with special reference to male production cycles (Hym., Apidae, Meliponinae). *Boletim Zoologica de Universidade de São Paulo*, **7**, 181–196.

Bego, L. R. 1990. Social regulation in *Nannotrigona* (*Scaptotrigona*) *postica* Lat., with special reference to productivity of colonies (Hymenoptera, Apidae, Meliponinae). *Revista Brasileira Entomolica*, **39**, 721–739.

Beig, D. 1972. The production of males in queenright colonies of *Trigona* (*Scaptotrigona*) *postica*. *Journal of Apicultural Research*, **11**, 33–39.

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

Boomsma, J. J. & Ratnieks, F. L. W. 1996. Paternity in social Hymenoptera. *Proceedings of the Royal Society of London, Series B*, **351**, 941–975.

Bourke, F. G. A. 1988. Worker reproduction in the higher eusocial Hymenoptera. *Quarterly Review of Biology*, **63**, 291–311.

Bourke, F. G. A. & Franks, N. R. 1995. *Social Evolution in Ants*. Princeton, New Jersey: Princeton University Press.

Bourke, F. G. A. & Ratnieks, F. L. W. 1999. Kin conflict over caste determination in social Hymenoptera. *Behavioral Ecology and Sociobiology*, **46**, 287–297.

Camargo, C. A. 1972. Mating of the social bee *Melipona quadrifasciata* under controlled conditions (Hymenoptera, Apidae). *Journal of the Kansas Entomological Society*, **45**, 520–523.

Camargo, J. M. F. 1974. Notas sobre a morfologia e biologia de *Plebeia* (*Schwarziana*) *quadripunctata quadripunctata* (Hymenoptera, Apidae, Meliponinae). *Studia Entomologi*, **17**, 433–470.

Choe, J. C. 1988. Worker reproduction and social evolution in ants (Hymenoptera: Formicidae). In: *Advances in Myrmecology* (Ed. by J. C. Trager), pp. 163–187. Leiden: E. J. Brill.

Cole, B. J. 1986. The social behavior of *Leptothorax allardei* (Hymenoptera, Formicidae): time budgets and the evolution of worker reproduction. *Behavioral Ecology and Sociobiology*, **18**, 165–173.

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

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

Cunha, M. A. S., Gomes, G. M. & Oliveira Campos, L. A. 1986. Deselvolviamneto ovariano enm operarias adultas de *Frieseomelita silvestri languida* (Hymenoptera, Apidae) sob condiciones normais e de fandade. *Ciencia e Cultura*, **38**, 1725–1731.

Drumond, P. M., Zucchi, R., Mateus, S., Bego, L. R. & Yamahe, S. 1996. Oviposition behavior of the stingless bees. XVII. *Plebeia* (*Plebeia*) *droryana* and ethological comparision with other Meliponinae taxa (Hymenoptera, Apidae). *Japanese Journal of Entomology*, **64**, 385–400.

Drumond, P. M., Olroyd, B. P., Dollin, A. E. & Dollin, L. J. 1999. Oviposition behavior of two Australian stingless bees, *Austroplebeia symei* Rayment and *Austroplebeia australis* Friese (Hymenoptera: Apidae: Meliponini). *Australian Journal of Entomology*, **38**, 234–241.

Drumond, P. M., Olroyd, B. P. & Osborne, K. 2000. Worker reproduction in *Austroplebeia australis* Friese (Hymenoptera, Apidae, Meliponini). *Insectes Sociaux*, **47**, 333–336.

Engels, W. & Imperatriz-Fonseca, V. L. 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* (Ed. by W. Engels), pp. 166–230. Berlin: Springer-Verlag.

Fletcher, D. J. C. & Ross, K. G. 1985. Regulation of reproduction in eusocial Hymenoptera. *Annual Review of Entomology*, **30,** 319–343.

Foster, K. R. & Ratnieks, F. L. W. 2001. The effect of sex allocation biasing on the evolution of worker policing in hymenopteran societies. *American Naturalist*, **158,** 615–623.

Foster, K. R., Ratnieks, F. L. W. & Raybould, A. F. 2000. Do hornets have zombie workers? *Molecular Ecology*, **9,** 735–742.

Foster, K. R., Ratnieks, F. L. W., Gyllenstrand, N. & Thorén, P. A. 2001. Colony kin structure and male production in *Dolichovespula* wasps. *Molecular Ecology*, **10,** 1003–1010.

Goodnight, K. F. & Queller, D. C. 2000. *Relatedness 5.07*. http://Gsoft.smu.edu?Gsoft.html.

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

Hamilton, W. D. 1964. The genetical evolution of social behaviour, I, II. *Journal of Theoretical Biology*, **7,** 1–52.

Hamilton, W. D. 1972. Altruism and related phenomena, mainly in social insects. *Annual Review of Ecology and Systematics*, **3,** 193–232.

Hastings, M. D., Queller, D. C., Eischen, F. & Strassmann, J. E. 1998. Kin selection, relatedness, and worker control of reproduction in a large-colony epiponine wasp, *Brachygastra mellifica*. *Behavioral Ecology*, **9,** 573–581.

Imperatriz-Fonseca, V. L. 1973. Miscellaneaous observations on the behaviour of *Schwarziana quadripunctata* (Hym., Apidae, Meliponinae). *Boletim de Zoologia e Biologia Marina, N.S.*, **30,** 633–640.

Imperatriz-Fonseca, V. L. & Zucchi, R. 1995. Virgin queens in stingless bee (Apidae, Meliponinae) colonies: a review. *Apidology*, **26,** 231–241.

Inoue, T. & Roubik, D. W. 1990. Kin recognition in the stingless bee, *Melipona fasciata.* In: *Social Insects and the Environment* (Ed. by G. K. Veeresh, B. Mallik & C. A. Viraktamath), pp. 517–518. New Delhi: Oxford University Press.

Inoue, T., Roubik, D. W. & Suka, T. 1999. Nestmate recognition in the stingless bee *Melipona panamica* (Apidae, Meliponinae). *Insectes Sociaux*, **46,** 208–218.

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

Kikuta, N. & Kazuki, T. 1999. Queen and worker policing in the monogynous and monandrous ant, *Diacamma* sp. *Behavioral Ecology and Sociobiology*, **46,** 180–189.

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

Machado, M. F. P. S., Contel, E. P. B. & Kerr, W. E. 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. 1974. *The Social Behavior of the Bees: a Comparative Study*. Cambridge, Massachusetts: Harvard University Press.

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

Nogeira-Ferreira, F. H., Zucchi, R., da Silva-Matos, E. V. & Sakagami, S. F. 1998. Oviposition behavior of the stingless bees XXIII. *Schwarziana quadripunctata* with comments on the ethological diversity among representatives of the Plebeia complex (Hymenoptera, Apidae, Meliponinae). *Entomological Science*, **1,** 511–521.

Oldroyd, B. P., Halling, L. A., Good, G., Wattanchaiyingcharoen, W., Barron, A. B., Nanork, P., Wongsiri, S. & Ratnieks, F. L. W. 2001. Worker policing and worker reproduction in *Apis cerana*. *Behavioral Ecology and Sociobiology*, **50,** 371–377.

Oliveira Campos de Melo, G. A. R. 1990. Physogastric queen mating in *Melipona quadrifasciata*, lep. (Hymenoptera, Apidae). *Revista Brasileira de Genetica*, **13,** 491–508.

Owen, R. E. & Plowright, R. C. 1982. Worker–queen conflict and male parentage in bumble bees. *Behavioral Ecology and Sociobiology*, **11,** 94–99.

Peters, J. M., Queller, D. C., Imperatriz-Fonseca, V. L. & Strassmann, J. E. 1998. Microsattelite loci for stingless bees. *Molecular Ecology*, **7,** 783–792.

Peters, J. M., Queller, D. C., Imperatriz-Fonseca, V. L., Roubik, D. W. & Strassmann, J. E. 1999. Mate number, kin selection, and social conflicts in stingless bees and honey bees. *Proceeding of the Royal Society of London, Series B*, **266,** 379–384.

Pollock, G. B. 1996. Kin selection, kin avoidance and correlated strategies. *Evolutionary Ecology*, **10,** 29–43.

Queller, D. C. 1994. A method for detecting kin discrimination within natural colonies of social insects. *Animal Behaviour*, **47,** 569–576.

Queller, D. C. & Goodnight, K. F. 1989. Estimating relatedness using genetic markers. *Evolution*, **43,** 258–275.

Queller, D. C. & Strassmann, J. E. 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. *American Naturalist*, **132,** 217–236.

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

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

Sakagami, S. F. 1954. Occurrence of an aggressive behavior in queenless hives, with consideration on the social organization of honeybee. *Insectes Sociaux*, **4,** 231–243.

Sakagami, S. F. 1966. Techniques for the observation of behavior and social organization of stingless bees by using a special hive. *Deartmento Zoologico de Secretario Agricultura de São Paulo*, **19,** 152–162.

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

Sakagami, S. F. & Zucchi, R. 1963. Oviposition process in a stingless bee, *Trigona* (*Scaptotrigona*) *postica* Latr. (Hym.). *Studia Entomologia*, **6,** 497–510.

Sakagami, S. F. & Zucchi, R. 1974. Oviposition behavior of two dwarf stingless bees, *Hypotrigona* (*Leurotrigona*) *muelleri* and *H.* (*trigonisca*) *duckei*, with notes on the temporal articulation of oviposition process in stingless bees. *Journal of the Faculty of Science, Hokkaido University, Series VI, Zoology*, **19,** 361–420.

Sakagami, S. F., Beig, D., Zucchi, R. & Akahira, Y. 1963. Occurrence of ovary-developed workers in queen-right colonies of stingless bees. *Revista Brasileira de Biologia*, **23,** 115–129.

Sakagami, S. F., Montenegro, M. J. & Kerr, W. E. 1965. Behavior studies of the stingless bee, with specieal reference to the oviposition process. V. *Melipona quadrifasciata anthioides* Lepentier. *Journal of the Faculty of Science, Hokkaido University, Series VI, Zoology*, **15,** 578–607.

SAS Institute 1990. *SAS/STAT User's Guide. Release 6*. 4th edn. Cary, North Carolina: SAS Institute.

Schwarz, F. H. 1948. Stingless bees (Meliponidae) of the western hemisphere. *Bulletin of the American Museum of Natural History*, **90,** I–XVIII, 1–546.

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

Starr, C. K. 1984. Sperm competition, kinship, and sociality in the aculeate Hymenoptera. In: *Sperm Competition and the Evolution of Animal Mating Systems* (Ed. by R. L. Smith), pp. 427–464. Orlando, Florida: Academic Press.

Strassmann, J. E. 2001. The rarity of multiple mating by females in the social Hymenoptera. *Insectes Sociaux*, **48,** 1–13.

Strassmann, J. E., Solis, C. R., Peters, J. M. & Queller, D. C. 1996. Strategies for finding and using polymorphic DNA microsatellite loci for studies of genetic relatedness and pedigrees. In: *Molecular Zoology: Advances, Strategies and Protocols* (Ed. by J. D. Ferraris & S. R. Palumbi), pp. 163–180, 528–549. New York: Wiley–Liss.

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

Terada, Y. 1974. Contribuicao ao estudo da regulacao social em *Leurotrigona muelleri* e *Frieseomlitta varia* (Hymenoptera, Apidae). M.Sc. thesis, University of São Paulo.

Tóth, E., Queller, D. C., Imperatriz-Fonseca, V. L. & Strassmann, J. E. 2002a. Genetic and behavioral conflict over male production between workers and queens in the stingless bee *Paratrigona subnuda*. *Behavioral Ecology and Sociobiology*, **53,** 1–8.

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

Trivers, R. L. & Hare, H. 1976. Haplodiploidy and the evolution of the social insects. *Science*, **191,** 249–263.

Visscher, R. K. & Dukas, R. 1995. Honey bees recognize development of nesmates' ovaries. *Animal Behaviour*, **49,** 542–544.

Walin, L., Sundström, L., Seppä, P. & Rosengren, R. 1998. Worker reproduction in ants: a genetic analysis. *Heredity*, **81,** 604–612.

Wilson, E. O. 1971. *Insect Societies*. Cambridge, Massachusetts: Harvard University Press.

Winston, M. L. 1987. *The Biology of the Honey Bee*. Cambridge, Massachusetts: Harvard University Press.

Woyciechowski, M. & Lomnicki, A. 1987. Multiple mating of queens and the sterility of workers among eusocial hymenoptera. *Journal of Theoretical Biology*, **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, Comparative Sociology of Bees, Wasps and Ants* (Ed. by T. Inoue & S. Yamahe), pp. 207–249. Tokyo: Hakuhinsha.