

Mate number, kin selection and social conflicts in stingless bees and honeybees

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Microsatellite genotyping of workers from 13 species (ten genera) of stingless bees shows that genetic relatedness is very high. Workers are usually daughters of a single, singly mated queen. This observation, coupled with the multiple mating of honeybee queens, permits kin selection theory to account for many differences in the social biology of the two taxa. First, in contrast to honeybees, where workers are predicted to and do police each other's male production, stingless bee workers are predicted to compete directly with the queen for rights to produce males. This leads to behavioural and reproductive conflict during oviposition. Second, the risk that a daughter queen will attack the mother queen is higher in honeybees, as is the cost of such an attack to workers. This explains why stingless bees commonly have virgin queens in the nest, but honeybees do not. It also explains why in honeybees the mother queen leaves to found a new nest, while in stingless bees it is the daughter queen who leaves.

Keywords: kin selection; mate number; social conflict; microsatellite; *Apis*; Meliponini

1. INTRODUCTION

If honeybees (*Apis*) are one of the pinnacles of social evolution, the stingless bees (Meliponini) occupy a twin peak. Stingless bees also belong to the family Apidae, but far exceed honeybees in diversity and native distribution (hundreds of species versus under ten; pan-tropical versus Old World; Roubik 1989; Camargo & Pedro 1992; Otis 1996). Both are classified as highly eusocial, with large perennial colonies, morphologically distinct worker and queen castes and an intricate division of labour and recruitment to food sources. Both normally have a single egg-laying queen and reproduce by division of the colony between the mother queen and a daughter (swarming) (Kerr 1969; Sakagami 1982; Roubik 1989; Engels & Imperatriz-Fonseca 1990). However, they also have some striking and puzzling social differences with respect to worker oviposition, which eats worker-laid eggs, whether workers produce male offspring, oviposition rituals, tolerance of new queens and which queen leaves with the swarm (table 1). Here we suggest that all of these differences stem from a difference in mate number via kin-selected (Hamilton 1964*a,b*; Crozier & Pamilo 1996; Queller & Strassmann 1998) differences in worker tolerance of new queens and of the male progeny of other workers. Our argument takes three parts. First, we show that single mating is the rule in stingless bees, in contrast to the well-known multiple mating of honeybee queens (Estoup *et al.* 1994; Oldroyd *et al.* 1997). Second, we use

worker policing theory (Starr 1984; Woyciechowski & Lomnicki 1987; Ratnieks 1988) to account for a number of social differences surrounding who produces the male eggs. Third, we develop kin selection models of how mate number should affect tolerance of daughter queens and use these results to explain the remaining social differences in table 1.

2. SINGLE MATING IN STINGLESS BEES

Single mating has been suspected for stingless bees on the basis of sperm counts (Kerr *et al.* 1962) and mating plugs (da Silva *et al.* 1972) but neither of these provides conclusive evidence (Boomsma & Ratnieks 1996). Allozyme and colour marker studies provide better evidence for several species (Contel & Kerr 1976; Falcão 1984; Machado *et al.* 1984). However, because the social traits summarized in table 1 are widespread in stingless bees, they can be explained by single mating only if it too is pervasive. Therefore, we investigated the genetic structure of 13 species (ten genera) collected from Brazil and Panama.

Brazilian samples were collected by V.L.I.-F., D.C.Q. and J.E.S. Samples of young workers that had not yet left the nest were taken from hives of captive, free-foraging colonies. We chose young workers because colonies that are artificially close together might exchange older foragers. Panamanian samples of worker bees were collected from nest entrances of wild colonies by D.W.R.

Microsatellite genotyping of workers was carried out using standard techniques (Strassmann *et al.* 1996)

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Table 1. *Social differences between honeybees and stingless bees*
(References are given in the text.)

trait	honeybees	stingless bees
worker oviposition	rare	common
eats worker-laid eggs	other workers	usually queen
produces males in queenright colonies	queens	sometimes workers
ritualized conflict over oviposition	no	yes
worker and male cells same size	yes	no
reserve of adult daughter queens	no	yes
leaves nest during swarming	mother queen	daughter queen

employing PCR primers derived from a *Melipona bicolor* library (Peters *et al.* 1998). Depending on the species, two to six polymorphic microsatellite loci were genotyped, from the original 24 loci identified in *M. bicolor*. We used the Macintosh computer program, Relatedness 4.2c (<http://www.rice.edu/wasps>) to estimate genetic relatedness, inbreeding and the standard errors (by jackknifing over colonies) by the methods of Queller & Goodnight (1989).

Under single mating, workers will be full sisters which are three-quarters related, while multiple mating will produce half sisters which are one quarter-related (in the haplodiploid genetic system, all sisters share half their mother's diploid genome and, in addition, full sisters share their father's entire haploid genome; Hamilton 1964*b*). For the 12 single-queen species (*M. bicolor* is exceptional in sometimes having multiple laying queens; Nogueira-Neto 1970), relatedness among workers from the same colony averaged 0.739, very close to the 0.75 expected under single mating (table 2). All 95% confidence intervals of the single-queen species included 0.75 (figure 1).

In six out of the 12 single-queen species, all worker genotypes were consistent with a single, once-mated queen because, at each locus, they all shared a common allele (from the haploid father) and the other allele had no more than two forms (from the diploid mother). The small number of anomalies in the remaining species (table 2) could arise from null alleles, accidental inclusion of males in the sample, recent queen changes, workers drifting among nests, continuing connections between mother and daughter nests or multiple mating. In *M. bicolor*, the many anomalies were presumably due to the multiple queens. Whatever the cause, the effect on average relatedness is small and the kin selection arguments that follow are not qualitatively affected.

3. MALE PRODUCTION AND WORKER POLICING

Mate number affects relatedness and relatedness affects kin selection in ways that can explain the striking social differences between stingless bees and honeybees, as summarized in table 1. One effect is on collective worker

preferences for which should lay the unfertilized haploid eggs that will develop into males (Starr 1984; Woyciechowski & Lomnicki 1987; Ratnieks 1988). Under either mating system, each worker will maximize her gene transmission by substituting her own sons ($r=0.5$) for the queen's sons ($r=0.25$). However, under multiple mating, such behaviour should be suppressed by other workers, because they are more related to the queen's sons ($r=0.25$) than to the sons of half-sister workers ($r=0.125$). As predicted, honeybee workers police each other; the small fraction of male eggs laid by workers are eaten by other workers, so nearly all adult males are sons of the queen (Ratnieks & Visscher 1989; Visscher 1996). Under single mating, there should be no such suppression, because workers are more related to nephews than to the queen's sons ($r=0.375$ versus $r=0.25$) (Ratnieks 1988). Therefore, workers should be in direct competition with the queen.

By confirming that stingless bees are singly mated, our results support the view (Ratnieks 1988; Crespi 1992) that lack of worker policing will lead to disharmony between queen and workers in this group. The predicted reproductive competition may explain why worker laying is very common (though it is not universal) in stingless bees (Sakagami 1982; Engels & Imperatriz-Fonseca 1990). It is also consistent with the fact that worker-laid eggs are not generally eaten by other workers, but are often eaten by queens (Zucchi 1993). Worker-laid eggs are sometimes specialized as trophic eggs, possibly reflecting a history of reproductive conflict lost by workers (Crespi 1992), but often they are viable (Zucchi 1993). Workers can successfully reproduce, for example by laying an egg after the queen has oviposited, after which the worker's male larva kills its cell mate (Beig 1972).

The fraction of males produced by workers versus queens has been studied in only a few species and the worker fraction ranges from very low to very high (Beig 1972; Contel & Kerr 1976; Camillo-Atique 1977; Machado *et al.* 1984; Sommeijer & van Buren 1992; Inoue *et al.* 1998). While the winner of the conflict over male production remains to be discovered for most species, behavioural evidence for queen-worker conflict is pervasive. The cell provisioning and oviposition process is an elaborate and ritualized interaction between workers and queens (Kerr 1969; Sakagami 1982; Roubik 1989; Engels & Imperatriz-Fonseca 1990; Crespi 1992; Zucchi 1993), unlike anything in honeybees. It is often described in terms of conflict. Besides the worker egg laying and eating of eggs by the queen, in various species it includes acts of ritualized aggression such as workers darting at the queen with open mandibles, workers being violently tapped or seized by queens, mutual pushing, workers ignoring food solicitation by the queen and ritualized avoidance, and escape behaviours. The conflict seems rather stereotypical within species but is highly variable between species (Kerr 1969; Sakagami 1982; Engels & Imperatriz-Fonseca 1990; Zucchi 1993), exactly what one expects of an evolutionary arms race with many possible strategies and counter-strategies (Crespi 1992).

The active conflict in stingless bees may also explain why they rear workers and males in identical cells, because male eggs laid in identifiable cells would be more easily removed by the opposing party. In honeybees,

Table 2. Genetic relatedness among workers in colonies of stingless bees

(Standard errors (s.e.) are estimated by jackknifing over colonies. Inbreeding coefficients averaged 0.070 and only *P. subnuda* ($f=0.175$) was significantly greater than zero.)

species	relatedness	s.e.	no. of worker genotypes (no. of colonies)	no. of anomalous worker genotypes (no. of colonies)	no. of loci (maximum no. of alleles per locus)	collection location
<i>Scaptotrigona postica</i>	0.855	0.137	32 (4)	0 (0)	4 (5)	São Paulo, Brazil
<i>Scaptotrigona barrocoloradensis</i>	0.841	0.200	32 (4)	0 (0)	4 (8)	Panama
<i>Melipona quadrifasciata</i>	0.828	0.050	32 (4)	0 (0)	4 (4)	São Paulo, Brazil
<i>Partamona near cupira</i>	0.800	0.035	94 (12)	0 (0)	2 (12)	Panama
<i>Schwarziana quadripunctata</i>	0.792	0.072	32 (4)	0 (0)	3 (5)	São Paulo, Brazil
<i>Melipona panamica</i>	0.774	0.052	94 (9)	0 (0)	5 (7)	Panama
<i>Tetragona clavipes</i>	0.745	0.138	63 (7)	4 (2)	3 (4)	São Paulo, Brazil
<i>Trigona fulviventris</i>	0.683	0.068	64 (7)	2 (1)	4 (7)	Panama
<i>Nannotrigona perilampoides</i>	0.669	0.112	47 (7)	3 (1)	3 (4)	Panama
<i>Lestrimellita limão</i>	0.657	— ^a	24 (2)	0 (0)	2 (3)	Panama
<i>Paratrigona subnuda</i>	0.616	0.110	47 (5)	7 (2)	5 (7)	São Paulo, Brazil
<i>Melipona bicolor</i>	0.615	0.049	96 (11)	20 (8)	6 (7)	São Paulo, Brazil
<i>Plebeia near minima</i>	0.602	0.067	67 (5)	3 (3)	4 (6)	Panama

^a Small sample sizes precluded standard error estimation.

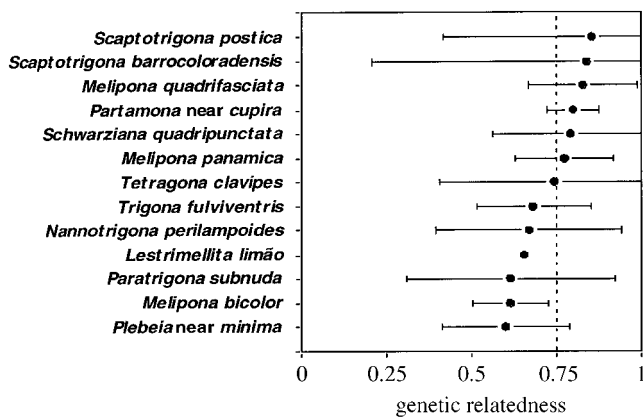


Figure 1. Average relatedness and 95% confidence intervals for worker colony mates in stingless bees.

where workers agree that queens should lay the male eggs, there is no such barrier to differentiation, and drone cells are larger than worker cells.

4. TOLERANCE OF NEW QUEENS

Mate number also affects fitness trade-offs between mother and daughter queens. Such trade-offs arise in the question of whether the old queen should be replaced and also in how colony resources should be divided between queens at the time of swarming. The mother queen, daughter queen and workers are predicted to be selected differently with respect to these issues (Visscher 1993; Crozier & Pamilo 1996), because they are differently related to the progeny of the two queens. According to Hamilton's (1964*a,b*) rule, a party will be selected to favour a benefit (b) to a daughter queen at a cost (c) to the mother queen when $b/c > r_M/r_D$, where r_M and r_D are that party's relatednesses to the progeny produced under the mother and daughter queens, respectively. Thus, each party may favour either the new queen or the old queen

depending on the threshold benefit:cost ratio determined by that party's relatednesses. The threshold, when plotted in benefit–cost space, is a straight line of slope r_M/r_D , with the daughter queen favoured above the line and the mother queen below the line (figure 2). Conflicts are therefore predicted in the regions between these indifference lines.

Consider how these arguments affect tolerance of virgin (not yet reproductive) queens in the colony. Such tolerance would be advantageous in allowing rapid replacement of a mother queen that dies, but tolerating daughter queens may also entail a threat. A daughter queen will always be selected to replace the old queen before the old queen is selected to yield (figure 2, M and D benefit:cost lines). She might therefore attack the old queen, but this risk differs with mate number.

Under the honeybee system of multiple mating, there is a large b/c space where the daughter queen is a potential threat. Worker interests coincide with the mother queen's interests (because both are twice as related to the mother queen's progeny as to the daughter queen's progeny) (figure 2*a*). Under single mating, two things change. First, there is a smaller region of conflict between the mother and daughter queens (figure 2*b,c*; because of the daughter queen's increased relatedness to the mother queen's progeny). Second, if males are produced by queens, worker interests shift somewhat away from the mother queen's interests in the direction of the daughter queen's interests (figure 2*c*; because of their higher relatedness to the sons of the daughter queen). The net result is a much narrower set of conditions under which a daughter queen should attempt to overthrow the mother queen against the workers' interests (shaded areas in figure 2*b,c*).

Therefore, daughter queens represent a serious threat to worker interests in honeybees and only a modest threat in stingless bees, consistent with the fact that stingless bees often keep a reserve of adult virgin queens (Kerr

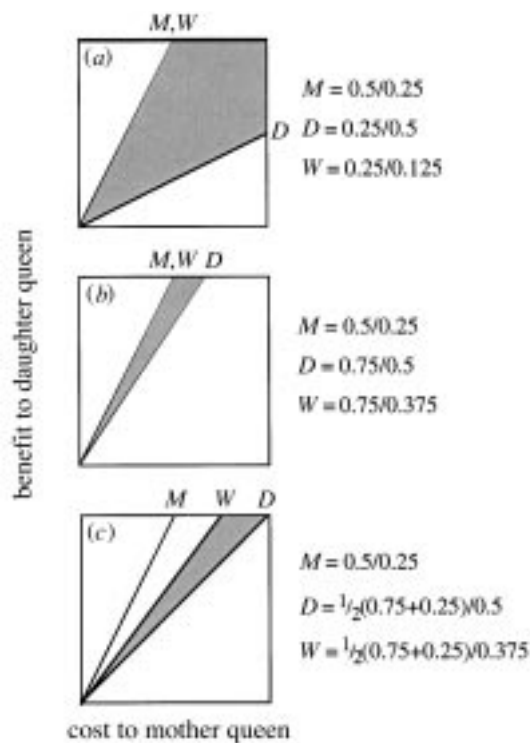


Figure 2. Conflicts of interest over behaviours that would cost the mother queen c progeny in return for one of her daughter queens obtaining b progeny. (a) Extreme multiple mating, where all sisters have different fathers, approximating the honeybee case. (b) Single mating with worker-produced males. (c) Single mating with queen-produced males.

'Progeny' are daughters and either sons (under queen laying) or grandsons (under worker laying). The lines show combinations of c and b where a particular party (M for mother queen, D for daughter queen and W for worker) is indifferent to the exchange. A party gains inclusive fitness above its line and loses below its line, so conflicts of interest occur in the regions between lines. The shaded regions show where daughter queens favour an exchange that workers oppose. The indifference lines are determined by the relatedness ratios r_M/r_D (see text). The ratios for each party are shown to the right of each graph (these are regression relatednesses multiplied by sex-specific reproductive value; see Crozier & Pamilo (1996)). When relatedness differs to female and male progeny (D and W in c), they are shown in that order and averaged (averaging is correct when all colonies have the same sex investment allocation; workers with a replacement queen could gain by rearing more males, but it can be shown that this further narrows the shaded conflict zone).

1969; Sakagami 1982; Engels & Imperatriz-Fonseca 1990; Imperatriz-Fonseca & Zucchi 1995), while honeybees never do. Even the very modest threat in stingless bees results in worker strategies for controlling daughter queens, for example imprisoning them in storage pots or special chambers (Imperatriz-Fonseca & Zucchi 1995). Ultimately, most virgin queens are not needed and are killed by the workers (Imperatriz-Fonseca & Zucchi 1995).

The much greater threat to honeybee worker interests posed by a daughter queen (shaded area in figure 2a) is defused by an avoidance strategy. First, there is no reserve of adult queens in case a replacement is needed. Second, even when a new queen is needed during colony division,

contact between the old and new queens is avoided. During swarming, it is the mother queen that leaves to found a new colony before the daughter queen has emerged from her cell (Visscher 1993). It seems counter-intuitive that the mother honeybee queen would abandon the valuable nest resource given that she values herself more than she values her daughter. However, it is acceptable to the mother queen because the workers share her interests (figure 2a) so she can count on them to apportion the colony's resources (particularly themselves) exactly as she would (figure 2a). This expectation is supported by the fact that most of the workers in *Apis mellifera* and disproportionately more of the valuable younger workers depart with their mother (Winston 1987). In contrast, in the stingless bee *Trigona laeviceps*, only 30% of the workers joined the daughter queen in the new colony and these were mostly older workers (Inoue *et al.* 1984).

The honeybee conflict cannot be defused by the alternative of the daughter queen leaving. From the daughter queen's point of view, the workers' division of resources would be unfavourable, making it tempting to kill the mother queen or to try to stay peacefully and usurp part of the mother colony's output.

5. DISCUSSION

Our data suggest that single mating is very widespread in the stingless bees, so that it can plausibly serve as the basis for kin selection explanations of their differences from multiply mated honeybees. Since we tested only one age cohort of workers, our results could also be consistent with extreme clumping of sperm from multiple mates. However, such extreme sperm clumping has never been reported in social insects and it is negligible in honeybees (Estoup *et al.* 1994).

This is not to say that single mating is universal. There were individual genotypes in our data seemingly inconsistent with single mating, though they might have been due to other factors, such as a recent queen turnover. However, in the context of the kin selection arguments, it is not necessary to establish that single mating is absolute, only that sister relatednesses are high and close to 0.75. It should be noted that several of the confidence intervals also include 0.5. This value, if it were the true mean relatedness, could arise from double mating and equal sperm use. We assessed the probability that one or more true means lie below 0.5 by drawing 5000 replicates of all species means, each from its sampled t -distribution. The majority of replicates had no species means under 0.5 and only 1% had more than two species in that range. Thus, our conclusion that worker relatedness is high in most species appears robust. Of course, whether multiple mating might occur elsewhere in the stingless bees is open to further study.

Visscher's (1993) kin selection model of honeybee swarming gave results essentially like our figure 2a. He argued that the delay before a daughter queen mates and begins reproducing may make the natal nest more than twice as valuable to her as to the mother queen, so that all parties may favour the daughter queen staying. However, this cost of delay could be largely avoided if daughter queens were simply raised earlier and allowed to

mature before leaving. We suggest that this does not happen because of the likelihood of queen fights and also note that the favourable division of resources by the workers makes it attractive for the mother queen to leave. We also show how the reduced potential for conflict in stingless bees allows for the opposite result.

Besides the relatedness factor, there are several constraints that might affect the difference in tolerance of daughter queens. An obvious one is that stingless bee daughter queens lack a sting and might, for that reason, be less of a threat to the old queen. However, stingless bees can certainly kill each other, if not as quickly as honeybees, and it is not clear which is the greater threat. A fight between stingless bee queens might be more costly if the risk of mutual injury is greater due to the lack of a quick-killing sting.

A difference in provisioning mode has also been suggested as an explanation for the presence or absence of daughter queens (Engels & Imperatriz-Fonseca 1990; Imperatriz-Fonseca & Zucchi 1995). Honeybees can alter the provisioning of a worker larva to make it develop into a queen if the old queen dies (Winston 1987). Stingless bees, in contrast, mass provision cells before oviposition. They may therefore be unable to initiate new queens when needed and may rely instead on reserves of adult queens. However, there are indications of ways in which selection could break this constraint. In some species, microqueens can emerge from worker cells, while in others, a worker larva can become a queen by chewing into another cell to gain extra food (Engels & Imperatriz-Fonseca 1990).

A final constraint is that stingless bee queens become physogastric and too heavy to fly, so the mother queen cannot leave. However, the causal path might well be the reverse. Physogastry might have evolved in stingless bees, but not honeybees, because stingless bee queens never leave the nest and honeybee queens do. If this is so, physogastry could be added to the list of differences explained by mate number in table 1.

Even if stingless bees are constrained by mass provisioning and physogastry, honeybees are not constrained in either way and our theory predicts honeybee behaviours that are otherwise difficult to explain. Why do honeybees not keep adult queens in reserve when the alternative of replacing a dead queen by converting a very young worker larva entails a cost of several weeks loss of production? It could be that the cost of the loss of production, which, to our knowledge, has never been measured, is not too serious. However, we suggest that it is because reserve queens impose a greater cost, arising from the danger that they will usurp the mother queen. Similarly, honeybee queens are not physogastric, so the mother could either stay or leave during swarming. So why does she make the extraordinary decision to leave, unless it is because this allows her to avoid potentially dangerous conflict while still obtaining her desired share of colony resources?

The third group of eusocial apids, bumblebees (*Bombus*), also seems to fit the theory. Bumble bees, like stingless bees, are normally singly mated (Estoup *et al.* 1995), are noted for queen-worker conflict (including killing of queens; Bourke 1994) and males are sometimes produced by workers (Crozier & Pamilo 1996). Since new

Bombus colonies are initiated by independent queens rather than by swarms, they cannot be compared for the other behaviours in table 1.

The far-reaching consequences of mate number in bees demonstrate the importance of relatedness and the power of kin selection. They also reinforce the conclusion from sex ratio studies (Crozier & Pamilo 1996; Queller & Strassmann 1998) that non-reproductive workers are not just feckless followers of the queens; they are independent evolutionary actors the distinct interests of which can powerfully mould the character of insect societies. Finally, they highlight the difference between potential and actual conflict (Ratnieks & Reeve 1992). Low relatedness creates the potential for conflicts, but in this case the actual conflicts are fewer in the low-relatedness honeybees because they have evolved conflict-reducing devices such as worker policing of male production and the departure of the mother queen.

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