The queen is dead—long live the workers: intraspecific parasitism by workers in the stingless bee *Melipona scutellaris*

D. A. ALVES,* V. L. IMPERATRIZ-FONSECA,* T. M. FRANCOY,† P. S. SANTOS-FILHO,*
P. NOGUEIRA-NETO,* J. BILLEN‡ and T. WENSELEERS‡

*Bee Laboratory, Bioscience Institute, University of São Paulo, Rua do Matão Trav. 14, 321, 05508-900 Sao Paulo, Brazil,
†School of Arts, Sciences and Humanities, University of São Paulo, Rua Arlindo Béttio 1000, 03828-000 Sao Paulo, Brazil,
‡Laboratory of Entomology, Zoological Institute, Catholic University of Leuven, Naamsestraat 59, B-3000 Leuven, Belgium

Abstract

Insect societies are well known for their high degree of cooperation, but their colonies can potentially be exploited by reproductive workers who lay unfertilized, male eggs, rather than work for the good of the colony. Recently, it has also been discovered that workers in bumblebees and Asian honeybees can succeed in entering and parasitizing unrelated colonies to produce their own male offspring. The aim of this study was to investigate whether such intraspecific worker parasitism might also occur in stingless bees, another group of highly social bees. Based on a large-scale genetic study of the species *Melipona scutellaris*, and the genotyping of nearly 600 males from 45 colonies, we show that ∼20% of all males are workers' sons, but that around 80% of these had genotypes that were incompatible with them being the sons of workers of the resident queen. By tracking colonies over multiple generations, we show that these males were not produced by drifted workers, but rather by workers that were the offspring of a previous, superseded queen. This means that uniquely, workers reproductively parasitize the next-generation workforce. Our results are surprising given that most colonies were sampled many months after the previous queen had died and that workers normally only have a life expectancy of ∼30 days. It also implies that reproductive workers greatly outlive all other workers. We explain our results in the context of kin selection theory, and the fact that it pays workers more from exploiting the colony if costs are carried by less related individuals.

Keywords: Meliponini, reproductive conflict, social insects, social parasitism, stingless bees, worker reproduction

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Introduction

Social insects such as ants, bees and wasps are well known for their high degree of cooperation, but the nonclonal structure of their colonies also sets the stage for various reproductive conflicts (Hamilton 1964; Trivers & Hare 1976; Ratnieks & Reeve 1992; Beekman & Ratnieks 2003; Ratnieks *et al.* 2006). One such conflict is queen-worker conflict over male parentage (Trivers & Hare 1976; Bourke 1988; Hammond & Keller 2004; Ratnieks *et al.* 2006; Wenseleers & Ratnieks 2006a). Workers, although generally being unable to mate, are usually capable of laying unfertilized eggs, which develop into male offspring if successfully reared. In addition, they are generally selected to do so, as workers are always most related to their own sons ($r = 0.5$) (Hamilton 1964; Trivers & Hare 1976; Cole 1986; Bourke

Correspondence: Denise Alves, Fax: +55 11 30917533;
E-mail: daalves@ib.usp.br

1988; Wenseleers *et al.* 2004b). Hence, a queen-worker conflict over the production of males ensues. Several factors, however, may help to resolve queen-worker conflict over male parentage and keep successful worker reproduction at a low level (Beekman & Ratnieks 2003; Hammond & Keller 2004; Ratnieks *et al.* 2006; Wenseleers & Ratnieks 2006a). First, the queen herself can counter worker reproduction by selectively eating any worker-laid eggs ('queen policing') (Oster & Wilson 1978; Ratnieks & Reeve 1992; Wenseleers *et al.* 2005a, b; reviewed in Ratnieks *et al.* 2006; Wenseleers & Ratnieks 2006a). Second, the workers may also cannibalize eggs laid by other workers ('worker policing', Ratnieks 1988; Ratnieks & Visscher 1989). This behaviour is seen particularly in species with multiple mated queens (such as honeybees, where worker policing was first discovered), due to the fact that the workers are more related to the queen's sons (brothers, $r = 0.25$) than to the sons of other workers in that situation (a mix of full- and half-nephews, $r < 0.25$) (Ratnieks 1988; Ratnieks *et al.* 2006; Wenseleers & Ratnieks 2006a, b; Ratnieks & Wenseleers 2008). By contrast, in species with a single-mated queen (such as some ants, bumblebees and stingless bees), workers are on average more related to the sons of other workers (full-nephews, $r = 0.375$), than to the sons of the queen ($r = 0.25$), and so collectively favour worker reproduction. Nevertheless, even in these species, worker reproduction may not always reach high levels, probably because of colony-level costs associated with worker reproduction, caused by the fact that reproductive workers in some species carry out less work in the colony (Cole 1986; Bourke 1988; Hillesheim *et al.* 1989; Wenseleers *et al.* 2004b) or because worker male production is traded off against worker production, thereby reducing future colony productivity (Ratnieks & Reeve 1992; Tóth *et al.* 2004; Wenseleers & Ratnieks 2006a; Ohtsuki & Tsuji 2009).

Recently, it has also been discovered that workers may not only exploit their own colony by laying male eggs, but that they may also parasitize and reproduce in other, unrelated colonies away from their natal nest (Beekman & Oldroyd 2008). For example, in the common bumblebee *Bombus terrestris*, workers may succeed in entering unrelated colonies, where they appear to reproduce earlier than normal, thereby insuring that their sons have a greater mating success than if they had produced such males during the normal male production period in their natal colony (Lopez-Vaamonde *et al.* 2004; Beekman & Oldroyd 2008). In addition, in two Asian honeybee species, *Apis florea* (Nanork *et al.* 2005) and *A. cerana* (Nanork *et al.* 2007), it has been shown that particularly queenless nests are prone to be parasitized by non-natal workers. The original interpretation was that this was because of workers from queenright colonies invading these nests, and that it represented a strategy to evade policing in their natal nest, as worker policing is generally switched off in queenless nests (Nanork *et al.* 2005, 2007; Beekman & Oldroyd 2008). More recent data, however, rather seem to suggest that the majority of the worker parasites appear to come from other queenless hives nearby (Chapman *et al.* 2009). In both bumblebees and Asian honeybees, non-natal workers also tend to reproduce more than natal ones, presumably because any concomitant costs of worker reproduction are carried by the unrelated workforce of the parasitized nest, thus limiting any inclusive fitness costs (Beekman & Oldroyd 2008).

The aim of this study was to test whether intraspecific worker parasitism might also occur in stingless bees, another major group of eusocial bees, using the Brazilian species *Melipona scutellaris* as a model. Like honeybees, stingless bees form perennial, swarm-founded colonies; although more similar to bumblebees, their colonies are headed by a single once-mated queen (Peters *et al.* 1999). Furthermore, in both wild colonies and colonies kept in apiaries, genetic studies have demonstrated the occasional occurrence of workers that could not be attributed to the current queen, which indicates the presence of either unrelated drifters or workers derived from a superseded queen (Paxton *et al.* 1999a, 2003; Peters *et al.* 1999; Palmer *et al.* 2002). As yet, however, it has not been determined whether such workers might also successfully reproduce, and if so, whether they might do more so than the rest of the colony. In general, worker reproduction in stingless bees, although favoured on relatedness grounds (Tóth *et al.* 2002b, 2004), can vary from low to high, with anywhere between 0% and 95% of the males being workers' sons depending on the species (see e.g. Contel & Kerr 1976; Sommeijer *et al.* 1999; Drumond *et al.* 2000; Palmer *et al.* 2002; Tóth *et al.* 2002a, b, 2003; Paxton *et al.* 2003; Koedam *et al.* 2005; Gloag *et al.* 2007; reviewed in Hammond & Keller 2004; Tóth *et al.* 2004; Velthuis *et al.* 2005; Wenseleers & Ratnieks 2006a). Although the cause of this variation remains unknown, it could perhaps be because of interspecific differences in the colony-level cost of worker reproduction or due to the queen suppressing worker reproduction to varying extents (Tóth *et al.* 2004; Wenseleers & Ratnieks 2006a; Ratnieks & Wenseleers 2008).

Given the occurrence of worker reproduction in stingless bees (Hammond & Keller 2004; Tóth *et al.* 2004; Velthuis *et al.* 2005; Wenseleers & Ratnieks 2006a) and the documented presence of less related or unrelated worker matrilines within colonies (Paxton *et al.* 1999a, 2003; Peters *et al.* 1999; Palmer *et al.* 2002), the specific

aims of this study were to test whether in *Melipona scutellaris* such workers might be able to successfully reproduce, and if so, to what extent. By genotyping colony samples before and after queen supersedure events, we also test for the first time if workers from a superseded queen could keep on producing male offspring, and if so, for how long. This allows us to determine whether workers might have the capacity to reproductively parasitize the next-generation workforce.

Materials and methods

Study species

Melipona scutellaris nests in cavities of tree trunks in the Atlantic rainforest and is widely distributed in the Northeast of Brazil (Camargo & Pedro 2007), where it is commonly kept by regional and traditional beekeepers for honey, pollen and wax (Cortopassi-Laurino *et al.* 2006). In addition, *M. scutellaris* is increasingly used for the pollination of various tropical crops (Heard 1999; Castro 2002). Colonies are perennial and swarm-founded, typically contain around 1500 workers and are headed by one single-mated queen (Tóth *et al.* 2002b). Workers, gynes and males are reared individually in similar-sized cells which are filled with larval food and sealed by workers immediately after an egg is laid. For this study, we used colonies of *M. scutellaris* maintained in free-foraging wooden nest boxes at Granja São Saruê (Igarassu, Pernambuco state, 7°50′3.74″S, 34°54′22.87″W), in the Bee Laboratory at the University of São Paulo (São Paulo, S. Paulo state, 23°33′37.41″S, 46°43′53.04″W) and at the Aretuzina Farm (São Simão, S. Paulo state, 21°26′25.97″S, 47°34′54.65″W). Colonies were spaced ~1–10 m apart, as is typical in most apiaries and also not uncommon in nature, where several nests can sometimes be found within a few metres of each other, either in the same tree or in different trees (Alves *et al.* 2005). During the time of our study, colonies were managed only by occasionally providing them with food.

Brood sampling

Between January 2006 and October 2008, we sampled brood for genetic analysis from a total of 16, 9 and 12 colonies in Igarassu, S. Paulo and S. Simão respectively (Table 1). This was carried out by collecting brood combs containing mature pupae and removing the cell cappings, after which 10 workers and any males present were preserved in absolute ethanol. The remainder of the brood was reintroduced into the natal nest. Brood samples that did not contain male brood were not retained for this study. In S. Paulo and S. Simão, we also repeated the sampling of brood when the mother queen, which was individually marked, was found to be replaced by a new one (Table 1). Such samplings were made at a median number of 206 days after the previous queen had died (range 77–737 days). Overall, we could collect male and worker brood produced by 45 distinct queens (Table 1).

Genetic analysis

For parentage analysis, we genotyped 10 worker pupae and an average of 13 haploid male pupae (range 5–36, total 576) for each of our 45 colonies (Table 1) at three microsatellite loci, *T4–171* (Paxton *et al.* 1999b), *Mbi-254* and *Mbi-201* (Peters *et al.* 1998). These loci were found to be most variable in this species in a preliminary screening of 10 loci. To facilitate parentage reconstruction and wherever necessary, we also noninvasively genotyped the mother queen from a wing tip sample (cf. Châline *et al.* 2004; Table 1). DNA was extracted using the Chelex method, whereby a single leg (for worker or male pupae) or wing tip sample (from mother queens) was frozen in liquid nitrogen and ground up using a plastic pestle, followed by an incubation at 95 °C for 15 min in 200 μL (50 μL for wing tip samples) of a 10% Biorad Chelex 100 resin solution. Samples were vortexed and centrifuged before use. Multiplex PCR reactions were carried out in a 10 μL reaction volume, and contained 0.5 μM of the forward and reverse primers of each locus, 0.2 mM of each dNTP, 1.5 mM $MgCl_2$, 1 μL of crude DNA extract, 0.4 units of Silverstar *Taq* polymerase (Eurogentec) and enzyme buffer supplied by the manufacturer. PCR was performed following a touch-down programme (Bonckaert *et al.* 2008), with an initial denaturation for 3 min at 94 °C, followed by 20 cycles consisting of 30 s at 94 °C, 30 s at 58 °C, but decreasing 0.5 °C in each step and 45 s at 72 °C; 10 cycles consisting of 30 s at 94 °C, 30 s at 46 °C and 45 s at 72 °C; and a final 10-min extension step at 72 °C. After amplification, 1 μL of the PCR product was mixed with 8.8 μL formamide and 0.2 μL Genescan 500 LIZ size standard (Applied Biosystems), denatured, and loaded onto an ABI-3130 Avant capillary sequencer. Alleles were called using the supplied GeneMapper software and checked by eye. Male genotypes that appeared to suggest they were the sons of drifted workers or sons of workers derived from a superseded queen (see below) were rePCRed, rescored and rechecked twice to eliminate the possibility of genotyping errors.

Analysis of male parentage

To infer male parentage, we first reconstructed the genotype of each mother queen and that of her mate

Table 1 Male parentage in *Melipona scutellaris* colonies kept in Igarassu (IG), São Paulo (SP) and São Simão (SS), with information on the sampling date, the number of diploid and haploid individuals genotyped, the number of males which was inferred to be sons of workers from either the current queen, a superseded queen or unrelated drifters and the percentages of males present in the sampled brood combs

Colony and queen	Sampling date	No. diploid individuals genotyped	No. haploid males genotyped	No. worker sons detected (%)			Power to detected sons of workers of the		% of males in sampled combs
				From workers of the current queen	From workers of the previous queen	From workers previous queen or drifted workers*	Current queen‡	Previous queen‡	
IG 01-a	Sep. 2006	10	14			3 (21.43)	0.50		36.05
IG 03-a	Jan. 2006	10	8				0.75		16.06
IG 05-a	Sep. 2006	10	9				0.88		18.48
IG 06-a	Sep. 2006	10	10				0.88		24.41
IG 08-a	Jan. 2006	10	9			1 (11.11)	0.50		8.36
IG 11-a	May 2007	10	9				0.75		45.08
IG 12-a	Sep. 2006	10	9				0.75		28.42
IG 13-a	Jan. 2006	10	9			8 (88.89)	0.00		28.73
IG 16-a	Jan. 2006	10	8				0.50		33.69
IG 19-a	Jan. 2006	10	8				0.00		21.10
IG 20-a	May 2007	10	10				0.50		17.93
IG 26-a	Sep. 2006	10	10	5 (50.00)			0.88		21.99
IG 27-a	Sep. 2006	10	10	1 (10.00)			0.50		6.37
IG 30-a	Sep. 2006	10	10				0.88		19.63
IG 36-a	May 2007	10	10				0.75		29.03
IG 39-a	May 2007	10	12				0.88		9.69
SP 01-a§	Oct. 2006	10	5			1 (20.00)	0.75		20.00
SP 01-d§¶	Jul. 2008	10	11				0.00	0.44	10.93
SP 02-b§¶**	Feb. 2007	10	13	1 (7.69)	5 (38.46)		0.75	0.58	90.00
SP 03-a	Jul. 2006	10	36			10 (27.78)	0.00		26.55
SP 03-b	Dec. 2006	10	9		3 (33.33)		0.50	0.25	2.94
SP 03-c§**	Jul. 2007	10	24				0.88	1.00	13.22
SP 04-a	Jul. 2007	10	27				0.00		28.21
SP 05-b¶	Nov. 2006	10	30				0.50	0.00	39.59
SP 06-a	Oct. 2008	10	6			4 (66.67)	0.75		3.55
SP 08-a	Dec. 2006	10	27			6 (22.22)	0.75		18.74
SP 09-c**	Sep. 2008	10	20				0.75	0.94	14.56
SP 10-b§¶	Jul. 2008	10	33				0.50	0.00	18.55
SS 01-a§	Mar. 2006	12††	7				0.75		66.31

Table 1 *Continued*

Colony and queen	Sampling date	No. diploid individuals genotyped	No. haploid males genotyped	No. worker sons detected (%)			Power to detected sons of workers of the		% of males in sampled combs
				From workers of the current queen	From workers of the previous queen	From workers previous queen or drifted workers*	Current queen†	Previous queen‡	
SS 02-b¶‖**	Feb. 2007	10	10				0.50	0.72	8.96
SS 04-a	Apr. 2006	10	10	1 (10.00)			0.50		13.77
SS 06-a	Jul. 2006	10	8				0.75		15.85
SS 06-b§	Dec. 2006	10	9		1 (11.11)		0.50	0.44	5.13
SS 08-a§	Mar. 2006	14††	5	2 (40.00)			0.88		61.89
SS 08-b§	Oct. 2006	10	10				0.75	0.00	17.02
SS 15-a§	Mar. 2006	10	11	3 (27.27)			0.75		21.01
SS 28-a	Aug. 2006	10	16				0.50		42.47
SS 36-a	Mar. 2006	10	7				0.50		5.65
SS 36-b	Nov. 2006	10	8		3 (37.50)		0.00	0.25	69.69
SS 43-a	Jul. 2006	10	10				0.50		19.41
SS 59-a	Sep. 2006	10	23			1 (4.35)	0.75		24.72
SS 59-b§**	Jan. 2007	10	20				0.75	0.63	38.06
SS 63-a	Apr. 2006	17††	7	1 (14.29)		1 (14.29)	0.50		55.39
SS 63-b§	Nov. 2006	10	9				0.50	0.44	10.00
SS 66-a	Sep. 2006	10	10				0.00		14.95
Total/average	Σ = 463	Σ = 576	Σ = 14	Σ = 12		Σ = 35	$\bar{x} = 0.56$	$\bar{y} = 0.44$	

The letters in the colony codes refer to the succession of different mother queens. The statistical power with which we could unambiguously detect sons of workers of the current queen and sons of workers from a previous queen is also given. The reconstructed parental genotypes and male genotypes are given in Table S1.

*The possibility of males being sons of workers of the previous queen or of a drifted worker could only be distinguished with certainty if the genotype of the previous, superseded queen and that of her mate was known.

†Probability of a worker son inheriting a unique paternal allele at at least one of the loci studied, calculated using the formula given in Foster et al. (2001).

‡Probability that a son of a worker of the previous queen could be distinguished from a son of a worker of the current queen at one of the loci studied at least (calculated on a case by case basis).

§Mother queen also genotyped.

¶Genotype of the superseded queen and her mate determined in another study (Wenseleers et al., unpublished data; Table S1).

**Queen was genetically unrelated to the previous, superseded queen (Wenseleers et al., unpublished data; Table S1).

††Diploid offspring genotyped comprised 10 workers plus 2, 4 and 7 diploid males respectively.

from the genotypes of the diploid brood she produced (worker pupae and occasionally also diploid males, Table 1) (cf. Foster *et al.* 1999). This was straightforward, given that in all cases, genotypes were consistent with the mother queens being singly mated and with just a single matriline being present. In a few cases, we also had the genotype of the queen herself available, which further facilitated parentage reconstruction (Table S1, Supporting Information). As a null hypothesis, we assumed that males were either sons of the queen or of workers derived from the present queen. These two possibilities could be distinguished if the paternal allele at any one locus was different from the maternal alleles (e.g. parental genotypes of the type ab × c or aa × b, but not ab × a). In that case, a worker's son could be detected if he inherited a unique paternal allele that was not present in the queen (cf. Foster *et al.* 2001). However, if a male carried an allele not present in either the mother queen or that of its mate, then it was clear that such a male was either the son of a drifted worker or of a worker derived from a superseded queen. The latter two possibilities could be distinguished either by comparison to the known genotypes of workers produced by the previous, superseded queen (Table 1), or by counting the total number of alleles present among such males at each locus in any one colony. The idea is that reproduction by a single superseded matriline of workers should never lead to the presence of more than three alleles, whereas the reproduction by workers that had drifted from multiple colonies might. Estimates of the percentage of males that were sons of workers from the current or previous queen were corrected according to the statistical power with which such males could be detected in our data set given the observed parental genotypes ($\bar{x}$ and $\bar{y}$, for details see Table 1, cf. Foster *et al.* 2001). Finally, we also calculated the probability that the son of a drifted worker would by chance be misclassified as the son of the current queen, a son of a worker of the current queen or a son of a worker of the superseded queen, by fortuitously having an identical multilocus genotype. This was carried out using the formula given in Nanork *et al.* (2005), using the population-specific allele frequencies.

Results

Allelic diversity and statistical power to detect worker reproduction

Loci were reasonably polymorphic, with a total of 7, 8 and 8 alleles detected (Igarassu: 7, 5, 8; S. Paulo: 6, 8, 7; S. Simão: 4, 3, 3) and mean expected heterozygosities of 79%, 71% and 73% (Igarassu: 74%, 64%, 78%; S. Paulo:

77%, 72%, 73%; S. Simão: 51%, 64%, 61%) at loci *T4–171*, *Mbi-254* and *Mbi-201* respectively. The mean statistical power to detect sons of workers derived from either the current queen or of the previous, superseded queen was $\bar{x} = 56\%$ and $\bar{y} = 44\%$ respectively (Table 1), with the latter figure based on 13 colony samples for which we also had the genotypes available of workers produced by the previous, superseded queen (Table 1). The mean probabilities of the son of a drifted worker fortuitously having the same genotype as the son of the current queen, a son of a worker of the current queen or a son of a worker of the superseded queen, were small, 3.81% (Igarassu: 1.86%; S. Paulo: 2.02%; S. Simão: 6.90%), 3.86% (Igarassu: 3.21%; S. Paulo: 2.65%; S. Simão: 4.76%) and 3.57% (S. Paulo: 2.06%; S. Simão: 4.58%) respectively. Hence, the probabilities of males being erroneously misclassified were small.

Male parentage

Out of the 576 males genotyped, 61 (10.59%) could not be assigned to the queen and were therefore definitely workers' sons (Table 1 and Table S1). Only a small percentage of these workers' sons (14 out of 61), 22.95%, however, were consistent with being sons of workers of the current queen, and the remainder, 47 out of 61 (77.05%), were therefore either derived from drifted workers or from workers produced by an earlier, superseded queen. Restricting ourselves to the subset of 13 colonies for which we had the genotypes available of workers produced by the superseded queen, however, we can see that all the anomalous males (12 out of 12 in four colonies, Table 1) were consistent with being the sons of workers from a superseded queen as opposed to being sons of unrelated, drifted workers (Table S1). In addition, for the remaining colonies where such anomalous male genotypes were found, we never sampled more than three alleles in males at any one locus in any of the colonies, again implying that such males were most probably produced by a single, superseded matriline of workers as opposed to workers that had drifted from several other, unrelated colonies (Table S1). For a few colonies, where only a few anomalous males were found, it remains possible, however, that these were the offspring of drifted workers. Assuming, nevertheless, that the majority of the anomalous males were the offspring of a superseded queen, and correcting our figures for nondetection, we estimate that 77.11% of the males were the queen's sons, 4.34% were the sons of the workers derived from the current queen and 18.54% were the sons of workers derived from a previous, superseded queen. Hence, using these corrected estimates, 81.03% of all workers' sons were the offspring of workers from superseded queens.

These figures are surprising given that for the São Paulo population, sons of workers deriving from the superseded queen could still be found in colonies SP 02-b and SP 03-b, despite the fact that these samples were taken 175 and 105 days after the previous queen had died, and that workers in *M. scutellaris* have a mean life expectancy of only 31 days (Oliveira & Kleinert-Giovannini 1991). Even accounting for the fact that some brood laid by the previous queen may have kept enclosing until ~40 days after her death (the development time from egg to adult in *Melipona*), and that the sampled brood was probably laid ~25 days before, we can see that reproductive workers must live a very long time, at least 175−40−25 = 110 days, which is 3.5 times as long as that of a normal worker, and in fact not too far off from the life expectancy of queens in this species, 175 days (Wenseleers *et al.*, unpublished data).

There was no significant correlation between the inferred percentage of males that were workers' sons and the percentage of males present in the combs from which the samples were taken (Spearman $R = 0.11$, $n = 45$, $P = 0.45$). This goes against the hypothesis that workers would reproduce mainly during periods in which the queen lays haploid eggs (Chinh *et al.* 2003; Sommeijer *et al.* 2003; Velthuis *et al.* 2005). In fact, in several cases (in colonies IG 08-a, IG 27-a, SP 03-b, SP 06-a and SS 06-b), we found evidence for worker reproduction even at times when fewer than 10% of the cells contained male pupae (Table 1). So although we concur with Chinh *et al.* (2003) and Velthuis *et al.* (2005) that stingless bee queens frequently lay male eggs in distinct batches (during 'male producing periods'), it does not appear to be the case that workers mainly reproduce during such periods, as worker reproduction was found both in periods in which many and in which few males were reared.

Discussion

Consistent with most previous genetic and behavioural studies on male parentage in stingless bees (reviewed in Hammond & Keller 2004; Tóth *et al.* 2004; Velthuis *et al.* 2005; Wenseleers & Ratnieks 2006a), our results show that a significant fraction, 22.88%, of the males in *Melipona scutellaris* are sons of the workers rather than of the queen. This is as expected from inclusive fitness theory, given that single mating in stingless bees collectively favours worker reproduction (Ratnieks 1988; Tóth *et al.* 2002b, 2004). Surprisingly enough, however, our results also show that ~80% of the workers' sons had genotypes that were incompatible with them being the sons of workers of the present queen. Based on sampling of colonies over multiple generations, before and after queen supersedure events, and based on the fact

that such males from any one colony never had more than three alleles at a given locus, we were able to show that these males were most probably produced by workers that were the offspring of a previous, superseded queen, rather than by workers that had drifted from other colonies. This represents the first demonstration of workers reproductively parasitizing the next-generation workforce for their own, selfish benefits.

That such a high percentage of the worker sons were produced by workers derived from a superseded queen is surprising, given that most colonies were sampled many months after the previous queen had died, and that workers in *M. scutellaris* normally only have a life expectancy of 31 days (Oliveira & Kleinert-Giovannini 1991; life expectancy of workers in five other *Melipona* species: 40–51 days, Giannini 1997; Biesmeijer & Tóth 1998). In fact, in one case, we estimated that reproductive workers had a life expectancy of ~110 days, which means they outlived the other workers by a factor of 3.5, and that they had a life expectancy which was almost comparable with that of the queen in this species, 175 days (Wenseleers *et al.*, unpublished data). The long life expectancy of laying workers is probably linked to the fact that reproductive workers usually do not carry out risky or energetically costly tasks, such as foraging, in order for them not to jeopardize their reproductive futures (Franks & Scovell 1983; Hartmann & Heinze 2003; Wenseleers *et al.* 2004b).

The high percentage of males produced by workers derived from superseded queens (~80%) was also surprising given that for colonies sampled at random points in time, such workers usually present only a very small fraction of the workforce. For example, the percentage of workers that had either drifted from other colonies or were descendants of a superseded queen was estimated at 3% across a sample of 12 species of stingless bees (Peters *et al.* 1999; excluding the facultatively polygyne *Melipona bicolor*), 10% in *Melipona beecheii* (Paxton *et al.* 1999a) and between 21% and 36% across three species of *Scaptotrigona* (Paxton *et al.* 1999a, 2003; Palmer *et al.* 2002). Overall, this probably means that, on a per capita basis, the workers from superseded queens specialize in reproduction. Partly, this may be due to the fact that during the brief period of queen-lessness following the death of the queen and preceding the establishment of a new laying queen, workers increase their rate of ovary activation as well as their egg-laying rate (Ferreira *et al.* 1989; Lacerda & Zucchi 1999; Kleinert 2005; Velthuis *et al.* 2005), and that this may give these workers a 'head start' relative to the workers produced later by the newly mother queen. However, the greater reproductive rate of workers derived from a superseded queen is also consistent with kin selection theory, given that it pays workers more

from exploiting the colony if costs are carried by less related individuals (Hamilton 1964; Wenseleers *et al.* 2004a, b; Ratnieks *et al.* 2006; Wenseleers & Ratnieks 2006b). In stingless bees, worker reproduction is probably to be costly, first because workers might carry out less work (but see Cepeda (2006) who shows that reproductive workers in *M. bicolor* do engage in some brood care), and second because the exclusively male eggs laid by workers will end up replacing some of the female, worker-destined eggs laid by the queen (Ratnieks & Reeve 1992; Tóth *et al.* 2004). Hence, worker reproduction in stingless bees will inevitably be traded off against worker production, and come at a cost to future colony productivity (Ratnieks & Reeve 1992; Tóth *et al.* 2004). From the perspective of workers from a previous queen, however, this cost will in inclusive fitness terms be lower as it will be carried by more distantly related individuals (nephews and nieces, $r = 0.375$, as opposed to sisters, $r = 0.75$), thereby causing them to be selected to exploit the colony more. These results are the first explicit demonstration that conflict over male parentage in insect societies is not just played out between the queen and workers and between the workers of one generation, but that the conflict may also spill over from one worker generation to the next. Future work will be required to test if such intergenerational worker reproduction conflict occurs more widely in other perennial insect societies.

Acknowledgements

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 Reconstructed parental genotypes and male genotypes observed in *Melipona scutellaris* colonies kept at Igarassu (IG), São Paulo (SP) and São Simão (SS), with information on whether the males were inferred to be sons of workers of the current queen (WSC), sons of workers of the previous queen (WSP) or sons of either workers of the previous queen or of drifted workers (WSP/D)

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