Behav Ecol Sociobiol (2005) 58: 486–496
DOI 10.1007/s00265-005-0939-1

ORIGINAL ARTICLE

Vincent Dietemann · Jürgen Liebig · Bert Hölldobler · Christian Peeters

Changes in the cuticular hydrocarbons of incipient reproductives correlate with triggering of worker policing in the bulldog ant *Myrmecia gulosa*

Received: 17 March 2004 / Revised: 23 February 2005 / Accepted: 1 March 2005 / Published online: 25 May 2005

Abstract In social insects, conflicts over male parentage can be resolved by worker policing. However, the evolution of policing behavior is constrained by the ability of individuals to identify reproductive nestmates, or their eggs. We investigated the occurrence of worker policing and its underlying chemical communication in the bulldog ant *Myrmecia gulosa*. Although workers have functional ovaries and can lay male-destined eggs, they do not reproduce in queenright colonies. To determine if their sterility is a consequence of worker policing, we experimentally induced worker reproduction in the presence of a queen. Some individuals were seized and immobilized by nestmates, and sometimes killed as a consequence. Although the ovarian development of immobilized individuals was variable, their cuticular hydrocarbon profiles were intermediate between reproductive and nonreproductive workers, indicating they were in the process of starting to reproduce. Approximately 29% of these incipient reproductive workers were successfully policed. To test for policing on eggs, we transferred viable worker eggs to queenright colonies and monitored their acceptance. Furthermore, we compared the surface hydrocarbons of the different types of eggs to determine whether these chemicals could be involved in egg recognition. We found that although there were differences in hydrocarbon profiles and discrimination between queen and worker-laid eggs, viable eggs were not destroyed. Our results strongly support the idea that cuticular hydrocarbons are involved in the policing of reproductive workers. A low level of worker policing appears sufficient to select for self-restraint in workers when few fitness benefits are gained by selfish reproduction. Policing of eggs may thus be unnecessary.

Keywords Worker reproduction · Worker policing · Aggression · Cuticular hydrocarbons · Oophagy

Communicated by L. Sundström

V. Dietemann (✉) · J. Liebig · B. Hölldobler
LS Verhaltensphysiologie und Soziobiologie, Biozentrum,
Würzburg University,
97074 Würzburg, Germany
e-mail: vdietemann@zoology.up.ac.za
Tel.: +27-12-4202548
Fax: +27-12-3625242

C. Peeters
CNRS UMR 7625, Laboratoire d'Ecologie, Université
Pierre-et-Marie Curie,
7 quai Saint Bernard,
75005 Paris, France

Present address:
V. Dietemann
Department of Zoology and Entomology, Pretoria University,
0002 Pretoria, South Africa

Introduction

Workers in social Hymenoptera typically forgo direct reproduction to help one or a few relatives produce all the offspring. In most species, workers retain functional ovaries and can lay male-destined eggs (Bourke 1988; Choe 1988). Even though workers might benefit from the production of their own offspring on relatedness grounds (Ratnieks 1988), worker-produced males are rare or absent when a queen is present in the colony (e.g. Walin et al. 1998; Hammond et al. 2003; Villesen and Boomsma 2003). This suggests that workers are prevented from reproducing. Workers benefit from suppressing each other's reproduction when this decreases their average fitness, i.e. when worker reproduction is costly in terms of colony productivity (Cole 1986; Ratnieks 1988; Kikuta and Tsuji 1999; Hartmann et al. 2003; Pirk et al. 2003) or when workers are more related to their brothers (the queen's sons) than to their nephews (their sisters' sons). The latter occurs in colonies headed by a multiply-mated queen or by several related queens (Ratnieks 1988; Pamilo 1991). If there is an effective mechanism of worker policing, workers that attempt to reproduce are at no selective advantage and self-restraint should be selected (Ratnieks 1988; Wenseleers et al. 2004).

Ratnieks (1988) described several proximate mechanisms by which worker policing could be achieved.

Among these, two have been studied in several social Hymenoptera: physical aggression on egg-laying workers (Gobin et al. 1999; Liebig et al. 1999; Hartmann et al. 2003) or destruction of worker-laid eggs (Ratnieks and Visscher 1989; Ratnieks 1993; Kikuta and Tsuji 1999; Foster and Ratnieks 2000, 2001; Halling et al. 2001; Oldroyd et al. 2001; Iwanishi et al. 2003; Endler et al. 2004; D'Ettorre et al. 2004; Helanterä and Sundström 2005). In both cases, the effectiveness of policing can be constrained by a lack of information on which workers can rely (Boomsma et al. 2003). Indeed, a necessary condition for the evolution of a policing mechanism is for workers to be able to identify accurately their targets: either reproductively active individuals, or their eggs. Cuticular hydrocarbons (CHCs) could be the basis of such a mechanism in social insects as they are putative signals used by workers to discriminate between reproductive and nonreproductive individuals (Monnin et al. 1998; Peeters et al. 1999; Liebig et al. 2000; Sledge et al. 2001; Cuvillier-Hot et al. 2001, 2004a, Hannonen et al. 2002; Heinze et al. 2002; Dietemann et al. 2003; Endler et al. 2004). To find a link between the occurrence of worker policing and the cuticular profile of target individuals would support the idea that workers use cuticular hydrocarbons to identify selfish adults, a causality which has not yet been shown. Similarly, egg-marking pheromones may allow police workers to discriminate between queen- and worker-laid eggs, and thus avoid destroying valuable sisters (Nonacs and Carlin 1990; Ratnieks 1995; Monnin and Ratnieks 2001). In female cockroaches, long-chained hydrocarbons produced by the oenocytes are transported both to the oocytes and to the cuticle (Schal et al. 1994; Gu et al. 1995). Thus, surface hydrocarbons of eggs could play a role in egg discrimination when CHC profiles of the egg-layers differ (e.g. Monnin and Peeters 1997; D'Ettorre et al. 2004). This was shown experimentally in *Camponotus floridanus*, as workers discriminate between queen- and worker-laid eggs on the basis of differences in hydrocarbon profiles (Endler et al. 2004).

Most studies on worker policing in ants (cf. above refs) are based on split-reunification of colonies. Thus, workers in queenright colonies are suddenly confronted with new reproductives that already have well-developed ovaries. This does not allow us to determine the point at which workers are able to discriminate 'selfish' individuals. In order to investigate this, it is necessary to monitor the changes in physiology or chemistry of the individuals in a biologically relevant context and at the time they become attacked. In this study, we adapted the protocol of Tsuji et al. (1999) to induce worker reproduction within queenright colonies of ants. This experimental setup simulates a more natural situation in which selfish workers are continuously in contact with police workers, and can be detected before they start laying eggs. Behavioral data about police workers can then be linked with chemical changes in the cues of selfish workers.

Until now, worker policing has not been investigated in the subfamily Myrmeciinae. *Myrmecia* ants exhibit several archaic morphological and biological traits (Ogata and Taylor 1991; Peeters 1997) of particular relevance to the conflict over male production (Bourke 1999). In *Myrmecia gulosa*, caste dimorphism is limited and colony size is small relative to 'higher' ants (colonies consist of 992± 551 workers, mean ± SD; Dietemann et al. 2002). *M. gulosa* is monogynous and queens are only slightly bigger than large workers, but have many more ovarioles (44 compared to 8–14 in workers; Dietemann et al. 2002). Queens are not aggressive toward workers and regulation of worker reproduction is based on pheromones (Dietemann et al. 2005). Rare instances of immobilization of dealated virgin queens and workers by their nestmates were observed in unmanipulated colonies (V. Dietemann, unpublished data), hinting that individuals may occasionally attempt to reproduce in the presence of a functional queen, and are punished by police workers. Furthermore, it has been demonstrated that workers can discriminate between different cuticular hydrocarbon blends associated with reproductive status (Dietemann et al. 2003).

We tested whether *M. gulosa* workers can discriminate between reproductive and nonreproductive workers and between queen-laid and worker-laid reproductive eggs. In addition, we induced worker reproduction in presence of a queen and extracted CHCs of workers and surface hydrocarbons of their eggs to investigate their potential role as recognition cues. We thus combined behavioral observations and chemical analyses to assess the information that is available for workers to perform policing. We hypothesize that hydrocarbon profiles specific to reproductive workers or their eggs can be detected and trigger worker policing (sensu Ratnieks 1988).

Methods

Collection and laboratory rearing

Colonies of *M. gulosa* ($n=10$, colony codes A, C-G, I-L) were excavated in sandstone areas close to Waterfall, New South Wales, Australia, between September 1999 and October 2000. These ants are large (14–23 mm in length), and for logistical reasons the number of individuals brought back to Germany was reduced: together with the queen and all the eggs, 500 workers, and up to 250 larvae and 100 cocoons were randomly selected. Ants were kept in plaster-of-Paris nests into which chambers had been molded. These nests were connected to foraging arenas where food (pieces of cockroach or entire crickets and honeywater) was deposited every 1–2 days. The temperature was maintained at 24±1°C, the photoperiod was set at 10:14 h (light:darkness) cycles and the plaster was regularly moistened. These conditions proved to be favorable for the ants since new adults emerged continuously in the laboratory.

Classification of reproductive activity in workers

Throughout all experiments workers exhibited different levels of ovarian activity. Nonreproductive workers either lay no eggs or lay trophic eggs (these fragile yolk sacs do not develop and are fed to larvae and nestmates; Dietemann et al. 2002). Trophic egg-layers could be identified by direct observation as they typically bent the gaster underneath the thorax and collected the egg in their mandibles. Upon dissection, nonlaying workers lack oocytes and trophic egg layers have oocytes longer than 1.25 mm with partly translucent yolk and no rigid chorion (see Dietemann et al. 2002).

After becoming orphaned, some workers start to reproduce. These could be recognized upon dissection because they had oocytes shorter than 1.25 mm with dense and homogeneous yolk (Dietemann et al. 2002). Some individuals had oocytes that we considered as intermediate between trophic and reproductive: they were shorter than trophic oocytes and yolk was dense, but they had a thin, flexible chorion. These oocytes were likely to develop in the unviable eggs that workers produce when they switch from trophic to reproductive eggs (Dietemann et al. 2002). We hypothesize that the individuals laying unviable eggs will eventually produce viable ones and therefore classified them as reproductive workers. Reproductive workers could not be recognized by direct observation as they neither adopted a typical posture when ovipositing nor collected their eggs in their mandibles. Although some workers stood high on their legs near the egg pile, with the sting protruding, this posture could be maintained for hours without any oviposition being observed. Thus, these individuals were isolated overnight and considered as reproductive workers only if an egg had been laid the next morning.

Experiment 1: Discrimination between reproductive and nonreproductive workers

We tested whether workers can discriminate between reproductive and nonreproductive workers by simultaneously introducing these in queenright colonies. If the reproductive workers are treated differently than the nonreproductive ones, then workers in queenright colonies are capable of discrimination. Reproductive workers were obtained by isolating groups of 100–150 individuals away from their queen (four colonies). Nonreproductive workers were identified in the same orphaned groups, because they laid trophic eggs. Workers were introduced only in alien colonies to level out the effect of colony odor. As only 45 reproductive workers were obtained, each was tested in up to three colonies, at intervals of at least 20 min.

A total of 80 tests were done using 7 discriminator queenright colonies. To evaluate the attention elicited by the introduced workers, the number of individuals antennating or biting either the reproductive or the nonreproductive worker was counted at 1-min intervals for 10 min. The antennation counts were cumulated for each introduced worker as were the bite counts.

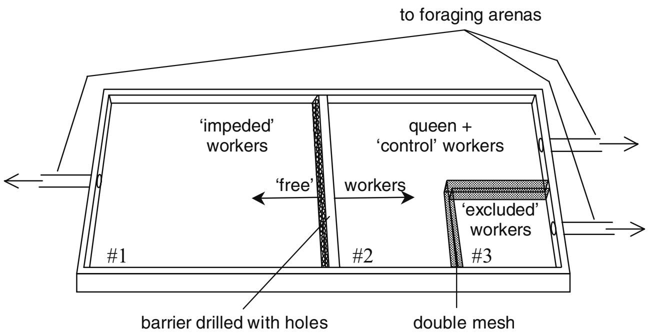

Fig. 1 Nest design in experiment 2. Movements of all individuals between compartments *#2* and *#3* are prevented by a double mesh, while *'impeded'* and *'control'* workers cannot cross the perforated barrier between compartments *#1* and *#2*

Experiment 2: Policing of reproductive workers and cuticular hydrocarbons

To investigate the link between the occurrence of worker policing and the cuticular profile of target individuals, we used Tsuji et al.'s (1999) experimental protocol to induce differentiation of reproductive workers in a queenright context, by preventing a proportion of nestmates from having physical contact with the queen. Experimental nests consisted of a single chamber (420×200 mm) covered with glass plates. A 15-mm-thick Perspex barrier drilled with 51 holes (8 mm in diameter) was placed in the middle of the chamber (Fig. 1). A third compartment was created using a double mesh (Fig. 1). Each of the three compartments was connected to its own foraging arena (185×185×90 mm).

Four replicates were used; two of the colonies resulted from splitting a field colony having two functional queens, 3 months earlier. *M. gulosa* workers exhibit a clear size dimorphism and colonies are composed of an equal number of small and large workers (Dietemann et al. 2002). These two types of workers are known to perform different tasks (Haskins and Haskins 1950) and have different reproductive outputs (Dietemann et al. 2002). Thus, to minimize behavioral and morphological biases and retain natural size distribution, we randomly selected 100 large and 100 small workers, together with their queen. Workers were individually marked with color dots on the thorax. All the brood was removed to exclude a possible effect on worker reproduction.

To regulate contact with the queen, we prevented most of the workers from crossing the Perspex barrier. A copper wire (0.2 mm diameter, 12 mm long for small workers, 14 mm for large workers) was tied around the thorax, including the queen's. A 2-week accommodation period without the Perspex barrier and without the double-mesh was allowed before starting the experiment. The workers were then randomly assigned to four treatments (n=25 large + 25 small workers per treatment in each of the four replicates): 'free' workers had the wire cut short after the accommodation period and could move freely between compartments #1 and #2; 'impeded' workers placed in #1 had no direct contact with the queen; 'control' workers were restricted with the queen in #2; 'excluded' workers in #3 lacked contact with both the queen and free workers (Fig. 1). Control workers allowed a check on the effect of the wire and lack of brood on ovarian development. Excluded workers approximated an orphaned condition (see Dietemann et al. 2002) and indeed started to behave aggressively; they are the benchmark against which aggressive interactions and reproductive activity of workers in #1 and #2 can be compared.

Observation bouts of 1–3 h were scheduled daily between 0900 and 1900 hours (total for each replicate of 22–31 hours over 13–22 days). We monitored the direction and frequency of aggression among workers: antennal boxing at the victim's head, or biting the victim's leg, mandible, head or antenna. Such antennal boxing differs from the antennal inspections recorded in experiment 1, and are a typical form of aggression in orphaned groups of workers (Dietemann et al. 2002). Immobilization was another aggressive behavior: two or more individuals bite and hold the victim for several hours to several days (e.g. Monnin and Peeters 1999). We monitored the identity of the workers participating in immobilizations and of their victims. The experiments ended 2–13 days after eggs started to accumulate in #3 (for a total of 22–27 days); oviposition did not occur in colony D and it was ended after 31 days. All workers (including those killed by their nestmates, when possible) were dissected to check reproductive activity. During the last 2 days of the experiments, cuticular hydrocarbons were extracted (see below) from the queens and from randomly chosen workers in three out of the four treatments (free, control and impeded). In addition, impeded workers were extracted on the first day of their immobilization.

Experiment 3: Egg policing and surface hydrocarbons

To assess whether workers can discriminate between eggs laid by the queen and reproductive workers, we transferred viable reproductive eggs into queenright colonies. Destruction of an egg takes several minutes as workers consume its yolk. It is thus possible to determine the fate of several eggs simultaneously. Depending on availability, batches of 1–20 worker-laid viable eggs were introduced in queenright colonies to test their acceptance. The receiver colonies were either kin (i.e. from which the orphaned egg layers originated, n=40 eggs, 2 colonies) or non-kin (n=123 eggs, 3 colonies). This allowed us to determine the effect of colony membership on egg destruction. As a control, batches of 1–20 queen eggs were collected and reintroduced into their colony of origin (kin, n=113 eggs, n=7 colonies) or into alien colonies (non-kin, n=73 eggs, n=6 colonies). Preliminary observations had shown that accepted eggs were deposited on the egg-pile within 10 min. Accordingly, we monitored the fate of the eggs for 10 min after their introduction. If the egg was deposited on the pile or was not destroyed during this period, it was considered as accepted. Additionally, we tested the acceptance of unviable eggs laid by workers just after orphanage (n=94, n=5 colonies) to determine the link between acceptance and surface hydrocarbon profile. Small workers normally destroy unviable eggs (Dietemann et al. 2002). Since large workers do not destroy them, we created newly orphaned groups of 20 large workers in order to collect these unviable eggs.

In a further experiment, we investigated the eventuality that workers can discriminate reproductive worker eggs without destroying them. For this, we compared the time elapsed before eggs were deposited in the pile (total of 15 worker-laid and 15 queen-laid eggs in 6 colonies).

Chemical analyses

The CHC profiles of workers and of eggs were characterized using the Solid Phase MicroExtraction/gas-chromatography technique (see Dietemann et al. 2003 for the extraction procedure of CHCs of adult ants). Eggs were collected from the nests with a mouth aspirator. During extraction, they were carefully held on a glass cover slide with smooth forceps cleaned with hexane. Four eggs were extracted for each sample in order to collect enough material. The SPME fiber (SUPELCO, coated with a 7-μm polydimethylsiloxane film) was rubbed for 10 s on each egg. SPME is a technique superior to solvent extraction for eggs, because chemicals present inside the eggs are not extracted. Extracts of 44 batches of 4 eggs originating from 7 colonies were collected.

The fiber was then directly inserted in the injection port of a Carlo Erba 8130 gas chromatograph equipped with a DB-1 nonpolar capillary column (J&W Scientific, Folsom, Calif.; 30 m × 0.32 mm × 0.25 μm). Helium was used as carrier gas with a column head pressure of 95 kPa. The fiber was desorbed at 260°C in the splitless mode for 4 min at a column temperature of 60°C. The temperature was then raised to 250°C at a rate of 20°C min^{-1} and from 250 to 300°C at 2.5°C min^{-1}. The flame ionization detector temperature was set at 310°C.

Statistical analysis

GC data

The CHCs of *M. gulosa* have been shown to represent the basis of recognition of reproductives (Dietemann et al. 2003), but it is not known which molecules are used by the ants. Accordingly, we selected the peaks present in all workers or eggs and representing more than 0.5% of the total peak area as variables. The relative areas of these peaks were restandardized to 100% and transformed using Aitchison's formula (1986):

$$Z_{ij} = \ln(Y_{ij}/g(Y_j))$$

where Z_{ij} is the standardized peak area i for individual j, Y_{ij} is the peak area i for individual j and $g(Y_j)$ is the geometric mean of all peaks for individual j. The homogeneity of variance of these variables was tested with Levene's test and Bonferroni's correction was applied. Only the variables with homogeneous variances were considered (n=14 peaks for CHCs, n=12 for eggs). These variables were entered in a backward stepwise discriminant analysis (DA). For this analysis, non-immobilized individuals were grouped according to their reproductive status (i.e. reproductives and nonreproductives, identified by dissection, no later than 2 days after extraction), independently of the treatment. Thus, the CHC profiles of three categories of workers were compared: nonreproductive workers (including impeded, control and free workers, n=29), reproductive impeded workers (n=9), and immobilized impeded workers (n=18). Queens (n=4) were included in the model as ungrouped cases.

For the eggs, the transformed areas were used as variables in a principal components analysis (PCA) to reduce the number of variables. The two extracted principal components together explained 56% of the variance and were then used as variables in a DA. Hydrocarbons on the surface of the eggs varied qualitatively compared to the cuticular profiles, with the eggs having more complex profiles (data not shown). However, the composition of cuticle and egg surface overlapped and hydrocarbons found on the cuticle also represented the major components of the egg surface. The relative proportions of hydrocarbons varied between egg surface and cuticle and our peak selection rule produced a different set of variables in the two analyses.

Behavioral data

In experiment 1, the antennation and bite counts received by reproductive and nonreproductive workers were compared in each colony using a Wilcoxon paired sample test. In order to reduce the probability of making a type I error owing to multiple comparisons, Bonferroni's correction was applied.

In experiment 2, we used a log-linear model to test our prediction that free workers will police impeded workers. We included three factors in the model: colony (D, I′, I″, J), attacker (free or impeded worker), and target (free or impeded worker). We predicted that the type of target should significantly contribute to our model explaining the variation observed in the aggressive interactions. If impeded workers activate ovaries and if this can be detected, they should be attacked by their free nestmates, while the latter should not attack other free workers. A second prediction is that impeded workers will compete together over male production, and thus they should mainly be aggressive towards other impeded individuals. Furthermore, there should be a difference in frequencies of aggressions between impeded and excluded workers, since impeded workers should be targeted by both free and other impeded workers. We thus predicted a two-way interaction between the factors "attacker" and "target".

The frequency of aggressions among workers in the different compartments were compared with Friedman's ANOVA. To confirm whether we successfully simulated orphaned conditions in compartment #1, we determined whether the proportion of reproductive and nonreproductive workers in #1 and #3 was similar. For this, the number of workers of each class in both compartments was compared with Fisher's exact test. The data are presented as averaged percentages across replicates for brevity.

To test our prediction that eggs laid by workers, but not those of queens, are destroyed by nestmates (experiment 3), we also used a log-linear model. We included three factors: fate (egg accepted or destroyed), colony membership (eggs laid by nestmates or nonnestmates), and egg type (queen laid, unviable worker-laid or viable worker-laid). We predicted that the factor "egg type" should significantly contribute to our model to explain the variation observed, and we thus expect a two-way interaction between the factors "fate" and "egg type". We used a Mann-Whitney U-test to compare the duration between the introduction and deposition in the pile of viable worker eggs and queen eggs. We used STATISTICA Version 6.0 (StatSoft, Tulsa, USA) for the analyses.

Results

Experiment 1: Discrimination between reproductive and nonreproductive workers

When introduced in queenright colonies, orphaned workers typically ran around the nest until they were stopped by the resident workers who antennated or bit them. Orphaned reproductive workers triggered significantly more antennation than orphaned nonreproductive workers in all colonies (Wilcoxon paired sample test, T_C=0.0, P_C <0.01, T_D=9.0, P_D=0.03, T_E=19.5, P_E <0.01, T_F=4.0, P_F <0.01, T_H=0.0, P_H <0.01, T_I=6.0, P_I=0.01, T_J=5.0, P_J <0.01; Fig. 2). Introduced reproductive workers were bitten significantly more often than nonreproductives in only two colonies (Wilcoxon paired sample test, T_C=0.0, P_C <0.01; T_D=26.0, P_D=0.53; T_E=71.0, P_E=0.33; T_F=4.0, P_F <0.01; T_H=41.0, P_H=0.16; T_I=8.5, P_I=0.02, NS after Bonferroni correction; T_J=28.0, P_J=0.04, NS after Bonferroni correction; Fig. 2). On some occasions, introduced reproductive workers came into contact with the queen, but she never attacked them.

Experiment 2: Policing of reproductive workers and cuticular hydrocarbons

In the queenless compartments (#1 and #3), attacks started 5.7±1.5 and 7.7±1.2 days (mean ± SD), respectively, after the beginning of the experiment (t-test, t=−0.74, df=6, P=0.48). These attacks preceded egg laying, since reproductive eggs were first laid in compartment #3 18.0± 3.6 days after separation from the queen. The number of aggressions occurring in #3 was higher than in #1. In the presence of the queen (compartment #2), there were very few aggressive interactions among the control and free workers (Table 1). These differences were significant (Friedman's ANOVA χ^2=8.0, df=2, P<0.02).

Table 1 Cumulative number of aggressive acts (antennal boxing, bites and immobilizations) in each nest compartment of the four replicates

Colony	Nest compartment		
	#1	#2	#3
D	75	1	126
I′	122	3	166
I″	311	3	312
J	199	0	541

Table 2 Directions of the immobilizations occurring in nest compartment #1

Attacker	Target	Colony			
		D	I′	I″	J
Impeded	Impeded	34	54	78	78
	Free	5	5	50	6
Free	Impeded	34	59	172	118
	Free	0	0	0	0

The most informative aggressive interactions occurred in #1. Free workers often crossed the barrier in both directions and frequently attacked impeded workers, but not other workers (either free or control; Tables 1, 2). In addition, impeded workers attacked each other at high rates. Some free workers were also targets of impeded workers, but at much lower frequencies. The two-way interaction of the factors "attacker" and "target", that confirms the occurrence of policing of impeded workers by free individuals, contributed to the log-linear model (partial association $\chi^2=109.6$, $df=1$, $P<0.01$; marginal association $\chi^2=102.1$, $df=1$, $P<0.01$). However, this was not sufficient to explain the pattern observed: the model fitting the distribution of the values requires that all single factors in our model need to be considered: colony (C), attacker (A) and target (T; partial association $\chi^2_\mathrm{C} = 172.5$, $df=3$, $P<0.01$; $\chi^2_\mathrm{A} = 7.6$, $df=6$, $P<0.01$; $\chi^2_\mathrm{T} = 516.5$, $df=1$, $P<0.01$; see Table 2). This indicates that patterns of aggressions varied among colonies, types of attackers and targets. Furthermore, each two-way interaction significantly contributed to the model (CA, TC, TA, maximum likelihood $\chi^2=3.36$, $df=3$, $P=0.34$). Removal of one of the interactions always resulted in a statistically significant deviation from the observations (CA TC, maximum likelihood $\chi^2=112.95$, $df=4$, $P<0.0001$; CA TA, $\chi^2=41.44$, $df=6$, $P<0.0001$; TC TA, $\chi^2=15.66$, $df=6$, $P<0.02$), demonstrating that these factors influenced each other.

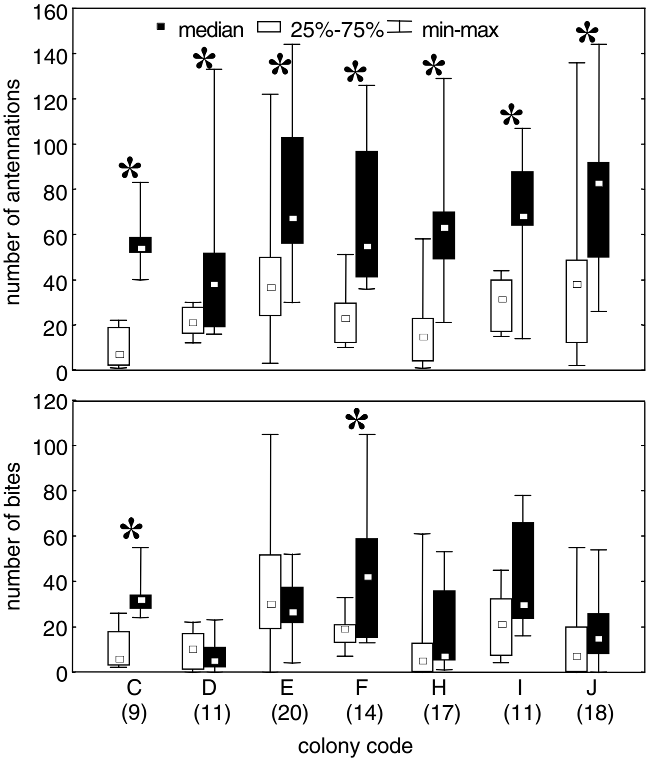

Fig. 2 Cumulative number of antennal inspections or bites directed at orphaned nonreproductive (*open boxes*) and reproductive (*solid boxes*) workers introduced in queenright colonies. The number of pairs of workers introduced is given in *parentheses*. *Asterisk* Wilcoxon test, $P<0.05$, after Bonferroni correction

Most of the immobilizations (75.0±4.8%) occurring in #1 were performed by free workers (Table 2). The immobilizations lasted several hours or days. A mean ± SD of 25.6±21.0% of the immobilized workers (eight individuals in total) were killed in the process. Because of these deaths and because a few individuals were removed for dissection, only one or two workers were immobilized at any given moment. Between 2 to 12 large and small workers performed the immobilization at any given time and there was a turnover in the individuals involved. A total of 30.5±8.5 impeded and free workers performed immobilizations.

Reproductive eggs accumulated in compartment #3 in all colonies except D. We could not determine whether impeded workers laid reproductive eggs in #1, because their eggs could be carried across the barrier and mixed with the queen's; oviposition was never observed directly. Nonetheless, ovarian dissections revealed that 22.5±3.6% (mean ± SD for 3 colonies, $n=159$ individuals dissected in total) of impeded workers reproduced; there were none in colony D. Similarly, 18.6±9.6% of the excluded workers reproduced ($n=214$ individuals dissected). Excluding D, the proportion of reproductive workers was similar in #1 and #3 (two-tailed Fisher exact test, $P_{\mathrm{I}'}=0.78$, $P_{\mathrm{I}''}=0.82$, $P_\mathrm{J}=0.20$). Thus, impeded workers reacted to queen separation similarly to the excluded individuals and our treatment successfully simulated a queenless environment. In contrast, all the control and free workers had only trophic oocytes in their ovaries ($n=144$ and $n=194$ individuals dissected, respectively). This corresponds to the normal situation in nonmanipulated colonies, in which individuals have physical contact with their queen and do not reproduce. Importantly, 65.0±33.5% of individuals (18 out of 27 in total) with reproductive oocytes in #1 were not observed to be immobilized. To determine the ovarian status of impeded workers at the time they became immobilized, we dissected 15 individuals 2.2±1.6 days after the start of their immobilization. They had variable ovarian status: their

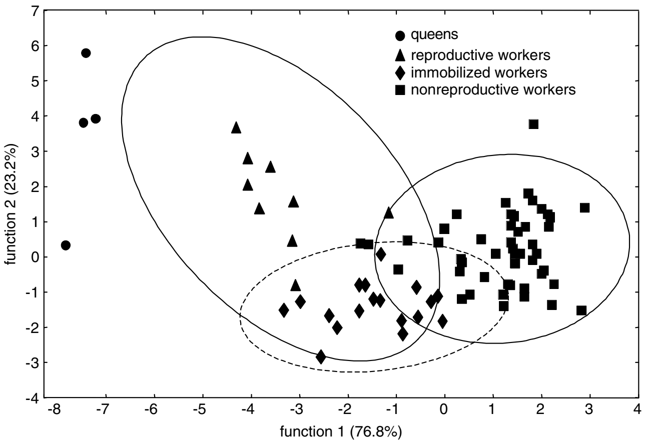

Fig. 3 Discriminant analysis of the cuticular hydrocarbons from three categories of workers in experiment 2. Categories are nonreproductive workers (including impeded, control and free workers, $n=29$), reproductive impeded workers ($n=9$), and immobilized impeded workers ($n=18$); *ellipses* represent 95% confidence intervals. Queens ($n=4$) are included in the model as ungrouped cases

ovarioles could be undeveloped ($n=7$), or contained either trophic ($n=4$) or reproductive oocytes ($n=4$; the latter included intermediate oocytes). Another 8 immobilized impeded workers were only dissected after they were released by nestmates, and 6 had reproductive oocytes. Across replicates, 8 workers have been killed following immobilization, whereas 2 survived and were nonreproductive. Thus 10 selfish individuals were successfully policed out of a total of 34 incipient reproductive workers (29.4%).

Although immobilized impeded workers exhibited variations in ovarian development, their CHC profiles were relatively similar. In the DA of the CHC profiles, immobilized workers formed a group that was intermediate between reproductive and nonreproductive workers (Fig. 3). The CHC profiles of the latter two groups were well separated in the DA (Wilks' Lambda: 0.14, $F(6,140)=39.79$, $P<0.01$), thus confirming the results obtained by Dietemann et al. (2003). The first function represented 76.8% of the variance. The best classification score was obtained for the immobilized workers (100% correct assignment), whereas one reproductive and two nonreproductive workers were misclassified with immobilized workers. A total of 94.7% of the cases were correctly classified. The compounds selected by the model were: 3-methylheptacosane, 9, 11, 13, 15-methylheptatriacontane and 13, 23; 11, 23; 9, 23-dimethylheptatriacontane.

Experiment 3: Egg policing and surface hydrocarbons

The log-linear model that best explained the data included the two-way interaction FE (fate-egg type), together with the interaction CE (colony membership-egg type; maximum likelihood $\chi^2=1.76$, $P=0.62$). The first interaction indicates that eggs laid by the different groups are destroyed at different rates. Examination of the marginal tables reveals that only unviable eggs laid by recently orphaned workers are destroyed in significant proportion; viable worker eggs are accepted as often as queen-laid eggs (Table 3). The two-way interaction CE might be due to sampling bias included in the factor C: nestmates and nonnestmates might have produced different proportions of each type of eggs. Importantly, the model does not use the interaction FC, indicating that eggs are not discriminated against when laid by nonnestmates.

Table 3 Marginal table for "fate" by "egg type" as specified by the log-linear model. The model included the two-way interactions "fate" by "egg type" and "colony membership" by "egg type", demonstrating that eggs are destroyed differentially according to their origin and that workers of different colonies produced different proportion of viable or unviable eggs

Egg type	Fate		
	Accepted	Destroyed	Total
Queen-laid	213	7	220
Viable worker-laid	159	6	165
Unviable worker-laid	43	53	96

Worker-laid eggs were repeatedly carried and dropped in the brood chamber, and were thus deposited in the pile after a significantly longer handling time than queen eggs (335±312 vs 107±72 s respectively; Mann-Whitney test, $n_{\text{queen}}=15$, $n_{\text{worker}}=15$, $U=49.5$, $P<0.01$), indicating discrimination between the two types of eggs by workers. Queens sometimes inspected the introduced eggs, but never destroyed them.

The three types of reproductive eggs extracted (queen-laid, viable and unviable worker-laid) had statistically distinct surface hydrocarbon profiles, but there was overlap between groups and only 70.5% of eggs were correctly classified a posteriori (Fig. 4; Wilk's Lambda=0.43, $F(4,80)=10.4$, $P<0.01$). The profile of viable worker eggs was closer to that of queen eggs (squared Mahalanobis distances: queen/unviable worker eggs=4.1; queen/viable worker eggs=2.8; viable/unviable worker eggs=3.3).

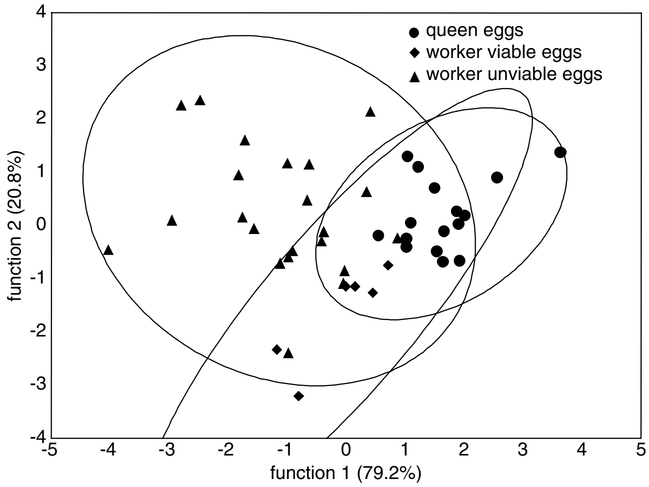

Fig. 4 Discriminant analysis of cuticular hydrocarbons of queen eggs ($n=16$), viable and unviable worker-laid eggs ($n=6$ and $n=22$, respectively); *ellipses* represent 95% confidence intervals

Discussion

Workers of *Myrmecia gulosa* can distinguish between orphaned nonreproductive and reproductive individuals when these are transferred to a queenright nest. Furthermore, using a protocol adapted from Tsuji et al. (1999), we demonstrated that workers can often identify and successfully police incipient reproductive workers by aggression. The latter did not always have reproductive oocytes, but they had a distinct pattern of cuticular hydrocarbons. This suggests that police workers use cuticular hydrocarbons to detect those individuals that become reproductively active. Differences in the surface hydrocarbons of viable queen and worker eggs may also be recognized, since worker eggs were handled longer than queen eggs. However, this information is not used for egg policing, since both types of eggs were accepted in the discrimination experiment.

Discrimination between reproductive and nonreproductive workers

When introduced in queenright colonies, reproductive workers were antennated more extensively than nonreproductive workers, indicating that discrimination according to reproductive status occurs. This result corroborates those of an earlier bioassay in which hydrocarbon extracts of reproductive workers elicited more interest than those of nonreproductive workers (Dietemann et al. 2003). In the experiment presented here, the frequency of bites received by reproductive individuals was significantly higher in only two colonies. However, bite frequency might have increased in the other colonies, if the experiment had run longer.

Queen presence and worker policing

A crucial result of experiment 2 is that we could successfully mimic queen absence in compartment #1, from which the queen was excluded. In #1, the impeded workers had no direct access to the queen but interacted with free workers that regularly crossed the barrier between #1 and #2. These impeded workers behaved as orphaned since they started antennal boxing and biting at the same time as the excluded workers, which had no direct contact with either the queen or free workers. Furthermore, an equal proportion of individuals developed their ovaries in these two groups in three out of four colonies. Thus, workers apparently need direct contact with the queen to recognize her presence, as was shown for other ants (e.g. Gobin et al. 1999; Liebig et al. 1999; Tsuji et al. 1999; Dietemann and Peeters 2000).

In colony D, impeded and excluded workers did not develop their ovaries during the 31 days of our experiment. Their behavior did not differ from that of the other colonies, except for the fact that attacks and immobilizations started later. This colony thus showed a slower response to the loss of queen contact. In *Camponotus floridanus*, workers also showed variable reaction delays to orphanage: out of 38 worker groups, 55% did not lay eggs after 158 days, whereas it took only 60 days for the first eggs to be laid in the fastest responding groups (Endler et al. 2004).

In all our replicates, some of the impeded workers were immobilized by free workers. Unlike impeded and excluded workers, the free workers were in a queenright context; they were not competing for direct reproduction and were not aggressive to each other. However, they recognized the presence of incipient reproductive workers and policed them. Only 29.4% of the incipient reproductives were prevented from reproducing as a result of successful policing. Furthermore, in each replicate some incipient reproductives were not observed to be immobilized. Their CHC profiles were typical for reproductive individuals, and their behavior was not observably different; we cannot explain why they escaped policing. Similarly, policing seems to be imperfect in some ants (Kikuta and Tsuji 1999; Iwanishi et al. 2003) and wasps (e.g. Foster and Ratnieks 2000).

Cuticular hydrocarbons and worker policing

In a previous study we showed that workers discriminate reproductive from nonreproductive individuals based on their cuticular hydrocarbons (Dietemann et al. 2003). We thus expected that cuticular hydrocarbons are also used in a policing context. Based on the hydrocarbon molecules selected for our discriminant analysis, the CHC profiles of immobilized individuals were distinct from those of nonreproductive workers and were intermediate between those of nonreproductive and reproductive workers (Fig. 3). A similar pattern was found in unmanipulated colonies (V. Dietemann, unpublished data), indicating that this is not an experimental artifact.

Although immobilized workers did not exhibit a consistent pattern of ovarian activity, they had similar CHC profiles. Thus, in *M. gulosa* as well as in other ants in which cuticular hydrocarbon profiles started to change soon after orphanage (e.g. Peeters et al. 1999; Liebig et al. 2000), the information encoded in cuticular hydrocarbons can provide accurate information about the physiological changes leading to reproduction although ovarian status might differ. Indeed, cuticular profiles are correlated to the level of gonadotropic hormones (Cuvillier-Hot et al. 2004b; Sledge et al. 2004) which can change well before the onset of oviposition (Cuvillier-Hot et al. 2004b).

The changes in the CHC profile and the behavioral pattern are closely linked in *M. gulosa*. Thus, it is likely that police workers compare the CHC profiles of queens and workers and detect incipient reproductive workers (i.e. individuals at an early stage of their reproductive activity when their CHC profile is still in transition toward that of fully reproductive workers). The discriminant analysis showed that enough information is encoded in the CHCs to identify incipient reproductives, and the low effectiveness of policing observed is not likely to be due to informational constraint (Beekman et al. 2003; Boomsma et al. 2003).

Egg policing

Viable worker eggs and queen-laid eggs were equally well accepted in our policing assay, indicating an absence of egg policing. A large proportion (47%) of the unviable eggs laid by incipient reproductives was immediately destroyed when introduced in queenright colonies. This cannot be considered as worker policing (sensu Ratnieks 1988), since the destruction of unviable eggs does not reduce the fitness of the egg-layer.

The absence of egg policing in *M. gulosa* contrasts with other studies in ants. In *Diacamma* sp. from Japan (Nakata and Tsuji 1996), *Camponotus floridanus* (Endler et al. 2004), *Pachycondyla inversa* (D'Ettorre et al. 2004), and *Formica fusca* (Helanterä and Sundström 2005) workers effectively police eggs laid by other workers. In the queenless species *Dinoponera quadriceps*, it is the dominant worker that destroys the eggs laid by subordinate individuals (Monnin and Peeters 1997). In *D. quadriceps* (Monnin and Peeters 1997), *C. floridanus* (Endler et al. 2004) as well as in *P. inversa* (D'Ettorre et al. 2004), surface hydrocarbon profiles of the eggs differ between the categories of egg-layers and this corresponds to the behavioral pattern of egg destruction observed. The use of surface hydrocarbons as egg recognition cues by workers has been demonstrated in *C. floridanus* (Endler et al. 2004).

In *M. gulosa* the hydrocarbon profile of unviable worker eggs is distinct from that of queen eggs. Although they are statistically separated from each other, there is a larger overlap of queen and viable worker egg profiles compared to the species cited above. Thus, the information encoded in the hydrocarbons may be insufficient to avoid recognition mistakes and destruction of sisters (Nonacs and Carlin 1990). As a result, workers might not be able to police eggs without great fitness costs.

Conclusion

Using Tsuji's protocol, incipient reproductive workers and police workers were continuously in contact with each other, simulating a natural situation where policing can occur as soon as information linked with selfish reproduction is detected. Although we had only a limited number of colonies and although the level of worker reproduction was variable, the results were consistent and demonstrated that incipient reproductives are policed. No evidence for queen policing was found.

A limitation of our experiment was the presence of a high number of impeded workers which simultaneously activated their ovaries and competed for reproduction. This is likely to affect the assessment of policing efficiency in an unpredictable manner. It could explain why some workers escaped policing (worker policing may be more effective when only one or a few individuals try to reproduce at one time) or caused an overestimation of policing as some impeded individuals participated in immobilizations.

Provided that our estimate is correct, approximately 29% of the incipient reproductive workers are successfully policed. Several models predict that worker policing in general may be relatively ineffective even though it is evolutionary stable (e.g. Ratnieks 1988; Wenseleers et al. 2004). The occurrence of worker reproduction is affected by its cost-benefit ratio. If the potential cost of being killed when attempting to reproduce is higher than the expected benefit of successful egg laying, a worker should not try to reproduce. Thus, we may expect higher levels of competition and policing in colonies where workers have a high potential benefit for attempting to reproduce (Foster 2004). This should be the case in small colonies with individuals having a high reproductive potential, such as queenless ants (e.g. Monnin and Ratnieks 2001). In *M. gulosa* however, workers have a relatively low reproductive potential and they cannot replace the queen. Their potential fitness gain is the production of a few sons that may not have the opportunity to mate if produced outside the mating season. This low benefit is counterbalanced by the 25.6% probability of being killed by nestmates, which may be sufficient to select for self-restraint and result in the absence of worker reproduction in a queenright context. The occurrence of self-restraint can thus explain the absence of policing on eggs independently of informational constraints. Indeed, in natural queenright conditions, individuals might never be exposed to workers' eggs and egg policing would not be selected.

The effectiveness of worker policing is an important factor in the regulation of reproduction in hymenopteran societies. However, understanding patterns of reproduction requires the knowledge of a whole set of parameters including the potential costs and benefits of attempted reproduction as well as informational constraints.

Acknowledgements We are grateful to Michael Schwarz, Katja Hogendoorn, Remko Leijs, Steve Shattuck, Archie McArthur and Russell for their hospitality and assistance during fieldwork. Ben Oldroyd, Thibaud Monnin, Liselotte Sundström and three anonymous referees gave valuable criticisms on earlier drafts. This work was funded by the Deutsche Forschungsgemeinschaft SFB 554 (C3) and the Graduiertenkolleg 'Grundlagen des Arthropodenverhaltens'. The experiments comply with the current laws of Germany. The ants were collected under scientific investigation license A2247 issued by National Parks and Wildlife Service of New South Wales, and exported under permits PWS P993042 and PWS 20002478 issued by Environment Australia.

References

Aitchison J (1986) The statistical analysis of compositional data. Monographs in statistics and applied probability. Chapman and Hall, London

Beekman M, Komdeur J, Ratnieks F (2003) Reproductive conflicts in social animals: who has power? Trends Ecol Evol 18:277–282

Boomsma JJ, Nielsen J, Sundström L, Oldham NJ, Tentschert J, Petersen HC, Morgan ED (2003) Informational constraints on optimal sex allocation in ants. Proc Natl Acad Sci USA 100:8799–8804

Bourke AFG (1988) Worker reproduction in the higher eusocial Hymenoptera. Q Rev Biol 63:291–311

Bourke AFG (1999) Colony size, social complexity and reproductive conflict in social insects. J Evol Biol 12:245–257

Choe J (1988) Worker reproduction and social evolution in ants (Hymenoptera: Formicidae). In: Trager JC (ed) Advances in myrmecology. Brill, Leiden, pp 163–187

Cole BJ (1986) The social behaviour of *Leptothorax allardycei* (Hymenoptera: Formicidae): time budgets and the evolution of worker reproduction. Behav Ecol Sociobiol 18:165–173

Cuvillier-Hot V, Cobb M, Malosse C, Peeters C (2001) Sex, age and ovarian activity affect cuticular hydrocarbons in *Diacamma ceylonense*, a queenless ant. J Insect Physiol 47:485–493

Cuvillier-Hot V, Lenoir A, Crewe R, Malosse C, Peeters C (2004a) Fertility signaling and reproductive skew in queenless ants. Anim Behav 68:1209–1219

Cuvillier-Hot V, Lenoir A, Peeters C (2004b) Reproductive monopoly enforced by sterile police workers in a queenless ant. Behav Ecol 15:970-975

D'Ettorre P, Heinze J, Ratnieks FLW (2004) Worker policing by egg eating in the ponerine ant *Pachycondyla inversa*. Proc R Soc Lond 271:1427–1434

Dietemann V, Peeters C (2000) Queen influence on the shift from trophic to reproductive eggs laid by workers of the ponerine ant *Pachycondyla apicalis*. Insect Soc 47:223–228

Dietemann V, Peeters C, Hölldobler B (2002) Caste specialization and differentiation in reproductive potential in the phylogenetically primitive ant *Myrmecia gulosa*. Insect Soc 49:289–298

Dietemann V, Peeters C, Liebig J, Thivet V, Hölldobler B (2003) Cuticular hydrocarbons mediate discrimination of reproductives and nonreproductives in the ant *Myrmecia gulosa*. Proc Natl Acad Sci USA 100:10341–10346

Dietemann V, Peeters C, Hölldobler B (2005) Role of the queen in regulating reproduction in the bulldog ant *Myrmecia gulosa:* control or signalling? Anim Behav 69:777–784

Endler A, Liebig J, Schmitt T, Parker J, Jones G, Schreier P, Hölldobler B (2004) Surface hydrocarbons of queen eggs regulate worker reproduction in a social insect. Proc Natl Acad Sci USA 101:2945–2950

Foster KR (2004) Diminishing returns in social evolution: the not-so-tragic commons. J Evol Biol 17:1058–1072

Foster KR, Ratnieks FLW (2000) Social insects—facultative worker policing in a wasp. Nature 407:692–693

Foster KR, Ratnieks FLW (2001) Worker policing in the common wasp *Vespula vulgaris*. Proc R Soc Lond 268:169–174

Gobin B, Billen J, Peeters C (1999) Policing behaviour toward virgin egg layers in a polygynous ponerine ant. Anim Behav 58:1117–1122

Gu X, Quilici D, Juarez P, Blomquist GJ, Schal C (1995) Biosynthesis of hydrocarbons and contact pheromone and their transport by lipophorin in females of the German cockroach (*Blattella germanica*). J Insect Physiol 41:257–267

Halling LA, Oldroyd BP, Wattanachaiyingcharoen W, Barron AB, Nanork P, Wongsiri S (2001) Worker policing in the bee *Apis florea*. Behav Ecol Sociobiol 49:509–513

Hammond RL, Bruford MW, Bourke AFG (2003) Male parentage does not vary with colony kin structure in a multiple-queen ant. J Evol Biol 16:446–455

Hannonen M, Sledge MF, Turillazzi S, Sundström L (2002) Queen reproduction, chemical signalling and worker behaviour in polygyne colonies of the ant *Formica fusca*. Anim Behav 64:477–485

Hartmann A, Wantia J, Torres JA, Heinze J (2003) Worker policing without genetic conflict in a clonal ant. Proc Natl Acad Sci USA 100:12836–12840

Haskins CP, Haskins EF (1950) Notes on the biology and social behaviour of the archaic ponerine ants of the genera *Myrmecia* and *Promyrmecia*. Ann Ent Soc Am 43:461–491

Heinze J, Stengl B, Sledge M (2002) Worker rank, reproductive status and cuticular hydrocarbon signature in the ant, *Pachycondyla* cf. *inversa*. Behav Ecol Sociobiol 52:59–65

Helanterä H, Sundström L (2005) Worker reproduction in the ant *Formica fusca*. J Evol Biol 18:162–171

Iwanishi S, Hasegawa E, Ohkawara K (2003) Worker oviposition in the myrmicine ant *Aphaenogaster smythiesi japonica* Forel. Anim Behav 66:513–519

Kikuta N, Tsuji K (1999) Queen and worker policing in the monogynous and monoandrous ant, *Diacamma* sp. Behav Ecol Sociobiol 46:180–189

Liebig J, Peeters C, Hölldobler B (1999) Worker policing limits the number of reproductives in a ponerine ant. Proc R Soc Lond 266:1865–1870

Liebig J, Peeters C, Oldham NJ, Markstädter C, Hölldobler B (2000) Are variations in cuticular hydrocarbons of queens and workers a reliable signal of fertility in the ant *Harpegnathos saltator*? Proc Natl Acad Sci USA 97:4124–4131

Monnin T, Peeters C (1997) Cannibalism of subordinates' eggs in the monogynous queenless ant *Dinoponera quadriceps*. Naturwissenschaften 84:499–502

Monnin T, Peeters C (1999) Dominance hierarchy and reproductive conflicts among subordinates in a monogynous queenless ant. Behav Ecol 10:323–332

Monnin T, Ratnieks FLW (2001) Policing in queenless ponerine ants. Behav Ecol Sociobiol 50:97–108

Monnin T, Malosse C, Peeters C (1998) Solid-phase microextraction and cuticular hydrocarbons differences related to reproductive activity in queenless ant *Dinoponera quadriceps*. J Chem Ecol 24:473–490

Nakata K, Tsuji K (1996) The effect of colony size on conflict over male-production between gamergate and dominant workers in the ponerine ant *Diacamma* sp. Ethol Ecol Evol 8:147–156

Nonacs P, Carlin NF (1990) When can ants discriminate the sex of brood? A new aspect of queen-worker conflicts. Proc Natl Acad Sci USA 87:9670–9673

Ogata K, Taylor RW (1991) Ants of the genus *Myrmecia* Fabricius: a preliminary review and key to the named species (Hymenoptera: Formicidae: Myrmeciinae). J Nat Hist 25:1623–1673

Oldroyd BP, Halling LA, Good G, Wattanachaiyingcharoen W, Barron AB, Nanork P, Wongsiri S, Ratnieks FLW (2001) Worker policing and worker reproduction in *Apis cerana*. Behav Ecol Sociobiol 50:371–377

Pamilo P (1991) Evolution of colony characteristics in social insects. II. Number of reproductive individuals. Am Nat 138:412–433

Peeters C (1997) Morphologically "primitive" ants: comparative review of social characters, and the importance of queen-worker dimorphism. In: Choe J, Crespi BJ (eds) The evolution of social behaviour in insects and arachnids. Cambridge University Press, Cambridge, pp 372–391

Peeters C, Monnin T, Malosse C (1999) Cuticular hydrocarbons correlated with reproductive status in a queenless ant. Proc R Soc Lond 266:1323–1327

Pirk CWW, Neumann P, Ratnieks FLW (2003) Cape honeybees, *Apis mellifera capensis*, police worker-laid eggs despite the absence of relatedness benefits. Behav Ecol 14:347–352

Ratnieks FLW (1988) Reproductive harmony via mutual policing by workers in eusocial Hymenoptera. Am Nat 132:217–236

Ratnieks FLW (1993) Egg-laying, egg-removal, and ovary development by workers in queenright honey bee colonies. Behav Ecol Sociobiol 32:191–198

Ratnieks FLW (1995) Evidence for a queen-produced egg-marking pheromone and its use in worker policing in the honey bee. J Apic Res 34:31–37

Ratnieks FLW, Visscher PK (1989) Worker policing in the honey bee. Nature 342:796–797

Schal C, Gu X, Burns EL, Blomquist GJ (1994) Patterns of biosynthesis and accumulation of hydrocarbons and contact sex pheromone in the female German cockroach, *Blattella germanica*. Arch Insect Biochem 25:375–391

Sledge M, Boscaro F, Turillazzi S (2001) Cuticular hydrocarbons and reproductive status in the social wasp *Polistes dominulus*. Behav Ecol Sociobiol 49:401–409

Sledge M, Trinca I, Massolo A, Boscaro F, Turillazzi S (2004) Variation in cuticular hydrocarbon signatures, hormonal correlates and establishment of reproductive dominance in a polistine wasp. J Insect Physiol 50:73–83

Tsuji K, Egashira K, Hölldobler B (1999) Regulation of worker reproduction by direct physical contact in the ant *Diacamma* sp. from Japan. Anim Behav 58:337–343

Villesen P, Boomsma JJ (2003) Patterns of male parentage in the fungus-growing ants. Behav Ecol Sociobiol 53:246–253

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

Wenseleers T, Helanterä H, Hart A, Ratnieks FLW (2004) Worker reproduction and policing in insect societies: an ESS analysis. J Evol Biol 17:1035–1047