Specialization in policing behaviour among workers in the ant *Pachycondyla inversa*

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Most animal societies are non-clonal and thus subject to conflicts. In social insects, conflict over male production can be resolved by worker policing, i.e. eating of worker-laid eggs (WLE) or aggression towards reproductive workers. All workers in a colony have an interest in policing behaviour being expressed, but there can be asymmetries among workers in performing the actual behaviour. Here, we show that workers of the ant *Pachycondyla inversa* specialize in policing behaviour. In two types of behavioural assays, workers developed their ovaries and laid eggs. In the first experiment, reproductive workers were introduced into queenright colonies. In the second experiment, WLE were introduced. By observing which individuals policed, we found that aggressive policing was highly skewed among workers that had opportunity to police, and that a similar tendency occurred in egg policing. None of the policing workers had active ovaries, so that policing did not incur a direct selfish benefit to the policer. This suggests that policing is subject to polyethism, just like other tasks in the colony. We discuss several hypotheses on the possible causes of this skew in policing tasks. This is the first non-primate example of specialization in policing tasks without direct selfish interests.

Keywords: division of labour; worker reproduction; worker policing; egg eating; aggression

1. INTRODUCTION

Division of labour, the specialization of cooperative labour in tasks to increase efficiency, is used throughout human and non-human societies (e.g. Taylor 1911; Oster & Wilson 1978; Sudd & Franks 1987). Insect societies are characterized by a reproductive division of labour. One or few individuals in a colony reproduce (the queen(s) and sometimes some of the workers), whereas most of the workers take care of all other tasks (Oster & Wilson 1978; Bourke & Franks 1995). Among workers, there is an additional division of labour, with different individuals specializing on a subset of the colony's tasks, for anything between a few days and their entire life. Three factors enhance the efficiency in division of labour in social insects. Firstly, experience improves skills when performing a particular task. For instance, foraging trails may be found more easily by a particular individual when it has already used the trail. Secondly, there may be improvement in spatial and temporal efficiencies (no time is lost in switching between tasks), and thirdly, morphological specialization can enhance individual efficiency, e.g. soldier castes for defence (Bourke & Franks 1995).

As in any cooperative group of non-clonal individuals, members of insect societies may pursue selfish interests, and hence are potentially involved in reproductive conflicts (Ratnieks *et al.* 2006). One of the most striking examples is the conflict over male production (Trivers &

Hare 1976; Starr 1984; Woyciechowski & Łomnicki 1987; Bourke 1988; reviewed by Ratnieks *et al.* 2006). Since workers of most species of social insects are capable of laying unfertilized male-destined eggs, and workers are more related to their own sons ($r=0.5$) than they are to the sons of other members of the colony ($r\leq 0.375$), worker–queen and worker–worker conflicts over the production of males can arise. This potential conflict can be resolved by worker policing (Ratnieks 1988), i.e. workers preventing each other from reproducing by eating worker-laid eggs or by aggressively suppressing reproductive workers. Worker policing can be selected for on relatedness grounds at an effective queen-mating frequency higher than two, as workers are then more closely related to the queen's sons ($r=0.25$) than to other workers' sons ($r<0.25$) (Ratnieks 1988). Worker policing may also be selected for at lower queen-mating frequencies, when worker reproduction reduces colony efficiency (cf. Ratnieks 1988; Pirk *et al.* 2003; Dampney *et al.* 2004; Hammond & Keller 2004; Wenseleers & Ratnieks 2006).

Worker policing by egg eating was first observed in the honeybee *Apis mellifera* (Ratnieks & Visscher 1989), and later in several species of bees, ants and wasps (e.g. Foster & Ratnieks 2000; Oldroyd *et al.* 2001; Helanterä & Sundström 2005; reviewed by Ratnieks *et al.* 2006). Worker policing by aggression towards reproductive workers has also been observed in several species of social insects, using a split–fusion method: splitting colonies into two parts and reunifying them again when queenless workers developed their ovaries (e.g. Visscher & Dukas 1995; Gobin *et al.* 1999; Kikuta & Tsuji 1999; Liebig *et al.* 1999; Monnin & Peeters 1999).

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All workers in a colony have an interest in policing behaviour being expressed. However, it can be expected that there is heterogeneity among workers in performing the actual behaviour, if resources or policing cost vary among individuals (Frank 1996). Hitherto, little information is available on which individuals actually are performing policing behaviour, except for studies on third-party policing in primates (Flack *et al.* 2006 and references therein). In social insects, Gobin *et al.* (1999) mentioned that policing in the ant *Gnamptogenys menadensis* was performed by workers with less developed ovaries. Similarly, in the ant species *Streblognathus peetersi*, several low-ranking workers immobilize overthrown gamergates (Cuvillier-Hot *et al.* 2004). On the other hand, Monnin & Peeters (1999) have shown that workers of the queenless ant *Dinoponera quadricaps* with ranks 3–10, all initiate immobilization at an equal rate, but they did not specify how workers with lower ranks behave. Recently, Wenseleers *et al.* (2005) found that in the wasp *Dolichovespula sylvestris*, there is policing with direct selfish interests, since the few workers that behave aggressively to other reproductive workers were laying eggs themselves. Saigo & Tsuchida (2004) suggested a similar mechanism in *Polistes chinensis antennalis*. Finally, Stroeymeyt *et al.* (2006) showed that policing by aggression is performed almost exclusively by high-ranking individuals in the ant *Temnothorax unifasciatus*.

The ponerine ant *Pachycondyla inversa* was one of the first ants in which worker policing by egg eating was shown (D'Ettorre *et al.* 2004), but policing by aggression towards reproductive workers has not been documented. The aim of the present study was to examine if policing by aggression occurs and if there is a division of labour in policing behaviour, with regard to egg eating or aggression towards reproductive workers.

2. MATERIAL AND METHODS

(a) Study organisms

Colonies of *P. inversa* (cf. Lucas *et al.* 2002) (Hymenoptera: Formicidae: Ponerinae) generally have a few dozen workers and several morphologically distinct queens. Polygyny in *P. inversa* is facultative and primary, i.e. when queens jointly found a colony they remain together after the workers hatch (Trunzer *et al.* 1998; Heinze *et al.* 2001; Kolmer *et al.* 2002; D'Ettorre *et al.* 2005). Moreover, monogynous colonies may contain workers which are daughters of queens that have died or left (Kellner *et al.* in press), which means that these colonies can be effectively polygynous, even if only one queen is actually present.

Ants were collected in November of two consecutive years from a cocoa plantation near Ilhéus, Bahia, Brazil. In 2004, the colonies were transferred to Regensburg, Germany (hereafter 'laboratory colonies'), where the experiments were performed; in 2005, the colonies were taken to a laboratory on the plantation (hereafter 'plantation colonies') and the experiments started almost immediately after collection.

(b) Laboratory colonies

Five monogynous and two polygynous colonies of *P. inversa* (mean $\pm$ s.e. = 28 ± 3 workers) were kept in a climate room under near-natural conditions (27°C and 60% humidity, 12 h L : 12 h D photoperiod) in plastic boxes (19.0 × 19.0 × 9.0 cm) with a moist plaster floor. A chamber (6.0 × 6.0 × 1.5 cm) in the plaster served as the nest cavity. Food (diluted

honey and cockroaches, *Nauphoeta cinerea*) was provided thrice a week, and water was provided *ad libitum*. Three of the monogynous colonies (L1–L3) were used in a pilot experiment, which indicated that workers can detect ovary development of other workers. The other four colonies (table 2, L4–L7) were exclusively used in aggressive-policing bioassays.

(c) Plantation colonies

Five monogynous, five polygynous and seven queenless colonies of *P. inversa* (mean $\pm$ s.e. = 39 ± 3 workers) were kept in round plastic boxes (ϕ , 21.1 cm and h, 6.9 cm) with a moist plaster floor under natural conditions for humidity, temperature and day–night cycle. Two chambers (2.8 × 2.8 × 0.9 cm) in the plaster served as nest cavities. Food (diluted honey, termites and crickets) was provided thrice a week, and water was provided *ad libitum*. In *P. inversa*, worker policing by egg eating occurs both in monogynous and polygynous colonies, but it has been shown to be slightly more efficient in polygynous colonies (D'Ettorre *et al.* 2004). Thus, only polygynous colonies (table 3, P6–P10) were used as discriminators in the egg-policing bioassays, whereas the monogynous colonies (table 2, P1–P5) were used as discriminators in aggressive-policing bioassays and as sources of eggs in the egg-policing bioassays. Queenless colonies were exclusively used as sources of eggs in egg-policing bioassays.

(d) Aggressive-policing bioassays

We used a protocol that was slightly different from previous split-and-fusion experiments. Usually, colonies are divided in a queenright and a queenless group, and after some workers in the queenless group start egg-laying, the two groups are reunited and policing behaviour is observed (Visscher & Dukas 1995; Kikuta & Tsuji 1999; Liebig *et al.* 1999; Hartmann *et al.* 2003). In these studies, all reproductive workers had to be assessed simultaneously. Instead, we opted for individual introduction of queenless workers into the queenright group, which gives a good estimate of how colonies react to rare occasions of workers with developed ovaries.

All the four laboratory colonies (L4–L7, details in table 2) were split into a queenright (QR) and a queenless (QL) part, and kept in the same closed box with a wired mesh between the two parts. In this way, the QL workers could detect any volatile pheromone produced by the queen, but not touch her. Similarly, the five monogynous plantation colonies (P1–P5, details in table 2) were split, but here the QR and QL parts were kept in separate boxes. All brood was kept in the QR parts, as it has been shown that ant larvae and eggs can inhibit ovary development (Heinze *et al.* 1996; Endler *et al.* 2004). Workers of both the QR and QL were marked individually with dots of enamel paint.

After egg-laying by the QL workers had started, workers of the QL part were introduced one by one into the QR part and observed for 5 min, during which the received aggression was quantified, and then they were removed again. QL workers were introduced approximately 5 cm from the brood area, but as a consequence of the manipulation, they usually moved fast throughout the entire box, so that all QR workers had a chance of encountering the introduced worker. Aggression was recorded as the number of bites and stings received (hereafter 'number of attacks'). Moreover, the individuals that were performing the aggressive-policing tasks were recorded for all colonies.

After all QL workers had been introduced into the QR part, the colonies were frozen at -20°C . The QL workers of both

laboratory and plantation colonies were dissected under a stereomicroscope to check ovary development, as were the QR workers of laboratory colonies. Ovary development was recorded as the sum of lengths of developing oocytes.

(e) Egg-policing bio-assays

The five polygynous plantation colonies (P6–P10, details in table 3) were used as egg-discriminator colonies in egg-policing trials, following the protocol of D'Ettore *et al.* (2004). This study showed that *P. inversa* workers discriminate between worker-laid eggs (WLE) and queen-laid eggs (QLE) regardless of the colony origin. Each discriminator colony received QLE from each of the five monogynous plantation colonies and WLE from each of the seven queenless plantation colonies. Hence, all the eggs were hetero-colonial. After introduction into the discriminator colony, ca 1 cm from a nest cavity, eggs could either be eaten (rejected) or transported to the brood area (accepted). All workers in these colonies were individually marked and it was observed which individuals antennated, manipulated and ate each egg, to see if policing behaviour was random or skewed among individuals.

(f) Division of policing tasks

In the both types of bio-assay, only workers that interacted with at least one reproductive worker or one egg that was policed at the end of the trial, and thus had the opportunity to police, were included in the analyses of skew (hereafter 'potential policers'). Workers that actually performed policing tasks are referred as 'actual policers' (AP), and those that had no interaction with any reproductive workers (in aggressive-policing assays) or eggs (in egg-policing assays) are referred as 'non-interacting workers'.

After the bio-assays were finished, all AP from the discriminator colonies (total=52) and non-policing workers (NP; both potential policers and non-interacting workers; total=35) were frozen at -20°C and dissected to assess the state of their ovaries.

(g) Statistics

In order to test if there was a correlation between ovary development of queenless workers and the number of attacks they received in the aggressive-policing bio-assays, we used a generalized linear model (GLZ) in the program SAS v. 9.1. The model used number of attacks a worker received ('attacks') as dependent variable, 'colony' as a grouping variable, and the degree of ovary development of a worker ('ovary') as a continuous explanatory variable. Since attacks is a discrete variable, and the data were not normally distributed, the model assumed a negative binomial distribution.

We used the binomial skew index (*B*-index; Nonacs 2000) in the program SKEW CALCULATOR 2003 (www.obee.ucla.edu/Faculty/Nonacs), to test if the observed distribution of policing tasks among workers was different from random sharing of tasks. Apart from calculating the *B*-value (range, 0–1; 0, random sharing; 1, monopoly by one individual), it calculates a probability level for each colony that their observed *B*-values are due to random chance by simulation. Furthermore, the program simulates a random distribution across all colonies to determine the probability level of the observed mean *B*-values. All of these probabilities are one-tailed. In our case, this method is more suitable than fitting the data to a Poisson-distribution using a χ^2 -test, as the *B*-index allows single colonies to be analysed. The other analyses were done with STATISTICA v. 7.1.

Table 1. Results of a generalized linear model to test for the correlation between ovary development and the number of received attacks in aggressive-policing bio-assays.

variable	deviance	d.f.	χ^2	<i>p</i>
<i>laboratory colonies</i>				
null model	1304.53	52		
full model	55.55	45		
ovary		1	38.38	<0.0001
colony		3	9.82	0.0201
ovary $\times$ colony		3	8.12	0.0437
<i>plantation colonies</i>				
null model	2116.57	100		
full model	102.2	91		
ovary		1	51.10	<0.0001
colony		4	70.32	<0.0001
ovary $\times$ colony		4	24.98	<0.0001
<i>all colonies</i>				
null model	3442.62	153		
full model	157.79	136		
ovary		1	83.58	<0.0001
colony		8	91.36	<0.0001
ovary $\times$ colony		8	35.57	<0.0001

3. RESULTS

(a) Queen signal

In the laboratory colonies (L4–L7), more workers had developed ovaries in the QL than in the QR (Fisher's exact tests (two-tailed); colony L4: QL: 9/14 workers, QR: 0/17 workers, $p=0.0001$; colony L5: QL: 6/15, QR: 0/15, $p=0.0169$; colony L6: QL: 3/10, QR: 1/10, $p=0.5820$; colony L7: QL: 6/15, QR: 1/17, $p=0.0330$; Fisher's combined probabilities: CP=34.4866, d.f.=8, $p<0.0001$). Hence, even though the workers were separated from the queen only by a wire mesh, they developed their ovaries and started laying eggs, which suggests that the queen signal is not a volatile chemical, and that it is only effective when workers can touch or are very close to the queen or the brood.

(b) Policing by aggression

Both in laboratory and plantation colonies, workers that developed their ovaries were attacked more than workers with undeveloped ovaries, suggesting that workers in *P. inversa* can detect each other's ovary development and police reproductive workers by attacking them. The pilot experiment with three colonies (L1–L3) demonstrated that there was a good correlation between received aggression and ovary development (effect of ovary; $\chi^2_1=19.04$, $p<0.0001$). Table 1 shows that for colonies L4–L7 and P1–P5 ovary, 'colony' and the interaction term 'ovary $\times$ colony' all gave significant contributions to the explained deviance in the full model (GLZ), which means that there is a correlation between the degree of ovary development and the number of received attacks, but that the level and slope of the correlation differs among colonies.

(c) Policing by egg eating

After an egg had been introduced into a discriminator colony, it usually was neither detected immediately, nor was it policed immediately after its presence was detected. The average time for eggs to be found and investigated by antennation was 40 ± 5 s (mean $\pm$ s.e.) and it took a colony

Figure 1. Frequency distribution of (a) aggressive-policing acts and (b) egg-policing acts among workers. Only workers that had the opportunity to police (i.e. workers that interacted with at least one reproductive worker (a) or egg; (b) potential policers) are included and they performed one policing act when they attacked one reproductive worker or ate an egg. The observed sharing of policing tasks is significantly more skewed than would be expected from random chance in both aggressive policing ($B=0.109$, $p<0.0001$) and egg policing ($B=0.025$, $p=0.004$).

Table 2. Policing by aggressing reproductive workers and skew in policing behaviour among potential policers. (Colonies L4–L7 are laboratory colonies, colonies P1–P5 are plantation colonies; n.s., not significant; n.a., not available. *Probability level obtained using Fisher's combined probabilities of the nine different colonies.)

	queenless part	queenright part						
colony	no. of workers	no. of queens	no. of workers	no. of potential policers ^a	no. of policers	mean $\pm$ s.e. no. of workers antennating, but not policing	<i>B</i> -index	probability level <i>B</i> -index is due to random chance ^b
L4	14	1	17	12	6	1.83 $\pm$ 0.55	0.123	<i>p</i> < 0.0001
L5	15	2	15	11	3	3.00 $\pm$ 0.45	0.264	<i>p</i> = 0.0093
L6	10	3	10	10	3	2.75 $\pm$ 1.38	0.050	<i>p</i> = 0.2870
L7	15	1	17	13	3	3.14 $\pm$ 0.14	0.201	<i>p</i> < 0.0001
P1	20	1	30	28	18	6.81 $\pm$ 1.18	0.029	<i>p</i> < 0.0001
P2	16	1	16	15	12	2.73 $\pm$ 0.72	0.031	<i>p</i> < 0.0001
P3	18	1	20	17	7	4.11 $\pm$ 0.93	0.161	<i>p</i> < 0.0001
P4	27	1	33	10	6	2.33 $\pm$ 0.33	0.010	<i>p</i> = 0.3504
P5	20	1	29	26	10	4.25 $\pm$ 0.64	0.115	<i>p</i> < 0.0001
mean	17.2	1.3	20.8	15.8	7.6	3.82 $\pm$ 0.35	0.109	<i>p</i> < 0.0001 <i>p</i> < 0.0001*

^a All workers are included that antennated at least one reproductive worker (i.e. a worker that was policed by themselves or others).

^b Probability levels were obtained with 100 000 simulations; if a Bonferroni correction for multiple tests was applied, the significance level becomes $p=0.05/9=0.0055$.

3.2 ± 0.6 min to police WLE, in which period usually several potential policers would antennate the egg, take it in the mandibles and drop it again (see below). QLE and WLE were different in their acceptance rates in the five discriminator colonies, with QLE being significantly better accepted (table 3; Fisher's combined probabilities (two-tailed); $CP=72.9465$, d.f. = 10, $p<0.0001$). This confirms previous research showing that workers of *P. inversa* police WLE in preference to QLE (D'Ettore *et al.* 2004, 2006).

(d) Specialization in policing tasks

(i) Aggressive policing

The distribution of policing tasks among potential policers is highly uneven as the B -index gave significantly higher skew than would be expected from a random sharing of tasks ($B=0.109$, $p<0.0001$), consistent with the result of

Fisher's combined probabilities ($CP=124.47$, d.f. = 18, $p<0.0001$). When colonies were considered separately, there was a significant skew in seven of the nine colonies (table 2), which implies that aggressive policing is non-random. Figure 1a shows the frequency distribution of aggressive-policing tasks among potential policers and the expected random Poisson distribution over all colonies.

Only 68 of the 142 potential policers (47.9%) performed any policing acts (table 2), and 50 of these 68 AP (73.5%) attacked more than one reproductive worker. On an average, a reproductive worker was antennated by 3.82 ± 0.35 (mean $\pm$ s.e.) potential policers that did not attack her (i.e. excluding the AP). Most AP (57/68 = 83.8%) had at least one interaction with a reproductive worker in which she did not attack her. On an average, they had 2.66 ± 0.27 interactions with reproductive workers.

(ii) *Egg policing*

For the analyses of skew in policing by egg eating, we only took WLE into account. Policing is not perfect due to possible overlapping recognition cues in queen-laid and WLE (Reeve 1989; Sherman *et al.* 1997), and using WLE, we analyse policing in its more strict sense. QLE made up no more than 12.9% (11/85) of the policed eggs and similar results concerning skew were found if both types of eggs were used for the analyses.

The observed sharing of policing tasks was not as highly uneven among potential policers as in the aggressive-policing bio-assays, but it was significantly skewed in two out of five discriminator colonies (table 3). Moreover, the mean skew across all colonies was significant ($B=0.025$, $p=0.004$), as was the result of Fisher's Combined Probabilities (CP=22.60, d.f. = 10, $p=0.012$). Figure 1b shows the frequency distribution of egg-policing tasks among potential policers and the expected random Poisson distribution over all colonies.

A worker-laid egg was usually not eaten by the first potential policers that encountered it, as would be expected under random sharing of policing tasks, but was on average antennated by 2.85 ± 0.48 (mean $\pm$ s.e.; table 3) potential policers before it was actually eaten. In general, 52 of the 150 potential policers (34.7%) ate any of the 73 eggs (one egg was eaten by a queen). In total, 14 of these 52 AP (26.9%) ate at least two eggs, and together these 14 workers ate 35 of the 73 eggs (47.9%). In 2 of the 73 trials, the actual policer would antennate the egg, leave it and come back later to eat it. However, several AP (31/52=59.6%) were found to only briefly antennate an egg before it was policed by another actual policer. On an average, they antennated 0.87 ± 0.12 eggs.

(iii) *Ovary development of policers*

Dissections showed that all AP in the four laboratory colonies of the aggressive-policing experiment had undeveloped ovaries under queenright conditions. In the five plantation colonies of the egg-policing experiment, there were some queenright workers with slightly developed ovaries, but never to a functional degree and the frequency of slightly developed ovaries was not significantly higher among the AP than among the NP (including both potential policers and non-interacting workers) that were dissected (Fisher's Exact Test, one-tailed; colony P6: AP: 2/11, NP: 2/9, $p=0.625$; colony P7: AP: 3/7, NP: 1/6, $p=0.343$; colony P8: AP: 6/13, NP: 3/8, $p=0.528$; colony P9: AP: 6/12, NP: 1/4, $p=0.392$; colony P10: AP: 4/9, NP: 2/8, $p=0.373$; Fisher's Combined Probabilities: CP=8.200, d.f. = 10, $p=0.609$).

4. DISCUSSION

We have shown that in the ant *P. inversa*, apart from policing by egg eating, aggressive behaviour towards reproductive workers is also operational to resolve reproductive conflict. Furthermore, there is a subset of workers that performs most of the policing tasks in both forms of policing. In particular, for aggressive policing, the distribution of policing tasks was highly asymmetrical, with some individuals interacting with reproductive workers but not policing at all, and others doing most of the police work. This is, to our knowledge, the first

non-primate example of specialization in policing behaviour without direct selfish motives.

Both aggressive- and egg policing were found to be significantly skewed among workers that had the opportunity to police (potential policers), although the skew in egg policing was not as apparent as the skew in aggressive policing. However, the same trend can be observed in egg policing as in aggressive policing, with more than the expected number of individuals not engaging in policing, and others policing more than expected. The skew in both forms of policing is also supported by reproductive workers and WLE being investigated by multiple individuals of which many did not police. The most logical explanation for these differences among workers seems to be that they would have different thresholds for either detection of WLE or reproductive workers, or for performing policing behaviour. Just as in other forms of division of labour, like pollen hoarding in honeybees (Page & Fondrk 1995), differences among workers in their thresholds for particular tasks could thus explain the observed patterns.

A hypothesis which is not mutually exclusive is that the polyethism in policing is associated with an already established division of labour like guarding or nursing. The aggressive policers might in fact be guards or patrollers that attack workers with a mismatch in their cuticular chemical profile (probably containing the recognition cues; Heinze *et al.* 2002), whereas the egg policers could be nurses that sort out mismatching eggs. We do not have substantial evidence to support or reject this hypothesis, but preliminary results from three scan samplings of which workers were present in the brood area suggest that at least the aggressive policers are hardly ever found among the nurses. For the egg policers this pattern was less clear, which begs the question whether egg policing is associated with any tasks in the colony.

Division of labour for policing tasks does not contradict the idea of policing having a general function in the resolution of reproductive conflict, as it is in the worker collective's interest to have some and not necessarily all nestmates being able to control selfish individuals. Policing is expected to occur in *P. inversa*, both on efficiency and relatedness grounds (D'Ettorre *et al.* 2004; Kellner *et al.* in press). A lower productivity of reproductive workers or an excess of males to rear are factors that could reduce colony efficiency, and hence fitness (Ratnieks *et al.* 2006; Wenseleers & Ratnieks 2006). In addition, colonies of *P. inversa* are typically headed by multiple unrelated queens that can be multiple mated, reducing the relatedness between workers and worker-derived males (Kellner *et al.* in press). Furthermore, due to colonies of *Pachycondyla* inhabiting multiple nests (i.e. polydomy; D'Ettorre *et al.* 2005; J. S. van Zweden & M. A. Fürst 2005, unpublished data) and the queen signal being non-volatile, workers may have ample possibility to develop their ovaries and attempt oviposition. Fertility and the associated fitness benefits of egg-laying workers can be substantial, as workers in the split plantation colonies laid 24–56 eggs per colony in four weeks of separation from the queen. Thus, policing behaviour may be highly rewarding for the worker collective in the QL part of the nest. This leads to the hypothesis that specialization in policing could be an emerging property of the social system driven by positive

Table 3. Policing by egg eating and skew in policing behaviour among potential policers. (Colonies P6–P10 are plantation colonies; n.s., not significant; n.a., not available. *Probability level obtained using Fisher's combined probabilities of the five different colonies.)

colony	no. of queens	no. of workers	egg type ^a	no. of eggs introduced	no. of eggs policed (%)	policing significance level ^b	total no. of potential policers policing 0–4 worker-laid eggs					mean $\pm$ s.e. no. of workers antennating an egg, but not policing	B-index	probability level B-index is due to random chance ^c
							0	1	2	3	4			
P6	2	52	QLE	10	1 (10.0%)	$p < 0.0001$	30	9	1	1	0	4.80 ± 1.24	0.018	$p = 0.164$
			WLE	16	15 (93.8%) ^d									
P7	3	24	QLE	10	3 (30.0%)	$p = 0.0085$	12	3	2	1	1	2.43 ± 0.64	0.063	$p = 0.017$
			WLE	16	14 (87.5%)									
P8	2	46	QLE	10	1 (10.0%)	$p < 0.0001$	25	11	2	0	0	3.66 ± 1.62	−0.007	$p = 0.635$
			WLE	16	15 (93.8%)									
P9	2	39	QLE	10	3 (30.0%)	$p = 0.0002$	12	9	2	1	0	0.94 ± 0.31	0.000	$p = 0.538$
			WLE	16	16 (100.0%)									
P10	4	41	QLE	10	3 (30.0%) ^d	$p = 0.0085$	19	6	2	0	1	2.50 ± 0.85	0.049	$p = 0.013$
			WLE	16	14 (87.5%)									
total/mean			QLE	50	11 (22.0%)	$p < 0.0001^*$	19.6	7.6	1.8	0.6	0.4	2.85 ± 0.48	0.025	$p = 0.004$
			WLE	80	74 (92.5%)									$p = 0.012^*$

^a QLE, queen-laid eggs; WLE, worker-laid eggs.^b Fisher's exact test (two-tailed), policing of worker-laid eggs in preference to queen-laid eggs.^c Probability levels were obtained with 100 000 simulations; if a Bonferroni correction for multiple tests was applied (the significance level becomes $p = 0.05/5 = 0.01$), then none of the colonies had a significant skew in policing behaviour.^d One egg was eaten by a queen.

feedback (Sudd & Franks 1987), i.e. a worker that has policed once and associates a specific cue (e.g. a chemical) to her policing act, is more likely to police again when encountering the same cue. Alternatively, specialization could be a cost-efficient solution to a Tragedy of the Commons problem (Hardin 1968), i.e. colonies with some workers specializing in policing are selected owing to higher efficiency.

Frank (1996) developed a general model to analyse which individuals should perform most policing behaviour. One of the outcomes was that individuals should police more when they have control over more resources or when the cost associated with policing is lower. This has indeed been found in the pigtailed macaque, *Macaca nemestrina*, where several high-ranking individuals perform most third-party policing (Flack et al. 2005). These individuals are apparently also the ones to control most resources and have a low cost associated with policing due to their status in the group. In social insects, differences among workers in their control over resources are unlikely to explain the heterogeneity in policing, as resources are fairly evenly distributed among all workers. A more probable explanation is that the cost associated with policing varies among individuals or rather that the implication of the same costs might vary. In general, older individuals are less valuable to an insect society due to their lower future life expectancy, so that older individuals might be expected to do most of the policing. Similarly, fertile workers could refrain from policing to avoid the high associated cost, but our data suggests that neither policing nor NP are fertile (have fully developed ovaries). Unequal impact of policing costs might also explain some of the observed difference in the degree of specialization between the two forms of policing, because policing by aggression can result in being stung and possibly in death, whereas policing by egg eating does not incur such a risk.

In some permanently queenless ants, such as *D. quadricaps* (Monnin & Peeters 1999) and *Harpegnathos saltator* (Peeters & Hölldobler 1995), policing by a non-reproductive worker may have the delayed selfish benefit of allowing that worker to later inherit the nest and become the reproductive (gamergate). However, this does not seem to apply to *P. inversa*, both because workers cannot mate and because a colony usually has multiple queens, so there would be little chance for workers to become a reproductive dominant. Even though workers could lay male eggs, we did not find workers with functional ovaries under queenright conditions. Thus, there is no evidence that our findings are a form of policing with selfish interests, as has been found in the tree wasp *D. sylvestris*, where policing individuals are laying eggs themselves (Wenseleers et al. 2005; see also Stroeymeyt et al. 2006). Policing in *P. inversa* should rather be viewed as a conflict management task that, as other tasks in a colony, is not performed at an equal rate by all workers.

We are grateful to J. H. C. Delabie, R. Rodrigues da Hora and employees of CEPLAC for providing us with logistic support in Brazil, and to K. Dietl for helping with the dissections. We would like to thank J. J. Boomsma, F. L. W. Ratnieks, D. R. Nash, G. Nachman and four anonymous referees for their helpful comments on the manuscript, and members of the Copenhagen Centre for Social Evolution for providing a stimulating working environment. This work was supported by the EU Marie Curie Excellence Grant CODICES-EXT-

CT-2004-014202 and the Deutsche Forschungsgemeinschaft grant DE 893/1-2, both assigned to P.D.E.

REFERENCES

- Bourke, A. F. G. 1988 Worker reproduction in the higher eusocial Hymenoptera. *Q. Rev. Biol.* **63**, 291–311. (doi:10.1086/415930)
- Bourke, A. F. G. & Franks, N. R. 1995 *Social evolution in ants*. Princeton, NJ: Princeton University Press.
- Cuvillier-Hot, V., Lenoir, A. & Peeters, C. 2004 Reproductive monopoly enforced by sterile police workers in a queenless ant. *Behav. Ecol.* **15**, 970–975. (doi:10.1093/beheco/arh072)
- D'Ettorre, P., Heinze, J. & Ratnieks, F. L. W. 2004 Worker policing by egg eating in the ponerine ant *Pachycondyla inversa*. *Proc. R. Soc. B* **271**, 1427–1434. (doi:10.1098/rspb.2004.2742)
- D'Ettorre, P., Kellner, K., Delabie, J. & Heinze, J. 2005 Number of queens in founding associations of the ponerine ant *Pachycondyla villosa*. *Insectes Soc.* **52**, 327–332. (doi:10.1007/s00040-005-0815-z)
- D'Ettorre, P., Tofilski, A., Heinze, J. & Ratnieks, F. L. W. 2006 Non-transferable signals on ant queen eggs. *Naturwissenschaften* **93**, 136–140. (doi:10.1007/s00114-005-0075-9)
- Dampney, J. R., Barron, A. B. & Oldroyd, B. P. 2004 Measuring the cost of worker reproduction in honeybees: work tempo in an 'anarchic' line. *Apidologie* **35**, 83–88. (doi:10.1051/apido:2003067)
- Endler, A., Liebig, J., Schmitt, T., Parker, J. E., Jones, G. R., 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. (doi:10.1073/pnas.0308447101)
- Flack, J. C., de Waal, F. B. M. & Krakauer, D. C. 2005 Social structure, robustness, and policing cost in a cognitively sophisticated species. *Am. Nat.* **165**, E126–E139. (doi:10.1086/429277)
- Flack, J. C., Girvan, M., de Waal, F. B. M. & Krakauer, D. C. 2006 Policing stabilizes construction of social niches in primates. *Nature* **439**, 426–429. (doi:10.1038/nature04326)
- Foster, K. R. & Ratnieks, F. L. W. 2000 Convergent evolution of worker policing by egg eating in the honeybee and common wasp. *Proc. R. Soc. B* **268**, 169–174.
- Frank, S. A. 1996 Policing and group cohesion when resources vary. *Anim. Behav.* **52**, 1163–1169. (doi:10.1006/anbe.1996.0263)
- Gobin, B., Billen, J. & Peeters, C. 1999 Policing behaviour towards virgin egg layers in a polygynous ponerine ant. *Anim. Behav.* **58**, 1117–1122. (doi:10.1006/anbe.1999.1245)
- Hammond, R. L. & Keller, L. 2004 Conflict over male parentage in social insects. *PLoS Biol.* **2**, E248. (doi:10.1371/journal.pbio.0020248)
- Hardin, G. 1968 The Tragedy of the Commons. *Science* **162**, 1243–1244. (doi:10.1126/science.162.3859.1243)
- Hartmann, A., Wantia, J., Torres, J. A. & Heinze, J. 2003 Worker policing without genetic conflicts in a clonal ant. *Proc. Natl Acad. Sci. USA* **100**, 12 836–12 840. (doi:10.1073/pnas.2132993100)
- Heinze, J., Trunzer, B., Oliveira, P. S. & Hölldobler, B. 1996 Regulation of reproduction in the neotropical ponerine ant *Pachycondyla villosa*. *J. Insect Behav.* **9**, 441–450. (doi:10.1007/BF02214021)
- Heinze, J., Trunzer, B., Hölldobler, B. & Delabie, J. H. C. 2001 Reproductive skew and queen relatedness in an ant with primary polygyny. *Insectes Soc.* **48**, 149–153. (doi:10.1007/PL00001758)

- Heinze, J., Stengl, B. & Sledge, M. F. 2002 Worker rank, reproductive status and cuticular hydrocarbon signature in the ant, *Pachycondyla* cf. *inversa*. *Behav. Ecol. Sociobiol.* **52**, 59–65. (doi:10.1007/s00265-002-0491-1)
- Helanterä, H. & Sundström, L. 2005 Worker reproduction in the ant *Formica fusca*. *J. Evol. Biol.* **18**, 162–171. (doi:10.1111/j.1420-9101.2004.00777.x)
- Kellner, K., Trindl, A., Heinze, J. & D'Ettorre, P. In press. Polygyny and polyandry in small ant societies. *Mol. Ecol.*
- Kikuta, N. & Tsuji, K. 1999 Queen and worker policing in the monogynous and monandrous ant, *Diacamma* sp. *Behav. Ecol. Sociobiol.* **46**, 180–189. (doi:10.1007/s002650050608)
- Kolmer, K., Hölldobler, B. & Heinze, J. 2002 Colony and population structure in *Pachycondyla* cf. *inversa*, a ponerine ant with primary polygyny. *Ethol. Ecol. Evol.* **14**, 157–164.
- Liebig, J., Peeters, C. & Hölldobler, B. 1999 Worker policing limits the number of reproductives in a ponerine ant. *Proc. R. Soc. B* **266**, 1865–1870. (doi:10.1098/rspb.1999.0858)
- Lucas, C., Fresneau, D., Kolmer, K., Heinze, J., Delabie, J. H. C. & Pho, D. B. 2002 A multidisciplinary approach to discriminating different taxa in the species complex *Pachycondyla villosa* (Formicidae). *Biol. J. Linn. Soc.* **75**, 249–259. (doi:10.1046/j.1095-8312.2002.00017.x)
- Monnin, T. & Peeters, C. 1999 Dominance hierarchy and reproductive conflicts among subordinates in a monogynous queenless ant. *Behav. Ecol.* **10**, 323–332. (doi:10.1093/beheco/10.3.323)
- Nonacs, P. 2000 Measuring and using skew in the study of social behavior and evolution. *Am. Nat.* **156**, 577–589. (doi:10.1086/316995)
- Oldroyd, B., Halling, L., Good, G., Wattanachaiyingcharoen, W., Barron, A., Nanork, P., Wongsiri, S. & Ratnieks, F. L. W. 2001 Worker policing and worker reproduction in *Apis cerana*. *Behav. Ecol. Sociobiol.* **50**, 371–377. (doi:10.1007/s002650100376)
- Oster, G. F. & Wilson, E. O. 1978 *Caste and ecology in the social insects*. Princeton, NJ: Princeton University Press.
- Page, R. E. & Fondrk, M. K. 1995 The effects of colony-level selection on the social organization of honey bee (*Apis mellifera* L.) colonies: colony-level components of pollen hoarding. *Behav. Ecol. Sociobiol.* **36**, 135–144. (doi:10.1007/s002650050133)
- Peeters, C. & Hölldobler, B. 1995 Reproductive cooperation between queens and their mated workers: the complex life history of an ant with a valuable nest. *Proc. Natl Acad. Sci. USA* **92**, 10 977–10 979. (doi:10.1073/pnas.92.24.10977)
- Pirk, C. W. W., Neumann, P. & Ratnieks, F. L. W. 2003 Cape honeybees, *Apis mellifera capensis*, police worker-laid eggs despite the absence of relatedness benefits. *Behav. Ecol.* **14**, 347–352. (doi:10.1093/beheco/14.3.347)
- Ratnieks, F. L. W. 1988 Reproductive harmony via mutual policing by workers in eusocial Hymenoptera. *Am. Nat.* **132**, 217–236. (doi:10.1086/284846)
- Ratnieks, F. L. W. & Visscher, P. K. 1989 Worker policing in the honeybee. *Nature* **342**, 796–797. (doi:10.1038/342796a0)
- Ratnieks, F. L. W., Foster, K. R. & Wenseleers, T. 2006 Conflict resolution in insect societies. *Annu. Rev. Entomol.* **51**, 581–608. (doi:10.1146/annurev.ento.51.110104.151003)
- Reeve, H. K. 1989 The evolution of conspecific acceptance thresholds. *Am. Nat.* **133**, 407–435. (doi:10.1086/284926)
- Saigo, T. & Tsuchida, K. 2004 Queen and worker policing in monogynous and monandrous colonies of a primitively eusocial wasp. *Proc. R. Soc. B* **271**, S509–S512. (doi:10.1098/rspb.2003.2640)
- Sherman, P. W., Reeve, H. K. & Pfennig, D. W. 1997 Recognition systems. In *Behavioural ecology: an evolutionary approach* (eds J. R. Krebs & N. B. Davies), pp. 69–96. Oxford, UK: Blackwell Science.
- Starr, C. K. 1984 Sperm competition, kinship, and sociality in the aculeate Hymenoptera. In *Sperm competition and the evolution of animal mating systems* (ed. R. L. Smith), pp. 427–464. Orlando, FL: Academic Press.
- Stroeymeyt, N., Brunner, E. & Heinze, J. 2006 Selfish policing controls worker reproduction in *Temnothorax unifasciatus*. In *XV Congress IUSSI Proc.* pp. 158–159. Washington, DC.
- Sudd, J. H. & Franks, N. R. 1987 *The behavioural ecology of ants*. New York, NY: Chapman & Hall.
- Taylor, F. W. 1911 *The principles of scientific management*. New York, NY: McMaster University Archive for the History of Economic Thought.
- Trivers, R. L. & Hare, H. 1976 Haplodiploidy and the evolution of the social insects. *Science* **191**, 249–263. (doi:10.1126/science.1108197)
- Trunzer, B., Heinze, J. & Hölldobler, B. 1998 Cooperative colony founding and experimental primary polygyny in the ponerine ant *Pachycondyla villosa*. *Insectes Soc.* **45**, 267–276. (doi:10.1007/s000400050087)
- Visscher, K. P. & Dukas, R. 1995 Honey bees recognize development of nestmates' ovaries. *Anim. Behav.* **49**, 542–544. (doi:10.1006/anbe.1995.0074)
- Wenseleers, T. & Ratnieks, F. L. W. 2006 Comparative analysis of worker policing and reproduction in eusocial Hymenoptera supports relatedness theory. *Am. Nat.* **168**, E163–E179. (doi:10.1086/508619)
- Wenseleers, T., Tofilski, A. & Ratnieks, F. L. W. 2005 Queen and worker policing in the tree wasp *Dolichovespula sylvestris*. *Behav. Ecol. Sociobiol.* **58**, 80–86. (doi:10.1007/s00265-004-0892-4)
- Woyciechowski, M. & Łomnicki, A. 1987 Multiple mating of queens and the sterility of workers among eusocial Hymenoptera. *J. Theor. Biol.* **128**, 317–327.