

Worker policing limits the number of reproductives in a ponerine ant

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Reproductive division of labour is an essential feature of insect sociality, but the regulation of sterility among colony members remains incompletely understood. Ant workers and queens are morphologically divergent and workers are only capable of producing males in a colony, although they usually do not do so. Worker policing is one mechanism proposed for their infertility and it can be expressed as either aggressive inhibition of ovarian activity among workers or destruction of worker-laid eggs. A few studies have shown that workers with developed ovaries are preferentially attacked by nest-mates, but adequate demonstration of worker policing also requires evidence that these attacks result in the suppression of ovarian activity or death. We investigated worker policing in the ponerine ant *Harpegnathos saltator* in which workers are able to mate and replace the founding queen. Five colonies were each divided into two groups, one of which consisted exclusively of infertile workers. Some individuals in the orphaned groups began laying eggs during the three-week separation and upon reunification these were vigorously attacked by infertile workers of the other groups. The ovarian activity of these new egg layers became inhibited, as revealed by subsequent dissection of marked individuals. Worker policing in *H. saltator* appears to function primarily in preventing an excess of reproductive workers.

Keywords: worker policing; regulation of reproduction; dominance; social insects; Formicidae; *Harpegnathos saltator*

1. INTRODUCTION

Insect societies are characterized by a distinct division of labour between a few reproductive and many sterile colony members (Wilson 1971; Michener 1974; Gadagkar 1994; Sherman *et al.* 1995). In ants, reproduction is often monopolized by the morphologically specialized queen while her numerous worker daughters refrain from reproducing. Kin selection theory explains that, as long as the workers help to increase the offspring production of close relatives sufficiently, they gain enough inclusive fitness in exchange for sterility and helper behaviour (Hamilton 1964*a,b*). Nevertheless, in most ant species and other social Hymenoptera, workers retain their reproductive options. The death or experimental removal of the reproductive queen of a colony often leads workers to produce haploid male-determined eggs (Bourke 1988; Choe 1988).

What prevents workers from increasing their direct fitness by producing some of the male offspring? Two basically different concepts provide answers to this important question in the understanding of sociality in insect colonies. The queen control hypothesis argues that the queen can either physically prevent her workers from reproducing (queen policing, *sensu* Ratnieks 1988) which is expected to work in small colonies, or produce pheromones to inhibit workers' egg laying (e.g. Fletcher & Ross 1985). On the other hand, workers should either voluntarily refrain from egg laying (Cole 1986; Woycie-

chowski & Lomnicki 1987; Ratnieks 1988) or they should prevent each other from reproducing to increase their inclusive fitness (worker policing, *sensu* Ratnieks 1988).

Worker policing can be exhibited by differentially eating eggs or directing aggression towards workers with developed ovaries (Ratnieks 1988). These mechanisms are expected to work when the relatedness between the workers is low as a consequence of multiple mating by the queen. However, such mutual antagonism is costly and affects the overall colony reproduction negatively. Therefore, it has been argued that selection should favour workers relinquishing their own reproduction in favour of colony productivity in the presence of a healthy queen that signals her fertility (Cole 1986; Ratnieks 1988; Pamilo 1991; Keller & Nonacs 1993).

Despite the importance of the worker policing concept in the Hymenoptera, good experimental evidence for the mechanism of selective oophagy has only been presented in honeybees (Ratnieks & Visscher 1989; Ratnieks 1993, 1995; Visscher 1996). Worker policing taking the form of behavioural inhibition of ovarian development has not yet been shown. In several investigations (e.g. Sakagami 1954; Hölldobler & Carlin 1989; Crosland 1990; Van der Blom 1991; Visscher & Dukas 1995) workers directed their aggression towards ovary-developed workers. However, these observations are not enough to demonstrate worker policing, since two steps are necessary: (i) aggression should be exclusively directed towards ovary-developed workers and (ii) aggression should result in the suppression of ovarian activity or death.

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Table 1. *Characteristics of the experimental colonies at the beginning of the experiment and at the time of reunification*

(IW, infertile worker; OW, new ovipositing worker; EG, established gamergate.)

colony code	colony size in number of IWs	number of reproductives	eggs in orphaned group	number of OWs	IWs dissected at the end
A ^a	2 × 50	ten EGs	81	9	71
B	2 × 50	seven EGs	48	4	82
C	2 × 53	two EGs	73	9	81 ^b
D ^a	2 × 50	five EGs	78	17	52 ^c
E	2 × 72	one queen	112	10	122
mean	—	—	—	10	—

^aThe colony became queenless at least six months before the beginning of the experiment.^bNot including nine workers that were infertile by the time of reunification but revealed active ovaries by the end.^cNot including three previously infertile workers with active ovaries by the end, as in colony C.

The ponerine ant *Harpegnathos saltator* is particularly well suited for investigating worker policing. Its colony size is small (mean \pm s.d. = 64 ± 30 workers, $n = 41$; Liebig 1998) so that whole colonies can be studied experimentally. Workers regularly mate which enables them to produce female-determined eggs (Peeters & Hölldobler 1995). Such reproducing mated workers are termed gamergates (Peeters & Crozier 1988). Furthermore, the workers have a high reproductive potential relative to the single queen of a colony due to a relatively small size dimorphism between the female castes. In fact, the egg-laying rate of an individual worker can be half that of a queen (Liebig 1998). Hence, every colony member is capable of taking over sexual reproduction in the colony and this increases the potential for reproductive conflicts. Worker policing can accordingly be studied in *Harpegnathos* in the general sense of mutual policing (including also female production) as it is now already used by other authors (e.g. Frank 1995; Reeve & Keller 1997).

In the natural life cycle two types of colonies occur: (i) those with a single queen as sole reproductive and non-laying workers, some of which are mated and (ii) queenless colonies where a few gamergates reproduce (Peeters & Hölldobler 1995). Both colony types were used for the experiment. When the founding queen of a colony dies some mated workers become gamergates while the others remain infertile. During the differentiation of the gamergates two different dominance behaviours occur: (i) duelling where two gamergates mutually box each other's antennae while running back and forth (Heinze *et al.* 1994) and (ii) the biting of body parts, mostly followed by violent jerking, usually directed from gamergates to infertile workers (Liebig 1998). These conspicuous behaviours were routinely used by us for correct identification of egg layers, since observation of oviposition is inappropriate due to their low fertility (less than one egg produced per day; Liebig 1998).

One way to observe worker policing in action is 'to stimulate attempted worker reproduction by appropriate manipulations' (Ratnieks 1988, p. 231). In *Harpegnathos*, infertile workers can be induced to develop their ovaries by separating them from the queen or gamergates (the term 'infertile' here refers to lack of ovarian activity at the time of study (e.g. dissection) and does not mean lifetime sterility). In colonies that are experimentally divided into

two groups with and without established reproductives, infertile workers in the latter start laying eggs after eight to 11 days (Liebig 1998). When these groups are reunited, the infertile workers that remained with the queen or gamergates will encounter newly egg-laying workers. If worker policing occurs, it is expected that these new egg layers will be attacked by the infertile workers and their ovarian activity will subsequently be inhibited.

2. MATERIAL AND METHODS

In May 1994 and October 1995 41 colonies were collected in Jog Falls, Karnataka, in southern India. Two gamergate and three queen colonies were used for this experiment while the other colonies were used for further behavioural observations and experiments about queen signalling (Liebig 1998). Two of the founding queens of the selected colonies died and were replaced by gamergates (table 1). Colonies were housed in plastic boxes (19 cm × 19 cm) containing a floor of plaster with a pre-formed nest chamber (15 cm × 15 cm) covered with a glass plate. The ants were kept at a temperature of 25 °C and a 12 L:12 D cycle. Twice a week the colonies were provided with live crickets which were readily hunted by the ants.

In order to eliminate any potential effect of mating status, even though such an effect did not become evident in other experiments (Liebig 1998), colonies were initially provided with male cocoons. Emerging males regularly mated with workers inside the nest (Peeters & Hölldobler 1995). Consequently, there were many mated workers at the beginning of the experiment, although no more than observed in natural colonies.

Before starting the experiment, established gamergates were behaviourally identified. This identification method is accurate, as has been shown in another experiment. Five colonies were each controlled for established gamergates for four consecutive days by video monitoring (Liebig 1998). In these colonies all egg layers were correctly identified on behavioural grounds. As a further criterion we used direct observation of egg laying in the present experiment if there was any doubt about the status of an individual. Established gamergates were marked with colour spots (Revell enamel paint) and thin wire rings around their petioles (in case the paint was lost). Subsequently, the colonies were divided into two groups of similar size by randomly selecting the same number of individuals, first from workers outside the nest and then from inside to obtain a similar age distribution in both groups. All gamergates or the queen, respectively, remained in one group. All eggs and cocoons were removed and the

larvae equally distributed between both groups. To investigate the presence of a volatile inhibitory pheromone we used a nest (10 cm × 11 cm) with two chambers separated by a double metal mesh (mesh size, 1 mm). The gap between the two screens (16 mm) prevented any antennal contact between members of different groups, which might have had an influence on the ovarian development of workers in the orphaned groups. Both sides were connected to a different foraging arena.

In two of the colonies (C and E) all workers were used in the experiments, whereas the other colonies were first reduced to 100 infertile workers in order to equalize the size across colonies (table 1). This size was above the colony average of 64 ± 30 workers ($n = 41$, Jog Falls population; Liebig 1998) and, accordingly, the sizes of the experimental groups with 50 workers were only slightly below average.

The colonies were separated for 19–23 days because preliminary observations indicated that workers can produce some oocytes and at least one mature egg in their ovaries during that period. New ovipositing workers, mated or unmated, were identified based on their duelling behaviour and oviposition. They also elicited crouching behaviour in other workers. Subsequently they were individually marked with colour spots and wire. All other workers were marked with one colour specific to each group. In addition, five workers in the group without established gamergates or a queen were marked with wire as a control for any potential artefact of the wire. Colours and wires were generally randomly assigned between groups of different colonies.

When the two groups were reunited all colony members were first placed into their respective foraging arenas and, after the mesh screen was removed, the ants re-entered the nest chamber. The behaviour of infertile workers, new ovipositing workers and established gamergates was subsequently monitored by time-lapse video recording for 24 h and regular visual checks. Thirty-six to 40 days after reunification (76 days in group C), the colonies were again observed for 24 h. Subsequently, all ants were frozen and dissected in insect Ringer's to check their ovarian development. Reproductive status (i.e. gamergates or infertile workers) was identified by counting yolky oocytes and by checking whether sperm was present in the spermatheca. Dissection of all workers was necessary to confirm that there were no egg layers in addition to those recognized on behavioural grounds.

3. RESULTS

During the separation period many workers began ovipositing in the orphaned groups and between 48 and 112 eggs were present at the time of reunification (table 1). This ovarian activity was possible despite the presence of the queen or the established gamergates on the other side of the mesh. Only four to 17 egg-laying workers (mean, 10) had regular duels and elicited crouching behaviour in other workers. Such individuals generally lay eggs in orphaned colonies and, thus, are termed new ovipositing workers (OWs) (Liebig 1998).

Less than 4 h after reunification, OWs, established gamergates and some infertile workers, chiefly from the queen or gamergate groups, engaged in duels. However, more overt aggression started 1–6 h after reunification. Infertile workers approached OWs from the side and, with their mandibles slightly open, jumped 1–2 cm towards the OWs and grabbed them with the distal tips of their jaws at the thorax, neck or petiolus (figure 1). This was apparently a firm grip because the victims were

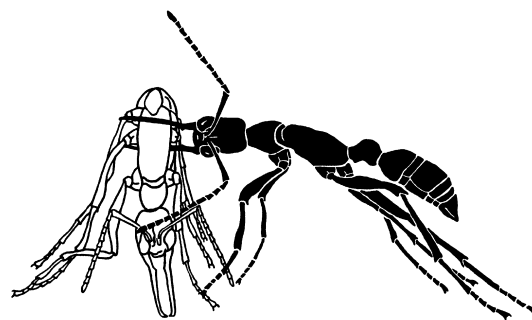


Figure 1. The jump-and-hold behaviour in *H. saltator*: one ant jumps at another from the side and grabs her at the thorax, using the distal tips of her own mandibles. The helpless victim can then be dragged around and held for hours. This behaviour is used to police workers with partly developed ovaries.

usually completely helpless and rarely escaped, despite strong resistance, and were sometimes dragged around and held for hours. This behaviour was not observed in any colony before starting the experiment or at its end, while it occurred with a mean frequency of 8.2 times per hour in the period 4–52 h after reunification ($n = 5$ colonies, with 1 h of observation per colony). This value underestimates the intensity of policing since several individuals escaped to the foraging arenas as a consequence of the aggression directed towards them.

In all colonies, infertile workers eventually jumped at and held all OWs (table 2). The 'jump-and-hold' behaviour of infertile workers towards OWs was gradually replaced by biting and jerking over the following days. Between 9 and 74 h after reunification OWs were victims of jump-and-hold or biting for, on average, 27% of the time (colonies A, B, D and E; $n = 10$ OWs, i.e. two and three OWs per colony with 1 h video observation time per individual). Between one and three infertile workers simultaneously took part in biting the OWs (mean, 1.3). Aggression towards the OWs continued for days before it finally stopped. None of the OWs were killed during these interactions. Neither the queen nor any established gamergate participated in these antagonistic activities. The wire marking of the new egg layers cannot have triggered the attacks, since the control workers were not jumped at.

By the end of the experiment all OWs had lost their status and all had stopped laying eggs, except five individuals from colony D with its exceptionally high number of OWs. These five individuals behaved like established gamergates, duelling with them, eliciting crouching in nearby infertile workers and laying eggs regularly (table 2).

Dissection of the ovaries of all colony members (figure 2) 36–40 days after reunification (76 days in C) confirmed the behavioural data. The ovaries of established gamergates and of the queen were fully developed. All OWs were no longer capable of laying eggs since their ovarian development did not differ from that of infertile workers except the five OWs of colony D, which still behaved like established gamergates at the end. Besides these ants, three workers from colony D and nine from colony C that were apparently infertile at the time of reunification had developed ovaries at the end of the experiment. Nine of these workers originated from the group with gamergates,

Table 2. *Behaviours directed at the reproductive individuals and their respective fate after 20 days of separation*

	colonies with gamergates				colony with a queen
	A	B	C	D	E
established gamergates or queen					
jumped at shortly after reunification	0	0	0	0	0
egg laying and dominant until the end	10	7	2	5	1
subordinate at the end	0	0	0	0	0
new ovipositing workers					
jumped at shortly after reunification	9	4	9	17 ^a	10
egg laying and dominant until the end	0	0	0	5	0
subordinate at the end	9	4	9	12	10

^a Due to their exceptionally high number, it is not clear whether all OWs had been the target of jump-and-hold behaviour.

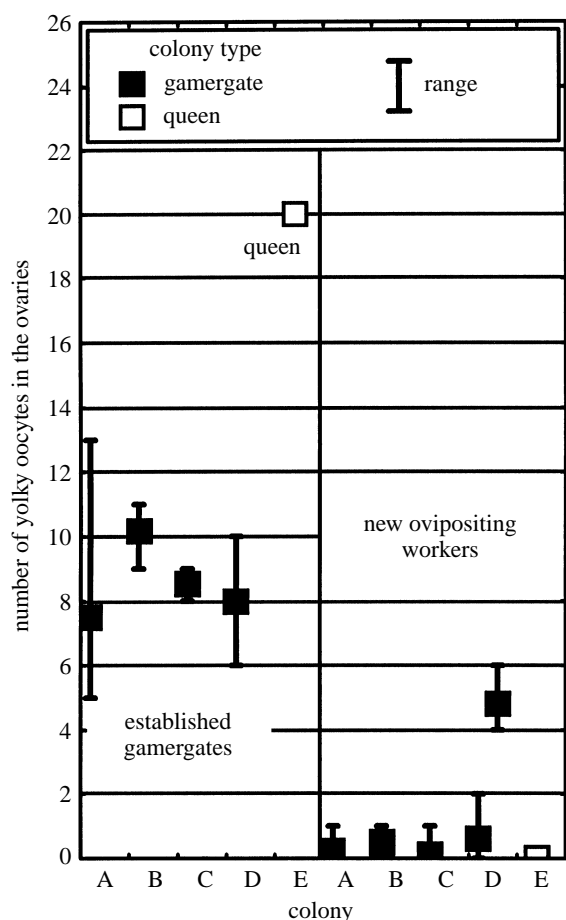


Figure 2. Ovarian development of established gamergates, queen and OWs at the end of the experiment. All the OWs of four colonies showed undeveloped ovaries, while the OWs of colony D segregated into two groups—workers that continued to lay eggs and those that became infertile again. One OW of the queen colony, which is not included in the figure, was removed two days after reunification. Her ovaries contained four yolky oocytes. The established gamergates and the queen all had active ovaries, which confirmed their status.

two were from the orphaned group and one could not be attributed to either group due to the loss of its colour code.

All OWs were mated in colonies B, C and D, while in colony A only five out of nine and in colony E only six out

of nine were mated. Despite this difference in mating status all OWs were similarly policed by infertile workers.

4. DISCUSSION

One of the possible mechanisms of worker policing is antagonism towards ovary-developed workers (Ratnieks 1988), which is exactly what the observations and experimental results in *H. saltator* show. Some workers developed their ovaries during the separation, as indicated by the presence of many eggs in the orphaned groups. Since the orphaned groups were exposed to eventual volatile pheromones from the queen or gamergates, we can rule out their involvement in the suppression of ovarian activity. This result is supported by another 12 colonies that were divided with a mesh for three months and in which queens or gamergates did not suppress the ovarian activity of workers across the mesh (Liebig 1998). In another two species with small colony sizes, a *Diacamma* species from Japan, which is a permanently queenless ponerine ant, as well as in *Leptothorax* sp. A, it has recently been shown that gamergate or queen signals are also non-volatile and only perceived by direct contact (Ortius & Heinze 1999; Tsuji *et al.* 1999).

After reunification of the *Harpegnathos* colonies, the OWs were recognized by infertile workers of the queen and gamergate groups and were subsequently the target of jump-and-hold behaviour. This antagonism towards the OWs was very efficient in inhibiting egg-laying activity, since in three gamergate colonies and in the queen colony all OWs again became subordinate and infertile after several weeks (table 2 and figure 2). Nevertheless, in colony D only 12 OWs became inhibited, while five remained egg layers and continued to behave like gamergates. Inhibition seems to be a direct consequence of aggression towards OWs and the effectiveness of this presumably depends on the number and intensity of attacks experienced. Thus, the differential inhibition in colony D was probably due to the unusually high number of OWs, which the infertile workers of the gamergate group were unable to police adequately. However, this exception also confirms that inhibition is not a consequence of any olfactory influence from the queens or the established gamergates, but indeed results directly from physical aggression by policing infertile workers. Jump-and-hold is a specialized behaviour used exclusively to

police workers that have partly developed ovaries in the presence of gamergates or a queen. These workers are not killed but their reproductive status changes to infertility as a consequence of the aggression directed towards them. Furthermore, queens and established gamergates did not police the OWs after reunification which excludes queen policing as an inhibitory mechanism.

The incomplete mutual policing in periods of intense aggression is also demonstrated by three workers from colony D and nine from colony C which had well-developed ovaries at the end. These workers were presumably infertile at the time of reunification since they chiefly originated from the gamergate side and, in the presence of gamergates, infertile workers do not usually activate their ovaries. The periods of intense policing provided the opportunity for some workers to become reproductive. In fact new gamergates only developed during periods of intense aggression between infertile workers which occurred irregularly in a three-year study of five colonies (Liebig 1998).

Aggression among workers has often been described in the context of ranks and hierarchies (e.g. Heinze 1993). One may think that the aggression in our experiment is similar to antagonistic interactions in terms of rank disputes. However, there are some differences. If the manipulation had led to a split in an eventual hierarchy and to changes in ranks among infertile workers then we would have expected an individual response to the OWs according to their different ranks after reunification. However, there was a collective response to the ovarian development of the OWs which resulted in consistent inhibition in four colonies while the exception in colony D was explained above. Thus, we can exclude disputes over rank as an explanation for the attacks on OWs in this experiment.

Workers are very sensitive to each others' level of ovarian activity, either in order to control each other or to single out less fertile reproductives. This also became evident in a further experiment in which *Harpegnathos* workers were kept isolated for longer (Liebig 1998). These workers with fully active ovaries were accepted as gamergates in gamergate colonies but not in queenright colonies. The situation in that experiment resembles more that of a polygynous society where differences in the fertility of reproductives lead to attacks of less fertile individuals. Ovary-developed workers are probably recognized as a consequence of changes in their olfactory characteristics resulting from profound physiological changes. In fact, it has already been demonstrated in *Dinoponera quadricaps* (Monnin *et al.* 1998; Peeters *et al.* 1999a) and *H. saltator* (Liebig 1998) that the cuticular hydrocarbon pattern changes according to ovarian development. Although the antagonism observed between infertile workers and OWs implies that worker policing in Ratnieks' (1988) sense occurs as a proximate mechanism in *H. saltator*, the social system of this species deviates from the underlying assumptions of the model and its relatedness-based predictions in gamergate colonies due to inbreeding and the ability of workers to produce females. Although workers should prefer their sons (life-for-life relatedness; $r=0.5$) and nephews ($r=0.375$) over their brothers ($r=0.25$), in queenright colonies of *Harpegnathos* the situation differs for female

offspring. Workers are more closely related to their sisters ($r=0.75$) than to their inbred nieces ($r=0.625$). The best relatedness-based strategy for workers is therefore to allow other worker nest-mates to produce male offspring but prevent them from producing females. However, this differential response to worker reproduction may include errors at the level of recognizing ovary-developed workers or destroying eggs. Similar costly errors in the correct identification of the sex of eggs or early larvae have been suggested to be a further explanation for the absence of worker reproduction in queenright colonies (Nonacs & Carlin 1990; Nonacs 1993). In gamergate colonies of *Harpegnathos* the relatedness pattern becomes even more complex due to the presence of several matriline and patriline, which increases the potential for making errors in the same decision.

When the production of males by workers reduces colony efficiency, male production by the queen is often selected for (Cole 1986; Ratnieks 1988; Pamilo 1991). The limitation of the number of reproductives in a colony is probably an important factor that promotes worker policing behaviour in *H. saltator*. Without control of their ovarian activity many mated workers could become gamergates. The regulation of the number of reproductives is only possible when the ovaries of the workers are still partly developed. As soon as ovarian activity is similar to that of the gamergates, infertile workers no longer differentiate between them (Liebig 1998). Although the proportion of gamergates in a colony is variable and their number is quite stable (table 1; Liebig 1998), colony efficiency is likely to decrease when a disproportionately high number of workers reproduce. This would result in surplus egg production, i.e. in a waste of resources, since not all of them can be raised to adulthood. Furthermore, it is probably more efficient to increase the workforce than to maintain surplus reproductives. In this experiment the addition of ten OWs on average as new reproductives would reduce the workforce by 10% (table 1), because gamergates only lay eggs and do not usually engage in other tasks. Assuming that these costs are important for colony productivity, worker policing is selected for in *H. saltator* because it maintains colony efficiency, which is in the interest of every individual in the colony.

Apis mellifera is the current model organism for worker policing in colonies with low worker relatedness, but it is exceptional among social insects because of its high degree of polyandry (seven to 20 males; e.g. Estoup *et al.* 1994). In contrast, *H. saltator* provides good opportunities for investigating the relative importance of relatedness aspects versus other factors such as perceptual errors or costs for colony efficiency, because it exhibits, unusually, within-colony variation in its social and genetic structure due to the transition from queen reproduction and monogyny to gamergate reproduction and polygyny.

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