

Research article

Social mutilation in the Ponerine ant *Diacamma*: cues originate in the victims

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Summary. In the queenless ponerine ant genus *Diacamma*, all workers eclose with a pair of innervated thoracic appendages termed gemmae. The gamergate (= mated egg laying worker) maintains reproductive monopoly by mutilating the gemmae of all eclosing individuals. Such mutilation leads to irreversible behavioural and neurological changes such that the individual lacking gemmae becomes incapable of appropriate sexual calling and mating. In one population related to *Diacamma ceylonense* from India, *Diacamma sp.* from Nilgiri (hereafter referred to as 'nilgiri'), gamergates do not mutilate their nestmates and yet maintain reproductive monopoly. To understand what triggers mutilation, we exchanged cocoons between the mutilating *D. ceylonense* colonies and the non mutilating 'nilgiri' colonies. 'nilgiri' callows were not mutilated even in *D. ceylonense* colonies while *D. ceylonense* callows were mutilated even in 'nilgiri' colonies, suggesting that the cues for mutilation originate in the victims (callows), presumably in the gemmae themselves. This finding should facilitate understanding the proximate mechanism and evolutionary significance of mutilation of gemmae as a method of resolution of reproductive conflicts in the genus *Diacamma*.

Key words: *Diacamma*, queenless ponerine ants, social mutilation, gemmae, cocoon exchange.

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Introduction

Colonies of queenless ponerine ants are headed by one or more mated egg laying workers, also called gamergates. Gamergates function like queens in that they not only produce male and female offspring but also maintain reproductive monopoly by inhibiting reproduction by other workers, using physical dominance and chemical signals (Peeters, 1993; 1997; Cuvillier-Hot et al., 2002).

In the genus *Diacamma*, the mechanism by which the gamergate maintains reproductive monopoly is most unusual. Every worker ecloses with a pair of innervated thoracic appendages termed gemmae, structures that are not present in any other ant genus. The gemmae contain glandular cells that presumably release an exocrine signal (Peeters et al., 1991). The gamergate systematically mutilates the gemmae of all individuals who eclose after her (Fukumoto et al., 1989; Peeters et al., 1989). Mutilation of the gemmae leads to irreversible neurological changes in the victims, (Gronenberg et al., 1993) preventing them from mating and thus effectively 'castrating' them. As a result, mutilated workers cannot challenge the gamergate's position as the sole mated reproductive of the colony. Mutilated workers can potentially lay haploid eggs but seldom do so (except in very large colonies, Nakata et al., 1996) in the presence of a healthy gamergate (Fukumoto et al., 1989; Peeters et al., 1989). It has been speculated that gemmae may release exocrine secre-

tions that may elicit or aid mutilation (Peeters et al., 1991; Tsuji et al., 1998). Mutilated workers appear not to mutilate others so that, after the death of a gamergate, the first worker to emerge retains her gemmae. This mechanism thus provides an automatic way of ensuring monogyny in *Diacamma* colonies. These facts appear to hold for all species of *Diacamma* studied so far, with one exception.

The exception concerns an Indian population of *Diacamma*, tentatively called *Diacamma* sp. from Nilgiri (hereafter referred to as 'nilgiri') (originally mislabeled as *Diacamma vagans* by Peeters et al., 1992) that occurs in parts of the states of Karnataka and Tamil Nadu, in Southern India. 'nilgiri' is morphologically indistinguishable from *Diacamma ceylonense*, except that almost all workers of 'nilgiri' retain their gemmae. And yet, reproduction is restricted to a single gamergate who establishes her reproductive monopoly, by dominance interactions (Peeters et al., 1992). This provides a unique opportunity to test whether *D. ceylonense* callows have cues eliciting mutilation while the 'nilgiri' callows do not, or whether *D. ceylonense* gamergates are capable of mutilation while 'nilgiri' gamergates are not. In other words, is mutilation dependent on cues emitted by the callows themselves or whether it is a behaviour that is characteristic of the gamergates of all species of *Diacamma* except 'nilgiri'. To distinguish between these alternatives, we exchanged cocoons between *D. ceylonense* and 'nilgiri' colonies. If mutilation depends on cues emitted by the callows, we might predict that *D. ceylonense* callows would be mutilated even in 'nilgiri' colonies while 'nilgiri' callows would escape mutilation even in *D. ceylonense* colonies. On the other hand, if mutilation depends on species-specific behaviour of the adult ants, we might predict that *D. ceylonense* callows would escape mutilation in 'nilgiri' colonies while 'nilgiri' callows would be mutilated in *D. ceylonense* colonies.

Here we report that 'nilgiri' callows are not mutilated even in *D. ceylonense* colonies, while *D. ceylonense* callows are mutilated even in 'nilgiri' colonies, suggesting that the cues for mutilation originate in the callows, presumably in the gemmae, themselves.

Materials and methods

Six *D. ceylonense* colonies were collected from the campus of the Indian Institute of Science, Bangalore (13°00'N, 77°32'E) (Karpakakunjaram et al., 2003) and six 'nilgiri' colonies were collected from Triambakapura (11°47'N, 76°45'E) (162 km from Bangalore) near Gundlupet, Karnataka, India. The *D. ceylonense* colonies contained 93–461 (212 ± 142) adult females, 0–15 (3 ± 6) males, 10–50 (37 ± 15) eggs, 4–25 (15 ± 9) larvae and 17–150 (61 ± 50) cocoons (pupae). The 'nilgiri' colonies contained 230–393 (318 ± 62) adult females, 0–14 (2 ± 5) males, 30–90 (62 ± 20) eggs, 0–43 (15 ± 14) larvae and 35–104 (67 ± 25) cocoons. Every colony collected had one, and only one, gamergate, identified by the presence of gemmae in *D. ceylonense* and by egg laying in 'nilgiri'. All colonies were housed in artificial plaster of Paris laboratory nests at 23°C–27°C and 69–81% relative humidity and provided with a diet of *Corcyra cephalonica* larvae, other insects, honey and water from the same source. All ants were marked with spots of coloured paint for individual identification.

A pair of *D. ceylonense* colonies and a pair of 'nilgiri' colonies were used for each of the three experiments. Cocoons from both *D. cey-*

lonense colonies were removed from their nests and pooled. Similarly all the cocoons from both 'nilgiri' colonies were pooled separately. One of the two *D. ceylonense* colonies was designated as control (DC[C]) and the other was designated as experimental (DC[E]). Similarly one of the two 'nilgiri' colonies was arbitrarily designated as control (DN[C]) and the other as experimental (DN[E]). Half the cocoons available from the two *D. ceylonense* colonies were offered to DC[C] and the other half were offered to DN[E]. Similarly half the available cocoons from the two 'nilgiri' colonies were offered to DN[C] and the other half were offered to DC[E]. Thus each control colony received conspecific cocoons and each experimental colony received heterospecific cocoons. The cocoons were placed, all at once, on the glass roof of the nests, near the entrance, and the ants themselves retrieved the cocoons and carried them into the nest chambers; every cocoon offered was carried into the nest, in both control and experimental colonies.

Behavioural observations were made using a dissection microscope on each of the nests, with special attention to the manner in which the gamergate and the workers treated the eclosing callows. These observations were made opportunistically, especially upon eclosion of callows, but were made for several hours every day for 13–26 days after the cocoons were first offered to the colonies. At the end of the experiment, 1 to 11 days after the last cocoon eclosed, a final census was made of the number of callows that had been mutilated or not. Of the callows eclosed, the proportion escaping mutilation in each colony was compared using two-tailed Fisher's exact test. The whole experiment was repeated three times with fresh sets of colonies. Since the results of the three experiments are essentially similar, certain minor differences between the procedures used for the three experiments are not mentioned, for the sake of brevity. Results from all the three experiments were analysed together by Fisher's method of combining probabilities from independent tests of significance (Sokal and Rohlf, 1995).

Results

In all colonies of *D. ceylonense* and 'nilgiri', both control and experimental, eclosing callows were subjected to rather aggressive behaviour by the workers and gamergates. The callows were often immobilized by groups of 4–8 workers, and aggressively groomed. On most occasions this served the purpose of cleaning the callow and ridding it of larval skin. Mutilation however was seen to be performed only by the gamergate, both in *D. ceylonense* as well as 'nilgiri'. This involved using her mandibles to scoop the free end of the gemma out of the gemmarium and pulling the gemma away from its base of attachment. Gamergates sometimes performed this while the callow was immobilized by the workers. However on other occasions, no worker was holding the callow when mutilation occurred because the callow had just escaped from the workers and the gamergate successfully chased, caught and mutilated her. The complete process of mutilation from the time of eclosion of the cocoon was observed, and in some cases filmed, for 29 callows, the process lasting from 10 to 180 (65 ± 29) min.

In experiments two and three, the proportions of *D. ceylonense* callows escaping mutilation in conspecific colonies (DC[C]) were statistically indistinguishable from the proportion escaping mutilation in heterospecific (DN[E]) colonies. Similarly, the proportions of 'nilgiri' callows escaping mutilation in conspecific (DN[C]) colonies were statistically indistinguishable from the proportion escaping mutilation in heterospecific (DN[E]) colonies. However, the

proportions of *D. ceylonense* callows escaping mutilation in conspecific and heterospecific colonies were significantly less than the proportion of 'nilgiri' callows escaping mutilation in conspecific (DN[C]) or heterospecific (DC[E]) colonies (Fisher's exact test with Bonferroni correction, Fig-

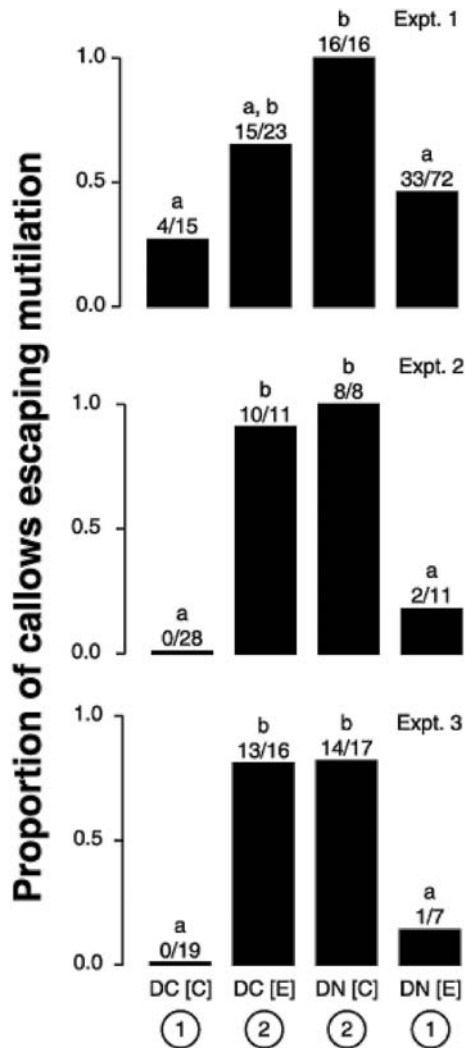


Figure 1. Proportions of callows escaping mutilation in *D. ceylonense* and 'nilgiri' colonies receiving conspecific (control) and heterospecific (experimental) cocoons. The numbers above the bars are the number of callows escaping mutilation/number eclosing. Bars having the same letters (above the bars) are not significantly different from each other while bars having different letters are significantly different from each other. Statistical comparisons of data in each of the three experiments are by two-tailed Fisher's exact test with Bonferroni correction ($p < 0.008$, the level of α set due to Bonferroni correction). Note that statistical comparisons are restricted to bars within each panel only. DC[C] = *D. ceylonense* control, DC[E] = *D. ceylonense* experimental, DN[C] = 'nilgiri' control and DN[E] = 'nilgiri' experimental colonies. Similar statistical comparisons are also performed using Fisher's method of combining probabilities from independent tests of significance (Sokal and Rohlf, 1995). The results of these comparisons are indicated by numbers below the X-axis labels such that experiments indicated by the same number are not significantly different from each other while those indicated by different numbers are significantly different from each other ($p < 0.001$).

ure 1). Similar trends were also obtained in experiment 1 although the comparisons here were not all statistically significant. This we suspect (with hindsight) to be due to the fact that we had introduced rather large numbers of cocoons in *D. ceylonense* as well as 'nilgiri' experimental colonies (23 and 72 cocoons respectively). In experiments two and three we therefore limited the number of cocoons introduced in the experimental colonies (7 to 16 cocoons). Using Fisher's method of combining probabilities from independent tests of significance (Sokal and Rohlf, 1995), we combined probability values obtained for each kind of comparison (e.g., DC[C] versus DC[E]) across the three experiments to find that the combined probabilities yield the same results as in experiments two and three. Thus our data show that *D. ceylonense* callows are mutilated even in 'nilgiri' colonies and 'nilgiri' callows escape mutilation even in *D. ceylonense* colonies. Hence mutilation is dependent on the species of the callow and not on the species of the gamergate or of the host colony.

Discussion

It is remarkable that *D. ceylonense* gamergates which normally mutilate all callows emerging in their nests, and which left only 6% of conspecific callows unmutilated in the present study, nevertheless failed to mutilate 76% of 'nilgiri'-callows. It is equally remarkable that 'nilgiri' gamergates left only 40% of *D. ceylonense* callows unmutilated. In natural colonies, 'nilgiri' gamergates leave about 94% of their workers unmutilated (L. Cournault and C. Peeters, unpublished) and in our experiments they left 93% of conspecific callows unmutilated. On the other hand, the finding that mutilation depends on the species of the callow and not the species of the colony or gamergate, is not so surprising after all. The gamergate is presumably responding to a chemical cue present on the callows. Given that the *D. ceylonense* gemmae are rich in glandular cells which presumably release an exocrine secretion (Peeters et al., 1991), the most likely hypothesis is that the cues for mutilation originate in the gemmae themselves (Peeters et al., 1991; Tsuji et al., 1998). Gamergates of 'nilgiri' showed much attention (licking) to the gemmae of conspecific newly eclosed workers, but they did not bite them off. This suggests that in 'nilgiri' also, the gemmae emit a chemical signal, but it may not be strong enough to elicit mutilation.

Because all other known species of *Diacamma* exhibit mutilation, it might be argued that the non-mutilating behaviour of 'nilgiri' is a derived condition. This argument is further supported by a molecular phylogenetic study using 228 codons of the Cytochrome Oxidase II mitochondrial gene. A comparison of *D. ceylonense*, *D. australe*, 'nilgiri', *D. indicum*, *D. cyaneiventre* and two unrelated ponerine ants, *Dinoponera quadriceps* and *Pachycondyla obscuricornis* confirms that, 'nilgiri' originates from the most recent divergence in the tree, thus confirming that the lack of mutilation in 'nilgiri' is a derived condition in the genus (<http://www.biologie.ens.fr/fr/ecologie/phylogenie.html>). In a discussion

of possible reverse social evolution, one of us has speculated that the loss of the queen caste in ponerine ants might represent an intermediate step in the eventual loss of eusociality (Gadagkar, 1997; 2001). In spite of the lack of a queen caste, workers in genera such as *Diacamma* are under effective control of the gamergate who irreversibly mutilates them immediately after their eclosion and renders them incapable of mating and replacing her (Fukumoto et al., 1989; Peeters et al., 1989). If the absence of mutilation in 'nilgiri' is a further evolutionary innovation, that ties in very well with the idea of reverse social evolution: the finding that cues for mutilation originate in the victim rather than in the aggressor, makes it plausible that potential victims can affect such lack of mutilation by gamergates by altering the cues required for mutilation. It must be noted however, that 'nilgiri' callows are also suppressed by their gamergates by some other means and they remain virgin and infertile. Whether this alternate mechanism of suppression by the 'nilgiri' gamergate is as effective or less so than mutilation remains to be studied. Further work on identifying the cues which trigger mutilation and the neurophysiological effects of mutilation are necessary in order to understand how the transition from mutilation to non-mutilation, without affecting the ability of the gamergate to maintain reproductive monopoly, became possible and to better appreciate the evolutionary implications of such a transition.

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