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## Gamergates in the Australian ant subfamily Myrmeciinae

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**Abstract** Ant workers can mate and reproduce in a few hundreds of species belonging to the phylogenetically basal poneromorph subfamilies (*sensu* Bolton 2003). We report the first occurrence of gamergates (i.e. mated reproductive workers) in a myrmeciomorph subfamily. In a colony of *Myrmecia pyriformis* that was collected without a queen, workers continued to be produced over a period of 3 years in the laboratory. Behavioural observations and ovarian dissections indicated that three workers were mated and produced the diploid offspring. The Myrmeciinae are thus another taxon in which the selective benefits of sexual reproduction by workers can be investigated.

### Introduction

A fundamental characteristic of ants is the existence of two female morphologies. Queens monopolize reproduction and can exhibit various degrees of specialization to start new colonies alone. Workers are always wingless and incapable of surviving alone, and they perform all the non-reproductive tasks necessary for colony survival. Size and ovarian differences between queen and worker castes vary over a wide range (Hölldobler and Wilson 1990; Peeters 1991a, 1997; Heinze 1998; Peeters and Ito 2001). Although workers retain the ability to lay eggs in most

species (Bourke 1988; Choe 1988), they usually lack a functional spermatheca and are thus restricted to producing males (Bourke and Franks 1995, p 227; B. Gobin, F. Ito, J. Billen and C. Peeters, unpublished data). However, in about 200 poneromorph species, workers are able to mate, store sperm and lay fertilized eggs (Peeters 1991b; Peeters and Ito 2001). Mated reproductive workers are termed ‘gamergates’ (Peeters and Crewe 1984) and can only exist in lineages where workers retain a functional spermatheca and are able to mate. This condition is considered to be symplesiomorphic, i.e. fully sexual workers are ancestral for the Formicidae as a whole.

The genus *Myrmecia* is endemic to the Australian region and has 89 described species (Ogata and Taylor 1991). Subfamily Myrmeciinae was considered to have a basal position in the Formicidae (Brown 1954; Wilson 1971; Hölldobler and Wilson 1990), but a phylogenetic analysis using both morphological and molecular data demonstrated that Myrmeciinae is nested within a grouping that includes the morphologically more derived ant subfamilies (Ward and Brady 2003). Both *Myrmecia* and *Nothomyrmecia* (also placed in this subfamily; Ward and Brady 2003) show many archaic biological traits, including a limited queen–worker divergence (McAreavey 1948; Haskins and Haskins 1950; Clark 1951; Ito et al. 1994; Peeters 1997; Dietemann et al. 2002). Accordingly, several authors have speculated about the occurrence of gamergates in *Myrmecia* (Crosland et al. 1988; Peeters 1991b). Here we show that gamergates exist in one species belonging to the *gulosus* group.

### Materials and methods

One colony (187 workers and 106 larvae of all sizes as well as 40 eggs) of *M. pyriformis* was excavated in October 1998 near Calga, New South Wales, Australia. No other nests were found in this immediate area. Alate queens have been reported in this species (Haskins and Haskins 1950; Clark 1951), but there was none in our colony. Ants were kept in a plaster-of-Paris nest into which chambers had been moulded and covered with glass plates to allow observation. The nest was connected to a foraging arena where food (pieces of cockroaches or entire crickets and honeywater) was de-

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posited every 1–2 days. The temperature was maintained at  $24\pm 1^\circ\text{C}$ , the photoperiod was set at 10:14 h (light:dark) cycles and a high humidity was maintained inside the nests by regularly moistening the plaster.

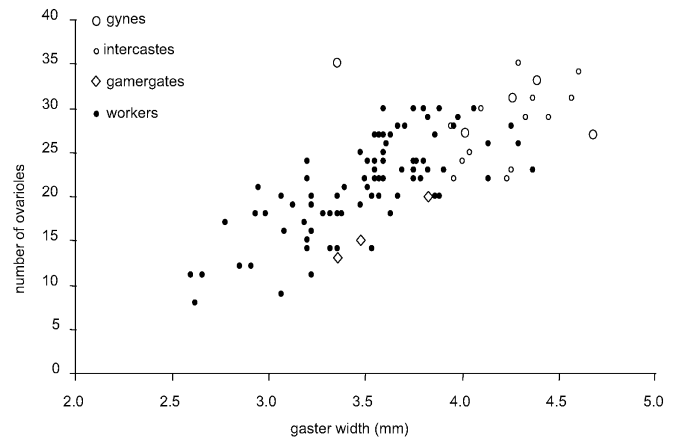
## Results

Although a queen had not been found, adult workers continued to be reared for a total of 3 years in the laboratory. To eliminate the possibility that some diploid eggs had remained from the field, we removed all eggs. Oviposition soon resumed and about 30 eggs accumulated in a pile. These developed into three kinds of adult females after about 2 months: workers, gynes and intercastes (*sensu* Peeters 1991a), confirming that diploid individuals were produced by the workers. Only two males were reared in the laboratory over 3 years. Direct observations allowed the identification of a worker displaying behaviours typical of a reproductive. She was the first individual to react by fleeing upon disturbance of the nest, and importantly, she induced crouching after antennal encounters with nestmates.

We subsequently divided this colony into two equal groups with similar age structure. In the group containing the suspected reproductive, eggs appeared in the 48 h following colony splitting. In the other group, eggs appeared after 6 days, and adult females (mostly workers) emerged. This indicates that there was more than one inseminated workers present in the original colony. In this orphaned group, one worker could also be identified as a presumed egg-layer, although oviposition was never observed directly. When both suspected egg-layers were removed for dissection, one more worker started laying eggs in one of the groups.

We verified whether these three workers were gamergates (mated workers) or reproduced by thelytokous parthenogenesis (production of diploid offspring from unfertilized eggs). Firstly, dissections indicated that sperm was present in the spermathecae of two individuals (in the third individual, we could not determine with certainty whether the spermatheca was empty or contained only a small amount of sperm). All three workers had yolky oocytes and numerous large yellow bodies, indicative of past egg-laying. Secondly, isolation of 20 callow workers for several months (with no opportunity to mate) showed that virgins produced neither diploid offspring nor males.

Gynes ( $n=5$ ) and intercastes ( $n=13$ ) emerged from the worker-produced brood. We were thus able to compare the external and internal morphology of gynes with that of the egg-layers. Gynes were winged and had typical large thoraces, whereas gamergates had the simple thoraces characteristic of all ant workers. Intercastes had an intermediate thoracic structure between that of gynes and workers. There was considerable size variation among the workers, and the gamergates had smaller gasters than the larger workers (Fig. 1). The gynes were not much larger than the biggest workers. Gaster size was highly corre-



**Fig. 1** Correlation between gaster size (maximal width of abdominal segment IV, measured dorsally; Ogata and Taylor 1991) and ovariole numbers in workers ( $n=85$ , gamergates included), intercastes ( $n=13$ ) and gynes ( $n=5$ ) of *M. pyriformis*. Unlike the gamergates, all gynes and intercastes were reared in the laboratory (together with most of the workers)

lated with ovarian structure ( $r_{n=103}=0.74$ ,  $P<0.01$ , Fig. 1). Gamergates had fewer ovarioles (mean  $\pm$  SD,  $16.0\pm 3.6$ ,  $n=3$ ) than gynes ( $33.3\pm 2.1$ ,  $n=5$ ), but also relative to the average for non-reproductive workers ( $21.4\pm 5.4$ ,  $n=82$ ). Importantly, all the workers dissected ( $n=85$ ) had a spermatheca. The three mated workers lived at least 3 years, which is interesting information about ant worker longevity.

## Discussion

The mated egg-layers described in *M. pyriformis* clearly belong to the worker caste and are true gamergates. As is typical of many *Myrmecia* species (Haskins and Haskins 1950; Higashi and Peeters 1990; Ito et al. 1994; Dietemann et al. 2002), *M. pyriformis* workers are markedly polymorphic in size; minor workers are almost half the size of majors (Fig. 1) but they all have a spermatheca. The three gamergates were intermediate in size. Our limited data suggest that only one gamergate reproduces at any one time, and that other workers can be inseminated but remain infertile. The coexistence of one or few gamergates with inseminated but infertile workers has been found in several Ponerinae, such as *Pachycondyla tridentata* (Sommer and Hölldobler 1992), *Pachycondyla (= Bothroponera)* sp. from Java (Ito 1993) and *Harpegnathos saltator* (Peeters and Hölldobler 1995).

This is the first clear documentation of gamergates in the subfamily Myrmeciinae. McAreavey (1948) described a male and a worker of *M. tarsata* locked together by their genitalia in a seemingly successful mating, although the spermatheca was not dissected. In *M. gulosa*, belonging to the same species-group as *M. pyriformis* (Ogata and Taylor 1991), males were sometimes observed attempting to mate with workers in the laboratory (V. Dietemann, unpublished results). The latter were reluctant and ag-

gressive towards the insistent males. No successful mating could be observed, and inseminated workers were never found (579 workers from 14 colonies dissected; Dietemann et al. 2002). This suggests that males are attracted to any females (either gyne or worker), and can initiate copulation if females are willing.

The occurrence of queens has been reported in most species of *Myrmecia*. Unlike some poneromorph ants, there is no evidence that the queen caste has ever been permanently replaced by gamergates. Haskins and Haskins (1950) found alate queens as well as queenright colonies in *M. pyriformis*. A dealate queen may have been absent in our colony due to natural death, although we cannot exclude the possibility that she may have escaped during excavation. The presence of inseminated workers suggests that they do sometimes produce diploid offspring. *M. pyriformis* could have a similar reproductive system to that in the Ponerinae ant *H. saltator*, where lone queens start new colonies and are replaced by gamergates after senescence (Peeters and Hölldobler 1995; Peeters et al. 2000).

Based on literature records and our own fieldwork (V. Dietemann, unpublished results), only about 140 complete colonies belonging to 21 species of *Myrmecia* have been collected. Only some of these have been reared in the laboratory and even fewer have been dissected. Such a low sample size reduces the chance of finding gamergates. The *aberrans* species group in *Myrmecia* exhibits a much smaller caste dimorphism than in *M. pyriformis* (e.g. *M. froggatti*, Ito et al. 1994), which increases the likelihood of gamergates existing. It is conceivable that workers can only mate in orphaned colonies, e.g. if a founding queen cannot be replaced by a younger queen. In *Nothomyrmecia macrops*, newly mated queens are adopted (Sanetra and Crozier 2002), and this could restrict the opportunities for workers to mate. Caste dimorphism is very small in *N. macrops* (Taylor 1978), and dissections of 100 workers from six colonies failed to find gamergates or inseminated individuals (V. Dietemann and C. Peeters, unpublished results; see also Hölldobler and Taylor 1983).

Gamergates have also been described in the genus *Metapone* (Hölldobler et al. 2002). This genus is currently classified in subfamily Myrmicinae, although it has an unusual combination of features present in the Myrmicinae, Cerapachyinae and poneromorph subfamilies (Shattuck 1999; R. Keller, personal communication). *Metapone* has been moved back and forth between these three subfamilies during its taxonomic history (Bolton 2003), and the claim that gamergates exist in the Myrmicinae needs to be treated with caution at this stage.

The existence of gamergates in the Myrmecinae further increases the number of independent lineages of ants that exhibit this alternative reproductive strategy; gamergates also occur in three poneromorph subfamilies (Amblyoponinae, Ectatomminae and Ponerinae; Peeters and Ito 2001). Gamergates are thus restricted to taxa with a limited queen–worker divergence, which is the ancestral condition in ants. It is necessary to test the hypothesis that

reproduction by poorly specialized queens was selected against in particular ecological contexts (Peeters 1997).

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## References

- Bolton B (2003) Synopsis and classification of Formicidae. (Memoirs of the American Entomological Institute, vol 71) American Entomological Institute, Gainesville, Fla.
- Bourke AFG (1988) Worker reproduction in the higher eusocial Hymenoptera. *Q Rev Biol* 63:291–311
- Bourke AFG, Franks NR (1995) Social evolution in ants. Princeton University Press, Princeton, N.J.
- Brown WL Jr (1954) Remarks on the internal phylogeny and subfamily classification of the family Formicidae. *Insectes Soc* 1:21–31
- Choe J (1988) Worker reproduction and social evolution in ants (Hymenoptera: Formicidae). In: Trager JC (ed) *Advances in myrmecology*. Leiden EJ, Leiden, pp 163–187
- Clark J (1951) The Formicidae of Australia. CSIRO, Melbourne
- Crosland MWJ, Crozier RH, Jefferson E (1988) Aspects of the biology of the primitive ant genus *Myrmecia* F. (Hymenoptera: Formicidae). *J Aust Entomol Soc* 27:305–309
- Dietemann V, Hölldobler B, Peeters C (2002) Caste specialization and differentiation in reproductive potential in the phylogenetically primitive ant *Myrmecia gulosa*. *Insectes Soc* 49:289–298
- Haskins CP, Haskins EF (1950) Notes on the biology and social behaviour of the archaic ponerine ants of the genera *Myrmecia* and *Promyrmecia*. *Ann Entomol Soc Am* 43:461–491
- Heinze J (1998) Intercastes, intermorphs, and ergatoid queens: who is who in ant reproduction? *Insectes Soc* 45:113–124
- Higashi S, Peeters C (1990) Worker polymorphism and nest structure in *Myrmecia brevinoda* Forel (Hymenoptera: Formicidae). *J Aust Entomol Soc* 29:327–331
- Hölldobler B, Taylor RW (1983) A behavioural study of the primitive ant *Nothomyrmecia macrops* Clark. *Insectes Soc* 30:384–401
- Hölldobler B, Wilson EO (1990) The ants. Belknap, Cambridge, Mass.
- Hölldobler B, Liebig J, Alpert GD (2002) Gamergates in the myrmecine genus *Metapone* (Hymenoptera: Formicidae). *Naturwissenschaften* 89:305–307
- Ito F (1993) Functional monogyny and dominance hierarchy in the queenless ponerine ant *Pachycondyla* (= *Bothroponera*) sp. in West Java, Indonesia (Hymenoptera: Formicidae: Ponerinae). *Ethology* 95:126–140
- Ito F, Sugiura N, Higashi S (1994) Worker polymorphism in the red-head bulldog ant (Hymenoptera: Formicidae), with description of nest structure and colony composition. *Ann Entomol Soc Am* 87:337–341
- McAreavey J (1948) Some observations on *Myrmecia tarsata* Smith. *Proc Linn Soc NSW* 73:137–141
- Ogata K, Taylor RW (1991) Ants of the genus *Myrmecia* Fabricius: a preliminary review and key to the named species (Hymenoptera: Formicidae: Myrmecinae). *J Nat Hist* 25:1623–1673

- Peeters C (1991a) Ergatoid queens and intercastes in ants: two distinct adult forms which look morphologically intermediate between workers and winged queens. *Insectes Soc* 38:1–15
- Peeters C (1991b) The occurrence of sexual reproduction among ant workers. *Biol J Linn Soc* 44:141–152
- Peeters C (1997) Morphologically “primitive ants”: comparative review of social characters, and the importance of queen-worker dimorphism. In: Choe J, Crespi B (eds) *The evolution of social behaviour in insects and arachnids*. Cambridge University Press, Cambridge, pp 372–391
- Peeters C, Crewe RM (1984) Insemination controls the reproductive division of labour in a ponerine ant. *Naturwissenschaften* 71:50–51
- 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* 92:10977–10979
- Peeters C, Ito F (2001) Colony dispersal and the evolution of queen morphology in social Hymenoptera. *Annu Rev Entomol* 46:601–630
- Peeters C, Liebig J, Hölldobler B (2000) Sexual reproduction by both queens and workers in the ponerine ant *Harpegnathos saltator*. *Insectes Soc* 47:325–332
- Sanetra M, Crozier RH (2002) Daughters inherit colonies from mothers in the “living-fossil” ant *Nothomyrmecia macrops*. *Naturwissenschaften* 89:71–74
- Shattuck S (1999) *Australian ants: their biology and identification*. CSIRO, Melbourne
- Sommer K, Hölldobler B (1992) Coexistence and dominance among queens and mated workers in the ant *Pachycondyla tridentata*. *Naturwissenschaften* 79:470–472
- Taylor RW (1978) *Nothomyrmecia macrops*: a living-fossil ant rediscovered. *Science* 201:979–985
- Ward PS, Brady SG (2003) Phylogeny and biogeography of the ant subfamily Myrmeciinae (Hymenoptera: Formicidae). *Invertebr Syst* 17:361–386
- Wilson EO (1971) *The insect societies*. Harvard University Press, Cambridge, Mass.