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# Functional morphology of the Dufour gland in the queenless ant *Dinoponera quadriceps*

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## Abstract

Colonies of the queenless ponerine ant *Dinoponera quadriceps* are characterized by a social hierarchy among the workers, in which the gamergate occupies the alpha rank. She may be challenged, however, by a beta worker in an attempt to take over reproductive control in the colony. The gamergate does not engage in a direct conflict, however, but only smears secretion from her Dufour gland onto the beta worker. This secretion then stimulates the low-ranked workers to immobilize the beta worker and thus prevent it from overthrowing the gamergate. Our histological and ultrastructural examination of the Dufour gland in gamergates, one-week-old workers that still have reproductive potential and low-ranked foragers clearly shows that the gamergate has the most developed gland containing secretory cells displaying abundant smooth endoplasmic reticulum and an obvious microvillar differentiation of the apical cell membrane. These findings are in line with the already existing chemical and behavioural observations of the peculiar pretender punishment in this species.


## Introduction

It is a common strategy in crime movies and real-life mafia organizations that the top level criminals do not eliminate their opponents directly by themselves, but rely upon the help of lower accomplices to carry out the dirty work. A comparable approach also exists in some permanently queenless ant species in which a hierarchy among the workers determines which individual(s) will be in charge of heading the colony and producing new offspring. In such species, workers have the potential to mate and, after being inseminated, become egg-layers that are referred to as gamergates (Peeters 1993). This phenomenon of mated workers is only known in about 100 species among the three ant subfamilies Amblyoponinae, Ectatomminae and Ponerinae (Monnin and Peeters 2008).

A well-documented example of a gamergate species is the large Neotropical ponerine *Dinoponera quadriceps*, that has colonies with on average 80 adult workers, including a single gamergate that occupies the alpha rank in a dominance hierarchy of 3-5 high-ranking workers (Monnin and Peeters 1998, 1999; Monnin et al. 2002). These high-ranking workers, with the highest chance for the beta individual, have the potential to replace the gamergate when she dies. Instead of waiting for the gamergate to die naturally, a high-ranking worker can increase her inclusive fitness by overthrowing the gamergate and produce her own offspring. In case of such conflict between a gamergate and a challenging high-ranking worker, short fights between both ants occur. The gamergate may perform ‘sting smearing’ against the pretender worker, during which the gamergate smears Dufour gland secretion onto her opponent. This results in the pretender being immobilized by low-ranking workers, which can last for several days, after which the pretender loses her high rank (Monnin et al. 2002). In this way, similar to crime movies, the gamergate relies on a cooperation with the subordinate workers to eliminate her pretenders.

Chemical analysis of the Dufour gland revealed that the hydrocarbon composition in gamergates is clearly different from that in the beta and low-ranking workers. Gamergates contain more high molecular mass hydrocarbons than low-ranking workers with beta workers having intermediate values. Bioassays in which workers were smeared with Dufour gland secretion moreover showed that the gland chemicals of the gamergate do act as a signal that elicits immobilization (Monnin et al. 2002).

The secretion of the gamergate's Dufour gland thus has a clear effect on the pretender immobilization as evidenced both by chemical analysis and behavioural bioassays (Monnin et al. 2002). We here aimed to examine the histological and ultrastructural appearance of this gland in gamergates and compare it with high- and low-ranked workers. For this, one-week old workers are chosen as hopeful reproductives since they typically have a high rank (Monnin and Peeters 1999), while foragers as old individuals represent low-ranked workers.

## Material and methods

Two colonies of *Dinoponera quadriceps* were excavated near Sambaíba, in Bahia state, Brazil, in October 1994. The collected ants were kept in the laboratory in plaster nests with various chambers. All ants as well as the newly eclosed workers were individually marked with numbers glued onto the thorax in order to record individual behaviour at known age as well as social status. Dufour glands of such individuals from two different colonies (2 gamergates, 4 one-week-old workers, 4 foragers) were dissected and fixed in cold 2% glutaraldehyde, buffered at pH 7.3 with 50 mM Na-cacodylate and 150 mM saccharose. Postfixation occurred in 2% osmium tetroxide in the same buffer. Dehydration took place in a graded acetone series (50-70-90-100-100%), followed by embedding in Araldite and sectioning with a Leica EM UC6 ultramicrotome. Semithin sections with a thickness of 1 µm were stained with methylene blue and thionin and viewed in an Olympus BX-51 microscope; double stained 70 nm thin sections were examined in a Zeiss EM900 electron microscope.

## Results

The Dufour gland appears as a long tubular sac with a central lumen that opens through the sting bulb, ventral to the opening of the venom gland (Billen 1987). Its epithelial wall is formed by class-1 glandular cells (following the gland classification by Noirot and Quennedey 1974). Semithin histological sections show a clear difference in the appearance of the gland epithelium in gamergates compared with that of other workers: the gamergate gland displays a thick epithelial with tall columnar cells with a height of up to 100 µm (Fig. 1a) while the epithelium in 1-week-old young (Fig. 1b) and forager workers (Fig. 1c) is much more wrinkled and has a mean thickness of 25 and 15 µm, respectively (range of epithelial thickness in gamergates 60-100 µm; in 1-week-old week old workers 15-30 µm and in foragers 5-

20 µm). The gland is surrounded by muscle fibres, that appear more conspicuous in foragers probably because of the wrinkled condition of the gland (Fig. 1a-c).

Ultrastructural examination illustrates the tall cylindrical cells with basally located round nuclei in gamergates (Fig. 1d) and the wrinkled appearance in the other workers (Fig. 1e,f). At higher magnification, the gamergate gland is characterized by additional differences. One is the occurrence of a regular border of microvilli with a length of 1 µm that represent the modified apical cell membrane (Fig. 1g). The microvilli, however, are much more dispersed in 1-week-old workers (Fig. 1h) or are even absent in old foragers (Fig. 1i). The other difference is the presence of abundant smooth endoplasmic reticulum in gamergates (Fig. 1g), whereas this is much less prominent in the other workers (Fig. 1h,i).

## Discussion

Information on the exocrine system of *Dinoponera* ants is available for morphological descriptions of the (post)pharyngeal gland (Schoeters and Billen 1997), pygidial gland (Billen et al. 1995) and venom gland (Schoeters and Billen 1995). Oldham et al. (1994) performed a chemical analysis of the mandibular gland of all 14 individuals of a colony of *D. australis*, taking into account their social role in the colony. Although the function of the mandibular gland secretion in *Dinoponera* is not known, it is interesting that this study revealed that the gamergate contained by far the smallest amount of secretion of all individuals, while the most active forager had the largest with 2,5-dimethyl-3-(3'-methylbutyl)pyrazine as the major component (Oldham et al. 1994). This finding is very different for the cuticular hydrocarbons in *D. quadriceps*, that are most abundant in the gamergate with the major component 9-hentriacontene acting as a signal for fecundity (Monnin et al. 1998; Peeters et al. 1999). Clear differences also exist in the Dufour gland composition of *D. quadriceps*, with the gamergate and beta worker having significantly more hydrocarbons than low-ranking workers, but the gamergate having the highest proportion of high-molecular-mass compounds (Monnin et al. 2002).

Our histological and ultrastructural examination of the Dufour gland confirms the chemical and behavioural differences according to social status as the gamergate gland clearly represents the highest metabolic activity. The abundant presence of smooth endoplasmic reticulum in the gamergate is entirely in line with the production of hydrocarbons, while the well-developed apical microvilli indicate high transportation activity (Billen and Morgan 1998). These features appear much more reduced in the glands of foragers that belong to the group of low-ranking workers. One-week-old workers show an intermediate development of their Dufour gland, which can be explained by their young age that makes them hopeful workers in terms of reproduction, as they may have the potential to reach the beta rank and then challenge the gamergate. Having a reasonably well-developed Dufour gland therefore can be understood as their being in standby for an eventual increase of their position in the social hierarchy. Our current data thus provides clear morphological support for the known chemical and behavioural role of the Dufour gland in the peculiar pretender punishment in *D. quadriceps* (Monnin et al. 2002).

The Dufour gland secretion plays a similar role in other ant species. In *Aphaenogaster cockerelli* it triggers worker aggression against replacement-queen pretenders (Smith et al 2012), and in incipient colonies of *Cataglyphis piliscapa* it possibly triggers aggression between young queens vying for colony ownership, i.e. when monogyny is restored after colony foundation (Monnin et al. 2018). Determining whether the Dufour gland of these species shows similar queen/worker differences as observed in *D. quadriceps* would be interesting, in particular because of the variability of function this gland plays across the ants, e.g. in foraging (Morgan 2009, Witte 2007), alarm (Lenz et al. 2013), appeasement (Billen et al. 2001; Grasso et al. 2005), defense (Billen et al 2009) or production of sex pheromones (Walter et al 1993).

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## Figure legends

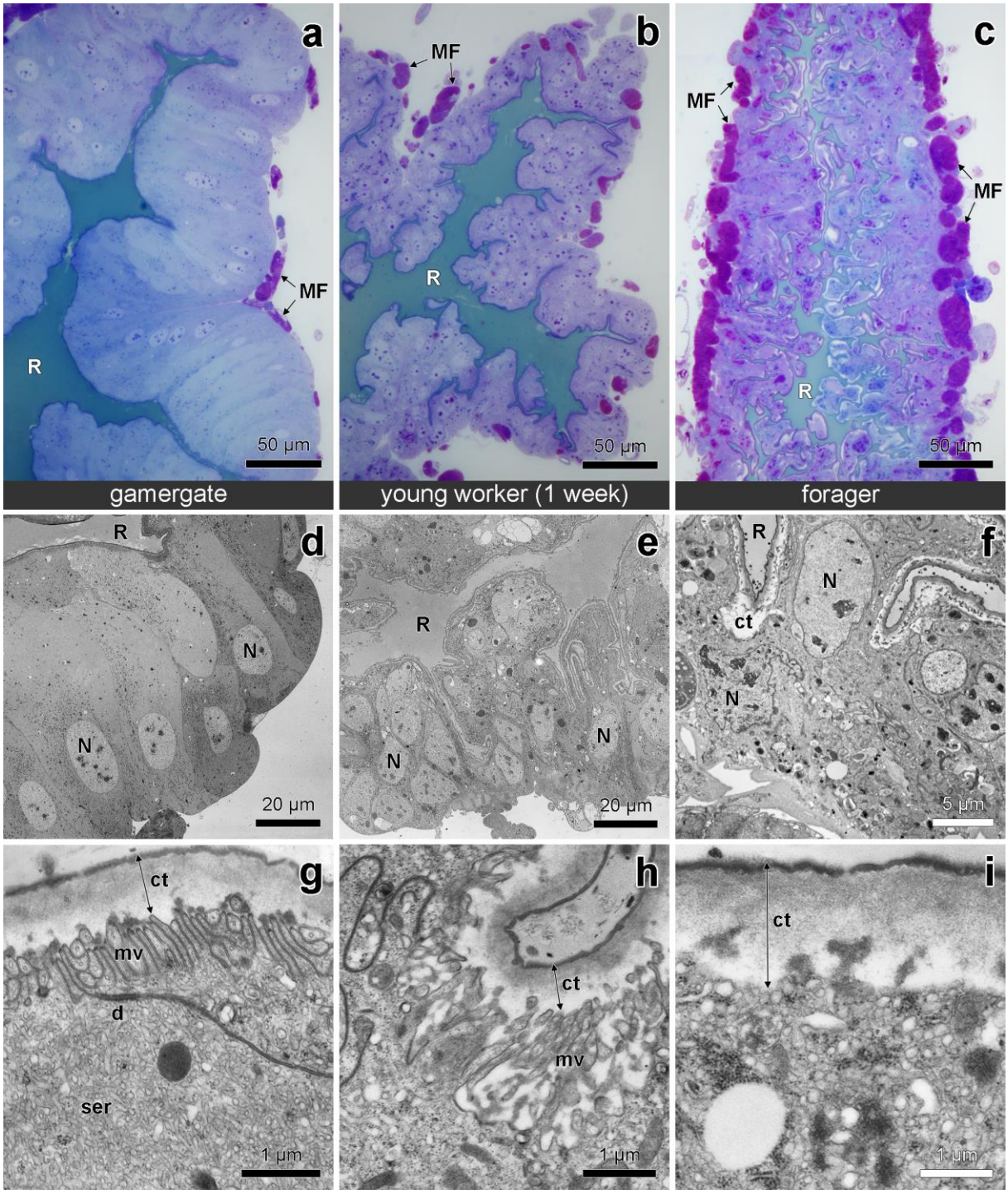


Fig. 1. Comparison of Dufour gland appearance in gamergate (left column: a,d,g), one-week-old worker (middle column: b,e,h) and forager worker (right column: c,f,i). **a**-**c**: semithin sections of gland epithelium, **d**-**f**: electron micrographs of gland epithelium, **g**-**i**: detail views of cuticle (ct) and apical cytoplasm. d: desmosome, MF: muscle fibers, mv: microvilli, N: nucleus, R: reservoir, ser: smooth endoplasmic reticulum.