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0014-4754/91/030227-03\$1.50 + 0.20/0

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A novel exocrine gland inside the thoracic appendages ('gemmae') of the queenless ant *Diacamma australe*

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Received 17 October 1989; accepted 8 August 1990

Summary. In the queenless ant *Diacamma australe*, all workers eclose with a pair of tiny thoracic appendages ('gemmae'). These are sac-like and exhibit a distinct cuticular sculpturing, with minute pores opening on the outer surface. These pores are connected to glandular cells which completely fill the appendages, and thus an exocrine signal is likely to be released. We discuss the social context of this signal: only one worker in each colony retains the gemmae.

Key words. Ant; Ponerinae; reproduction; exocrine glands; queenless.

The dorsal appendages found on the thorax of workers of *Diacamma* do not exist in any other ant genus. Although they were originally described as vestigial wings¹, they differ from the wing rudiments which W. M. Wheeler² has described in rare abnormal worker forms. In order to eliminate confusion, we advocate that the dorsal appendages of *Diacamma* be termed 'gemma' (pl. 'gemmae'), meaning a bud or jewel. This neutral description leaves aside the question of whether the gemma is homologous with the forewing or the tegula.

In *Diacamma* and in various other ponerine species, queens have disappeared and gamergates (= mated workers) reproduce instead^{3–5}. In *D. australe* as well as *D. rugosum*, there is a single gamergate per colony, which can be recognized externally because it is the only individual which retains the gemmae^{4,5}. In all the other workers of a colony, the gemmae are pulled off ('mutilated') soon after the cocoons eclose. The gemmae have an important social function, because their mutilation determines an individual's behaviour, reproductive activity

and opportunity to mate⁴. The gemmae in *D. australe* are filled with glandular cells which open to the outside via minute ducts. Here we describe the external and internal morphology of gemmae, using scanning and transmission electron microscopy.

Material and methods

A colony of *D. australe* was excavated near Townsville, north Queensland, Australia, in March 1989. The ants were taken to Sydney and distributed into several experimental groups. When cocoons eclosed in the absence of a worker with gemmae, one of the callows would remain unmutated. Callows with gemmae were kept alive for a few days before they were sacrificed. Strips of cuticle with the gemmae attached were cut away and fixed in cold 2% glutaraldehyde, buffered with 50 mM sodium cacodylate and 150 mM saccharose. Tissues were kept in buffer until arrival in Belgium. Postfixation in 2% osmium tetroxide in the same buffer preceded dehydration in a graded

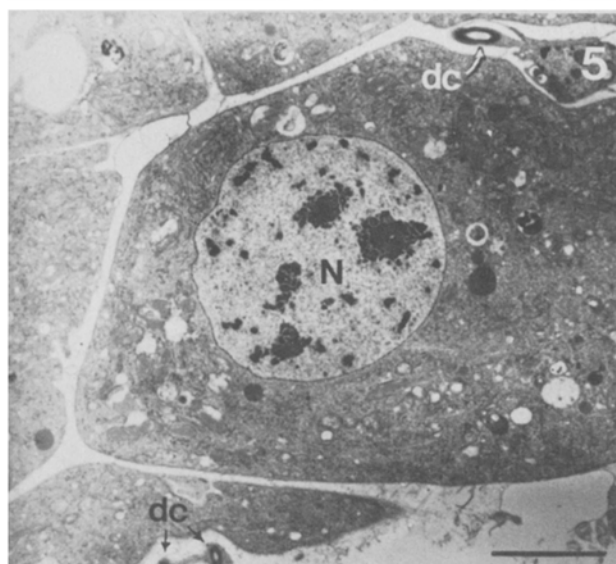
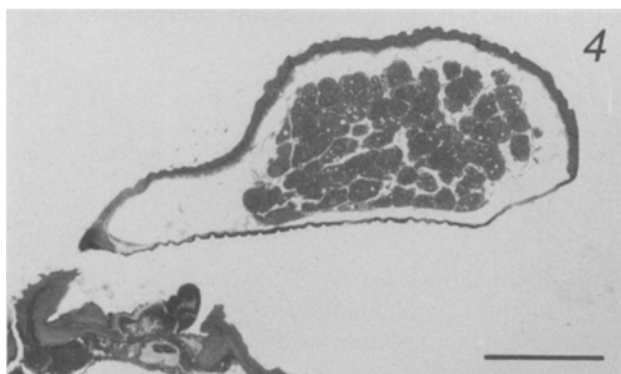
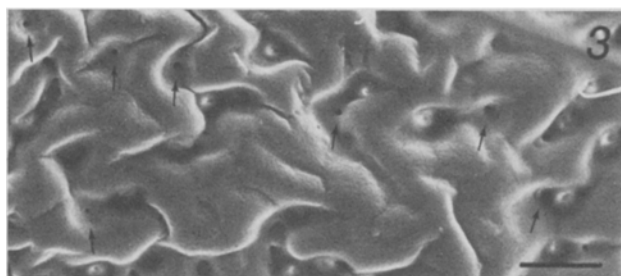


Figure 1. Dorsolateral view of worker thorax of *D. australe* showing the position of the left gemma (scale bar 1 mm).

Figure 2. Surface of the outer side of gemma, with sculptured cuticle and long hairs near the proximal end (scale bar 100 μ m).

Figure 3. Detail of pore openings (arrows) on the outer side (scale bar 10 μ m).

Figure 4. Semi-thin histological section through gemma, showing closely-packed glandular cells (scale bar 100 μ m).

Figure 5. Electron micrograph of a secretory cell (N = nucleus) and duct cells (dc) (scale bar 5 μ m).

acetone series and embedding in 'Araldite' resin. Sections were obtained with a Reichert Ultracut E microtome. Semi-thin sections for light microscopy were stained with methylene blue and thionin. Specimens for scanning electron microscopy were coated with gold-palladium and viewed with a Cambridge 360 instrument. Transmission electron microscopy was performed with a Zeiss EM 900 instrument.

Results and discussion

Each gemma is a sac-like mesothoracic appendage (fig. 1), that articulates to the thorax via a distinct stalk. A gemma measures approximately 450 μ m in length (without stalk 300 to 350 μ m) and 200 μ m in width, and

can reach a thickness of up to 150 μ m. During pupal development, the two gemmae extend outwardly from the body wall, while in the adult stage they are recessed into a distinct pleural cavity, which can be designated as a 'gemmarium' for convenience. In adults, the gemmae are orange in striking contrast to the dark body wall. The convex outer surface has a very wrinkled appearance with a number of long hairs (up to 100 μ m) at its proximal end (fig. 2). The tegumental cuticle of the outer surface has a thickness between 10 and 12 μ m, and has numerous minute pores with a diameter around 0.3 μ m (fig. 3). The concave inner surface, on the other hand, has a cuticular lining of only 2 μ m in thickness, and there are no hairs or cuticular pores.

Histological examination of the gemmae in *D. australe* (fig. 4) revealed that they are completely filled with glandular cells, each with an intracellular end apparatus and an accompanying duct cell, corresponding to the 'secretory unit' type common in insect glands⁶. The efferent ducts all individually open through the numerous pores on the outer surface, after penetrating the thick cuticular lining. The number of glandular cells, based on counts from serial sections, is estimated to be in the range of 500 per gemma. The glandular cells are polygonal with a rounded nucleus (fig. 5). Their cytoplasm contains numerous mitochondria, a well-developed Golgi apparatus and scattered free ribosomes. The very weak development or absence of granular endoplasmic reticulum, as determined by ultrastructural observations, indicates that the secretions may be mainly non-proteinaceous. The cytoplasmic appearance suggests the elaboration of volatile substances, which then may have a pheromonal function.

It is likely that an exocrine signal is released from the gemmae. This was initially suspected on behavioural grounds, because a worker with gemmae is immediately recognized by another. Indeed, when two such unmutated workers (irrespective of whether virgin or mated) were placed into an experimental group, they quickly found each other and proceeded to fight as well as attempt mutilation. However, as soon as a worker possessing gemmae is mutilated (naturally or surgically), it no longer elicits these attacks.

Does the gemma signal have another function besides identification? Newly-emerged workers are surrounded by nestmates and held captive for a variable number of hours⁴. This attention gradually decreases with time, and then the callows become free. It seems unlikely that the secretions from the gemmae are responsible for this attraction, because callows continue to be held after they have been mutilated, while others are sometimes free to walk around before they are mutilated. Thus it may rather be the callow characteristics (e.g. chemical cues from the incompletely-sclerotized cuticle) which attract the workers' attention, and the gemmae signal is superimposed on that. This signal may trigger the attempts at mutilation, and it may also be used to localize the gemmae. The absence of a reservoir indicates that the secretions may be continuously released.

Two further social phenomena are associated with the retention of gemmae⁴, and thus possibly with the pro-

duction of a signal: 1) ovarian inhibition – when a gamergate is present in a colony, the ovaries of almost all unmated nestmates are undeveloped. However, if the gamergate is removed, many of these workers soon begin to lay eggs. This suggests that a primer pheromone⁷ is produced by the gamergate. This inhibitory pheromone could be released from the gemmae, or it could be produced elsewhere in the body. 2) sexual activity – mutilated young workers never mate when foreign males visit the nests. Indeed, there is a strict association between the presence of sperm in the spermatheca and retention of the gemmae. In a few ponerine species without queens, secretions from the pygidial gland in the abdomen are known to attract foreign males⁸. A pygidial gland has been found in another species of *Diacamma*⁹, but its involvement in sexual attraction has not been studied. It is possible that a variety of exocrine glands function in sexual communication in the Ponerinae, including the gemma gland.

The presence of gemmae is associated with both the production of an identification signal, and a change in individual behaviour (from aggressive to timid) which occurs upon mutilation. These two processes seem to be physiologically separate, and further experiments (e.g. blocking signal production, without mutilation) are necessary to disentangle their behavioural consequences. Gemmae are unique to the genus *Diacamma*, and thus the existence of an exocrine gland within them reveals the likely *de novo* evolution of a glandular organ.

Acknowledgments. We thank W. L. Brown Jr for suggesting the term 'gemma', M. Manly for his help with the SEM, and R. Crewe, R. Crozier, M. Elgar and P. Kukuk for comments on this manuscript. C. P. was supported by an Australian Research Council grant to R. H. Crozier.

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0014-4754/91/030229-03\$1.50 + 0.20/0
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