THE STING BULB GLAND IN *MYRMECIA* AND *NOTHOMYRMECIA* (HYMENOPTERA : FORMICIDAE): A NEW EXOCRINE GLAND IN ANTS

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Abstract — A new exocrine gland has been discovered within the sting of the endemic Australian ants of the genera *Myrmecia* and *Nothomyrmecia* (Hymenoptera : Formicidae). It consists of approximately 20 secretory cells with their accompanying duct cells, located between the ducts of the venom and Dufour glands in the proximal part of ths sting bulb, hence my suggestion to designate it as the sting bulb gland. Ultrastructural examination reveals the development of both granular and smooth endoplasmic reticulum in the glandular cells, which possibly may indicate the elaboration of a rather complex secretion. Although the function of the gland remains unknown, its exclusive presence in these ants provides another argument for a closer phylogenetic relationship between both genera than is reflected by their actual classification.

Index descriptors (in addition to those in title): *Myrmecia*, *Nothomyrmecia*, morphology, ultrastructure, phylogeny.

INTRODUCTION

THE STING in social insects has evolved, from its original function as an ovipositor, towards an effective weapon for colony defense. The usually very sharp posterior end of the heavily sclerotized sting shaft and lancets provide the mechanical equipment for piercing the prey or enemy, while exocrine glands produce the venom compounds to be injected in the victim. The glandular apparatus of the sting comprises the venom gland and Dufour's gland, which are the modified accessory glands of the female reproductive system, and both of which open through the anterior side of the sting base in the Formicidae (Billen, 1987a). The mostly proteinaceous defensive secretion generally originates from the venom gland, while the Dufour's gland often produces volatile hydrocarbons with a pheromonal function.

Apart from these major and common sting glands, smaller and much less ubiquitous glands may also be found in association with the sting. In several Ponerinae, a number of glands are situated in the region of the sting apparatus sclerites, with a paired sting chamber gland opening through the membrane connecting the sting base and the last abdominal sternite (Jessen et al., 1979; Jessen and Maschwitz, 1983). Leaf-cutting ants, as well as some other Myrmicinae, possess an unpaired sting valve gland, which opens in very close proximity to the dorsal wall of the sting base (Bazire-Bénazet and Zylberberg, 1979).
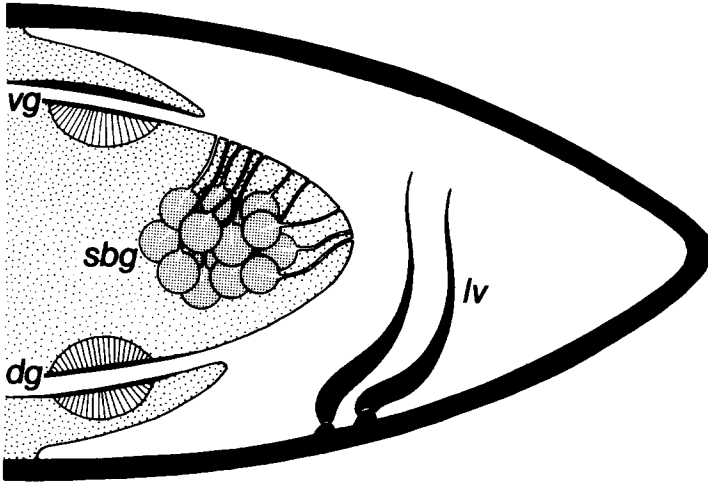
FIG. 1. Schematic longitudinal section through the sting representing the location of the sting bulb gland (sbg), relative to ducts of venom gland (vg) and Dufour gland (dg) and lancet valves (lv).

In the course of my survey of the sting associated glands, however, I discovered a gland, which, in contrast to all other known glands, entirely appears within the sting. I found it only in the primitive Australian genera *Myrmecia* and *Nothomyrmecia*, and here report on the morphology and ultrastructure of this new exocrine gland.

MATERIAL AND METHODS

Foraging workers of *Nothomyrmecia macrops* and *Myrmecia nigriceps* were collected at Poochera, S.A., and Canberra, A.C.T., respectively. Foragers and the queen of *M. pilosula* were obtained from a nest in Hobart, Tasmania. Their stings with associated sclerites and glands were fixed in 2% cold glutaraldehyde and postfixed in 2% cold osmium tetroxide, both buffered at pH 7.3 with 50 mM sodium cacodylate. Tissues were dehydrated through a graded acetone series and embedded in Araldite. Semithin sections for light microscopy were stained with methylene blue and thionin, double stained thin sections were examined with a Philips EM 400 electron microscope.

RESULTS

The sting in aculeate Hymenoptera consists of a robust ventrally open sting shaft and 2 needle-shaped sting lancets that articulate with the former over its entire length by means of a tongue and groove system. Both lancets bear one or 2 pairs of valves (Figs 1, 2) in their proximal region, whose function may be related with the propulsion of the venom. A very short distance anterior to the lancet valves, a bulbous tissue projection into the sting base is found, which in the Formicidae comprises the ducts of both the venom gland (dorsally) and the Dufour gland (ventrally) (Fig. 1).

In workers of *Myrmecia* (Fig. 2) and *Nothomyrmecia* (Fig. 3), however, an additional cluster of glandular cells, situated between the 2 main ducts, occurs within this tissue bulb. It is formed by approximately 20 secretory cells, each accompanied by its own duct
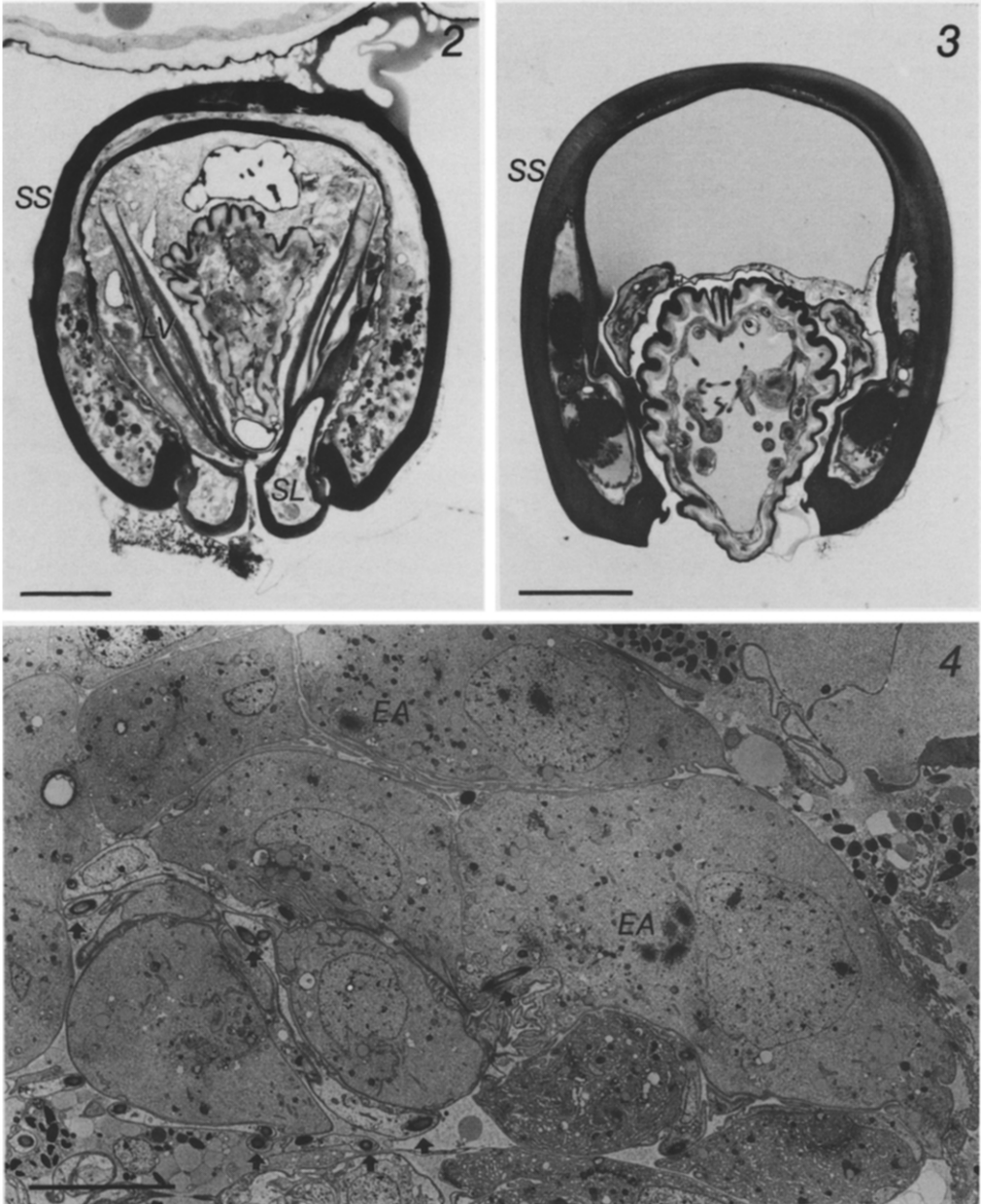
FIGS 2, 3. Semithin cross sections through sting base in *Myrmecia nigricips* (Fig. 2) and *Nothomyrmecia macrops* (Fig. 3), with tissue bulb containing some secretory cells and duct cells in centre of picture. Sting lancets (SL) and their lancet valves (LV) were lost during dissection of *Nothomyrmecia* sting. SS = sting shaft. Scale bar in both figures is 50 μm.
FIG. 4. Electron micrograph of sting bulb gland secretory cells in *M. nigriceps*. EA = end apparatus. Arrows point to efferent ducts. Scale bar = 10 μm.

cell. The efferent ducts open into the venom canal through the most distal wall and the adjacent dorsal region of the tissue projection (Figs 1–3). In close proximity to the glandular cells are nerve fibres (Fig. 5) and tracheoles, the latter sometimes penetrating them (Fig. 6). The gland also occurs in the sting of the queen of *Myrmecia pilosula* that I had available for examination, although the incomplete section series did not allow me to estimate its development relative to the situation in workers.

The secretory cells have a rounded to polygonal appearance, with an average diameter of approximately 20 μm. The nuclei are also rounded or slightly lobate, and measure 8–10 μm in diameter (Figs 4, 5). Each cell is characterized by a presumably short but sinuous end apparatus, which, therefore, may appear more than once in a plane section of a particular cell (Figs 4, 6), or not at all (Fig. 5). At the ultrastructural level, the end apparatus is formed by a cuticular collecting ductule and a surrounding sheath of robust microvilli. Its internal diameter is approximately 0.4 μm, and is lined with a discontinuous inner epicuticular layer and granular peripheral layer (Fig. 6). The cytoplasm shows both granular and smooth endoplasmic reticulum, although the granular form is mostly restricted to a few randomly dispersed strands (Fig. 7), or occurs as a loosely arranged network amidst an abundance of ribosomes (Fig. 6). Numerous mitochondria are scattered in the cytoplasm, while occasionally a lamellar inclusion, comprising a concentric arrangement of membranes, is found (Fig. 8). Each secretory cell is invested with an amorphous basement membrane of approximately 0.1 μm thickness (Fig. 6).

The cuticular ductule of the end apparatus upon leaving the secretory cell continues within a separate duct cell, which eventually will open into the venom canal, as could be ascertained from serial sections. Its internal diameter is 0.5 μm, with a continuous and homogeneous epicuticular lining of approximately 0.1 μm (Fig. 9). The duct cell cytoplasm contains numerous microtubules, but very few other organelles. Nuclei are small and mostly elongated.

DISCUSSION

Although social insects are to be considered as miniaturized walking glandular batteries with exocrine glands virtually in all parts of their tiny bodies, the occurrence of yet another gland within the sting of some Australian ants has remained unknown up to now. According to its particular location, I propose to name this newly discovered gland the "sting bulb gland". This designation refers to its location within the projecting tissue bulb, as well as to the most proximal part of the sting where it is situated, and which is known as the sting bulb (Kugler, 1978).

The cellular arrangement of the gland corresponds to the secretory unit type, in which each glandular cell is associated with its own duct cell that will carry secretion to the outside. The ultrastructural organization of this gland type is characterized by the occurrence of an end apparatus in the secretory cell, which represents a specialized intracellular region where the secretory products are transferred from the secretory cell to the duct cell (Noirot and Quennedey, 1974; Billen, 1987b). The microvilli to this purpose provide an increased surface for the molecules to pass through, while the fenestrated cuticle also facilitates their transport. The chemical nature of the secretion is unknown, although it possibly is partly proteinaceous, considering the occurrence of the moderately developed granular endoplasmic reticulum and the abundant ribosomes. The
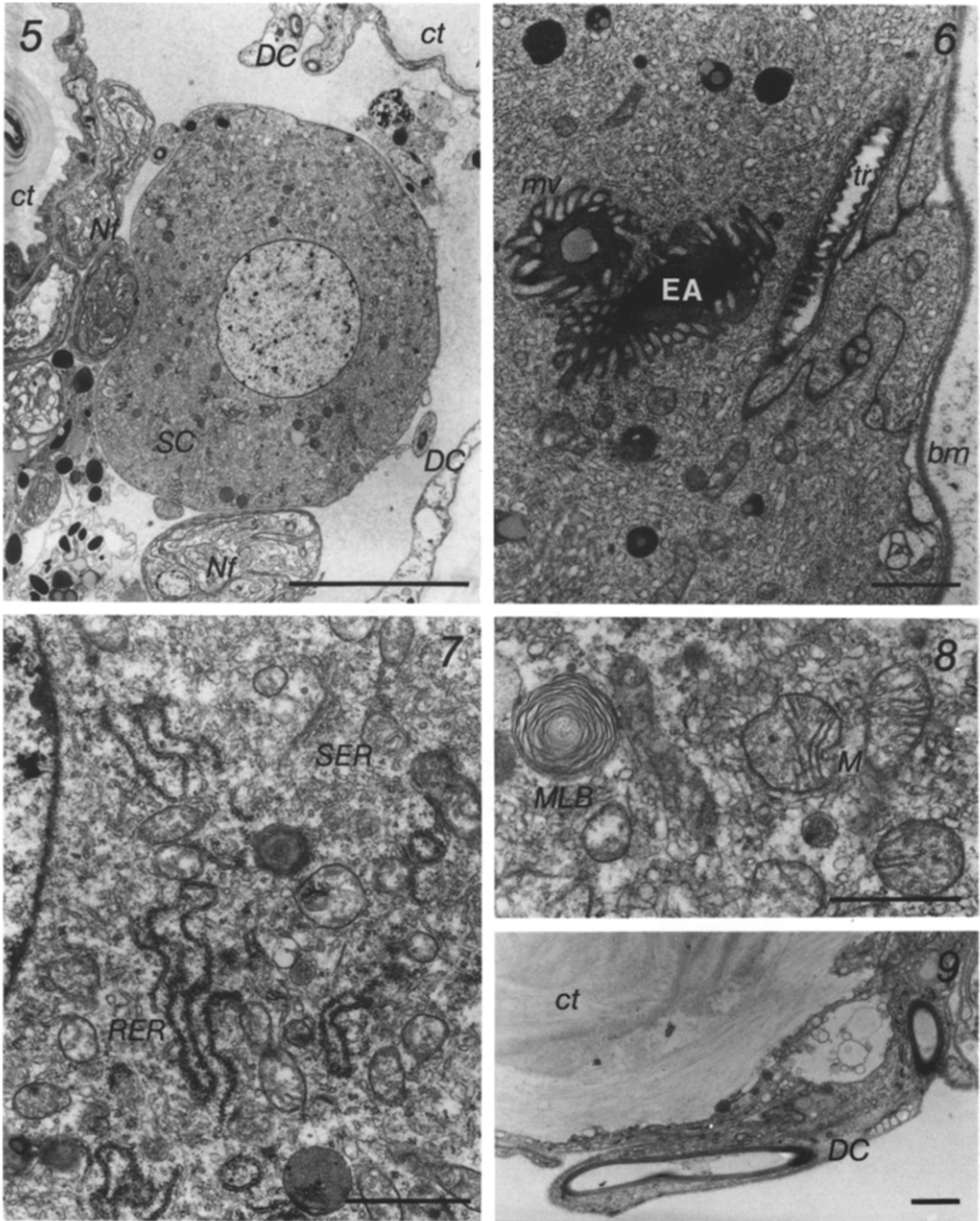
FIG. 5. Secretory cell (SC) and sections through duct cells (DC) of sting bulb gland in *N. macrops*. ct = cuticle lining projecting tissue bulb, Nf = nerve fibres. Scale bar = 10 μm.

FIG. 6. Peripheral portion of secretory cell with surrounding basement membrane (bm), end apparatus (EA) and microvilli (mv) in *M. nigriceps*. Note penetration of tracheoles (tr). Scale bar = 1 μm.

FIGS 7, 8. Cytoplasmic details showing both smooth (SER) and granular endoplasmic reticulum (RER), multilamellar body (MLB) and numerous mitochondria (M). Scale bar = 1 μm.

FIG. 9. Detail of efferent ducts (DC) adjacent to cuticular lining (ct) of tissue bulb. Scale bar = 1 μm.

equally present smooth endoplasmic reticulum, however, may be indicative of the elaboration of a nonproteinaceous fraction too, which eventually might turn out to be volatile compounds.

The chemical composition of its secretion and thus the function of the sting bulb gland, remains speculative. The small size of the gland and the lack of a storage reservoir for its secretion necessarily result in a reduced secretory capacity when compared with most other exocrine glands, although this should not minimize its significance. The occurrence of both granular and smooth endoplasmic reticulum in the secretory cells leaves open a number of possible roles, as does also the location within the sting. An eventual pheromonal role cannot be excluded, nor a merely mechanical function, such as producing a lubricating secretion for the nearby sting lancet valves. More research is clearly needed for a better understanding of the significance of the sting bulb gland, although the very close proximity of the ducts of the much bigger Dufour and venom glands will make it difficult in this regard to avoid contamination with their respective secretions.

A very peculiar aspect of the sting bulb gland is its very restricted presence among the Formicidae. After its discovery in *Myrmecia* and *Nothomyrmecia*, I checked for its existence in a number of stinging species representing Aneuretinae, Ecitoninae, Myrmicinae, Ponerinae and Pseudomyrmecinae, but never found it. The exclusive presence in the monogeneric subfamilies Myrmeciinae (comprising approximately 90 *Myrmecia* species (Taylor, 1987) and Nothomyrmeciinae (*Nothomyrmecia macrops* as the single species (Taylor, 1978)) therefore may be of particular phylogenetic interest. Both endemic Australian subfamilies are presently classified as considerably different and unrelated groups (Taylor, 1978; Baroni Urbani, 1989), mainly because of the tubulate abdominal segment IV in *Myrmecia* versus its non-tubulate appearance in *Nothomyrmecia*. Earlier classifications, however, put both genera together within the Myrmeciinae (Brown, 1954; Brown and Wilson, 1959). Although these older reports were based on the only 2 worker specimens of *Nothomyrmecia* then available, we too are in favour of a closer relationship between *Myrmecia* and *Nothomyrmecia* (Billen, 1988). Both genera, in fact, show a surprisingly similar chemical secretion in their exocrine glands (Billen *et al.*, 1988a, b), the exocrine system in general is very alike, and also the morphology of the sting apparatus supports a close relationship (Kugler, 1980). The exclusive sharing of the sting bulb gland by the 2 genera, therefore, provides another argument to revise their actual distant classification in 2 separate subfamilies, and bring them closer together.

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