

Histology of structures used in territorial combat by Borneo's 'exploding ants'

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Abstract

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Histological studies of the hypertrophied structures of Borneo's 'exploding ants' confirm homology with mandibular glands; reservoirs (only) are hypertrophied as storage structures. The general anatomical organization and ultrastructure of the glands is similar to that in other ant species. Development of secretory structures is not unusually great, and secretory cells are apparently confined to the head. These results suggest gradual production of mandibular gland compounds and filling of reservoirs and are consistent with known and hypothesized roles of mandibular glands and their products.

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Introduction

In ants (family Formicidae), exocrine glands and their secretions play numerous, diverse and important roles in, e.g. communication among nestmates, reproductive signalling and defence of colonies and their foraging territories (Hölldobler and Wilson 1990; Billen and Morgan 1998). Although specific individual glands tend to perform similar functions in related taxa, novel functions arise occasionally. Changes in product chemistry, and in glandular size and ultrastructure, can be indicators of altered function across taxa (reviewed in Hölldobler and Wilson 1990; Jones *et al.* 2004; Billen *et al.* 2008) and even castes (Grasso *et al.* 2005; Niculita *et al.* 2007). A possible constraint on the evolution of broader or novel function is the relatively narrow distribution of glands over body parts. Thus, except for pedestal hair glands, subepithelial glands and the glandular tegumental epithelium, exocrine glands and their reservoirs (if present) tend to occur in just one body part: head, thorax (or legs), and abdomen (= gaster plus petiole and postpetiole, if present) (Billen 2009). They are neither replicated nor extend into additional body parts.

South-east Asian camponotines currently classified as the *Camponotus* (*Colobopsis*) *cylindricus* complex (hereafter 'COCY' spp.) are well known for pronounced hypertrophy of cephalic glands through the thorax to the abdominal tip, and for novel use of glandular products in territorial combat (Jones *et al.* 2004). Maschwitz and Maschwitz (1974) first

described how grasping a worker's leg with forceps instantly induced individuals to expel contents of the hypertrophied structures suicidally through the intersegmental wall of the gaster. Similar behaviour was observed by these authors when the Asian ants were introduced to European ants in the laboratory. In COCY species with self-sacrificing workers, suicidal release of glandular product occurs after a COCY worker grasps the leg or antenna of an intruder into the colony's foraging territory (Jones *et al.* 2004; Cook 2008). Wrapping itself around an opponent's ventral side or doubling the dorsal gaster onto the face of a weaver ant (*Oecophylla smaragdina*), the worker expels adhesive product from the mouth and gaster and adheres to its opponent in a permanent 'death grip'. Subsequent study revealed that self-sacrifice was a derived trait in the COCY species complex and that both glandular hypertrophy and unique glandular products (Jones *et al.* 2004) likely evolved under selection for some other functions (Cook 2008). In the case of 'exploding' COCY species, glandular hypertrophy across body parts has been accompanied by novel function: voluntary self-sacrifice (autothysis) in defence of territory.

As yet, no histological or other morphological evidence has documented whether the hypertrophied structures are actually mandibular glands, as reasonably proposed by Maschwitz and Maschwitz (1974), versus some other paired ant cephalic gland, or even a novel gland altogether. Nor have detailed studies determined whether glandular ultrastructure differs from that in homologous glands of other ants, or whether the

structures are glandular throughout their length, rather than possessing typical amounts of glandular tissue but hypertrophied reservoirs. Later investigators of these taxa have nevertheless deferred to the early identification of the glands, although some (Davidson *et al.* 2007, 2009) have assumed that extensions of these structures through the thorax and abdomen might actually be reservoirs lacking secretory capacity. Further, although abdominal pumping by workers may suffice to force reservoirs through the gastral wall (Cook 2008), musculature in the walls of glandular reservoirs themselves could play some role in product expulsion, although this remains to be determined by comparison with similar musculature surrounding homologous reservoirs of other ants. In these contexts, histological study has the potential to advance our understanding of glandular function.

Here, we present the results of histological studies designed to answer the following four questions: (1) Are the hypertrophied glands homologous with the mandibular glands of other ants? (2) Are secretory cells confined to the head, with just glandular reservoirs extending into posterior segments, or are those cells also distributed through the thorax and abdomen? (3) Does histological evidence suggest possible involvement of reservoir musculature in expulsion of glandular product? (4) If the glands indeed are homologous with mandibular glands, does novel function in defence depend on glandular (or reservoir) hypertrophy alone, or also on changes in glandular ultrastructure?

Material and Methods

Ants were collected from lowland dipterocarp rain forest surrounding the Kuala Belalong Field Studies Centre (KBFSC), in the Temburong District of Brunei Darussalam (04°32'N, 115°10'E). From the 16 COCY species present there, we chose to work with the small-bodied *Camponotus [Colobopsis]* sp. 'YG' ('yellow goo [product]'; voucher # DWD KB02-108, in the Natural History Museum of Los Angeles County). This species exhibited the highest frequency of gastral rupture in trials with forceps, and it has the greatest whole-body 'investment' in product on the basis of concentration: weight (Cook 2008). Smaller-bodied ants also present fewer barriers to histological study than do their larger-bodied counterparts.

Body parts of workers were cut so as to expose tissues sufficiently to penetration of chemicals used during tissue processing for histological examination. Cuts were made to obtain anterior heads, posterior heads (with prothorax attached), posterior thorax (with petiole and anterior gaster attached) and posterior gaster. Tissues were fixed in cold 2% glutaraldehyde, buffered at pH 7.3 with 50 mM Na-cacodylate and 150 mM saccharose and postfixed in 2% osmium tetroxide in the same buffer. Dehydration was carried out in a graded acetone series. Araldite-embedded tissue blocks were sectioned with a Reichert Ultracut E microtome. Semithin 1- μ m sections were stained with

methylene blue and thionin and viewed in a Zeiss Axioskop microscope. Double-stained 70-nm thin sections were examined in a Zeiss EM900 electron microscope.

Results

Histological sections of *Camponotus (Colobopsis)* sp. 'YG' workers confirm that the paired large sacs extending from the head through the thorax into the abdomen are hypertrophied mandibular gland reservoirs. This conclusion follows both from the general anatomical organization, with class-3 secretory cells opening in the reservoir (Fig. 1A), and from the characteristic appearance of a narrow duct linking the reservoir to a dorsal slit-like groove at the base of the mandible (Fig. 1B). The two cephalic reservoir sacs continue as two narrow tubes through the thorax and petiole (Fig. 1C) and upon entering the gaster, widen again to become two voluminous, blind-ending sacs that occupy more than half of the gaster volume (Fig. 1D). Based on a single good series of sections, the reservoirs would appear to be equal in length. In its entire range from head to gaster, the reservoir wall is very thin (0.5–1.5 μ m) with cells containing flat nuclei. It is lined internally with a thin cuticle of 0.1–0.3 μ m thickness (Fig. 2E). No muscle fibres were observed in association with any part of the reservoir.

Secretory cells are restricted to the cephalic portion only of the mandibular gland, where they occur in clusters next to the reservoirs (Fig. 2A,B). They belong to class-3, according to the standard classification of Noirot and Quennedey (1974); thus, each secretory cell is associated with a duct cell that carries the secretion to the reservoir. The ovoid-to-polygonal secretory cells measure approximately 30 \times 20 μ m and contain a rounded central nucleus with a diameter of about 10 μ m. The duct cell continues into the secretory cell through an end apparatus, which is formed by a porous central cuticular ductule surrounded by microvilli. The microvilli can appear in a regular pattern (Fig. 2C) or as considerably distorted (Fig. 2D), and both situations can be found in cells of the same ant. The cytoplasm contains numerous mitochondria and some dark-staining secretory droplets (Fig. 2C).

Discussion

Our histological data clearly illustrate that the hypertrophied glands in workers of *Camponotus (Colobopsis)* sp. 'YG' are homologous with mandibular glands and that the reservoirs are hypertrophied as storage structures. The opening of the glandular ducts at the upper proximal base of the mandibles through a slit-like groove corresponds with the typical anatomical organization for ant mandibles (Grasso *et al.* 2004). The development of secretory structures is not unusually great, however, as secretory cells are apparently confined to the head. Thus, the reservoirs likely fill gradually, as a continuous supply of precursor molecules (fatty acids, see Jones *et al.* 2004) is absorbed from the haemolymph.

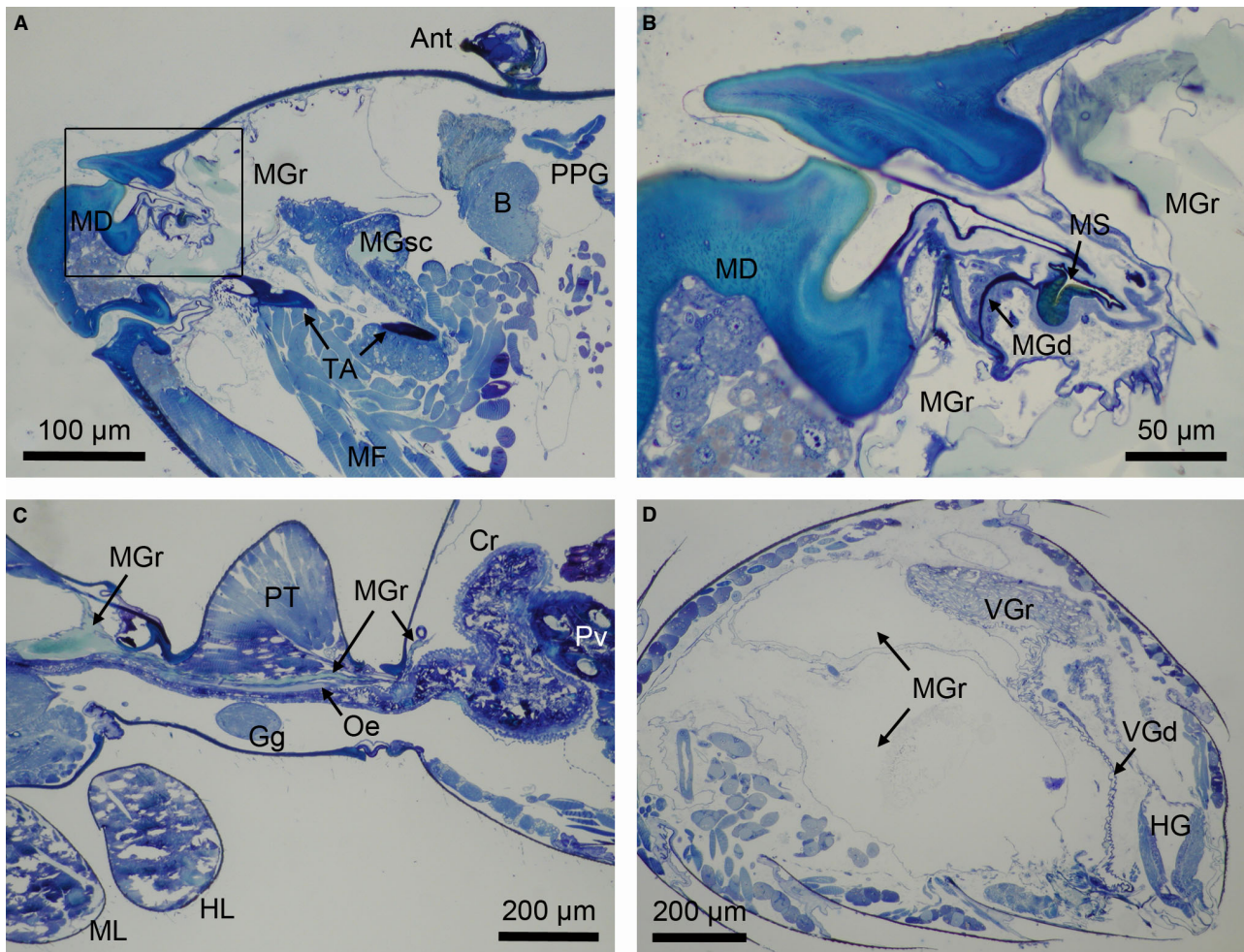


Fig. 1—Semithin sections showing the general histological appearance and development of the mandibular gland in workers of *Camponotus* (*Colobopsis*) sp. ‘YG’ workers. In all pictures, the anterior side is to the left. — **A**. Longitudinal section through the anterior part of the head, showing the large mandibular gland reservoir (MGr) and secretory cells (MGsc). The inset with the opening of the mandibular gland duct is enlarged in **B**. — **B**. Detail of mandibular base with opening of the mandibular gland duct (MGd) at upper proximal region of mandible. — **C**. Longitudinal section through petiolar region, illustrating narrow passage of mandibular gland reservoir (MGr) from thorax into abdomen. — **D**. Longitudinal section through abdomen, showing large mandibular gland reservoirs (MGr). Ant, antenna; B, brain; Cr, crop; Gg, ganglion; HG, hindgut; HL, hindleg; MD, mandible; MGd, mandibular gland duct; MGr, mandibular gland reservoir; MGsc, mandibular gland secretory cells; MF, muscle fibres; ML, midleg; MS, mandibular slit; Oe, oesophagus; PPG, postpharyngeal gland; PT, petiole; Pv, proventriculus; TA, tentorial arms; VGd, venom gland duct; VGr, venom gland reservoir.

Gradual filling of reservoirs with mandibular gland product is consistent with known and hypothesized uses of these secretions, including ‘cephalic digestion’ of microbial foods (D. W. Davidson, D. E. Edwards, and A. S. Kamariah, unpublished data). As a group, COCY species forage by grazing microbes (mainly spores) on plant surfaces, and they apparently subsist on these solids, too large to pass through the proventriculus to the midgut, by digesting them *in situ* in the buccal chamber (see also Hansen *et al.* 1999; for other camponotines). The latter usage may explain why glandular reservoirs are hypertrophied even in non-exploding species in this clade, as well as in non-exploding major workers (Cook 2008). For species

with m-cresol as a major mandibular gland product, a role of secretions in regulating nest pathogens has also been suggested (Cook 2008; Davidson *et al.* 2009). Consistent with lack of reservoir musculature for product expulsion and of hypertrophy of secretory structures and capacity, use of mandibular gland product in both digestion and nest hygiene is likely to be gradual.

Although the mandibular gland reservoirs of these ants are unusual in size, the glands and reservoirs are not atypical in their general anatomical organization nor in their ultrastructure. As class-3 glands, according to Noirot and Quennedey (1974), they consist of variable numbers of

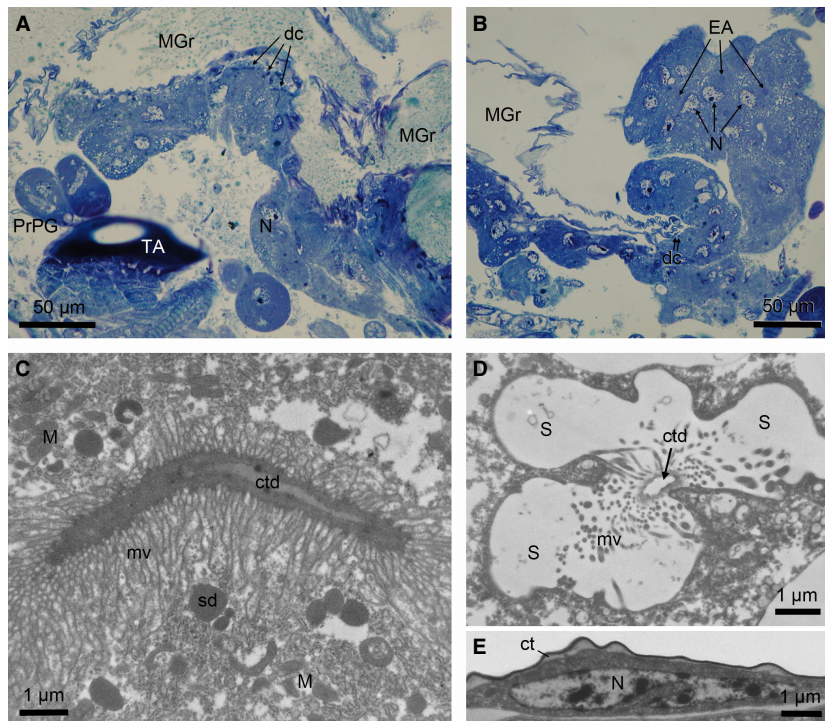


Fig. 2—**A,B.** Semithin sections of mandibular gland secretory cells opening into the reservoir in the head. — **C,D.** Electron micrographs of the end apparatus with regular and distorted microvillar pattern, respectively. — **E.** Electron micrograph detail of the flat reservoir wall. ct, cuticle; ctd, cuticular ductule; dc, duct cells; EA, end apparatus; M, mitochondria; MGr, mandibular gland reservoir; MF, muscle fibres; mv, microvilli; N, nuclei; PrPG, propharyngeal gland; S, extracellular space; sd, secretory droplet; TA, tentorial arm.

two-cell units with one secretory and one duct cell, and they likely function much as described for secretory structures in mandibular glands in other ant species (Billen and Schoeters 1994). Precursor molecules are taken up from the haemolymph through the secretory cell's peripheral membrane. The microvilli of the end apparatus provide a considerable surface increase that, together with the porous cuticular lining, allows the secretory products to leave the secretory cell (Noirot and Quennedey 1974; Billen 1991). Accumulation of secretion in the end apparatus can lead to a distorted appearance of the microvilli, which is also known for the mandibular gland of *Formica* ants (Billen and Schoeters 1994).

We found no sign of any reservoir musculature; pumping of the gaster, through the local abdominal muscles, may therefore suffice for forceful expulsion of the reservoir contents. Secretion release through strong contractions of the gaster leading to its bursting has also been suggested by Buschinger and Maschwitz (1984).

Prior depictions of the enlarged 'glands' or glandular reservoirs in the literature have been inconsistent. Some (Hölldobler and Wilson 1990) show the reservoirs extending in parallel throughout the gaster and equal in length, whereas other investigators (Maschwitz and Maschwitz 1974) have drawn one of the reservoirs apparently shorter than the other and ending just past the centre of the gaster. This difference may be biologically significant, because suicidal sacrifice typically results when one of the reservoirs bursts through the dorsal intersegmental membrane of the anterior gaster (Maschwitz and Maschwitz 1974; D. W. Davidson, personal

observations), as well as at the gastral tip (noted frequently in our studies, but not in earlier publications). By emerging from two different parts of the gaster, mandibular gland secretions may contaminate more of an enemy's body. Thus, when a worker grasps the antenna of a weaver ant (*Oecophylla smaragdina*) and bends the gaster ventrally against its face, both the eyes and the mandibles can be contaminated by adhesive product (D. W. Davidson, personal observations). Our histological results are consistent with the reservoirs being approximately equal in length and with asymmetry in product emergence site being positional rather than anatomical. However, just one series of sections was adequate to investigate asymmetry, and additional study would be required to investigate the generality of our result.

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