Exocrine glands of the ant *Myrmoteras iriodum*

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Abstract

This paper describes the morphological characteristics of 9 major exocrine glands in workers of the formicine ant *Myrmoteras iriodum*. The elongate mandibles reveal along their entire length a conspicuous intramandibular gland, that contains both class-1 and class-3 secretory cells. The mandibular glands show a peculiar appearance of their secretory cells with a branched end apparatus, which is unusual for ants. The other major glands (pro- and postpharyngeal gland, infrabuccal cavity gland, labial gland, metapleural gland, venom gland and Dufour gland) show the common features for formicine ants. The precise function of the glands could not yet be experimentally demonstrated, and to clarify this will depend on the availability of live material of these enigmatic ants in future.

Key word: Formicidae, intramandibular gland, mandibular gland, metapleural gland, venom gland.

INTRODUCTION

Ants exist in various sizes and shapes, and many species are intensively studied (Hölldobler & Wilson 1990). One of the most remarkable and enigmatic genera is the tropical Asian *Myrmoteras* Forel. These formicine species are characterized by their extremely big eyes and very long and slender mandibles, that can be held open at 280 degrees during foraging, which is the record value so far recorded among the ants (Fig. 1). Our knowledge on the biology of these ants mainly goes back to a paper by Moffett (1986), that describes their trap-jaw predation as well as a number of other field and laboratory behavioural observations of *Myrmoteras toro* Moffett and *M. barbouri* Creighton.

As we could collect live material of the Malaysian *Myrmoteras iriodum* Moffett, we undertook a morphological study to describe the workers' exocrine system, as no such information is available for this ant genus so far. Particular

attention was given to the mandibles, as an intramandibular gland had been reported as a common feature inside ant mandibles (Schoeters & Billen 1994), but so far could never be studied in species with such elongate mandibles. In this paper, we therefore focus on the light and electron microscopical analysis of the mandibles, and briefly survey the general histological characteristics of the other major exocrine glands in this species. The order in which the glands are described is from 'head to tail'; all longitudinal sections are shown with the anterior side to the left.

MATERIAL AND METHODS

Three small queenright monogynous colonies of *Myrmoteras iriodum* containing 1, 4 and 6 workers, respectively, were collected from fallen twigs in Ulu Gombak, peninsular Malaysia. Anterior head parts (with the mandibular distal portions cut off to allow penetration of fixative solution from that side), anterior as well as posterior thorax halves, and posterior abdomens of workers were fixed in cold 2% glutaraldehyde, buffered at pH 7.3 with 50 mM Na-cacodylate and 150 mM saccharose. This was followed by postfixation in 2% osmium tetroxide in the same buffer. Dehydration was achieved in a graded acetone series, and Araldite-embedded tissues were sectioned with a Leica EM UC6 ultramicrotome. Serial semithin sections with a thickness of 1 µm were stained with methylene blue and thionin and viewed with an Olympus BX-51 microscope. Double stained 70 nm thin sections were examined with a Zeiss EM900 electron microscope. Material for scanning microscopy was critical point dried in a Balzers CPD 030 instrument and examined in a JEOL JSM-6360 scanning microscope.

RESULTS AND DISCUSSION

Intramandibular gland

Histological sections through the mandibles show, along their entire length and both at the upper and lower side, the presence of abundant ovoid gland cells of 20–30 μm diameter with rounded nuclei that are wedged in between the epidermal cells of the mandibular tegument (Fig. 2A,C). Scanning microscopy of the mandibular surface reveals the presence of numerous pores with a diameter around 0.5 μm on both the upper and lower mandibular surface; approximately 200 pores can be given as a rough estimate count per mandible per side. The pores are most abundant near the inner edge, where each pore is associated with a slit of approximately 10 μm long, that is oriented lengthwise on the mandibular surface (Fig. 2B). Pores occur along the entire length of the mandible, but are slightly more abundant in the proximal region. The gland cells correspond with class-3 following the standard classification of Noirot and Quennedey (1974), and are thus each associated with an accompanying duct cell. The ducts carry the secretion to the exterior, where they open through the pores that are visible under the scanning microscope. The gland cells show a vesicular cytoplasm (Fig. 2C), which is very clear at the ultrastructural level. The majority of the spherical electron-lucid secretory vesicles have a diameter between 1 and 3 μm . They concentrate around the end apparatus (Fig. 2D,E), which illustrates their secretory nature. The vesicles often contain lamellar inclusions, especially near the end apparatus (Fig. 2D), that represent lipidic secretory products (Schmitz & Müller 1991). The epidermal class-1 cells of the mandibular tegument have a thickness between 10–20 μm , and appear to have glandular activity as well, as can be inferred from the clear presence of a Golgi apparatus and of apical microvilli and basal invaginations (Fig. 2F).

The *Myrmoteras* mandibles thus harbour a conspicuous intramandibular gland, that contains both class-3 secretory cells, as is common for the intramandibular gland of ants in general (Schoeters & Billen 1994; Grasso *et al.* 2004), but also class-1 gland cells. The presence of an epithelial class-1

intramandibular gland in ants has so far been reported only for *Pyramica* (*Strumigenys*) *membranifera* (Emery) (Billen & Espadaler 2002), some attine and ponerine species (Amaral & Caetano 2006; Martins & Serrão 2011), and for *Protanilla* Taylor (Billen *et al.* 2013); some stingless bees also contain epithelial intramandibular glands (Costa Leonardo 1978). In the very rare agroecomyrmecine *Tatuidris tatusia* Brown and Kempf, a different kind of class-3 intramandibular gland has been described, in which the ducts form one large bundle that opens through a sieveplate at the ventral proximal side of each mandible (Billen & Delsinne 2013).

The peculiar slits associated with the pores of the class-3 openings in *Myrmoteras* may represent a structural adaptation to regulate the release of secretion, as evaporation of volatile components may be slowed down when these are contained in the narrow slits. The presence of the gland over the entire length of the mandibles indicates it must have a significant function, while the main concentration of the pores at the masticatory inner margin may indicate it is related with food processing. Moffett (1986) describes how prey is held between the mandibles, the teeth of which are pressed into the prey as a kind of chewing. It remains unknown whether the intramandibular gland may be involved in this behaviour.

Mandibular gland

The paired mandibular gland consists at each side of a thin-walled reservoir and a cluster of 25–30 secretory cells near its inner ventral side. The cylindrical secretory cells have a height of 40–45 μm and a width around 25 μm , with rather flattened basally located nuclei. The gland corresponds with class-3 following the classification of Noirot and Quennedey (1974). The secretory cells have a peculiar shape with a big apical cuticular cup that represents an extension from the reservoir. The bottom of each cup connects with a bulbous

proximal portion of the end apparatus, from which a few straight branches start towards the deeper regions of the cell (Figs 3,4A). As we unfortunately had no material available for ultrastructural examination, we are unable to comment on the microvillar organization in the end apparatus. The occurrence of a branched end apparatus is exceptional for ant glands, but is a common characteristic for class-3 exocrine glands in wasps (Delfino *et al.* 1979, 1983). Details of these unusual cellular features of the mandibular gland of *Myrmoteras* remain unknown. As in most ants, its secretions may form part of the alarm-defence system (Maschwitz 1964; Hefetz & Lloyd 1985), although experimental evidence for such function in *Myrmoteras* so far is not yet available.

Propharyngeal gland

The paired propharyngeal gland at each side contains approximately 25 rounded secretory cells with a diameter of 45–50 µm. The large class-3 cells, with centrally located round nuclei of 10–12 µm diameter, are situated underneath the pharynx, and at each side open through their accompanying duct cells into a small ventrolateral atrium which in turn opens into the pharyngeal lumen (Fig. 4B). This opening site of the propharyngeal gland can be linked with its function of producing digestive enzymes, as has been reported for other ant species (Ayre 1967; Gama & Cruz Landim 1982; Billen & Peusens 1984).

Infrabuccal cavity gland

The wall of the infrabuccal cavity is differentiated into a glandular epithelium of class-1 cells that reaches a thickness between 15 and 25 µm (Fig. 4B,E: IGE). A similar infrabuccal cavity gland has also been reported in *Monomorium pharaonis* Linnaeus (Eelen *et al.* 2004) and in *Protanilla* (Billen *et al.* 2013). The location of the gland presumably is in line with a digestive function.

Postpharyngeal gland

The postpharyngeal gland (class-1) corresponds with the usual glove-shape, with 8–10 tubular fingers occurring next and underneath the pharynx, and opening in the posterior region of the pharyngeal lumen. The glandular cells have a thickness of 15–20 μm (Fig. 4C). The postpharyngeal gland is only known in the Formicidae, and is generally involved in species recognition through the cuticular hydrocarbons it contains (Bagnères & Morgan 1991).

Labial gland

The labial (= salivary) gland opens at the upper side of the labium (Fig. 4D: arrows). The unpaired labial duct from there runs posteriorly through the head, and bifurcates in the neck region. As is common for formicine ants (Gama & Cruz Landim 1982), the two labial glands are situated in the anterior half of the thorax, and represent simple epithelial tubules made up of class-1 secretory cells with a thickness around 15 μm (Fig. 4E).

Metapleural gland

Together with the postpharyngeal gland, the metapleural gland is a neoformation that only occurs in ants. Its main function is the production of antibiotics, that protect the ants and their nests against microorganisms (Hölldobler & Engel-Siegel 1984). The paired gland is situated in the metathorax, and at each side contains 40–50 spherical secretory cells (class-3) with a diameter around 35 μm (Fig. 5A). From each secretory cell, a slender duct cell with a cuticular canal with internal diameter of 1 μm connects to a sieve-plate at the anterior side of a clearly sclerotized and hence rigid reservoir chamber or bulla (Fig. 5A,B). The two lateral reservoir chambers have a diameter around 50 μm , and at their posterior side show a permanently open

and wide slit-like contact with the outside. Cuticular bristle hairs protrude from the reservoir chambers (Fig. 5B,C), and probably help is the dispersal of the secretory products. The permanently open contact with the outside can be understood from the gland's production of antibiotics, that have to be constantly discharged to protect the ants (Hölldobler & Engel-Siegel 1984). The occurrence of fairly well developed metapleural glands in *M. iriodum* is in line with their nesting in twigs or subterranean nests, where protection against microorganisms is an evident requirement.

Venom gland

As in other formicine ants (Schoeters & Billen 1998), the venom gland is by far the largest abdominal gland in *Myrmoteras iriodum*. Its major part is formed by the pulvinated convoluted gland, that forms a dorsal cap overlaying a muscle-lined reservoir sac. The convoluted gland appears as a compact mass of the extremely lengthy and tortuous secretory filament, of which the very distal portion bifurcates and occurs as the two free secretory filaments of the venom gland (Fig. 5D). This complex compartmentalization of the formicine venom gland allows biosynthesis and storage of the highly concentrated formic acid without the risk of self-toxication (Hefetz & Blum 1978). In its posterior region, the reservoir tapers down into the venom gland duct (Fig. 5D: VD), that eventually opens through a slit-like orifice into the cloacal chamber, dorsally to the equally slit-like Dufour gland orifice (Fig. 5E). Upon contraction of the reservoir musculature (Schoeters & Billen 1996), the formic acid is forcibly squeezed out of the gland and is sprayed through the acidopore (Fig. 5E).

Dufour gland

The Dufour gland appears as a long tube with a length around 500 µm and a width around 50 µm (Fig. 5E). The central lumen is lined with irregular epithelial

class-1 cells with a thickness that varies between 15 and 20 μm . In its posterior region, the gland narrows to form a slit-like duct, that opens ventrally of the venom gland duct in the cloacal chamber (Fig. 5E). No clear and general function could so far be attributed to the Dufour gland in formicine ants, of which the secretion is often dominated by undecane or tridecane (Morgan 2008). Also for *Myrmoteras*, the function remains unknown, although its fairly large size should go along with a significant function.

CONCLUSION

Our survey of the exocrine system in workers of *Myrmoteras iriodum* revealed the presence of the common exocrine structures in ants. The pygidial gland, which is a widespread gland in most ant subfamilies, however, was not found. This is in line with the usual absence of this gland in formicine ants (Hölldobler & Engel 1978; its presence in *Polyergus rufescens* Latreille is rather exceptional, see Hölldobler 1984). The most striking finding was the presence of well-developed intramandibular gland cells of both class-1 and class-3 along the entire length of the slender mandibles. Such conspicuous intramandibular gland must go along with an important function, although no experimental data are available so far. The peculiar structural organization of the secretory cells of the mandibular gland has not been found in other ant species. Its functional significance for the time being remains unknown, and equally forms a challenge to be tested as soon as these fascinating ants will become available again for further study.

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FIGURE LEGENDS

Figure 1 Scanning micrographs of *Myrmoterias iriodum* worker in lateral view (A), and of the head in rest position (B) and with open mandibles as during foraging (C).

Figure 2 Intramandibular gland: (A) Longitudinal section through anterior head region (framed part is enlarged in figure 2C). (B) Scanning micrograph detail of inner upper part of mandible in its proximal region. Note parallel slits, each slit containing the opening of a gland duct (arrows). (C)

Longitudinal section through mandible, showing class-3 intramandibular gland cells (arrows) embedded between epidermis. (D) Electron micrograph of class-3 secretory cell and junction to its accompanying duct. (E) Ultrastructure of cytoplasm of intramandibular gland secretory cell (class-3), note lamellar inclusions associated with end apparatus. (F) Ultrastructure of intramandibular epidermal cell (class-1). AB: antennal base, Bi: basal invaginations, Ct: cuticle, EA: end apparatus, GA: Golgi apparatus, LI: lamellar inclusions, M: mitochondria, Md: mandible, MF: muscle fibres, MN: mandibular nerve, Mv: microvilli, N: nucleus.

Figure 3 Schematic drawing of organization of mandibular gland cells, with apical cuticular cups (CC), bulbous proximal part (B) and branched distal portion of end apparatus (EA). Nd: nucleus duct cell, Nr: nucleus reservoir cell, Ns: nucleus secretory cell, R: reservoir.

Figure 4 (A) Mandibular gland section. White arrows indicate bulbous proximal part of end apparatus, black arrows indicate branched appearance of end apparatus. (B) Cross section through anterior pharynx, showing opening of propharyngeal gland cells (arrows) in left side atrium (right side atrium not shown on this section). (C) Cross section through head, showing opening of postpharyngeal gland tubules (asterisks) into pharynx. (D) Longitudinal section through anterior portion of head. Arrows show proximal part of labial gland duct; double arrow shows location and angle of cross section indicated in figure 4B. (E) Detail of labial gland tubules. Atr: atrium, CC:

cuticular cups, CE: compound eye, Lb: labium, LE: labial gland epithelium, IBC: infrabuccal cavity, IGE: infrabuccal gland epithelium, N: nucleus, Ph: pharynx, R: reservoir, Ts: tracheolar sac.

Figure 5 (A) Metapleural gland secretory cells and duct cells opening through perforated anterior tip of bulla. White arrows indicate end apparatus. (B) Longitudinal section through posterior part of thorax, showing metapleural

gland opening through bulla (white arrows show end apparatus, black arrows indicate duct cells). Double grey arrow shows permanently open orifice of metapleural gland bulla. (C) Scanning micrograph of posterior thorax and petiole. Dashed line indicates location of metapleural gland. Note permanently open bulla and hairs protruding from it. (D) Proximal part of multicompartmentalized venom gland. (E) Longitudinal section through abdominal tip along body midline, showing opening of venom gland and Dufour gland. The upper and lower arrow indicate the slit-like opening of the venom gland duct and Dufour gland duct, respectively. AH: acidopore hairs, B: bulla, bh: bristle hairs, DC: duct cells, DD: Dufour gland duct, DG: Dufour gland, HG: hindgut, HL: hindleg, MF: muscle fibres, ML: midleg, MS: metathoracic spiracle, Ovd: oviduct, Pt: petiole, SC: secretory cells, VCG: convoluted gland portion of venom gland, VD: venom gland duct, VST: venom gland secretory tubules.