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ORIGINAL ARTICLE

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The intramandibular gland, a novel exocrine structure in ants (Insecta, Hymenoptera)

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Summary A novel glandular structure is described within the mandibles of a series of representatives of the Formicidae. It consists of a variable number of secretory cells with their accompanying duct cells, located in the vicinity of the mandibular cuticle. Because of its localization inside the mandibles, we propose to designate this structure as the intramandibular gland of ants. Ultrastructural observations reveal the development of smooth endoplasmic reticulum in the glandular cells. Several electron-lucid and electron-dense inclusions occur in the cytoplasm. Until now, the function of this widespread gland among the Formicidae remains unknown.

A. Introduction

Ants possess a well-developed social organization with a communication system based on pheromonal secretions originating from exocrine glands. Because of their primordial role in social life, an impressive number of these glands is found. According to their morphological appearance and anatomical localization, up to 38 different glands can be distinguished among the Formicidae (Billen 1994). Following their ectodermal origin, glands are found in close association with the sclerotized exoskeleton (Billen 1987), which for a long time has made them almost inaccessible for morphological research. Among the special glandular structures with restricted occurrence among the Formicidae that have already been discovered are the cloacal gland in *Camponotus* (see Hölldobler 1982) and *Cataglyphis* species (Billen 1989), the gemma gland in *Diacamma australe* (see Peeters and Billen 1991), the sting bulb gland in representatives of *Myrmecia* and *Nothomyrmecia* (see Billen 1990) and the tibial gland in *Crematogaster ashmeadi* (see Leuthold 1968). Jessen et al. (1979) described a new series of abdominal glands in Ponerinae. Males and females of many species of the major subfamilies were surveyed for exocrine tergal and sternal glands by Hölldobler and Engel (1978, 1982). A survey of the metapleural gland, a thoracic gland unique to the ants, is provided by Hölldobler and Engel-Siegel (1984).

Inside the head of social insects, special glandular structures in association with the mouthparts have been reported in *Cerceris rybyensis* (malar gland, Ågren 1978) and some social wasps (Landolt and Akre 1979). The obvious occurrence of pores on the mandibular surface in several ant species led us to a comparative histological and ultrastructural study of their mandibles, which revealed the common existence of the intramandibular gland as a novel exocrine structure in this family. A preliminary report on intramandibular glands in ants was provided by Toledo (1967) for *Atta sexdens rubropilosa*. The aim of the present study is to get an overview of the internal histology, ultrastructure and the external morphology (SEM) of the intramandibular glands in ants.

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B. Materials and methods

The ants investigated in the present study represent the major subfamilies and are listed in Table 1. Whole heads and cut mandibles were fixed in 2% glutaraldehyde, buffered at pH 7.3 with 0.05 M sodium cacodylate and 0.15 M saccharose. Cut mandibles were prepared in order to obtain a better penetration of the cold fixative. After postfixation in 2% osmium tetroxide, the samples were dehydrated in acetone and embedded in Araldite. Semithin sections (1 μm) were stained with methylene blue and thionin. Thin sections for TEM were double stained with an LKB 2168 Ultrostainer and examined with a Zeiss EM 900 electron microscope. Mandibles for scanning microscopy were coated with gold and viewed with a Philips SEM 515 microscope.

C. Results

1. Localization and general morphology

The intramandibular gland consists of a series of secretory cells with their corresponding duct cells. A secreto-

Table 1 Occurrence of intramandibular gland openings in worker ants belonging to several subfamilies. The presence of the gland was observed by using histological sections (*H*) or by scanning microscopy (*S*). Also indicated is whether we are dealing with grouped pores (*gp*), double pores (*dp*) or single pores (*sp*)

Aneuretinae	**Myrmicinae**
Aneuretus simoni Emery, 1893S	*Atta bisphaerica* Forel, 1908S, sp, dp, gp
Dolichoderinae	*Atta cephalotes* Linnaeus, 1758H and S, sp
Azteca lanuginosa Emery, 1893S, dp	*Atta laevigata* Fr. Smith, 1858H and S, sp
Azteca muelleri Emery, 1893S, dp	*Atta sexdens sexdens* Linnaeus, 1758H and S, sp
Dorylinae	*Atta sexdens rubropilosa* Forel, 1908H and S, sp
Aenictus sp.H and S, dp	*Monomorium pharaonis* (Linnaeus, 1758)H
Anomma molestum Gerstaecker, 1858H and S, dp	*Myrmica sabuleti* Meinert, 1860S, sp
Ecitoninae	*Orectognathus* sp.S, sp
Eciton burchelli urichi Forel, 1899S	*Pheidole* sp.No pores observed
Labidus praedator (Fr. Smith, 1858)H and S	*Solenopsis invicta* Buren, 1972S
Neivamyrmex nigrescens (Cresson, 1872)S	*Trachymyrmex* sp.S
Formicinae	**Nothomyrmeciinae**
Camponotus cruentatus (Latreille, 1802)H	*Nothomyrmecia macrops* Clark, 1934H and S, gp sp
Camponotus abdominalis (Fabricius, 1804)H	**Ponerinae**
Formica fusca (Linnaeus, 1758)S, dp, sp	*Amblyopone* sp.H
Formica pratensis Retzius, 1783S	*Euponera* (*Brachyponera*) *nigrita chinensis* Emery, 1894H and S
Formica sanguinea Latreille, 1798H and S, dp, sp	*Diacamma rugosum* (Le Guillou, 1840)H
Lasius fuliginosus (Latreille, 1798)S	*Dinoponera australis* (Emery, 1901)S
Leptothorax acervorum (Fabricius, 1793)S, dp	*Ectatomma quadridens* (Fabricius, 1793)S
Oecophylla longinoda (Latreille, 1802)H	*Ectatomma ruidum* Roger, 1860H
Oecophylla smaragdina (Fabricius, 1775)H and S, dp, sp	*Harpegnathos saltator* (Jerdon, 1851)H and S, sp
Leptanillinae	*Megaponera foetens* (Fabricius, 1793)No pores observed
Leptanilla japonica Baroni Urbani, 1977No pores observed	*Pachycondyla obscuricornis* (Emery, 1890)S, sp
Myrmeciinae	*Pachycondyla striata* Fr. Smith, 1858S, sp
Myrmecia gulosa (Fabricius, 1775)H	*Platythyrea cribrinodis* (Gerstaecker, 1858)S, dp, sp
Myrmecia nigriceps Mayr, 1862H and S, sp	**Pseudomyrmecinae**
Myrmecia pilosula Fr. Smith, 1858H	*Pseudomyrmex ferrugineus* (Fr. Smith, 1877)S, sp

ry unit is formed of one secretory cell and its accompanying duct cell. In most Formicidae the secretory units occur scattered inside the mandible. The appearance of the intramandibular gland in representatives of the major subfamilies is illustrated in Figs. 1–5 (semithin sections). In general, few light microscopic details in the cytoplasm of the secretory cells can be seen, except dark-staining spots which correspond to end apparatuses (Fig. 1). In other individuals, these end apparatuses do not correspond to dark staining spots. More details concerning the morphology of these end apparatuses will be provided in the section on ultrastructure. The secretory cells were often found to stain faintly (Figs. 1, 3). The chitinous ducts inside the duct cells, conducting the secretion to the outside, were rarely observed light microscopically, both due to their small size and small numbers. In *Oecophylla smaragdina*, the ducts are visible under the light microscope (Fig. 2). A gland reservoir is absent, since each secretory unit (a secretory cell and its accompanying duct cell) opens directly to the outside. When we consider species belonging to the Formicinae and Myrmicinae, especially of the genera *Oecophylla* and *Atta*, a clustered distribution of glandular cells seems to exist. In several species of *Atta*, for instance, a well-defined group of secretory units can be observed filling up to the anterolateral portion of the mandible (Fig. 1). In *Oecophylla smaragdina* (minor and major workers), we were able to demonstrate a series of secretory units, all of them situated next to each other (Fig. 2). Semithin longitudinal sections through the mandibles of *O. smaragdina* demonstrated that the duct cells penetrate the mandibular cuticle at an oblique angle and that they point towards the tip of the mandible (Fig. 2).

Inside the mandibles of *Nothomyrmecia macrops*, a "living fossil" among ants, we observed a lot of fat cells containing large lipid droplets. In between these cells the intramandibular gland cells are visible, but are not very numerous. Near the mandibular tip a cluster of pores was seen. *Myrmecia gulosa*, *M. nigriceps* and *M. pilosula* workers also possess large fat cells. There are only a few rounded intramandibular gland cells, but they have conspicuous duct cells (Fig. 4). The gland cells are usually found near the outer margin of the mandible. The position of the intramandibular gland cells in representatives of the subfamilies Aneuretinae and Ecitoninae is indicated in Figs. 3, 5.

2. Scanning electron microscopy

The intramandibular gland openings can be recognized as single or grouped pores (Table 1). Usually, single duct openings were found (Figs. 6–8), whereas in some species paired duct openings were more frequent (Fig. 8). In some cases even more than two ducts were seen together (probably corresponding to clusters) (Figs. 6, 9; Table 1). Single and paired duct openings can also be seen together in individuals of the same species, such as *Formica sanguinea* (Fig. 8; Table 1).

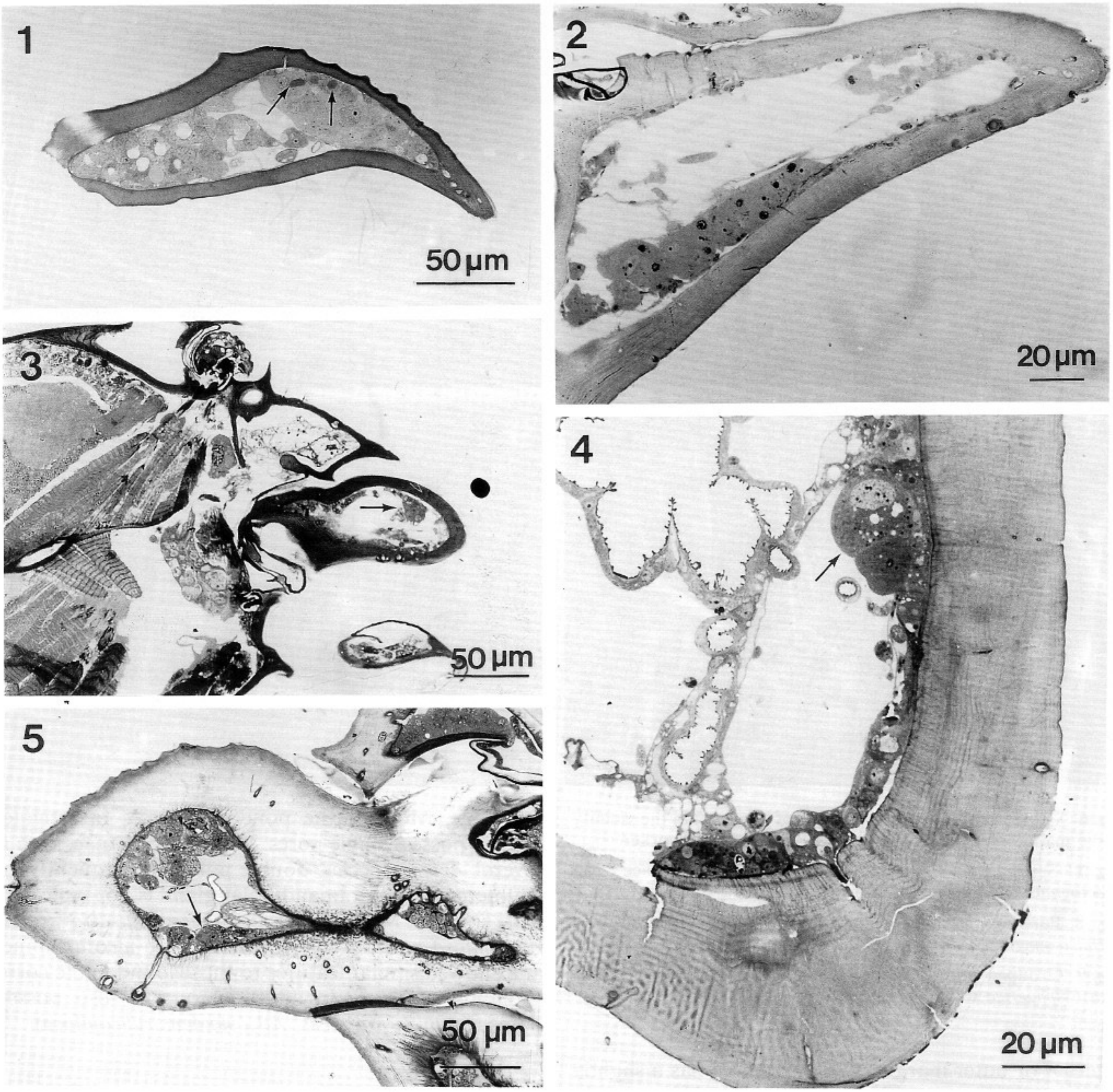


Fig. 1 Transverse semithin section through the mandibles of an *Atta sexdens sexdens* minima worker, with special reference to the dark-stained end apparatuses (*arrows*)

Fig. 2 Longitudinal semithin section through a mandible of an *Oecophylla smaragdina* worker. Note the fairly large group of secretory cells and duct cells penetrating the mandibular cuticle at an oblique angle

Fig. 3 Longitudinal semithin section through the head of an *Aneuretus simoni* worker. The secretory cells (*arrow*) are situated close to the upper mandibular cuticle

Fig. 4 Gland cells (*arrow*) near the mandibular cuticle on transverse semithin section through mandible of *Myrmecia nigriceps* female (callow)

Fig. 5 Longitudinal semithin section through the mandible of a *Labidus praedator* worker. The secretory cells are indicated (*arrow*)

Some representatives of the Formicinae, such as *Formica fusca* and *F. sanguinea* and also *Camponotus* species possess conspicuous rows of often paired pores. One large row of paired pores can be found in the vicinity of the mandibular teeth forming the masticatory margin of the mandible. This is also the case for *Myrmica sabuleti* (Myrmicinae). In several cases, the pores occur near the base of these teeth at the dorsal surface of the mandible (Fig. 8). In addition, an extra row of pairs was observed almost parallel to the basal margin of the mandible of *Formica fusca.* A third row of pores was observed following the curved line drawn by the edge (outer margin) between the dorsal and ventral mandibular surface and is situated more ventrally. A similar arrangement was investigated in *Oecophylla longinoda* and *O. smaragdina.* In these, the row of pores near the

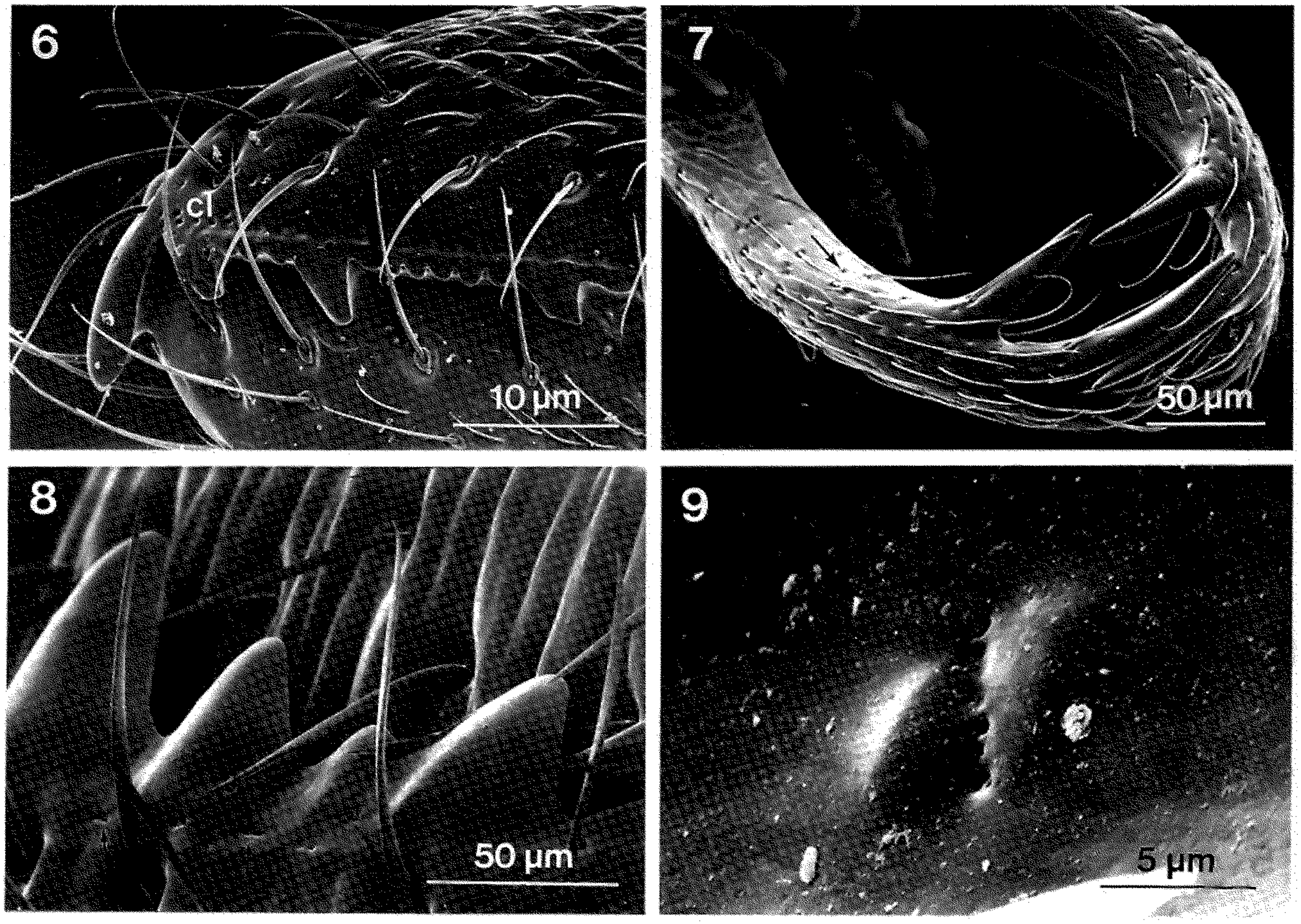


Fig. 6 Single pores (*arrow*) and cluster of pores (*cl*) in the vicinity of the mandibular tip of a *Nothomyrmecia macrops* worker

Fig. 7 Series of pores (*arrows*) on the mandibular surface of an *Orectognathus versicolor* worker

Fig. 8 Pores appearing as pairs (*arrows*) in the mandible of a *Formica sanguinea* worker

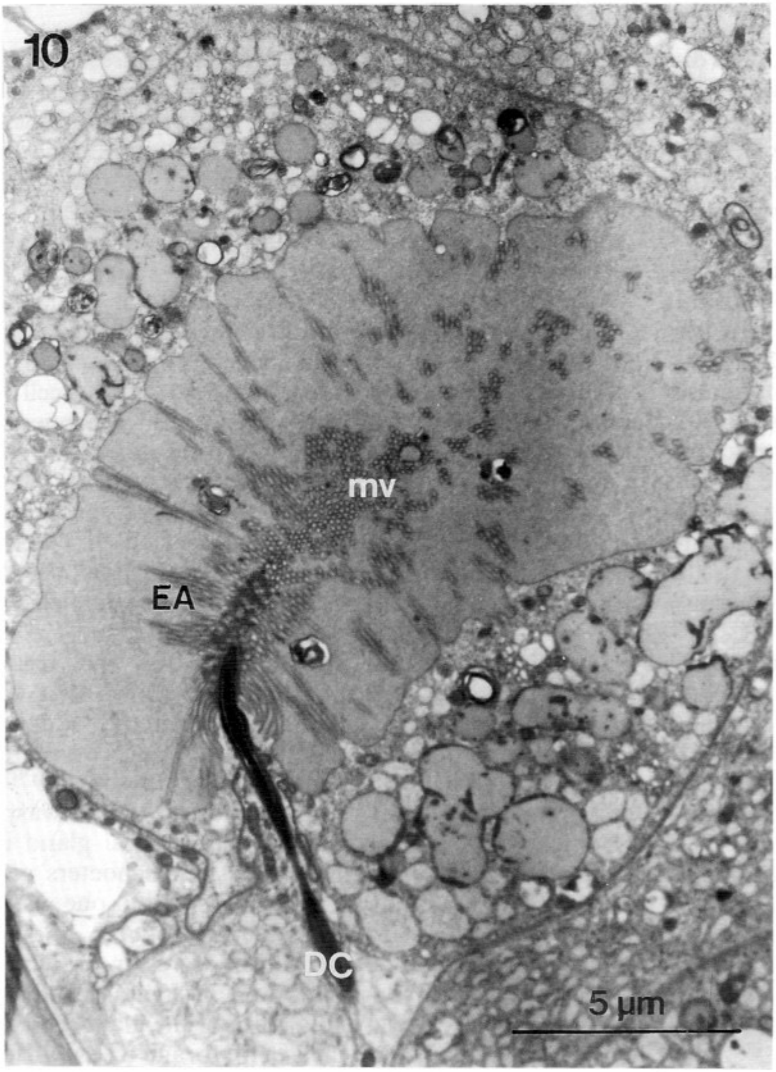
Fig. 9 Grouped pores (clusters) in the mandibular cuticle of an *Atta Bisphaerica* soldier

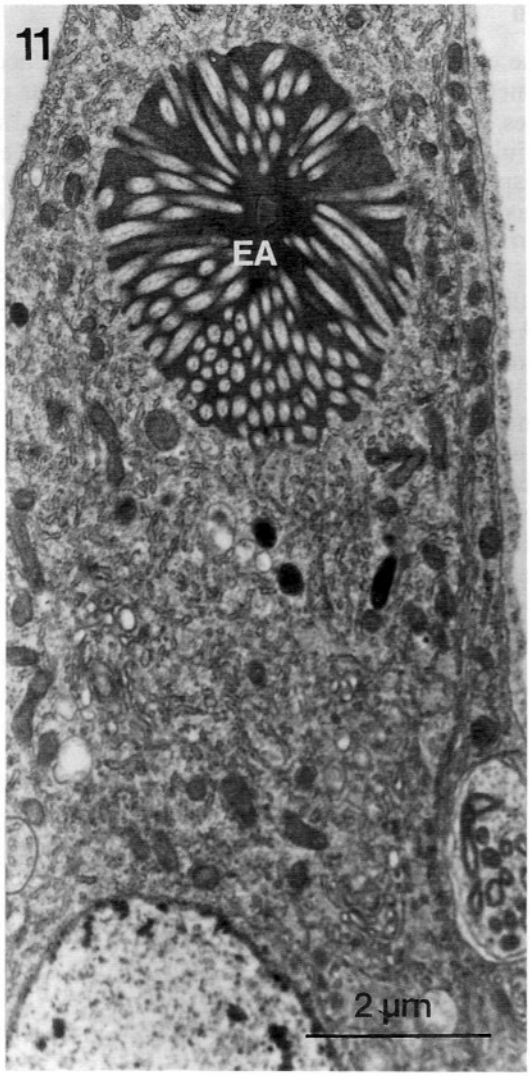
external or outer margin of the mandible has a slightly curved appearance, with the individual pairs not in a straight line. In many cases, the pores are situated more or less in the vicinity of small sensillae and/or hairs, frequently encountered on the mandibular surface (Figs. 6–9).

In *Nothomyrmecia macrops*, a fairly large concentration (clustered organization) of pores was observed near the tip at the upper surface of the mandible (Fig. 6). A field of single pores was also found near the tip on the upper surface of the mandible in *Trachymyrmex* sp. In the Australian bulldog ant *Myrmecia nigriceps*, single pores were found in the vicinity of the outer edge of the mandible. This is also the case for several representatives of the leaf-cutting ants belonging to the genus *Atta* (*A. bisphaerica* soldier, *A. laevigata* and *A. sexdens rubropilosa*). In these species, an extra row of paired pores was observed near the masticatory margin of the mandible. Most of the ponerine species investigated usually possess single pores. In Dolichoderinae, such as several *Azteca* species, double pores are frequently encountered. The intramandibular gland is not typical for workers only. Males of *Formica sanguinea*, *Atta sexdens rubropilosa* and *Solenopsis invicta* were also examined and showed similar features to those found in the corresponding workers. In addition, queens also possess pores (*Formica pratensis*, *Atta sexdens rubropilosa*).

3. Ultrastructure of the intramandibular gland

In all individuals examined, each secretory cell is accompanied by a duct cell (Fig. 10). The most conspicuous feature, indicating that we are dealing with a glandular structure, is the occurrence of enlarged extracellular spaces which can be found in the glandular end apparatus (Fig. 10). Such extracellular spaces can be found in gland cells belonging to the secretory unit type, type-3 according to Noirot and Quennedey (1974). Each cell shows one or two sections through such an extracellular space. The secretion probably accumulated in these spaces can be obviously stained with thionin and results in pink or purple spots in the secretory cells. The secretory cells (diameter 15–20 µm) release their secretion through a duct which penetrates through the mandibular cuticle. Therefore, pores (diameter 0.5–1 µm) occur in





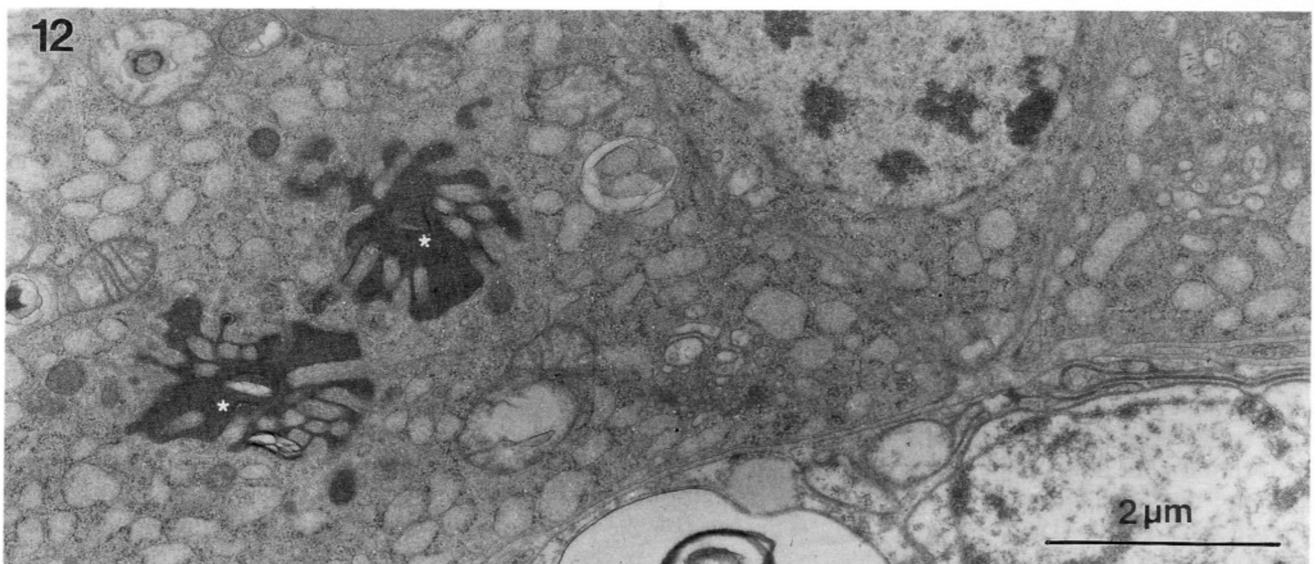


Fig. 10 Secretory cell of an *Atta sexdens rubropilosa* worker, showing end apparatus (*EA*) and accompanying duct cell (*DC*). Note the enlarged extracellular space in between the microvilli (*mv*)

Fig. 11 Cytoplasm and end apparatus (*Ea*) in an *Atta sexdens rubropilosa* callow worker. Note occurrence of electron-dense material between the microvilli

Fig. 12 Microvillar distortion in an *Aneuretus simoni* worker. The extracellular space has a rather electron-dense content (*)

the cuticle, opening at the mandibular surface. The diameter of these pores is almost constant for every species. A remarkable aspect of this novel gland is that (in adults) the extracellular spaces, without any exception, display a certain degree of microvillar distortion. This has clearly been demonstrated for *Aneuretus simoni* and *Atta sexdens* (Figs. 10–12).

The intramandibular gland cells in most species typically have a globular appearance, except in both *Oecophylla* species, where the polygonal secretory cells are closely packed together. In the cytoplasm of the gland cells in representatives of *Atta*, rounded electron-dense and electron-lucent inclusions were observed (Fig. 10), some of them probably corresponding with secretion vesicles. The presence of a smooth endoplasmic reticulum in the secretory cells is a general feature for the intramandibular gland. Further ultrastructural examination reveals a probably poorly active glandular tissue in adult ants, since mitochondria are not numerous (Figs. 10–12).

We also examined the gland cells during the pupal stage of *Atta sexdens*. Before eclosion, the microvillar pattern shows almost no signs of distortion. Microvillar distortion begins to appear in callow workers (Fig. 11). In older ants, the microvilli appear in a different arrangement, probably distorted by considerable accumulation of secretion or its precursors (Fig. 10). This widening of the spaces between microvilli can become of considerable size, when compared with the actual diameter of the whole glandular cell (Fig. 10).

D. Discussion

It was found that the intramandibular gland is present in the majority of Formicidae investigated, but does not always show the same general morphological features. In each mandible a series of glandular cells was found, each cell being connected to the outside through the mandibular cuticle by its accompanying duct cell. The presence of an intramandibular gland in other social Hymenoptera, i.e. in representatives of the Apoidea, has already been demonstrated for the Meliponini and *Bombus* species by Nedel (1960) and Costa Leonardo (1978); scanning microscopy of the mandibular pores corresponding to the openings of the ducts of the intramandibular glands of *Scaptotrigona postica* (Latreille) workers has been reported by Stort et al. (1986).

Morphological data on the intramandibular gland of ants were not available until now, except the preliminary report of Toledo (1967). When looking at the intramandibular gland of other social insects, we see that Nedel (1960) investigated the gland in Meliponinae, some representatives of *Apis* and *Vespa*, but found that species belonging to both *Apis* and *Vespa* lack the gland. Costa Leonardo (1978) considered other social bee species belonging to the genera *Apis*, *Bombus*, *Melipona*, *Plebeia*, *Scaptotrigona* and *Schwarziana*. Rounded intramandibular gland cells were found to be present in all species, but not in *Apis mellifera*. This corresponds to the observations of Nedel (1960). Apart from these spherical cells with their own duct cell, Costa Leonardo (1978) found a hypertrophied epithelium in mandibles of Meliponinae. Similar observations were made by Cruz-Landim (1962). Combining these morphological data with those mentioned in the present study, it seems that more than one type of intramandibular gland exists. Every example of the intramandibular gland in ants dealt with in the present paper belongs to the secretory unit type, each glandular cell with its own duct cell in connection with the outside (Noirot and Quennedey 1974). For other social insects, such as termites, Deligne et al. (1981) mention the presence of numerous glandular pits opening at the surface of the inflated part of the mandibles in soldiers of *Machadotermes inflatus* Weidner, 1974 (Isoptera). According to these authors, the secretion could be toxic and enhance the negative effects after the mandibles have made a wound.

Our ultrastructural investigation of the intramandibular gland in leaf-cutting ants shows that the gland cells possess a synchronous secretory activity. Earlier observations on the sting valve gland in a representative of *Atta* (Bazire-Bénazet and Zylberberg 1979), on secretory activity of venom glands of *Polistes* wasps (Delfino et al. 1983) and on the metapleural gland in *Diacamma rugosum* and *D. vagans* (see Schoeters and Billen 1992), show that secretory cells within one gland often have an asynchronous secretory activity.

The chemical composition of the secretion produced by the intramandibular gland and its function remain unknown. The relatively small size of the gland and its cells, combined with the lack of a storage reservoir for the secretion, make it rather inconvenient for chemical research. When comparing with other glands that have a large reservoir, this observation suggests that its secretory capacity is small. By saying this we certainly do not intend to minimize its importance, since we are dealing with a gland inside the mandibles, structures that are frequently used in the ants life for different things.

An important aspect of the intramandibular gland is its widespread presence among the Formicidae, but the localization of pores on the mandibles is often subject to variation. After its discovery in leaf-cutting ants (Toledo 1967), a number of species representing all major subfamilies have been checked. The sharing of this gland by several species probably represents an important glandular source in the biology of ants. Nevertheless we observed that in representatives of certain genera, such as *Oecophylla*, a degree of specialization has occurred. In the two known species belonging to this genus the gland is very well developed.

A role in nest building has been postulated by Nedel (1960) for Meliponini and species belonging to *Bombus*. Nedel (1960) also suggests that the gland could be involved in the production of a lubricant for the mandibles. A lubricating function has already been formulated for a different array of ant exocrine glands, such as certain abdominal glands in Ponerinae (Jessen and Maschwitz 1983) and Dufour glands (Hefetz 1987).

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