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Review

Occurrence and structural organization of the exocrine glands in the legs of ants

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ABSTRACT

Apart from their obvious locomotory function and hence the presence of muscle fibres, ant legs are also endowed with an astonishing variety of exocrine glands. This paper reviews the presence and structural variety of the 20 different glands that have so far been found in the legs of ants. Four of these glands are described for the first time in this paper. Glands have been described in the three leg pairs, although considerable differences may exist. Glands occur in the various leg segments. A number of glands, especially those located in the hindlegs, may have a function in the production of trail pheromones. Other possible functions that have been reported deal with antenna cleaning, production of lubricant substances and sex pheromones.

1. Introduction

The exocrine system in ants is formed by an impressive plethora of glands that occur all over the body, and exemplifies the wide variety of functions exocrine secretions play in the social communities of these insects (Hölldobler and Wilson, 1990; Billen and Morgan, 1998). As exocrine glands are always associated with a cuticular component, difficulties with sectioning have often put a technical limitation to the structural study of the exocrine system. Using plastics as embedding medium and glass or diamond knives for sectioning, however, it became possible to section through the very hard tegumental cuticle, even through heavily sclerotized structures such as stings, mandibles and appendages like antennae and legs. As a result, these body parts became also accessible for histological examination, and have revealed that exocrine glands also occur here. Examples include the sting bulb gland in *Nothomyrmecia* and *Myrmecia* (Billen, 1990a), the intramandibular gland in the majority of ant species (Schoeters and Billen, 1994; Grasso et al., 2004), as well as glands in various antennal segments (Billen, 2000; Isidoro et al., 2000). The first exocrine gland discovered in ant legs was the tibial gland in the hindleg of *Crematogaster* (Leuthold, 1968). For more than two decades, however, this metatibial gland was the only known leg gland of ants. In the 1990s, the existence of several additional glands in ant legs was reported, some of these being very common, others having a much more restricted occurrence.

In this paper, we aim to review the actual knowledge on the occurrence and structural organization of the various leg glands in ants, including the description of four previously unknown glands. For clarity and convenience, the order in which the 20 known glands will be treated is from most proximal (coxa) to most distal in the leg (pretarsus). The descriptions in this article deal with the situation in workers, unless specified otherwise. Longitudinal sections are shown with the distal side to the right. All figures in this review are the result of our own histological and ultrastructural work, and therefore followed the same preparation methodology. This comprised fixation of leg parts in 2% cold glutaraldehyde (buffered at pH 7.3 with 50 mM Na-cacodylate and 150 mM saccharose) and post-fixation in 2% cold osmium tetroxide in the same buffer. Tissues were dehydrated through a graded acetone series and embedded in araldite. Semi-thin sections with a thickness of 1 μm for light microscopy were stained with methylene blue and thionin, thin sections at 70 nm for electron microscopy were double stained with lead citrate and uranyl acetate, and examined in a Zeiss EM900 electron microscope. Legs prepared for scanning electron microscopy were critical point dried in a Balzers CPD 030 instrument and examined in a Philips XL30 ESEM scanning microscope.

2. Survey of the exocrine glands in the legs of ants

In spite of their impressively high number, insect glands can be classified into two main groups according to their cellular organization, as described in the pioneer paper by Noirot and Quennedey (1974). In their classification, class-1 glands are formed by a simple monolayered epithelium that can either be part of the tegumental epidermis or can occur as the lining of an invaginated reservoir (Fig. 1A,B). In both cases, uptake of precursor molecules from the haemolymph takes place through the basal plasmalemma, which for this purpose often displays surface-increasing basal invaginations. The secretory products leave the cells at the apical

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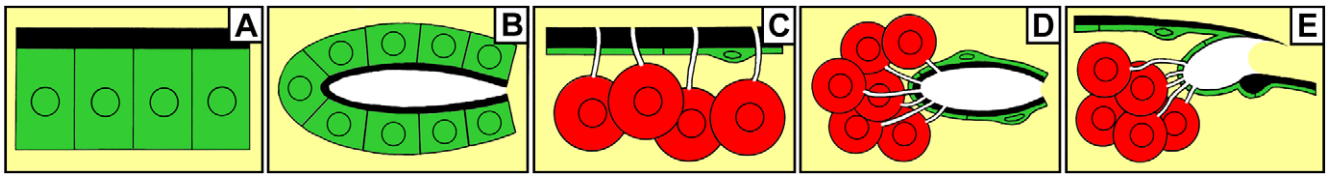

Fig. 1. Schematical illustration of the five main anatomical types of insect glands. (A) Epithelial glands without reservoir. (B) Epithelial glands with reservoir. (C) Bicellular unit glands without reservoir. (D) Bicellular unit glands with reservoir. (E) Bicellular gland units opening through articulation membrane. All gland types except D are represented in ant legs (see also Table 1).

side, where microvilli provide an enlarged surface for this discharge activity. The cuticle can be sufficiently porous to allow the secretory products to reach the exterior or the reservoir, or can display very narrow cuticular pores for passage of the secretion (Billen and Morgan, 1998; Quennedey, 1998). Class-3 glands are formed by one or generally more bicellular units that each comprise a secretory cell and its accompanying duct cell. Similar to the situation in class-1 glands, the bicellular unit glands can either open directly through the tegumental cuticle, or may be arranged around a central reservoir (Fig. 1C,D). Class-3 glands can also open through the articulation membrane between adjacent segments (Fig. 1E). The junction between the secretory cell and the duct cell characteristically shows the presence of an "end apparatus", which is formed by a porous cuticular ductule surrounded by microvilli. This structural arrangement allows efficient transport of the secretory products when they leave the producing gland cell in order to reach the duct cell, which will carry them towards the outside or to the reservoir. Of these five anatomical gland types, all are represented among the leg glands in ants, except for bicellular unit glands with reservoir (Fig. 1D). Fig. 2 summarizes the presence and location of the various exocrine glands that are presently known in ant legs.

2.1. Basicoxal gland

Workers and queens of several poneromorph species possess an epithelial basicoxal gland in the proximal part of the coxae of their mid- and hindlegs (Billen and Ito, 2006). The gland occurs at the outer dorsal part of the coxa, and represents a thickened differentiation of the tegumental epidermis (Fig. 3A). Its position and ultrastructural characteristics are indicative for a lubricant function for the ball-and-socket junction of these legs with the thorax: apical microvilli and transcuticular pores facilitate transportation of the secretory products, while the presence of smooth endoplasmic reticulum is in agreement with an oily composition of the secretion (Fig. 3B). The gland is absent in the forelegs, however, which can be understood by the different articulation of the rather immobile fore coxae, compared to the higher manoeuvrability of the coxae of the mid- and hindlegs (Billen and Ito, 2006). We found no literature data on the existence of similar glands in other insects.

2.2. Coxal gland

Class-3 glandular cells have been reported inside the coxae of the three leg pairs of workers of *Pachycondyla obscuricornis* and a few other species, both in the proximal part near the articulation with the thorax, and in the distal part near the articulation with the femur (Schoeters and Billen, 1993; Fig. 3A). As in the basicoxal gland, the cells contain numerous free ribosomes, but no granular endoplasmic reticulum. The accompanying duct cells open through the corresponding intersegmental articulation membranes (Fig. 3C), where the secretion is thought to serve a lubricant function for the heavily sclerotized species in which they occur (Schoeters and Billen, 1993). Similar bicellular gland clusters were later also described in the proximal dorsal part of the coxa in stingless bee species (Cruz Landim et al., 1998), and have also been found in the coxae of *Prorhinotermes* termites (Šobotník et al., 2003).

2.3. Trochanter gland

The proximal ventral part of the trochanter displays an obvious glandular epithelium (Fig. 3A,D), which is very similar in its cellular organization to the basicoxal glands. It occurs in the three leg pairs, and has been found in species belonging to various phylogenetically distant genera such as *Atta*, *Camponotus*, *Oecophylla*, *Diacamma*,

Table 1
Occurrence of the exocrine glands in the foreleg (F), midleg (M) and hindleg (H)

	No.	Gland	Occurrence			Reference	Type
Coxa	1	Basicoxal gland	–	M	H	Billen and Ito, 2006	A
	2	Coxal gland	F	M	H	Schoeters and Billen, 1993	E
Trochanter	3	Trochanter gland	F	M	H	Billen, 2008	A
Femur	4	Apicofemoral gland	F	M	H	Bolton, 1999; Billen et al., 2000a	A
	5	Distal femoral gland	F	M	H	This article	E
	6	Distal femoral sac gland	F	M	H	This article	B
Tibia	7	Proximal tibial gland	F	M	H	This article	A
	8	Tibial tendon gland	F	M	H	Leuthold, 1968; Billen, 1984	B
	9	Metatibial gland	–	–	H	Hölldobler et al., 1996	A
	10	Apicotibial gland	F	M	H	Bolton, 1999; Billen et al., 2000a	A
	11	Distal tibial gland	F	M	H	Hölldobler et al., 1996; Billen, 1997	C,E
	12	Tibial spur gland	F	?	H	Hölldobler et al., 1992; Tijskens et al., 2002	A
Basitarsus (t1)	13	Antenna cleaner gland	F	–	–	Schönitzer and Dott, 1989; Schönitzer et al., 1996	A
	14	Basitarsal gland	F	M	H	Hölldobler and Palmer, 1989a; Hölldobler et al., 1992	C[a]
Tarsomeres (t2, t3, t4)	15	Proximal tarsomere glands	F	M	H	Billen, 1997; this article	A
	16	Tarsomere glands	F	M	H	This article	C
	17	Third tarsomere gland	–	–	H	Billen et al., 2000b	C
	18	Distal tarsomere glands	F	M	H	Billen et al., 2000b	A
Pretarsus (t5)	19	Footprint gland	–	–	H	Hölldobler and Palmer, 1989b; Billen et al., 2005	A
	20	Arolium gland	F	M	H	Hölldobler and Palmer, 1989b; Billen, 1990	B

(–) indicates gland absence, (?) indicates that presence or absence could not be verified. "Type" indicates gland type according to the five types shown in Fig. 1.
[a] The assignment of the basitarsal gland (14) to type C remains unclear (see text).