

Morphology and ultrastructure of the Dufour gland in workers of social wasps (Hymenoptera, Vespidae)

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Abstract

The Dufour gland in workers of vespine wasps appears as an unpaired tubiform gland that opens in close proximity to the sting base. The epithelial cells that line the central reservoir are characterized by apical microvillus-like projections and deep basal invaginations. Their cytoplasm contains a well-developed Golgi apparatus, numerous mitochondria, as well as strands of smooth endoplasmic reticulum. The Dufour gland duct occurs ventrally to the venom gland duct, and bends downward near the sting base to open in the dorsal vaginal wall. In this region, the duct is dorsoventrally flattened, and shows conspicuous bundles of parallel microtubules in the epithelial cells, that transmit the pulling forces of the myofilaments of the underlying muscular supply to the cuticle. This results in an active opening mechanism regulated by muscular contraction, while passive closure probably results from the return of the cuticular intima to a rest position.

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1. Introduction

Social wasps are characterized by a notorious sting that serves as an effective weapon in prey capture and colony defence. The heavily sclerotized sting shaft ensures the mechanical penetration into the victim, while the highly specialized venom gland delivers the powerful venomous secretion. For this purpose, an impressive muscular supply surrounds the gland's reservoir (Fig. 1). Upon muscle contraction, the secretion is forced into the venom gland duct, that carries it straight through the sting and injects it into the victim (Hermann and Krispyn, 1975). In contrast to the well known role and structure of the venom gland in these wasps (Delfino et al., 1983; Schoeters and Billen, 1995) is the limited knowledge on the Dufour gland (in old literature often designated as the alkaline or basic gland). Together with the venom gland, it represents the former accessory glands of the female reproductive system (Robertson, 1968).

In the south-east Asian Stenogastrinae, the secretion of the Dufour gland is used in larval nutrition and nest defence (Turillazzi, 1985; Keegans et al., 1993; Sledge et al., 2000). For the two other major and cosmopolitan social wasp subfamilies Polistinae and Vespinae, various functions have been suggested for the Dufour gland, such as being the source of sting lubricants (Spradbery, 1973) or of egg marking substances with a role in dominance interactions (Downing and Jeanne, 1983). It has also been reported to be involved in male attraction during mating (Reed and Landolt, 1990; Fratini et al., 1996), and in nestmate recognition (Dani et al., 1996).

Apart from the Stenogastrinae (Delfino et al., 1988), the morphological knowledge of the Dufour gland in the Vespidae is surprisingly limited, and mainly goes back to an old although remarkably precise contribution by Schlusche (1936). This general lack of information dealing with the vespine Dufour gland is also reflected in the superficial anatomical and morphological descriptions of the gland in the common textbooks on vespine wasps (Spradbery, 1973; Edwards, 1980; Matsuura and Yamane, 1984; Downing, 1991).

Morphological studies of the Dufour gland in bees have been performed in the primitive megachilid and anthophorid

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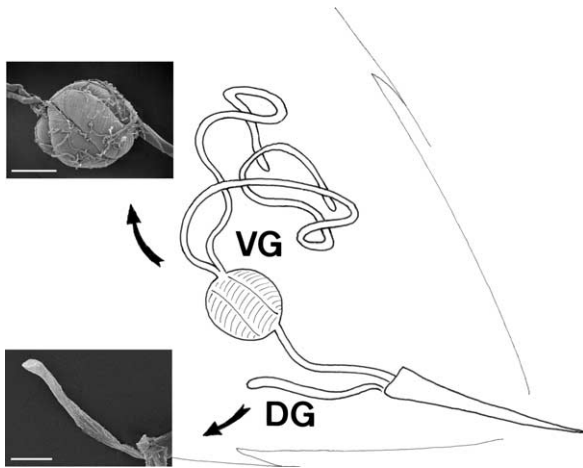


Fig. 1. Schematic view of the sting apparatus of a wasp worker, showing the position of the Dufour gland (DG) and venom gland (VG). The insets show scanning electron micrographs of the tubiform Dufour gland (*P. vulgaris*) and of the venom gland reservoir surrounded by a conspicuous muscular supply (*Dolichovespula media*). Scale bars 0.5 mm.

bees (Chapman and Barrows, 1986; Barrows et al., 1986), in bumblebees (Abdalla et al., 1999a,b), the stingless Meliponini (de Lello, 1976), and the honeybee (Abdalla, 2002; Abdalla and da Cruz-Landim, 2001a,b,c). In earlier work, we carried out a comparative study of the morphology and fine structure of the Dufour gland among the various subfamilies of the Formicidae (Billen, 1986), including its muscular apparatus in the region where the gland opens through the sting (Billen, 1982, 1987). Considering the lack of this kind of information among the social wasps, we have undertaken a study of the general morphology and the ultrastructural organization of the Dufour gland in workers of several polistine and vespine wasp species. We here report on our examination and compare the appearance of the wasp Dufour gland with that in the ants and bees.

2. Material and methods

Workers of the following polistine and vespine species have been included for both light and electron microscopy in the present study: *Polistes annularis* from Athens, GA (USA), *Polistes fuscatus* from Madison, WI (USA), *Polistes variabilis* from Brisbane (Australia), *Ropalidia plebeiana* from Nelligen, NSW (Australia), *Dolichovespula saxonica* and *D. sylvestris* from Diepenbeek (Belgium), *Paravespula germanica* and *Paravespula vulgaris* from Mechelen (Belgium), *Vespa analis* from Takamatsu (Japan), and *Vespa crabro* from Yvoir (Belgium).

Abdominal tips with the sting and its associated glands attached in situ as well as dissected Dufour glands were fixed in 2% glutaraldehyde (buffered at pH 7.3 with 50 mM sodium cacodylate and 150 mM saccharose) and postfixed in 2% osmium tetroxide in the same buffer. Dehydration was carried out in a graded acetone series and preceded

embedding in Araldite and sectioning with a Reichert Ultracut E microtome. Thin sections of 1 μ m for light microscopy were stained with methylene blue and thionin. Ultrathin sections of 70 nm were double stained (lead citrate and uranyl acetate) and examined in a Philips EM400 electron microscope. Dissected glands for scanning electron microscopy were critical point dried in a Bal-Tec CPD030 instrument and examined in a JEOL JSM 6360 microscope.

3. Results

The Dufour gland is formed by a single slender tube (Fig. 1), of which the central lumen is formed by a simple columnar epithelium with microvilli and a cuticle. In its proximal region near the sting base, the duct diameter narrows while the shape becomes dorsoventrally flattened.

3.1. Dufour gland epithelium

The epithelium has a thickness between 10 and 15 μ m, and shows conspicuous foldings, which give the gland an accordion-like appearance (Fig. 2A). In callow individuals, the opposite epithelial walls touch each other as the lumen is still collapsed at that stage. After a few days, accumulation of secretory products results in a more pronounced lumen. The cuticular lining of the epithelium has a thickness of approx. 0.5 μ m, and consists of a thin electron-dense outer epicuticle of 50 nm and an electron-lucent fibrillar procuticle (Fig. 2C). The apical cell membrane is differentiated into an obvious border of microvillus-like projections (Fig. 2B and C), while the basal cell membrane shows conspicuous invaginations, that penetrate for 2–3 μ m into the basal region of the cells (Fig. 2A and D). The cytoplasm is characterized by the presence of a well developed Golgi apparatus (Fig. 2E), while mitochondria are scattered in the cytoplasm (Fig. 2A, B and E). The smooth endoplasmic reticulum is well represented (Fig. 2C and E), with strands typically extending into the microvillar fingers (Fig. 2C). The epithelium rests on an amorphous electron-dense basal lamina with a thickness of approx. 200 nm. Isolated muscle fibres occur underneath the epithelium.

3.2. Dufour gland duct and opening site

The tubular Dufour gland becomes dorsoventrally flattened when it approaches the sting base, and is characterized by an extensive muscular supply in this most proximal region (Fig. 3B and C). Posterior to this muscular region, the duct bends downward and exits the sting base through its ventral lining (Fig. 3D and F). The venom gland duct with its thick cuticular lining, on the contrary, continues through the sting shaft, in which it is accompanied by conspicuous musculature. In the proximal part of the sting base, slender transverse muscle fibres connect the ventral

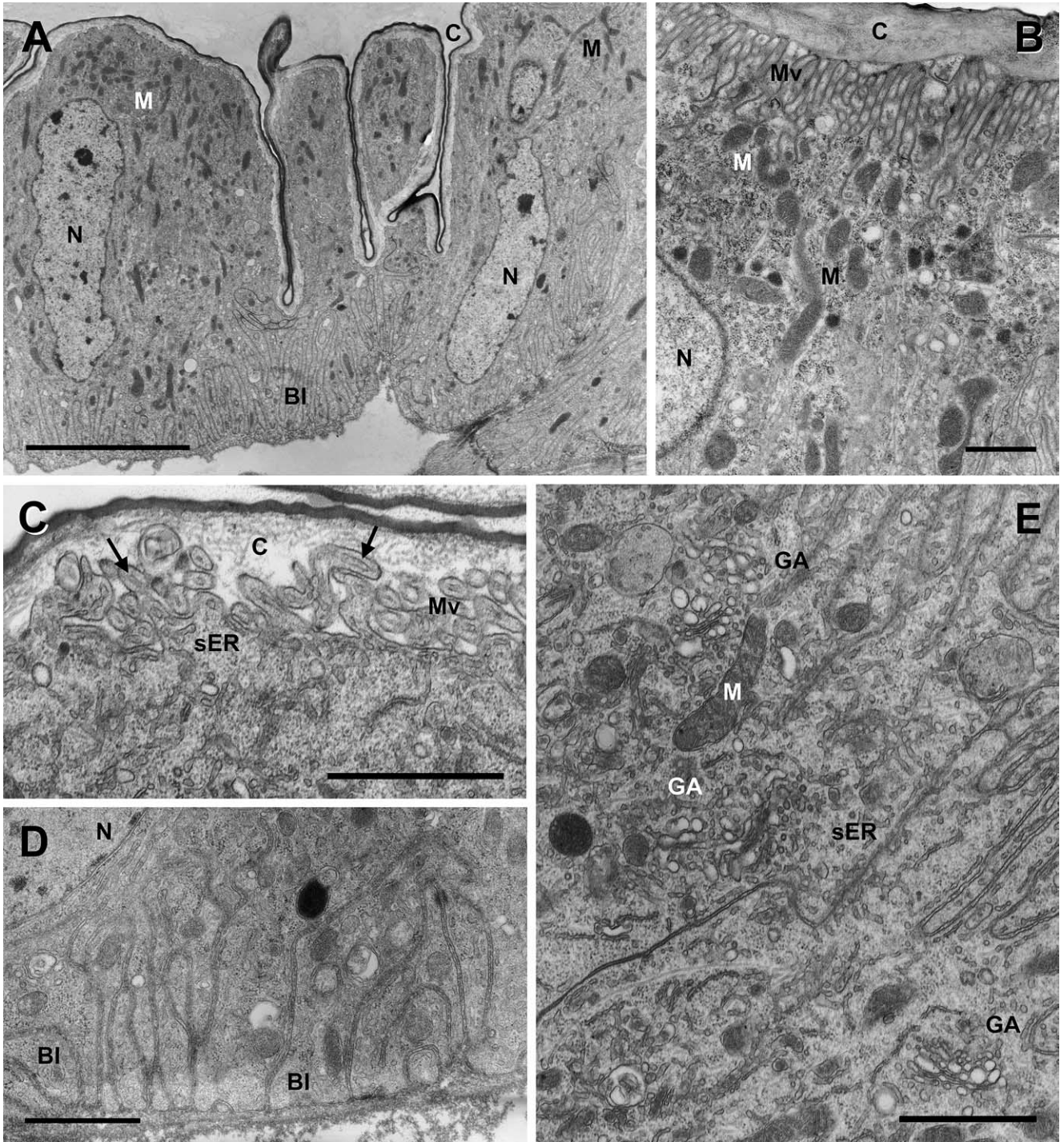
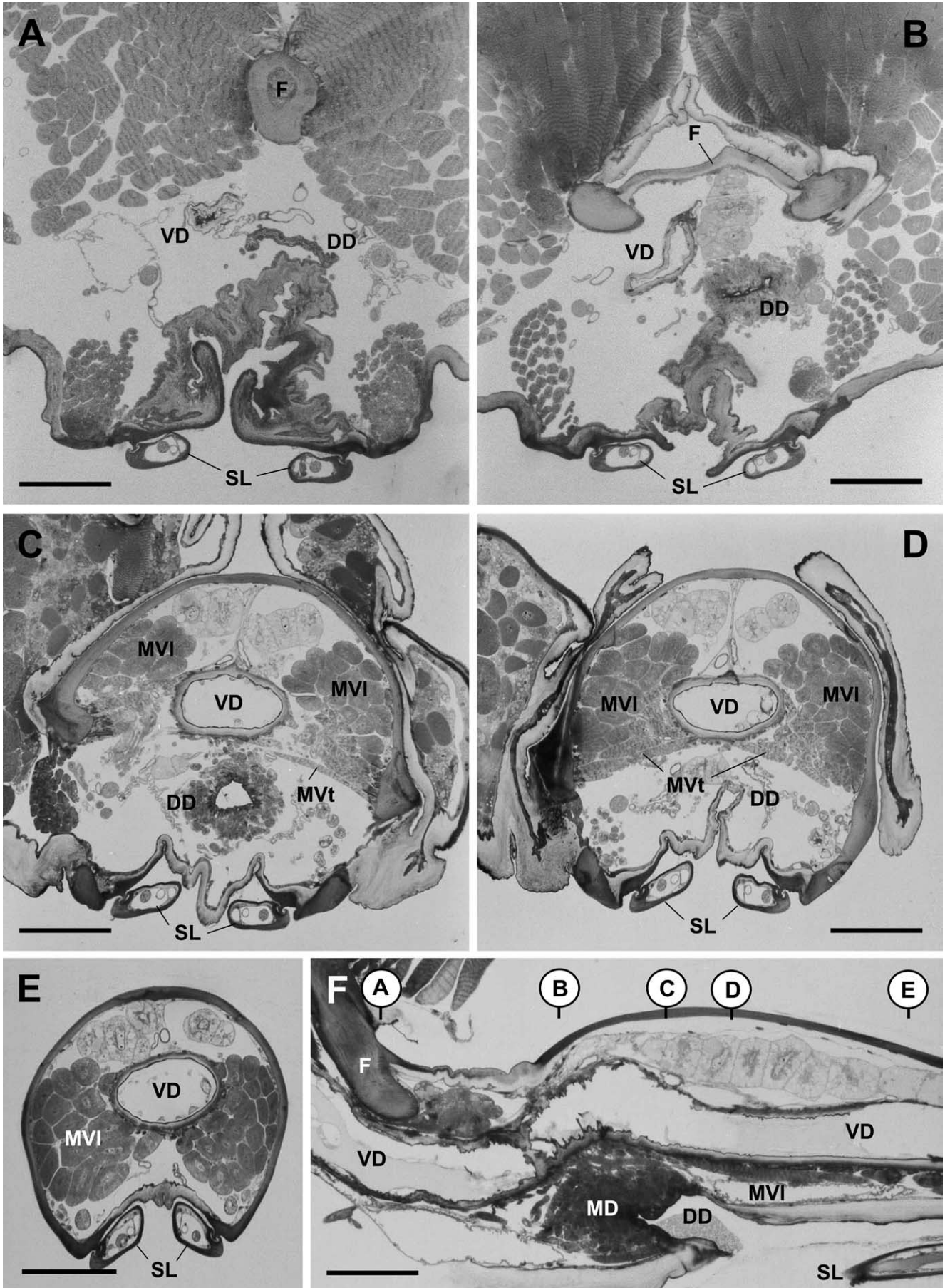


Fig. 2. Electron micrographs of Dufour gland epithelium. A. Folded epithelium of *P. annularis* worker (scale bar 5 μ m). B. Apical region of epithelium showing microvilli (*V. crabro* worker, scale bar 1 μ m). C. Detail of apical microvilli containing tubules of smooth endoplasmic reticulum (arrows) (*P. fuscatus* callow worker, scale bar 1 μ m). D. Basal region of epithelium showing extensive basal invaginations (*P. variabilis* worker, scale bar 1 μ m). E. Cytoplasmic detail *P. fuscatus* callow worker with conspicuous Golgi apparatus and smooth endoplasmic reticulum (scale bar 1 μ m). BI, basal invaginations; C, cuticle; GA, Golgi apparatus; M, mitochondria; Mv, microvilli; N, nucleus; sER, smooth endoplasmic reticulum.

venom duct with the inner lateral wall of the sting shaft (Fig. 3C and D), while two main longitudinal muscle bundles run through the sting shaft along both sides of the duct (Fig. 3C–F).

In its muscular region, the cells of both the dorsal and ventral epithelium of the Dufour gland duct show parallel bundles of microtubules that run perpendicular to the slit-like lumen (Fig. 4A–C). In this way, the microtubules are



oriented in the same direction as the myofilaments that adhere underneath the epithelium. The contact region between the muscle fibre and epithelial duct cell shows a very irregular appearance of the basal lamina, onto which both the myofilaments and the microtubules adhere through hemidesmosomes (Fig. 4C). Hemidesmosomes likewise occur where the microtubules reach the cuticle at the apical side of the epithelium. In this region, the cuticle has a thickness of approx. 1.3 μm , which is more than twice the thickness it has in the tubular secretory part. The cuticle also shows a more electron-dense appearance in its duct region in comparison to the secretory part (Fig. 4A and B).

4. Discussion

The Dufour gland from a structural point of view is one of the most simple glands in wasps. The single epithelial layer is formed by secretory cells that are characterized by a well-developed Golgi apparatus, smooth endoplasmic reticulum and numerous mitochondria. This cytoplasmic composition is consistent with that of the Dufour gland in other Hymenoptera (Delfino et al., 1988 for wasps; Abdalla and da Cruz-Landim, 2001a,c for bees; Billen, 1986 for ants), and is indicative of the elaboration of a non-proteinaceous secretion (Billen and Morgan, 1998). The presence of well-developed apical microvillus-like projections and deep basal invaginations provide a significant increase of the surface area of the secretory cells, enabling them to transport substances efficiently. Also the continuation of tubules of smooth endoplasmic reticulum into the microvillar projections probably contributes in an efficient discharge of the secretory products of the glandular cells. The special organization in the duct region, with parallel bundles of microtubules in the duct cells, corresponds with the common organization in insects where muscle fibres adhere to cuticle (Lai-Fook, 1967; Caveney, 1969), and results in an active opening of the gland duct through muscular contraction, while duct closure is achieved by a passive returning of the thick rigid cuticle to a rest position when muscular contraction comes to an end (see also Billen, 1982, 1986).

The precise function of the Dufour gland secretion in social wasps still remains disputable (Dani, 1996), except for the Stenogastrinae, where it is used in larval nutrition and nest defence (Turillazzi, 1985; Keegans et al., 1993; Sledge et al., 2000). In primitive bees, the Dufour gland produces substances for nest entrance marking (Hefetz, 1987). Also in vespine wasps like *D. saxonica*, chemical marking of the nest

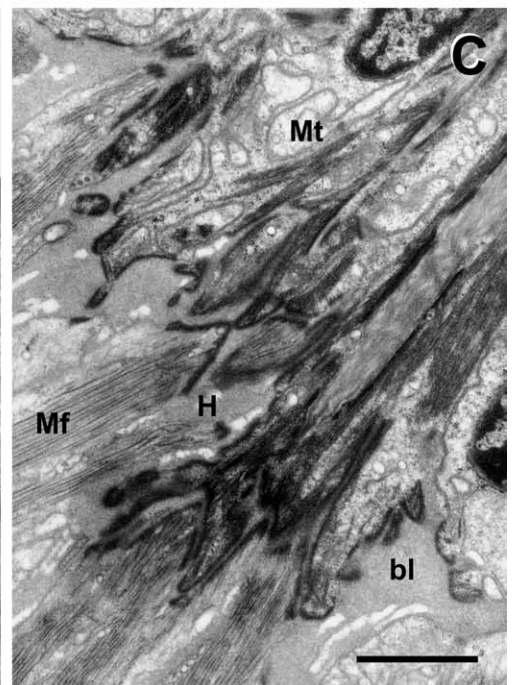
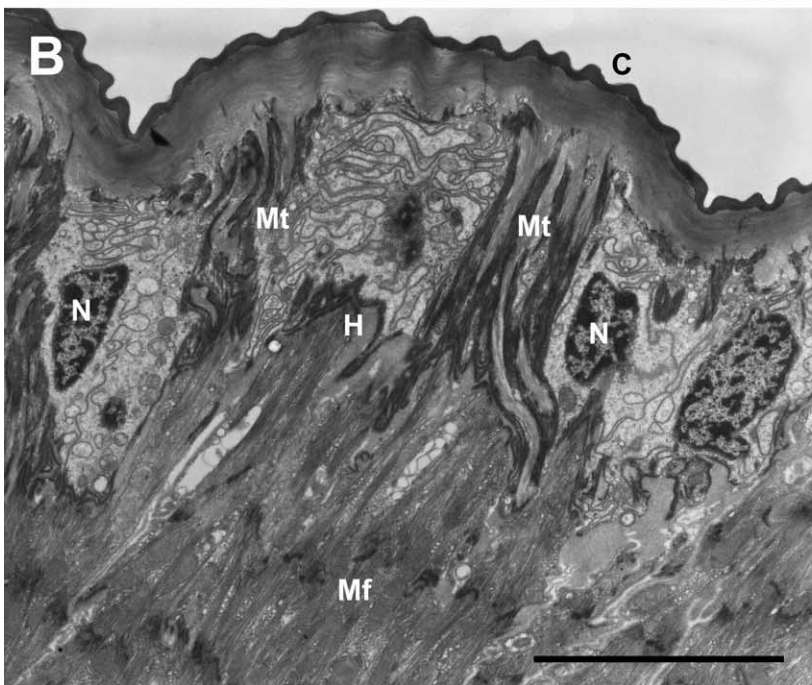
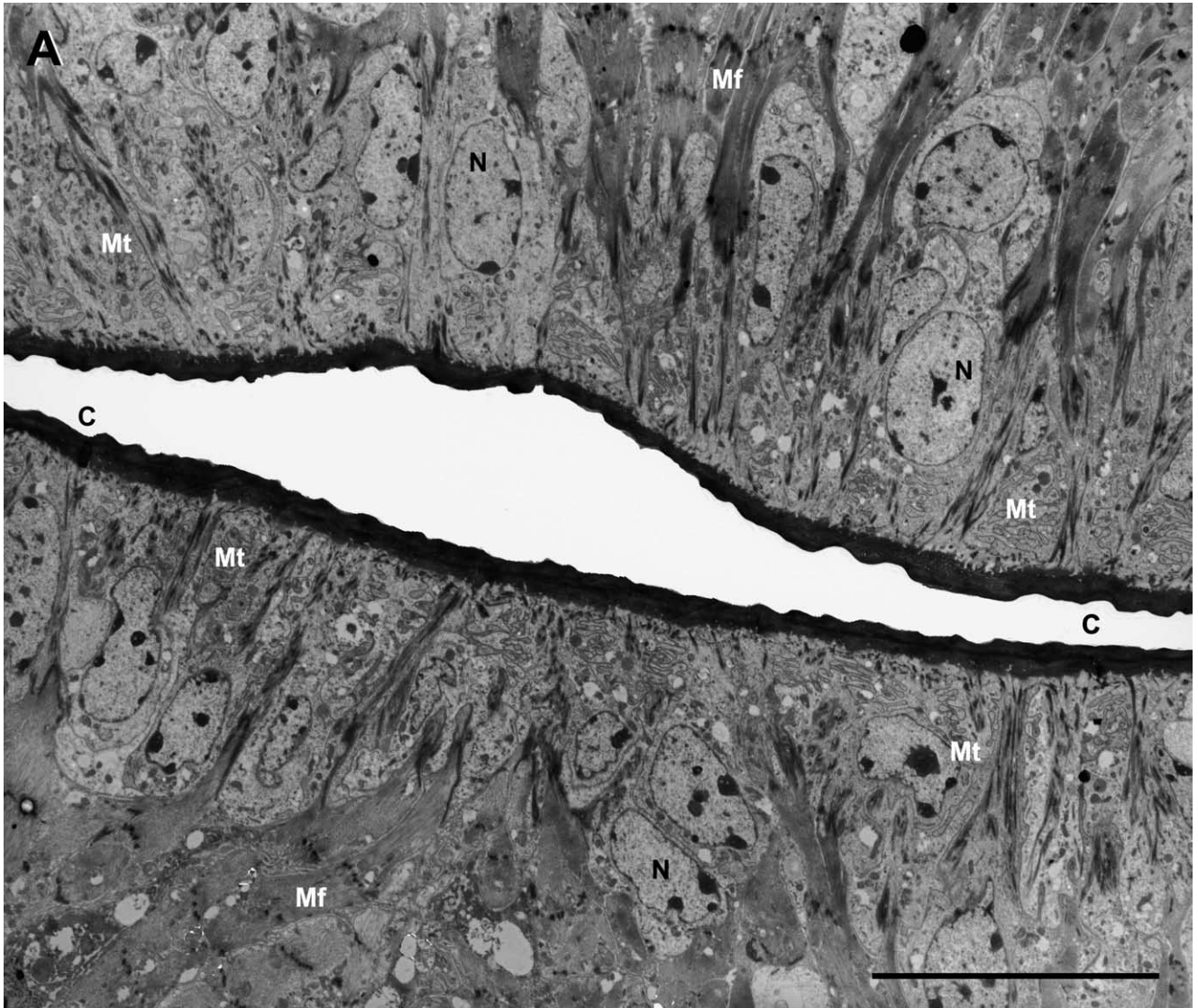
entrance has been reported, although the origin of the substances involved remains unknown (Steinmetz and Schmolz, 2003). Other authors refer to it as the source of the wasps' sting lubricants (Spradbery, 1973), of egg marking substances that play a role in dominance interactions (Downing and Jeanne, 1983), of male attractants during mating (Reed and Landolt, 1990; Fratini et al., 1996) and of substances involved in nestmate recognition (Dani et al., 1996).

Two of the most recent review articles on wasps claim our knowledge of the 17 vespid glands is reasonably certain, with the Dufour gland believed to be an egg marking substance (Downing, 1991; Jeanne, 1996). Such function finds direct support in the functional morphology of the gland's opening site. Contrary to the situation in ants, where the Dufour gland opens through the sting (Billen, 1986, 1987), in wasps (as well as bees and bumblebees: Billen, 1987; Martin et al., 2005) the posterior part of the Dufour gland duct bends downward when approaching the sting, and thus opens into the dorsal vaginal wall. As a result, the gland anatomy makes it well suited for a function of egg marking, as eggs to be laid have to pass the gland exit. Also the ultrastructural features of the secretory cells, that indicate the elaboration of non-proteinaceous products, is in line with the chemical nature of the known egg-marking substances in honeybees (Oldroyd et al., 2002—although the precise involvement of the Dufour gland in honeybee egg marking is still debatable: see Katzav-Gozansky et al., 2002; Martin et al., 2002).

The eventual role of the wasp Dufour gland secretion in egg-marking goes along with inter- as well as possibly intra-caste differences between individuals, as it is due to the coating with Dufour gland substances that eggs laid by workers or subordinate or non-nestmate females will have a significantly higher chance to be eaten by the dominant female (Downing, 1991). Morphological differences between castes and/or related to age were already documented for honeybees (Abdalla and da Cruz-Landim, 2001b,c) and for bumblebees (Abdalla et al., 1999a,b). Also in ants, caste-dependent differences in Dufour gland development and function have been reported, especially in socially parasitic species, where the parasite queens often have a very well developed Dufour gland that plays a crucial role in the usurpation process (Billen et al., 2001; Lenoir et al., 2001; Grasso et al., 2005).

The present article provides a first general report describing the ultrastructural organization of the Dufour gland in vespid workers, that can serve as a basis for more specific studies dealing with interspecific, caste or age-dependent characteristics.

Fig. 3. Thin 1 μm sections through sting base region in *D. saxonica* workers (all at same magnification, scale bar 100 μm). A–E. Serial transverse sections at subsequent positions. F. Longitudinal section through sting and associated Dufour and venom gland ducts, with indication of position of transverse sections in figures A–E. DD, Dufour gland duct; MD, muscles of Dufour gland duct; MVl, longitudinal muscles of venom gland duct; MVt, transverse muscles of venom gland duct; F, furcula; SL, sting lancets; VD, venom gland duct.



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Fig. 4. Electron micrographs through Dufour gland duct. A. Survey of dorsal and ventral epithelium with slit-like lumen in *P. fuscatus* worker (scale bar 10 μ m). B. Ventral duct epithelium in *P. vulgaris* worker (scale bar 5 μ m). C. Detail of contact region between muscular myofilaments and epithelial microtubules in *P. vulgaris* worker (scale bar 1 μ m). bl, basal lamina; C, cuticle; H, hemidesmosomes; Mf, myofilaments; Mt, microtubules; N, nucleus.

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