Structural Examination of the Dufour's Gland of the Solitary Bees *Osmia lignaria* and *Megachile rotundata* (Hymenoptera: Megachilidae)

Author(s): Theresa L. Pitts-Singer, James S. Buckner, Thomas P. Freeman, and Christelle Guédot


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ABSTRACT  The Dufour’s gland of two solitary cavity-nesting bees, Osmia lignaria Say and Megachile rotundata (F.) (Hymenoptera: Megachilidae), was examined with microscopy to determine the structure and arrangement of the gland in the sting apparatus. The appearance and relative size of the Dufour’s gland of these two bee species are similar. Unlike the termination of the Dufour’s gland at the base of the sting in the honey bee, Apis mellifera L. (Apidae), the posterior portion of the Dufour’s gland in these megachilids enters into the sting bulb along with the venom duct. Within the sting bulb, the Dufour’s gland is ventral to and longer than the venom duct. The following evidential findings presented here and elsewhere are in support of the hypothesis that the Dufour’s gland is the source of an individual nest recognition cue in these two bee species: 1) the presence of a duct and exit pore at the posterior end of the Dufour’s gland that may release glandular secretions, 2) the location of thick, brushy metasomal setae and the setosa membrane that could be used to apply a secretion to a substrate, and 3) the observed dragging of the tip of the abdomen during nest-marking.

KEY WORDS  Apoidea, Dufour’s gland, Megachilidae, microscopy, solitary bee

The Dufour’s gland is an accessory exocrine gland associated with the sting apparatus in most female aculeate hymenopterans. The biochemistry, morphology, and function of the Dufour’s glands of eusocial honey bees, Apis mellifera L. (Apidae), and several species of primitively eusocial bumble bees, Bombus spp. (Apidae), have been examined extensively (e.g., reviewed in Duffield et al. 1984, Billen 1987, Abdalla and da Cruz-Landim 2001a-d). The morphology and structure of at least one species of the eusocial stingless bees, Melipona bicolor Lepeletier (Apidae), also has been studied (Abdalla and da Cruz-Landim 2004). Although the Dufour’s glands of representatives from many tribes and major genera of nonsocial bees have been investigated in one way or another, the characteristics of glands of most of the 16,000 or more species of solitary bees in the world (Michener 2000) remain unknown. On account of the broad diversity of bees, the study of additional species would allow for comparisons of glands at different taxonomic levels and for discovery of any possible novel characteristics and uses.

Many studies of Dufour’s gland chemistries include solitary bees in Apidae, Colletidae, Halictidae, and Megachilidae (e.g., Hefetz et al. 1979; Albans et al. 1980; Norden et al. 1980; Cane and Brooks 1983; Brooks and Cane 1984; Cane and Carlson 1984; Tengo et al. 1985, 1992; Shimron et al. 1985; Williams et al. 1986). The known or possible functions of the Dufour’s gland have been described for several solitary bee species including Xylocopa virginica texana Cresson (Apidae), Eucerast palestinae Friese (Apidae), Colletes spp. (Colletidae), Halictus hesperus Smith (Halictidae), Sphecodes spp. (Halictidae), Megachile integra Cresson (Megachilidae), and M. mendica mendica Cresson (Vinson et al. 1978, Albans et al. 1980, Brooks and Cane 1984, Shimron et al. 1985, Williams et al. 1986). Examples of the study of structural characteristics of the Dufour’s gland (internal, external, or both) are few and include that of X. virginica texana, X. v. virginica, Sphecodes spp., and Osmia cornifrons Radoszkowski (Megachilidae) (Cane and Brooks 1983, Barrows et al. 1986, Chapman and Barrows 1986, Tengo et al. 1992).

The structure and its relevance to the function of the Dufour’s gland of two solitary bees, Osmia lignaria Say and Megachile rotundata (F.) (the alfalfa leafcutting bee), are the focus of work presented here. Osmia lignaria is native to North America, and M. rotundata is native to Europe but accidentally imported into North America, where it established and then later was commercialized (Stephen 2003). These two bee species readily nest in artificial cavities in large aggregations in commercial settings and are managed to pollinate temperate tree fruits and almonds (O. lignaria) and alfalfa

1 USDA–ARS Bee Biology & Systematics Laboratory, Utah State University, Logan, Utah 84326.
2 theresa.pitts-singer@ars.usda.gov.
3 USDA–ARS Red River Valley Agricultural Research Center, Insect Genetics & Biochemistry Research Laboratory, Fargo, North Dakota 58105.
4 Electron Microscopy Center, Department of Plant Pathology, North Dakota State University, Fargo, North Dakota 58105.
5 USDA–ARS Yakima Agricultural Research Laboratory, Wapato, Washington 99351.

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THERESA L. PITTS-SINGER,1,2 JAMES S. BUCKNER,3 THOMAS P. FREEMAN,4 AND CHRISTELLE GUE´DOT5


KEY WORDS  Apoidea, Dufour’s gland, Megachilidae, microscopy, solitary bee
(M. rotundata) (Bosch and Kemp 2001, Pitts-Singer 2008, Pitts-Singer and Cane 2011). Like other nonsocial, mass-provisioning bees, females assemble individual pollen-nectar provisions, and upon each provision one egg is laid. The nesting female bee uses mud (O. lignaria) or leaf pieces (M. rotundata) to separate mass provisions that occur sequentially in a cavity; in M. rotundata leaf pieces also are used to make the lateral cell lining. If artificial cavities are provided for newly emerged bees near an appropriate flowering crop, female bees will provision nest cells with local forage and establish nests at commercial sites. After foraging trips, females are able to repeatedly locate their nests among the thousands of artificial cavities and will defend them from intruders. Studies have shown that O. lignaria (Guédot et al. 2006) and M. rotundata (Guédot et al., in review) females use olfactory cues to discriminate between their own nests and nests of others nearby. The behavior of a nest-building female inside her cavity suggests that she applies an abdominal substance to the walls of the cavity, and this substance appears to be used as a nest recognition cue (Gerber and Klostermeyer 1972, Guédot et al. 2006, Guédot et al., in review). Although components of the chemical residue in nests have been identified for O. lignaria (Guédot et al. 2006) and M. rotundata (Guédot et al., in review), the original source of a marking substance is unknown. One obvious possibility is that the marking chemicals originate from the Dufour’s gland.

The main suggested function of the lipid-containing secretions of Dufour’s glands is to serve as a nest cell lining, especially in ground-nesting bees for which a hydrophobic secretion forms a protective barrier to safeguard brood cells (reviewed in Hefetz 1998). Although the chemistry and function of the Dufour’s glands of some megachilid species have been studied previously, most studies have investigated the massive glands of groundnesting species. However, nests that are made above-ground, like those of our species of interest, are free of the risks of excessive soil moisture. The Dufour’s gland ultrastructure of only one cavity-nesting megachilid, the European O. cornifrons (Barrows et al. 1986), has been described thus far. In addition to safeguarding cells, Dufour’s gland secretions have been shown to provide a rich food source for Anthophora abrupta Say (Apidae) larvae (Norden et al. 1980) and have been suggested as a food source for M. integra and M. m. mendica larvae (Williams et al. 1986). The secretions also may have pheromonal activity for aggregating, mate-seeking, or recognizing individual nests and conspecifics (Duffield et al. 1984; Hefetz 1990,1992; Guédot et al. 2006). Xylocopa v. texana females use Dufour’s gland secretions to mark passion flowers to signal that the flower has been visited previously (Vinson et al. 1978).

To discover the function of the Dufour’s gland secretion of O. lignaria and M. rotundata, the secretory nature of the gland should be confirmed, and the location of its release for delivery should be determined. In this study, we examine the Dufour’s gland of O. lignaria and M. rotundata to reveal its 1) ultrastructure, 2) position relative to the sting, and 3) secretory product release point.

**Materials and Methods**

Populations of O. lignaria (2005, 2007–2010) and M. rotundata (2007, 2008, and 2010) were maintained at the USDA ARS Bee Biology & Systematics Laboratory (BBSL), Logan, UT (Richards 1984, Bosch and Kemp 2001, Frank 2003). For adult bee emergence, O. lignaria were incubated at 25°C during the spring, and M. rotundata were incubated at 29°C during the summer. Dufour’s glands were dissected from young adult female bees collected as pre-emergent adults (O. lignaria) or as 1- to 3 d-old adults (O. lignaria and M. rotundata). Also, for several other bees of each species that were emerged in the laboratory, maintained on a diet of honey-water (10% honey by volume) in the presence of males, and freezer-killed at 3–4 d old, the length of both the dissected Dufour’s gland and the intertegular span were measured using an ocular micrometer. Because the intertegular span relates to dry body weight (Cane 1987), the ratio of Dufour’s gland length to the estimated dry weight (estimated from the measure of the intertegular span) was calculated and used to reveal a proportional relationship between the relative bee body size and Dufour’s gland size.

At the North Dakota State University (NDSU) Electron Microscopy Center in Fargo, ND, whole intact bees of both species were fixed in 2.5% phosphate-buffered glutaraldehyde or placed in clearing agent to be examined with light and scanning electron microscopy for location of surface ducts. At the BBSL, the Dufour’s glands and venom ducts still attached to the sting apparatus were dissected from fresh bees, immediately fixed in 2.5% phosphate-buffered glutaraldehyde, and shipped overnight to the NDSU Electron Microscopy Center where they were rinsed in sodium phosphate buffer and prepared for microscopic examination.

Samples for light and transmission electron microscopy were postfixed in buffered osmium tetroxide (2%) for 4 h, dehydrated in a graded acetone series and critical point dried in a Tousimis Autosamdri 810 critical point drier (Tousimis, Rockville, MD) or an Olympus BH2 (Olympus America, Center Valley, PA) light microscope.

Some of the dissected Dufour’s glands and venom ducts with attached sting were dehydrated in a graded ethanol series and critical point dried in a Tousimis Autosamdri 810 critical point drier (Tousimis, Rockville, MD) using liquid carbon dioxide as a transitional
Dried samples were mounted on aluminum stubs with double stick carbon tape, silver paint, or both, coated with gold or gold-palladium (60:40) in a Balzers SCD 030 sputter coater (Oerlikon Balzers, Elgin, IL), and examined with a JEOL JSM-6300 or JEOL JSM-6490LV scanning electron microscope (JEOL USA Inc., Peabody, MA).

For study of the external morphology of whole bees, intact *O. lignaria* and *M. rotundata* females were fixed in buffered glutaraldehyde and critical-point dried or air dried, then examined with a SZH Olympus (Olympus America, Center Valley, PA) dissecting light microscope or mounted and coated as above for scanning electron microscopy. Intact *O. lignaria* also were fixed by being immediately submerged in 2,2-dimethoxypropane (DMP; acidified with 1–2 drops of HCl) for 1 h. The DMP was evaporated in a fume hood, and the bees were mounted and coated for scanning electron microscopy. In all, at least 10 specimens per species were examined.

**Results**

No surface ducts were found on the inner or outer ventral surfaces of the bees’ abdomens that would suggest that any sternal exocrine glands are present for use in nest-marking. However, abdominal setae of different types were present. Located ventrally along a megachilid’s abdomen on each sternite are rows of setae that are used for pollen transport (Fig. 1a–e). The scopal setae are scalloped in *M. rotundata* (Fig. 1b, inset), but are smooth in *O. lignaria* (Fig. 1e, inset). However, in both species the dense rows of setae on the sixth metasomal sternite (last complete sternite)
(Fig. 1a–f) contain much stouter, plumose setae than the scopal setae and are located such that they may function collectively as a brush for smearing any secretion delivered through the sting. Male bees do not bear this dense row of setae. Also, surrounding the sting bulb is the setosa membrane with its many short, stout setae. The location of this membrane suggests that it could function as a platform or brush for application of the secretory product onto a surface (Fig. 2a). When the sting is extended from the tip of the bee’s abdomen, it can deliver venom as a defensive response. Because the Dufour’s gland is an internal component of the sting apparatus (Fig. 2) and the end of the Dufour’s gland enters the sting bulb (Fig. 2b, c), the glandular secretions could be delivered via the sting and spread with the aid of the brush on the sixth metasomal sternite and the setosa membrane (Figs. 1 and 2).

The Dufour’s glands of *O. lignaria* and *M. rotundata* are very similar in their structure, arrangement, and tubular, sac-like appearance (Fig. 2). In both species, the gland is dorsal-ventrally flattened and enters the sting bulb with the venom duct (Fig. 2). Inside the sting bulb, the Dufour’s gland is positioned beneath the venom duct and extends beyond it (Figs. 2c, 3). *Osmia lignaria* is a larger bee (average female fresh

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**Fig. 2.** Sting and associated structures, which are identical in orientation for *O. lignaria* and *M. rotundata*; anterior is top of figure. (a) entire sting of *M. rotundata*, depicting the setosa membrane (SM) and the longitudinal seam between the sting lancets (SL). (b) light micrograph of intact sting apparatus dissected from *O. lignaria* female. (c) drawing of sting apparatus including the Dufour’s gland (DG), venom gland (VG) and venom duct (VD), sting bulb (SB) and sting shaft (SS), and showing approximate planes of sections for Figs. 4b, 4d, and 5c. (drawing by James P. Pitts, Utah State University). Bars 200 μm (for a), 250 μm (for b, c).

**Fig. 3.** Stereo pair from scanning electron micrographs of partial sting apparatus from *O. lignaria* female (ventral view) showing the relative positions of the Dufour’s gland (DG, left) and venom duct (VD, top) as they enter and continue into the sting bulb. Arrow indicates the Dufour’s gland terminus, also shown in Fig. 5a. A positive-curvature (magnifying) stereoscope is helpful for three-dimensional viewing. Bar 100 μm.
weight ± SE = 0.110 ± 0.005 g; n = 11; T.L.P., unpublished data) than *M. rotundata* (average female fresh weight ± SE = 0.037 ± 0.002 g; n = 20; T.L.P., unpublished data). Although the Dufour’s gland structure and position in the bee bodies are quite similar for each species, the gland of *M. rotundata* is proportionally longer compared with its body size than that of *O. lignaria*. The gland to body size proportion is slightly more variable in the laboratory-reared *O. lignaria* than in *M. rotundata* (Table 1). For both species, the longest glands were not present in the largest bees.

In cross section, the lumens of the Dufour’s gland and the venom duct are obvious in both *M. rotundata* (Fig. 4a) and *O. lignaria* (Fig. 4b). The outer border of the Dufour’s gland is formed by repeated invaginations of the gland’s surface resulting in a undulating pattern (Fig. 4a, b). The gland’s secretory cells are simple class 1 cells that have direct contact with the gland cuticle (Noirot and Quennedey 1974).

The Dufour’s gland lumen is lined by cuticle and subcuticle. Muscle is present near the lumen as well as smooth endoplasmic reticula and mitochondria (Fig. 4c). Toward the outer surface of the gland are smooth endoplasmic reticula and many mitochondria (Fig. 4d). The tip of the Dufour’s gland within the sting bulb (Fig. 5a) contains a duct (Fig. 5b) that ends in a pore (Fig. 5c) from which the Dufour’s gland secretion can exit into the sting between the lancets.

**Discussion**

The position, shape, and relative size of the Dufour’s gland in *O. lignaria* and *M. rotundata* are similar. Although the termination point of the Dufour’s gland of at least some social apid bees is at the sting base (Billen 1987), the Dufour’s glands of these megachilids extend into the sting bulb. Thus, the Dufour’s gland contents of the megachilids, and likely other bees, may have different functions and delivery systems than those of social apids. The posterior end of the megachilid Dufour’s gland lies ventral to the venom duct, and reaches further into the sting shaft than does the venom duct. The secretory activity of the Dufour’s gland is indicated by the presence of secretory cells found along the length of the Dufour’s gland. Secretory cells are characterized by their abundance of smooth endoplasmic reticula and mitochondria that are likely involved in biosynthesis of lipid and other intermediary metabolites (Noirot and Quennedey 1974, Billen 1986). We did not identify specific cells that contained an accumulation of materials, e.g., lipids, that could be considered a secretory product, and therefore, the exact source of any secretory product could not be determined from our investigation. However, our unpublished chemical analyses of Dufour’s gland contents confirm the presence of lipids.

The presence of the terminal duct and pore at the end of Dufour’s gland presumably allows the glandular secretion to exit into the sting bulb and then be ratcheted by the sting lancets to the tip of the sting, or be squeezed or allowed to ooze from the sting’s ventral seam between the lancets (Fig. 5b, c). Muscles around the gland lumen may aid in forcing secretory material out of the gland and allow for the control of secretion for designated use.

Abdominal setae located above and below the sting may serve as brush-like applicators for spreading Dufour’s gland secretion to coat the inner surface of a nest cavity, although these setae also may play other roles in the nest-provisioning behavior. The ability of a bee to apply Dufour’s gland secretion by pressing the posterior portion of the abdomen to the walls of a nest cavity matches the behavior that has been observed during nest construction. Manipulations of newly marked nest cavities of *O. lignaria* and *M. rotundata* elicited behaviors indicating that chemical cues are important for nest recognition (Guédot et al. 2006, Guédot et al., in review). Although visual orientation cues are used by these solitary bees to find the approximate location of their nest cavity (Fauria et al. 2004; Guédot et al. 2005, 2007), chemical cues are important once the bee is in contact with the nest cavity (Guédot et al. 2006, Guédot et al., in review). As demonstrated in an artificial setting, a bee that entered a “wrong” nest cavity quickly withdrew from that cavity and investigated surrounding cavities searching for her own. Removing a previously marked piece of a cavity and replacing it with an unmarked piece caused the bee to explore other cavities (Guédot et al. 2006, Guédot et al., in review). Thus, the presence of an individual nest signal is implied and is the means by which a bee can distinguish her own nest cavity from that of others.

For solitary bees that individually select a nest cavity in which they provision cells for brood production, it is important to indicate ownership of that cavity. Nest-marking is a method to satisfy this need. Our unpublished data has confirmed that *O. lignaria* and *M. rotundata* Dufour’s gland contents contain components similar to those found on the bees’ cuticles and their nest walls and that individual bees vary in the proportions of those components. As such, application of a cavity-nesting bee’s Dufour’s gland secretion to nest walls could provide a unique signal that is rec-

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**Table 1.** The average (± SE) Dufour’s gland length, intertegular span, estimated dry weight, and gland length: dry weight ratio for *Osmia lignaria* and *Megachile rotundata* females

<table>
<thead>
<tr>
<th>Species (n)</th>
<th>Dufour’s gland (mm)</th>
<th>Intertegular span (mm)</th>
<th>Est’d dry wt. (mg)</th>
<th>Ratio (mm:mg)</th>
<th>Ratio range</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>O. lignaria</em> (7)</td>
<td>2.45 ± 0.25</td>
<td>2.95 ± 0.09</td>
<td>27.90 ± 2.04</td>
<td>0.09 ± 0.02</td>
<td>0.05-0.16</td>
</tr>
<tr>
<td><em>M. rotundata</em> (8)</td>
<td>2.25 ± 0.15</td>
<td>2.35 ± 0.01</td>
<td>15.57 ± 1.80</td>
<td>0.15 ± 0.01</td>
<td>0.09-0.18</td>
</tr>
</tbody>
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Dry weight estimated from formula in Cane 1987.
ognized by its bee occupant. The Dufour’s gland secretion of *A. mellifera* has compositions that are caste-specific and indicate mating status, and they may function as a component of the queen’s signal (Katzav-Gozansky et al. 2000). However, unlike previously thought, the *A. mellifera* Dufour’s gland secretion is not an egg-marking pheromone (Ratnieks 1995, Katzav-Gozansky et al. 2002, Martin et al. 2005). Therefore, the Dufour’s gland is probably not used in egg-marking in the cavity-nesting bees either.

**Fig. 4.** (a–b) Light micrographs of thick cross sections. (a) section through the sting bulb, Dufour’s gland (DG), and venom duct (VD) of *M. rotundata*. (b) section (plane indicated in Fig. 2c) through the sting bulb, Dufour’s gland (DG), and venom duct (VD) of *O. lignaria*; box on DG indicates area of Fig. 4c. (c–d) transmission electron micrographs of sectioned Dufour’s gland of *O. lignaria*. (c) near the constricted lumen (L) are muscle (Mu), smooth endoplasmic reticulum (SER), mitochondria (Mi), nuclei (N), and nucleoli (Nu); lumen is lined by cuticle (C) and subcuticle (SubC). (d) near the outer margin of the gland are smooth endoplasmic reticulum (SER) and many mitochondria (Mi); plane of section indicated in Fig. 2c. Bar 50 μm (for a and b), 1 μm (for c), 2 μm (for d).
The morphological variation of the sting apparatus and associated glands of bees also is important for understanding bee phylogenetics and systematics (Cane 1983; Packer 2003). Compared with the glands of apids and ground-nesting bees that have been studied, the Dufour’s gland of *O. lignaria* and *M. rotundata* are quite small. The position of the megachilid Dufour’s gland relative to the venom duct in *O. lignaria* and *M. rotundata* is the same as the sphecid wasp *Liris niger* Fabricius (Apoidea: Sphecidae) (Gnatzy et al. 2004). Although the megachilids and sphecids share a common ancestor with the apids (Danforth et al. 2006, Pilgrim et al. 2008), the location of the Dufour’s glands of *O. lignaria*, *M. rotundata*, and *L. niger* are similar to the location of the Dufour’s gland in Formicidae (Billen 1987). Thus, the position of the Dufour’s gland in the megachilids and sphecids may be pleisiomorphic, whereas the position in the vesvids and apids are likely apomorphic. Once the Dufour’s gland and venom duct of more bee (and wasp) species are examined for apomorphies. Once the Dufour’s gland and venom glands of bees also is important for understanding bee phylogenetics and systematics (Cane 1983; Packer 2003). Compared with the glands of apids and ground-nesting bees that have been studied, the Dufour’s gland of *O. lignaria* and *M. rotundata* are quite small. The position of the megachilid Dufour’s gland relative to the venom duct in *O. lignaria* and *M. rotundata* is the same as the sphecid wasp *Liris niger* Fabricius (Apoidea: Sphecidae) (Gnatzy et al. 2004). Although the megachilids and sphecids share a common ancestor with the apids (Danforth et al. 2006, Pilgrim et al. 2008), the location of the Dufour’s glands of *O. lignaria*, *M. rotundata*, and *L. niger* are similar to the location of the Dufour’s gland in Formicidae (Billen 1987). Thus, the position of the Dufour’s gland in the megachilids and sphecids may be pleisiomorphic, whereas the position in the vesvids and apids are likely apomorphic. Once the Dufour’s gland and venom duct of more bee (and wasp) species are examined for their relative positions at or in the sting base, the origin of such arrangements will become clear.

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