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Occurrence of Antennal Glands in Ants

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Abstract

A previous report of the discovery of exocrine glands in the antennal club of queens and workers of *Solenopsis invicta* Buren, 1972 (Isidoro et al., *Insectes Sociaux* 47: 236-240, 2000) left open the question of the extent to which similar glands occur in the Formicidae family. We wanted to know if these antennal glands are unique to *Solenopsis*, or if they are found in a wider taxonomic group. Using scanning electron microscopy, we examined the antennae of 41 ant species. Presence of the antennal glands was indicated by a characteristic circumferential ring of pores in a distal antennal segment of workers. Pores were found in the 9th antennal segment of all 26 species of *Solenopsis* examined. Pores were absent in the following: *Monomorium minimum*, *M. pharaonis*, *Pheidole* sp., *Crematogaster* sp., *Linepithema humile*, *Forelius* sp., *Dorymyrmex* sp., *Paratrechina* sp., *Oecophylla smaragdina*, *Campanotus* sp., *Ectatomma ruidum*, *E. tuberculatum*, and *Pseudomyrmex ferruginea*. However, pores were found in the antennal club of *Tetramorium bicarinatum* workers and queens. After KOH digestion of *T. bicarinatum* antennae, internal canals were observed in both workers and queens, and the canals connected to spherical reservoirs in queens. *T. bicarinatum* was only non-*Solenopsis* species examined which showed evidence for antennal glands in the distal funiculus.

Keywords

Formicidae; *Solenopsis*; *Tetramorium*; glands; antenna

Introduction

The antennae of insects are considered important sensory structures (Keil, 1999), but glands were first reported following a light microscopic examination of the antennae of *Melittobia australica* (Eulophidae) (Dahms, 1984). Bin and Vinson (1986) reported an antennal sex gland in males of another parasitoid, *Trissolcus basalis* (Scelionidae). Since then antennal glands have been reported in a number of Hymenoptera (Isidoro et al., 1996; Goettler et al., 2006) and beetles (Giglio et al., 2005; Weis et al., 1999). Although glands were reported from the antennal club of female red imported fire ants, *Solenopsis invicta* Buren, 1972 (Isidoro et al. 2000) and the scape of an army ant, *Eciton burchelli* (Billen 2000), the flagellum of a primitive Ponerinae, *Dinoponera lucida* (Marques-Silva et al., 2006) and the antennae of a slave-making formicine ant (Romani et al., 2006) little is known about other genera. The glands of *S. invicta* are located in the 9th of 10 antennal segments in workers and in the 9th and 10th of 11 segments in queens. The outlets of the gland canals appear as a ring of pores in the cuticle near the antennal segment joints. Occasionally, secretions are observed coming from the glands in queens. The gland cells are penetrated by cuticular

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canals which terminate in external pores (Isidoro et al., 2000 and are thus classified as class 3 cells, in contrast to classes 1 and 2, in which the gland cell lacks a cuticular canal (Noirot and Quennedy, 1974). The function of these glands is unknown. There is some evidence for involvement of the gland secretion in queen recognition by workers (Kuriachan and Vinson, unpublished). Aside from the observations of Isidoro et al. (2000), there are no other published reports of similar antennal glands in ants, although a thorough survey of antenna morphology from about 50 ant species (not including *Solenopsis*) was presented by Hashimoto (1990). We have now examined a wide range of ant species to determine the distribution of type 3 antennal glands in the distal funiculus.

Materials and Methods

Solenopsis and *Ectatomma* specimens were obtained from the collection of the Smithsonian Institution National Museum of Natural History. Species assignments were made by the Museum. All other specimens were from laboratory colonies maintained at Texas A&M, with the exception of field-collected specimens of *Pogonomyrmex* sp., *Monomorium pharaonis*, and *Oecophylla smaragdina*. Antennae from dried museum specimens were transferred directly to double-sided carbon tape on aluminum stubs. Live specimens were cold-anesthetized, fixed overnight in formalin, dehydrated through an ethanol series, and dried in a desiccator. Field specimens collected in alcohol were dried, rehydrated, fixed in formalin, and then dried after dehydration. Specimens for views of the inner surface of the antennal cuticle were treated with KOH by the method of Bin and Vinson (1986). Stubs were coated with gold *in vacuo* for 40 sec. to 1 min. Scanning electron microscopy (SEM) was done with a JEOL 840 microscope with a LAB-6 filament operated between 5 and 20 kV, or a Leo 435VP operated at 15 kV. For *Solenopsis invicta* and *Tetramorium bicarinatum*, both worker and queen antennae were examined. All other specimens in this study were workers. In most cases, six antennae from three ants were examined.

Results

The species examined in this study are listed in table 1. For species which displayed external pores in the antenna viewed by SEM, the name is followed by an asterisk. When external pores as well as internal canal structures were observed SEM, the species name is followed by two asterisks. Representative electron micrographs are shown in the figures.

Antennal glands in *Solenopsis invicta*

Pores on the external surface are visible where canals from the antennal glands pass through the cuticle, as previously shown (Isidoro et al., 2000). Figures 1A and 1B show pores in workers and queens, respectively, near antennal segment joints. Occasionally secretions are observed coming from queen antennal glands (figure 1C).

Pores in antennae of other *Solenopsis* species

Worker antennae of twenty-five other species of *Solenopsis* were examined by SEM (table 1 and fig. 2). All were dried museum specimens, ranging in age from 46-70 years after collection. In every case, at the joint between segments 8 and 9, pores were observed, similar to those seen in *S. invicta* workers. This suggests that antennal club glands are likely to be present in all species of *Solenopsis*.

Antennae of other ant species

The antennae of fifteen non-*Solenopsis* species were examined by SEM. The specimens included ants which were field-collected, taken from lab colonies, or from museum collections. A close examination was made of the segment joints in the club, if present, or

the distal three segments of the funiculus, in the absence of a club (fig. 3). In all but one species, no pores were observed (except for obvious openings of *sensilla coeloconica* or *s. ampulacea*.)

Antennal glands in *Tetramorium bicarinatum*

Surprisingly, we observed pores in the antenna of *T. bicarinatum* workers and queens (fig. 4). The pores were observed in the distal segment of the club, at the joint with the proximal segment (fig. 4D). Some antennae were cut longitudinally and digested with KOH for imaging of the inner surface of the antennae (fig. 4A, B and C). This treatment reveals the gland canals, which have a number of features that are different from the *S. invicta* gland canals. The *T. bicarinatum* canals cross directly through the flat cuticle surface into the external pores. By contrast, the *S. invicta* canals enter an apodeme (fig. 5A) before connecting with the external pores. The *T. bicarinatum* canals are narrower than the *S. invicta* canals (approx. 0.2-0.3 μm , compared with 0.5 μm). Examination of the KOH-treated *T. bicarinatum* antennae revealed that the glands occurred in all club segments, unlike *S. invicta*, which lacks glands in the distal club segment. The external pores in *T. bicarinatum* were difficult to observe in the proximal club segments due to their placement in the middle of the segments, where they could be mistaken for broken sensilla. The pores in the *T. bicarinatum* distal club segment, and the pores in *Solenopsis*, are more obvious because they are located in the sensilla-free region near the joint with adjacent segments. A further difference between *T. bicarinatum* and *S. invicta* antennal glands is that in *T. bicarinatum* queens, the pore canals terminate in spherical cuticle-covered reservoirs (fig. 4A, B and C). The reservoirs were not observed in *S. invicta* queens or workers, nor were they observed in *T. bicarinatum* workers.

Discussion

Cuticular pores were observed in antennal segment 9 near the joint with segment 8 in workers of all twenty-six species of *Solenopsis* examined (table 1 and figs. 1 and 2). This implies that type 3 glands (Noirot and Quennedy, 1974) are a characteristic of the antennal club of all members of this genus. The presence of antennal club glands does not extend to all members of the tribe Solenopsidini. Two species of *Monomorium* lack this type of antennal gland. We also failed to find evidence for antennal glands in the distal funiculus of a wide variety of other ant species (table 1 and fig. 3). However, we did observe antennal club glands in *Tetramorium bicarinatum*, a Myrmicine ant in a different tribe from *Solenopsis* (fig. 4). The glands differ from those in *Solenopsis invicta* as follows: i) they occur in all club segments; ii) in queens, they contain cuticular reservoirs; iii) the canals penetrate the antennal wall through a flat surface, whereas *S. invicta* canals exit through an apodeme (fig. 5). The recently published molecular phylogeny of ants (Moreau et al., 2006) shows a wide separation between *Tetramorium* and *Solenopsis*. We found no evidence of antennal club glands in *Crematogaster*, which is closer to *Tetramorium*; and we found no evidence of antennal club glands in *Monomorium*, which is closer to *Solenopsis*. Thus, we conclude that antennal club glands have arisen more than once in ants.

In addition to the glands of the distal funiculus described here and previously (Isidoro et al., 2000), glands have been observed in the scape of three ant species: *Eciton burchelli* (Billen, 2000), and *Polyergus rufescens* and *Formica cunicularia* (Romani et al., 2006). Also, both type 1 and type 3 glands were observed in all antennomeres except the scape of the primitive ant *Dinoponera lucida* (Marques-Silva et al., 2006). We examined antennae of two species of *Ectatomma* and saw no evidence for the external pores of type 3 glands. However, Moreau et al. (2006) raised questions about whether this genus belongs with the Ponerine ants. The distribution and variability of ant antennal glands suggests multiple evolutionary origins for these glands.

Our results have some bearing on a long-standing taxonomic controversy. The thief ants have been sometimes classified as a separate genus *Diplorhoptrum*, or as a subgenus, *Solenopsis* (*Diplorhoptrum*). We examined three thief ants, *Solenopsis molesta*, *Solenopsis molesta validiuscula*, and *Solenopsis fugax*. In all three, the gland pores characteristic of other members of the *Solenopsis* genus were observed. This could be taken as a piece of evidence for the similarity of thief ants to the rest of the genus *Solenopsis*.

We do not yet know the functions of glands in the clubs of female ants. The species distribution suggests multiple evolutionary origins, so there may also be different functions in different species. It is possible that the glands are vestigial in workers. No secretions have been observed from antennal glands in *S. invicta* workers, in contrast to queens. Also, gland reservoirs are present in *T. bicarinatum* queen antennae, but not in worker antennae. Further understanding of antennal gland function awaits more complete behavioral and biochemical studies.

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Figure 1. Surface pores from antennal glands in *Solenopsis invicta*

A. Worker, junction between antennal segments 8 and 9. Scale bar, 10 µm. **B.** Queen, segment 9 including junctions with segments 8 and 10. Scale bar, 10 µm. **C.** Secretions from queen antennal glands. Scale bar, 1 µm. Top of each SEM image is distal.

Figure 2. Surface pores on antennae from *Solenopsis* species

SEM images show the joint between segments 8 and 9 of worker antennae. Left side of each image is distal. A. *S. aurea*; B. *S. clytemnestra*; C. *S. corticalis*; D. *S. electra*; E. *S. gayi*; F. *S. interrupta*; G. *S. latastei*; H. *S. molesta validiuscula*; I. *S. pergandei*; J. *S. quinquecupis*; K. *S. richteri*; L. *S. wasmannii*. One pore is identified by an arrow in each micrograph. Scale bars: A and C-L, 30 μm ; B, 10 μm .

Figure 3. Worker antennae from representative ant species

SEM images of the joint between segments 9 and 10 worker antennae of A. *Forelius sp.*; B. *Linepithema humile*; C. *Monomorium pharaonis*; and D. the joint between segments 10 and 11 of a worker antenna of *Oecophylla smaragdina*. No gland pores are evident. Left or bottom of each image is proximal. Scale bars: A-C, 20 µm; D, 10 µm.

Figure 4. Antenual glands in *Tetramorium bicarinatum*

A. Cut-away view of segment 12 (tip segment) of KOH-treated *T. bicarinatum* queen antenna. Cluster of spherical gland reservoirs (arrows) visible at proximal end of segment. **B.** Higher magnification view of reservoirs, showing gland canals (arrows) leading to pores.. **C.** Close-up view of reservoir (*r*), canal (*c*) and pore (*p*) in segment 12 of queen antenna. **D.** External view of segment 12 of worker antenna showing pores (arrows) which lead into canals. Only canals without reservoirs are observed in worker antennae (not shown). Scale bars, 10 μm (A, B and D) or 3 μm (C).

Figure 5. Inside surfaces of *Solenopsis* and *Monomorium* antennae

A. *Solenopsis invicta* worker antennal segment 9. Gland canals (arrows) penetrate the wall of the antenna through an apodeme. **B.** *Monomorium pharaonis* worker antennal segment 12. No canals are observed. Scale bars, 10 µm.

Table 1

Species examined in this study.

Dolichoderinae*Dorymyrmex* sp.*Forelius* sp.*Linepithema humile* Mayr, 1868**Formicinae***Campanotus* sp.*Oecophylla smaragdina* Fabricius, 1775*Paratrechina* sp.**Pseudomyrmecinae***Pseudomyrmex ferruginea* F. Smith, 1877**Myrmicinae**Solenopsidini

Solenopsis aurea Wheeler, 1906^{*}, *S. clytemnestra* Emery, 1896^{*}, *S. corticalis* Forel, 1881^{*}, *S. electra* Forel, 1914^{*}, *S. fugax* Latreille, 1798^{*}, *S. gayi* Spinola, 1851^{*}, *S. geminata* Fabricius, 1804^{*}, *S. globularia litoralis* Creighton, 1930^{*}, *S. globularia desecheoensis* Mann, 1920^{*}, *S. interrupta* Santschi, 1916^{*}, *S. invicta* Buren, 1972^{**}, *S. krockowi* Wheeler, 1908^{*}, *S. latastei* Emery, 1895^{*}, *S. macdonaghi* Santschi, 1916^{*}, *S. molesta* Say, 1836^{*}, *S. molesta validiuscula* Emery, 1895^{*}, *S. pergandei* Forel, 1901^{*}, *S. picea* Emery, 1896^{*}, *S. picta* Emery, 1895^{*}, *S. punctaticeps* Mayr, 1865^{*}, *S. quinquecupis* Forel, 1913^{*}, *S. richteri* Forel, 1909^{*}, *S. tennesseensis* M.R. Smith, 1951^{*}, *S. texana* Emery, 1895^{*}, *S. wasmannii* Emery, 1895^{*}, *S. xyloni* McCook, 1879^{*}, *Monomorium minimum* Buckley, 1867, *M. pharaonis* Linnaeus, 1758

Myrmicine tribe group*Pogonomyrmex* sp., *Tetramorium bicarinatum* Nylander, 1846^{**}, *Pheidole* sp.Other*Crematogaster* sp.**Ponerinae (or Ectatomminae)***Ectatomma ruidum* Roger, 1860, *E. tuberculatum* Olivier, 1792

* external evidence for antennal glands from pores at segment joints, viewed by SEM

** evidence for antennal glands from both external SEM and internal observations from TEM sections and/or SEM of KOH-treated antennae