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Origin of the trail pheromone in Ecitoninae : a behavioural and morphological examination

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Abstract - *The trail pheromone of the New World army ant* Eciton burchelli *was found to originate from the 7th abdominal sternite, on the inner side of which a conspicuous glandular epithelium is located. The trail substance retains its activity for several days, and elicits an extremely strong following reaction in both workers and soldiers, that run inceasingly along a trail made from a 7th sternite extract at an average speed of 7.5 cm per second.*

Key words : *Eciton burchelli*, army ants, trail pheromone, sternal gland, ultra-structure.

Introduction

Social insect communities are well known for their often very ingenious communication systems that regulate the various interactions between the numerous individuals. A particular example is recruitment, in which nestmates are guided to a specific place, e.g. where better nesting conditions or food have been discovered. Honeybees in this regard have developed their unique dance system based upon a sun compass (von Frisch, 1967), while the terrestrial ants and termites mostly rely on a pheromonal communication, in which chemical trails originating from a variety of glands are laid down onto the substrate (reviewed in Morgan, 1990). The most spectacular trails undoubtedly are found in the Dorylinae and Ecitoninae, the army ants from the Old and New World, respectively. These ants form impressive raiding columns with thousands of excited individuals on the march, although very little is known about the underlying pheromonal regulation. That pheromones are used by the virtually blind army ants is a generally accepted idea, with observations of the ants in the forefront of the raid in Ecitoninae rubbing their abdomen against the ground, thereby most probably releasing a trail extending substance (Schneirla, 1971). A number of pioneer papers claimed the hindgut to be the major source for the active compound(s) in Ecitoninae (Blum and Portocarrero, 1964 ; Watkins *et al.*, 1967 ; Torgerson and Akre, 1970). Later reports repeated this information, or raised the additional possibility that the pygidial gland might equally contribute to the production of trail substances (Hölldobler and Engel, 1978).

The present paper reports on our recent examination of trail-following in *Eciton burchelli* in order to verify and update the present knowledge, and provides a morphological description of the glandular tissue that produces the trail pheromone.

Material and Methods

Raiding workers and soldiers of *Eciton burchelli* (Westwood, 1842) were collected in the rain forest at the Reserva Ducke of the INPA (Instituto Nacional de Pesquisas da Amazônia) near Manaus, Brasil, in March 1991.

In the INPA-laboratories, a few tens of ants were dissected and their glands fixed in cold 2% glutaraldehyde, buffered at pH 7.3 with 50 mM Na-cacodylate and 150 mM saccharose. Postfixation was carried out in 2% osmium tetroxide in the same buffer, and was followed by dehydration in a graded acetone series and embedding in Araldite resin. Semi-thin sections for histology were stained with methylene blue and thionin, double-stained thin sections were used for ultrastructural examination in a Zeiss EM 900 electron microscope.

Trail-following was assayed by drawing a circle of diameter 10 cm with a hexane extract (50 μl) of two glands in a 0.55 Standardgraph pen on a sheet of paper, and presenting this paper to individual ants (Pasteels and Verhaeghe, 1974). A square frame (13 x 18 cm) with fluon-coated inner walls was used to keep ants within the testing arena. The distance run along the trail was used as a measure of activity. Before each test, the drawing pen and tissue grinder were thoroughly cleaned in acetone, and the bioassay carried out as a control using only 50 μl of hexane.

Results

It was virtually impossible to investigate any kind of trail following by *E. burchelli* within the first hours or day after they were collected, as the ants were far too excited. When presented with an artificial trail on a sheet of paper they continued to run very quickly along the inner walls of the surrounding frame, and paid no attention whatsoever to the trail.

Only after 2 days, when the ants have become at least a bit more quiet, were we able to start the bioassay. A first trail made of 2 venom glands was followed for a few laps by one worker (a medium worker with a head width of 10 mm ; the other ants were still too excited to be of any value in the bioassay). A "control" of an extract of an abdominal tip from which the venom gland had been removed, however, elicited an extremely strong following by this same worker, which continually ran laps at an average speed of 16 laps per minute (i.e. approx. 8 cm/sec). Subsequent attempts to localize the precise source of the pheromone, still using the same worker to test following activity, gave entirely negative results with trails made from either Dufour gland or hindgut extracts. A trail with an extract of the 7th abdominal sternite of 2 medium workers, however, caused the same spectacular and high speed response of 16 laps per minute as reported before. The worker was running non-stop along the trail for 1 hour, after which it was removed and fixed for morphological examination.

Three 3 days later (day 5 after collecting), the trail made with this same 7th sternite had retained its full activity, and was now followed by all surviving workers as well as soldiers (average speed workers 7.5 ± 1.5 cm/sec (N=8), speed of 2 soldiers 8.1 and 11.7 cm/sec, resp.). Speed measurements of individual *E. burchelli* on a 1 meter strip of smooth surface in the field resulted in 6.3 ± 0.4 cm/sec for minor workers (N=22), 7.5 ± 0.5 cm/sec for medium workers (N=22) and 8.8 ± 1.1 cm/sec for soldiers (N=22) (A. Harada, pers. comm.).

Morphological examination of the abdomen of Ecitoninae revealed the presence of a conspicuous glandular epithelium on the 7th sternite, which represents a spe-

Fig. 1. - *Semi-thin longitudinal section through the posterior part of the abdomen of an* E. burchelli *worker. Note the conspicuous glandular epithelium (GE) on the inner side of the 7th sternite (S7). PG = pygidial gland, St = sting, scale bar 100 µm.*

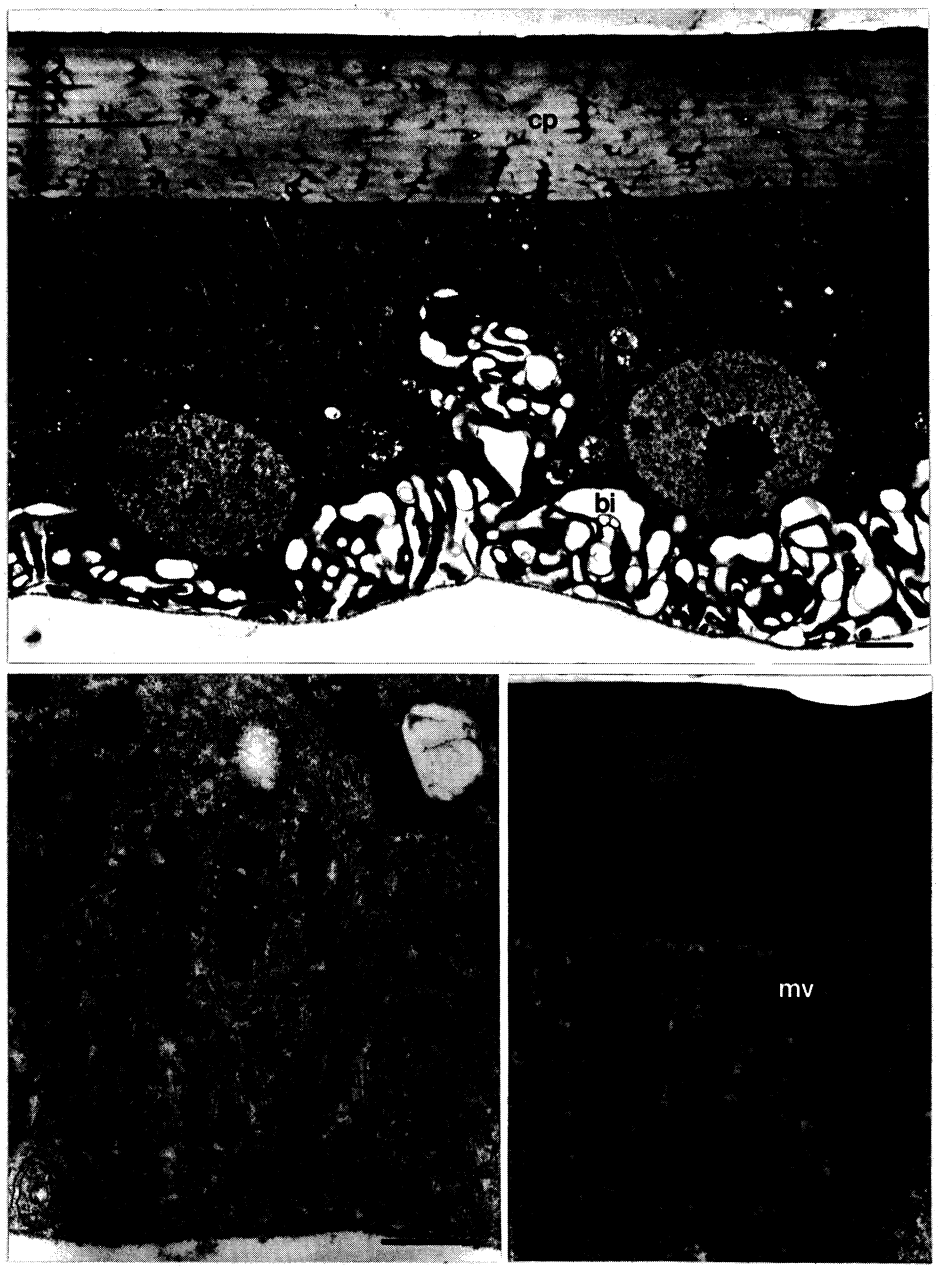
cialized modification of the epidermis of the upper surface of the sternite lining the cloacal chamber (Fig. 1). The epithelium has an average thickness between 10 and 15 μm, and covers an area with a length of approx. 250 μm and a width of approx. 220 μm. At its basal side, a clear basement membrane with a thickness around 50 nm is found. Nuclei generally are rounded, and occupy a basal position in the glandular cells (Fig. 2). The basal cell membrane forms extensive invaginations (Figs. 2 and 3) which may eventually give rise to considerable extracellular spaces (Fig. 2). Mitochondria and abundant free ribosomes are randomly scattered in the cytoplasm. Endoplasmic reticulum is very rare, both in its smooth and granular form. The apical cell membrane is modified into a clear but irregular microvillar border (Fig. 4). The epithelium is covered with a cuticular layer with a uniform thickness of 3 μm. The latter is characterized by numerous pores, in which granular material with the same electron density as the material in the intermicrovillar spaces is found (Figs. 2 and 4).

We found this gland in all workers we investigated of *Eciton burchelli*, *Labidus praedator* and *Neivamyrmex nigrescens*.

Discussion

Our results clearly illustrate that trail following in *E. burchelli* relies on a pheromonal mechanism. The inherent excited nature of these ants, however, makes it much more difficult to study this behaviour. Their extremely pronounced tendency for directionalized mass movement was already noticed by Schneirla (1944) with the spectacular observation of circular milling of ants, cut off from their road, for more than a day until they had all died. After calming down for a few days, the ants we collected became susceptible to the bioassay, from which the origin of the active substance could be determined as being the 7th abdominal sternite. The absolute inactivity of a trail made from hindgut extract questions an earlier report on *E. hamatum* by Blum and Portocarrero (1964), in which the reaction elicited by the hindgut may be due to contamination from the 7th sternite, which in those days will not have been considered as a possible source for the trail substance. The apparent activity we observed with a trail laid with venom gland extract, might equally be due to contamination with material from the 7th sternite. Although the chemical nature of the trail pheromone is still unknown, it turns out to be an extremely active and long-

Figs. 2-4. - *Electronmicrographs of the sternal gland in* Eciton burchelli *workers, scale bar 1 μm.*
2 : epithelium with considerable basal invaginations (bi), apical microvilli (mv) and cuticular pores (cp). 3 : basal cytoplasm and invaginations (bi), showing scattered mitochondria (M) and free ribosomes. 4 : apical cytoplasm and cuticle. Note microvilli (mv) and cuticular pores (cp), in which electron-dense material is found.


cp
bi
mv

lasting substance, the activity of which persists for several days. This ability of *E. burchelli* workers to recognize trails that are up to a week old has already been noted by Franks and Bossert (1983). Also in contrast with most other trail pheromones is the extremely high activity it elicits, as the traditional quantification in counting the number of 1 cm arcs followed in the bioassay (Pasteels and Verhaeghe, 1974) seems of a different dimension compared with the continuously running of laps at an average speed of approximately 7.5 cm/sec by our *Eciton*. This speed is very similar to the speed of *E. burchelli* running in the field (Franks, 1985 ; A. Harada, pers. comm.). According to Franks *et al.* (1991), the velocity of running of *E. burchelli* shows a gradual increase as a function of the strength of the pheromone trail, speeding up as they saturate their own trail.

The conspicuous glandular epithelium, which secretes the active trail substances, represents a specialized portion of the epidermis lining the inner wall of the 7th sternite. The obvious basal invaginations (uptake of metabolites), apical microvilli and cuticular pores (discharge of secretory products) provide a clear indication of a transporting tissue. To what extent the main steps in the elaboration of the final secretory products take place within the gland cells or whether these are mostly active in transporting the active compounds from the haemolymph to the outside remains unclear. The discharge of the secretory products into the cloacal chamber brings these compounds in close contact with the sting, which probably forms the device for depositing the pheromones onto the substrate.

Although the gland seems to be characteristic for workers of Ecitoninae in general, its occurrence outside the Ecitoninae has also been reported for *Novomessor* (Hölldobler and Engel, 1978) and *Aenictus* (Jessen, 1987). Especially the glandular similarity between *Aenictus* and the Ecitoninae offers the opportunity for phylogenetic speculation dealing with the origin and evolution of the various army ant groups.

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