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THE PYGIDIAL GLAND AND CHEMICAL RECRUITMENT COMMUNICATION IN *Pachycondyla* (= *Termitopone*) *laevigata*

BERT HÖLLDOBLER and JAMES F.A. TRANIELLO

Museum of Comparative Zoology Laboratories, Harvard University
Cambridge, Massachusetts 02138

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Abstract—Termite predation by the ponerine ant *Pachycondyla* (= *Termitopone*) *laevigata* is regulated by a recruitment trail pheromone which originates from the pygidial gland and not, as previously assumed, from the hindgut. The pygidial gland opens between the 6th and 7th abdominal terga and is associated with a distinct cuticular structure which obviously serves as a glandular applicator.

Key Words—*Pachycondyla* (= *Termitopone*) *laevigata*, Hymenoptera, Formicidae, chemical communication, termite predation, pygidial gland.

INTRODUCTION

Pachycondyla (= *Termitopone*) *laevigata* is an obligate termite predator distributed throughout the New World tropics. Colonies are nomadic and change the location of their nests periodically. One of the most outstanding and conspicuous features of the biology of *P. laevigata* is the organization of their predatory forays in which columns of 500–1700 workers file out of the nest after suitable termite prey has been discovered (Downing, 1978). *Pachycondyla laevigata* preys on a variety of termite species, including *Neocapritermes*, *Microcerotermes*, *Coptotermes*, and *Armitermes* (Wheeler, 1936; Downing, 1978; personal observations). Both termite soldiers and workers are attacked by the raiding ants, which paralyze the prey by stinging, then quickly retrieve the immobilized termites along the reversed raiding route to the nest. Additional details on the biology of *P. laevigata* and related species are provided by Wheeler (1936), Hermann (1968), and Downing (1978).

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During raiding, workers move along a powerful trail pheromone laid down by the leading scout ants. Blum (1966) has identified the hindgut as the source of this recruitment trail pheromone. We have not been able to confirm these findings, and we consequently report here the results of our own experimental analysis of the trail pheromone communication in *P. laevigata*.

METHODS AND MATERIALS

Two colonies of *P. laevigata* were collected on BCI, Panama. Their loosely structured soil or leaf-litter nests were cautiously excavated and transported to our laboratory at Harvard University, Cambridge. In the laboratory colonies were housed in plexiglass nests (30 × 75 cm) with a layer of gypsum covering the bottom. A depression was excavated in one end of the nest and then covered with a red glass plate to provide a dark, moist nest cavity. The nests were connected to one or two arenas, each measuring 140 × 70 cm, in order to observe and record foraging and recruitment behaviors. The ants were fed with termites (*Microcerotermes* and *Reticulitermes flavipes*).

For histological investigation live specimens were fixed in alcoholic Bouin, embedded in methyl methacrylate, and sectioned 8 μm thick with a Jung Tetrander I microtome. The staining was Azan (Heidenhain). The SEM pictures were taken with an AMR 1000 A scanning electron microscope.

RESULTS

A typical case history of the organization of a raid by *P. laevigata* is given in Figure 1. A nest was connected to a large foraging arena, in which a small colony fragment of the termite *Reticulitermes flavipes* had been placed. Scout ants immediately began exploring the new area, and 4 min later the first termites were discovered and instantly paralyzed. When successful scouts returned to the nest, they obviously were laying trails. The gaster was bent down and slightly forward, so that the last tergum, not the abdominal tip, was dragged over the surface (Figure 2). During this procedure the gaster was moved sideways from the right to left side and then in the reversed direction at irregular intervals. Shortly after the first scouts entered the nest the first raiding column emerged from the nest. It was led by one or more trailing ants, presumably the recruiting scout ants, but many of the recruited ants in the column also showed trail-laying behavior.

Downing's (1978) photographs and our own observations confirmed that *Pachycondyla laevigata* exhibits the same characteristic trail laying behavior under natural conditions.

This stereotyped behavioral pattern of trail laying made it seem very

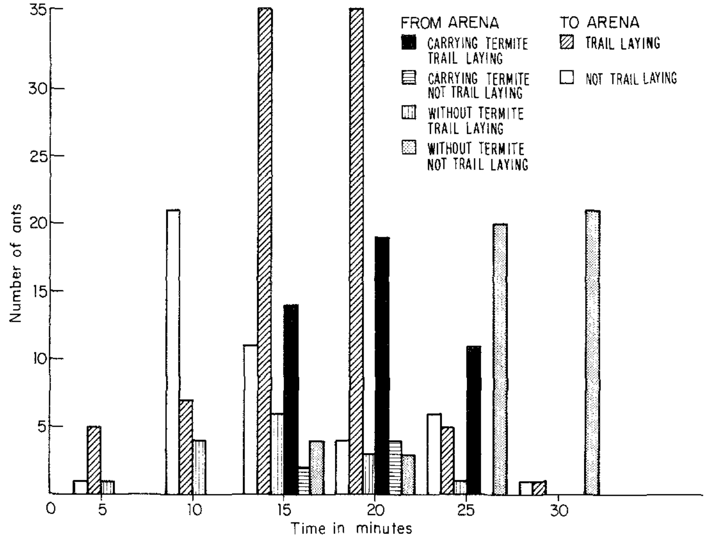

FIG. 1. A case history of the recruitment process after a fragment of a termite colony was discovered by scout ants.

unlikely to us that the trail pheromone is being released from the hindgut. In fact, all tests conducted with artificial trails drawn with hindgut material failed completely; not one *P. laevigata* worker could be induced to follow a hindgut trail. On the other hand, the trail-laying posture strongly suggested that the trail pheromone originates from the pygidial gland, a tergal gland recently described in a variety of ant species belonging to several subfamilies (Hölldobler and Engel, 1978).

Pachycondyla laevigata has a very well-developed pygidial gland, consisting of paired clusters of glandular cells located dorsolaterally in the 6th abdominal segment. Each cell sends a duct through the intersegmental membrane between 6th and 7th terga. The gland is associated with an elaborate cuticular structure on the 7th tergum. The glandular secretion is apparently stored in the many cavities of this structure (Figure 3). When trailing, the ant obviously exposes this structure, which is normally covered by the 6th tergum, and rubs it with its special applicator surface over the ground, and thereby apparently deposits the trail pheromone. The following experiments were designed to test this hypothesis.

We dissected in Ringer solution the following body parts from *P. laevigata* workers: Dufour's gland, poison gland, hindgut, and pygidial gland. The freshly dissected organs of single workers were smeared with the tips of hardwood applicator sticks over a cardboard floor of the arena to create artificial trails of 40 cm in length. The number of workers that followed each

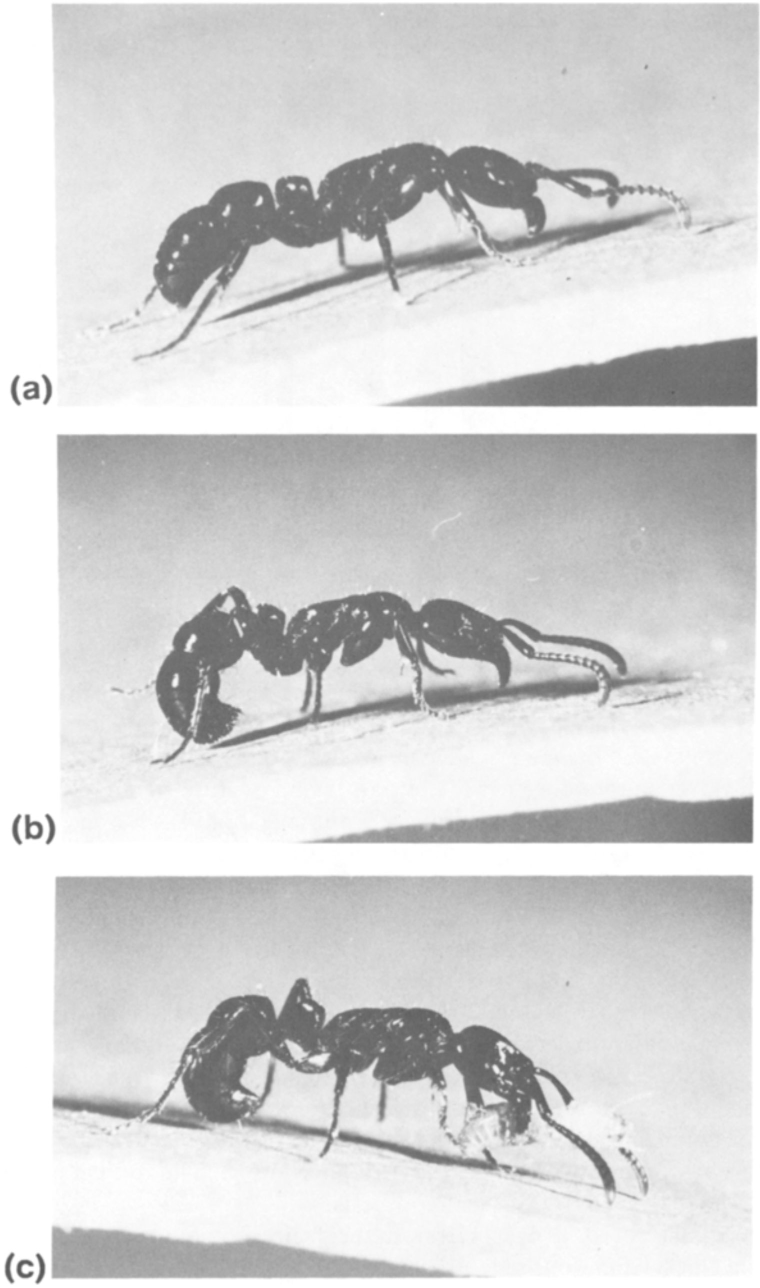

FIG. 2. Trail-laying behavior in *Pachycondyla laevigata*. (a) Normal locomotory behavior. (b) Trail-laying worker, dragging the applicator surface of the pygidial gland over the ground. (c) Worker retrieving termite prey and simultaneously laying trail.

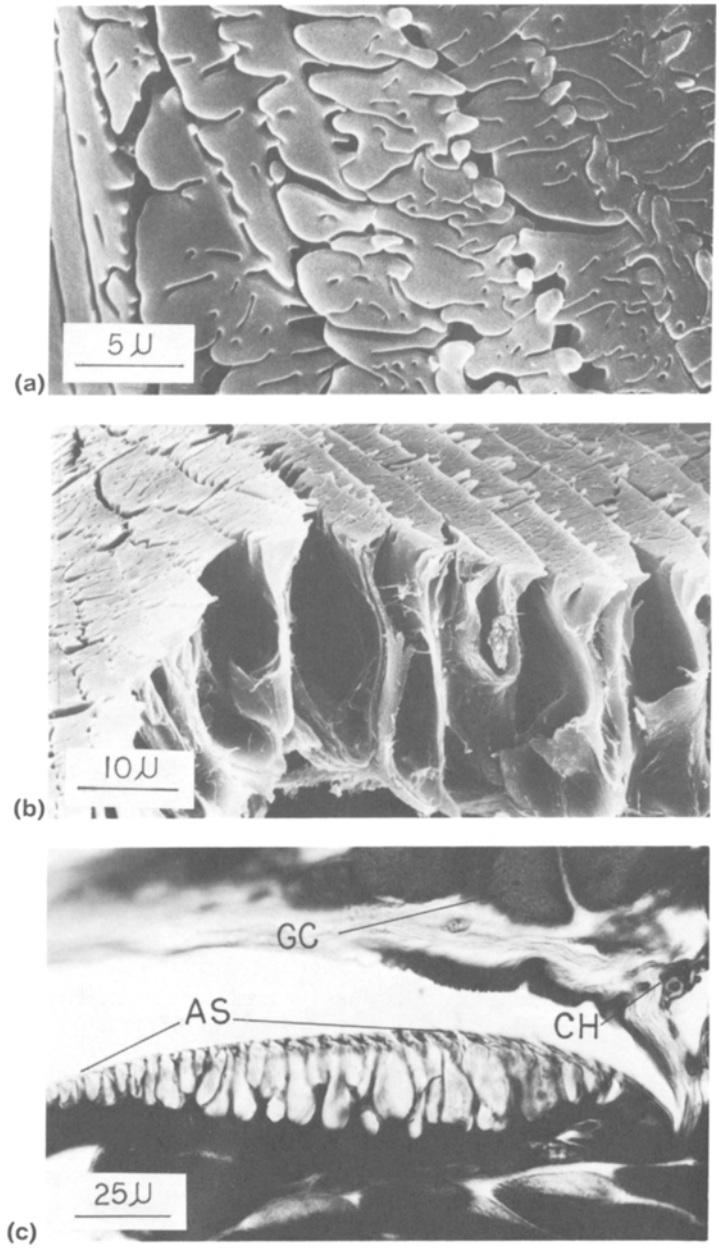

FIG. 3. The pygidial gland of *Pachycondyla laevigata.* (a) SEM photograph of the glandular applicator surface on the 7th tergum of a *P. laevigata* worker. (b) SEM photograph of the glandular applicator surface; the cut open area shows the large cavities associated with the structure. (c) Sagittal section through the pygidial gland, showing the cuticular structure of the applicator surface (AS), glandular cells (GC), and glandular channels (CH) in the intersegmental membrane.

trail during the first 2 min was then recorded. During the test, a test trail and a control trail were offered simultaneously, both starting at the same spot at the nest entrance, but then deviating, so that they were 15 cm apart. The sides of the trails were regularly alternated, and for each test a new cardboard surface was used.

The results presented in Table 1 were quite conclusive. Hindgut contents and Dufour's gland secretion elicited almost no noticeable behavioral response. Poison gland secretion caused an outrush of workers from the nest entrance and many of them displayed aggressively open mandibles and forward pointed stinger. This suggests that the poison gland secretion contains an alarm pheromone. Trail-following behavior, however, was released solely by the secretions from the pygidial gland. Almost all ants leaving the nest followed the pygidial gland trail to its end. When two trails, one drawn with pygidial gland secretion and the other with hindgut contents, were offered simultaneously, only the pygidial gland trail was followed by the ants (Figure 4).

Pygidial gland secretions not only released trail following, they also seemed to stimulate trail-laying behavior in *P. laevigata* workers. We observed that many workers following an artificial trail exhibited the typical trail-laying behavior, thereby apparently reinforcing the chemical trail. These observations led us to the questions: How durable are the pygidial gland trails? Does the reinforcing trail-laying behavior increase with increased evaporation of the trail pheromone?

The persistence of the recruitment trail pheromone was tested by smearing the secretion of one pygidial gland along a 40-cm-long trail on a piece of cardboard and allowing periods of 5, 15, 30, and 35 min to elapse before the cardboard was placed into the arena. To avoid adaptation effects between each test, we waited at least 60 min before a colony was tested again. In addition, the different samples were offered in a randomized fashion.

The results clearly demonstrated that the trail remains effective up to 30 min, although it gradually loses power during this period (Figure 5). On the

TABLE 1. MEAN NUMBER OF ANTS (± STANDARD DEVIATION, $N = 6$) LEAVING THE NEST (A) AND FOLLOWING ARTIFICIAL TRAILS (B)[a]

Pygidial gland		Hindgut		Poison gland		Dufour's gland	
A	B	A	B	A	B	A	B
37.5 ± 6.5	29.2 ± 5.8	3.5 ± 1.0	0	13.8 ± 2.1	0	5.3 ± 0.9	0

[a]Trails were drawn with the secretions of various body parts. Counts were taken during periods of 2 min. As a control, a trail drawn with Ringer solution was offered simultaneously. No ant ever followed the control trail.

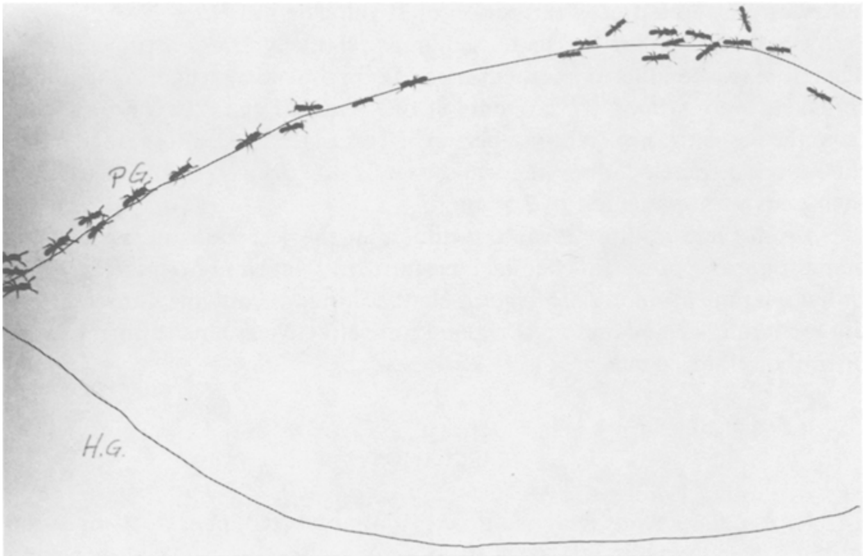

FIG. 4. *Pachycondyla laevigata* workers following an artificial trail. Two trails, one drawn with pygidial gland secretion (PG) and the other with hindgut contents (HG), were offered simultaneously. Only the pygidial gland trail was followed by the ants.

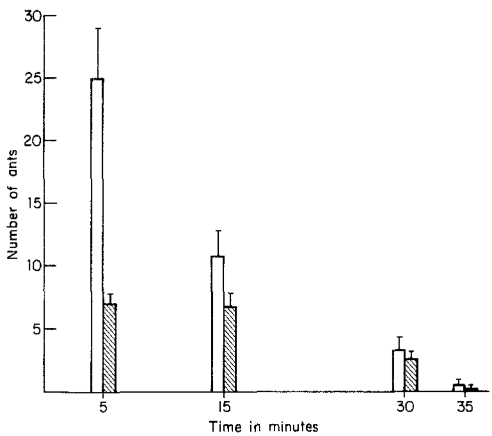

FIG 5. Persistence of the pygidial gland trail pheromone. Trails (40 cm long) were drawn with the secretion of one gland of a *Pachycondyla laevigata* worker. The trails were introduced into the test arena after periods of 5, 15, 30, and 35 min had elapsed. During the following 2 min the number of ants following the trail to its end were counted. The mean and standard deviations of six replications for each period are given.

other hand, the percentage of trail-following ants that showed trail-laying behavior increased during the period of 30 min (Figure 5).

Up to this point we had used only relatively short artificial trails. Therefore, we conducted another series of experiments in which 350-cm-long trails, each containing the contents of one pygidial gland, were drawn out from the nest entrance. Within a period of 2 min 41.3 ± 6.9 ants rushed out of the nest and traveled along the trails, and 32.0 ± 5.3 ants followed them to their ends within a period of 2.5 min ($n = 3$).

From these results we can conclude that the secretions of the pygidial gland serve as a powerful chemical recruitment signal and orientation cue in trail communication in *P. laevigata*. Neither hindgut contents nor secretions of the Dufour's gland and poison gland were effective as a recruitment signal or trail orientation cue.

DISCUSSION

Pachycondyla laevigata conducts well-organized predatory raids on termites. During raiding the workers move in a single file, one closely behind another, along a trail pheromone laid down by leading scout ants. Our laboratory study revealed that a single termite is enough to elicit recruitment behavior in scout workers foraging in the laboratory arena. The scout returns to the nest, laying a trail with pygidial gland secretions. Shortly afterwards a column of nestmates arrives at the area where the termite was discovered. In many ant species the recruitment efforts by individual ants are correlated with the amount of food discovered or with the size of the prey objects that have to be retrieved (Hangartner, 1969; Hölldobler, 1978; Hölldobler et al., 1978). On the other hand, for a termite raiding species it appears reasonable that a single termite should trigger a massive recruitment response, because the presence of a single termite usually indicates the presence of a broken gallery or access to a termite nest.

Although the peculiar trail-laying behavior of *P. laevigata* does not suggest that the sting glands or the hindgut are involved in the discharge of the trail pheromone, Blum (1966) has identified the hindgut as the source of the recruitment trail pheromone in *P. laevigata*. Since Blum was not aware of the existence of the pygidial gland, it is possible that in his experiments the hindgut extracts were contaminated with secretions from the pygidial gland. This would explain why he was able to release trail-following behavior with artificial trails drawn with hindgut contents.

The pygidial gland seems to play a major role in the chemical communication of other ponerine ants. Maschwitz and Schönegge (1977) demonstrated that the pygidial gland secretions of *Leptogenys chinensis* serve as a recruitment trail pheromone in conjunction with poison gland substances. Earlier we analyzed the signals involved in tandem running recruitment in *Pachycondyla*(*Bothroponera*) *tesserinoda* (Hölldobler et al., 1973; Maschwitz et al., 1974). We discovered that the cues responsible for "binding" the follower to the leader ant include both a surface pheromone and mechanical stimuli. Although we were able to extract this surface pheromone, we could not identify its anatomical source; all experiments with secretions from the known exocrine glands had negative results. Following the discovery of the pygidial gland in *Pachycondyla* (Holldobler and Engel, 1978), we began to conduct tandem running experiments with *P. crassa* and *P. harpax*, using dummies contaminated with pygidial gland secretions. Our preliminary results strongly suggest that the pygidial gland is the anatomical source of the tandem running recruitment pheromone in these species.

A quite different function of the pygidial gland has been discovered in the ponerine ant *Rhytidoponera metallica*. Here the wingless virgin females attract males by the release of a pheromone from the pygidial gland (Hölldobler and Haskins, 1977). Since *Rhytidoponera* workers also have well-developed pygidial glands and are attracted to its secretions, it is possible that they might also function in *Rhytidoponera* as a recruitment signal.

With respect to the evolution of chemical communication in different ant subfamilies it is interesting to note the striking analogy of the functional spectrum of the pygidial glands in Ponerinae to that of the poison glands in Myrmicinae. In several species of the myrmicine genera *Leptothorax* and *Harpagoxenus* the poison gland secretions serve as a tandem running recruitment pheromone (Möglich et al., 1974; Möglich, 1979; Buschinger and Winter, 1977). Many other myrmicine species lay chemical recruitment trails with poison gland secretions (for review see Hölldobler, 1978). In a different context, however, the poison gland secretions of some myrmicine ants also function as sex pheromones (Hölldobler, 1971; Buschinger, 1972).

Finally, preliminary experimental evidence suggests that the pygidial and postpygidial glands also play an important role in the chemical trail communication in army ants. Both *Neivamyrmex* and *Eciton* have large pygidial glands with distinct reservoirs. The postpygidial gland is smaller, but still considerably larger than in most of the other investigated species (Hölldobler and Engel, 1978). Our first preliminary tests demonstrated that *Eciton* workers follow artificial trails drawn with the crushed tergal glands. When we simultaneously offered trails drawn with hindgut contents and with secretions of the tergal glands, the latter were significantly preferred during the first minute. Chadab and Rettenmeyer (1975) demonstrated that besides the relatively long-lasting hindgut trail-substance other signals are involved in the organization of mass recruitment in *Eciton*. Similar results were obtained by Topoff and Mirenda (1975). Topoff et al. (1980) recently provided circumstantial evidence that pheromone trails deposited by recruiting *Neivamyrmex* workers are "qualitatively different from the ants' exploratory trail." Our first bioassays of the tergal gland secretions suggest that the pygidial gland produces a recruitment trail pheromone in *Eciton hamatum*; however, the postpygidial gland might also be involved in this chemical communication process. Both glands are anatomically so closely associated that it is almost impossible to dissect them individually without contaminating them with secretion of the other gland. Thus, we have to stress that these experiments with army ants must be considered pilot tests. The preliminary results, however, are striking enough to warrant a more detailed investigation in the future.

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