A new type of exocrine gland and its function in mass recruitment in the ant *Cylindromyrmex whymperi* (Formicidae, Cerapachyinae)

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Abstract:

Workers of the ant *Cylindromyrmex whymperi* display mass trail recruitment. Bioassays show that the trail pheromone originates from a unique gland between abdominal sternites 6 and 7. The gland has a hitherto unknown structural organization. Upon leaving the secretory cell, the duct cell widens to form a sclerotized pear-shaped reservoir chamber, lined with multiple duct cells. Each duct thus forms a miniature reservoir for the secretions of each single secretory cell, a novel structural arrangement in exocrine glands of social Hymenoptera.

Article:

Introduction

Communication among colony members is one of the most important traits in social insects. In ants, chemical communication through the emission of pheromones plays a major role in all aspects of colony life. Many species of ants use trail pheromones to recruit nestmates to new nests or food sources. The active substances can originate from a variety of exocrine glands, located either in the abdomen or on the legs (Billen and Morgan *1998*). According to their structural organization, two major gland types can be distinguished among the social insects: epithelial glands and glands formed by bicellular units. The latter comprise one secretory cell and one duct cell, with the duct cell invariably containing a narrow cuticular channel that transports the secretory products from the secretory cell to a common reservoir for temporary storage or directly to the outside (Billen and Morgan *1998*).

Little is known about the biology of ants of the neotropical cerapachyine genus *Cylindromyrmex*. These rare ants (Delabie and Reis *2000*) generally nest in cavities in decaying wood or live stems (Brown *1975*; Andrade *1998*). Workers are often found in termite galleries, and at least one species, *Cylindromyrmex striatus*, is a known specialist termite predator (Overal and Bandeira *1985*). Since termites are social, they represent a prime example of a clumped resource. However, the resource is probably short-lived, since termites cover their foraging paths with soil galleries, and any gaps are quickly repaired. Additionally, termite galleries are well-defended by

termite workers and soldiers, so a solitary ant predator would quickly be outnumbered (Stuart *1969*). Ants preying on other social insects often possess a mass recruitment system, such as chemical trails to the food source (Wilson *1958*; Hölldobler et al. *1994*). Therefore, we expect a form of recruitment in *Cylindromyrmex*. We discovered the presence of massive trail recruitment in *Cylindromyrmex whymperi*, and elucidated the glandular origin and the putative chemical nature of its secretions.

Materials and methods

About 70 workers and one dealate queen were collected from a raiding column with about 100 workers on the forest floor at Rincon de la Vieja NP, Acguanacaste, Costa Rica, and kept in a plastic nestbox (7x7 cm) with a moist plaster floor, connected to a foraging arena (40x50 cm). Food items (sugar water and freshly killed crickets, cockroaches and termites) were placed in the arena, about 40 cm from the nest entrance. Preliminary bioassay trail tests indicated that workers readily follow trails made with abdomen extracts. To test for the exact origin of trail substances, we killed ants by freezing and several abdominal glands were dissected. We extracted possible trail substances by squashing two glands, originating from two workers, in 20 µl of hexane, after which we laid 10 cm-long artificial trails on plain white paper. We always waited 2 min for the solvent to evaporate before allowing ants on the trail. Each such trail was presented only once at the nest entrance, and the response of the first ant to encounter such a trail was recorded. An ant was recorded as following the trail when it followed it for more than 5 cm. Replicates were therefore the trails of gland extracts of different pairs of workers. Once we had determined the sternal gland to be the origin of the trail pheromone, we presented the extracts of the sternal gland and different glands in a pairwise comparison (two trails angling away from each other at 45°; the side of the sternal gland extract was changed in each trial) to avoid possible effects of contamination during dissection. Ants were allowed on the trail by placing the nestbox with the entrance in front of the start of the V-shaped trails. Again, each double trail was presented to the ants only once, and the response of the first worker was recorded and analysed with a one-tailed binomial test.

The posterior part of the abdomen as well as dissected sternites of workers for morphological examination were fixed in 2% glutaraldehyde in sodium cacodylate buffer. After post-fixation in 2% osmium tetroxide and dehydration in a graded acetone series, tissues were embedded in araldite. Semithin sections for light microscopy were stained with methylene blue and thionine. Thin sections for electron microscopy were viewed in a Zeiss EM900 microscope. Tissue arrangement was examined in a Philips XL30 ESEM microscope on air-dried material as well as on material prepared overnight in a 5% KOH solution to macerate soft tissues.

Sternal glands were dissected and sealed in a soft glass capillary, as described by Morgan (*1990*) and analysed by gas chromatography using the solid-sampling method described by Morgan and Wadhams (*1972*). Chromatography was carried out with a Hewlett-Packard 5890 gas chromatograph directly coupled to a 5970B Mass Selective Detector and HP 5970C Chemstation.

Results

We observed mass recruitment once in the field. A column of *C. whymperi* was moving along a distinct "street", although it was unclear whether this served for foraging or nest relocation. In

the laboratory, however, when dead termites were discovered by a single *Cylindromyrmex* worker, it returned to the nest and recruited nestmates (n=3). Within 10 min, all workers had left the nestbox and were investigating the area around the termites. However, not a single dead termite was taken or carried towards the nest in the next 30 min. Only when we placed dead termites directly into the nest were they quickly eaten. We never observed recruitment to crickets, cockroaches or sugar water (n=10), nor were these eaten when placed in the nestbox (n=5).

Workers leaving the nestbox were frequently followed in their exact paths by subsequent workers. All workers continuously drag their abdomen over the substrate as we could see when they walked over a glass surface stained with candle smoke. All tested workers clearly followed artificial trails made from extracts of the conspicuous sternal gland between sternites 6 and 7 (n=5 trails). Workers never followed extracts of the venom gland (n=5), the tergites (n=5), the hindgut (n=5) or pure hexane controls (n=10). However, extracts from the Dufour gland and of sternite 7 without the sternal gland attached elicited short (i.e. less then 5 cm) following responses in 1 and 2 out of 5 trials, respectively.

To avoid effects of possible contamination during dissection, we presented the gland extracts in a choice experiment. The sternal gland extract was invariably chosen over all other extracts by foragers leaving the nest as well as over the hexane control (binomial test: 6 out of 6 choice trails, P<0.015). In detail, ants always preferred sternal gland extract over both Dufour gland extract (binomial test: 6 out of 6, P<0.015) and sternite 7 extract (sternal gland removed, 5 out of 5, P<0.031). Unfortunately, we could not make enough replicates with venom gland, tergites and hindgut extracts to obtain statistical significance (each 4 out of 4, P=0.062), but we feel confident that these glands can be omitted as a possible source of the trail pheromone since they were never followed in the single trail trials.

The sternal gland appears as a large paired structure at the articulation between the 6th and 7th abdominal sternites. The left and right parts of the gland closely touch each other, which results in their appearance as one large glandular cluster (Fig. 1a). The gland contains numerous rounded secretory cells with a diameter of around 30-35 µm. Each cell has a rounded nucleus and an end apparatus that continues in a sclerotized duct as is usual in class-3 gland cells (Noirot and Quennedey *1974*). The numerous duct cells open to the outside through the intersegmental membrane between the 6th and 7th sternite (Fig. 1b, c). The most peculiar feature of the gland is that each duct, upon leaving the secretory cell, abruptly widens to form a sclerotized pear-shaped reservoir chamber with a diameter of about 20 µm and a length of 30-40 µm (Fig. 1d, e). Each secretory cell thus has its own individual reservoir. The epithelial wall of the reservoir chambers has a thickness of roughly 2 µm, of which 0.5 µm is occupied by the apical cuticular lining. It appears that more than one cell is involved in the formation of the reservoir chamber/duct complex. No muscles are associated with these ducts. Ultrastructural examination of the secretory cells revealed the presence of a vesicular cytoplasm due to the development of smooth endoplasmic reticulum.

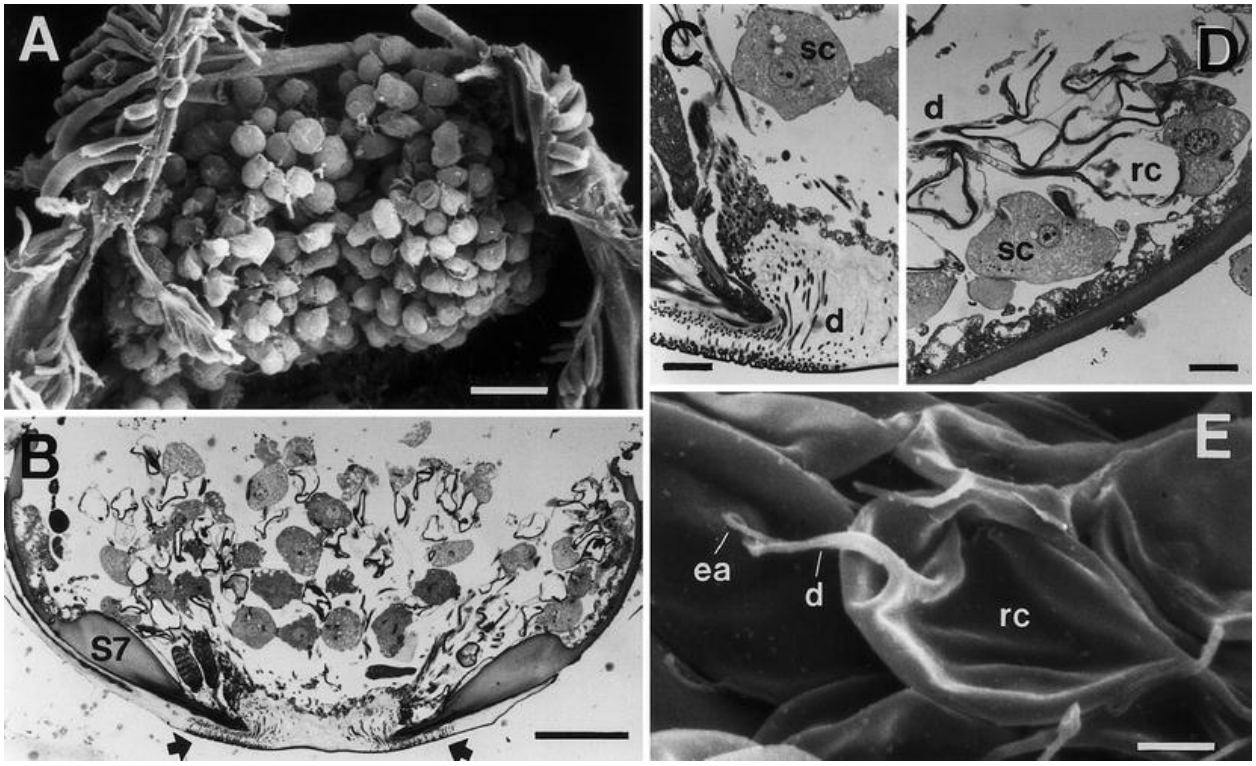

Fig. 1. a Scanning micrograph of anterior portion of 7th sternite showing the sternal gland (*scale bar* 100 µm). **b** Semithin cross section through anterior part of 7th sternite showing secretory cells, reservoir chambers and ducts opening through intersegmental membrane (*arrows*), *scale bar* 100 µm. **c** Detail of ducts opening through intersegmental membrane between 6th and 7th sternite (*scale bar* 20 µm). **d** Detail showing arrangement of secretory cells, reservoir chambers and ducts (*scale bar* 20 µm). **e** Scanning micrograph of cuticular part of lining reservoir chambers and end apparatus after maceration of soft material with KOH treatment (*scale bar* 10 µm). *d* Ducts, *ea* end apparatus, *rc* reservoir chamber, *sc* secretory cells, *S7* 7th sternite

Gas chromatographic analysis of duplicate samples of the sternal gland secretion of *C. whymperi* showed very close agreement. Seven compounds (*A* to *G*) could be quantified in the secretion. In order of elution, the percentage composition was: *A*, 2.9%; *B*, 1.4%; *C*, 73.9%; *D*, 7.0%; *E*, 0.7%; *F*, 1.1%; *G*, 12.9%. Compound *A* was identified as 2,7-dimethyl-7-octenoic acid. Compounds *B* and *C* were clearly a pair of isomers, molecular mass 196. Compound *C*, by far the major constituent, had a mass spectrum showing strongest ions at m/z 57, 43, 85 and 82, in order of decreasing intensity, and a most probable molecular formula of $C_{12}H_{20}O_2$ Compounds *D* and *G* appear to belong to the same group as *B* and *C*, but are of higher mass and again a pair of isomers (M^+ 210). Compounds *E* and *F* were too weak to identify. Unfortunately, we could not further identify or test any of these compounds in bioassays to determine which are active in trail recruitment.

Discussion

Our experimental observations clearly revealed that *C. whymperi* workers use trail pheromones to recruit large numbers of nestmates. Indeed, the ants were collected while walking in a distinct column. This suggests raiding as a predominant foraging style, which makes sense in a genus thought to be foraging mainly on termites (Brown *1975*; Overal and Bandeira *1985*). Termites

are an abundant, but ephemeral resource, and as much prey as possible must be collected before the termite gallery is sealed or defended (Hölldobler and Wilson *1990*). During our observations, *C. whymperi* workers fed only on termites, although we cannot exclude the possibility that in nature other prey is hunted for.

Unlike true army ants that always forage in groups (Gotwald *1995*), *C. whymperi* shows group predation induced by a single returning forager. Similar mass recruitment is found in the related genus *Cerapachys* (Hölldobler *1982*). Legionary behaviour has two fundamental components: migration and group predation (Wilson *1958*). Our results suggest that a certain degree of legionary behaviour is common in the entire cerapachyine tribe, supporting morphological and molecular evidence (Baroni Urbani et al. *1992*; Sullender *1998*) that they are a sister group of the true army ants.

We found that *C. whymperi* workers readily follow artificial trails made of sternal gland extracts. Some minor trail-following response was elicited by Dufour gland and pure sternite 7 (without the sternal gland) extracts. This mild response might be due to contamination during dissection, since these structures lie in close contact with the sternal gland. The choice experiment confirmed that the sternal gland is the source of the trail pheromone. Although only measured on a single colony, we feel confident that the sternal gland secretes the trail substance in *C. whymperi*, since the use of trail pheromones and their glandular sources are species-specific traits (Billen and Morgan *1998*). In related *Cerapachys*, an orientating component of the trail pheromone originates from the poison gland, while some additional stimulatory effects are released from the pygidial gland (Hölldobler *1982*). The source of the trail substance in the true army ants varies equally (postpygidial gland in Aenictinae, venom gland in Dorylinae, and an epithelium internally lining the 7th sternite in Ecitoninae; Billen and Gobin *1996*). The ecitonine epithelium is functionally similar to the sternal gland in *C. whymperi*, but structurally very different. In the taxonomically more remote ponerine ant, *Onychomyrmex*, an unpaired sternal gland between the 5th and 6th sternites - without a reservoir - secretes the trail pheromone (Hölldobler et al. *1982*). The sole fully identified chemical component of the sternal gland secretion bears no resemblance to known pheromones (Billen and Morgan *1998*) while, unfortunately, the identification of the other compounds is too tentative to allow structural comparison.

Although sternal glands are known in many ants (Hölldobler and Engel *1978*), not all have reservoirs. A sternal gland with reservoir between sternites 6 and 7 is known in only three other ponerine genera: *Leptogenys*, *Harpegnathos* (Hölldobler and Engel *1978*; Jessen et al. *1979*) and *Myopias* (J. Billen and F. Ito, unpublished). The general pattern in such glands is that several secretory cells discharge their secretion through ducts into a common reservoir. This reservoir is an invagination of the intersegmental membrane and consists of cuticle lined with flattened epidermal cells. The sternal gland in *C. whymperi* is very unusual, however, in that each secretory cell has its own reservoir chamber (Fig. 2). The secretory cells contain large amounts of vesicular endoplasmatic reticulum, which is in accordance with the secretion of pheromones. The reservoir chambers appear to be more than a mere widening of the proximal parts of the duct cells, as more than one cell occurs in the epithelial lining of each reservoir chamber and duct. This unique type of gland organization has so far never been found among ants, as ducts in species of this family are always formed by one single duct cell. In other insect groups more cells

can be found, in some cases the canal may even have a small inflated ampulla or bulb (Quennedey *1998*), but the occurrence of a conspicuous reservoir chamber such as we found in *Cylindromyrmex* has not been found before. There are no muscles associated with the reservoirs, suggesting that an active control mechanism for release of secretion is absent. The size of each reservoir would, however, allow temporary storage of the trail pheromone through capillary forces. Dragging the glandular pores over a substrate should alter these forces, thus releasing the pheromone. This agrees with the observation of continuous dragging of the abdomen during trail-laying in this species.

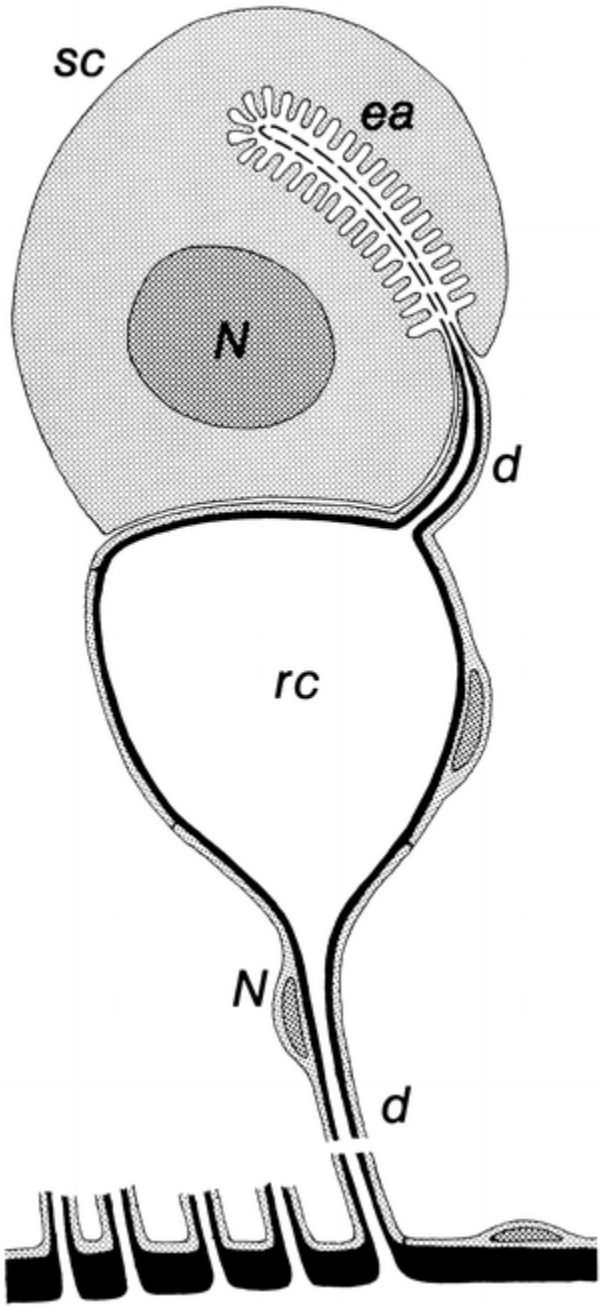

Fig. 2. Schematic survey showing organization of glandular unit of sternal gland. *d* Duct, *ea* end apparatus, *N* nucleus, *rc* reservoir chamber, *sc* secretory cell

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References

Andrade ML de (1998) Fossil and extant species of *Cylindromyrmex* (Hymenoptera: Formicidae). Rev Suisse Zool 105:581-664

Baroni Urbani C, Bolton B, Ward PS (1992) The internal phylogeny of ants (Hymenoptera: Formicidae) Syst Ecol 17:301-329

Billen J, Gobin B (1996) Trail following in army ants (Hymenoptera, Formicidae). Neth J Zool 46:272-280

Billen J, Morgan ED (1998) Pheromone communication in social insects - sources and secretions. In: Vander Meer RK, Breed MD, Espelie KE, Winston ML (eds) Pheromone communication in social insects: ants, wasps, bees, and termites. Westview, Boulder, Colo., pp 3-33

Brown WL Jr (1975) Contributions toward a reclassification of the Formicidae. V. Ponerinae, tribes Platythyreini, Cerapachyini, Cylindromyrmecini, Acanthostichini, and Aenictogitini. Search Agric 5:1-115

Delabie JHC, Reis YT (2000) Sympatry and mating flight synchrony of three species of *Cylindromyrmex* (Hymenoptera: Formicidae) in southern Bahia, Brazil, and the importance of Malaise trap for rare ants inventory. Rev Bras Entomol 44:109-110

Gotwald WH (1995) Army ants. Comstock, Ithaca, N.Y.

Hölldobler B (1982) Communication, raiding behavior and prey storage in *Cerapachys* (Hymenoptera: Formicidae). Psyche 89:3-23

Hölldobler B, Engel H (1978) Tergal and sternal glands in ants. Psyche 85:285-330

Hölldobler B, Wilson EO (1990) The ants. Belknap Press/Harvard University Press, Cambridge, Mass.

Hölldobler B, Engel H, Taylor RW (1982) A new sternal gland in ants and its function in chemical communication. Naturwissenschaften 69:90-91

Hölldobler B, Braun U, Gronenberg W, Kirchner WH, Peeters C (1994) Trail communication in the ant *Megaponera foetens* (Fabr.) (Formicidae, Ponerinae). J Insect Physiol 40:585-593

Jessen K, Maschwitz U, Hahn M (1979) Neue Abdominaldrüsen bei Ameisen. I. Ponerini (Formicidae, Ponerinae). Zoomorphologie 94:49-66

Morgan ED (1990) Insect trail pheromones: a perspective of progress. In: McCaffery AR, Wilson ID (eds) Chromatography and isolation of insect hormones and pheromones. Plenum, New York, pp 259-270

Morgan ED, Wadhams LJ (1972) Gas chromatography of volatile compounds in small samples of biological materials. J Chromatogr Sci 10:528-529

Noirot C, Quennedey A (1974) Fine structure of insect epidermal glands. Annu Rev Entomol 19:61-80

Overal WL, Bandeira AG (1985) Nota sobre hábitos de *Cylindromyrmex striatus* Mayr, 1870, na Amazônia (Formicidae, Ponerinae). Rev Bras Entomol 29:521-522

Quennedey A (1998) Insect epidermal gland cells: ultrastructure and morphogenesis. In: Harrison FW (ed) Microscopic anatomy of invertebrates, vol 11A. Wiley-Liss, New York, pp 177-207
Stuart AM (1969) Social behaviour and communication. In: Krishna K, Weesner FM (eds) Biology of termites, vol 1. Academic Press, New York, pp 193-232
Sullender B (1998) A molecular phylogeny for the Formicidae. Version updated 14 April 1998. http://www.ruf.rice.edu/~bws/molecular_phylogeny.html (accessed 3 August 2001)
Wilson EO (1958) The beginnings of nomadic and group-predatory behaviour in the ponerine ants. Evolution 12:24-36