Netherlands Journal of Zoology
46 (3-4): 272-280 (1996)

TRAIL FOLLOWING IN ARMY ANTS (HYMENOPTERA, FORMICIDAE)

by

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ABSTRACT

Army ants form impressive columns that rely on the following of trail pheromones. We could study trail following in three army ant species and locate the glandular source of the respective active pheromones. *Eciton burchelli* (Ecitoninae) produces very long lasting trails from a specialized glandular epithelium associated with the 7th abdominal sternite, while in *Dorylus molestus* (Dorylini) short-lasting trails are elaborated from the venom gland. In *Aenictus* sp. near *laeviceps* (Aenictini), the secretion of the well developed postpygidial glands forms the long-lasting trail pheromone, which in this species could be chemically identified as a two-component system with methyl nicotinate (1% of gland secretion) initiating the ants to follow a trail, and methyl anthranilate (99%) causing the real trail following.

KEY WORDS: trail following, *Aenictus* sp., *Dorylus molestus*, *Eciton burchelli*, postpygidial gland, venom gland, sternal gland.

INTRODUCTION

Army ants represent one of the most spectacular features of wildlife in the tropics (GOTWALD, 1995). The New World army ants are classified in the Ecitoninae, the Old World army ants in the Dorylinae, which include the mainly African Dorylini and the mainly Australasian Aenictini (HÖLLDOBLER & WILSON, 1990). According to BOLTON (1990) and GOTWALD (1995), the Aenictinae are to be elevated to the subfamily status. Characteristic for all army ants is their nomadic behaviour and group predation (WILSON, 1958). With high speed, the blind workers follow a strong chemical trail that ensures raid cohesion (Dorylini and Aenictini are entirely blind, Ecitoninae have very much reduced eyes). Distinction is made between the exploratory trail, which orientates the ants along the trail on their way during raids, and the recruitment trail, which is laid down by workers that have located prey and convey this information to their nestmates (TOPOFF *et al.*, 1980). Although the existence of chemical trails has been the subject of several pioneer papers on ecitonine army ants (TOPOFF, 1984; TOPOFF & MIRENDA, 1975; TOPOFF & LAWSON, 1979; TOPOFF *et al.*, 1972, 1973, 1980; TORGERSON & AKRE, 1970; WATKINS *et al.*, 1967), almost no information was known on the origin of the pheromones involved. The only data on this subject deal with the genus *Eciton*, where the hindgut was reported as the origin of the trail substances (BLUM & PORTOCARRERO, 1964).

BIOASSAY EXPERIMENTS

The experimental determination of the glandular origin of trail pheromones involves bioassay studies in which extracts of the potentially active glands are presented to the ants. The behavioural response of the ants can be quantified through circular trail tests, where the distance followed is equivalent to trail activity (PASTEELS & VERHAEGHE, 1974), or can be qualitatively investigated in Y-tests, where the preference for a particular substance or mixture of compounds can be evaluated (for instance BILLEN *et al.*, 1992). A peculiar problem for behavioural laboratory research on army ants is the difficulty to keep them in captivity, as well as their extremely excited behaviour when being handled. From our experience, we felt army ants were over-excited the first few days after being collected, and could only be studied the following days. When in captivity for more than approximately a week, the ants become less responsive, after which they soon die.

For our work, we collected army ant workers from their natural raiding column, and immediately brought them to the laboratory for trail following experiments. The species we had available were *Eciton burchelli* (Westwood) from Manaus, Brasil, *Dorylus* (*Anomma*) *molestus* Gerstaecker and *Aenictus rotundatus* Mayr from Nairobi, Kenya, while workers of another *Aenictus*-species were collected in Hong Kong, and appear to be taxonomically near to *A. laeviceps*. Extracts were made by crushing dissected hindguts, venom glands, Dufour glands, postpygidial glands, sternal glands or hindlegs in hexane, and applied along the circumference of a circle with a diameter of 10 cm on a sheet of white typing paper. Hexane was used as a control. After evaporation of the hexane, ants were introduced onto the test paper for examination of their behaviour (PASTEELS & VERHAEGHE, 1974). Using this bioassay, we could recently determine the glandular origin of the trail substances in *Eciton burchelli* (BILLEN, 1992) and an *Aenictus* species (OLDHAM *et al.*, 1994a), and were also able to demonstrate the use of trail pheromones and localize their glandular source for *Dorylus molestus*.

RESULTS

Eciton burchelli

Extracts of the various tissues tested did not elicit trail following, except for the 7th abdominal sternite, of which an extract caused extremely intensive and long-lasting following activity (BILLEN, 1992). This sternite is characterized by the presence of a very well developed glandular epithelium underneath the cloacal side. An extract of this tissue elicits trail following at a speed similar to that observed in the field, with a higher speed for soldiers than for medium-sized workers (Fig. 1). Literature data on the running speed of this species in laboratory experiments reveal higher speeds for mature adult workers compared with callows, which is probably due to callow workers being more sensitive to tactile stimuli inside the bivouac (TOPOFF *et al.*, 1972). On the other hand, intermediate sized workers appear to follow trails at higher speed than do majors (TOPOFF *et al.*, 1973), which the authors explain by a behavioural difference of the majors that are more responsive to alarm rather than to trail pheromones.

Dorylus molestus

Trail following experiments using glandular extracts of *Dorylus* (*Anomma*) *molestus* gave a very active result only when venom gland extract was

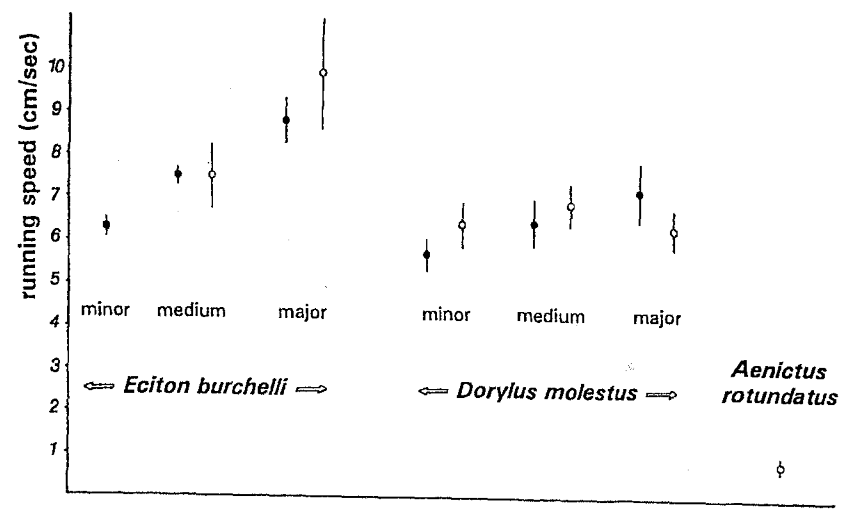

Fig. 1. Running speed measurements (mean and standard deviation) of minor, medium and major workers (soldiers) of *Eciton burchelli* and *Dorylus molestus*, and of the smaller monomorphic workers of *Aenictus rotundatus* (• = natural raiding trail in the field, o = following gland extract in the laboratory).

presented to the ants. The activity of the trail, however, did not last very long, and more or less disappeared after more than 45 minutes. A fresh venom gland extract was followed at a speed more or less comparable to that of trail following *Eciton*, without significant differences between minor, medium and major workers (Fig. 1). Field measurements of the running speed of this species (H. COENE, pers. comm.) showed that medium-sized workers run at a pace intermediate between that of the faster majors and the slower minors (Fig. 1).

Aenictus sp. near *laeviceps* and *A. rotundatus*

The long-lasting trail pheromone of the Asian *Aenictus* sp. near *laeviceps* is elaborated in the very well developed postpygidial glands, of which an extract induces very conspicuous trail-following. Chemical analysis of this gland revealed the presence of two compounds, with methyl anthranilate representing 99% of the contents and methyl nicotinate as the minor compound (OLDHAM *et al.*, 1994a). Behavioural experiments nicely showed that the nicotinate acts as a primer pheromone that is needed to stimulate the ants to follow the anthranilate that is the orientating substance.

Although the postpygidial glands are equally well developed in the African species *Aenictus rotundatus* and equally contain methyl anthranilate, their extract appears to be inactive in this species. Trail following can be obtained, however, with extracts of the venom gland and the seventh abdominal sternite (B. GOBIN, unpubl. obs.). Running speed measurements in the laboratory for this species gave much lower values when compared with the larger *Dorylus* and *Eciton* (Fig. 1). Even minor workers of the latter species, that have approximately the size of *Aenictus*, still run much faster than *Aenictus*.

The study of army ant behaviour in general, and of trail following in particular, is very difficult because of the extremely pronounced interactions between individuals (TOPOFF & LAWSON, 1979). Workers appear more reluctant to follow trails when devoid of contact with nestmates. In no trail following ants is tactile contact as intense as it is in the army ants, where hundreds of individuals are rushing along the trail. If they are allowed these tactile contacts with fellow workers during experiments, however, they become extremely active trail followers. When presented with extracts of the appropriate glands, the ants will run along the trail with a speed similar to that observed in the field on their natural raids (Fig. 1). Due to their enormous colonies and peculiar raids for food, detailed army ant behaviour can hardly be studied in laboratory conditions, except for species with reasonably small colonies such as *Neivamyrmex nigrescens* (TOPOFF, 1984). In these studies, clear distinction could be made between exploration

or foraging pheromones and recruitment substances, the latter being laid by workers after contact with prey. Laboratory experiments on this model species by TOPOFF and co-workers (1980) indicated that both substances are qualitatively different, although no information is available on the glandular origin of the chemicals involved.

DISCUSSION

Our data confirm the use of trail pheromones in army ants, and for the first time we could determine the glandular source from which the active substances originate in the species of the three main army ant groups investigated. Although the glands involved are located in the posterior part of the abdomen, which is the most common site for glands producing trail substances, they are very different in these species (Fig. 2). The most common exocrine glands known to produce trail pheromones in ants in general are the venom gland and Dufour's gland (ATTYGALLE & MORGAN, 1985), which are present in the workers and queens of every ant species and that both discharge their respective secretions through the sting (BILLEN, 1987). For army ants, however, we found the venom gland to be the source of the trail substances only in *Dorylus molestus* with also some activity released in *Aenictus rotundatus*.

Among the other abdominal glands involved in the production of trail pheromones are a number of sternal glands, that all have an obvious position for deposition of the pheromonal substances when the abdomen touches the substrate (the Pavan's gland in the Dolichoderinae, WILSON & PAVAN,

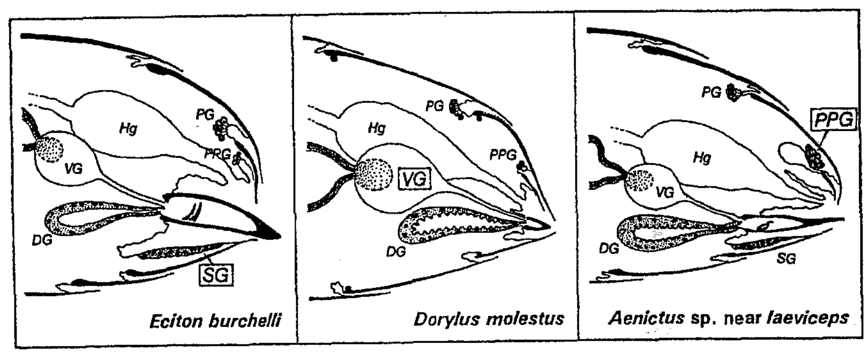

Fig. 2. Schematical representation of the posterior part of the abdomen in *Eciton*, *Dorylus* and *Aenictus*, showing the existing exocrine glands with indication of the glands producing the trail pheromones (DG: Dufour's gland, Hg: hindgut, VG: venom gland, PG: pygidial gland, PPG: postpygidial gland, SG: epithelial gland 7th sternite).

1959; the unpaired gland underneath the 5th abdominal sternite in *Onychomyrmex*, HÖLLDOBLER *et al.*, 1982). *Eciton* and *Aenictus* are characterized by a conspicuous glandular epithelium underneath the cloacal side of the 7th abdominal sternite, whereas this gland does not occur in *Dorylus* (JESSEN, 1987). In *Aenictus rotundatus*, this gland shows some trail following activity, while it clearly is the source of the trail pheromone of *Eciton burchelli* (BILLEN, 1992). The old literature report by BLUM & PORTOCARRERO (1964) indicating the hindgut as the source of the trail substance probably is based on contamination of hindgut tissue with the, at that time unknown, sternal epithelium.

The role of tergal glands in the elaboration of trail pheromones seems rather unusual. Only the pygidial gland in the dolichoderine *Tapinoma simrothi* (SIMON & HEFETZ, 1991) and the ponerine *Pachycondyla laevigata* (HÖLLDOBLER & TRANIELLO, 1980) have been reported to elaborate orientation trail substances, which in the latter species are deposited by a downward bent gaster thus bringing the gland opening in contact with the substrate. HÖLLDOBLER & ENGEL (1978) mention that crushed pygidial glands cause trail following in *Eciton hamatum*, although they stress these tests are to be considered as pilot tests only. Postpygidial glands are present in all three army ant groups, but are especially well developed in the genus *Aenictus*. The opening of the gland's reservoir very close to the abdominal tip makes it a perfect candidate for the source of trail substances (Fig. 2), as could be demonstrated for the Asian *Aenictus* sp. near *laeviceps* (OLDHAM *et al.*, 1994a). On the other hand, the African *Aenictus rotundatus*, in spite of an equally well developed postpygidial gland and similar chemical contents, appears to follow chemical trails originating from the venom gland and the epithelial gland of the 7th sternite (B. GOBIN, unpubl. obs.).

The various glandular sources involved in the production of trail pheromones in army ants reflect the complex phylogenetic position of these ants. Although originally put all together within the single subfamily Dorylinae, the army ants generally are classified into the New World Ecitoninae and the Old World Dorylinae (HÖLLDOBLER & WILSON, 1990). The two doryline genera *Dorylus* and *Aenictus*, however, display a number of common characters but equally show several substantial differences (Fig. 3). These have led to the hypothesis of a triphyletic origin of the army ants (GOTWALD, 1979), which has resulted in the establishment of the Aenictinae as a new subfamily, next to the Dorylinae and Ecitoninae (BOLTON, 1990; GOTWALD, 1995). We want to emphasize that our data on trail following refer to one species per group only and therefore may not be generalized. The situation in *Aenictus* shows that different glands can be involved in trail following in different species of the same genus even when they have the same exocrine glands with a similar chemical composition. This first determination of the

	Dorylini	Aenictini	Ecitoninae
			
distribution	mainly Africa	mainly Australasia	New World
worker eyes	blind	blind	vestigial
petiole	single	double	double
sting	reduced	functional	functional
gland sternite 7	no	yes	yes
Dufour's gland	crenellate tricosene, tricosane	crenellate geranylgeraniol	smooth geranyllinalool
trail pheromone	venom gland *(Dorylus molestus)*	postpygidial gland *(Aenictus* sp.)	gland sternite 7 *(Eciton burchelli)*

Fig. 3. Survey of the main characteristics of the three main army ant groups. The Aenictini share characters both with the Ecitoninae and the Dorylini, which illustrates their questionable phylogenetic position. Characters dealing with the Dufour gland include both the morphological appearance of the gland's epithelium (BILLEN, 1985; BILLEN & GOTWALD, 1988) and the chemical composition of its secretion (BAGNERES *et al.*, 1991; KEEGANS *et al.*, 1993; OLDHAM *et al.*, 1994b).

glandular origin of trail substances in the three army ant groups hopefully forms the basis for further work on the communication system that regulates their raiding behaviour.

ACKNOWLEDGEMENTS

We are very grateful to Dr. M. Crosland, Dr. A. Harada, Dr. E. Martens, H. Coene, J. Stallaert and T. Wenseleers for their help in collecting army ants, to Prof. W. H. Gotwald, Jr. and Prof. J. van Boven for taxonomic advice, and to E. Jacques for help in trail tests on *Dorylus*. BG acknowledges the support through a specialization grant of the Flemish Institute for promotion of scientific research (IWT). This research was supported through grant N° G.0254.96 from the Belgian National Fund for Scientific Research.

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