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*Museum of Comparative Zoology Laboratories, Harvard University, Cambridge*

# Recruitment and Other Communication Behavior in the Ponerine Ant *Ectatomma ruidum*

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*With 5 figures*

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## Abstract

The ponerine ant *Ectatomma ruidum*, though previously reported to possess only rudimentary recruitment ability, was found to lay chemical trails for mass recruitment to rich or difficult food sources. The pheromone originates from the Dufour's gland, a new source of trail pheromones in the primitive ant subfamily Ponerinae. During nest emigrations, *E. ruidum* practices stereotyped social carrying in the myrmicine mode. The discovery of this form of social carrying and of a recruitment pheromone in the Dufour's gland secretions support the hypothesis that the subfamily Myrmicinae is derived from an ectatommine ancestor. Other communication behaviors exhibited by *E. ruidum* include exchange of liquid food carried between the mandibles, chemical alarm communication, nest entrance marking, and an additional social carrying posture previously unknown in ants.

## Introduction

*Ectatomma ruidum* is a moderately large (8—10 mm long), ground-nesting ant common in forests, savannahs, and cultivated areas from central Mexico to northern Brasil (KUGLER & BROWN 1982). Although many reports have stressed its local abundance, and WEBER (1946) pointed out its potential ecological and economic importance, it has received surprisingly little attention from behavioral ecologists (LEVINGS & FRANKS 1982; KUGLER & BROWN 1982). Previous studies of *E. ruidum* have indicated a largely solitary hunting strategy, with evidence of search site fidelity by individual workers (LACHAUD et al. 1984). In addition, LACHAUD (1985) described a primitive form of recruitment, in which the return to the nest of a scout which had encountered a rich tuna bait was followed by an outpouring of ants in the general direction of the bait. LACHAUD did not observe the laying or following of a recruitment trail, but hypothesized that the emerging ants had been excited by the scout through some unknown pheromonal or mechanical signal. On the other hand, true mass recruitment by pheromone trails

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has been found in *Ectatomma quadridens,* a larger South American species (OVERAL 1986). The trail is apparently deposited from the tip of the gaster, but its glandular origin has not been identified.

In the present study I found that *E. ruidum* does in fact lay recruitment trails, and I investigated their glandular source. I also examined the species' foraging ecology and other aspects of its communication behavior, including alarm, nest entrance marking, and food exchange. The results reveal a remarkably diverse array of communication behaviors, including a mass recruitment system well adapted to the species' particular foraging strategy.

## Study Site and Methods

Field studies were carried out at the Smithsonian Tropical Research Institute on Barro Colorado Island, Republic of Panama, during the rainy season months of April—August, 1987. Vegetation at the field site consisted of scrubby young tropical moist forest (FOSTER & BROKAW 1982). Most behavioral observations were made during the peak activity period between early morning and mid-afternoon. Only undisturbed field colonies were observed.

Several colonies were excavated and transported to the Museum of Comparative Zoology Laboratories, Harvard University, where they were housed in test tubes containing water held in by a cotton plug, and fed frozen crickets or live cockroaches and a specially prepared diet (BHATKAR & WHITCOMB 1970). Voucher specimens were deposited in the Museum of Comparative Zoology at Harvard University, the Invertebrate Museum at the University of Panama and the National Museum of Natural History in Washington, D.C. (S. PRATT, Series 47). The four largest colonies, each containing 60—120 workers, one or two queens and several dozen eggs, larvae, and pupae, were placed in 30 × 75 × 12-cm plastic boxes which served as foraging arenas. These colonies were used for experiments between November 1987 and June 1988. Descriptions of individual laboratory and field experiments are given in the appropriate sections below.

Glandular dissections were performed in distilled water on ants killed by placing them for a few min in a freezer. The mandibular gland was dissected by removing the mandible along with its associated muscle tissue, some of the adjacent exoskeleton of the head capsule, and the gland. To test for possible pheromone sources in the legs, hindlegs of 25 to 50 ants were extracted for several days in approximately 1 ml of diethyl ether, and the extract was used in pheromone assays. The hindgut, Dufour's gland and poison gland were large and distinct enough to be removed independently of any sclerites or other organs. The existence of sternal and pygidial glands in this species is not established, but since both are widespread in ponerines (HÖLLDOBLER & ENGEL 1978), they were tested by dissecting out the sixth and seventh abdominal sternites and their intersegmental membrane (possible sternal glands) and the sixth and seventh abdominal tergites and their intersegmental membrane (possible pygidial gland).

Data were analyzed with the statistical program package SPSS/PC+. Data samples were tested for normality via one-sample Kolmogorov-Smirnov goodness of fit tests and for homogeneity of variances by F-tests or Cochran's C-tests. Data showing homogeneity of variances and goodness of fit to a normal distribution were tested by the parametric methods described in each section below. When data failed one or both criteria, they were tested by both parametric and nonparametric methods and the results of both are presented.

## Results

### Field Studies

#### *1. Diet*

Food items included a wide variety of protein and sugar sources. Workers exploited fruit of *Astercarium* sp., *Virola* sp. and *Randia armata* either by collecting bits of pulp or carrying home droplets of juice. This liquid, as well as

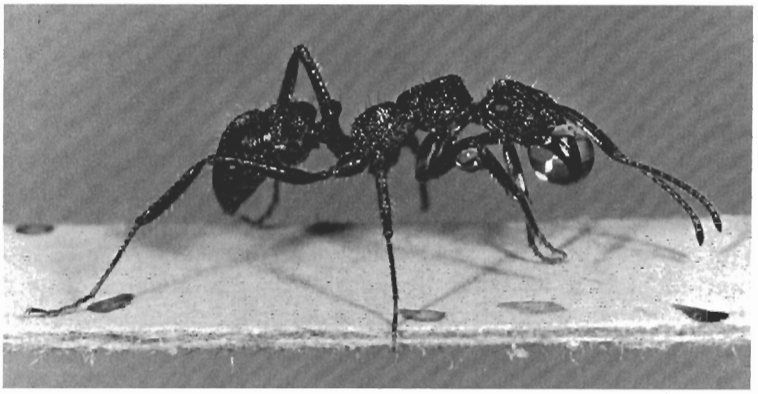

*Fig. 1:* *E. ruidum* worker carrying a droplet of sweet liquid in its mandibles

liquid retrieved from aphids, membracids, psyllids, extrafloral nectaries, and drops of rainwater on leaves, was carried in droplets held between the worker's open mandibles and against the edge of its clypeus (Fig. 1). Solid food items included carrion (an unidentified rodent), but most of the observed retrievals were of small to medium-sized arthropods and annelid worms. By far the most common prey items were fresh, and often still-living, isopods (*Philoscia muscorum*) and small annelids. Workers in the field and in the laboratory captured live, healthy isopods. Most prey was approximately the same size as the ants or a little bigger, and nearly all items were retrieved by single workers. When two or more ants cooperated to bring home a large annelid, they often worked at crosspurposes and generally proceeded very slowly.

### 2. *Demographics*

A 5 by 10 m rectangular plot was laid out at the Barro Colorado Island study site, all the leaf litter was cleared away from the area, and every *E. ruidum* nest entrance was located and marked. The area was rechecked daily over the next three days until it seemed reasonably certain that all entrances had been found. A total of 53 active nest entrances gave a total density of 1.06 entrances per $m^2$. 13 colonies from this plot and the area within 7 m of its borders and one colony from a similar location approximately 1 km away were collected and their adult populations censused within one day of collection. 6 were queenless, while one had two and another had three queens; the remainder had one queen each. Worker populations ranged from 14 to 106. Queenless worker groups were smaller on average than queenright groups at the 10 % significance level, but showed no difference at the 5 % level (queenless: $\overline{X} \pm SD = 47.5 \pm 22.2$; queenright: $\overline{X} \pm SD = 76.4 \pm 27.4$; independent samples t-test, $p = .057$). Small queenless groups may correspond to the colony annexes noted by LACHAUD et al. (1984). Although small size and queenlessness could both be characteristics of incompletely collected colonies, excavations were fairly thorough, and the large and active ants are easy to locate in their relatively shallow nests.

### *3. Temporal Foraging Rhythms*

Temporal rhythms in foraging activity were investigated by periodic observation of a field nest throughout a 24-h cycle. A nest in the Allee Creek ravine of Barro Colorado Island was observed from 7.00 h July 3 to 3.00 h July 4. Heavy rainfall then interrupted observation, so the remaining part of the cycle was observed from 4.00 to 7.00 h July 5. The number of ants entering and leaving the nest during each 30-min observation session was recorded. The colony was watched for 30 min of each h except for the least active period from 1.00 to 5.00 h when observations were made for 30 min of every 2 h. Nighttime and twilight observations were taken under red light to minimize disturbance of the ants. Aside from the above-mentioned interruption and one rainfall from 14.00 to 14.30 h, the skies were clear to partly cloudy and free of rain throughout the observation period.

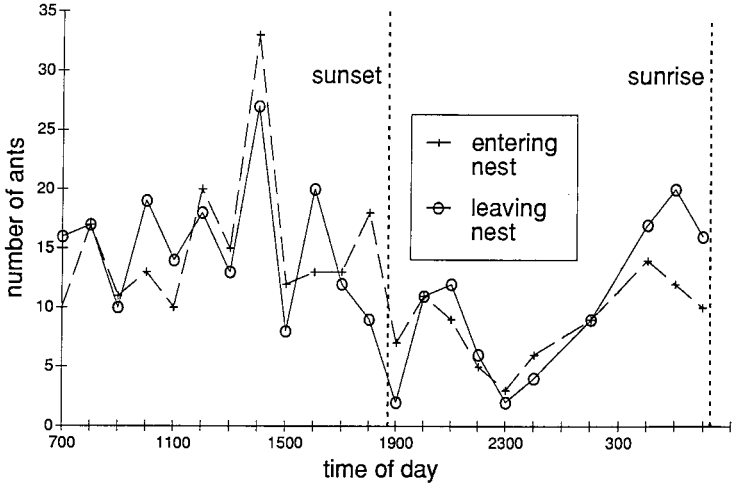


*Fig. 2:* Foraging activity of *E. ruidum* during a 24-h period. Data were taken from 7.00 h 3 July to 3.00 h 4 July and from 4.00 h to 7.00 h 5 July

The results (Fig. 2) show that *Ectatomma ruidum* forages throughout the photocycle but shows distinctly greater daytime than nighttime activity. The daytime mean number of ants entering and leaving the nest significantly exceeds the nighttime mean (daytime: $\overline{X} < SD = 28.0 \pm 5.7$, $N = 11$; nighttime: $\overline{X} \pm SD = 18.5 \pm 9.5$, $N = 10$; independent samples t-test, $p = .011$). This activity pattern differs notably from that observed in *Ectatomma tuberculatum*, a sympatric species also common on Barro Colorado Island. It is predominantly active at night, with peaks at dawn and dusk, although it continues to forage at reduced levels throughout the day (WHEELER 1986). Both species exploit similar food sources, and staggered foraging activity peaks may represent niche specialization to reduce competition.

### *4. Recruitment to Food Sources*

To determine whether colonies recruit to food sources, large (2 cm long) freshly killed *Pelidnota* sp. beetles were pinned to the ground, approximately 30—50 cm from a nest entrance. Typically, within a few min of discovering the bait, a forager walked straight toward the nest entrance while tapping its gaster tip repeatedly to the ground. Within 1 min of its reentry into the nest, approximately 5—10 workers emerged and followed the path of the initial forager to arrive at the bait. This behavior suggested that the successful scout laid chemical trails along which nestmates were recruited to the food source. I frequently observed that the recruits, when returning to the nest, also exhibited the gaster-tapping behavior. Trail following was generally slow and often inaccurate. Each ant appeared to follow the trail independently. There was no indication that they followed each other or a leader ant. Tandem running never occurred in this or any other context.

In a second, more rigorous series of bait experiments, the baits (either sugar water, 50—100 freshly killed termites, or a large freshly killed *Pelidnota* sp. beetle) were offered at 50, 100, or 200 cm from the nest entrance. One nest was used for all of the observations, and the number of workers at the bait was monitored at 1-min intervals. Fig. 3 shows the results of a typical episode. Ants recruited nestmates by trails to baits of sugar water or beetles 2 m away from the nest, but they rarely laid trails from termite baits (Table 1). Ants discovering termite baits antennated the whole pile for several s before quickly grabbing a few termites and carrying them to the nest. Over the course of each observation period only a few workers were seen on the pile or travelling between it and the

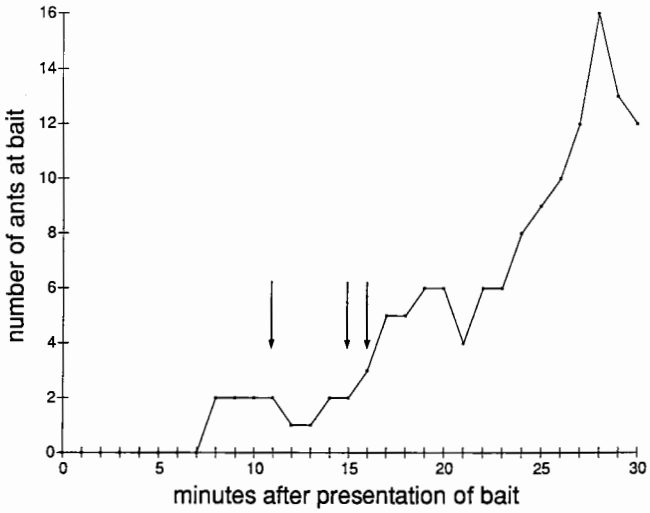


*Fig. 3:* Mass recruitment by *E. ruidum*. A saturated sugar water solution was set out on the ground in a small (1.5 cm diameter) plastic bottle cap 50 cm from an *E. ruidum* nest entrance. Two workers discovered the bait within a few min. When the first scouts returned to the nest, laying recruitment trails (arrows), the number of ants at the bait increased dramatically

*Table 1:* Recruitment to baits presented near *E. ruidum* nest entrances in the field. The upper value of each ratio gives the number of trials in which trails were laid; the lower value gives the total number of trials

| Distance from entrance (cm) | Bait: whole beetle | sugar water | termites |
|---|---|---|---|
| 50 | 6/6 | 6/7 | 1/6 |
| 100 | 5/5 | 5/5 | 0/6 |
| 200 | 2/2 | 5/5 | 2/5 |

nest, suggesting that a relatively small number of foragers exploited the bait by making several trips back and forth. Foragers that discovered beetles, on the other hand, left the bait only after struggling for several min to budge it or pull off a small piece for retrieval. Whether or not they eventually tore off a piece, they usually deposited a trail when they returned home. In supplementary experiments, workers also laid trails to fallen *Virola* sp. fruit (two out of three trials), to freshly killed isopods pinned to the ground (four out of five trials), and to *Pelidnota* sp. pronota (6 out of 6 trials). On one occasion, a worker deposited a trail over a distance of approximately 7 m, but no response to it was observed.

The number of ants leaving the nest within 1 min of the entry of the first trail-layer differed significantly from the number of ants leaving in 1 min control samples taken before each experiment (control: $\overline{X} \pm SD = 1.9 \pm 1.6$, $N = 37$; post-recruitment: $\overline{X} \pm SD = 5.5 \pm 3.7$, $N = 37$; paired-samples t-test, $p < .001$; Mann-Whitney U-test, $p < .0001$). This indicates that the rush of ants emerging after the entrance of a trail-layer is in response either to the trail itself or to recruitment behavior by the trail-layer inside the nest.

The effectiveness of recruitment in summoning nestmates to the bait was investigated by counting the number of workers at the bait when the first trail was laid. This control value indicated the number of ants finding the bait without recruitment. I then recorded the increase in the number of workers at the bait after a time period equal to the interval between bait presentation and the laying of the first trail. This value indicated the number of ants finding the bait after recruitment had occurred. The first number differed significantly from the second, indicating a real recruitment effect (before recruitment: $\overline{X} \pm SD = 1.8 \pm 1.0$; after recruitment: $\overline{X} \pm SD = 3.7 \pm 4.0$; paired-samples t-test, $p = .006$, $N = 38$; Mann-Whitney U-test, $p = .0354$).

Colonies did not appear to match numbers of recruited workers to the sizes of baits. The maximum number of ants at a bait within 20 min after the first trail was laid was compared for two sizes of protein bait: whole beetles and beetle pronota only. The mean for whole beetles did not differ significantly from the mean for pronota (whole beetle: $\overline{X} \pm SD = 10.5 \pm 5.85$, $N = 13$; pronotum:

$\bar{X} \pm SD = 11.0 \pm 5.87$, $N = 6$; independent samples t-test, $p = .87$). However, the small sample size for pronota tends to weaken the conclusiveness of this test.

I investigated several parameters of foraging timing and recruitment response in order to detect effects of bait type and distance of bait from the nest. Because of the small sample size for termite baits, only data for whole beetle and sugar water baits were analyzed. Of the parameters examined, only the mean time between discovery of a food source and laying of a trail to it showed any significant variance. It did not vary with distance, but did vary with bait identity (two-way ANOVA, effect of distance: $p = .304$, effect of bait: $p = .039$). This may reflect the relatively short time required for ants to retrieve sugar water droplets compared to the amount of time spent manipulating the large and hard-bodied beetles. The mean number of ants at a bait when the first trail to it was laid did not vary significantly with bait identity or distance (two-way ANOVA, effect of distance: $p = .183$, effect of bait: $p = .491$), nor did the maximum number of ants at a bait within 20 min of the first trail (two-way ANOVA, effect of distance: $p = .406$, effect of bait: $p = .843$), or the mean number of ants leaving the nest within 1 min after the entry of the first trail-layer (two-way ANOVA, effect of distance: $p = .106$, effect of bait: $p = .428$).

## Laboratory Studies

### *1. Recruitment to Food Sources*

In order to determine with certainty that the recruitment trail consisted of a pheromone and in order to pinpoint the anatomical origin of this pheromone, a variety of glandular organs were dissected out of a freshly killed worker, placed

*Table 2:* Numbers of ants following artificial trails laid with secretions from crushed glands or with distilled water (controls).

Each trail originates at the nest entrance. Values give mean number ($\bar{X} \pm SD$) of ants following each trail within a period of 10 min. Sample size for all trials was 10 except for the experiment with hindgut material and its control ($N = 9$). See text for details of procedure

| Poison and Dufour's glands | | Dufour's gland | | Abdominal sternites VI & VII (Sternal glands?) | | Abdominal tergites VI & VII (Pygidial gland?) | |
|---|---|---|---|---|---|---|---|
| exp. | control | exp. | control | exp. | control | exp. | control |
| 5.7 ± | * 0.0 | 4.0 ± 3.2 | * 0.0 | 2.6 ± 3.4 | * 0.10 ± .32 | 1.8 ± 1.5 | * 0.0 |
| Poison gland | | Mandibular gland | | Hindgut | | Hindlegs | |
| exp. | control | exp. | control | exp. | control | exp. | control |
| 1.5 ± 1.7 | 0.0 | 0.60 ± 0.84 | + 0.0 | 0.22 ± 0.44 | n.s. 0.0 | 0.10 ± 0.32 | n.s. 0.0 |

* $p < .05$, paired samples t-test and Mann-Whitney U-test.

\+ $p = .051$, paired samples t-test; $p = .030$, Mann-Whitney U-test.

n.s. ($p > .05$) by both tests.

One-way ANOVA and Kruskal-Wallis one-way ANOVA detect significant differences among experimental means ($p < .001$).

*Table 3:* Numbers of ants following artificial trails laid with secretions from crushed glands offered on paired paper bridges. Values give mean number ($\bar{X} \pm SD$) of ants following each trail, and the sample size (N) of each comparison. See text for details of procedure

| Dufour's gland | versus | Poison gland | Dufour's gland | versus | Poison and Dufour's glands | Dufour's gland | versus | Abdominal tergites VI & VII (pygidial gland?) |
|---|---|---|---|---|---|---|---|---|
| 12.3 ± 7.9 | * N = 12 | 3.3 ± 2.7 | 9.2 ± 3.6 | n.s. N = 10 | 9.5 ± 3.9 | 10.4 ± 6.8 | * N = 12 | 4.1 ± 1.9 |
| Dufour's gland | versus | Water | Poison gland | versus | Water | Poison and Dufour's glands | versus | Water |
| 11.4 ± 5.3 | * N = 12 | 1.9 ± 1.3 | 3.6 ± 2.4 | * N = 14 | 1.4 ± 1.3 | 10.5 ± 1.9 | * N = 6 | 1.5 ± 1.9 |
| Dufour's gland | versus | Abdominal sternites VI & VII (sternal glands?) | Abdominal sternites VI & VII (sternal glands?) | versus | Abdominal tergite III | Abdominal tergites VI & VII (pygidial gland?) | versus | Abdominal tergite III |
| 15.4 ± 6.1 | * N = 8 | 3.4 ± 2.1 | 5.5 ± 3.2 | ** N = 8 | 2.1 ± 1.5 | 6.0 ± 5.3 | + N = 8 | 2.6 ± 1.8 |

* $p < .01$, paired samples t-test.
** $p < .05$, paired samples t-test and Mann-Whitney U-test.
\+ $p = .047$, paired samples t-test; $p = .2001$, Mann-Whitney U-test.
n.s., paired samples t-test, $p = .847$.

on the tip of an applicator stick, and rubbed along a 10 cm long pencil-drawn line on a small piece of typing paper. A parallel control trail, starting at the same point as the experimental trail and verging off at approximately a 70° angle, consisted of distilled water. The paper was placed in the foraging arena with the trail origins approximately 5 mm from the nest entrance. Over the next 10 min, a record was made of the number of ants following either trail and the distance and direction (toward or away from the nest) each one walked. The number of ants who antennated the trail without following it was also recorded.

The results (Table 2) include only ants following the trail for at least 4 cm in either direction. Response to controls was virtually nonexistent in all trials. Only the hindlegs and hindgut failed to show a significant difference from their controls. Although some response was seen to all sources except the hindlegs, and no source consistently drew a strong response, a one-way analysis of variance and Scheffé procedure showed that only the two strongest sources (Dufour's gland and the combination of Dufour's gland and the poison gland) drew significantly larger responses than any of the others ($p < .05$). This suggests that Dufour's gland is the principal source for a trail pheromone, with a possibility that the poison gland may also play a role. The poison gland alone did not, on average, produce a strong result, but the variance of responses was high, with a few very strong responses occurring. Furthermore, the pygidium and sternites both induced some responses.

Since these data were equivocal, further experiments were performed to look more closely at Dufour's gland, the poison gland, the Dufour's gland/poison gland combination, the terminal sternal region and the pygidial region. In these experiments, the nest tubes were transferred to a 23 × 15 × 5 cm plastic box within the foraging arena. Ants could enter the arena only by crossing a V-shaped pair of paper bridges, 29 cm long. On each branch of a pair of bridges a trail was drawn with crushed glands or body parts, or with water. In addition, the sternal and pygidial regions were tested against the first gastric tergite to determine whether the observed effects were actually due to intersegmental glands and not simply to surface chemicals. The marked pair of bridges was substituted for the bridges already in the test colony's nest, and the number of ants crossing from the nest to the arena during the next 10 min was recorded. Counts included only ants making a complete crossing, and only journeys from nest to arena. Within each series, the left-right arrangement of pairs of bridges was alternated from test to test to take into account possible artifactual preferences for one side or another.

The results (Table 3) show that Dufour's gland trails attracted significantly more followers than trails made from distilled water, the poison gland, the sternites, or the pygidium. Although poison gland trails elicited a significantly better response than trails drawn with water, the combination of poison and Dufour's gland trails did not attract a significantly different number of followers from trails made with the Dufour's gland alone. The weak preference demonstrated for poison over water trails may simply reflect the venom's action as an alarm pheromone (see below). The other trials clearly show the unique strength of Dufour's gland secretions in inducing trail-following. Furthermore, in nearly all trials with a Dufour's gland trail, a group of 5 to 12 workers gathered at the

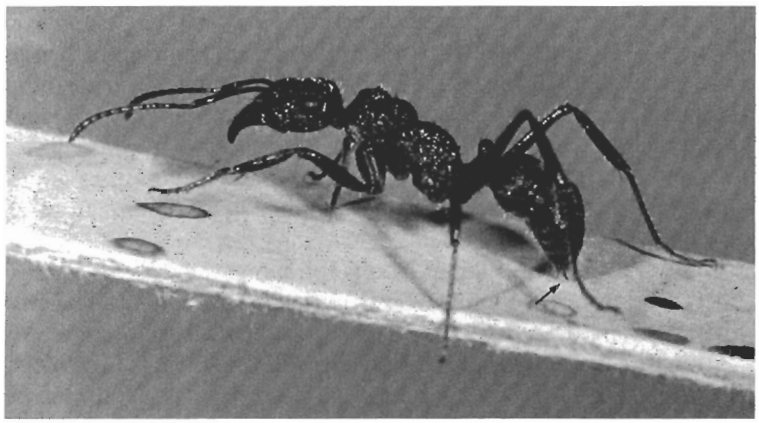

*Fig. 4:* After discovering a large prey object, an *E. ruidum* worker returns to the nest extruding its sting (arrow) and tapping it against the ground

arena end of the Dufour's gland trail, milling around and antennating the bridge. No other trails induced such gatherings. Finally, observation through an operation microscope (Technoscope, Zeiss) and macrophotography of trail-laying ants show clearly that the sting is extruded during trailing (Fig. 4), indicating that the sting-associated glands are involved in trail-laying. Both the Dufour's gland and the poison gland secrete their contents through the sting. Since artificial trail experiments indicate that the poison gland does not play a role in trail-laying and that Dufour's gland does, I conclude that Dufour's gland is the source of the trail pheromone.

The results of field experiments showed that the entry of trail-layers into the nest signficantly increased the number of workers emerging (see above), suggesting that trail-layers engage in recruitment behavior inside the nest. In the laboratory I observed that, immediately upon entering the nest, trail-layers ran excitedly among their nestmates, vigorously antennating every worker encountered. Antennated workers became excited, and nearby workers were also attracted to the recruiter, possibly by a pheromone, or the smell of food adhering to the forager's body. On some occasions, successful foragers performed such excitation behaviors without having first laid a trail.

In order to determine whether this behavior, rather than the trail itself, is responsible for the recruitment effect, I made the following comparisons. Single paper bridges were placed between the nest box and the foraging arena, and a fresh insect bait was pinned to the arena floor. When a forager found the bait, returned to the nest, and laid a pheromone trail on the bridge, I promptly removed the bridge and replaced it with one on which I had previously made an artificial trail with a crushed Dufour's gland. The number of ants crossing all the way over this bridge from the nest to the arena was counted over the next 10 min. In controls performed 1.5 to 5 h before each experiment, bridges marked with artificial Dufour's gland trails were presented and the number of ants crossing over the next 10 min counted. In these controls, with no bait and no trail-layer, recruitment events did not occur inside the nest. Means from the two series

differed significantly, indicating that the recruiter's behavior inside the nest strongly stimulates the response of nestmates to the trail pheromone (control: $\overline{X} \pm SD = 17.4 \pm 7.2$; experiment: $\overline{X} \pm SD = 27.2 \pm 10.5$; paired-samples t-test, $N = 7$, $p = .022$).

The colony specificity of the trail pheromone was examined in bridge choice tests comparing trails made from nestmate Dufour's glands with trails made from alien Dufour's glands. The mean number of ants crossing nestmate-marked bridges was virtually identical to the mean number crossing alien-marked bridges (nestmate: $\overline{X} \pm SD = 13.4 \pm 4.0$; alien: $\overline{X} \pm SD = 13.4 \pm 7.7$; paired-samples t-test, $N = 10$, $p = 1.000$). Workers gathered at the arena end of both trails. This indicates that the trail pheromone is not colony specific.

### *2. Recruitment During Nest Emigration*

Recruitment behavior during nest-moving was investigated in the laboratory by observing the ants' return to the nest after knocking the contents of a nest tube containing the queen onto the foraging arena, 15—40 cm from the nest location. Three queenright colonies, one with about 25 workers and two with about 100 workers, were each tested once. Within a few min of the displacement, some workers began returning brood to the nest. Over the next 20 to 90 min, depending on the size of the colony and the distance of the displacement, a light but steady traffic of brood carriers gradually returned the colony to its original tube. The carriers were all fully pigmented adult workers which moved swiftly back and forth between the dumping site and the nest.

Carriers laid no recruitment trails, but they carried queens, a majority of callows and some adult workers in a stereotyped posture very similar to that described for myrmicine ants (MÖGLICH & HÖLLDOBLER 1974). The carried ant

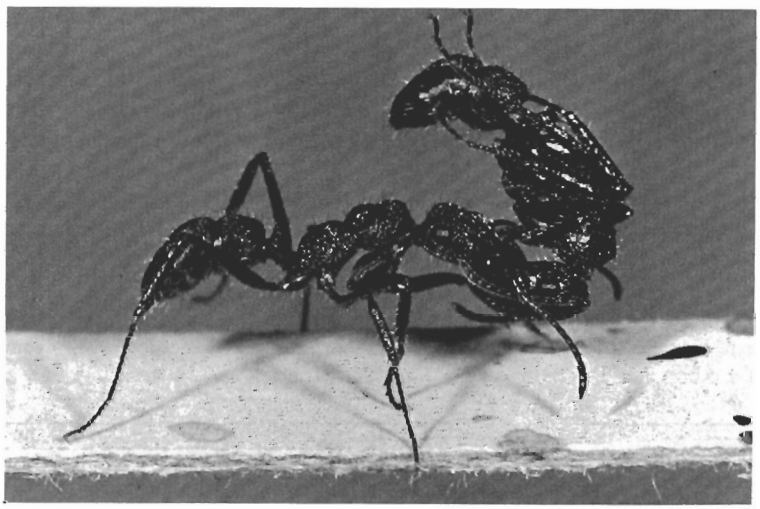

*Fig. 5:* Social adult transport in *E. ruidum*. The carried ant assumes a "pupal posture" and is curled over the back of the carrier

was held by the cheeks or mandibles, with its legs and antennae folded in and its body curled over the carrier's back (Fig. 5). Carriers generally initiated the behavior by approaching a nestmate and rapidly antennating it on the head. The nestmate usually reciprocated the antennation, and the carrier turned its head sideways, seized the nestmate by the head and lifted it up into the stereotyped posture. In a few cases, a callow initiated the event by antennating an adult, who then reciprocated antennation and picked up the solicitor. Carrying was very frequent during nest-moving; in one case nearly 50 instances were observed in a colony of about 100 workers. Social carrying was also used in the laboratory and in the field to return single, apparently lost, foragers to the nest.

During one nest-move a curious "hitch-hiking" behavior was noted. A callow solicited an adult already carrying brood or another worker and was therefore not picked up; it then grabbed onto the adult by a leg or antenna, usually a hind leg, and walked along behind or next to the leading ant. Thus, while the callow used its own power to return to the nest, it was oriented by the leader. In one case, the hitch-hiker lost contact with the ground while the leader ant crossed a narrow cardboard bridge, pulled in its legs and adopted the pupal posture typical of carried ants, except that in this case the hitch-hiker was holding the carrier. Hitch-hiking was observed 8 times in this colony.

### *3. Alarm Communication*

*E. ruidum* has been reported to exhibit aggression, attraction, and increase in speed when presented with filter papers on which the gaster or head of a conspecific has been crushed (JAFFE & MARQUEZ 1987), suggesting that the gaster and head may contain alarm pheromones. I offered crushed body sections and dissected glands to colonies to investigate the source of alarm and attraction responses. The potential glandular sources tested were Dufour's gland, the poison gland, the mandibular gland, the hindgut, the pygidium, and the sixth and seventh sternites.

A 23 mm diameter circle was drawn with a pencil on a round piece of filter paper (9 cm in diameter), which was placed in the foraging arena so that the edge of the circle was 10 mm from the nest entrance. After allowing the ants a few days

*Table 4:* Alarm pheromone assays. Mean numbers of ants ($\bar{X} \pm$ SD, N) approaching crushed body sections of nestmates. Crushed sections were presented on wooden applicator sticks. Controls were clean applicator sticks. See text for details of procedure

| Head | | | Alitrunk | | | Gaster | | |
|---|---|---|---|---|---|---|---|---|
| exp. | | control | exp. | | control | exp. | | control |
| 10.8 ± 5.0 | * | 1.2 ± 1.3 | 3.8 ± 3.1 | n.s. | 1.2 ± 1.8 | 12.6 ± 3.2 | * | 1.6 ± .55 |
| 6 | | 6 | 5 | | 5 | 5 | | 5 |

* $p < .01$, independent samples t-test and Mann-Whitney U-test.
n.s. by independent samples t-test ($p = .154$) and by Mann-Whitney U-test ($p = .1116$).
One-way ANOVA detects significant differences among experimental samples, $p = .009$.
Scheffé procedure detects significant differences between the alitrunk and both the head and the gaster ($p < .05$), but none between the head and gaster ($p > .05$).

to become acclimated to the paper's presence, I presented a freshly cut body part or gland from a freshly killed ant on the tip of a wooden applicator stick and held it over the center of the circle for 1 min at a height of about 1 cm. All ants entering the circle in this period were counted. If an ant left the circle and then re-entered, this was scored as two entries. A brief description of the behavior of the ants in response to the stick was also recorded. In a control carried out prior to or after each experiment, a clean applicator stick was held over the circle for 1 min, and entries and behavior were noted as described above. Two series of experiments were carried out, one testing whole body sections and another testing individual dissected glands.

The results with whole body sections showed a significant difference between control and experiment in number of ants approaching the head and the gaster, but not for the alitrunk (Table 4). A one-way analysis of variance and Scheffé procedure revealed a significant difference ($p < .05$) between the number of ants approaching the alitrunk and the number approaching both the gaster and the head, but no difference between the gaster and head. The alitrunk never elicited more than antennation and threatening with open mandibles by workers, while four out of five head trials and five out of five gaster trials induced considerable excitement. Ants were attracted to the sticks from several cm away, ran around rapidly near them, and left the nest to approach, vigorously antennate and bite them. This suggests that the head and gaster both contain excitatory substances, but that the alitrunk does not.

The results of the experiments with exocrine glands (Table 5) showed that the mandibular gland and poison gland attracted the largest numbers of ants. Only the hindgut response failed to differ from its control at the .02 significance

*Table 5:* Alarm pheromone assays. Mean numbers of ants ($\bar{X} \pm SD$, N) approaching crushed glands of nestmates. Crushed glands were presented on wooden applicator sticks. Controls were clean applicator sticks. See text for details of procedure

| Mandibular gland | | | Poison gland | | | Abdominal tergites VI & VII (pygidial gland?) | | |
|---|---|---|---|---|---|---|---|---|
| exp. | | control | exp. | | control | exp. | | control |
| $10.1 \pm 7.1$ | * | $1.3 \pm 1.3$ | $6.6 \pm 4.9$ | * | $1.6 \pm 1.5$ | $4.0 \pm 3.8$ | * | $0.89 \pm 1.5$ |
| 12 | | 12 | 14 | | 14 | 18 | | 18 |
| Abdominal sternites VI & VII sternal glands?) | | | Hindgut | | | Dufour's gland | | |
| exp. | | control | exp. | | control | exp. | | control |
| $3.5 \pm 1.8$ | * | $1.9 \pm 1.6$ | $3.2 \pm 2.4$ | n.s. | $2.3 \pm 1.8$ | $2.9 \pm 1.7$ | * | $1.5 \pm 1.6$ |
| 16 | | 16 | 17 | | 17 | 16 | | 16 |

* $p < .01$, independent samples t-test; $p < .02$, Mann-Whitney U-test.
n.s. by independent samples t-test ($p = .236$) and by Mann-Whitney U-test ($p = .2619$).
One-way ANOVA and Kruskal-Wallis one-way ANOVA detect significant differences among experimental samples, $p < .05$.
Scheffé procedure detects significant differences between mandibular gland and all other sources except poison gland; no other differences are detected.

*Table 6:* Alarm pheromone assays. Frequencies of trials in which excitement behavior was shown toward crushed glands. Crushed glands were presented on wooden applicator sticks. Observed values are significantly different from values expected if all glands are equally likely to induce excitement behavior ($\chi^2$ test, $p < .001$). "All other glands" includes hindgut, Dufour's gland and possible sternal glands (abdominal sternites VI and VII)

| | Mandibular gland | Poison gland | Abdominal tergites VI & VII (pygidial gland?) | All other glands |
|---|---|---|---|---|
| Excitement | 7 | 7 | 1 | 1 |
| No excitement | 5 | 7 | 17 | 48 |

level, but only the mandibular gland, poison gland, and pygidium responses differed at the .01 significance level. A one-way analysis of variance and Scheffé procedure showed that only the response to the mandibular gland differed significantly from the responses to other sources, although it did not differ from the poison gland.

Behavior clearly distinguished the mandibular and poison glands from the other sources (Table 6). Excitement reactions to both were very frequent, and occurred significantly more often than excitation responses to other sources. Although the pygidium and some other sources can attract ants, they do not release a real alarm response. Attraction to these body parts may result from the odor of crushed fat bodies, muscle tissue, or hemolymph adhering to them. Thus, it can reasonably be concluded that both the poison gland and mandibular gland act as alarm pheromones by inducing aggression, attraction, and excitement. These results suggest that the poison and mandibular glands, and possibly the pygidium, release alarm signals.

### *4. Nest Entrance Marking*

Nest entrance marking is a phylogenetically widespread phenomenon occurring in many ant genera (MASCHWITZ et al. 1986; HÖLLDOBLER 1987). Hindgut pheromones deposited in fecal droplets around the nest entrance contribute to this marking in some species. JAFFE & MARQUEZ (1987) reported evidence of territorial marking in *E. ruidum,* and I noted that colonies in laboratory nests liberally spot the floors of their nests with fecal droplets. I therefore tested the hypothesis that these droplets or some other signal act as colony-specific nest entrance markers.

Filter paper circles (9 cm diameter) were placed at the nest tube entrances of six queenright colonies with worker populations of 50—120. After seven weeks, the papers were speckled with fecal droplets and I removed them. Each filter paper was used to line a test tube containing water held in by a cotton plug. Similar control test tubes were lined with clean filter paper. Two test tubes, one control and one marked, were placed in a 19 × 14 × 8 cm plastic box, one on each side of the box. 10 workers and 10 larvae and/or pupae from the colony which marked the filter paper were introduced to the box and their location monitored every h for at least 3 h or until all the brood and most of the workers had moved

into the tubes. A total of 10 trials were made (four of the papers were tested twice). In order to establish that the marking is colony specific, 13 similar experiments were performed, presenting a worker group with a filter paper marked by their own colony and one marked by an alien colony. At the time of the experiments, all colonies had been fed the same diet and housed under the same conditions for nine months.

In all but one of the marked-unmarked choice tests, all the brood and a majority of the workers moved into the marked tube within 5 h. The last group moved into the marked tube within 19 h. In no case was any brood moved into the unmarked tube, and workers were only rarely seen there. Thus, with 10 out of 10 cases showing a positive response to the marked tubes, no statistical analysis was necessary or possible to conclude the existence of a preference for marked over unmarked papers.

In 11 of the 13 familiar-alien choice tests, the worker groups chose their own colony's paper within 12 h. The other two groups chose the alien tube. A sign test indicated a significant difference between the distributions of groups choosing the two kinds of tubes ($p = .0225$), supporting the hypothesis that ants mark their nest entrances with colony-specific markers.

### *5. Communication during Food Exchange*

Previous authors have reported an unusual type of food exchange among *Pachycondyla* spp. ponerine ants which do not practice regurgitation (LENOIR & JAISSON 1982; HÖLLDOBLER 1985). Foragers retrieve liquid food by carrying large droplets between their mandibles, and exchange all or part of these droplets in the nest when solicited with a stereotyped series of antennation signals. HÖLLDOBLER labelled this technique of food exchange the "social bucket" method. Since *E. ruidum* workers also habitually transport liquid in mandibular droplets (Fig. 1), I made the following observations to detect whether they employ the social bucket method.

Three laboratory colonies were starved for five days and then provided with a semi-liquid laboratory-prepared diet (BHATKAR & WHITCOMB 1970). Foragers returned to the nest with mandibular droplets. Approximately 1.5 h of observation indicated that *E. ruidum* does not regurgitate liquid from the crop, but does engage in behaviors virtually identical to those described for *Pachycondyla* spp. Droplet-laden foragers returned immediately to the nest tube and, after a few s of excitation behavior, either stood still or walked slowly about the nest with mandibles open and mouthparts usually retracted. They were generally approached within a few s by unladen workers who gently antennated the clypeus, mandibles, and labium of the drop-carrier, using the tips of their antennae. The carrier then opened its mandibles wide and pulled back its antennae, while the solicitor opened its mandibles, extruded its mouthparts and began to drink. During feeding, it continued to antennate the donor, who remained motionless. Usually the solicitor also rested one or both front legs on the head or mandibles of the donor. It generally drank for several s, and then took all or part of the drop into its own mandibles. Solicitors could then act as donors

themselves in subsequent exchanges and distribute the forager's load throughout the colony.

On two occasions, workers were seen feeding drops to larvae, although no mechanical signalling by either partner was detected. Males frequently fed from donors, but they performed no antennation or other solicitation behaviors. They simply put their feet on the mandibles or head of the donor and began drinking. Donors, in turn, did not open their mandibles as wide for males as they did for workers and often backed away from feeding males. One instance of queen feeding was observed, but I could not clearly determine what kind of signalling the queen or the donor used.

## Discussion

Although *E. ruidum* workers generally forage individually, they do employ recruitment behavior when encountering rich or difficult food sources. The recruitment system consists of orienting pheromone trails and a stimulating behavior performed inside the nest. This contradicts previous reports indicating only a rudimentary recruitment ability in this species (LACHAUD 1985; LACHAUD et al. 1984). LACHAUD may have observed instances of recruitment in the nest unaccompanied by orienting trails, similar to those which I sometimes noted in laboratory colonies and which have also been reported for *E. quadridens* (OVERAL 1986). The difference in responses to beetle and termite baits may also have a bearing on LACHAUD's failure to find trail communication. Although termite and whole beetle baits used in my experiments were approximately the same size and mass, only the latter consistently stimulated recruitment, suggesting that unwieldiness and difficulty of exploitation, rather than bait size, are the cues stimulating differential recruitment to solid food sources. As long as individuals can easily and rapidly retrieve all or part of a find, they do not recruit nestmates, even if the source is large and requires several trips to retrieve entirely. This hypothesis is further supported by the fact that small protein baits like isopods, normally retrievable by a single worker, will induce recruitment if pinned to the ground. LACHAUD's tuna bait may have been analogous to my termite baits in that it could easily be torn apart and retrieved piecemeal by one worker. My observations suggest that an ant discovering such a bait might perform the stimulating behavior inside the nest, but would rarely deposit a trail.

Sugar water baits readily induce recruitment behavior in *E. ruidum,* even though individual workers can easily gather up a droplet of liqid and return immediately to the nest. Since natural sugar sources, such as nectaries and groups of homopterans, are relatively long-lasting and potentially very productive, they may constitute particularly prized resources. They are also well-suited to exploitation and defense by large numbers of ants, which may be the reason why they so readily elicit a recruitment action in the ants.

Mass recruitment serves *E. ruidum* well in exploiting widely spaced, concentrated food resources, including nectaries and large prey items. *Paraponera clavata,* a ponerine ant which also makes extensive use of rich nectar sources,

employs very similar recruitment communication (BREED & BENNETT 1985). *Paltothyreus tarsatus* likewise supplements its generally individual foraging strategy with mass recruitment to concentrated sources, in this case termite colonies (HÖLLDOBLER 1984 b). Another factor making recruitment particularly useful to *E. ruidum* is the extremely high density of colonies, a potential source of considerable intraspecific competition for large food sources. The ability to recruit a small group of workers to a resource may allow its rapid exploitation and defense against conspecifics. High population density may also make nest-entrance marking particularly important, in order to avoid confusion of entrances and potentially costly aggressive interactions.

BROWN (1950, 1958) hypothesized on morphological grounds that the Myrmicinae are derived from an ectatommine ancestor, but this claim has not been supported previously by much behavioral or physiological data. Since the Dufour's gland, heretofore unknown as a source of recruitment pheromones in the Ponerinae, frequently serves this role in myrmicine ants (HÖLLDOBLER & WILSON 1990), my findings strongly support BROWN's hypothesis. Moreover, secretions from the pygidial gland, which have proven to serve as a recruitment pheromone in some non-ectatommine ponerines (HÖLLDOBLER & TRANIELLO 1980, MASCHWITZ & SCHÖNEGGE 1977), play no major role in *E. ruidum*. Pygidial gland secretions elicited a limited alarm effect, but this did not nearly approach that induced by secretions of the mandibular and poison glands. Careful microscopic dissections of several workers revealed no well-developed pygidial gland (HÖLLDOBLER, pers. comm.). Finally, the method of social carrying employed by *E. ruidum* is typical of myrmicine species, but rare in ponerines (MÖGLICH & HÖLLDOBLER 1974). Besides *E. ruidum*, the only other ponerines known to perform social carrying in this manner are also ectatommines: *Rhyditoponera metallica* (MÖGLICH & HÖLLDOBLER 1974), *E. quadridens* (OVERAL 1986), *Gnamptogenys* sp. (HÖLLDOBLER, pers. comm.), and *Proceratium silaceum* (PRATT, unpubl. obs.).

As is frequently the case in both subfamilies, *E. ruidum* employs social carrying in colony emigrations. The sample size of three experimentally-induced nest moves is too small to conclude that trails are never laid in this context, but social carrying alone seemed a very effective means of colony relocation. Workers in migrating colonies also performed an interesting behavior which I refer to as "hitch-hiking", a previously unknown variation of social carrying in ants. It is noteworthy that many of the social carrying episodes, including all of the hitch-hiking ones, were instigated by the carried ant rather than the carrier, the reverse of the usual sequence in ants.

The Ectatommini are the second ponerine tribe in which the social bucket method of food exchange has been described. Ponerines do not generally employ regurgitation, and the social bucket constitutes an analogous method of distributing liquid food throughout the colony. Further research on the many ponerine species known to carry mandibular droplets may show the behavior to be even more widespread.

The impressive communication abilities of *E. ruidum* expand the already large repertory of communication behaviors known from the Ponerinae. In

particular, the fact that Dufour's gland is the fifth known ponerine recruitment gland further supports the hypothesis that recruitment by pheromone trails has evolved several times in this subfamily (HÖLLDOBLER 1984 a). This diversity reflects the polyphyly of the taxon, which includes some of the most primitive living genera, as well as many species exhibiting very sophisticated social behaviors. Further studies of chemical communication mechanisms and the glandular sources of the pheromones will shed light on the phylogenetic relationships of ponerine tribes and the evolution of social behavior in ants in general.

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