

Recruitment behaviour in the ponerine ant, *Plectroctena mandibularis* F. Smith (Hymenoptera: Formicidae)

K.J. Wilkins, K. Harman & M.H. Villet*

Department of Zoology and Entomology, Rhodes University, Grahamstown, 6140 South Africa

Although workers of *Plectroctena mandibularis* laid trails with their stings while foraging, the trails appeared to be for individual orientation, because they never recruited nestmates to prey. However, both workers and queens laid trails when recruiting nestmates of either caste to new nest sites. During trail-laying, fluted hairs on the posterior edge of tergite VI were dragged along the ground, presumably applying a pheromone to the substrate. Anatomical and behavioural evidence suggests that pygidial gland secretions moved from the intersegmental pygidial gland between tergites VI and VII into a fingerprint-like, lamellar cuticular reservoir on the pygidium, and from there *via* the hairs to the substrate. These results suggest that recruitment may be crucial to moving nests but of value only to certain types of foraging, and that recruitment might even have originated in the Formicidae in the context of colony relocation, and then secondarily evolved to assist foraging.

Key words: trail laying, nest relocation, foraging, pygidial gland, tandem running, Ponerinae.

INTRODUCTION

Plectroctena mandibularis (Smith) forms colonies containing less than 300 workers (Villet 1990), and it therefore could be a model species for studies of how ant societies coordinate and organize themselves. Communication is central to coordination, and recruitment signals are an important means of communicating the location of food sources and new nest sites to nestmates (Hölldobler & Wilson 1990). However, workers of *P. mandibularis* forage alone, and queens appear to found new colonies alone (Villet 1991, 1999), so there is little evidence of routine recruitment in this species. Despite this, small columns of workers of *P. mandibularis* are occasionally observed in the field, indicating that some form of recruitment does occur, but the function and mechanisms of such recruitment remain obscure. Research on recruitment in *P. mandibularis* might offer more insight into the social activities to which recruiting behaviour is indispensable. In turn, this may enhance our understanding of how small societies of ants function, and fuel constructive speculation on how ancestral ant societies were organized. This work therefore investigated the function and mechanism of recruitment in *P. mandibularis*.

MATERIAL AND METHODS

Two whole colonies of *Plectroctena mandibularis*

were excavated in the Grahamstown area (30°17'S 26°30'E). There were 47 ants in the first colony, 18 of which were queens, identified by their reduced ocellar pits and larger size (Bolton 1974; Villet *et al.* 1999), and 23 ants, including one queen, in the second colony. The colonies were established in the laboratory in three-chambered Lubbock nests containing soil and covered with glass lids and red plastic. The ants from the first colony were marked using dots of enamel paint to make them individually identifiable. The second colony was not marked and served as a control for the effects of marking. After the behavioural experiments, ants from the second colony were used for anatomical study because they were free of paint.

Behavioural observations

We tried to induce recruitment to food sources in a large, glass-topped 0.7 × 0.6 m arena using prey that was too large for one ant to handle alone. The species is a specialized millipede hunter (Arnold 1914; Villet 1991), so millipedes 5–8 cm long were released in the arena. Note was made of how prey were retrieved to the nests, and what postures the ants showed during the foraging process. Over 22 man-hours, and substantially more ant-hours, of observations were made.

We induced recruitment to new nest sites in the same arena. At the start of each trial, the floor of the arena was covered with fresh newsprint paper to

*To whom correspondence should be addressed.
E-mail: m.villet@ru.ac.za

mask any existing chemical cues, and a fresh, washed, unoccupied Lubbock nest was placed near one corner of the arena. The colony's nest was then placed in the opposite corner of the arena, with glass and plastic lids removed. Observations were made until all of the ants had moved to the new nest. This experiment was repeated seven times, with a few variations: the new nest was moved into various positions within the arena, always away from the arena wall to prevent the ants from simply following the wall until they found the new nest. Recruitment behaviours, particularly trail-laying postures, were photographed. Over 10 man-hours of observations were made.

Smoked glass was used to determine the parts of the body in contact with the ground during trail-laying (Hangartner 1969). Pieces of glass thinly coated on one side with candle soot were placed in the path of recruiting ants and solitary ants on their way out of the nest. Soot left in the soot was lit from below and photographed with a Wild M400 Photomakroskop camera mounted on a stereomicroscope.

To verify the involvement of a scent, a worker was allowed to lay a trail over a clean sheet of white paper and the paper was then rotated 90°. Other workers were then allowed to walk across the paper until they encountered the putative trail, and their responses, if any, were noted. This procedure was repeated four times.

To determine whether queens could recruit, and to determine their preference between following another queen or a worker, four queens were isolated in a clean glass vial in a small arena and provided with a distant plastic vial containing soil. Observations were made on their emigration to the soil-filled container before and after the introduction of a single worker ant. The experiment was repeated seven times with different queens and workers.

Scanning electron microscopy

Abdominal tergites VI and VII of a sample of workers and queens were removed and prepared for scanning electron microscopy, *i.e.* washed in water, mounted on glass microscope slides, dried at room temperature, mounted on brass stubs, and coated with gold. Scanning electron micrographs were taken using a JEOL JSM 840 scanning electron microscope.

RESULTS

Behaviour

The following behaviours were seen in both marked and unmarked ants, with no qualitative differences. Inevitably, there were quantitative differences even within the same colony or with individuals on different days.

Foraging. Individual workers ($n = 22$) successfully caught and retrieved the smaller millipedes unassisted, and attacked and killed the larger ones but could not drag them back to the nest alone. Recruitment to prey was never seen, although workers were seen to drag their stings while foraging alone, as though laying a trail. Ants leaving their nest immediately after it was disrupted by removing its roof also touched their stings to the ground periodically as they walked about. This was also seen in ants moving about the arena in day-to-day activities *e.g.* refuse removal ($n = 7$ individuals), and in ants placed on an unfamiliar surface such as a tabletop ($n = 10$ individuals). Such ants were able to retrace their steps, holding their antennae to the ground. The sting-dragging behaviour was interpreted as the laying of a trail. No other ants were attracted to these trails.

Nest relocation. Once the new nest had been located, the discoverers ($n = 17$ individuals, not all of which were seen foraging in the days before the experiment) returned to the original nest with their abdomens held high or touching their stings to the ground. Once back at the original nest, a discoverer would approach a potential recruit and tug at its mandibles and forelegs for a second or so, and then turn around and set off with its gaster curled downwards. The tugged ant would usually follow. While leading recruits, the leading ant curled the tip of its abdomen ventrally and the surface of tergite VII was apparently dragged across the ground (Fig. 1) as though laying a trail. Recruited ants followed close behind the leading ant, but not in contact with it. As they moved along the trail, they held their antennae to the ground and seemed to tap the ground with their forelegs in a more exaggerated way than was observed in other situations. As many as five or six ants were seen to follow a leader in a line at one time. Some of the recruits returned to the original nest and recruited other ants to the new nest themselves. Both workers and queens were seen to scout and recruit to the new nest. Some queens recruited other queens and workers. Recruitment of queens



Fig. 1. Worker laying a recruiting trail. On the left of the ant is a segment of a millipede, the food source of this species.

by queens took longer and needed more frequent encouragement from the leader than their recruitment by a worker. Recruits were able to follow the trail even after the leading ant was out of sight behind an object.

Ants detecting this type of trail on a sheet of paper rotated 90° after the trail was laid, followed it for up to 20 cm and became hesitant and disorientated at the edge of the paper. When this happened, they returned to the paper, moving their heads from side to side, holding their antennae to the paper and tapping it with their forelegs. When they crossed the trail again they set off in one or other direction along the trail.

Analysis of spoor left on soot-covered glass

revealed three different types of marks. In the first footprints were visible. The second was produced when the ant touched its sting to the ground periodically. Footprints with a row of well-spaced dots between them were visible. The third type was produced by recruiting ants, *i.e.* while curling the abdomen and dragging tergite VII over the ground. Only the hairs on the posterior edge of tergite VI, and not the cuticle of the pygidium, were in contact with the ground. Footprints were visible on either side of four parallel lines made by the hairs (Fig. 2).

Microanatomy

Scanning electron micrographs of the cuticle of

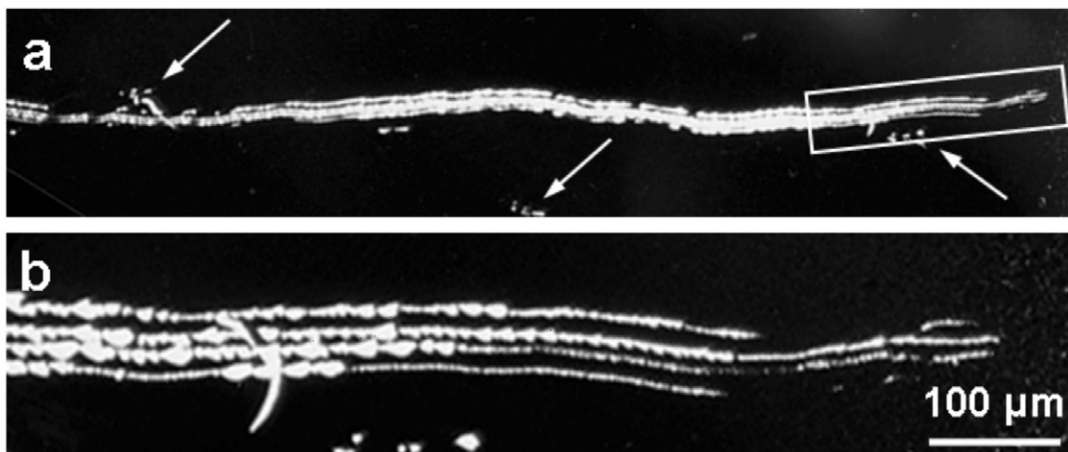


Fig. 2. Marks left on smoked glass by a worker of *Plectroctena mandibularis* laying a recruitment trail. **a**, Footprints (marked by arrows) can be seen on either side of the four parallel lines left by the hairs on the posterior edge of tergite VI. **b**, Enlargement of the area marked by dotted lines in Fig. 2a.

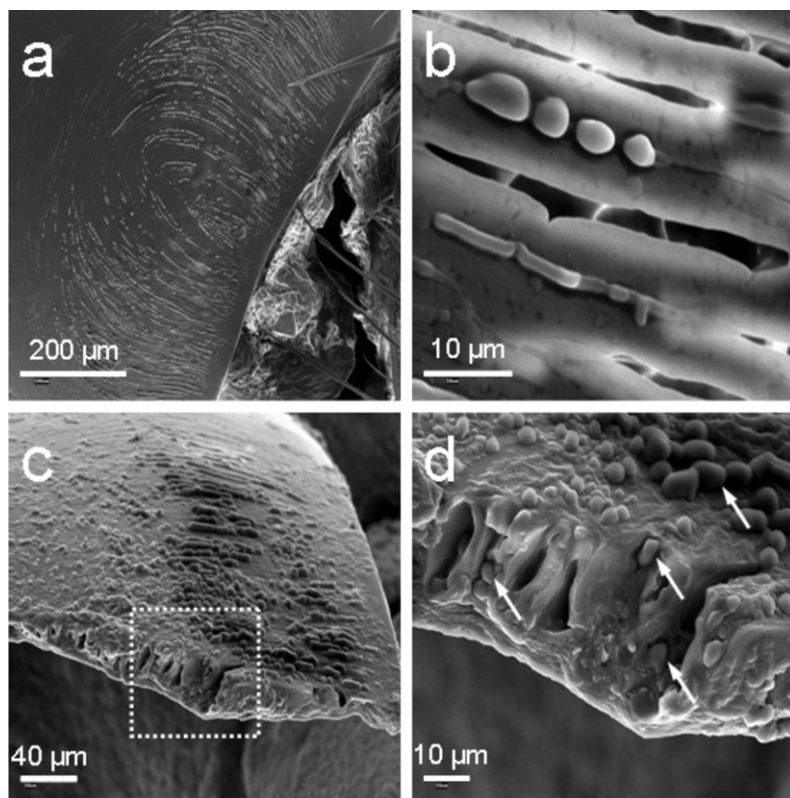


Fig. 3. Cuticular structure of tergite VII, the pygidium of *Pectroctena mandibularis*. **a**, The anterior edge of the tergite lies to the right. Note the setae, some of which are more robust, on the hind margin of the previous segment. **b**, Surface of tergite VII, showing openings of cavities in the cuticle, some of which have globular material protruding from them. **c**, The fractured edge of tergite VII of *P. mandibularis*, showing cavities within the cuticle. **d**, Enlargement of the area marked by dotted lines in Fig. 3c, showing globular material (marked by arrows) inside the cavities and protruding from their openings.

tergite VII, the pygidium, revealed a median, semicircular, fingerprint-like structure on the anterior edge (Fig. 3a), just posterior to a narrow band of smooth cuticle. This smooth band and the lamellar structure were exposed only during trail-laying; when not in use they were covered by tergite VI. The lamellae were the openings of crevices (Fig. 3b–d) that often had globular material protruding from them, giving the impression of ridges (Fig. 3). The openings were somewhat larger nearer the anterior margin (Fig. 3a). Fracturing the tergite across the lamellae revealed that the crevices were deep and restricted to the lamellar region (Fig. 3c,d).

Scanning electron micrographs of the four hairs, on the posterior edge of tergite VI, responsible for trail-laying, revealed grooves running the length of the hairs (Fig. 4a). Fluted hairs were not observed on other parts of the body.

DISCUSSION

Both queens and workers of *P. mandibularis* are clearly capable of recruiting nestmates of either caste. However, this behaviour occurs only during colony relocation, and not during foraging. This suggests that the evolutionary origin of recruiting behaviour in the Formicidae in general may have been more driven by the need to move nests than by the needs of foraging. Foraging involving recruitment appears to be associated with larger colony sizes (Traniello 1989), probably not a characteristic of ancestral ant societies.

Although workers lay a trail when they are foraging or exploring new areas, this seems to be for their personal orientation only. Such individualized trails have been reported in *Pachycondyla tesserinoda* (Jessen & Maschwitz 1985). In *P. mandibularis*, the sting appears to be involved in trans-

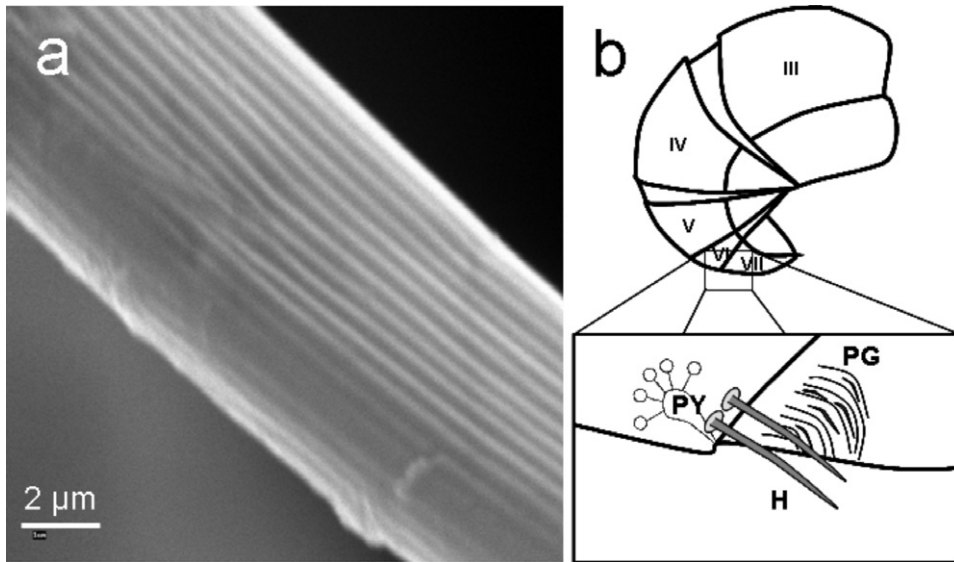


Fig. 4. **a**, Grooves on one of the hairs on the posterior edge of tergite VI of *Plectroctena mandibularis*. **b**, Schematic lateral view of abdominal segments III–VII of *P. mandibularis*. The pygidial gland (PY) releases pheromone that moves into the cavities in the cuticle of the pygidium (PG). During recruiting, pheromone is released onto hairs (H) on the margin of the previous tergite, which apply it to the surface being marked.

ferring the trail pheromone to the ground, so that the source of the trail could be the Dufour's gland, the poison gland, the hindgut, or some combination of these (Hölldobler & Wilson 1990; Vander Meer *et al.* 1998).

We postulate that the trail marker is a pheromone produced by the pygidial gland, which opens on the intersegmental membrane between tergite VI and VII (Hölldobler & Engel 1978; Villet *et al.* 1984). In *Plectroctena strigosa* a similar structure with deep cavities is present on the pygidium (Hölldobler & Engel 1978). When the abdomen is in its normal resting position, contact between the duct openings in the intersegmental membrane and the openings of the lamellae transfer pheromone from the pygidial gland into the cavities within the cuticle of the pygidium (Fig. 4b). The crevices thus act as a reservoir. When the abdomen is inverted during recruitment trail-laying (Fig. 1), gravity could cause the pheromone to leak from the exposed reservoir onto the four fluted hairs (Fig. 4b), which transfer it to the ground as they are dragged along.

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The fluting on the hairs might assist this process.

Plectroctena is closely related to *Pachycondyla* (Bolton 1974). The pygidial gland is used during tandem running in *Pachycondyla obscuricornis* (Traniello & Hölldobler 1984), and to recruit foragers in *Pachycondyla laevigata* (Hölldobler & Traniello 1980). We suggest that tandem running is an evolutionarily derived behaviour that may have developed from trail-following behaviour like that observed in *P. mandibularis*. Tandem running would save pheromones because they are not deposited on the substrate.

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