Glandular sources of recruitment, trail, and propaganda semiochemicals in the slave-making ant *Polyergus rufescens*

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Received 14 August 2001, accepted 9 October 2001

The chemical sources involved in raiding activities of the slave-making ant *Polyergus rufescens* were investigated in the laboratory. In particular, we identified the sources of substances the raiding workers employ to convey information to nestmates and to disrupt reactions by defending workers during host nest invasion. Different secretions were offered to the dulotic ants on the tip of a blotting paper strip inserted a few centimetres into the nest entrance. The Dufour’s gland secretion induced the characteristic mass exit from the nest and is likely the source of a recruitment signal. Moreover, laboratory tests with secretions from different anatomical structures revealed that the trail signals for homing raiding workers are conveyed by their hindgut contents released during the outbound trip. Finally, to ascertain if *P. rufescens* raiding workers use “propaganda” pheromones, the behavioural response of the *Formica (Serviformica) cunicularia* host workers to different secretions of the slave-makers was investigated. Only mandibular secretions caused the typical panic reactions shown by residents at the moment of nest invasion. This probably facilitates both nest invasion and brood sacking by raiding workers.

**KEY WORDS:** *Polyergus rufescens*, recruitment, trail-following, propaganda, Dufour’s gland, hindgut, mandibular glands, pygidial gland.

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INTRODUCTION

The complex organization of ant societies depends on the efficiency of different forms of communication, involving a great diversity of cues, such as chemical, acoustical, tactile and visual (Hölldobler & Wilson 1990). Olfaction is the principal sensory modality involved in integration and social coordination in ants. Hence, the complex interactions among nestmates in a colony are either partially or completely dependent upon semiochemicals (Attygalle & Morgan 1985, Traniello & Robson 1995). Chemicals discharged from various exocrine glands that induce a specific behavioural response in members of the same colony are referred to as "pheromones" (Karlson & Lüscher 1959).

Like most communication patterns, recruitment and trail-following are mediated by pheromones. The definition of recruitment has varied greatly: here, we refer to recruitment as the induction of nestmates to leave their nest, assemble and perform a task at a target area. In this context, a chemical signal may stimulate ants that are inactive or engaged in some other work to leave their nest and form part of a newly-mobilized group.

Ants have evolved an astonishing array of strategies to recruit nestmates. The behavioural mechanisms involved in recruitment communication have been investigated and different organizational levels of recruitment behaviour have been described and arranged in a series of increasing complexity (Hölldobler 1978, Hölldobler & Wilson 1990). Various ant species rely on idiosyncratic combinations of different cues to achieve this aim; however the chemical cue most likely prevails (Hölldobler & Wilson 1990).

In trail communication, already excited individuals are led (along a trail) to the target area where their cooperation is required (Hölldobler & Wilson 1990, Traniello & Robson 1995). This pheromonal communication system is based on the release of chemicals from a variety of glands which then form a trail (Morgan 1990). Recruitment and trail-following behaviour are often used improperly as synonyms. This is because the chemical trace can serve (but not always) as a recruitment signal and an orientation cue. In the present investigation, we refer to trail-following behaviour as an activity in which the ants are able to follow chemical traces previously laid down by nestmates.

The aim of our investigations was to determine the sources of recruitment and trail semiochemicals in the formicine social parasite Polyergus rufescens, an obligate slave-making ant distributed throughout the European Palaearctic regions. This species exploits the labour of workers obtained as pupae during raids conducted against neighbouring colonies of related species (subgenus Serviformica) (Emery 1909). Hundreds of workers (specialized for this function) file out of their nest and conduct highly organized incursions against host colonies to pillage resident brood, from which they will obtain the slaves (Mori et al. 1991a, 1991b; Le Moli et al. 1994).

To attack and exploit newly discovered target colonies, P. rufescens requires both communication and orientation signals. Raiding activity begins early in the afternoon with a small number of workers (the scouts) that leave the nest searching for suitable host colonies. When they return home after a successful trip, they mobilize their nestmates for the raid organization. This phase is followed by pre-raid activity during which recruitment occurs: the excitation of a few workers (the first to get in touch with the scouts) provokes the mass exit of other raiding workers. A few minutes later, hundreds of workers mill frantically around the nest.
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entrance until they converge on a distinct direction and form a close-packed outbound column (Mori et al. 1991a).

The ethological aspects of raid organization of *P. rufescens* have been studied intensely and extensively in the field (Czechowski 1975; Dobrzanski & Dobrzanska 1978; Mori et al. 1990, 1991a, 1991b; Grasso et al. 1994, 1997; Le Moli et al. 1994). However, to date there have been no studies concerning the strategies adopted by *P. rufescens* scouts to elicit recruitment of nestmates. In particular, little is known about the sources of chemical signals that trigger and coordinate this behaviour. Recruitment could be influenced by the contact rate, as demonstrated in other ant species (Holldobler 1999). However, we believe that chemical signalling plays a more important role. In fact, we suggest that the pre-raid phase is mediated by chemical signals inducing raiding workers to start out on the incursion (Le Moli et al. 1994).

As demonstrated by our previous studies, raiding columns of *P. rufescens* orientate by means of trail pheromones, in addition to celestial cues. Whereas during the outbound trip the raiding workers do not follow any chemical trail, during the inbound trip they strongly rely on the trail deposited during the outbound journey (Le Moli et al. 1994).

The quickness of brood pillage in the invaded colony prompts us to hypothesize the use of “propaganda” substances (sensu Regnier & Wilson 1971) by the invaders. In fact, in several slave-making species, the use of overt aggression has been replaced by chemical weapons. This kind of strategy is known in *Formica subintegra*, *F. pergandei* (Regnier & Wilson 1971) and *Harpagoxenus* spp. (Allaway 1979). Raiding workers of these species only rarely attack the workers of the raided colony. Instead, they induce panic in the target nest and any defenders that do not attack are subsequently attacked by their own nestmates (Bradshaw & Howse 1984). Chemical substances employed in this context are defined as “propaganda” pheromones, chemicals utilised in interspecific communication which induce a response by the receiver that is adaptive only for the emitter (Brown et al. 1970). A further aim of this work was to determine the source of probable propaganda substances used by the *P. rufescens* raiding workers to facilitate invasion of the host nest and sacking of the brood.

Different anatomical structures have been identified in various ant species as sources of recruitment and trail pheromones. It is assumed that these communication patterns have evolved several times independently in the different ant subfamilies (Holldobler & Wilson 1990).

In Formicidae, the exocrine glands involved in the production of recruitment substances are the pygidial gland (Maschwitz & Schönegge 1977, Holldobler 1982), poison gland (Käib & Dittebrand 1990, Holldobler et al. 1995), Dufour’s gland (Wilson 1962, Cammaerts-Tricot 1974) and the hindgut (Robson & Tranello 1998). Sources of trail pheromones are the venom gland in Myrmicinae (except for the genera *Monomorium* and *Solenopsis* in which Dufour’s gland is involved), pygidial gland in Ponerinae, Pavan’s gland in Dolichoderinae, postpygidial gland in Aenictinae, and the hindgut in Formicinae (Biller & Morgan 1998). However, while recruitment and trail communication have been described in several species, propaganda pheromones are known only for a few slave-making ants belonging to the subfamilies Formicinae and Myrmicinae, the workers of which employ these substances from their Dufour’s glands to subdue the target colonies.

To date, little is known about these communication patterns in *P. rufescens*. In particular, no attempt has been made to identify the sources of chemical signals
involved in these contexts. In this paper, we present a detailed investigation of the recruitment, trail-following and propaganda behaviour of this slave-making species.

MATERIALS AND METHODS

Recruitment

For laboratory studies on chemical recruitment communication, two colony fragments of *P. rufescens* were collected in the Apennines at an altitude of about 700 m (in the Province of Parma, Northern Italy). Each fragment was composed of about 500 slave-makers, 400 slaves (*Formica cunicularia*) and brood. In the laboratory, they were kept in elliptical plaster arenas (50 × 70 cm); a nest chamber was excavated in one end of the arena and then covered with a red glass plate to provide the ants with a darkened nest cavity. The inside of the vertical plastic walls lining the arenas was coated with paraffin oil to prevent the ants from escaping. Colonies were kept in the laboratory at about 24 °C with a natural light/dark cycle. When not being tested, the ants were supplied with a sucrose solution, dead arthropods and water.

To test the recruiting effect of the contents of some potential sources, we recorded the response of *P. rufescens* workers to extracts of the hindgut and Dufour's, poison, pygidial, and mandibular glands. Six specimens of each type of organ were dissected out of *P. rufescens* workers, which had been killed by freezing, and placed in 300 µl hexane (a suitable solvent to extract the different glandular contents). Five µl of extract (0.1 gland equivalent) were deposited at the end of a blotting paper strip (10 × 0.5 cm), which was then presented at the nest entrance for 5 min. During this period, the number of ants leaving the nest was counted. As control, a blotting paper strip treated only with hexane was presented to the ants. Each type of experiment was repeated 8 times.

Trail-following

For the behavioural tests, we collected a colony fragment consisting of approximately 700 *P. rufescens* workers, 200 slaves (*F. cunicularia*) and brood in a field near Piacenza (Northern Italy). The colony fragment was brought immediately to the laboratory and housed in a circular plastic container, which served as a nest (Ø 20 cm), covered by a red-glass plate. The nest was placed at one end of a big plastic arena (450 × 70 cm) while, at the other end, a colony fragment of the host species *F. cunicularia* (300 workers and brood) was housed in another plastic nest and replaced daily after the raid. This apparatus allowed us to re-create the necessary conditions for raiding activities (LE MOI et al. in press). Colonies were maintained as previously described.

The experimental determination of the origin of the trail pheromones involved bioassay studies in which suspensions of the potential sources were presented to the ants. The following organs were tested: pygidial, poison and Dufour's glands, hindgut and tarsi. The tarsi were tested because raiding workers rub their legs against the tip of their gaster before leaving the nest. In this way, they might imbue their tarsi with a secretion from a gastral gland and deposit it on the ground (as footprints) during the outbound trip. Artificial trails (about 10 cm) were made using three specimens of each type of organ (but 18 tarsi corresponding to three individuals), crushed in 100 µl distilled water and applied to the substratum with a fine brush (along a pencil line) in front of the inbound raiding workers (perpendicular to their direction). The arena floor was covered with a new paper sheet after each raid. The behavioural response of the ants was recorded with a VHS video system. This allowed us to count all ants that were induced to deviate from their route and to follow the artificial trail. For each trial, a mean of 218 (± 44) individuals was tested. Trails of pure water were also tested.
Propaganda

Three artificial colony fragments of *Formica (Serviformica) cunicularia*, each containing 350 workers and abundant brood, were housed in the laboratory in cylindrical plastic boxes (Ø 20 cm, height 4 cm), covered by a red-glass plate. Colonies were maintained as described in the previous section.

The artificial nests were divided into four identical sectors, each provided with a fissure. In order to test the panic effect of some secretions of *P. rufescens* raiding workers, the response of *F. cunicularia* workers to the hindgut material and to the mandibular, Dufour's, poison and pygidial gland contents was recorded. Three specimens of each type of organ were crushed at the end of a blotting paper strip (5 × 0.5 cm), which was then introduced into one of the fissures, chosen randomly. In this case, no solvent was used because a dilution of the glandular secretions was not needed. For 3 min thereafter, the reaction of the residents was video-recorded. The orientation of the reaction (expressed by the number of individuals counted every 30 sec in the experimental sector) and excitement level (expressed by the linear speed of three ants randomly chosen from those in the experimental sector at the beginning of the trial) were recorded.

Controls were performed by introducing an untreated blotting paper strip into the fissure. For each type of test, six trials were conducted.

RESULTS

Recruitment

The recruitment effect of the extracts is presented in Fig. 1. The number of recruited ants differed significantly among treatments (0.01 < *P* < 0.02; Kruskal-Wallis test). In particular, the Dufour's gland secretion caused a significant increase of the number of exits (72.75 ± 13.22) compared to controls (14.37 ± 3.99; *P* < 0.05; Two-tailed Multiple Comparisons test). By contrast, the responses to extracts from the hindgut (59.25 ± 21.23) and the poison (15.12 ± 1.87), pygidial (35.87 ± 11.13) and mandibular (17.25 ± 2.69) glands did not differ from controls (Two-tailed Multiple Comparisons test). Moreover, only the Dufour's gland contents elicited the typical patterns of behaviour performed by the ants in the field before a raid (see Mōri et al. 1991a, 1991b; Le Molî et al. 1994). The ants appeared very excited and left the nest *en masse* to mill around. Most of the recruited raiding workers performed a peculiar movement (also recorded in the field during the frantic pre-raid phase) consisting in rubbing their legs against the tip of their gaster.

Trail-following

Trail-following responses of the ants to different secretions are illustrated in Fig. 2. The percentage of ants following artificial trails made from extracts of the pygidial gland (4.7%, 7/148), poison gland (2.3%, 5/213), Dufour's gland (2.3%, 6/262) and tarsi (1.8%, 4/217) were not significantly different from controls (0.4%, 1/264) (χ² test). By contrast, the percentage of ants following the artificial trail was
Fig. 1. — Mean number of *P. rufescens* workers recruited by hexane (controls), hindgut contents, or poison, mandibular, pygidial or Dufour’s gland secretions.

Fig. 2. — Percentage of *P. rufescens* workers induced to abandon natural trails and to rely on artificial ones made of pure water (controls), hindgut contents, tarsi, or poison, pygidial or Dufour’s gland secretions.
significantly higher in response to the hindgut contents (47.3%, 61/129) than to controls and the other secretions ($P < 0.001$; $\chi^2$ test). Almost half of the raiding workers encountering the hindgut artificial trail left the natural path to move along the artificially marked trail, performing the typical trail-following behaviour, while the other half continued to move on the natural trail.

**Propaganda**

The results are presented in Figs 3 and 4. All the secretions (except the hindgut contents) induced an increase of locomotor activity of the resident ants (*Formica cunicularia*). The linear speed of the ants differed significantly among treatments ($P < 0.001$; Kruskal-Wallis test). In particular, extracts of the mandibular glands caused a strong panic reaction in resident workers. In this case, not only the linear speed ($0.75 \pm 0.05$ cm/sec) (see Fig. 3), but also the number of workers that abandoned the experimental sector (see Fig. 4) was statistically higher [$P < 0.05$ (Two-tailed Multiple Comparisons test) in the former case, $P < 0.005$ (t-test) in the latter] than in the controls and the other treatments. Moreover, before fleeing, several *F. cunicularia* workers seized cocoons with their mandibles, as usually observed in the field when the raiding workers enter the target host nest (see MÔRI et al. 1991a, 1991b).

Quantitatively and qualitatively different reactions were induced with the other secretions. Although the linear speed of *Formica* workers recorded in trials

![Fig. 3. — Mean speed of Serviformica workers in response to untreated blotting paper strips (controls), hindgut contents, or poison, mandibular, pygidial or Dufour’s gland secretions.](image-url)
with Dufour’s (0.28 ± 0.04 cm/sec), poison (0.27 ± 0.03 cm/sec) and pygidial gland secretions (0.33 ± 0.06 cm/sec) was high (0.04 ± 0.01 cm/sec; \(P < 0.05\); Two-tailed Multiple Comparisons test) (see Fig. 3), the direction of the ants’ reaction (towards or away from these secretions) was not statistically different from control trials (t-test) (see Fig. 4).

**DISCUSSION**

The survival of a dulotic society is based on a particular resource: the host colonies. To better exploit this resource, *Polyergus rufescens* has evolved both morphologically and behaviourally. This parasitic species has evolved a fast and efficient communication system whereby workers are mobilized for raiding activities. The results of our experiments demonstrate that this recruitment process is mediated by the emission of chemical signals produced by Dufour’s gland and released by successful scouts and probably also by recruited workers during raiding column organization (secondary recruitment).

By introducing Dufour’s gland extracts into the artificial slave-maker nest, we were able to induce the typical exit flow of the raiding workers, which previously were quite inactive and grouped together. Once outside the nest, the artificially recruited raiding workers performed a peculiar pattern of movements consisting in rubbing their legs against the tip of their gaster, a behaviour typical of *P. rufescens* raiding workers just before the raid (Grasso 1993).
If group recruitment is generally considered intermediate — in evolutionary terms of efficiency and complexity — between tandem-running and mass recruitment (Wilson 1971, Möglich 1979, Hölldobler 1984a), the case of P. rufescens could be considered an additional level of recruitment between group and mass recruitment. In fact, the gathering of hundreds of raiding workers involved in column formation is similar to typical mass recruitment. However, recruits are led to the target colony by a “signaller ant” (the scout), which is typical of group recruitment (Mori et al. 1990, 1991, 1991b; Grasso et al. 1994; Le Moli et al. 1994).

The relatively high number of ants recruited with hindgut contents (cf. Fig. 1) could be explained by the results of the experiments on trail markers (cf. Fig. 2). They show that the hindgut contains the trail pheromones by which workers of P. rufescens mark the outbound path of slave-raids, which is subsequently followed during the return trip.

Although there are considerable differences in the sources of pheromones in the different subfamilies, the trail pheromones of most Formicinae ants (to which P. rufescens belongs) originate from the hindgut (Hölldobler & Wilson 1990). Comparative studies suggest that Formicinae ants have evolved chemical trail communication by a gradual ritualization of the defecation process (Hölldobler 1984a). Generally, the ants discharge their hindgut contents at frequent intervals. However, in many species, workers do not defecate randomly but defecate preferentially at specific sites (certain locations within the nest, nest borders, garbage dumps and the borders of trunk trails leading to permanent food sources), where chemical cues can be perceived successively and used for home range orientation and trail communication (Hölldobler & Wilson 1990).

The results of experiments conducted with tarsi suggest that the characteristic “leg rubbing” shown by raiding workers before the raid is not to imbue their legs with some gastral secretions, as suggested by Hasegawa & Yamaguchi (1994) for P. samurai. Instead, this behaviour could be to clean the bristles surrounding the tip of their gaster which might function in the trail-laying process.

In a comparative study of the strategies adopted by different slave-making ant species in the organization of their raiding activities, Buscinger et al. (1980) hypothesized that Polyergus spp. could also employ propaganda pheromones to disrupt host colony defence. Our previous field studies supported the hypothesis that Polyergus workers use pheromones to prevent the organization of defence by the host species and to disperse the defenders (Mori et al. 1991a, 1991b; Le Moli et al. 1994). In fact, raiding workers generally spend only a few minutes pillaging the brood inside the attacked nest, while residents escape by scattering in all directions.

Our present results show that the mandibular gland secretion of P. rufescens raiding workers provokes a typical strong panic reaction among the resident Serviformica workers. During tests with this glandular extract, they became alarmed and rushed away, avoiding the experimental blotting paper as soon as the secretion was offered to them. Moreover, in accordance with our observations in the field, several resident ants seized cocoons and escaped with them. No significant reactions were induced by secretions from the other sources examined. Hence, we suggest that mandibular glands are the source of propaganda pheromones (sensu Regnier & Wilson 1971) by which P. rufescens raiding workers avoid fierce attacks and disturbance by residents during host colony invasion. It is also possible that, in addition to dispersing the resident defenders, the compounds of this glandular secretion also attract the slave-makers, as demonstrated by Regnier & Wilson (1971) for two dulotic species of the Formica sanguinea group. Finally, we cannot exclude that at
least part of the panic reaction of the raided Serviformica colonies is caused by an alarm pheromone discharged by resident workers themselves, as suggested by Topoff et al. (1989) for slave-raids of the American species P. breviceps.

Holldobler (1984b) first discovered a pygidial gland in Polyergus spp., the only case known to date in formicine ants; its function could have evolved in connection with the highly specialized slave-raiding behaviour of these parasitic species. The author suggested that this glandular secretion might function as a kind of propaganda pheromone or as an arousal signal discharged by the leader ant at the front of the raiding column. In our experiments we found no propaganda effect of this glandular secretion. However, we cannot exclude an involvement of the pygidial gland in raid organization. Given its peculiar anatomical position (between the last two gastral tergites), the pygidial gland of P. rufescens probably evolved to produce semiochemicals secreted by raiding workers during the outbound trip in order to maintain close-packing of the swarming column and ultimately acting as short-range attractants for neighbouring nestmates.

The use of this kind of propaganda pheromone may be regarded as an additional specialization in the slave-making habitus evolved by P. rufescens to reduce casualties and, above all, to make invasion of a colony and brood sacking an extremely rapid process (on average only a few minutes) (Mori et al. 1991a, 1991b; Le Moli et al. 1994). Bradshaw & Howse (1984) suggested that the metabolic cost to the raiding workers of releasing such large amounts of chemicals must be high and that this could be counterbalanced by a reduction of casualties. Thus, a main advantage of this chemical warfare could be conservation of resources in proximity to the dulotic nest: workers from the raided colony are not killed and can return to their nest to rear the young brood left behind by the slave-makers.

Hence, also in P. rufescens — as in other ant species (see Attygalle & Morgan 1985, Holldobler 1995) — a multisource system produces various semiochemicals, which act synergistically or consecutively to control a complex pattern of behavioural processes such as slave-raiding activities.

ACKNOWLEDGEMENTS

We are grateful to C. Baroni Urbani and an anonymous referee for their helpful comments and suggestions on an early draft of the manuscript. We wish to thank Miss A. Negri for helping us to collect the ants in the field and to record some data in the laboratory. This research has been supported by grants from the Ministero dell’Università e della Ricerca Scientifica e Tecnologica (ex-40% and ex-60% funds) assigned to F. Le Moli.

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