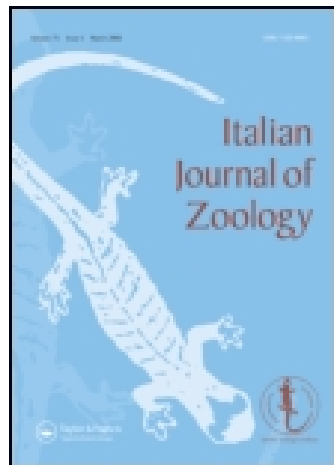


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The mandibular glands as a source of sexual pheromones in virgin queens of *Polyergus rufescens* (Hymenoptera, Formicidae)

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INTRODUCTION

Hölldobler & Bartz (1985) designated two types of mating strategies in ants: the 'male-aggregation syndrome' and the 'female-calling syndrome'. The former is typical in ant species that have nuptial flights where numerous colonies simultaneously release young winged queens and males over a large area. Often, the males gather in specific mating sites where they attract conspecific females through sexual pheromones (Hölldobler, 1976; Bourke & Franks, 1995; Ayasse *et al.*, 2001). One consequence of this mechanism is the low probability of inbreeding owing to the large scale and to the infrequency of nuptial flights. This strategy may furthermore serve as a defensive strategy against predators, or have a dilution effect (Bourke & Franks, 1995). The female-calling syndrome is characterized by females that disperse a short distance from the natal nest, and then emit sexual pheromones from the ground or low vegetation to attract conspecific males (Hölldobler & Wilson, 1990; Bourke & Franks, 1995; Ayasse *et al.*, 2001). This pattern is typical of phylogenetically primitive species with relatively small, scattered colonies that usually do not produce a large number of sexuals, as is the case of some ponerine and leptothoracine species (Bourke & Franks, 1995). This strategy probably maximizes the female's chance of finding a mate when both sexes are at low densities. The female-calling syndrome may also be adaptive for scattered colonies associated with a patchy habitat; female-calling enables a low dispersal rate and so reduces the need for leaving a habitat that is good in terms of resources. This probably explains why this behaviour is widespread among the socially parasitic ants, which require colonies, an essential resource for their survival (Buschinger, 1971, 1975; Buschinger & Alloway, 1979).

The slave-making genus *Polyergus* is one of the most specialised social parasites (cf. Topoff, 1990; Mori *et al.*, 2001). Ants of this genus (the Amazon ants) are all obligatory slave-makers, which periodically organize group raids against colonies of the related genus *Formica* to sack the resident brood. A significant portion of the plundered brood is reared to eclosion and integrated into the work force of the parasitic colony to perform all domestic tasks (Buschinger *et al.*, 1980; Le Moli, 1980; Hölldobler & Wilson, 1990; Mori *et al.*, 1991).

Colony founding is another biological context where the socially parasitic nature of this species is evident. Because queens are not able to rear their own brood and start a new colony alone, they must invade a *Formica* colony, kill the resident queen(s) and become accepted by the resident workers (Topoff *et al.*, 1988; Mori *et al.*, 1994, 1995, 2001). It could thus be adaptive for newly-mated queens to follow a raid and exploit the panic and disorganization of the host colony during *Polyergus* raiding swarms (Talbot, 1968; Topoff & Greenberg, 1988; Mori *et al.*, 2001).

Winged queens of *P. breviceps* for the most part do not take part in nuptial flights, but instead they take

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ABSTRACT

'Female-calling syndrome' is the reproductive strategy adopted by the European slave-making ant *Polyergus rufescens*, where a winged female disperses a short distance from the nest, ascends to an elevated position (at the top of a blade of grass) and is joined shortly thereafter by conspecific males. This suggests the probable use of very effective sexual calling pheromones by females. Here, the role of the mandibular glands of winged females is demonstrated to be a source of sexual pheromones. The secretion of these glands (among the structures tested: the mandibular, Dufour, poison and pygidial glands and the head without mandibular glands) appears to have a strong attractive effect on males and also elicits male copulatory behaviour.

KEY WORDS: Slave-making ant - Female-calling syndrome - Sexual pheromones - Mandibular glands - *Polyergus rufescens*.

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part in raids, where they release mandibular pheromones to call conspecific males. After mating, queens shed their wings and rejoin the raiding swarm (Topoff & Greenberg, 1988). In *P. lucidus*, the queens perform nuptial flights, and, after mating, search for a conspecific colony, not necessarily their natal one, where they then follow a slave-raid (Talbot, 1968; Marlin, 1971; Kwait & Topoff, 1984). Dealate *P. samurai* females have also been observed to join raiders after mating flights (Hasegawa & Yamaguchi, 1994).

Polyergus rufescens Latreille 1798 females, however, adopt a more complex strategy, because they may copulate after mating flights, during slave raids or on the ground a few meters from their nest. In any case, females show the same typical female-calling behavioural pattern: they ascend a blade of grass and then open their mandibles. A short time after, males arrive and attempt to mate (Grasso *et al.*, 1994; Mori *et al.*, 1994). This makes mandibular glands a possible source of sexual attractants as shown by Topoff & Greenberg (1988) in *P. breviceps*. Because multiple mating has rarely been observed, *P. rufescens* reproductive behaviour may be considered similar to the "female-calling syndrome". After any of the mating strategies adopted, *P. rufescens* females may then take part in slave-raids (Mori *et al.*, 1994).

The present study was designed to identify the glandular source involved in the production and release of sexual pheromones used by *P. rufescens* virgin queens when attracting mates. Therefore, the behaviour of *P. rufescens* males exposed to the influence of different female glandular secretions was studied.

MATERIALS AND METHODS

Winged females of *P. rufescens* were collected during the nuptial flights period (July) from a colony situated in a forage field in the Apennines in the Province of Parma (northern Italy) at an altitude of ca. 700 m a.s.l. Males of *P. rufescens* were collected from a different colony located in the same area. In addition to the reproductives, individuals which included *P. rufescens* workers, *F. cunicularia* host workers, and brood from both colonies were also taken. In the laboratory, colony fragments were kept at 24-26 °C with 60-80% relative humidity. Ants were fed honey and insects; water was provided daily.

The experimental device consisted in a Y-shaped wind tunnel made of transparent Plexiglas (Fig. 1). Each arm ended in a sliding wall with a round hole that was closed by a thin meshed wire net to prevent ants escaping while, at the same time, allowing air to pass through. At the end of the lateral arms, supports were placed for the substances to be tested. At the beginning of each experimental trial, two electric fans (diameter 11 cm) provided a slight and continuous one-way current of air (velocity 2 m/s) moving from the two lateral arms to the central one and then going out through the hole in its end wall.

The behaviour of the males was recorded in response to the secretions from the following structures of winged females: the head (without mandibular glands), mandibular glands, Dufour, poison, and pygidial glands. Prior to dissection (in distilled water), ants were killed by freezing for a few minutes. Dissected structures were then crushed in 1 µl of distilled water on a slide. One microliter of distilled water was used as control. For each condition, 12 replications were run.

The experimental procedure of a single trial was carried out as follows:

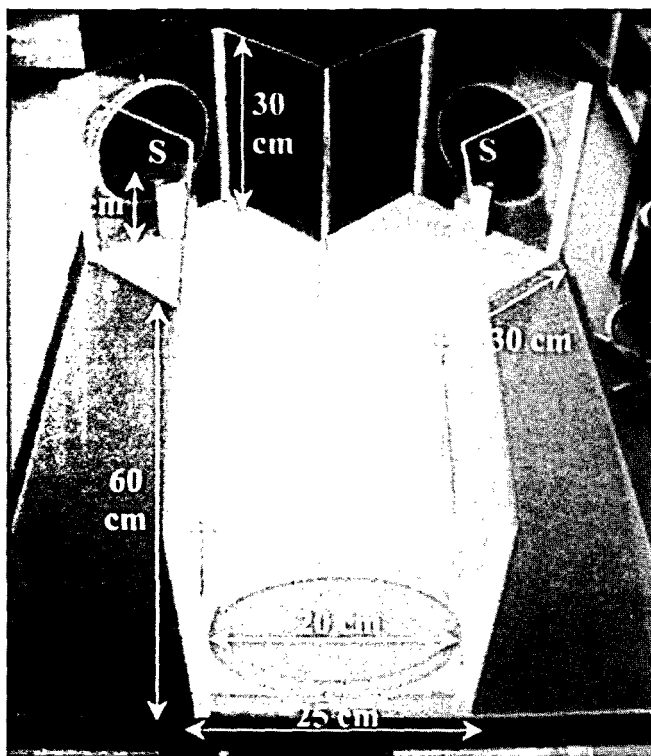


Fig. 1 - Experimental device. Y-shaped wind tunnel made with transparent Plexiglas. Dimensions of the device and the two supports (S) for the tested structures and controls are shown.

- i. after being confined in a transparent plastic jar for 5 min, a male of *P. rufescens* was introduced into the central arm of the wind tunnel;
- ii. the two slides containing the tested structure and the water control were placed each on top of one support in the lateral branches (Fig. 1). The sides of treatments and controls were alternated regularly, so that, for each condition, 6 males were tested with the experimental substance in one arm of the tunnel, and 6 males in the other arm;
- iii. the two electric fans were turned on and the tested structure was crushed in the distilled water droplet on the slide;
- iv. the male was allowed to exit the jar and its behaviour was observed. Primarily, a binary-choice test was performed on the particular arm chosen by the male and then it was recorded whether the male climbed the support to reach the slide once the particular arm had been chosen. Data were analysed by one-sample χ^2 -test.

Tests (each lasting 10 min) were conducted early in the afternoon, the period during which the daily activity is highest for both males and queens (see Mori *et al.*, 1994). At the end of each trial, the tested male was removed and the experimental device was cleaned thoroughly. At least 30 min elapsed between trials and a new male was used for each test ($n = 60$).

RESULTS

Males of *P. rufescens* showed a strong attraction towards mandibular gland secretions of conspecific virgin females. Highly significant results were obtained in the arm-choice tests involving mandibular glands (in comparison with controls) (χ^2 -test, $P = 0.004$). In these cases, 11 males (91.7%) chose the treatment arm (Fig. 2).

In the experimental series with secretions from pygidial, poison, Dufour glands and using the head, males did not show any preferences but indifferently reached both the experimental and control branches (45-55%; χ^2 -test, $P = 0.763$ throughout, $n = 48$) (Fig. 2).

When attracted by mandibular gland secretions, males appeared excited, rapidly moving their antennae and increasing their flight activity and locomotion. The experimental slides were the subject of great interest. In particular, 81.8% of males that chose the arm with mandibular secretions (9 out of 11 individuals; χ^2 -test, $P = 0.035$) climbed the support, and upon reaching the slide, explored it incessantly with their antennae. Four males (33.3%) were clearly observed performing copulatory movements: a leaning forward of the abdomen and an extrusion of the genital organs. None of these reactions were observed with controls or the other tested structures. In the latter experimental situations, after randomly choosing the arm, the males wandered about without reaching the slide.

DISCUSSION

The first step of colony life cycle in ants is the 'founding stage'. Generally, this phase begins with nuptial flights through which a colony disseminates its reproductives. Females and males, however, need to meet during this time, and their union is achieved via different strategies, with either the males or the females calling potential mates (Hölldobler & Wilson, 1990; Bourke & Franks, 1995).

In *P. rufescens*, virgin females call the males (Mori *et al.*, 1994). Results of the present investigation clearly demonstrate that mandibular glands are the source of signals with which females attract males for mating. This is consistent with data reported for the American species *P. breviceps* (Topoff & Greenberg, 1988) and with our previous field studies on the reproductive biol-

ogy of *P. rufescens* (Mori *et al.*, 1994). Once outside their natal nest, winged queens tend to reach elevated positions (e.g. the top of grass blades) where they open their mandibles, at the base of which the mandibular gland openings are situated (see Billen & Schoeters, 1994). This behaviour leads to the prompt arrival of a male and, immediately after, mating. Thus, it is likely that the sexual-calling behaviour of females involves the release of mandibular pheromone(s) which attract(s) their reproductive partners and can aptly be considered a 'female-calling syndrome' that is common among many primitive and socially parasitic ants (Hölldobler & Bartz, 1985; Hölldobler & Wilson, 1990; Bourke & Franks, 1995). This reproductive strategy is associated with some peculiar features: the number of reproductives produced per year tends to be relatively low; virgin queens do not disperse widely but stay relatively close to their natal nest and produce male-attractant pheromones, and nuptial flights are not well synchronized between sexes (Buschinger, 1975).

To date, the glandular origin of calling pheromones involved in the reproductive context has been identified for only a few species of ants (Hölldobler & Wilson, 1990). For example, in *Harpagoxenus canadensis* and *H. sublaevis*, both parasitic myrmicine ants, the male-attracting pheromones are produced in the female's poison gland and released from an extruding sting (Buschinger & Alloway, 1979). In *Rhytidoponera metallica*, queens call conspecific males by sexual pheromones produced in the pygidial gland (Hölldobler & Haskins, 1977), whereas in *Monomorium pharaonis*, the female calling-pheromones come from the Dufour gland (Hölldobler & Wüst, 1973). Generally, when the mandibular glands are involved as source of sex pheromones in ants, males are responsible for their emission. In species of the genus *Pogonomyrmex*, males meet in 'leks' and females reach them later, attracted by mandibular pheromones discharged by all the gathered males (Hölldobler, 1984). In the case of some formicine species such as *Camponotus berkeleyanus*, winged queens are induced to perform mass nuptial flights by secretions from male mandibular glands (Hölldobler & Maschwitz, 1965). By contrast, we verified that in *P. rufescens* mandibular sex attractants are released by females.

Our experiments also suggest further functions of the mandibular secretions of winged queens of *P. rufescens*. In addition to attraction towards a source of signals, mandibular gland secretion acts as a sort of aphrodisiac substance and elicits male copulatory patterns. As soon as males reached the experimental slide on which the glands had been crushed, they extruded their genital organs and performed typical gastral copulatory movements.

We demonstrated previously that mandibular gland secretions are used by workers of *P. rufescens* as propaganda allomones during slave-raids on host colonies of *F. cunicularia*. These secretions provoke a typical strong panic reaction among workers of *F. cunicularia*

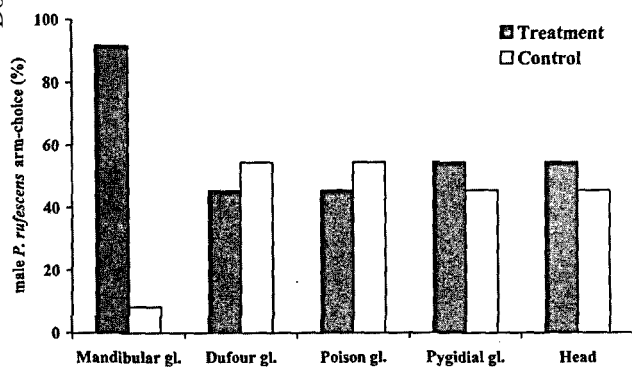


Fig. 2 - Graphical representation of male *P. rufescens* arm-choice (treatment vs control). In the y-axis, the percentage of males choosing the two alternative arms is reported. The five different tested structures are listed in the x-axis. The difference between choices is statistically significant only in trials with the mandibular glands as treatment (χ^2 -test, $P = 0.004$).

preventing them from organizing an effective defence against invaders (Visicchio *et al.*, 2001). It thus appears that the contents of the same anatomical structure (mandibular glands) is used by queens of *P. rufescens* in the homospecific context of reproduction, while it is used by workers of the same species in the heterospecific context of slavery. The next step will be the chemical identification of these signals in order to verify whether in *P. rufescens* the same substances may have different effects in different contexts (depending on the receiver), or whether behavioural responses of males and host workers are mediated by different semiochemicals produced by the mandibular glands of queens and workers, respectively.

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