Research article

Colony founding in *Polyergus rufescens*: the role of the Dufour’s gland

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**Summary.** In the European slave-making ant *Polyergus rufescens*, the occurrence of chemical strategies during the initial phase of dependent colony foundation or usurpation was investigated. To test this idea, we analysed the effect of the secretion of different glands (Dufour’s, poison, pygidial, rectal, and mandibular) on the behaviour of workers of its common host species, *Formica cunicularia* (subgenus *Serviformica*). Workers of another species, *Formica rufibarbis* (*Serviformica*), were daubed with these extracts, and introduced into colony fragments of *F. cunicularia*. The results of a set of laboratory aggression tests showed that the secretion of the mandibular, pygidial, rectal, and poison glands do not alter the characteristic aggressive reactions generally performed by resident workers against alien ants. By contrast, the Dufour’s gland seems to play a crucial role in the appeasement of residents of the target host colony. In fact, its secretion drastically lowers the degree of overt attacks shown by *F. cunicularia* workers against the intruders. This chemical strategy probably allows an easier invasion and usurpation of host colonies by newly mated females of *P. rufescens*.

**Key words:** Ants, *Polyergus rufescens*, colony usurpation, Dufour’s gland, appeasement allomone.

**Introduction**

Slavery (dulosis) in ants is a form of social parasitism (facultative or obligatory) occurring in eleven genera of the subfamilies Myrmicinae and Formicinae (Hölldobler and Wilson, 1990). These slave-making species regularly conduct raids against neighbouring colonies of their host or slave species. Typically, a swarm of raiders penetrates a target nest, repels or attacks the residents, and pillages all or part of the brood. In the dulotic nest, a portion of it is reared, and the slaves which eclose form a social attachment to the slave-makers, accepting them as nestmates (Le Moli and Mori, 1985, 1987a, b; Hare and Alloway, 1987). In the obligatory slave-makers, the worker caste has become so specialized for raiding activity that it is wholly dependent on slaves for colony maintenance. Moreover, slave-makers possess both morphological and behavioural adaptations which improve their raiding efficiency but, at the same time, attenuate their ability to carry out the normal domestic tasks (Sakagami and Hayashida, 1962; Wilson, 1971; Dobrzanska, 1978a, b; Buschinger et al., 1980; Stuart and Alloway, 1983; Mori and Le Moli, 1988).

The formicine ant genus *Polyergus* comprises five species, all obligatory social parasites, which conduct amazing raids against colonies of the related genus *Formica*. In particular, *Polyergus rufescens*, the so-called European Amazon ant, exploits the labour of *Serviformica* workers, belonging to the *Formica fusca* group, such as *F. cunicularia* and *F. rufibarbis*.

As is the rule in obligatory slave-makers, *P. rufescens* queens are unable of founding new colonies independently (because they are unable of rearing even their first brood) and must rely on a form of dependent colony foundation (Bourke and Franks, 1995). Before laying eggs, a newly-mated female must locate and invade a colony of the host species and usurp the role of reproductives. In particular, after the mating flights, dealate *P. rufescens* queens often return to a dulotic nest and follow raiding swarms (Mori et al., 1994). This is an advantageous strategy, because the invasion and subsequent usurpation of the host colony may be facilitated by the panic and disorganisation produced by raiding swarms (Talbot, 1968; Topoff and Greenberg, 1988; Mori et al., 1994).

The first critic phase of usurpation is the entrance into the target colony, since the parasitic queen must avoid the defensive behaviour of *Serviformica* workers. Previous laboratory experiments (Mori et al., 1995) have shown that, when a newly-mated queen of *P. rufescens* is introduced into an artificial nest of the host species *F. cunicularia*, the adult workers fiercely attack the intruder. The parasite female counter-attacks and, in a successful usurpation, detects the resident queen and kills her in a short time by using the sharp mandibles. The lack of the resident queen causes a change in
the behaviour of the resident workers. In fact, their attacks against the alien queen begin to alternate with grooming and, in few hours, the Polyergus female stands on the host brood surrounded with a court of Serviformica workers. These results are in agreement with those recorded in P. breviceps by Topoff and co-workers (1988). Nevertheless, the formation of an interspecific bond is an enigmatic matter and the capacity to gain acceptance of adult slave workers is one of the crucial steps in the evolution of slavery.

It is known that the cuticular hydrocarbon pattern of the P. rufescens queen before entering the Serviformica nest differs from that shown after the usurpation. In fact, the cuticular profile becomes more similar to that of the host species (D’Ettorre et al., 1996; D’Ettorre and Errard, 1998), and this strategy allows her not to be recognized inside the foreign nest. But another question is how she is able to avoid the violent aggressive reaction of the resident workers at the time of entrance. Surely, the panic caused by a raiding swarm and the probable overt attacks performed by intruders towards the residents make the usurpation process easier, but further mechanisms are likely to be involved.

The aim of this work was to clarify the chemical strategies adopted by the newly-mated females of the European Amazon ant during the initial phase of usurpation. In fact, it is possible that the P. rufescens queens rely on a process of code breaking in the chemical communication of their hosts. In this context, the effect of the secretion of the main gastral glands and of the mandibular gland of P. rufescens on the behaviour of workers of the common host species F. cunicularia was analysed. In particular, laboratory experiments were conducted to verify if these glandular secretions reduce the level of aggression displayed by Serviformica workers against alien ants.

Materials and methods

Colony fragments of Formica cunicularia, consisting of adult workers and brood (larvae and pupae within cocoons) were collected from a pure colony in Casola (province of Parma, Northern Italy). In the same area, newly-mated females of Polyergus rufescens and adult workers of F. rufibarbis were collected. Both slaves and slave-makers were kept separately under controlled conditions of temperature (24–26 °C) and relative humidity (60–80%), and were fed with water, honey and pieces of insects.

The extracts of Dufour’s, poison, pygidial, rectal, and mandibular glands were prepared crushing 4 glands of each kind in 200 μl of distilled water. The pygidial gland, which is difficult to dissect cleanly, was removed after separating the last two tergites of the ant’s gaster. Because of the same reason, the mandibular gland was removed by cutting the forepart of the ant’s head, including the mandibles.

Aliquots of 3 μl of each extract were put onto the thorax of workers of F. rufibarbis which under normal conditions would evoke overt attacks by F. cunicularia ants. According to the procedure followed by Topoff and co-workers (1988), we chose this indirect method of valuation because it was impossible to use P. rufibarbis queens for bioassays of the pheromones’ effectiveness; in fact, we could not keep them from secreting their own pheromones.

Afterwards, the daubed F. rufibarbis ants were individually introduced into artificial colony fragments of F. cunicularia consisting of about 50 workers and housed in circular plastic boxes (20 cm of diameter). As controls, F. rufibarbis daubed with distilled water were tested in the same way.

A 10-min “aggression test” (cf. Le Moli and Parmigiani, 1981) was conducted to record the behaviour of resident workers towards the intruder. In particular, using a series of electronic counters, the following indices were measured in seconds: mutual investigation (MI), i.e. the time spent in reciprocal inspection by the intruder and resident workers; latency to attack (LA), i.e. the time from the first contact to the first attack (when no attack occurred, 10 min latency was allocated); accumulated attacking time (AAT), i.e. the total time spent attacking.

The frequencies of some elements of aggressive behaviour were also recorded (Wallis, 1964; De Vroe, 1980; Le Moli and Parmigiani, 1981): startle response, threat with open mandibles, upright posture, gaster flexing, seizing and dragging. In particular, startle response, threat and upright posture are patterns peculiar to a ritualized fighting (agonistic behaviour), whereas the other items characterize the overt aggression (conflict behaviour). Moreover, the number of attacks (No. A) delivered was counted.

A different group of F. cunicularia and F. rufibarbis workers were used for each test.

Results

The effect of five glandular secretions on the aggressive behaviour of F. cunicularia towards treated F. rufibarbis is illustrated in the diagrams depicted in Figure 1 and in Table 1.

In particular, F. cunicularia ants always showed fierce aggressive reactions towards F. rufibarbis workers daubed with poison, pygidial, rectal, and mandibular gland secretions. In fact, the intruder (used as a vehicle of these substances tested one at a time) was immediately attacked by the residents. In these trials the time spent in mutual investigation and, consequently, the duration of latency to attack were very short. By contrast, the accumulated attacking time and the number of attacks delivered attained very high values. Generally, the differences between mutual investigation, latency to attack, accumulated attacking time and number of attacks values recorded in these trials and the controls are not statistically different (Two-tailed Mann-Whitney ‘U’ test; cf. Fig. 1).

Only the Dufour’s gland secretion protected the intruding ants from the incessant aggression by resident workers. F. rufibarbis smeared with this secretion were free to move inside the artificial colony and even allowed on the top of the brood. In particular, it is worth nothing that in these trials the time spent in the attacks (AAT) towards the intruder resulted statistically lower (p < 0.002) in comparison with the control, as were the number of attacks delivered (p < 0.02; median values: 2.75 in experimental test; 6.25 in control test). In fact, the attacks towards the F. rufibarbis intruders were short and rare. Moreover, a longer reciprocal inspection and prolonged latency to attack were recorded, resulting statistically higher (for both p < 0.002) in comparison with the control. Two-tailed Mann-Whitney ‘U’ test was used for all these comparisons.

Concerning the elements of aggressive behaviour, the secretion of the poison, pygidial, rectal, and mandibular glands did not alter the characteristic aggressive reaction generally performed by Serviformica workers against alien ants. In fact, these extracts showed results statistically similar to the controls (Fisher exact probability test). By contrast, the use of the Dufour’s gland secretion caused a more fre-