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Postpharyngeal Gland Secretion as a Modifier of Aggressive Behavior in the Myrmicine Ant *Manica rubida*

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The role of postpharyngeal gland secretion in nestmate recognition was tested in Manica rubida. *Behavioral tests comprised two consecutive encounters between five ants and their color-marked nestmate. The first encounter utilized an untreated ant constituting a control, whereas in the second encounter the marked ant was treated with postpharyngeal gland exudate. Scoring was done using an aggression index obtained by direct observation and an agitation index deduced from a frame-by-frame analysis of videotapes of the various tests. When the glandular secretion originated from an alien ant, the ants became very agitated and were aggressive toward their nestmate. When the exudate originated from a nestmate, the ants generally remained calm, although their rates of self-grooming increased.*

KEY WORDS: postpharyngeal gland; nestmate recognition; aggressive behavior; agitation index; *Manica rubida;* Formicidae.

INTRODUCTION

Nestmate recognition constitutes one of the most complex behaviors in ants. Generally speaking, it allows the cohesiveness of the ant colony and protects it from exploitation by conspecific alien ants. Much attention has been given in recent years to the chemical nature of the signals that are responsible for nestmate recognition, leading to the conclusion that these cues reside among the chemicals

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found on an individual's cuticle (Howse, 1975; Bradshaw and Howse, 1984; Blum, 1987). This conclusion stems from two major observations: the signal seems to be a contact pheromone, since recognition is generally attained only after antennation of the encountered individual; and the signal is spread throughout the body, since recognition is instant irrespective of the body part that is antennated.

Indeed, various groups have demonstrated that in ants nestmate recognition cues reside on the cuticular surface. It is further postulated that cuticular hydrocarbons, due to their dominant presence in the epicuticle, on the one hand, and the correlation between similarities in their patterns and recognition between alien societies, on the other hand, constitute the specific chemical signature (Bonavita-Cougourdan *et al.*, 1987, 1989; Henderson *et al.*, 1990; Nowbahari *et al.*, 1990). However, the hypothesis that cuticular hydrocarbons serve as recognition cues requires testing using purified hydrocarbons or synthetic mixtures.

The nature of the template is less understood. An individual may use its own cuticular chemistry as a reference signal to which it compares the signal of the encountered ant. In these cases the template would be dynamic and subject to signal exchange among the colony members. A dynamic system seems to be obligatory in cases, e.g., *Solenopsis invicta*, in which temporal changes in cuticular chemistry occur (Vander Meer *et al.*, 1989). On the other hand, the template may be embedded in the ant's memory (whether innate or early learned). In this latter case the template will become more fixed with time and may change only through learning.

There is direct as well as indirect evidence implying that the postpharyngeal gland, idiosyncratic to ants, plays an important role in nestmate recognition. There is a large congruency between the composition of the glandular secretion and the epicuticular lipids (Bagnères and Morgan, 1991). Moreover, ants reared in mixed species groups obtain a unified hydrocarbon pattern both on their cuticle (Bagnères *et al.*, 1991) and in the postpharyngeal gland (Hefetz *et al.*, 1992). This hypothesis was recently supported by direct experiments with *Cataglyphis niger* in which the postpharyngeal exudate of a nestmate applied onto an alien ant resulted in reduction in aggression. In the converse experiments, the postpharyngeal gland exudate from a foreign ant applied onto another ant elicited aggression toward her by her nestmates (Soroker *et al.*, 1994). Recently it was demonstrated in several species of ants, including *M. rubida*, that these hydrocarbons are sequesters by the postpharyngeal gland and that the ants apply them onto their cuticular surface by self-grooming. It was further found that members of a colony constantly exchange the content of their postpharyngeal gland through trophallaxis and allogrooming (Soroker *et al.*, 1995a,b; Vienne *et al.*, 1995).

In the present paper we demonstrate that the postpharyngeal gland secretion modifies the aggressive behavior of the myrmicine ant *Manica rubida*. This species was selected due to the vast information available on its aggressive behavior and its well-defined cuticle and postpharyngeal gland chemistry (Hefetz *et al.*, 1992; Errard, 1994). The fact that it is a myrmicine ant allows further generalization on the function of the gland in ants. We also introduce a new method for measuring aggressive behavior using video frame-by-frame analyses.

MATERIALS AND METHODS

Ants

Colonies of the olygogynous ant *Manica rubida* (Myrmicinae) were collected from the French Alps, at an altitude of 800 m, in June 1994 and taken to the laboratory in Paris. Each (colony containing a queen, brood, and workers) was reared in a plastic box (280 × 275 × 85 mm) at 20 ± 3°C and subjected to the natural photoperiod. The boxes were equipped with a watering place and a nest tube (180 × 17 mm) covered with a black cardboard and fitted at one end with a water container. The plastic box itself provided the external foraging arena in which food was placed. The colonies were fed with a honey/apple mixture and mealworms *ad libitum*.

Tests of Aggression

Tests were composed of two consecutive encounters between five ants of *Manica rubida* and a marked nestmate which was either untreated (first encounter—control) or treated with a postpharyngeal gland exudates from different sources, i.e., alien's or nestmate's postpharyngeal gland secretion (second encounter—test). For the application of PPG exudates, ants were dissected in water under stereomicroscope and their glands removed, dried, and placed on the tip of an entomological pin. This was immediately applied on the test ant by crushing it on the thorax and smearing the secretion as evenly as possible over the entire body of the ant. A single gland was used for each test.

Encounters were conducted for 3 min in a petri dish (90-mm diameter). Before each test, the test ant was allowed to calm down by secluding it in a glass tube for 1 min in the test arena. Tests began by removing the glass tube and recording the reaction of the ants toward the marked nestmate using an event recorder according to the following index of aggression: 0 = inspection and antennal contact, 1 = threat as indicated by mandibular opening, 2 = biting, and 3 = curling the abdomen in stinging attempts. The frequencies and duration of each behavioral component were registered and the overall aggression exhibited in each encounter was calculated as follows:

$$\sum_{i=0}^{N} \frac{I_A t}{n}$$

where I_A, t, and n are the aggression index, the duration of each act, and the number of acts, respectively.

The data within a manipulation, i.e., control versus test, were compared using a nonparametric paired sign test. For comparison between treatments, the control encounter was subtracted from the respective test encounter, and this new variable served for comparison among the various treatments using ANOVA. The number of replicate experiments was 13 for the treatments with a nestmate's postpharyngeal gland and 19 for the experiments in which the ants were treated with an alien postpharyngeal gland.

The same encounters were also videotaped and later analyzed for assessment of the ants' agitation as a function of the treatments, using frame-by-frame analysis. Samples of 27 seconds of each of the 3 min of the test were selected by capturing a frame each 0.75 s. The positions of the ants in each frame were registered while differentiating between the treated ant (distinguishable by its colored mark) and its nestmates. The distance of the ants from the treated ant as well as their velocity was automatically registered. According to these data an index of agitation was constructed combining two formulas; the first taking into account the distances between the ants and the second their speed.

The formula for calculating the distance component that contributed to the agitation index was

$$d(x) = \frac{1}{1 + 10 \cdot x^2}$$

where $x = (d/d_{\max})$; and d is the distance between one of the ants and the marked ant.

The velocity component was calculated using the formula

$$s(x) = x^2$$

where $x = (s/s_{\max})$, and s is the velocity of one ant. These functions, $d(x)$ and $s(x)$, vary between 0 and 1 for values of x that vary between 0 and 1.

For each frame the parameter $P(x_j)$ was measured for each of the ants ($N = 5$), from which an index I_i for the i^{th} image was calculated as follows (Fig. 1):

$$P(x_j) = d(x_j) + s(x_j); \qquad I_i = \frac{1}{C} \sum_{j=1}^{N} \frac{P(x_j)}{N}; \qquad C = P_{\max}$$

For a sequence of n frames comprising one encounter, a primary index r was constructed as follows:

$$r = \frac{\mu}{\sigma}, \qquad \text{where} \qquad \mu = \sum_{i=1}^{n} \frac{I_i}{n} \qquad \text{and} \qquad \sigma^2 = \sum_{i=1}^{n} \frac{(I_i - \mu)^2}{n}$$

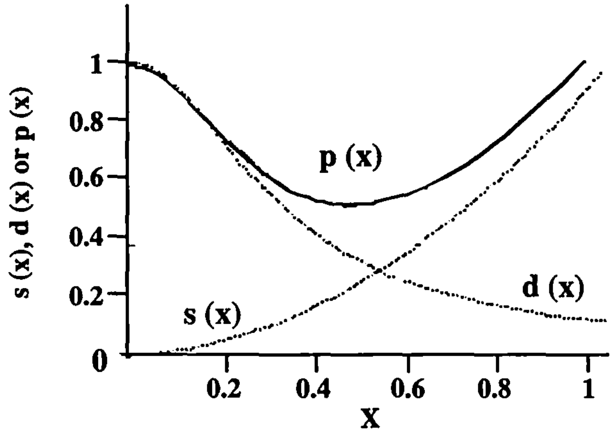

Fig. 1. Functions for describing the agitation index of *Manica rubida* toward nestmates treated with postpharyngeal gland secretion from alien ants or nestmates. The formulae for $d(x)$, $s(x)$, and $P(x)$ are listed under Materials and Methods.

The index of agitation (R) equals $R = r_t - r_c$ taking into account that each test was composed of a control encounter (r_c) and a test encounter (r_t).

RESULTS

When placed in the arena, workers of *Manica rubida* were in general calm and moved around the arena. In the first encounter, the behavior of the ants toward their introduced, untreated, nestmate was indifference. If one of the ants was nearby when the glass tube was removed from the arena, it approached the introduced ant and there was a short mutual antennation, after which the ants continued their walking in the arena. The behavior of the ants altered when the introduced ant was treated with a postpharyngeal secretion. As soon as the treated ant was encountered by one of its nestmates, it was antennated extensively. Further reaction was provoked only if there was physical contact between the ants, with its character depending on the nature of the glandular deposit. In the case of an alien postpharyngeal gland secretion, the untreated ant became progressively aggressive. This aggression was often expressed as threats with opened mandibles and occasional biting. In a few of the cases it culminated with flexion of the gaster in an attempt to sting the treated ant. The interactions between the treated ant and the ant it encountered apparently provoked the other ants in the arena, since they started to move quickly in the arena and often recruited to the encountering spot, in many cases joining the attack against the treated ant. The reaction of the ants toward a nestmate treated with a postpharyngeal secretion excised from another nestmate was completely different. The ants that encountered the treated ant antennated her for a short while, but without demonstrating any overt aggression. In a few of the experiments the treated ant was allogroomed by her nestmates.

Following a contact between the treated ant and its nestmates, we often observed intensive self-grooming, which was even more intensive if the test ant was treated with an alien postpharyngeal gland secretion.

A quantitative assessment of aggressive behavior in the two types of encounters was done by comparing the behavior of the workers toward the test ant before and after the application of the glandular exudates (Table I). When a nestmate's postpharyngeal gland secretion was applied to the test ants, there were no differences in the behavior of her nestmates before or after the application (nonparametric paired signed test; $n = 13$, $P = 0.58$, for the direct observation data and $P = 0.27$ for the video data analysis). When, on the other hand, the ant was treated with an alien postpharyngeal gland, her nestmates were much more aggressive to her compared to a few minutes before the application ($n = 19$, $P < 0.001$, for the direct observation data and $P = 0.012$ for the video analysis data, $n = 17$).

In order to obtain a more precise estimation of how the nature of the secretion affects the aggressive behavior of the ants, the aggression indices were corrected by subtracting the index in the control encounter from that in the test encounter. The results of the aggressive behavior obtained from direct observation and the agitation index calculated from the videotapes analyses are depicted in Fig. 2. Application of the postpharyngeal gland of an alien *M. rubida* clearly elicited aggression of the ants toward their treated nestmate. This reaction was also clear from the general agitation of the ants in the arena and their

Table I. The Behavior of Worker *Manica rubida* Toward Nestmates that Were Color Marked but Otherwise Left Untreated (Control Encounter) and Subsequently Treated with Exudates of the Postpharyngeal Glands from Another Nestmate (MHm/MHm-s) or an Alien Ant (MHm/MHm-d)[a]

Experiment	Encounter	Direct observations aggression index (*n*)	*P*	Video analyses agitation index (*n*)	*P*
MHm/MHm-s	Control	0.41 ± 0.05 (13)	0.58	3.81 ± 0.24 (13)	0.27
MHm/MHm-s	Treatment	0.64 ± 0.16 (13)		4.21 ± 0.27 (13)	
MHm/MHm-d	Control	0.85 ± 0.18 (19)	<0.001	8.41 ± 1.45 (17)	0.012
MHm/MHm-d	Treatment	4.64 ± 0.68 (19)		3.26 ± 0.23 (17)	

[a] Direct observations were done using an event recorder, whereas the agitation index was calculated from videotapes using frame by-frame-analyses. For details of the calculations of the different indices see Materials and Methods.

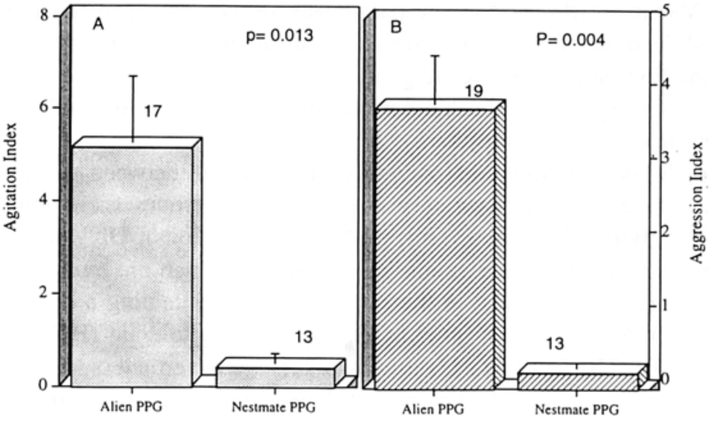

Fig. 2. Agitation (A) and aggression (B) (expressed as treatment − control) of *Manica rubida* toward nestmates treated with postpharyngeal gland exudates from an alien ant or a nestmate.

recruitment toward the treated ant (ANOVA; $F = 6.9$, $P = 0.013$, for the agitation index; $F = 16.1$, $P = 0.004$, for the aggression index).

DISCUSSION

The results of the behavioral assays presented above indicate that the secretion emanating from the postpharyngeal gland secretion of *M. rubida* contributes, at least in part, to the colony specific chemical label. This was evident from both direct observations and the video data analysis. Utilization of the latter is especially significant because it allows a more objective and quantitative estimate of the reaction of the ants to the glandular secretion. Direct description of the aggressive behavior is more subjective and liable to observer's interpretation. Even with an objective indexing of the reactions of the ants and continuous computerized recording of the duration of each act, it is hard to standardize the data for the purpose of comparison with other studies. This is especially true if the studies are made with different species and different rearing regimes.

The postpharyngeal gland is also the source of the colony label in the formicine ant *Cataglyphis niger* (Soroker *et al.*, 1994). The position of the gland in the head and its opening to the buccal cavity facilitates the application of its secretion onto the ant's body by selfgrooming. Moreover, there is a continuous flow of the glandular secretion between nestmates, mainly via trophallaxis, but also through allogrooming (Soroker *et al.*, 1995a,b). Similar exchange between nestmates was found to exist in *M. rubida* or *Formica selysi* from homospecific colonies and between individuals from both species reared in mixed species groups (Vienne *et al.*, 1995). The parallelism in the biological function of the gland and the dynamics of its hydrocarbon constituents in two disparate subfamilies of the Formicidae allow us to generalize that this idiosyncratic gland is one of the gestalt organs responsible for the unification of colony odor, thereby mediating nestmate recognition.

The ability of workers *M. rubida* to discriminate between nestmates and alien ants is especially interesting in view of the olygynous social structure of this species (Bernard, 1968; Errard, personal observations). Olygynous species in general have colonies containing several queens which are mutually aggressive and, apparently, coexist in the same nest by maintaining a distance from each other. Workers, on the other hand, are mutually tolerant (Hölldobler and Wilson, 1990). The fact that *M. rubida* workers are nevertheless hostile to alien ants suggests that, although they may originate from various matrilines, they still possess a common chemical signature. This unified colony odor is made possible by constantly exchanging postpharyngeal gland secretions between nestmates (Vienne *et al.*, 1995). Noteworthy is the intensive self-grooming that took place when the ants contact a treated ant. It is possible that the contact with the extra rich postpharyngeal gland secretion that was deposited on the tested ant's body contaminated the antennae, thereby altering its recognition capacity. We postulate that the extensive cleaning of the antennae while passing them through the mouthparts serves for reapplication of its own postpharyngeal secretion, i.e., reestablishing the reference signature. If this holds true, it follows that, rather than having a neural template, the ants use the reference signature that exists on the antennae.

The distinction between the reference hypothesis and the memory hypothesis can be experimentally tested using mixed species group of ants. By altering the ant's behavior through the application of the postpharyngeal gland exudates from various sources, we can test whether recognition is group specific (lending credence to the reference model) or can be generalized also toward the parent homospecific colonies, thus lending credence to the memory hypothesis.

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