



Aggregation Role of Mandibular Gland Secretions in the Giant Tropical Ant *Paraponera clavata* (Formicidae: Paraponerinae)

Andrade TT¹, Cristaldo PF², Araujo VA³, Delabie JHC⁴ and Martins LCB^{1*}

¹Department of Chemistry and Biology, State University of Maranhão, Brazil

²Department of Agronomy, Rural Federal University of Pernambuco, Brazil

³Institute of Biodiversity and Sustainability (NUPEM), Federal University of Rio de Janeiro (UFRJ), Brazil

⁴Laboratory of Myrmecology, Federal University of Southern Bahia, Brazil

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***Corresponding author:** Luiza Carla Barbosa Martins, Post-Graduation Program in Biodiversity, Environment and Health, Department of Chemistry and Biology, State University of Maranhão, Alecrim Hill, Caxias, Maranhão, Brazil, Email: luizamartinsuema@hotmail.com

Abstract

Social insects exhibit sophisticated behavioral repertoires to maintain communication and regulate colony activities. To elucidate the behavioral mechanisms of social insects is essential a thorough understanding of morphophysiological characteristics, such as the functionality of exocrine gland secretions. In this study, it was analyzed whether the mandibular gland secretions influence the aggregation behavior of workers of the ant *Paraponera clavata*. Associated behavioral acts were also studied. Bioassays indicated that mandibular gland extract triggers aggregation behaviors in *P. clavata* workers. The observed behavioral acts include mandible opening, raising of the antennae, antennal contact with treated filter paper, self-grooming, cleaning of the abdominal tip, and allogrooming. These observations demonstrate the potential multifunctionality of compounds secreted by mandibular glands within the behavioral repertoire of *P. clavata*.

Keywords: Chemical Communication; Aggregation Pheromone; Exocrine Gland; Social Insect

Abbreviations: GLM: Generalized Linear Model; LAMIR: Laboratory of Myrmecology.

Introduction

Ants are the most diverse group of eusocial Aculeata. There are substantial morphophysiological differences between mechanisms of social interactions of different ant societies. For instance, at least 85 types of exocrine glands have been described in ants; these glands produce compounds that are essential for chemical communication

[1-4]. Exocrine gland secretions are used for interaction between individuals, territory demarcation and defense, and also to recognition of sexual partners [5-6].

Mandibular gland secretions are also highly diverse [7] and are known to trigger a series of behaviors (e.g., mandible opening, aggression) associated with colony defense. Pheromones produced by mandibular glands are the channel of chemical communication which trigger inter-individual recognition within the colony [1,8] and induce specific surveillance and alert behaviors in workers [9-11]. The

response of workers to the pheromone is characterized by an initial alarm behavior, followed by aggregation to defend the colony [12-14]. Thus, pheromones produced by mandibular glands have two evident functions: (1) they serve as an alarm at high concentrations and (2) lead to recruitment of workers at low concentrations.

For the giant tropical ant, *Paraponera clavata* (Fabricius), few studies have investigated the role of exocrine glands, whose functionality is not yet fully understood [15-17]. The species *P. clavata* is the only living of the subfamily Paraponerinae. It is distributed in the Neotropical region, mainly in the tropical forests of western Brazil [18,19] and in several other countries in South and Central America [20]. This extremely aggressive species is large (up to 2.5 cm in length) and well known for its painful sting. Nests of *P. clavata* are generally found at the base of trees [21]. The species is an opportunistic predator but can also exploit other resources, such as extrafloral nectars, acting mainly at night, although it can occasionally be seen foraging during the day. Recruitment of workers is achieved using pheromones for the foraging of both soil and tree canopy [22-25]. Recently, Andrade, et al. [17] observed morphophysiological variations in the contents of *P. clavata* mandibular gland reservoirs, which contain solid, high-protein secretions. Such variations may indicate that mandibular gland secretions in *P. clavata* have a variety of functions in the communication between individuals.

In the present study, we test the hypothesis that mandibular gland secretions in *P. clavata* trigger behaviors associated with aggregation (include what type of behavior is associated). Ultimately, we aim to evaluate the behavioral role of mandibular secretions in *P. clavata* workers.

Material and Methods

Ants

Adult workers of *P. clavata* were manually collected from nests (N=3) located in the Inhamum Environmental Protection Area (04°53'S 43°24'W), Caxias, Maranhão, Brazil, and transferred to the Laboratory of Myrmecology (LAMIR) of the State University of Maranhão (UEMA), Brazil. Workers were maintained in plastic boxes lined with soil and substrate collected from the original nests. Artificial nests were kept under controlled conditions (27±2 °C and a photoperiod of 12 h light and 12 h darkness). Ants were fed a diet of apples, honey, and locust nymphs.

Preparation of Mandibular Gland Extract

Mandibular glands of *P. clavata* workers (N=20) were dissected in saline (125 mM NaCl). Then, extraction was

performed by immersing and grinding the glands in 400 µL of hexane (98.5%). Extracts were used immediately after preparation. In all bioassays, workers had contact with extracts produced by individuals from their own colony.

Study of Aggregation Behaviors

Bioassays were performed to verify the effects of mandibular gland secretions on the aggregation behavior of *P. clavata* workers. For this, two parallel artificial trails measuring 60 cm in length and 3 cm in width were made in an acrylic arena. One trail was treated with mandibular gland extract and the other with hexane (control). Treatments were applied at predetermined points (10 µL per point) spaced 10 cm apart, as depicted in Figure 1. A release area (20 cm length × 6 cm width) at the base of the arena provided access to both trails, enabling ants to choose their preferred route. The side of treatment and control trails was randomly changed in each trial. At every trial, the arena was cleaned on both sides with hexane.

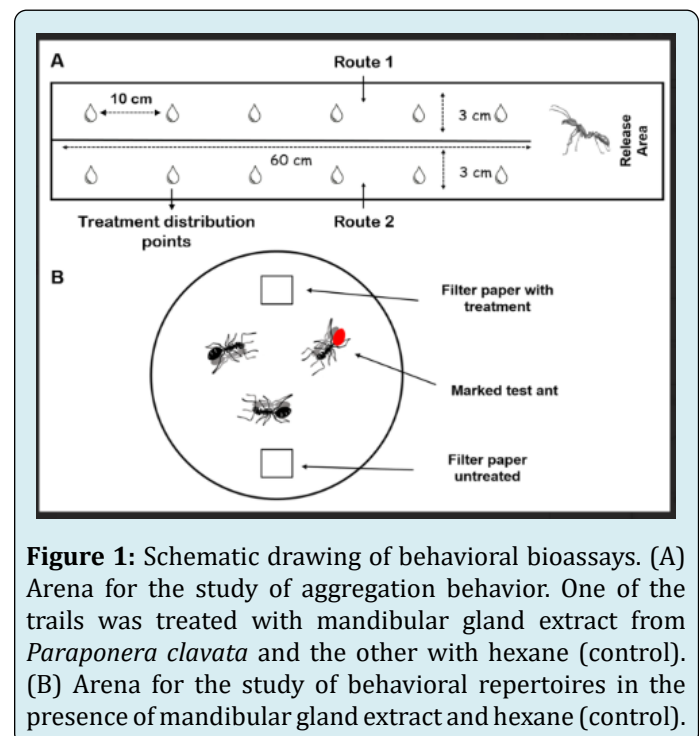


Figure 1: Schematic drawing of behavioral bioassays. (A) Arena for the study of aggregation behavior. One of the trails was treated with mandibular gland extract from *Paraponera clavata* and the other with hexane (control). (B) Arena for the study of behavioral repertoires in the presence of mandibular gland extract and hexane (control).

Ants were placed, one at a time, in the release area and observed for 5 min. or until they traveled to the end of one of the routes. Altogether, 30 workers from each nest (N = 3) were tested, totaling 90 bioassays. All ant movements were filmed with a digital camera (Sony Carl Zeiss, 14.1 megapixels).

Study of Behavioral Repertoires

Manipulative bioassays were performed under laboratory conditions to investigate the potential of

mandibular gland secretions in triggering other behavioral repertoires in *P. clavata* workers. Groups of three nestmate workers were placed in arenas consisting of a plastic container (20 cm diameter × 10 cm height) lined with filter paper. Before placing the ants in the arena, one of the workers was marked with water-based paint (liquid, non-toxic) at the gaster level in order to monitor its behavior individually. The behavior of non-marked ants was not analyzed. Two pieces of filter paper (1 cm²) were introduced into the arena, of which one was untreated and one was treated with 10 μL of mandibular gland extract or hexane (control). Each test was started immediately after solvent volatilization (a few seconds). For each test combination (control and mandibular gland extract), 30 repetitions were performed per nest (N=3), totaling 180 bioassays. Bioassay analysis consisted in the observation of behavioral acts directed at treated and nontreated filter papers and nestmates. All ant movements were filmed with a digital camera (Sony Carl Zeiss, 14.1 megapixels).

Statistical Analysis

Data were analyzed in the R statistical software [26]. The effect of mandibular gland extract (x-variable) on the distance traveled by workers (y-variable) was assessed by a Generalized Linear Model (GLM). The effect of extract (x-variable) on ant behaviors (y-variable) was examined by a Generalized Linear Model (GLM) with Binomial distribution. The response variable was the proportion of a specific behavior in relation to the total number of behaviors observed. Analysis for each y-variable was conducted independently for each nest. Residual analysis was performed to assess the adequacy of error distribution and model fitting.

Results

The distance followed by *P. clavata* workers on artificial trails was significantly affected by the presence of extract in all tested nests (GLM; nest 1: $F_{1,28} = 8.68$, $P = 0.004$; nest 2: $F_{1,28} = 17.05$, $P = 0.0001$; nest 3: $F_{1,28} = 8.55$, $P = 0.004$). Ants who followed the trail containing mandibular gland extract traveled a greater distance than ants who followed the trail treated with solvent only (control) (Figure 2).

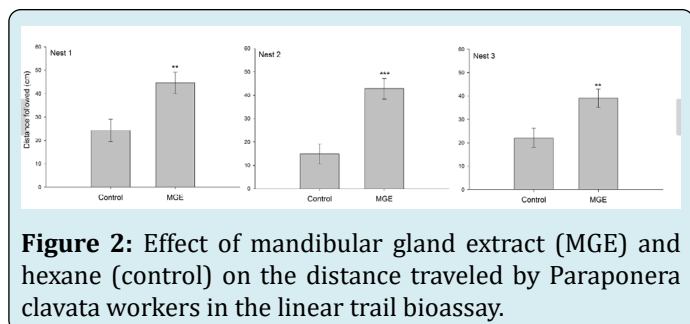


Figure 2: Effect of mandibular gland extract (MGE) and hexane (control) on the distance traveled by *Paraponera clavata* workers in the linear trail bioassay.

Six behaviors were frequently observed during the alarm response bioassay: mandible opening, raising of the antennae, antennal contact with filter paper, self-grooming, cleaning of the abdominal tip, and allogrooming. In all nests, the proportion of mandible opening, raising of the antennae, and antennal contact with filter paper was significantly higher in workers exposed to mandibular gland extract compared with control (Figure 3). The other behaviors (self-grooming, cleaning of the abdominal tip, and allogrooming) were not significantly affected by the presence of mandibular gland extract.

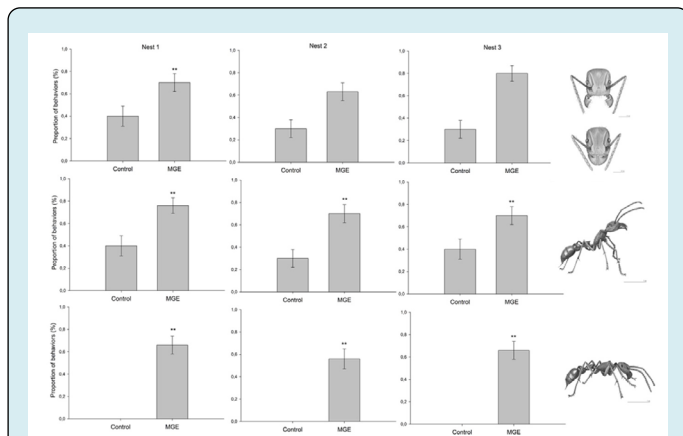


Figure 3: Effect of mandibular gland extract (MGE) and hexane (control) on the proportion of behaviors exhibited by *Paraponera clavata* workers in the alarm bioassay for each tested nest. (A) Mandible opening, (B) raising of the antennae, (C) antennal contact with filter paper.

Discussion

In ants, the mandibular gland is predominantly associated with the production of defense pheromones [13,27,28]. Worker recruitment can be considered a secondary function of mandibular gland secretion, as evidenced by the results of bioassays with *P. clavata*. The mandibular gland extracts triggered aggregation behavior in workers.

Previous studies have shown that ant workers release a small amount of alarm pheromone, usually stored in mandibular glands. This immediately causes an increase in movement rate; nearby workers open their mandibles and then more workers are recruited to defend the colony, leading to aggregation [14,29]. This behavioral repertoire may explain the results of the bioassay of the present study, in which *P. clavata* workers were more prone to follow the trail that contained mandibular gland extract than the path treated with only solvent (Figure 2).

In other ant species, pheromones responsible for aggregation behavior are generally secreted by abdominal glands, as in *Linepithema humile* Mayr [30,31] and *Camponotus pennsylvanicus* De Geer [29]. However, in *P. clavata*, this effect seems to be produced by mandibular gland secretions.

According to Ghosh A, et al. [32], ants detect areas with the highest concentrations of aggregation pheromones and move in that direction. In the present study, we observed that *P. clavata* workers were in constant search for the origin of the odor, walking toward the path that contained the mandibular gland extract. This was possible because aggregation pheromones of ants are stable, unlike those of other insect groups, which are highly volatile [33]. As a result, the time span in which olfactory cues remain available and can be followed by ants is increased. Andrade TT, et al. [17] demonstrated that *P. clavata* individuals store mandibular secretions as protein-rich solids. However, for an effective alarm response, secretions generally need to be highly volatile. This characteristic explains the lack of aggressive alarm behavior in the test arena, such as sting exhibition, attack, and agitation. In bioassays conducted by Hermann, et al. [15] under natural conditions, mandibular gland extracts had no significant effects on alarm reactions of *P. clavata* workers.

The most common defensive responses of ants are mandible opening and sting exhibition [1,27]. In the bioassays carried out in this study, *P. clavata* workers did not show their sting, but mandible opening was frequently observed when ants were exposed to mandibular gland extract. Mandible opening has been associated with the triggering of alarm behavior in several social hymenopterans [1,14,34-36].

Raising of the antennae and antennal contact with treated filter paper were also more frequent in ants exposed to mandibular gland extract than in those exposed to the control. These behaviors were reported in other ant species as an initial part of a series of defensive responses [35,36]. However, only mandible opening was observed in the tested *P. clavata* workers as a defensive act; other behavioral patterns characteristic of an alarm response was not identified.

Self-grooming is one of the most common behaviors described in ethograms for ants exposed to different extracts [37-41]. In this study, although self-grooming and allogrooming were observed, these behaviors were not significantly altered by exposure to mandibular gland extract.

According to Napper, et al. [42], defensive behavior triggered by pheromones secreted from mandibular glands is a precursor of other pheromonal responses, such as alarm (high concentration) and aggregation (low concentration).

The responses of *P. clavata* workers observed here corroborate this affirmation.

Although behavioral patterns were not characteristic of an obvious alarm effect, mandibular gland extract showed potential to attract nestmates [43-50].

In the present study, we demonstrated that mandibular gland extract triggers behaviors associated with aggregation in *P. clavata* workers. Other behaviors were also observed, indicating the importance and multifunctional potential of compounds secreted by mandibular glands in *P. clavata* [51-56].

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Author Contributions

Thito Thomston Andrade: Investigation, Formal analysis, Writing - Original draft.

Paulo Felliipe Cristaldo: Formal analysis, Writing - Original draft - Review & Editing.

Vinícius Albano Araújo: Formal analysis, Writing - Review & Editing.

Jacques Hubert Charles Delabie: Conceptualization, Methodology, Writing - Review & Editing. Luiza Carla Barbosa Martins: Conceptualization, Methodology, Investigation, Writing - Review & Editing.

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