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Research Article

Behavior of *Paussus favieri* (Coleoptera, Carabidae, Paussini): A Myrmecophilous Beetle Associated with *Pheidole pallidula* (Hymenoptera, Formicidae)

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Several specimens of the myrmecophilous beetle *Paussus favieri* were reared in ant nests of *Pheidole pallidula*. Their interactions were recorded and all behaviors observed are described. Duration and frequency of five behaviors of *P. favieri* were analyzed with ANOVA and post hoc Tukey tests; these comprised rewarding, antennal shaking, antennation, escape, and "no contact". Significant differences both in duration and in frequency among behaviors were detected. The main result is that the rewarding behavior, during which the beetle provides attractive substances to the host, is performed significantly more frequently than all others. This result strongly supports the hypothesis that the chemicals provided by the beetles and licked by the ants are of great importance for the acceptance and the full integration of *P. favieri* in the ant society. This result also suggests that, contrary to previous findings and interpretations, the myrmecophilous strategy of *P. favieri* is very similar to the symphilous strategy described for *P. turcicus*. The occasional interactions of some beetle specimens with the *P. pallidula* queen were recorded, illustrated, and discussed, indicating the possibility of a more complex strategy of *P. favieri* involving a chemical mimicry with the queen. In addition, the courtship performed by the beetle is described for the first time, together with a peculiar "cleaning" behavior, which we hypothesize functions to spread antennal chemicals over the body surfaces.

1. Introduction

Ant nests are very attractive for many organisms, because they represent well-protected and stable environments that are rich in various resources (ants, their brood, stored food, waste materials, etc.). In particular, a large number of insects establish relationships with ants for a considerable part of their life cycle [1–3] and are classified as true myrmecophiles [4]. Insect-ant interactions range from commensalism to specialized predation, parasitism, and mutualism [1]. The most specialized myrmecophiles are able to deceive the complex communication and recognition systems of the ants, infiltrating their societies and exploiting their resources [1, 4, 5]. These ant parasites represent about 10% (∼ 10,000 species) of known myrmecophilous insects and most are members of Coleoptera, Lepidoptera, Orthoptera, and Diptera [6]. They show several refined adaptations (e.g., chemical and morphological mimicry, specialized feeding behaviors, structural modifications) to avoid ant attacks, to be accepted by ants, and to develop and reproduce within ant nests [7].

All members of the ground beetle tribe Paussini (Coleoptera, Carabidae, Paussinae) are myrmecophiles and are considered to be ant parasites [8]. Like many other parasites of ants, they show striking adaptations, such as greatly modified antennae (flattened, enlarged, lenticular, globular, concave, elongate, etc.), slender or compact bodies, elongate or flattened legs, and peculiar "myrmecophilous organs" composed of trichomes (tufts of hairs) connected to exocrine glands for the release of chemical secretions.

Paussini (known as "ant nest beetles") are typically rare insects living in concealed environments which makes it difficult to observe their behavior in nature [7]. Therefore, while they have been extensively studied from a taxonomic point of view [8], information about their interactions with hosts and their life cycle is limited and largely indirect (i.e., inferred from their structural adaptations) with few ethological observations [9]. Although several attempts have been made to rear Paussini with their host ants, this has proven to be particularly difficult, and promising results have been achieved only for a few species (5 out of the currently recognised 572 Paussini species) [8].

The first observations of Paussini behaviors in captivity were reported by Péringuey [10, 11] for *Paussus lineatus* Thunberg, in 1781, and *P. linnaei* Westwood, in 1833, and, to a lesser extent, for *P. burmeisteri* Westwood, in 1838. Other early ethological notes were reported by Escherich [12] for *P. turcicus* Frivaldszky, in 1835, *P. favieri* Fairmaire, in 1851 [13], and *P. arabicus* Raffray, in 1885 [14]. These authors carefully reported their annotations mainly emphasizing the obligate association of these beetles with the ants (especially the ant genus *Pheidole*), their feeding strategy on larvae of the host ants, and some interactive behaviors between beetles and ants (e.g., dragging, grooming, aggressive behaviors). According to this first, though limited and speculative set of information gathered in captivity, and to previous anecdotal observations in nature reported by several authors (e.g., [15–19]), Escherich [14] tentatively categorized the strategies of the members of the genus *Paussus* in three main levels of interactions, referring to Wasmann's [20, 21] myrmecophilous categories: synectrans (e.g., *P. linnaei*), synecoetes (e.g., *P. arabicus* and *P. lineatus*), and symphilous (e.g., *P. turcicus*). Later, Le Masne [22–24] successfully reared *P. favieri*, adding valuable and detailed information to the knowledge on the biology of this species which is a guest of the facultatively polygenic ant *Pheidole pallidula* (Nylander, 1849). Le Masne mainly focused his observations on the predatory strategy of *P. favieri* while feeding on adults and ant larvae [22, 24], and on the mechanisms of adoption of the beetle inside the nest [23]. More recently, Escherich's [14] classification has been reviewed and updated by Geiselhardt et al. [8], and three different strategies have been identified, exemplified by three *Paussus* species: (1) the strategy of *P. arabicus* reported by Escherich [14] which is considered the most basal, since the initial contact with the ants triggers their aggression; however, the attacks cease after the contact with the ants [10, 12, 14, 25], and for this reason, the authors speculated that a chemical camouflage might occur in this species [8]; (2) the costly strategy of *P. turcicus*, which is actively groomed by its host ants, to which the beetle supplies an attractive and possibly rewarding antennal secretion [12, 13]; and (3) the strategy of *P. favieri*, which is considered the most derived, since it has no apparent costs. According to the observations by Escherich [13] and Le Masne [23], this beetle is readily accepted and fully integrated within the colony without hostility. It is usually ignored by the ants, only rarely touched, quickly groomed, and dragged, and it moves undisturbed within the nest, free to feed on brood and adults. Probably, an advanced chemical mimicry mediates the mechanism of this association [8].

Paussus favieri was also the object of recent researches, being one of the most common species of Paussini in Northern Africa and one of the two species present in Europe. Cammaerts et al. [26–28] showed that *P. favieri* preferentially follows the pheromone trail produced by the poison glands of its host ant, discriminating this from pheromones of nonhost ant species. Lastly, Di Giulio et al. [7] reared and described the first instar larva of *P. favieri* that, like other *Paussus* larvae, shows remarkable adaptations to a myrmecophilous lifestyle (e.g., shortened and degenerated head capsule, reduced mouthparts, partial atrophy of legs, fused terminal disk), with specialized feeding behaviors that suggest that the larvae are fed by the ants through trophallaxis.

To clarify the mechanisms underlying host-parasite relationships between *P. favieri* and its host ant *P. pallidula*, we investigated the interspecific and intraspecific behaviors performed by the beetles inside the ant nests maintained in laboratory. In particular, our aims were (1) to describe the main behaviors performed by *P. favieri* and its host ant, (2) to analyze the duration and frequency of the behaviors performed by the beetles, and (3) to discuss the possible functional and adaptive significance of the observed behaviors.

2. Materials and Methods

2.1. Material Examined and Rearing Conditions. During an expedition to Morocco (High Atlas Mountains) in May 2010, adults of *P. favieri* were collected under stones, in nests of *P. pallidula* (Figure 1). Beetles and ants were then transported to the laboratory for behavioral experiments. Each beetle was reared with the ants from the nest in which it was found; when multiple specimens of *P. favieri* were found in the same nest, all specimens were reared together. Ants and beetles were housed in transparent glass boxes (32 × 22 × 15 cm) lined with a layer of plaster, and the walls were coated with fluon to prevent ants from escaping. Colonies were kept under controlled conditions (21–24°C; 12 h : 12 h light : dark; 60% humidity), following the procedures described by Detrain and Deneubourg [29], and maintained on a diet of sugar or honey, and fruit flies or moth caterpillars provided three times per week. The boxes were kept open to facilitate observations. After the ants and beetles were acclimated to these new conditions (about for 10 days), behavioral observations were made.

Ten colonies were established but we used only five, well-structured colonies with at least 100 nestmates (70% minors, 30% majors and queen) for behavioral observations.

2.2. Descriptions of Behaviors. Host-parasite interactions and intraspecific behaviors (cleaning and mating) were observed under natural light. Video was recorded with an NV GS120EG Panasonic video camera for a total of 20 hours. Because manipulation could have unpredictable effects on

Figure 1: *Paussus favieri* with minor and major worker of *Pheidole pallidula* (photo by P. Mazzei).

the host-parasite interactions, beetles and ants were not marked and beetles were not sexed. For the analysis of the host-parasite interactions, we selected 14 beetles for which recording sessions of at least 15 minutes were available.

All behaviors of both the beetles and the ants were described and classified into five categories (see Sections 3.2 and 3.3). The behaviors performed by *P. pallidula* during the interactions with the beetles were described following the behavioral repertoire suggested by Hölldobler and Wilson [1], Passera and Aron [30], and Sempo and Detrain [31]. Beetle cleaning and sexual behaviors were described after analyzing the videos in slow motion.

2.3. Statistical Analyses of Behaviors. We statistically analyzed five behaviors performed by the beetle while interacting with the host ant (see Section 3.2). Recording sessions were analyzed using the observation transcription tool EthoLog 2.2 [32] to continuously record the time that the beetle spent performing different behaviors. We tested whether different behaviors of beetles have significantly different durations, that is, if there are differences in the amount of time a beetle spends engaged in different behaviors when it interacts with ants. Differences between behavior duration were tested using a main effect ANOVA. A total of 1030 measurements of behavior duration (dependent variable) were analyzed. Because the beetles were not obtained by rearing but were collected from ant nests in the field, we have no information about possible interindividual variation due to genotypic differences, or previous experience with ants, age, days of fasting, and so forth. Thus, we combined all of these unknown factors into the concept of “individuality”. To control for this “individuality”, beetles were numbered from 1 to 14 and “beetle identity” was introduced as a second factor in the ANOVA. Therefore the identity of the beetle, which exhibited a behavior, and the type of behavior (classified into five categories, A–E, see Section 3.2) were used as categorical predictors (factors). Post hoc comparisons were performed using Tukey HSD tests. To determine whether different behaviors were performed more frequently than others, we executed analogous analyses on the recorded frequency of the behaviors. Statistical analyses were performed with Statistica for Windows version 7.2 (StatSoft Inc., Tulsa, OK, USA).

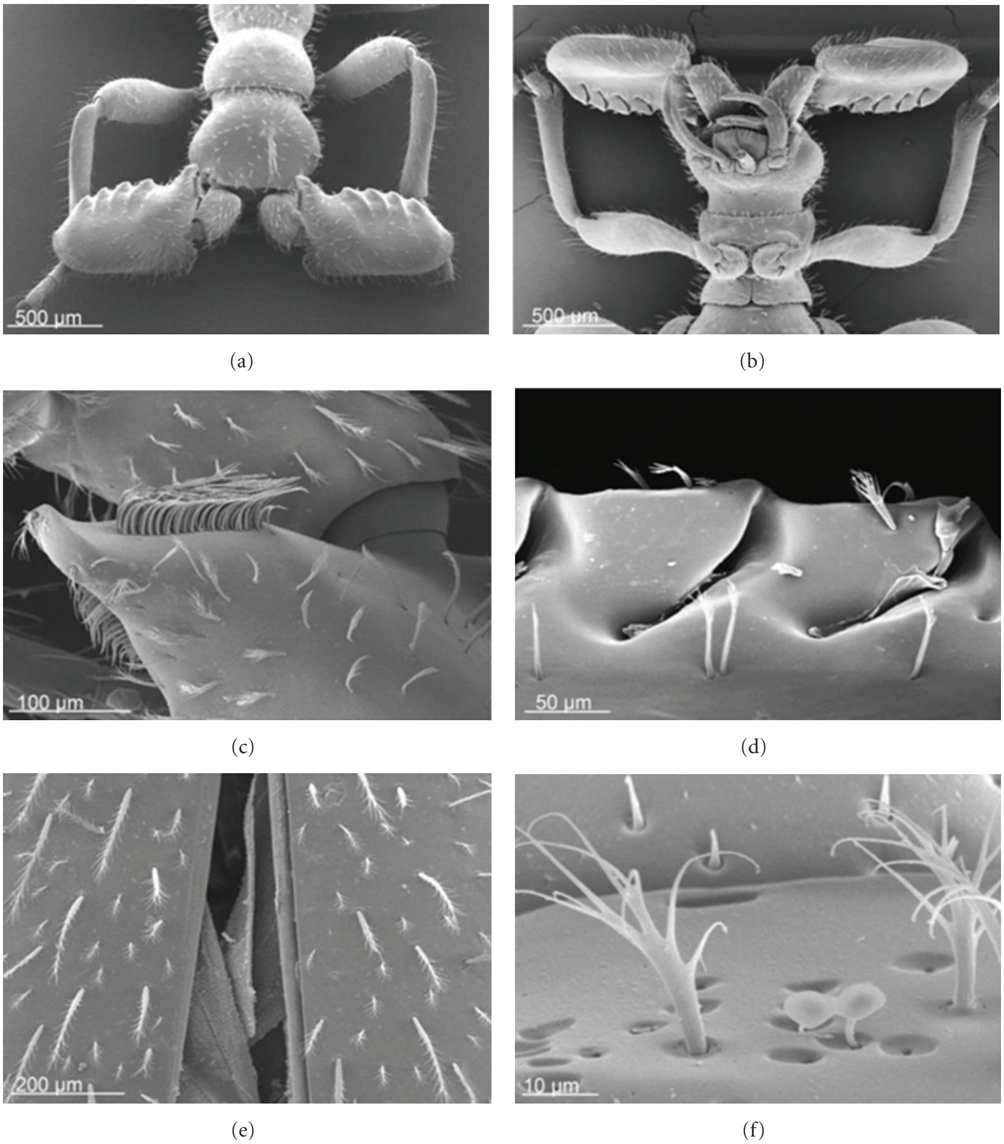
2.4. Scanning Electron Microscopy. Morphological structures of *P. favieri* (Figure 2) involved in the interactions with host ants and with others conspecifics were studied using a Philips XL30 scanning electron microscope at L.I.M.E. (Interdepartmental Laboratory of Electron Microscopy, University “Roma Tre”, Rome). Specimens used for morphological study were kept overnight in a detergent water solution, cleaned by ultrasounds for 15 seconds, rinsed in water, dehydrated through a series of EtOH baths of increasing concentration (70, 80, 90, 95, and 100%), critical point dried (Bal-Tec CPD 030), mounted on a stub (by using self adhesive carbon disks), and sputter-coated with gold (Emitech K550 sputter coater).

3. Results

3.1. General Morphology of Paussus favieri. The beetle is small (length ~ 4 mm), much bigger than minor workers of *P. pallidula*, with intermediate dimensions between majors and queen (Figures 1 and 3). The body is slim with slender elongated legs and bulged modified antennae. The body color is light brown, similar to that of minor and major workers of the host ant, with shining, oily appearance. The head is subhexagonal with elongate palpi and dark eyes, bearing dorsally a long medial tuft of trichomes (Figures 2(a) and 2(b)). The antennae are particularly modified, composed by three joints: (1) a cylindrical and slightly elongated scape; (2) a globular, ring-like pedicel; and (3) a single segment “antennal club” (resulting from the fusion of 9 flagellomeres) that is wide, sub-triangular, swollen, and strongly asymmetrical (Figures 2(a) and 2(b)). The scape and the antennal club are covered by several modified trichomes and glandular pores (Figure 2(d)), while chemoreceptors are mainly distributed apically. The antennal club has a pointed basal spur with two tufts of trichomes (myrmecophilous organs, Figures 2(a) and 2(c)), and ventral pockets (Figure 2(d)) where glandular secretions are stored. The prothorax is elongated, of about the same width as that of the head, strongly constricted in the middle, without tufts of trichomes. Like the other *Paussus* species, a stridulatory organ is present on the ventral side, composed of finely ridged pars stridens on the hind femora and a plectrum (row of cuticular spines) on the basal part of the abdomen. The elytra are parallel and covered with elongate, branched trichomes. The pygidium is truncate with short-fringed trichomes. The ventral side of the body is smooth, without trichomes.

3.2. Description of Paussus favieri Behaviors When Interacting with Host Ant

(a) *Rewarding.* The beetle remains still, while it is antennated and actively licked by ant minor and major workers (Figure 1; (see Supplementary Material 1 available online



(a) (b) (c) (d) (e) (f)

Figure 2: SEM micrographs of *Paussus favieri*: (a) anterodorsal view of head and thorax; (b) ventral view of head and thorax; (c) basal spur of the antennal club, dorsal view; (d) ventral antennal pockets with visible secretion; (e) elytra with modified sensilla chaetica; (f) modified sensilla chaetica on head with glandular pores.

at doi: 10.1155/2012/940315). This behavior is generally associated with movements of the beetle's hind legs, either singly or in combination.

(b) *Antennal Shaking.* The beetle vibrates the antennae, quickly shaking them forward and backward in the vicinity of the ants. This behavior mostly occurs after a long period of rewarding (see above).

(c) *Antennation.* The beetle moves its antennae in a slow, alternate, vertical way, oriented toward the object of interest. The beetle usually explores an ant's body with the apices of the antennae, which are particularly rich in sensorial structures.

(d) *Escape.* The beetle tries to elude the host ant in a temporary negative reaction. This behavior is not connected with aggression by the host, but rather in most cases it is a consequence of the presence of a high number of excited ants antennating and licking the beetle, or after an extended rewarding period.

(e) *No Contact.* The beetle does not interact with the ants. This state includes many different activities like exploring, resting, cleaning, interacting with partners, mating, and so forth.

Feeding and mating behaviors were observed rarely. The beetle feeds on ant larvae by piercing the integument with its mandibles and carrying around the victim while sucking blood and soft tissues from the abdomen. In these situations,

(a) (b)

Figure 3: Interactions between *Paussus favieri* and queen of *Pheidole pallidula* (photo by P. Mazzei).

the ants do not react aggressively toward the beetle. These behaviors never occurred in the movies selected for analyses.

Beetles were observed directly interacting with the queen (Figures 3(a) and 3(b)). In a few cases, the beetles remained in the queen's chamber for some days, antennating and rubbing against the queen's body without any aggressive reaction from the queen or the workers.

3.3. Description of *Pheidole pallidula* Behaviors When Interacting with Beetles

(a) *Antennation.* The ants touch the beetles with their antennae on all exposed parts of the body, but especially on the beetle's antennae (Figures 2(a) and 2(b)).

(b) *Alarm.* The ants antennate frenetically and widely open their mandibles, similarly to alarm behaviors performed during dangerous situations [1, 31]. This behavior is rarely observed against beetles, but when it is, it is not followed by biting.

(c) *Licking.* The ants lick all exposed parts of the beetle's body that are rich in trichomes (antennae, head, legs, elytra and pygidium) (Figures 2(a), 2(e), and 2(f)). This licking behavior is very similar to the ants' allogrooming behavior ([31] and references therein). The ants spend most time licking the trichomes on the basal spur and the pockets of the antennae (Figures 2(c) and 2(d)). This activity can be performed simultaneously on one beetle by many ants (minor and major workers), and it is the reciprocal behavior to "rewarding" by *P. favieri* (see Section 3.2).

(d) *Dragging.* The ants, mostly minors, occasionally bite the antennal club of *P. favieri* and quickly drag the beetle around the nest. The beetles, though much bigger than ants, do not resist being dragged around. In two cases, we observed minor workers dragging a beetle inside the queen's chamber.

3.4. Cleaning Behavior of Paussus favieri. The cleaning behavior is characterized by the following phases (see Supplementary Material 2).

(1) Antennal Cleaning. The forelegs clean the antennae one at time, starting from ventral to dorsal side of antennal surface. In particular, the tarsus and the hairy apical part of tibia rub the apex and the posterior part of the antennal club, the ventral pockets (Figures 2(b) and 2(d)), and the posterolateral teeth (Figure 2(a)), with numerous quick movements. The tarsus also rubs against the whole antennal club, moving laterally from base to apex with slow movements. Additionally, the tibia cleans the dorsal side of antennal surface, with a single movement. During this phase, the antenna is highly movable and it is rotated according to the side to be cleaned.

(2) Head Cleaning. One of the forelegs moves over the head, rubbing the apical tuft of long sensilla (Figure 2(a)). This behavior has been rarely observed.

(3) Leg Cleaning. This cleaning is performed mutually by pairs of legs of the same side, the fore against the middle, and the middle against the hind legs. The tarsus and the tibia of one leg slowly rub the reciprocal leg from the base to the apex. In addition, the tarsi are rubbed together (fore-middle, middle-hind) repeatedly.

(4) Elytral Cleaning. The elytra are cleaned in the antero-posterior direction with slow repeated movements of the middle and hind tibiae and tarsi of the same side. The tarsi of the middle and posterior legs also rub the lateral surface of the abdomen.

3.5. Mating Behavior of Paussus favieri. The mating behavior of *P. favieri* is characterized by two distinct phases: courtship and copulation (see Supplementary Material 3).

Courtship. Males actively search for females, approaching them by antennal contact (antennal approach) in one of two different ways: (a) with a slow alternate vertical movements of his antennae touching the female's antennae (frontal approach), and (b) his antennae touching laterally the side of the female's elytron (lateral approach). After the lateral antennation, the male forelegs are moved up and down, touching the female elytra and pronotum. The female replies by moving her antennae and the hind legs. After this preliminary antennal approach, the male climbs upon the female's body, dorsally positioning himself in the opposite direction of the female, touching his antennae the apex of the female's abdomen. This dorsal inverted phase lasted a few seconds; afterwards the male turns 180°, reaching the typical mating dorsal phase. During this phase, the partners reciprocally touch their antennae, and the female often moves her hind legs. In the 10 sequences analyzed, the dorsal phase lasts from 5 to 12 minutes and, in a few cases, it was followed by copulation attempts.

Copulation. From the dorsal phase, the male of *P. favieri* slides backwards, bends the abdominal apex downward, extrudes the aedeagus, and tries to insert it into the female's genitalia. The antennae of the male are frenetically moved up and down. The copulation with complete insertion of genitalia was observed only once. In fact, the female often rejects the male and avoid copulation.

During mating, the ants frequently interact with the beetles, antennating them and/or actively licking their antennae and legs.

3.6. Analyses of the Behaviors of Paussus favieri during Interactions with Its Ant Host. The following behaviors of *P. favieri* were analyzed statistically: (A) rewarding, (B) antennal shaking, (C) antennation, (D) escape, and (E) no contact. We detected significant differences in the time a beetle spends performing different behaviors (Table 1). Post hoc Tukey tests showed significant differences between E versus A, B, C, and D ($P < 0.0001$ in all pairwise comparisons). Individuality was not significant, which indicates that behavioral patterns do not vary significantly among individuals. Differences in the mean duration of different behaviors are shown in Figure 4.

We found that significant differences among the frequencies that different behaviors were performed (Table 2). Post hoc Tukey tests showed significant differences between A versus B, C, D and E ($P < 0.0001$) and between C versus B and D ($P < 0.05$). Differences in the mean values of frequencies of different behaviors are shown in Figure 5.

Table 1: Results of a main effect ANOVA for values of times spent performing different behaviors by beetles when interacting with ants. d.f.: degrees of freedom; SS: sum of squares; MS: mean sum of squares; *F*: Fisher; *P*: probability.

Effect	d.f.	SS	MS	*F*	*P*
Individuality	13	6183.000	475.620	0.848	0.6090
Behavior	4	27389.200	6847.310	12.201	**0.0001**
Error	1012	567925.800	561.190		

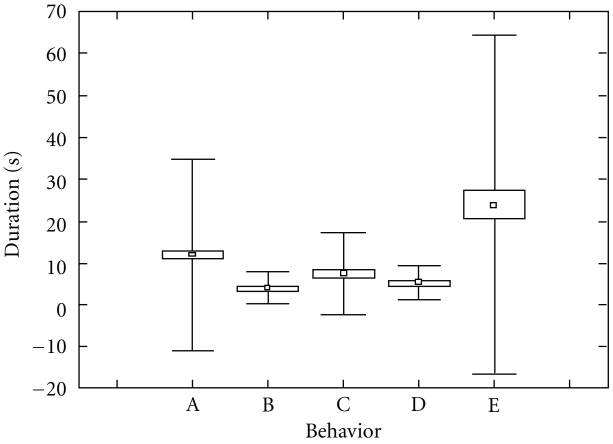


Figure 4: Differences in the duration of behaviors performed by beetles when interacting with ants. Mean values are shown by squares, standard errors as boxes, and standard deviations as whiskers. A: rewarding; B: antennal shaking; C: antennation; D: escape; E: no contact.

Table 2: Results of a main effect ANOVA for values of frequency of different behaviors performed by beetles when interacting with ants. d.f.: degrees of freedom; SS: sum of squares; MS: mean sum of squares; *F*: Fisher; *P*: probability.

Effect	d.f.	SS	MS	*F*	*P*
Individuality	13	643.238	49.480	1.027	0.4370
Behavior	4	4498.345	1124.586	23.336	**0.0001**
Error	66	3180.655	48.192		

4. Discussion

According to Wasmann [33, 34], two defensive structural types are generally recognized in myrmecophile morphology: the "protective" type, characterized by a compact body with hard and smooth surfaces, and retractable appendages; and the "symphilous" type, characterized by slim bodies with long slender appendages and many trichomes covering the body and/or crowded in myrmecophilous organs [8, 35]. These body forms suggest different strategies both for entering the host nests and for avoiding ant attacks. Both body types are present in the Paussini, sometimes with intermediate forms, with the symphilous type generally present in the most derived taxa that are considered to be the best integrated into ants' colonies [1, 36]. *Paussus favieri* is clearly assignable to the latter type, showing all the distinctive characters noted previously. Our observations confirm that *P. favieri* is fully integrated in the host ant society since almost no aggressive behaviors against the beetles were observed. On the contrary, ants were strongly attracted by the beetle's secretions.

The results of our statistical analyses show that beetles and ants spend a significantly longer amount of time not interacting (no contact, E) than the time they spend interacting with one another in a specific behavior. The state of no contact (E) can be the effect of a temporary withdrawal of the beetle, or the absence of caring by the ants. This is an expected result, since it is reasonable that the beetle spends most time in a number of activities that do not involve host interactions (i.e., exploring, mating, cleaning, resting, etc.).

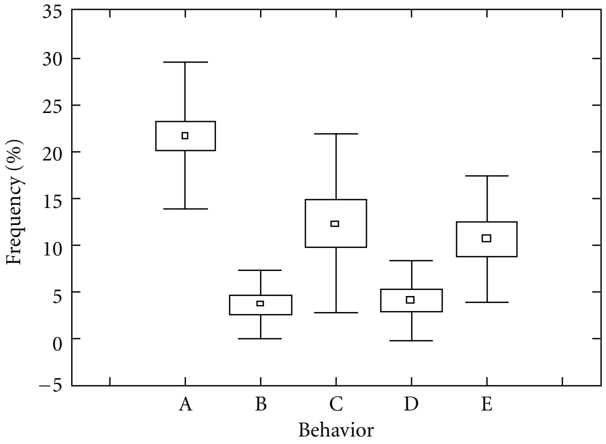


Figure 5: Differences in the frequencies of behaviors performed by beetles when interacting with ants. Mean values are shown by squares, standard errors as boxes, and standard deviations as whiskers. A: rewarding; B: antennal shaking; C: antennation; D: escape; E: no contact.

Concerning the behaviors performed by the beetle during the interactions with its host ants, the analysis of duration showed that rewarding (A), antennal shaking (B), antennation (C), and escape (D) are performed for similar amounts of time. However, it is notable that frequency of the rewarding behavior (A) is significantly greater than that of all other behaviors. During the rewarding behavior, *P. favieri* is antennated and actively licked by the ants, especially near the antennal symphilous organs (Figure 2(c)). This is consistent with the fact that the primary role of the highly modified antennae of *P. favieri* is glandular, producing substances that are highly attractive to the ants [37]. These substances are mostly stored inside the antennal pockets (Figure 2(d)). The chemical nature of this secretion is unknown, but it seems to be important for the acceptance and survival of the beetles within the ant nest [37] and for the success of the parasitic interaction. It has been speculated that, similar to other social parasites [1, 38], the chemicals secreted by Paussini beetles may have an appeasing function [8, 34]. Another hypothesis is that these substances provide a protective or rewarding food for the ants and their brood [8]. The rewarding behavior is generally associated with movements of the beetle's hind legs, an action possibly connected to the emission of stridulations. The high frequency of the rewarding behavior recorded in our experiments is quite in contrast with the previous observations by Escherich [13] and Le Masne [23], who reported that the ants only occasionally groom the beetle. According to our observations, the myrmecophilous strategy of *P. favieri* seems very similar to that of *P. turcicus* [12, 13], and the supposition that this strategy corresponds to a more derived (less costly) level of integration for *P. favieri* [8] seems unjustified.

The quick shaking of the antennae (antennal shaking (B)), not noted by Le Masne [22–24] and never recorded for any other coleopteran genera, has been occasionally observed in another species of *Paussus* [15]. Our observations suggest that antennal shaking might be correlated with the glandular activity of the antennae, facilitating the spread of the viscous exudates from the antennal surface, or, most probably, with the spray of volatile allomones whose presence needs to be confirmed.

The antennation behavior (C) was described by Le Masne [22, 24], who interpreted it as a precursor to predation. Le Masne [24] observed that through antennation the beetle finds the ant's abdomen. Once found, the beetle pierces the abdomen with its sharp mandibles and feeds on the ants' hemolymph. However, in the videos analyzed for the present work, we never observed predation following the antennation behavior.

The occasional observation of some beetles interacting with the queen (Figures 3(a) and 3(b)), also for a prolonged time, is particularly interesting. We hypothesize that the physical interaction could supply a queen-specific chemical camouflage to the beetle and/or that the beetle could spread some of its attractive substances on the queen's body. In both cases, a chemical combination of beetle and queen odors could be reached, resulting in a deception of the hosts, allowing the beetle to achieve a higher social status inside the nest. The dragging of *P. favieri* inside the nest by *P. pallidula* minor and major workers is a behavior that this species (Maurizi and Di Giulio pers. obs.) and other *Pheidole* species [39] usually reserve for the queen [1, 8] and could be related to this possible mimicry. However, further research is required to confirm that this is a regular interaction, and that an exchange of cuticular hydrocarbons or other substances is involved.

The cleaning and mating behaviors performed by *P. favieri* inside the nest of *P. pallidula* have been observed and described in this work for the first time. Péringuey [10] mentioned a similar "brushing" behavior by fore and hind legs performed by males of *P. lineatus* after copulation. The complex cleaning behavior of *P. favieri* is quite different from the simple cleaning of other Carabidae [40] which mainly involves rubbing the comb organ of the forelegs (a row of spines positioned in an emargination of the inner edge of the fore tibiae) against antennae and mouthparts. In fact, the typical comb organ of ground beetles is vestigial or absent in Paussini [40, 41]. In *P. favieri*, the antennae have primarily a glandular function [37] and secrete a large amount of attracting substance. We interpret the rubbing of the forelegs against the antennae and then against middle and hind legs, head, elytra and abdomen, as a means of spreading antennal substances all over the body. This is also supported by the fact that the ants actively lick not only the antennae but also the head, legs, and elytra, suggesting that the attractants are present also on these body parts.

Little is known about the sexual behavior of Carabidae [42, 43], while no information is available for the Paussini except for a brief note of Péringuey [10] on *P. lineatus*. In this species, the male fixes his mandibles in the prothoracic excavation of the female and, with the hind legs, pulls the abdominal apex of the female towards him; in order to strengthen his position on the female's back, the male passes his antennae under the females antennae, keeping this position for several hours. Serrano et al. [44] observed in

Portugal two specimens of *P. favieri* in copulation in an ant nest of *P. pallidula*, confirming that this beetle mates inside the colony, as is reported for other myrmecophilous beetles [45]. In captivity we observed the specimens of *P. favieri* mating in the ant nests several times and for a long duration. Precopulatory behavior includes exchanging tactile signals with antennae and legs, though it is possible that chemical signals are also involved. Unlike observations of *P. lineatus* [10], in both precopulatory and copulatory behaviors the mandibles are not used by *P. favieri*, while the dorsal position is maintained only by the male's legs. Of particular interest is the presence of an "inverted" dorsal phase (not noted in *P. lineatus*) that may be unique within the Carabidae.

Our experiments also suggest that acoustic signals are probably exchanged during the precopulatory behavior, since the female has been observed repeatedly moving the hind legs, a behavior possibly connected to the emission of stridulations (see Section 3.2(a)). However, the actual role of the acoustical communication in intra- and interspecific behaviors remains unknown.

In conclusion, the importance of the rewarding behavior confirms the primary role of the antennal secretions, possibly spread by a complex "cleaning" behavior, for the successful acceptance and integration of *P. favieri* inside the host colony. The identification of the secretions would be very important to verify their appeasing/rewarding properties, providing a more complete understanding of the myrmecophilous strategy of *P. favieri* and of other members of this tribe.

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