

ECOLOGY, BEHAVIOR AND BIONOMICS

Why do Male *Xylographus contractus* Mellié (Coleoptera: Ciidae) Present Abdominal Fovea? Evidence of Sexual Pheromone Secretion

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Por que Machos de *Xylographus contractus* Mellié (Coleoptera: Ciidae) Apresentam Fóvea Abdominal? Evidências de Secreção de Feromônio Sexual

RESUMO - A maioria dos machos de ciídeos possuem uma fóvea no primeiro urosternito. Foram testadas duas hipóteses alternativas para explicar a função da fóvea abdominal em machos de *Xylographus contractus* Mellié: (i) a fóvea secreta feromônio sexual, e (ii) a fóvea secreta feromônio de agregação. Para isso, avaliou-se a resposta de 59 indivíduos, separadamente, a dois estímulos olfativos (extrato da fóvea e controle) em olfatômetro tipo Y. Nas análises, consideraram-se as respostas dos 47 indivíduos que se dirigiram a um dos braços do olfatômetro. A probabilidade de se deslocar em direção ao extrato de fóvea foi maior para fêmeas do que para machos ($\chi^2 = 3,94$, $P = 0,047$). A proporção de machos que se dirigiram para cada um dos braços do olfatômetro foi a mesma ($\chi^2 = 0,29$, $P = 0,59$). A fóvea abdominal dos machos secreta feromônio sexual, não sendo secretado nenhum feromônio de agregação por essa mesma estrutura. Sugere-se que o feromônio sexual seja usado em comunicação a curta distância, mediando mecanismos pré-copulatórios.

PALAVRAS-CHAVE: Besouro micetobionte, corte copulatória, tangorreceptor

ABSTRACT - Most male ciids present a fovea in the first urosternite. We tested two alternative hypotheses for the function of male abdominal fovea in *Xylographus contractus* Mellié: (i) the fovea secretes sexual pheromone, and (ii) the fovea secretes aggregation pheromone. For this, 59 specimens were submitted, separately, to two stimuli (fovea extracts and control) in an y-shaped glass olfactometer. For the analyses, we considered the 47 individuals that moved toward one of the longer olfactometer branches. The probability of moving toward the fovea extract was greater for females than for males ($\chi^2 = 3.94$, $P = 0.047$). The proportion of males that moved toward any of the olfactometer branches was the same ($\chi^2 = 0.29$, $P = 0.59$). We concluded that the male's abdominal fovea secretes a sexual pheromone and that there is no aggregation pheromone being secreted by this structure. We suggested that this sexual pheromone is used for short-range communication, acting in pre-copulatory courtship.

KEY WORDS: Mycetobiont beetle, copulatory courtship, tangoreceptor

Ciids are cosmopolitan minute fungus beetles (Lawrence 1965, Lawrence 1987) that live in association with the mycelia and fruiting bodies of wood-rotting fungi, especially bracket fungi (Basidiomycetes, Polyporaceae *s. lat.*). They are considered mycetobionts, because both larvae and adults are entirely dependent on a fungus for food and shelter (Scheerpeltz & Höfler 1948, Lawrence 1973, Navarrete-Heredia 1991, Navarrete-Heredia & Burgos-Solorio 2000). Despite their small body size, their populations represent a considerable biomass and actually contribute to the degradation of Polyporaceae (Lawrence 1973). Their population density may be very high, reaching 10,000 individuals per fungus (Navarrete-Heredia & Burgos-Solorio 2000). Ciids have already been recognized as dried fungi pests, both in commercial products and in herbarium

collections (Lawrence 1971, Lawrence 1991, Madenjian *et al.* 1993).

In most Ciidae species, the males have a pubescent fovea in the first urosternite (Lawrence 1971), whose function is not clear. As this character is exclusive to males, it could be suggested that the abdominal fovea secretes sexual pheromone. An alternative hypothesis to explain the function of this fovea is that it could secrete an aggregation pheromone. Similar abdominal structures are found in males of many different beetles (Lawrence 1971), such as in *Aegithus melaspis* (Coleoptera: Erotylidae), which have gregarious behavior (Navarrete-Heredia & Novelo-Gutiérrez 2000). Aggregation pheromone attracts both sexes, maximizing resource exploitation, overcoming host resistance, enhancing the

probability of sexual meetings and may include security from predation (Gullan & Cranston 2000). This kind of pheromone has already been found in several insect orders, such as Coleoptera (e.g., bark beetles), Blattodea and social Hymenoptera (Vilela & Della Lucia 1987). A third hypothesis would be that the male abdominal fovea secretes alarm pheromones. Such pheromones are characteristic of most social and several subsocial insects (Gullan & Cranston 2000). As far as Ciidae do aggregate inside their resource (Navarrete-Heredia & Burgos-Solorio 2000), alarm pheromones could have high adaptive value. To our knowledge, since *X. contractus* was described (Mellié 1848), there has been no other study on its biology nor pheromone production.

In the present study, we tested three hypotheses: (i) the abdominal fovea of *Xylograpus contractus* Mellié (Ciidae) secretes sexual pheromone; (ii) this fovea secretes aggregation pheromone; (iii) the fovea secretes alarm pheromone.

Material and Methods

Ciid Collection and Maintenance. *X. contractus* was collected in bracket fungus at Viçosa (Mata do Paraíso), Minas Gerais State, Brazil (20°45' S, 42°50' W). The colonies were bred under controlled conditions ($26 \pm 1^\circ\text{C}$, without light) from October 1999 to January 2001. This species develops quickly under laboratory conditions. A great number of adults (>500) were obtained each time, due to generation overlapping. This ciid species does not have any conspicuous sexual dimorphism, which allowed us to carry out a blind experiment, i.e., we only knew an individual's sex after its use in the experiment.

Scanning Electron Microscopy. The ultrastructure of the urosternite was analyzed to (i) confirm fovea presence in *X. contractus*, because it is absent in some *Xylographus* species (Lawrence 1971), and (ii) evaluate the presence of pores in other parts of the urosternite, which could be related to pheromone secretion.

Some specimens were covered with gold in a sputtering (Balzers MED 010) and photographed in a Scanning Electron Microscopy (Zeiss DSM 940 A).

Extract Preparation. Dissections were made in physiological solution (NaCl 0.09%) at low temperatures (circa 0°C), to avoid loss of volatile substances. The first urosternite of thirty males was cut and put in separate tubes with 200 μl of bi-distilled hexan to obtain the extracts. Samples were stored in 2 ml tubes with teflon-lined lids until use, and kept at -30°C until their use in the experiments.

Olfactometer Experiments. We exposed 59 specimens to two stimuli in an olfactometer: (i) extract of the first male urosternite, without visceral contents; (ii) pure bi-distilled hexan. The apparatus used consisted of a Y-shaped glass tube with one shorter and two longer branches (Eiras & Mafra Neto 2001). In each of the longer branches we put an air pump (to produce airflow), a filter with activated coal (to avoid contaminants), a fluxometer (SHO -RATE; $I_n/h \text{ AIR @ } t 20^\circ\text{C}$ and $p_a 1.013 \text{ bar}$) to control airflow, a container with water and

another with a stimulus, directly connected to the branch. Filter papers with 40 μl of a stimulus (extract or pure hexan) were placed in each of the latter containers. The airflow was calibrated in $0.1 \text{ m}\cdot\text{s}^{-1}$. A track of filter paper, Y-shaped, was placed through the short branch, reaching 15cm of each of the longer branches to provide a substrate for walking. This procedure also was adopted to make the replacement of the insects easier. The track was changed for each tested specimen to prevent contaminants or interference of any substance. Each specimen was used only once.

The experiment was conducted with one specimen each time. Each specimen was placed 5cm in front of the olfactometer bifurcation. Specimens that did not present any response within 10 min. were discarded. Specimens that presented a response, i.e., moved to one of the longer olfactometer branches, were sacrificed in carbon dioxide gas and dissected to allow sex determination.

Predictions. If the first hypothesis (the abdominal fovea secretes sexual pheromone) were correct, females would be more attracted to fovea extracts than to the control. If the second hypothesis (the fovea secretes aggregation pheromone) were correct, then not only females, but also males would be attracted to fovea extracts. In this case one could not discard simultaneous secretion of sexual pheromone. If the third hypothesis (the fovea secretes alarm pheromone) were correct, both male and female would avoid fovea extracts.

Statistical Analyses. All statistical analyses were done with the GLIM 4 statistical package (Francis et al. 1994). Generalized linear models were used in place of the usual nonparametric statistical tests to ensure greater statistical power (Siegel 1975). To test the hypothesis that the fovea secretes sexual pheromone, a generalized linear model was used, analogous to logistic regression, with binomial errors and logit link function (Crawley 1993). The prediction of this hypothesis was that sex would affect the probability choosing one olfactometer branch, and that there should be a greater number of females that moved to the branch with the fovea extract.

To test the hypothesis that the fovea secretes aggregation pheromone, we analyzed a contingency table with the Chi-squared test. Even if there were a sexual pheromone, an aggregation pheromone could also be secreted, leading to a greater number of males moving towards the branch with the fovea extract. If there were only secretion of aggregation pheromone, there should be a greater number of specimens, irrespective of sex, moving towards the branch with the fovea extract.

Results

Pores were not found around the male's abdominal fovea or in any of the urosternites (Fig. 1). Several bristles surround the fovea, forming a setose patch, and make the discrimination of the fovea itself difficult (Fig. 2). Fovea or pores were not found in the female's urosternites (Fig. 3).

In the experiment, 47 individuals (31 males and 16 females) moved toward one of the longer olfactometer branches. The other 12 specimens, that moved to the minor

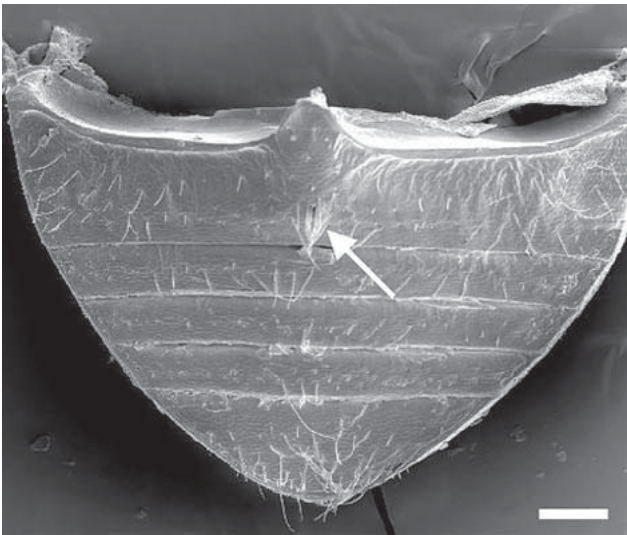


Figure 1. Urosternites of a male *Xylographus contractus*. Arrow indicates a setose patch in the middle of the first urosternite. Note that there are not any other setose patches on the abdomen, but only some sparsely distributed bristles. Scale bar: 100 μ .

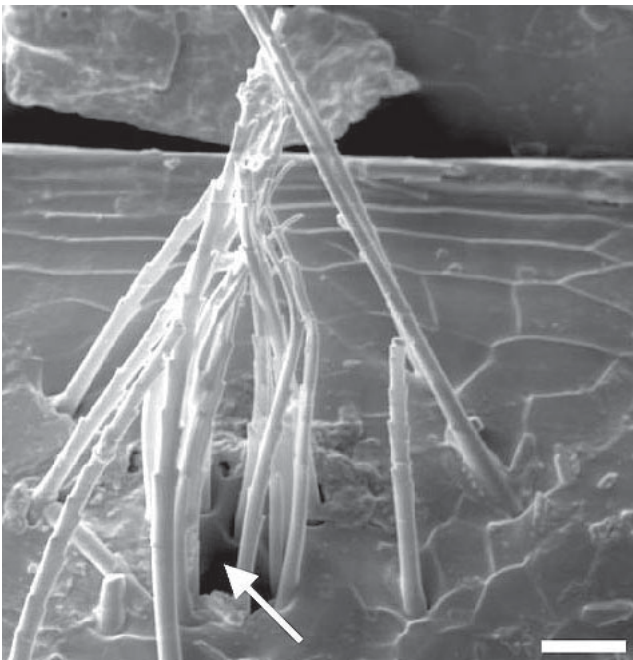


Figure 2. First urosternite (third sternite) of a male *Xylographus contractus*. Arrow indicates the fovea, which is surrounded by bristles, forming a setose patch. Scale bar: 10 μ .

branch or did not move, were not considered in the analyses. The probability of moving to the branch with extract was higher for females (12 in 16) than males (14 in 31; $\chi^2 = 3.94$, $P = 0.047$). Thus the hypothesis that the fovea secretes sexual pheromone was accepted. The number of males that moved toward the branch with extract did not differ from the number of males that moved toward the other branch ($\chi^2 = 0.29$, $P = 0.59$). Thus the hypothesis that the fovea secretes aggregation pheromone was rejected.

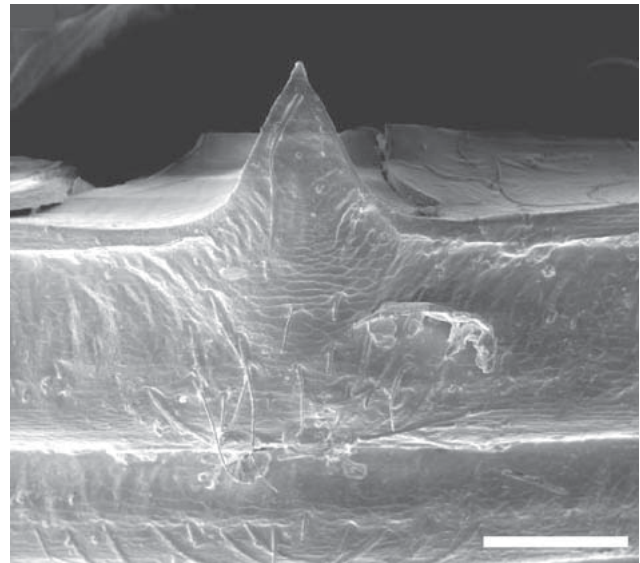


Figure 3. First urosternites of a female *Xylographus contractus*. Note that there is no fovea or pore in the middle of the first urosternite (third sternite). Scale bar: 100 μ .

Discussion

Our results revealed that (i) the male's fovea is the only structure that could secrete pheromones in the abdominal sternites of *X. contractus*, (ii) the male abdominal fovea secretes sexual pheromone and (iii) that it does not secrete aggregation or alarm pheromones. Why are bristles arranged around the fovea? Males are generally found outside the fungus, while females stay inside it for longer, laying their eggs in chambers of the galleries' walls (Lawrence 1974). To copulate, either the males should produce some pre-copulatory stimulus to attract the females to the surface, or the females should leave the fungus and find their mates by chance. Males outside the fungus scratch their abdomens on the surface before copulation (Lopes-Andrade pers. obs.). Therefore, the foveal bristles may be some kind of tangoreceptor sensilla (Snodgrass 1935), which stimulates pheromone secretion. Thus, the sexual pheromone of males may be important in pre-copulatory courtship, attracting females to the fungus surface, where copulation takes place. Therefore, the pheromone produced by males may be courtship, and not a long-range attraction pheromone. Additionally, the fovea bristles may serve to increase pheromone spreading, facilitating the dispersion of the odor plume. This mechanism may enhance the area covered by the odor plume in a short distance. Jonsson *et al.* (1997) suggested that long-range pheromones are absent in Ciidae. For long-range attraction, fungus volatiles play the most important role (Jonsell & Nordlander 1995, Fossli & Andersen 1998, Fäldt *et al.* 1999).

As ciids present gregarious behavior (Lawrence 1974), there could be an aggregation pheromone being secreted. Lawrence (1973) suggested that such a pheromone would be secreted together with the feces. An abdominal fovea is found in almost all ciids, but this does not mean that in all these species the fovea has the same function, *i.e.*, secreting a sexual

pheromone. For instance, in some species of *Xylographus* Mellié and *Cis* Latreille, it was suggested that the fovea is vestigial or completely absent (Lawrence 1971). Experiments to evaluate the presence of other pheromones, in addition to the sexual, are still necessary.

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