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SCIENTIFIC NOTE

Behavioral Evidence of an Ectoparasitic Interaction between *Triatoma pseudomaculata* Corrêa & Espínola (Heteroptera: Reduviidae) and *Periplaneta americana* (L.) (Blattodea: Blattidae)

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Abstract

The present work tested whether *Triatoma pseudomaculata* Corrêa & Espínola shows behavioral traits indicating that it is capable of feeding on arthropods. We consistently observed nymphs extending their proboscis in the direction of cockroaches and attempting to bite. Insects presented a preference for biting specific cockroach body parts. Our results suggest that arthropod hemolymph represents an alternative source of food that increases the survival of *T. pseudomaculata* individuals undergoing long starvation.

Triatoma pseudomaculata Corrêa & Espínola is a hematophagous insect capable of transmitting *Trypanosoma cruzi,* the etiological agent of Chagas disease, to humans (Silveira & Vinhaes 1998). It is frequently captured in human-associated ecotopes and its distribution includes most of the semiarid northeast of Brazil. As for human environments, this species is mostly found in peridomiciliary settings (Dias & Diotaiuti 1998).

Triatoma pseudomaculata has been found associated with cockroaches inside chicken-coops (Freitas *et al* 2005), and precipitin tests confirmed the presence of cockroach hemolymph in the intestinal contents of some of the sampled insects. Our work tested whether *T. pseudomaculata* presents behavioral traits indicative of arthropod feeding. We performed experiments in which nymphs of this species were confronted with those of *Periplaneta americana* (L.) and their behavior analyzed to characterize feeding interactions.

Starved fifth instars of *T. pseudomaculata* were used for these experiments 30-35 days after ecdysis. Different instars of cockroach were fed *ad libitum* on rabbit food pellets and presented as potential hosts. For each assay, three *T. pseudomaculata* nymphs were individually weighed, marked with paint and transferred to a Petri dish (10 cm diameter) that was used as an experimental arena (15 replicates, 45 insects). A cockroach nymph (n = 15) was subsequently introduced in the arena and the assay initiated.

The assays were conducted during the first 6h of the scotophase. An infrared sensitive CCD video camera (HDL, HM 30PB, Brazil) connected to a time-lapse video-recorder (Samsung SVR 960NRT, Korea) recorded bug behavior in the experimental arena. Triatomine nymphs were re-weighed after the assays to determine whether they had ingested hemolymph. The following parameters were analyzed: a) number of times that the rostrum of nymphs contacted a cockroach, b) proportion of these contacts on antennae, head, thorax, legs or abdomen of *P. americana*, c) duration of contacts and d) final state of cockroach nymphs, *i.e.*, alive or dead. Bugs from seven out of 15 assays, i.e., 21 bugs, were afterwards separated in two groups: 1) insects that had increased their weight (7) and 2) insects that had not (14) and, their survival rate was evaluated for two months. The duration of bites was analyzed sorting them in a total of eight 30s intervals ranging from less than 30s (< 30s) to more than 210s (> 210s), e.g. 30-60s.

We observed nymphs extending their proboscides in the direction of cockroaches and carrying out biting attempts in 266 occasions distributed through all assays (Fig 1). The minimum number of bites per assay was six, while the maximum was 57 (33.2 ± 16.40). As a consequence, 29% of the insects (13/45) increased their weight after the experiment. Interestingly, fed bugs presented an initial weight lower than those that did not feed (32/45, unpaired *T* test, *t*-value = -3.68, df = 42 n = 45, P = 0.0006), indicating that low nutritional status might have motivated them to bite arthropods. After 60 days all triatomines in group 1 (n = 7) were still alive. Nevertheless, only 29% of group 2 (n = 4/14) survived (difference between proportions test, P = 0.0069).

Triatomines directed their bite attempts to all parts of the cockroach body, but the choices were not random (Fig 2, Kruskal-Wallis, P = 0.035). The most frequently bitten body parts were the legs and head, but a significant difference was only observed between the number of bites on the legs and the thorax (Fig 2, Dunn's *post hoc* multiple comparison, P < 0.05). The head, antennae and legs are structures that exhibit many sensory, integrative and motor components of the nervous system and concentrated 80% of the bites. Additionally, it is known that triatomine saliva contains substances that affect nerve cell responses (Dan *et al* 1999). Our data suggest that the pharmacological effects of triatomine salivary components might not be exclusively directed to facilitate vertebrate blood ingestion, but also hemolymph intake through the manipulation of arthropod motility.

Fig 1 *Triatoma pseudomaculata* fifth instar nymph attempting to feed on a cockroach, *Periplaneta americana*.

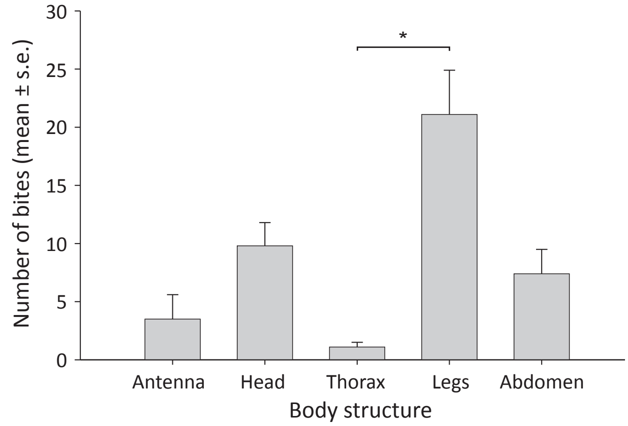

Fig 2 Distribution of bites (mean ± s.e.) of *Triatoma pseudomaculata* fifth instar nymphs on different parts of the body of cockroaches (Kruskal-Wallis test, P = 0.006).

Two out of 15 cockroaches died after the trials and the number of triatomine bites was highest in both assays (57 and 53). The death of *P. americana* nymphs may have been caused by specific effects on neurons (Dan *et al* 1999) or by unspecific toxic or proteolytic effects when saliva was injected into their hemolymph; the last being the least probable due to the fact that most cockroaches survived (13/15). Shortest and longest bite duration intervals were the most frequent categories (< 30 s = 86 bites, > 210 s = 76 bites, respectively). The remaining six duration intervals concentrated only 104 of the bites. This bimodal distribution was probably due to the fact that the bugs probed the body of the cockroach in short bursts (< 30 s) in order to find an adequate area to insert their mouthparts and subsequently inject saliva. Hemolymph ingestion would demand longer contact periods and accordingly, the frequency of contacts longer than 210 seconds was also considerable. These long rostral contacts suggest that triatomines intended to feed on *P. americana*.

The proboscis extension reflex of triatomines is a behavior of extreme importance that frequently reflects the start of the feeding process (Ferreira *et al* 2007), which was observed in all the assays carried out. The fact that some of the nymphs increased weight after assays confirms this interpretation of prolonged biting behavior. This suggests that arthropod hemolymph represents an alternative source of food that increases the survival of *T. pseudomaculata* individuals undergoing long starvation. Therefore, triatomine bugs may have partially conserved their entomophagous habits during their evolution to the haematophagous feeding specialization.

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