New evidences supporting trophobiosis between populations of Edessa rufomarginata (Heteroptera: Pentatomidae) and Camponotus (Hymenoptera: Formicidae) ants

Daniel Paiva Silva a,*, José Antônio Marin Fernandes b

a Department of Biological Sciences, Instituto Federal Goiano, Uruataí, GO, Brazil
b Instituto de Ciências Biológicas, Universidade Federal do Pará, Belém, PA, Brazil

ABSTRACT

Despite its important effect on the maintenance of tritrophic interactions among plants, insect herbivores, and ants, there is still a paucity of natural history and basic biology information involving trophobiosis among Heteroptera stink bugs. Here, based on previous observations of a new trophobiotic interaction between Edessa rufomarginata (De Geer, 1773) and Camponotus rufipes (Fabricius, 1775) ants, we describe the chemical profile of the honeydew obtained by Gas Chromatography–Mass Spectrometry. There were mainly three different sugars (trehalose, glucose, and sorbose) within our samples. The extratropical nectaries of Caryocar brasiliense Camb., the host plant of E. rufomarginata, attracts a wide assemblage of Cerrado ants with varying aggressiveness toward herbivores. Therefore, this facultative trophobiotic interaction may allow the survival of the stink bug while feeding on the risky, highly visited plant. Given the rarity of trophobiotic interactions between Pentatomidae species and ants and considering a zoological perspective within this family, here we discuss the ecological and evolutionary routes that may allow the rise of these interactions.

© 2016 Sociedade Brasileira de Entomologia. Published by Elsevier Editora Ltda. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Introduction

Ants comprise a very diverse and abundant group of organisms in terrestrial ecosystems that feed on a variety of food resources, including liquid sugary substances produced by myrmecophilous plants and insects (Davidson, 1997; Hölldobler and Wilson, 1990). Foliage-dwelling ants are the main visitors of plants bearing extratropical nectaries (EFNs hereon) and of honeydew-producing insects, and their patrolling activities around such food sources have been repeatedly reported to protect the plants from herbivores, as well as the trophobiont insects from natural enemies (Stadler and Dixon, 2008; reviewed by Rico-Gray and Oliveira, 2007).

Generally, trophobiosis with ant partners involves costs (physiological and developmental constraints) and benefits for the trophobionts (enemy-free space, higher reproductive success), some of which have already been assessed for ant-tended riodinid and lycaenid butterflies (Kaminski, 2008; Kaminski et al., 2009, 2010); as well as for auchenorrhynchous and sternorrhynchous hemipterans (Stadler and Dixon, 2008; for comprehensive reviews see Hölldobler and Wilson, 1990). Among the Hemiptera, the vast majority of trophobiont interactions are found in the Auchenorrhyncha and Sternorrhyncha suborders, and just a few have been reported for the Heteroptera (Table 1). Myrmecophily is also poorly documented among heteropterans (Table 1; Delabie, 2001) and Pentatomidae has only two cases reported in the literature supporting myrmecophily. Stahel (1954) observed the potential trophobiotic interaction of Lincus spathuliger (Bredin) (Heteroptera: Pentatomidae) feeding from the roots of coffee plants and Pheidole ants found in Surinam. Guerra et al. (2011) successfully described the obligatory trophobiotic interaction between Eurystethus microlobothus Rudbeck 1966 (Heteroptera: Pentatomidae) feeding exclusively on individuals of the mistletoe Psittacanthus robustus Mart. (Loranthaceae) and several Camponotus (Formicidae: Formicinae) species, with C. rufipes (Fabricius) also showing the most aggressive behavior while securing these bugs.

Field observations regarding the biology and ecology of Pentatomidae species are scant, not commonly reported, and too focused to those species causing economic damages to crop plants species (Callan, 1944; Eberhard, 1975; Iannaccone et al., 2007; Santos and Albuquerque, 2001a, 2001b). Among Edessinae species, there are few studies considering their biological and ecological aspects,
especially because the taxonomic boundaries between species are still confusing, but in the few studies considering these aspects, trophobiosis is not usually mentioned (De Fortes and Grazia, 1990; Iannacone et al., 2007; Lima et al., 2010; Rizzo, 1971; Rizzo and Saini, 1987; Silva and Oliveira, 2010). Studies by Rizzo (1971) and Rizzo and Saini (1987) are particularly important because of their extensive review on the biological and ecological aspects of the two most important Edessa species causing economic problems to crop plant species when considering agriculture perspectives.

In the Brazilian savanna (also known as Cerrado), the stink bug Edessa rufomarginata De Geer (Heteroptera: Pentatomidae) commonly infests Caryocar brasiliense Cambessédes (Caryocaraceae), a shrub possessing extrafloral nectaries (EFNs) that is visited by a wide assemblage of ant species (Oliveira and Brandão, 1991). Ant-exclusion experiments with C. brasiliense revealed that ant visitation significantly reduced infestation by four species of insect herbivores, including E. rufomarginata (Oliveira, 1997).

Field observations of ant species from Cerrado showed that some of these species indeed present conspicuous aggressiveness toward herbivores on C. brasiliense host plants (Oliveira and Freitas, 2004; Sendoya et al., 2009). However, recent data indicate that the interaction between E. rufomarginata and some visiting ants species may not be necessarily antagonistic. Although aggressive ants, such as Pachycondyla villosa (Fabricius) (Formicidae: Ponerae), were already observed attacking E. rufomarginata nymphs (Oliveira, 1997), Silva and Oliveira (2010) frequently noticed C. rufipes ants tapping their antennae in the abdomen of third- to fifth-instar nymphs of the stink bug (n=30). During these interactions, honeydew consumption by the ants was observed twice (Fig. 1). Nonetheless, despite the aggressiveness of this ant species toward herbivorous insects, in none of these interactions the pentatomid nymphs seemed to be disturbed by the ant presence. In order to better understand this ant-pentatomid interaction, a chemical analysis of the honeydew produced by this pentatomid species was done. Once the presence of specific saccharides (i.e., melezitose) may enhance the specialization of the trophic interactions between some heteropterans and ants (Klós, 1981), describing the chemical compounds from the honeydew of E. rufomarginata is important to evaluate the interaction of this hemipteran with C. rufipes ants. Therefore, our aim here is to report this previously unknown interaction between pentatomids and from the Brazilian Cerrado Savanna and discuss the potential behavioral and evolutionary triggers that may have resulted in this interaction.

### Material and methods

Fieldwork was carried out in a Cerrado area near Itirapina, SE Brazil (22°15'S, 47°49'W) from September 2008 to February 2009, when E. rufomarginata individuals were observed in 75 blooming shrubs of C. brasiliense (0.5–2.0 m tall), three to four times a week (Silva and Oliveira, 2010). The phenotype of E. rufomarginata matches with that of C. brasiliense, and its immatures develop in the wet season (October to March), when there is increased production of both vegetative and reproductive plant tissues (Silva and Oliveira, 2010). Both the nymphs and adults of E. rufomarginata feed on stem parts, flower buds and fruits; females do not exhibit parental care and nymphs disperse in the third instar (Oliveira, 1997; Silva and Oliveira, 2010).

Eight E. rufomarginata nymphs (third- and fourth-instar) were hand-stimulated in the field to secrete honeydew on a small piece of filter paper. Once the honeydew was collected, the filter paper was immediately stored in ice (0°C) to avoid honeydew deterioration. The material was stored at −20°C for the chemical analysis in the laboratory. Gas Chromatography–Mass Spectrometry (GC–MS) was used to determine and estimate the relative amount of sugary substances in the honeydew.

The pieces of filter paper with honeydew were extracted in 5 mL Ethanol (EtOH). The extract was vacuum dried and then acetylated with 300 μL pyridine:AcOEt (1:1 ratio) for 48 h. The reaction was dried overnight on air flux and recovered in 300 μL Ethyl-Acetate (EtOAc) for the GC–MS analysis in the EI mode on a Hewlett Packard-6890 GC system, with a fused capillary column (30 m × 0.25 mm × 0.25 μm); HP-5MS, directly coupled to a selective mass detector Hewlett Packard 5973. Injection conditions: injector temperature was 250°C; oven temperature program was 160–300°C, with 4°C/min, 5 min at 300°C; transference line temperature was 300°C; injection mode split 20:1. Carrier Helium gas: 1 mL/min with constant flow; sample volume 1.0 mL. The sugars characterization was assessed by comparison of MS fragmentation with NIST Mass Spectral Search Program (Version 2.0f 2008), according to Biemann et al. (1963). The same procedures were applied to pieces of filter paper containing only distilled water (control treatment).

### Results and discussion

The honeydew samples of the nymphs of E. rufomarginata contained mainly three kinds of sugar (Fig. 2): a disaccharide (trehalose) and two monosaccharides (glucose and sorbose). Trehalose was the main sugar found, accounting for 94.1% of the carbohydrides in the samples. Glucose and sorbose accounted for 4.7% and 1.2% of the total sugar amount, respectively. The control filter paper did not present any sugars.

Honeydew composition and attractiveness to ants varies with the honeydew-producing species (Blüthgen et al., 2006), but trehalose usually makes up for more than 35% of the sugar contents.
Fig. 1. Record of a *Camponotus rufipes* ant consuming honeydew produced by a fourth instar nymph of *E. rufomarginata*. In (1), the ant is tapping its antennae onto the end of the nymph’s abdomen, a process that continues in (2). In (3), the ant finally receives a tiny droplet of sugary exudate from the nymph. However, after promptly consuming it, in (4) it continues to tap its antennae on the nymph’s abdomen. In (5), it received a bigger amount of exudate from the nymph prompt consumed in (6). After consuming the liquid, the ant continued to tap the nymph’s abdomen. The arrows within some of the photos indicate the presence of the honeydew droplets within the ant’s mandibles. Photos by Sebastián Sendoya.

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sorbose</td>
<td>1.2%</td>
</tr>
<tr>
<td>2</td>
<td>Glucose</td>
<td>4.7%</td>
</tr>
<tr>
<td>3</td>
<td>Trehalose</td>
<td>94.1%</td>
</tr>
</tbody>
</table>

Fig. 2. Chromatograms of the honeydew contents from *Edessa rufomarginata* third- and fourth-instar nymphs. The percentages refer to the amount of each sugar in the analyzed sample.
of honeydew, as it is the major sugar found in insects haemolymph (Hölldobler and Wilson, 1990). Therefore, the high concentration of this sugar in the honeydew of *E. rufomarginata* nymphs is not surprising. Following trehalose, monosaccharides are the main honeydew constituents of myrmecophilous hemipterans (Hölldobler and Wilson, 1990).

Contrary to other *C. brasiliense* herbivores (i.e. *Eunica bechinea* Talbot (Lepidoptera: Nymphalidae)), which possess several tactics to avoid ant attacks (Oliveira and Freitas, 2004; Sendoya et al., 2009). *E. rufomarginata* nymphs cannot fly and exhibit low mobility rates. Although they could quickly withdraw their proboscis from the plant tissues and spray their scent glands when approached by enemies, *E. rufomarginata* nymphs do not do it in the presence of *C. rufipes*. Although they are continuously exposed to threat of ant predation while feeding on a highly ant-visited plant such as *C. brasiliense* (Oliveira, 1997; Silva and Oliveira, 2010), secretion of honeydew may decrease attacks by foliage-dwelling ants. Trophobiosis with ants via honeydew offering is a widespread strategy among insect herbivores in Cerrado, some of which may even flick honeydew beneath the host plant to attract prospective ant attendants (Del-Claro and Oliveira, 1996, 2000). Nevertheless, the offer of honeydew cannot be considered as a universal adaptation against ant predation, since the consumption of honeydew producers by honeydew-attendant ant species occasionally may occur (Stadler and Dixon, 2008).

Some biological features of the sap-feeding herbivores are believed to determine the degree of specialization of this interaction, whether the interaction is facultative or obligatory, with ants. Gregariousness, low dispersal ability, specialized diet breadths, and the occurrence of parental care by the sap-feeding insects are generally believed to be strongly associated with the occurrence of trophobiosis (Delabie, 2001; Guerra et al., 2011). Such features also seem to determine the evolution of trophobiotic interactions among other hemipterans (Steiner et al., 2004; Souza and Francini, 2010). When we specifically consider *E. rufomarginata*, the lack of parental care, gregariousness, and the low dispersal rates of its specimens, when combined with the apparent dependence to feed only from *C. brasiliense* may determine the facultative status of this trophobiotic interaction, and the sugary content of the exudate of *E. rufomarginata*. Therefore, even though *E. rufomarginata* not necessary depend of the ant species to survive, some degree of interaction with them may provide them an increased protection when compared to other species also feeding from *C. brasiliense*.

In previous field observations of different populations of *E. rufomarginata*, from north to south of Brazil, this species usually did not obtained food from Solanaceae plant species with visiting ants (Fernandes, J.A.M., pers. comm.). The specimens composing the population analyzed here are recognized by a light brown coloration with a yellow dorsal margin of their abdomen, instead of the dark green with red abdominal margin that usually characterizes the majority of the populations of this species occurring elsewhere in the country. Given such differences, Silva et al. (2004) considered this variation as the form II of *E. rufomarginata*, which is only distributed in Cerrado savannas from the Brazilian states of São Paulo and Minas Gerais, and there are usually sampled on *C. brasiliense*. Therefore, we suppose that the present population of *E. rufomarginata* composes a cryptic complex of species, and justifies the low morphological differences and the absence of trophobiosis of other *E. rufomarginata* populations reported in the literature.

The resemblance of the honeydew of the nymphs of *E. rufomarginata* with that produced by hemipterans from Auchenorrhyncha and Sternorrhyncha suborders and the behaviors the nymphs exhibit in the presence of *C. rufipes* ants suggest that this trophobiotic interaction is not incidental, being new among Edessinae species. Therefore, even though these interactions were still not frequently described among the Pentatomidae species, the study of trophobiosis in the Heteroptera suborder of Hemiptera is promising and many other similar interactions may occur and be described in future studies. Therefore, we believe that further investigation is still necessary to determine whether this interaction represents an incidental interaction or a mutualism.

**Conflicts of interest**

The authors declare no conflicts of interest.

**Acknowledgements**

We thank P.S. Oliveira, P. Rodrigues, C. Schwertner, A.V.L. Freitas, R.R. Solar, and two anonymous reviewers for several suggestions provided to previous versions of this manuscript, which significantly improved our text. DPS thanks S. Sendoya for providing the photos of the ant consuming the nymph’s exudate. J.R. Trigo kindly provided the sugar analyses by GC–MS. We also acknowledge “Programa de Pós-Graduação em Ecologia” from UNICAMP for scholarships [from “Coordenação de Aperfeiçoamento de Pessoal de Nível Superior” (CAPES) and “Fundação de Amparo à Pesquisa do Estado de São Paulo” (FAPESP – no. 2008/52781-8)] that were offered to DPS during his Master’s Degree. A research grant from FAPESP to P.S. Oliveira (# 08/54058-1) also supported this study. José A. M. Fernandes thanks Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) grant 311403/2012-3. Finally, the authors are also grateful to Instituto Federal Goiano – Campus Urutai for the payment of the publication fees of this manuscript.

**References**


