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

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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 extrafloral 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 ant-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.

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 extrafloral 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* (Breddin) (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 microlobatus* Ruckes 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; Iannacone et al., 2007; Santos and Albuquerque, 2001a, 2001b). Among Edessinae species, there are few studies considering their biological and ecological aspects,

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Table 1
Records of trophobiotic interactions between ants and heteroperans.

Taxa	Location	Reference
Coreidae		
Hygia (Trichocolpura) cliens (Dolling)	Malaysia	Maschwitz et al. (1987)
Cloresmus spp.	Malaysia	Maschwitz et al. (1987)
Notobius affinis (Dallas)	Malaysia	Maschwitz et al. (1987)
Amorbus obscuricornis (Westwood)	Tasmania	Steinbauer (1996)
Undescribed coreid sp. 1	Borneo	Blüthgen et al. (2006)
Undescribed coreid sp. 2	Borneo	Blüthgen et al. (2006)
Pentatomidae		
Lincus spathuliger (Breddin)[a]	Surinam	Stahel (1954)
Eurystethus microlobatus (Ruckes)	Brazil	Guerra et al. (2011)
Edessa rufomarginata (De Geer)	Brazil	Present study
Plataspidae		
Coptosoma sp. (Laporte)	Sri Lanka	Green (1900) *apud* Waldkircher et al. (2004)
Coptosomoides myrmecophilus (China)	Sumatra	China (1931) *apud* Waldkircher et al. (2004)
Tropidotylus servus (Dolling)	Malaysia	Maschwitz et al. (1987)
Tropidotylus minister (Dolling)	Malaysia	Maschwitz et al. (1987)
Caternaultiella rugosa (Schouteden)	Cameroon	Dejean et al. (2000, 2002) Gibernau and Dejean (2001)
Tetrisia vacca (Webb)	Malaysia	Waldkircher et al. (2004)
Undescribed plataspid sp.	Borneo	Blüthgen et al. (2006)

[a] True trophobiosis not documented.

especially because the taxonomic boudaries between species are still confuse, 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* Cambessèdes (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: Ponerinae), 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 (Kiss, 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 phenology 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:AcO_2 (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 290 °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 dissacharide (trehalose) and two monossacharides (glucose and sorbose). Trehalose was the main sugar found, accounting for 94.1% of the carbohydrates 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