

Unexpected attraction of *Urostreptus* sp. (Spirostreptidae) to seed elaiosomes: a new resource record to millipedes' diet

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Abstract

We describe, for the first time, the attraction of an unidentified millipede species (*Urostreptus* sp.) to an elaiosomes of a Euphorbiaceae species (*Jatropha mutabilis* (Pohl) Baill.). After field observations of *Urostreptus* specimens feeding on elaiosomes' seeds of *J. mutabilis* in areas of Caatinga in Petrolina, northeast Brazil, we conducted experiments to test the hypothesis that this millipede is specifically attracted to *J. mutabilis* elaiosomes. In the first experiment, millipedes were offered a choice between pairs of seeds with and without elaiosomes. In each trial we recorded the animal choice and the time required to access the seed. In the second experiment, millipedes were offered a choice between pairs of pebbles similar in size to the seeds, one of which was soaked in elaiosome extract. The results showed that millipedes significantly preferred seeds with elaiosomes and were all attracted to pebbles soaked in the elaiosome extract. We also speculate some of the potential implications for seed dispersal and seedlings distribution.

Keywords: Brazil; Caatinga; Diplopoda; elaiosome-bearing seed; Euphorbiaceae.

Introduction

Over 3,000 angiosperm species from more than 80 families have evolved a fat body appendage called elaiosome (Beattie 1985), originating from several seed or fruit tissues, referred to in the literature as aril, ariloide and caruncle (see a review by Gorb & Gorb 2003). Elaiosome-bearing seeds attract a large number of ant species that carry the seeds to their nests,

remove and consume the elaiosomes, and discard/concentrate the intact seeds in refuse piles either within subterranean nest chambers or on the soil surface around nest entrances (Beattie 1985). Recent studies have identified the Caatinga vegetation, a mosaic of seasonally dry forests and scrub vegetation in northeast Brazil, as a hotspot of myrmecochory, where Euphorbiaceae species are the most important

group with elaiosome-bearing seeds (Leal et al. 2007; Leal et al. 2015).

Casual observation in the Caatinga vegetation indicated a high activity level in an unidentified millipede species of the genus *Urostreptus* Silvestri, 1985 (Diplopoda, Spirostreptidae) feeding on elaiosomes of the seeds of *Jatropha mutabilis* (Pohl) Baill. (Euphorbiaceae). Millipedes represent an important segment of the Neotropical fauna and are a key group within the saprophage macrofauna, most millipedes are detritivores that feed mostly on decomposing plant material, i.e. leaf litter, dead wood, and the microbiota associated with these substrates (David & Handa 2010; Hopkin & Read 1992). Recently, Koprdoová et al. (2010) showed the consumption of different small seeded species by one millipede species in laboratory experiments in Prague. Notwithstanding their important role in ecosystem functioning, little is known on the ecology and natural history of these invertebrates (Battirola et al. 2009), especially in the poorly studied Caatinga vegetation (Santos et al. 2005).

We did not find any scientific reference regarding more specific interactions between millipedes and plants in South America (except for a case of epizoic bryophytes on a platyrhacid millipede species in Colombia (Martínez-Torres et al. 2011). Here, we describe for the first time the attraction of a millipede species to the elaiosomes of *Jatropha mutabilis* seeds (a Euphorbiaceae species very common in Caatinga vegetation), which may have potential consequences in terms of plant distribution and seed germination. Additionally, we performed experiments in order to test the role of the elaiosome in the attraction of *J. mutabilis* seeds to *Urostreptus* sp. individuals.

Material and methods

Study area and species

Seeds from *Jatropha mutabilis* and *Urostreptus* sp. individuals were collected in an area of Caatinga vegetation located in the Campus of the Universidade Federal do Vale São Francisco (09°19'S, 40°32'W, 395 m.a.s.l.), Petrolina, northeast Brazil. The climate is hot

semi-arid, with an average annual rainfall of 401 mm and a marked annual dry season (< 60 mm per month) lasting 7-11 months (IBGE 1992). Predominant soils are aridisol and typic quartzipisamment (IBGE 1992). The vegetation consists of patches of seasonally dry forest (*sensu* Mooney et al. 1995) and sclerophyllous vegetation. This site is included among priority areas for biodiversity conservation in the Caatinga vegetation (MMA 2002).

The genus *Urostreptus* comprises 12 species occurring exclusively in South America (Pierozzi & Fontanetti 2006). The sole available information on this genus is based on taxonomic description of species, chromosome related aspects and records of urban invasion (Fontanetti et al. 2010; Pierozzi & Fontanetti 2006). The species studied here is still under taxonomical investigation. The genus *Jatropha* L. (Euphorbiaceae) is composed by 175 tropical and subtropical species, most of them presenting elaiosome-bearing seeds (Webster 1994). Thirteen species occur in Brazil (Cordeiro & Secco 2013), including *Jatropha mutabilis* (Pohl) Baill., an endemic and monoic shrub (~2.0 m high) of the Caatinga, frequently found in sandy soils with predominant scrub vegetation (Sátiro & Roque 2008). The reproductive phenology of this species is continuous, producing flowers and fruits all around the year (Santos et al. 2005). Fruits are capsules with three carpels (Fig. 1a), each one sheltering a single seed (Santos et al. 2005). The elaiosome is found on top of the distal portion of the seeds. When seeds are ripe, they are dispersed through a diplochoric system, i.e. autochory (ballistic dispersal of the explosive dehiscent capsules) followed by myrmecochory (seed dispersal by ants) (Leal et al. 2007; Leal et al. 2014).

Removal experiments

In order to investigate whether *Urostreptus* sp. individuals are attracted to *J. mutabilis* elaiosomes, we conducted experiments using seeds with and without elaiosomes (Fig. 1b). Mature fruits were collected directly from shrubs and oven dried for 30 min at 70°C to induce the release of seeds. Seeds (n = 90) were then randomly separated into two equal-sized

groups: intact seeds, and seeds with experimentally removed elaiosomes. Seeds of *J. mutabilis* and individuals of *Urostreptus* sp. (n = 45) were collected in the same area of Caatinga. The *Urostreptus* individuals were kept in plastic boxes until the removal experiments (for no longer than two hours), without being fed. One seed of each group (i.e. with and without elaiosome) were placed in two opposite sides of plastic chambers (8 cm x 21 cm) with a sand layer (ca. 2 cm thick). We then placed one randomly selected individual of *Urostreptus* sp. in the center of the chamber and recorded its behavior for five minutes. After this time, animals and seeds were replaced for the next trial. In each trial we recorded the animal choice and the time required to access to the seed. We considered that a choice had occurred when the animal manipulated any seed for more than 60 seconds. Positive choices referred to those targeting elaiosome-bearing seeds (Fig. 1c), while negative choices represented the cases in which animals manipulated a seed without elaiosome as well as any seed that was not manipulated. After each trial, animals and seeds were replaced, and seeds were placed in different positions. In order to verify if the seed biomass influenced the millipede's choice, we used similar-sized seeds that were weighted on an electronic balance.

A second experiment was performed to confirm the attraction of *Urostreptus* sp. to *J. mutabilis* elaiosome. An alcoholic extract was prepared with 40 ml of ethylic alcohol and 10 g of macerated elaiosome. To test the effect of this extract we selected 15 pairs pebbles with shape and size similar to *J. mutabilis* seeds. One pebble of each pair was soaked in the extract for 2 h and then exposed to direct sun during 15 min to dry up. We then offered to *Urostreptus* individuals one imbibed and one non-imbibed pebble using the same procedure described in the previous experiment. Positive choices referred to those targeting pebbles that were imbibed with extract, while a negative choice represented the sum of cases in which animals manipulated non-imbibed pebbles or that any pebble was manipulated. After each trial, animals and pebbles were replaced, pebbles (with and without extract) were placed in

interchangeable positions in each trial. The pairs of pebbles were weighted to verify that was not their mass that influenced the millipede's choice.

Statistical analyses

The frequency of positive and negative choices during both experiments was tested with Chi-square test. Differences in seed and pebble mass offered to millipedes during both experiments were tested using paired t tests. The time required by the millipede to access to the seeds with and without elaiosome and imbibed and non-imbibed pebbles were compared with Kruskal-Wallis tests. Normality and homoscedasticity were tested with Shapiro-Wilk and Levene tests respectively. All analyses were performed with PAST v1.75 (Hammer et al. 2001) and are described in Sokal & Rohlf (1995).

Results

Of the 45 trials of the first removal experiment, we recorded 37 positive choices (i.e. *Urostreptus* sp. chose *Jatropha mutabilis* seeds with elaiosome) and 8 negative choices (i.e. 3 cases in which animals manipulated a seed without elaiosome and 5 cases in which no seed was manipulated) thus indicating that millipedes were significantly more attracted to seeds with elaiosomes ($\chi^2 = 13.89$, $df = 1$, $p < 0.001$) as compared to seeds without elaiosomes. However, the encounter time of elaiosome-bearing seeds (52.9 ± 45.4 s, $n = 37$ (mean \pm SD)) was not significantly different (H (the K-W test statistic) = 3.85, $df = 1$, $p = 0.45$) from that time spent to encounter seeds without elaiosomes (46.3 ± 59.6 s, $n = 3$). There was no statistically significant weight difference between seeds with and without elaiosome ($t = 0.21$, $df = 41$, $p = 0.83$), excluding the possibility that animal attraction was related to differences in seed weight.

In the second experiment, all individuals of *Urostreptus* sp. tested ($n = 15$) were attracted to pebbles imbibed in the elaiosome extract (Fig. 1d). Animals took 63.9 ± 54.5 s to find imbibed pebbles and manipulated them for 48.9 ± 14 s. The time spent by animals to find imbibed pebbles (63.9

± 54.5 s) was not statistically different ($F = 1.42$, $p = 0.38$) from the time spent to encounter seeds with elaiosome (52.9 ± 45.4 s). Finally, there was no significant difference between the mass of imbibed and non-imbibed pebbles ($t = 1.32$, $df = 15$, $p = 0.20$).



Figure 1. (A) Detail of a *Jatropha mutabilis* (Euphorbiaceae) with fruits in different maturation stages (photo: G. Fernandes); (B) *J. mutabilis* seeds with (left) and without (right) elaiosome (photo: G. Fernandes); (C) An *Urostreptus* sp. individual eating a *J. mutabilis* elaiosome (photo: S. Ribeiro); and (D) individual of *Urostreptus* sp. attracted to a pebble imbibed in elaiosome extract (photo: G. Fernandes).

Discussion

For the first time we describe the attraction of millipedes to seed elaiosome. Our experiments demonstrate that *Urostreptus* individuals are attracted to elaiosome of *Jatropha mutabilis* seeds. Animals were attracted to the elaiosomes of the seeds, ate them and left the intact seeds in the spot. Only recently the first evidence that millipedes consume seeds were provided (Koprdoová et al. 2010), however solely the consumption of small seeded species by one millipede species (*Cylindroiulus caeruleocinctus* (Wood 1864)), in laboratory experiments, was recorded. Millipedes usually have small heads and mouthparts relative to its body size, what probably limits their ability to grasp and consume intact seeds, especially bigger ones. Different seed properties other than size such as seed shape, chemical composition, seed coat roughness and thickness

probably are related to their ability to consume those (Koprdoová et al. 2010). Elaiosomes on the other hand are relatively soft, fleshy structures that are relatively easier to be consumed by millipedes, rather than the seed itself. This is a relatively unexplored area, which needs further attention.

In the Caatinga vegetation, approximately 68% of all Euphorbiaceae species are dispersed by ants (Leal et al. 2015). These myrmecochorous Euphorbiaceae species are dispersed through a diplochory system, in which autochory (ballistic discharge of seeds from explosive dehiscent capsules) is followed by myrmecochory (Leal et al. 2007; Leal et al. 2014). As an adaptation to myrmecochory, these Euphorbiaceae species present seeds with their micropylar region covered by a caruncle type elaiosome (Gorb & Gorb 2003). Ants are attracted to the elaiosome and also use it as a handle for seed transportation. During the transport, some seeds are lost by the ants may germinate and establish in new sites (Leal et al. 2007). The seeds that reach the nests have their elaiosomes eaten by the ants and then are discarded mostly intact in nest galleries, outside into the refuse dump, or in the vicinity of the nest entrances. Nests are considered favourable microsites for seed germination and plant establishment (Leal et al. 2007; Lobo et al. 2011).

The results here described demonstrate that not only ants (and dung beetles) are attracted to elaiosome-bearing seeds in the Caatinga region. We observed millipedes eating the elaiosome not only of *J. mutabilis*, the species focused in this study, but also that of *J. mollissima*, another elaiosome-bearing Euphorbiaceae species commonly found in Caatinga vegetation. We believe that the decomposing plant litter poor diet that most millipede species have (Loranger-Merciris et al. 2008; David & Handa 2010), probably are enhanced, in the case of the sampled *Urostreptus* sp., with the consumption of elaiosome of the *J. mutabilis* seeds, that are structures usually rich in lipids, proteins, vitamins and carbohydrates (Gorb & Gorb 2003). These structures serve as a food reward for the ants that remove the

diaspores (seed + elaiosome) (Gorb & Gorb 2003). Recently, Iannuzzi et al. (2013) reported that dung beetles also consume the elaiosome of *Jatropha molissima* seeds. But in that case the dung beetles also transported the elaiosome with the seed (at least for some distance, or to their burial sites), serving probably as a secondary dispersal agent. But here, we didn't register the millipedes moving the seed more than one inch, thus clearly not acting as one dispersal agent.

The elaiosome of Euphorbiaceae species covers the micropyle, the structure responsible for seed imbibition (Webster 1994; Gorb & Gorb 2003). Therefore, the process of seed germination may be favoured by elaiosome removal by accelerating seed imbibition and, consequently, seed germination (Gorb & Gorb 2003). The seeds could be less predated if its germination rate is faster due to removal of the elaiosome by millipedes. But it also could be considered otherwise, that millipedes parasite an ant-plant relationship only, millipede-manipulated seeds (without the elaiosomes) would not be transported by ants to a microsite with a lower bulk density and higher water-holding capacity (inside or near ant nests (Leal et al. 2007)), resulting in seedlings with a more clumped distribution near the parent clone, where seedling mortality is usually higher (see Higashi et al. 1989), as well as the risks of predation (Lobo et al. 2011) and inter and intraspecific competition (Beattie 1985). Furthermore, although in general ants may benefit plants in several different ways, the actual benefits may vary across systems and are context-dependent (Giladi 2006). However, we are aware of the recent and important advancement in the study of myrmecochory that strongly suggest that, in many cases, only a few species of ants provide efficient seed dispersal, while other species of ants may be suspected to be parasites (Ness et al. 2004; Christianini et al. 2012; Leal et al. 2014, Leal et al. 2015). Further studies should be conducted to clarify this point.

As any new animal-plant interaction description, several questions are raised of this new millipede-plant interaction, as well as its influence on other animals such as ants. But

even with many gaps to fill, the present study reported a new and unexpected interaction between millipedes and plants in the Caatinga vegetation, where most interactions may be crucial for species persistence in such a harsh environment.

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