Research Article

Effects of the Trophobiont Herbivore *Calloconophora pugionata* (Hemiptera) on Ant Fauna Associated with *Myrcia obovata* (Myrtaceae) in a Montane Tropical Forest

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Many studies have investigated the mechanisms behind the structure of arboreal ant assemblages. In this study, the objective was to evaluate the effect of availability of honeydew-producing colonies of *Calloconophora pugionata* (Membracidae) on the structure of ant assemblages associated with the host plant *Myrcia obovata* (Myrtaceae) in an Atlantic forest of Minas Gerais (Brazil). Our experiment consisted in a gradual exclusion of hemipteran colonies out of the host plant crown and further record of the ant assemblage response (species richness, composition, and occurrence) to the presence and density of treehopper colonies. The hypothesis was that an increase in the number of trophobiont herbivores results in an increase in tending ant occurrence but a reduction in ant species diversity. Results corroborated our main hypothesis: membracids had a positive effect on the occurrence of ants but negative on species richness. Overall insect occurrence was also reduced with increasing in *C. pugionata* colonies, probably due to strengthening dominant ant species territory sizes and intensification of patrolling.

1. Introduction

The outstanding occurrences of large arboreal ant colonies result in the most remarkable animal biomass found in any tropical canopy [1]. Territory patrolling behaviour causes species dominance which reflects in nest sizes and intense search for food [2, 3]. The most common and rich food resource for ants is extrafloral nectaries secretion and exudates of sap-sucking herbivores, the honeydew [4–6]. These resources are predictable, highly energetic, and nutritive, leading to an increase of ant activity on the foliage [7] and ant fitness [8].

The trophobiosis is a relationship between ants and honeydew-producer insects [9]. It is a mutualistic interaction that involves consumption of honeydew by ants in return for protection of the hemipterans against natural enemies [10]. This kind of resource characterizes its consumers as indirect herbivores and provides energy necessary to achieve higher population sizes [3, 10]. However, the honeydew is a spatially limited resource, despite its importance, and consequently it may trigger interspecific competition [11].

Resource competition is one of the most important forces driving ant assemblage structure [2, 12]. In the canopy habitat, there are a small variety of resources for numerous ant species that share the same kind of preferred food, like honeydew or nectar, and canopy may be indeed one of the most competitive tropical environments [11, 13]. Studies with stable isotopes showed that many ant species frequently use honeydew and nectar as main food item, and thus these resources do shape arboreal ant community [3, 11, 14, 15].

The bottom-up effect caused by the sugar resources consumed in such interactions may lead to a cascade effect at multitrophic scales [15–17]. The resource monopolization by the dominant species may increase the interspecific
competition and the encounter frequencies between competitors, reducing the forage of nondominant ant species [18, 19]. In addition, when colony nutritional demands increase beyond the offer of honeydew, predatory behaviour became more frequent, along with the protective behaviour of removing any arthropod from the foliages close to the sugar resources, thus reducing the arthropods in the host plant [20–22]. The last case may generate an indirect benefit to the host plant by reducing herbivore occurrence and thus leaf chewing damage [20].

Trophobiosis has been largely studied in lowland, close tropical rainforests; and in the Brazilian Cerrado, among a few other ecosystems [17, 18, 20–22, 23, 24]. Although it is a well-described mutualistic system, likely to result in similar ecological output wherever it happens, it is necessary to investigate such interactions in other habitats [23]. For instance, climatically challenging conditions, or biogeographically isolated and intensely disturbed ecosystems, would result in harsh ecological conditions, capable for altering the expected output of increasing territories and competition around honeydew producers. Namely, how resilient a large colony would be to hemipteran density variation in space or time, under extreme environments?

In the present study we evaluated the effect of variation in the presence and density of hemipterans exudates producers among tree crowns on the arboreal ant species richness and occurrence, in a secondary montane forest ecosystem. We tested the hypothesis that the availability of the Calloconophora pugionata Driecht colonies positively affects the feeding behaviour and diversity of ants associated with the host plant Myrcia obovata (O. Berg) Nied (Myrsinaceae). Further, we expected that a high density of ants around the hemipteran colonies would affect other arthropods, from plant natural enemies, such as herbivore beetles, to membracid predators, such as spiders.

2. Material and Methods

2.1. Study Sites. The field work was conducted in Itacolomi State Park (20°26′26″S, 43°30′52″W) located between the cities of Ouro Preto and Mariana, Minas Gerais State, Brazil. This ecological reserve has an area of 7,000 ha covered with a mosaic of Atlantic montane forests and Cerrado. The rainfall regime varies between 1,500 to 2,000 mm per year and temperature between 19°C and 22°C. The rainy season extends from September to February and the dry season from March to August.

2.2. Model System. The tritrophic interaction is composed by Myrcia obovata (O. Berg) Nied (Myrtaceae), a common Atlantic forest tree, Calloconophora pugionata Driecht (Membracidae), a sap-sucking insect and honeydew producer, and the associated ant species, mostly Camponotus crassus Mayr, 1862 (Formicidae) and Camponotus rufipes Fabricius, 1775 (Formicidae), two of the numerical dominant ants in these forests [25]. This system occurs mostly in tropical montane forests established in the “canga”, an ironstone outcrop areas.

The field work was carried out in October and November of 2009 (wet season). Thirty-six individuals of M. obovata with similar structure (number of stems, height, crown circumference) were tagged in this site. From this subsample, 20 trees were randomly marked and divided in two groups of 10 plants named according to the presence of membracid as treatment (with membracid colonies) or control (without colonies). The colonies of C. pugionata hosted in each experimental plant had 25.4 ± 5.3 membracids (mean ± s.d.; n = 30), between adults and nymphs. Trees of M. obovata may occur isolated or in the same patch, but with a minimal distance of five meters between each one. Control and treatment plants were randomly selected from the original population, so their crowns are not grouped but mixed in the same canopy.

2.3. Experimental Design. The experiment was set by gradual removal of the membracid colonies (one colony per tree each time), and then we evaluate how the ant assemblage responds to that (Table 1). The procedure was carried out through four periods separated by three-day interval, which simulated a reducing resource gradient. At the end of each period all individuals of one colony of C. pugionata were removed from each treatment plant which were then monitored again straight way for the next days. In the last period, treatment plants had all colonies removed. The control plants were not manipulated and had no trophobioses in all periods. Hence, all ten plants of each group (independent variable) were successively observed (six events per period) along the resource gradient (period then used as repeated measures). These observations were made every two days on both control and treatment trees during two weeks for each period and last for 20 minutes per plant individual, and all visiting ants were recorded. Additionally, the other arthropods specimens observed were also quantified. Every species was classified using behaviour and morphological characteristics, as trophobiont ants, nontrophobiont ants, potential C. pugionata predator arthropods, potential M. obovata herbivore arthropods. Vouchers specimens of ant species were deposited at the insect collection of the Laboratory of Evolutionary Ecology of Canopy Insects and Natural Succession at Federal University of Ouro Preto, Ouro Preto, Brazil.

2.4. Data Analysis. The mean ant species richness (number of species per tree at each observation) and the mean occurrence of ants (number of ants per species observed in each tree) were analyzed using repeated measures ANOVA models, with the experimental groups as group factor (treatment and control) and the four periods as repeated measures (resource gradient). Repeated measure ANOVA was also used to compare the relative proportion of trophobiont ant species between the treatment groups for each period. A Fisher’s LSD post hoc test was used, performed as paired test.

The total ant species richness (total number of ant species observed at each period for both experimental groups) for every case was estimated using Jacknife I with 500 repetitions. After that, the values of total estimated
species richness were interpreted for the treatments with visual analyses on the overlap of the confidence intervals (95%) as indicator of difference. Additionally, the ant species composition was compared by discriminant analysis using the periods as grouping factor.

The mean occurrence of herbivores, predators, and all nonant arthropods grouped (including dipterans, cockroaches, etc.) was also analyzed using repeated measures ANOVA models as performed to ants data. Fisher’s LSD test was used after ANOVA as paired test. All analyses were performed with data transformed ($X' = \log_{10}X$), but graph was plotted with original data.

3. Results

We recorded a total of 1,897 ants visiting *M. obovata*, belonging to 10 species from four subfamilies, with 81.5% only at the treatment group (Tables 2 and 3). The subfamily Formicinae was the most representative group with *Camponotus* species as the numerically dominant group (89.4%). *M. obovata* was also visited by 1,125 nonant arthropods, including 62.5% in the control plants and 37.5% in the treatment plants (Table 3).

The presence and amount of *C. pugionata* colonies positively affected ant forage in the trees. In the treatment plants we observed an decrease in the ant number responding to reduced availability of honeydew sources, while nothing changed in the control (Repeated measures ANOVA: Group×Period: $F(3;354) = 4.4; P < 0.01; Figure 1$). Besides, the ant foraging was higher in the treatment plants except for trophobiose-lack period, when the ant activity was similar for both groups (LSD: $P < 0.05$). In fact, treatment plants were mostly occupied by honeydew-feeding ants, but again only at the periods when hemipterans colonies occurred (Repeated measures ANOVA: $F(3;354) = 3.6; P < 0.02; Figure 2$).

The mean ant species richness per plant, that is, the number of ant species foraging at the same time on each crown, was higher in the presence of mutualistic hemipteran colonies, although the result did not change in response to colony removal (Repeated measures ANOVA by Group: $F(1;118) = 10.47; P < 0.01; Figure 3$). Still, mean richness was always lower in the control than in treatment plants (LSD: $P < 0.05$). In opposite, the estimated total number of ant species was higher in the control plants than in the treatment but became similar to the control plants in the last period (Figure 4).

The ant species composition also changed in response to trophobiont hemipteran occurrence (Discriminant analysis: Wilk’s $\lambda = 0.206; \chi^2 = 110.47; d.f. = 70; P < 0.01$). Accordingly, with 75.9% of the variance explained by the first function and 8.6% by the second, the ant assemblage associated with plants hosting ant-hemipteran mutualism was significantly different from plants where hemipterans were totally removed or never existed (Figure 5). This difference was mainly associated with the massive presence of the two trophobiont ant species, *Camponotus crassus* and *C. rufipes*, when plants had colonies of *C. pugionata* (*C. crassus*: $F(7;72) = 5.46; P < 0.01$; *C. rufipes*: $F(7;72) = 3.31; P < 0.01$; other species did not contribute significantly for the model).
Table 2: List of ant species observed associated with Myrcia obovata and respective total number of occurrence.

<table>
<thead>
<tr>
<th>Family Formicidae</th>
<th>Total number of ants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subfamily Formicinae</td>
<td></td>
</tr>
<tr>
<td>Camponotus rufipes Fabricius 1775</td>
<td>635 23</td>
</tr>
<tr>
<td>Camponotus crassus Mayr 1862</td>
<td>1315 132</td>
</tr>
<tr>
<td>Camponotus novogranadensis Mayr 1870</td>
<td>122 113</td>
</tr>
<tr>
<td>Camponotus fastigatus Roger 1863</td>
<td>22 29</td>
</tr>
<tr>
<td>Subfamily Myrmicinae</td>
<td></td>
</tr>
<tr>
<td>Crematogaster sp1</td>
<td>0 118</td>
</tr>
<tr>
<td>Cephalotes pusillus Klug 1824</td>
<td>16 13</td>
</tr>
<tr>
<td>Pheidole sp1</td>
<td>23 19</td>
</tr>
<tr>
<td>Subfamily Pseudomyrmicinae</td>
<td></td>
</tr>
<tr>
<td>Pseudomyrmex gracilis Fabricius 1804</td>
<td>41 35</td>
</tr>
<tr>
<td>Pseudomyrmex pallidus Smith 1855</td>
<td>7 10</td>
</tr>
<tr>
<td>Subfamily Ponerinae</td>
<td></td>
</tr>
<tr>
<td>Gnamptogenys striatula Mayr 1884</td>
<td>2 0</td>
</tr>
</tbody>
</table>

Table 3: Total number of occurrences registered for each experimental group throughout the experiment.

<table>
<thead>
<tr>
<th></th>
<th>Treatment</th>
<th>Group</th>
<th>Control</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ants</td>
<td>1546</td>
<td>351</td>
<td>1897</td>
<td></td>
</tr>
<tr>
<td>Herbivores</td>
<td>276</td>
<td>384</td>
<td>660</td>
<td></td>
</tr>
<tr>
<td>Predators</td>
<td>102</td>
<td>253</td>
<td>355</td>
<td></td>
</tr>
<tr>
<td>Others arthropods</td>
<td>44</td>
<td>66</td>
<td>110</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1968</td>
<td>1054</td>
<td>3022</td>
<td></td>
</tr>
</tbody>
</table>

Arthropods other than ants visiting M. obovata showed a positive response to decrease honeydew source gradient (Repeated measures ANOVA group*periods: Non-ant arthropods: F(3;354) = 3.62; P < 0.05; Figure 6(a). The control group did not change through the periods despite low fluctuations (LSD: P > 0.05). A similar pattern was observed when herbivores and predators were analyzed separately (Repeated measures ANOVA group*periods: Herbivores: F(3;354) = 3.21; P < 0.05; Predators: F(3;354) = 4.66; P < 0.01; Figures 6(b) and 6(c). The occurrence of every other arthropod guilds in the treatment plants was lower than that in the control plants, except at the last period when both plant groups presented the same number of foreign visitors, as expected (LSD: P < 0.05).

4. Discussion

The bottom-up effect caused by C. pugionata was noticeable for both tending ants as well as for the rest of the associated fauna in M. obovata. The availability of exudate-producing herbivore colonies positively affected the occurrence of foraging ants on the plants. There was an increase in the numerical dominance of species collecting honeydew, along with a decreasing in species richness. Still, the number of species foraging at the same time was greater in the presence of membracid, probably due to the high frequency of trophobiont species. Furthermore, we found a low occurrence of potential host plant herbivores and likely predators when colonies of mutualistic hemipterans were present. Such findings suggest the presence of mutual benefit among the
Figure 3: Plants of the treatment group presented higher mean ant richness than control group in all periods (Repeated measures ANOVA: Group: F(1;118) = 10.47; P < 0.01). Different letters indicate significant differences according to post hoc test (LSD: P < 0.05).

Figure 4: Estimated total richness calculated for the four periods in both groups. Treatment group shows low richness compared to control group in the three first periods.

Figure 5: Discriminant analysis comparing the experimental groups (for each period of experiment) using ant species composition and relative occurrence. With 75.9% of the variance explained by the first and 8.6% by the second function, periods were discriminated in cases with trophobioses and without trophobioses by the high occurrence of the trophobiont ant species *Camponotus rufipes* and *C. crassus* in the first group (Wilks's $\lambda = 0.206; P < 0.01$; *C. crassus*: F(7;72) = 5.46; P < 0.01; *C. rufipes*: F(7;72) = 3.31; P < 0.01; other species did not contribute significantly for the model).

Mutualism actors (e.g. [17]), or at least some compensation for the sap-sucking damage on the host plant.

The existence of an ant dominance and patrolled territory in association with hemipteran colonies or extrafloral nectaries is a pattern extensively described for various tropical habitats, although it is relatively unknown for montane forests [3, 11, 26–28]. Clearly, ecology of arboreal ants is strongly related to plant hemipteran sweet exudates, and the dominant ants are the principal consumer of this sort of trophic resource [29]. Such phenomena may even explain the formation of large mosaic type of territories in the canopies of tropical native and cultivated forests [11, 26, 27].

It is common knowledge that the number of ants foraging in the canopy is proportional to the amount of resources, as sources of nectar or exudates [11, 15, 21]. When they engage in trophobiosis, they assume a primary consumers status, and their occurrence is equal or larger than the other herbivores in its community. In the absence of sugary secretions, predation becomes the primary feeding behaviour; then the situation is reversed, and the ants assume low occurrence [6, 10, 29]. Other studies also showed that the increased aggressiveness and occurrence of ants are related to the availability of honeydew sources [4, 5, 11, 21, 30].

Blüthgen et al. [15] showed that the ant species richness in host plants with trophobiosis was lower compared with plants carrying extrafloral nectaries or with non-myrmecophilous plants. Dominant ants are considered the most aggressive species, and they are also numerous in other food sources such an extrafloral nectaries [15]. This behaviour, along with high territoriality, affects negatively the ant species richness due to the exclusion of competing species, reduction of co-occurrences, and avoiding species replacement [11, 15]. Thus, the same pattern we observed in the system *M. obovata*-*C. pugionata*-ants was similar to...
Figure 6: Mean occurrence of the whole nonants arthropods (a) and for herbivores (b) and predators (c) separately, registered in the experimental groups (lines) at each period (x axis). These arthropods respond positively to decrease occurrence of ants and consequently the absence of *C. pugionata* (ANOVA: nonants arthropods: $F(3;354) = 3.62; P < 0.01$; Herbivores: $F(3;354) = 3.21; P < 0.01$; Predators: $F(3;354) = 4.66; P < 0.01$). Different letters indicate significant differences according to post hoc test (LSD: $P < 0.05$).
different ecosystems with other types of myrmecophily [11, 28, 31, 32].

The interaction between trophobiont herbivores and dominant ant species may alter the entire food web [9, 33–35]. This could generate benefits for the host plants and mutualistic treehoppers by killing or repelling their natural enemies [29, 30, 35]. Such protection has consequences as reducing of herbivory [17, 36] and increasing plant fitness [24, 37]. Rosumek et al. [22] and Schoereder et al. [28] reviewed the ant protective role and the importance of nectaries and trophobiont hemipterans, showing that most studies presented a negative correlation between ants and herbivores mainly when myrmecophilous resources are involved. In this interaction system, the host plant is the one taken some costs, due to the constant sap-sucking activity from the hemipterans, plus the damage caused by opportunistic phytopathologies [38]. However, such cost may be compensated by the protective action from the ants against the chewing herbivores [8, 24]. Further studies are needed to evaluate the actual damage caused by _C. pugionata_ and its effects on _M. obovata_ fitness.

The establishment of trophobioses can also result in an ecological cascade with further evolutionary consequences for the entire community [28]. For example, one quarter of the species and one third of the plant individuals in the Brazilian Cerrado have extrafloral nectaries and/or trophobiont hemipterans [39]. In addition, a third of the ant species that feed in the Cerrado vegetation are typically sugary exudates consumer [28, 40–42]. In one hand, the low, accessible tree crowns in the cerrado, montane forests, and rupestrian field’s vegetation allow such type of investigation and a precise quantitative analysis of the actual availability of such resources. Therefore, the investigation of how relevant trophobiosis would be for other tropical ecosystems is constrained mostly by restricted canopy access to a large enough area in order to produce comparative results. The present work was developed in relatively low and also accessible canopies of a secondary montane forest. This unique situation allowed us to explore the very mechanisms of the interactions plant-hemipteran-ants in this distinct forest habitat, where its relevance and ecological strength seem similar to that previously observed in the Cerrado.

Our study provides empirical evidence of bottom-up control exerted by the sap-sucking hemiptera _C. pugionata_ in their community, an effect clearly perceptible in the ant assemblage. By means of the trophobioses, the mutualistic membracid species are capable of altering not only the ant species richness and occurrence but also the structure of the entire arthropod community associated with its host plant. In the tritrophic system formed by _M. obovata_, _C. pugionata_, and ants, any species seem to benefit from the interaction [17]. In conclusion, myrmecophilous resources produced by several animals and plant species can be key elements for the configuration of hierarchically structured ant assemblage, as observed coherently among different ecosystems, and then affect all the arboreal community associated [3, 11, 14, 15, 28, 40].

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