Defining Habitat Use by the Parabiotic Ants *Camponotus femoratus* (Fabricius, 1804) and *Crematogaster levior* Longino, 2003

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**Abstract**

Ant-garden ants have a strong relationship with epiphytes that need light to grow, for these reasons, it has been previously documented in forest gaps. Moreover, larger gaps have more available area for nesting and habitats for use as forage. Thus we hypothesize that 1) canopy openness influence the presence of ant’s gardens in gaps, and 2) greater gaps will have more nests, and 3) both openness canopy and area determine the colony size in forest gaps. Furthermore, it is known that parabiotic ants foraging on the ground and in vegetation, the nests are arboreal. So, we also hypothesize that 4) parabiotic ants are more often sampled in arboreal strata and 5) increasing vegetation connectivity and the volume of accumulated litter in the soil increase the foraging of the ants in vegetation and ground, respectively, with the increase in canopy openness increasing the activity of the two species in both strata. Presence, number of Ant-gardens, as colony size, was affected by area and locality, but not by canopy openness. Nevertheless, there was not overall difference in the use of strata by *Camponotus femoratus*, neither by *Crematogaster levior*. On the other hand, frequency of *C. femoratus* on the ground decreases with canopy openness but is not affected by the vegetation connectivity. Also, *C. levior* frequency on the ground also decreases with the increase of complexity of vegetation and canopy openness. In addition, neither vegetation connectivity, nor canopy openness influence the frequency of foraging of these ants in understory.

**Introduction**

Ants are involved in many biological interactions with plants, microorganisms and other invertebrates (Izzo & Vasconcelos, 2009; Dâttilo et al., 2012; Vicente et al., 2012; Sanders et al., 2014; Koch et al., 2015; Puker et al., 2015). Among these interactions, ants can interact with other species of ants throughout their life history from mutualistic to parasitic relationship (Sanhudo et al., 2008; Gallego-Ropero & Feitosa, 2014; Powell et al., 2014). An example of mutualist interaction maintained by Neotropical ants *Camponotus femoratus* (Fabricius, 1804) (Formiciniae) and *Crematogaster levior* Longino, 2003 (Myrmicinae) are known as parabiosis (Orivel & Leroy, 2011). In this interaction, ants share the same foraging trails and their arboreal nests (Swain, 1980).

In the nests, they actively plant one or several epiphyte species, forming a complex nest, known as Ant-Gardens (hereafter AGs) (Davidson, 1988; Longino, 2003; Youngsteadt et al., 2009). This interaction includes nine species of ants (obligate AGs inhabitant) however, AGs with parabiotic association between *C. femoratus* and *C. levior* are among the most commonly found in the Amazon (Davidson, 1988; Orivel & Leroy, 2011). In exchange for support and humidity control for the nest and, in some cases, extrafloral nectar...
The study was carried out on two municipalities of the southern Amazonia as part of the Research Program in Biodiversity (PPBio). The standard sampling protocol used in PPBio is the RAPELD methodology (see Costa & Magnusson, 2010). In each of the two municipality a set of trails of 250m in length are installed at a minimum distance of 1 km each other, linked by access trails (henceforward Module). In Claudia municipality, three modules are installed close to each other (less than 20km), in a large fragment surrounded by plantations. The Claudia modules are Module I (11°34’S, 55°17’W) and Module II (11°35’S, 55°17’W) that are broadly connected, and Module III (11°39’S, 55°04’W) that is an area with about 900ha almost completely surrounded by plantations and narrowly connected to the rest of the fragment. The area of these three modules had selective logging more than eight years ago. Because of this, they have older gaps caused by the management of selective logging as wood storage yards and trails used for the dragging of cut trees (abandoned roads). One other area is the Parque Estadual do Cristalino Reserve, a continuous pristine forest in the municipality of Novo Mundo municipality, Mato Grosso States (9°28’S, 55°50’W).

According to the Köppen classification, the climate is tropical humid (Am) with annual average of 25°C. Precipitation is about 2,500 to 2,800 mm year in forest remnants of the municipality of Claudia and 2.800 to 3.100 mm year in Parque Estadual do Cristalino (Alvares et al., 2013). In both, there are two well-defined seasons, a rainy season between November and April and a dry season between May and October (Vicente et al., 2014). The local vegetation in Claudia is classified as area of ecological tension between Ombrophylous Forest and Savanna. For the Parque Estadual do Cristalino local vegetation is classified as Ombrophylous Forest and ecological tension areas between Ombrophylous Forest and Savanna, Savanna and Seasonal Forest, Seasonal Forest and Ombrophylous Forest (Instituto Brasileiro de Geografia e Estatística, 2004).

Data collection
Gap-occupation

We carried out the experiment about occupation of forest-gaps only in forest remnants in the three modules (I, II, III) of Claudia municipality in April 2014. In these areas, we surveyed all access trails that link plots, totaling approximately 32km. In these trails, all forest-gaps, including the gaps caused by selective logging, within the range of 10 meters of each side of the trails (20 m wide) were selected and we recorded the presence or absence of AGs, number of nests and colony size. Furthermore, we measured the canopy openness and the length of the two central perpendicular axes of the forest gap.

Canopy openness was measured with Concave Spherical Densiometer. It is considered a practical and cheap device that provides a reliable proxy as to the relative availability of light (Baudry et al., 2014). In the center of each forest-gap, we recorded four measurements in the cardinal directions to calculate an average availability of light (Dáttilo et al., 2013, 2014; Baudry et al., 2014). The first two major perpendicular measures of gaps were used as length and width to calculate the gap area, using the formula of the ellipse area (Runkle, 1981; Arihafa & Mack, 2013). In forest-gaps formed by abandoned roads, because of the difficulty in measuring their main axes, we considered a continuous habitat and we assigned an arbitrary value area, the double of our largest forest gap.

Vertical habitat use

To access the information about the use of the vertical habitat by parabiotic ants thirty four (34) plots of 250m, allocate in the four areas previously mentioned were investigated. Collections were made between November 2009 and February 2010 in Claudia modules and in November 2012 and May 2013 at Parque Estadual do Cristalino. In each plot, every 25 meters a collection of C. femoratus and C. levior ants on the ground and in vegetation was performed, totaling 680 samples (340 on ground and 340 in vegetation). For the collection of ground-dwelling ants, we installed pitfall-traps buried at ground level containing water and detergent. Pitfalls were on field for 48 hours. For sampling arboreal ants was used beating-tray. At
four points on the North, South, East and West, 2 meters away from each pitfall all vegetation within 1m² between 1 to 3 meters tall was sampled (more details: Vicente et al., 2016). Posteriorly we made comparisons with specimens deposited at the Laboratório de Ecologia de Comunidades from the Centro de Biodiversidade da Universidade Federal de Mato Grosso (UFMT) and the ant collection from the Laboratório de Sistêmática, Evolução e Biologia de Hymenoptera from the Museu de Zoologia da Universidade de São Paulo (MZSP). Vouchers were deposited in the collections mentioned above and in Setor de Entomologia da Coleção Zoológica da Universidade Federal de Mato Grosso, Departamento de Biologia e Zoologia, Cuiabá, Mato Grosso, Brazil (CEMT).

In these points, measurements of vegetation connectivity and litter height were taken, summed up and made the averages. Connectivity of the vegetation was accessed with a number of touches in the understory of a stem of 2 meters positioned one meter high, perpendicular to the ground. Litter height was measured with a rule and canopy openness with Concave Spherical Densiometer as explained earlier in the section gap-occupation.

**Data analysis**

**Gap-occupation**

To test which characteristics determine the presence and abundance of Ant-gardens in gaps, presence of AG and number of nests were the dependent variables and forest-gap area and canopy openness average were the independent variables. We tested the correlation between independent variables with Pearson correlation (Pearson: 0.026). Among the three sampled areas, Module III is almost totally isolated area and which has the least amount of gaps colonized by parabiotic ants (only 10.3%) while Module I and Module II showed more than twice the number of colonized gaps (24.2% and 25.5% respectively). This is an indication of the intrinsic characteristics of this locality, as area and edge effects, affecting the population dynamics of this ant species. Consequently, the location was inserted into the GLM analyses using Orthogonal Contrast established a priori, considering the principles described by Gotelli & Ellison (2011), being set up as Contrast: Module I (+1), Module II (+1) and Module III (-2). We used the R software to perform all analyzes (R Core Team, 2014).

To test whether the colony size, which indirectly is represented by the volume of the nests, was related to independent variables a GLM was performed. Since AGs are spherical, the variable colony size was calculated using the formula of the volume of spheres (V) (Khattar, 1968), where r is the radius from the ant-garden nest and π is the constant pi: 

\[ V = \frac{4}{3} \pi r^3 \]

The volume of each nest of each evaluated forest-gap was added, representing the size of the colony by forest-gap. Finally, to test whether the colony size was related to the amount of nests one Pearson correlation was made.

**Vertical habitat use**

To confirm the hypothesis that *C. femoratus* and *C. levior* have different preferences about the use of ground or vegetation a Test-t was performed for each species. In this analysis the frequency of each species per plot was the dependent variable and stratum was the independent factor. To access the information about what structural environmental variable affects the strata use of *C. femoratus* and *C. levior* a GLM with Negative binomial distribution was used. This error distribution is indicated when Poisson error distribution shows overdispersion (Hilbe, 2007; Lindén & Määttäniemi, 2011). Because a correlation between independent variables was detected with Pearson correlation with Bonferroni correction, we excluded litter height variable of analyses.

**Results**

**Gap-occupation**

We recorded 27 colonies of ant-garden ants in all 142 forest-gaps (125 in tree-fall gaps and 17 in abandoned roads). Regarding environmental variables of forest gaps, they had an average size of 215.125 m² (SD: 130.44m²) value established for abandoned roads). The canopy openness had an average of 32.94% (SD: 14.61).

Presence of Ant-gardens colony was affected by area (Z<sub>1.138</sub>: 2.470, p: < 0.05 – Table 1) and locality (Z<sub>1.138</sub>: 2.269, p: < 0.05), but not by canopy openness (Z<sub>1.138</sub>: -1.849, p: < 0.05).

Abundance of Ant-gardens nests was also affected by area (Z<sub>1.138</sub>: 2.090, p: < 0.05) and locality (Z<sub>1.138</sub>: 2.570, p: 0.010) and also not by canopy openness (Z<sub>1.138</sub>: -1.583, p: 0.113 – Table 1). Ants colony size was also influenced by the gap area (Z<sub>1.138</sub>: 3.494, p: < 0.05), the locality (Z<sub>1.138</sub>: 2.422, p: < 0.05)

**Table 1.** Influence of factors in gap occupation by Ant-Gardens nests of parabiotic ants *Camponotus femoratus* and *Crematogaster levior*.

<table>
<thead>
<tr>
<th>Presence of AGs</th>
<th>Z</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area</td>
<td>2.470</td>
<td>&lt; 0.05*</td>
</tr>
<tr>
<td>Locality</td>
<td>2.269</td>
<td>&lt; 0.05*</td>
</tr>
<tr>
<td>Canopy openness</td>
<td>-1.849</td>
<td>0.064</td>
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<th>Abundance of AGs</th>
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<tr>
<th>Colony size of AGs ants</th>
<th>Z</th>
<th>p-value</th>
</tr>
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<tbody>
<tr>
<td>Area</td>
<td>3.494</td>
<td>&lt; 0.05*</td>
</tr>
<tr>
<td>Locality</td>
<td>2.422</td>
<td>&lt; 0.05 *</td>
</tr>
<tr>
<td>Canopy openness</td>
<td>-1.230</td>
<td>0.221</td>
</tr>
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* Significative
and not the canopy opening ($Z_{1.33} = -1.230, p = 0.221$ – Table 1). Therefore, the abundance of Ant-gardens and the colony size present a strong correlation ($Pearson: 0.883$).

**Vertical habitat use**

*Camponotus femoratus* and *Crematogaster levior* were sampled in 21 of the 34 plots studied. At least one of the two species was collected in 69 samples (30 on the ground and 39 on vegetation) of the 420 samples (210 on the ground and 210 on vegetation) corresponding to 21 plots where these ants were found. *C. femoratus* was collected in 49 samples of 18 plots (30 samples in soil and 19 on vegetation) and *C. levior* on 30 samples (15 times in each stratum). We assumed that in all cases both species are co-occurring in the same area, but with differences in abundance and, thus, in the probably of being collected. Therefore, these species were collected in the same sample in 13 cases (8 on the ground and 5 on vegetation).

Regarding the environmental variables evaluated, they had an average of vegetation connectivity of 1.417 (SD: 0.645). The canopy openness had an average of 32.94% (SD: 14.61).

Nevertheless, there was no an overall difference in the use of vegetation and ground strata by *Camponotus femoratus* ($Z_{1.46} = -1.519, p = 0.129$ – Figure 1), nor by *Crematogaster levior* ($Z_{1.96} = 0.908, p = 0.364$ – Figure 1). *C. femoratus* occurs in average 1.67 (SD: 1.63) samples per plot in ground and 1.06 (SD: 1.47) on vegetation. *C. levior* occurs in average 1.25 (SD: 1.16) samples per plot in ground and 1.25 (SD: 1.23) on vegetation. On the other hand, the frequency of *C. femoratus* on the ground decreases with canopy openness ($Z_{1.31} = -2.435, p < 0.05$ – Table 2) but is not affected neither by the vegetation connectivity ($Z_{1.31} = -1.407, p = 0.159$) nor for interaction of two factor ($Z_{1.31} = 1.771, p < 0.05$). Also, *C. levior* frequency on the ground also decreases with vegetation connectivity ($Z_{1.31} = -2.243, p < 0.05$ – Table 2), but, in this case, a decrease was also noted in the frequency associated with canopy openness ($Z_{1.31} = -1.977, p < 0.05$) and an interaction between these factors ($Z_{1.31} = 2.565, p < 0.05$). In addition, neither vegetation connectivity (*C. femoratus*: $Z_{1.32} = 0.200, p = 0.841$; *C. levior* $Z_{1.31} = -1.225, p = 0.221$ – Table 2), nor canopy openness (*C. femoratus*: $Z_{1.32} = 0.580, p = 0.562$; *C. levior* $Z_{1.32} = 0.804, p = 0.421$) influence the frequency of foraging of these ants in understory.

**Discussion**

Our results demonstrate that the size and local intrinsic characteristics of the forest gaps are determinant to the occupation by the parabiotic ants *Camponotus femoratus* and *Crematogaster levior*. These factors influence the presence as well as the quantity of Ant-Gardens and the ants colony size. Also, larger gaps host more and larger AGs than smaller forest gaps. This can be explained because large forest gaps remains available for longer once the time of full restoration of a large forest gap, from its formation till the canopy closure is probably much longer than in small gaps. In fact,
larger patches implies in a higher probability of colonization and a lower risk of extinction (MacArthur & Wilson, 1967; Donner et al., 2010; McCarthy & Lindenmayer, 1999). Thus, a colony of parabiotic ants has more time to colonize, grow and cultivate its mutualistic epiphytes in large gaps. Consequently, larger gaps are probably a much more profitable resource to maintain C. femoratus and C. levior populations.

As in gaps the incident light in the lower vegetation is higher, there is an associated increase in the total primary productivity, including a major production by extrafloral nectar (Radhika et al., 2010; Bixenmann et al., 2011; Brenes-Arguedas et al., 2011). Several studies also demonstrate that this increase in primary production attracts more herbivores (Harrison, 1987; Coley & Barony, 1996; Louda & Rodman 1996; Sipura & Tahvanainen, 2000). The increase in both extrafloral nectar and herbivores means an increase in food resources for ants (Swain, 1980; Davidson, 1988; Vantaux et al., 2007). However, canopy openness of forest gaps has not influenced on AGs presence, neither quantity of AGs or colony size. Probably the lack of a relationship in the occupation of forest gaps and canopy openness can be explained by the low variation among the sampled gaps. Furthermore, the size of the forest gaps induces changes that go beyond the light intensity, changing moisture and biological properties of soil are characteristics responsible for the forest recovery (Muscolo et al., 2014).

Parabiotic ants do not show any preference for foraging in the understory plants or on the ground. In fact, these ants are widespread and found in the soil and in vegetation in the Amazon (Davidson, 1988; Dejean et al., 2000; Ryder Wilkie et al., 2010; Vicente et al., 2014, 2016). Nevertheless, canopy openness was a determining factor on the habitat use by C. femoratus and C. levior. With the increase of canopy openness that results in an increase in primary productivity and consequently of herbivores (Brenes-Arguedas et al., 2011; Coley & Barony, 1996), that increase food resources in vegetation (as discussed above) could induce a preferential foraging by ants, there was a reduction in the parabiotic ants frequency foraging on the ground. However, the decrease of ants on soil is not associated to an increase in the frequency of this ant species on vegetation.

Although the increased vegetation connectivity does not influence the use of the arboreal strata by C. levior, there was a decrease in soil use. The increase of the vegetation connectivity means a denser understory, forming bridges among plants. Thus, valuable resources for C. levior as extrafloral nectary (Davidson, 1988) are easier to access with a vegetation connectivity increase. The increase in the vegetation connectivity can provide a greater diversity of substrates, which may facilitate access to the resource or run away from ant predators and competitors (Clay et al., 2010). This reduction in the need of the ground use minimizes possible confrontations with other ant species, once the ground strata is naturally richer in ant species (Vasconcelos & Vilhena, 2006; Neves et al., 2013; Vicente et al., 2016). Competition between species for C. levior is damaging since it lost its chemical defense, depending on the defensive ability of C. femoratus (Longino, 2003) that show aggressive behavior (Vicente et al., 2014) as well as other species of Neotropical species of Camponotus (Yamamoto & Del-Claro, 2008; Santos & Del-Claro, 2009; Alves-Silva & Del-Claro, 2014; Anjos et al., 2016). Thus, C. femoratus did not show influence of vegetation connectivity in use of strata, showing a partition in the niche between the two ant species. This niche partitioning should go beyond the influence of plant connectivity in resource exploration demonstrated in this work. Davidson (1988) showed that C. levior accumulates more workers in sugary baits than C. femoratus. Also, while workers of C. levior did not show any preference among plants with extrafloral nectaries, C. femoratus actively choose plant species with larger nectaries and with greater concentration of nectar (Davidson, 1988). Therefore, plant traits are probably not associated to the complexity of the habitat.

Our results demonstrate that although these parabiotic ants occupy the same sites sharing their nests and maintenance activities of the colonies, they have divergences in their niche with regards to the exploitation of habitat. The use of habitat by C. femoratus and C. levior differ among each other depending on the stratum and environmental characteristics. It also shows that these dominant ants, with different biological characteristics, can influence both soil and vegetation communities differently. Therefore, the influence of both C. femoratus and C. levior on soil and vegetation ant communities should be investigated in order to understand whether these parabiotic ants alter the ant communities, where they occur and what the mechanisms involved in this alteration are.

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