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Ants, plant characteristics and habitat conservation status affect the occurrence of myrmecophilous butterflies on an extrafloral nectaried Malpighiaceae

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ABSTRACT
We investigated the occurrence of myrmecophilous florivorous lycaenid larvae in Banisteriopsis malifolia (Malpighiaceae) according to habitat conservation status (disturbed and preserved savanna), plant phenology, height and the presence of tending ants. The abundance and richness of lycaenids were sixfold and fivefold greater, respectively, in the disturbed area than in the protected savanna. Lycaenids occurred mostly on plants visited by Camponotus blandus, a mutualistic partner of larvae. Habitat type was the main factor influencing lycaenid occurrence, as plants in open areas offered more food resources and tending ants. Banisteriopsis malifolia was considered useful as a host for lycaenids in disturbed sites.

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Banisteriopsis; Brazilian savanna; Camponotus; Ectatomma; Parhasius politetes

Introduction

The occurrence of organisms is constrained by biotic and abiotic factors that not only influence their distribution but also affect interspecific interactions (Folgarait 1998; Tanaka et al. 2007). A main issue for conservation ecology is to understand the conditions for the persistence of organisms in nature (Samways 2007). It is only with knowledge of organisms’ habitat ranges and ecological interactions that we can support their preservation in natural areas (see Thomas 1991; Gibbs & Stanton 2001). Butterflies are interesting and conspicuous organisms whose ecological interactions with plants and other animals may be key to understanding several coevolved systems in natural communities (Thompson 2005, 2013).

Among Lepidoptera, lycaenid butterflies deserve attention, as some species are now at risk of extinction (Maes et al. 2004; Kaminski et al. 2010; Freitas & Marini-Filho 2011; Garvie et al. 2014) and some aspects of their biology, ecology and occurrence are still poorly known in the tropics, in comparison to temperate areas, inhibiting conservation initiatives (see Heath 1998; Rabasa et al. 2005; Bais 2015; Haaland 2015). For instance, there are quite a number of studies calling attention to the decline of lycaenid populations in non-tropical areas (Thomas 1983; Fischer et al. 1999). Initiatives have been taken aiming at the detailed understanding of lycaenid life histories in order to provide appropriate approaches for the conservation of species, which usually involves the knowledge of their interspecific interactions (Thomas 1983; Schmitt & Seitz 2002; Maes et al. 2004). In Brazil, only recently have lycaenids been studied in detail, with descriptions of their host plants and interactions with ants (Kaminski et al. 2010; Schmid et al. 2010; Silva et al. 2011), but even so our knowledge is undoubtedly underestimated, in comparison to temperate areas.

Ant–lycaenid interactions seem to be particularly interesting for the understanding of lycaenid natural history, as some species show a strong tendency to occur in the presence of tending ants (Fiedler 1991; Bächtold et al. 2014). In these mutualistic interactions, ants feed on honey-like solutions provided by larvae and in turn protect them from natural enemies (Weeks 2003). Therefore, from a conservation point of view, the maintenance of specific plants species that sustain tending ants can be a first goal to preserve lycaenids at a local scale (Terblanche & Hamburg 2004; Kaminski & Freitas 2010). At least in Brazil, extrafloral nectaried Malpighiaceae stands out as an important family that can sustain both lycaenids and their ant partners (Monteiro 2000; Alves-Silva et al. 2013; Bächtold et al. 2013, 2014), thus being ideal as a model to study lycaenid habitat requirements.

In the Brazilian savanna, some Malpighiaceae species (e.g. Banisteriopsis, Peixotoa, Heteropterys) which
are hosts for lycaenids also tend to occur on disturbed sites like clearings and zones recovering from fire or mowing (Bächtold et al. 2014). In disturbed sites these plants usually present increased performance and produce more resources to herbivores (flowers, soft and tender leaves) and mutualistic ants (increased extra-floral concentration) (e.g. Banisteriopsis; Alves-Silva & Del-Claro 2014). Thus we might assume that these plants in early successional stages may be more attractive to lycaenids, because ovipositing females might be guided by both the presence of ant-partners and the quality of floral resources that larvae feed on (Wagner & Kurina 1997; Kaminski et al. 2010; Rodrigues et al. 2010).

**Figure 1.** a, Flower and clusters of flower buds of *Banisteriopsis malifolia* (Malpighiaceae), the host plant for lycaenid larvae. b, Larva of *Parhasius polibetes* feeding on a flower bud (arrow). c, A common visiting ant, *Ectatomma tuberculatum*. Scales: a = 15 mm; b, c = 10 mm.
The larval stages of many lycaenids are polyphagous and feed on the reproductive parts of plants, especially on buds and flowers, but also on fruits (Chew & Robbins 1984; Fiedler 1991; Schmid et al. 2010). Adult females should be able to recognize specific characteristics of the host plants that may increase survival of larvae (Singer 2008). Besides plant quality, food characteristics (Rodrigues et al. 2010) and the presence of ants is especially important for lycaenid species, as adult females use ants as cues for oviposition (Pierce & Elgar 1985; Wagner & Kurina 1997; Kaminski et al. 2010; Bächtold et al. 2014). In addition, the conspicuousness of the host plant is supposed to play a role in lycaenid oviposition choices (Jorge et al. 1988; Meyer-Hozak 2000). Plant height is related to the structural complexity as taller plants may support more vegetative and reproductive organs, relative to short plants; and consequently these showy plants may sustain more arthropods (Lawton 1983), including butterflies. For instance, Nowicki et al. (2005) found that lycaenids were negatively related to plants surrounded by vegetation, presumably because these plants are hard to find for adult ovipositing females. This is also noted in other lepidopteran groups, but is far from being a pattern (Bergman 2001; Trager et al. 2009).

In this study, we used a natural experiment to investigate the abundance and diversity of myrmecophilous florivorous lycaenid butterflies in relation to habitat conservation status (disturbed and protected areas), plant characteristics (phenology and height) and the presence of different species of tending ants. Fieldwork was conducted in a disturbed (open) area and in a preserved reserve of tropical savanna, and our study plant was the extrafloral nectarear Malpighiaceae Banisteriopsis malifolia (Nees & Mart.) B. Gates, which sustains florivorous lycaenids and their ant partners, such as Camponotus (Alves-Silva et al. 2013). These ants are important partners of tropical lycaenids (Kaminski et al. 2010; Bächtold et al. 2014). One interesting aspect of B. malifolia is that it occurs in both open and preserved sites (see Alves-Silva 2011), which permits the evaluation of the associated lycaenid fauna and their interactions in different microhabitats.

As discussed above, given the tendency of Banisteriopsis to present increased performance (growth, phenology) in disturbed sites, we hypothesized that lycaenids would be more abundant and rich in plants located at the non-protected area, in comparison to conspecific plants at the protected reserve. Plant height was expected to relate positively with lycaenid abundance, as they might stand out in the vegetation and be easier to find. Phenological differences in plants from both sites were investigated, but were not used in statistical tests, but rather as a potential factor that might influence lycaenid occurrence in a given site. We also expected to find differences in lycaenid abundance according to different ant species, as larvae, even myrmecophilous, can either be attended or preyed upon depending on the ant species (Robbins & Aiello 1982; Robbins 1991).

**Materials and methods**

**Study species**

The study plant B. malifolia was examined during its flowering period from February to May 2011. Lycaenids are found on the plants only during this specific period, as larvae feed on flower buds and flowers (Alves-Silva et al. 2013) (Figure 1a, b). The plants were studied in a Brazilian savanna area in Uberlândia city (18°97′S, 48°29′W), where they grow mainly in disturbed/open sites, but are also found in undisturbed areas. Banisteriopsis malifolia is a much-branched shrub (<2 m tall). Flower buds grow along inflorescences, close to young leaves with active extrafloral nectaries. Buds are round (c.6 mm in diameter) and pinkish; leaves are up to 15 cm in length and 10 cm in width, and have small hairs on both sides. A pair of extrafloral nectaries is located at each side of the midrib and is visited by many patrolling ant species, especially Camponotus spp. and Ectatomma spp. (Alves-Silva 2011; Vilela et al. 2014; Figure 1c). Species within these two genera were observed in association with several myrmecophilous lycaenids (Robbins & Aiello 1982; Kaminski et al. 2010).

**Study sites**

The “open area” is a private site characterized by roughly 10 ha of short and secondary vegetation, with a predominance of shrubs (<2 m tall), especially Bignoniaceae, Malpighiaceae and Fabaceae, and a moderate cover of grasses. A dirt road about 3 m wide crosses the area and soil erosion is common. The flow of people, vehicles and animals on this road is constant, imposing injuries and sometimes killing seedlings and small shrubs. In addition, fires and mowing by bulldozers destroy the vegetation every two to three years. In this area, B. malifolia receives both vertical and lateral sunlight all day. Shrubs are not in contact with plant species other than grasses.

The “reserve” is a fenced protected natural site (cerrado sensu stricto) measuring about 230 ha with dense vegetation containing Ochnaceae, Caryocaraceae, Vochysiaceae and Myrtaceae, among other plant...
families. This area is also crossed by a narrow dirt road (<2 m) and the flow of people and vehicles is limited and requires permission. The site chosen for the study (approximately 10 ha within the protected area) had not been burned for the previous 10 years and mowing does not occur at all at this location. The distance between the protected and disturbed sites is approximately 1.7 km. Banisteriopsis malifolia grows mostly at the borders of the area and individuals are sometimes shaded by the canopy of large trees, thus receiving low direct and lateral light. Shrubs are also in constant contact with other plant species.

**Fieldwork and sampling design**

In early February 2011, we tagged 40 B. malifolia shrubs in the open area and the reserve (total of 80 plants). However, in the course of the study some plants died due to unknown causes or did not bloom at all. Therefore, the final sample sizes were standardized to $n = 29$ B. malifolia individuals in each area. Ants occur on the plants only during the period of extrafloral nectary activity, which coincides with the reproductive season (Alves-Silva et al. 2013). Since plants were tagged in February, i.e. before the reproductive season and ant colonization, we had no control of the species of ants that might later occur on them.

The frequency (number of plants on which they occurred) of ants was estimated once (February) in both areas as their presence on the plants was consistent throughout the study. Ant abundance was not used as a variable in this study, as we did not know the exact number of ants on a given shrub when lycaenid oviposition occurred.

Plants were visited weekly for lycaenid sampling, totaling 14 sampling occasions. On each occasion, flower buds and inflorescences were carefully examined. Larvae were captured, kept individually in transparent plastic pots (200 ml) and maintained in the laboratory (12 h L:12 h D, ±25°C). Larvae were fed whenever necessary with B. malifolia flower buds until pupation (following Bächtold et al. 2013). Adults were identified to species soon after emergence and released in the field whenever possible. During field sampling, the interactions between lycaenid larvae and ants were observed to investigate whether larvae were tended by ants or not.

Banisteriopsis malifolia phenology was recorded for all tagged individuals at both sites. Observations were made every two weeks, from March to May. The intensity of flowering phenophase was scored from 0 to 4: 1 = 1–25%; 2 = 26–50%; 3 = 51–75%; 4 = 76–100% of plants with flowers (Vilela et al. 2014), and the monthly average values were used in analysis. Plant size (in m) was estimated once at the start of the study. Measurements were made from the plant foot to the apex of the highest branch.

**Statistical analyses**

Quantitative data are presented as mean ± standard deviation whenever appropriate, and the use of parametric or non-parametric statistical procedures was based on normality tests. We performed a logistic regression to examine the odds of finding lycaenids in a given habitat type. Areas were designated as “0” or “1” and lycaenid presence/absence was employed as the intercept. The frequency of plants supporting lycaenids in each area was examined with Fisher’s exact test. A Student’s t-test was used to test for differences in plant size between areas. An analysis of covariance test was used to investigate whether lycaenid abundance was related to area and plant height (covariate).

The G test was used to investigate the frequency of B. malifolia supporting each ant species in both areas. Fisher’s exact tests were used to analyze the relation between lycaenids and plants with each ant species. A logistic regression analysis was used to test whether lycaenids were related to different ant species, but in this last test we did not consider the effect of area, that is, all data were pooled. Statistical procedures were performed in R statistical software version 3.2.4 (R Core Team 2016).

**Results**

**Lycaenids and plant characteristics**

Twenty-five lycaenid larvae (0.86 ± 0.99 per plant) were found on B. malifolia in the open area ($n = 29$ plants examined) whereas only four larvae were found at the reserve (0.14 ± 0.35 per plant, $n = 29$ plants examined) (Table 1). These differences were statistically significant in a logistic regression, showing that the odds of finding lycaenids in the open area were six times greater than in the reserve (odds-ratio = 6.6964, intercept = −1.8326, slope = 1.9016, $p < 0.01$). Furthermore, significantly more individual plants supported lycaenids in the open area ($n = 15$; 51.7%) than in the reserve ($n = 4$; 13.8%) (Fisher’s exact test $p < 0.05$).

Plant height was significantly different between areas, averaging 1.55 ± 0.29 m in the open area and 1.06 m ± 0.24 m in the reserve ($U = 76.0$, $p < 0.0001$; $n = 29$ plants per area). The analysis of covariance showed that lycaenids were significantly related to...
both plant height and the interaction effect between plant height + area (Table 2).

In the reserve, all four larvae were of Parrhasius polibetes. In the open area, P. polibetes was also abundant (n = 9 larvae, 36% of individuals), but four other lycaenid species were also found: Rekoa marius (24%; n = 6); Allosmaitia strophius (16%; n = 4); Rekoa stagna (4%; n = 1); and Tmolus venustus (4%; n = 1). Four larvae (16%) collected in the open area died in the laboratory from unknown causes and their identification was not possible.

Plants bloomed in the same period in both areas, from March to May, but the intensity of flowering was markedly greater in the open area (Figure 2). The greatest abundance of lycaenids at this site occurred in April, when 96% of larvae were collected. This period overlapped with the maximum intensity of flowering. In the reserve, lycaenids were only found in March and May (n = 2 larvae in each month), when flowering intensity was low (Figure 2).

### Lycaenids and ants

Only two ant species were frequently found in association with the plants, Camponotus blandus (Formicinae) and Ectatomma tuberculatum (Ectatomminae). In the open area most plants supported C. blandus while E. tuberculatum was not found as frequently (G = 58.6098, df = 1, p < 0.0001) (Figure 3a). In the reserve, the scenario was opposite, as E. tuberculatum was more frequently associated with B. malifolia plants than C. blandus (G = 9.9702, df = 1, p < 0.01) (Figure 3a). There was no overlap between ant species on any individual plants throughout the study period. Most lycaenids in the open area were found in association with C. blandus (Fisher’s exact test, p > 0.05) (Figure 3b). In the reserve lycaenids were found only on plants with C. blandus (Fisher’s exact test, p < 0.05) (Figure 3b).

At the lycaenid species level, there was a slight difference concerning larvae found together with C. blandus and E. tuberculatum (Table 1). Eighteen lycaenids were found in association with C. blandus and seven with E. tuberculatum at the disturbed site. Plants with C. blandus had significantly more larvae than plants with E. tuberculatum (Fisher’s exact test, p > 0.05). In addition, the logistic regression showed that the odds of finding lycaenids on plants with C. blandus were greater (odds-ratio = 3.5625, intercept = −1.5581, slope = 1.2705, p < 0.05). In the open area, both Rekoa species were attended by C. blandus. This ant species also attended P. polibetes in both study areas. Individuals of E. tuberculatum were not observed tending any larvae that co-occurred with them on the same individual plant. Other than lycaenids, two larvae of the myrmecophilous butterfly species Synargis calyce (Felder & Felder 1862) (Riodinidae) were also found in B. malifolia and were tended by C. blandus, but we did not include these riodinids in the analyses above.

### Table 1. Larvae of lycaenid species found together with the ant species Camponotus blandus and Ectatomma tuberculatum on Banisteriopsis malifolia at two Brazilian savanna areas with distinct characteristics.

<table>
<thead>
<tr>
<th>Lycaenidae species</th>
<th>Open area Mean</th>
<th>Reserve Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Camponotus blandus</td>
<td>Ectatomma tuberculatum</td>
</tr>
<tr>
<td>Allosmaitia strophius</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Parrhasius polibetes</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Rekoa stagna</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Rekoa marius</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Tmolus venustus</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Unidentified species</td>
<td>4</td>
<td>–</td>
</tr>
<tr>
<td>Total abundance</td>
<td>18</td>
<td>7</td>
</tr>
<tr>
<td>Total species richness</td>
<td>5</td>
<td>3</td>
</tr>
</tbody>
</table>

### Table 2. Occurrence of lycaenid larvae according to plant (Banisteriopsis malifolia) height and areas (disturbed and reserve).

<table>
<thead>
<tr>
<th>Effects</th>
<th>Df</th>
<th>Sum of squares</th>
<th>Mean of squares</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Main effects</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Area</td>
<td>1</td>
<td>0.4259</td>
<td>0.4259</td>
<td>18.4248***</td>
</tr>
<tr>
<td>Plant height</td>
<td>1</td>
<td>0.1429</td>
<td>0.1429</td>
<td>6.1847*</td>
</tr>
<tr>
<td>Interaction effects</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Area* + plant height</td>
<td>1</td>
<td>0.3231</td>
<td>0.3231</td>
<td>14.3674 ***</td>
</tr>
<tr>
<td>Residuals</td>
<td>54</td>
<td>1.2481</td>
<td>0.0231</td>
<td></td>
</tr>
</tbody>
</table>

Both the main and the interaction effects were statistically significant. Df = degrees of freedom; superscript asterisks indicate statistical significant differences, *** = p < 0.0001 and * = p < 0.05.

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**Note:** This text is a natural language representation of the content provided. It is based on the assumption that the original document contains valid and comprehensive data, without any errors or omissions that would affect the accuracy of the information presented. Any conclusions drawn from this representation are based solely on the information provided in the text and do not extend beyond what is explicitly stated or implied in the provided content. Additionally, this text does not include any figures, tables, or formulas that are not present in the original document, as they would require visual aids or computational analyses not feasible in this text-based format.
Discussion

Our results showed that ants (notably *C. blandus*), together with plant phenology, size and habitat affected the occurrence of larval lycaenids on *B. malifolia*. The lycaenid species found in this study also occur on other plant species, especially on Malpighiaceae (Silva et al. 2011; Bächtold et al. 2013, 2014). *Camponotus* species have been observed tending *P. polibetes* and *Rekoa* spp., but not *A. strophius* (Monteiro 2000; Kaminski & Freitas 2010). The biology and ecology of *T. venustus* larvae is unknown (but see Silva et al. 2014), since records are based on adult individuals (Pinheiro & Emery 2006).

In our study, most lycaenids were found in association with *C. blandus*, and in fact, this is common in lycaenid–ant interactions. In general, lycaenid abundance was up to threefold higher on plants with *C. blandus* than on plants with *E. tuberculatum*. These results comply with the literature of ant–lycaenid interactions, where authors state that *Ectatomma* ants may sometimes kill the larvae (Robbins 1991; Bächtold & Alves-Silva 2013). On the other hand, *Camponotus* ants play an important role in lycaenid life history, as adult females of butterflies use these ants as cues for oviposition, and ants also tend the lycaenid larval stages (Kaminski et al. 2010; Alves-Silva et al. 2013; Bächtold et al. 2014). This mutualistic interaction is
mediated by the sugared liquids produced by caterpillars (see Schmid et al. 2010), and in our study all of the lycaenid species sampled have ant-organs which are responsible for the association with ants to some extent (but see Kaminski & Freitas 2010).

In lycaenids, habitat selection is determined by the conditions required by the immature stages (Ellis 2003) and plants that offer more resources (e.g., food and mutualistic ant) may sustain a more abundant lycaenid fauna (see Rodrigues et al. 2010). In this context, *B. malifolia* is advantageous for the development and survivorship of larvae since it offers plenty of food (hundreds of flowers) and ant guards (Alves-Silva et al. 2013) which can provide enemy-free space for myrmecophilous larvae (Kaminski et al. 2010). In this study, most larvae were found in the open area where plants bloomed intensively. In general, florivorous lycaenids are well adapted to exploit the cycle of flowering seasons (New 1993), and the polyphagous habit of many species allows for their maintenance in time and space on different plants (Robbins & Aiello 1982; Kaminski & Freitas 2010; Silva et al. 2011).

In this study, habitat conservation status was an important factor influencing lycaenid occurrence. Some studies demonstrate that several lycaenid species are common in disturbed areas and stressed environments with open vegetation (New 1993; Ellis 2003). This is because larvae of some species can develop better in hosts adapted to disturbed and/or early successional habitats (Thomas 1985; Dover et al. 2009). *Banisteriopsis malifolia* has characteristics of pioneer/early successional species (Swaine & Whitmore 1988; Válio & Scarpa 2001), such as rapid growth, ability to resprout after disturbance and production of hundreds of fruits dispersed by wind (Alves-Silva & Del-Claro 2016). Because of its rapid growth, this plant stands out in open areas. This factor may account for the greater occurrence of lycaenids at the open site compared to the reserve, where *B. malifolia* is surrounded by dense vegetation. Plant conspicuousness and height are important for butterflies in general (Beccaloni 1997; Langan et al. 2001; Bächtold et al. 2014), and for lycaenids, they may be an indication of the availability of resources (Wynhoff et al. 2014) or the presence of mutualistic ants (Smiley et al. 1988). Taller individuals of *B. malifolia* support more patrolling ants and resources (Alves-Silva & Del-Claro 2014), thus may be more susceptible to lycaenid occupation.

This study showed that a lycaenid community occurred mostly in *B. malifolia* individuals located at an open/disturbed site, where plants were tall, bloomed intensively and supported more tending ants, especially *C. blandus*. This study contributes to the knowledge of the ecological factors responsible for the occurrence, maintenance and stability of the lycaenid fauna at a local scale (Brown 1993). Any information on lycaenid host range and interspecific interactions contributes to the understanding of these butterflies in natural areas, especially in the neotropics where deforestation imposes a serious risk for butterflies and their host plants (Oliveira & Marquis 2002; Freitas & Marini-Filho 2011).

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**Disclosure statement**

No potential conflict of interest was reported by the authors.

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