Influence of *Camponotus blandus* (Formicinae) and flower buds on the occurrence of *Parrhasius polibetes* (Lepidoptera: Lycaenidae) in *Banisteriopsis malifolia* (Malpighiaceae)

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**Abstract**

In the Brazilian savanna, myrmecophilous lycaenids are often found in many shrubs feeding on plant reproductive structures while are tended by ants, but only recently the role of both ants and food on the occurrence of lycaenids have received attention. In this study, we investigated the influence of *Camponotus blandus* (Formicinae) and flower bud abundance on the occurrence of *Parrhasius polibetes*, a florivorous lycaenid species that occurs in *Banisteriopsis malifolia* (Malpighiaceae). We also examined to what extent larval florivory was deleterious to plant reproductive outputs. Ant-exclusion experiments revealed that most *P. polibetes* individuals were found on branches with free *C. blandus* access. Nonetheless, the occurrence of larvae was not related to the abundance of ants and flower buds, indicating that the presence, rather than the abundance of mutualistic ants and food, influenced the occurrence of *P. polibetes*. Larvae were attended by *C. blandus*, which antennated frequently the dorsal nectary organ of larvae. Larval florivory was not deleterious to the plant. *Banisteriopsis malifolia* produces thousands of buds simultaneously and larvae feed only on a small portion of flower buds. The occurrence of *P. polibetes* in *B. malifolia* is advantageous for the larvae, since this plant supports mutualistic ants and plenty of food resources.

**Introduction**

Lycaenidae has one of the most remarkable interactions within the Lepidoptera – mutualistic associations with ants (Pierce et al., 2002). Larvae have dorsal nectary organs (DNOs) and perforated cupolas organs (PCOs) (Fiedler, 1991). The former releases a sugared substance while the latter pacifies the aggressive behavior of tending ants (Malicky, 1970). In this context, studies have shown that lycaenid female oviposition choices may be ant-mediated (Seufert & Fiedler, 1996; Wynhoff et al., 2008). This trend was observed in *Parrhasius polibetes* (Stoll), a facultative myrmecophilous species that is frequent in the Brazilian cerrado savanna (Silva et al., 2011). Kaminski et al. (2010a) showed that *P. polibetes* female oviposition was mediated by the presence of tending ants. Ants may increase larval performance and survivorship by protecting them from natural enemies (Weeks, 2003). In these cases, females will seek more fa-
The availability of food items also influences the occurrence of lycaenids (Wagner & Kurina, 1997) and by feeding on plant reproductive structures, larvae are supposed to exert negative effects on plant fitness (Oliveira & Del-Claro, 2005). For instance, Badenes-Pérez et al. (2010) discussed the use of lycaenids as biological control agents against the invasive species Miconia calvescens DC. (Melastomataceae) in Costa Rica, as larvae were observed to damage up to 30% of the reproductive structures of the plant (see also Jordano et al., 1990).

In the Brazilian savanna (Cerrado biome), the extrafloral (EFN) nectary shrub Banisteriopsis malifolia (Nees & Martius) B. Gates (Malpighiaceae) is patrolled by a wide range of EFN feeding ants (Alves-Silva, 2011). Camponotus blandus (Smith), Formicidae, is one of the most abundant ant species in B. malifolia, being very aggressive towards other arthropods (Alves-Silva et al., 2012).

In this study, we investigated the influence of i) ants (presence and abundance) and ii) flower buds (presence and abundance) on the occurrence of lycaenids in B. malifolia. We also examined the quantity of flowers buds consumed by larvae to investigate whether larvae negatively influenced plant fitness. To conclude, we conducted observations of the behavior of C. blandus towards P. polibetes larvae and towards other herbivores. An appreciation of the factors involved in the occurrence of lycaenid larvae in plants of common occurrence can be a tool for the understanding of ant-lycaenid mutualisms in the neotropics, especially in the Brazilian savanna (cerrado).

Material and Methods

Study area

The study was conducted in a strictu sensu cerrado area (18°59’ S – 48°18’ W) in Uberlândia city, Brazil, from March to May 2012, which corresponds to the reproductive season of B. malifolia. The cerrado covers about 230 hectares and is dominated by shrubs and trees ranging between 2 - 4m tall. The climate is markedly seasonal with a dry winter (May to September) and a rainy summer (October to April).

Plant species

Banisteriopsis malifolia is a small shrub (< 2 meters high). Leaves have small trichomes on both sides and bear a pair of EFNs at the base, near the petiole. Flower bud production starts in March and peaks in mid-April. Buds are on average 7-10 mm in diameter, pinkish and bear eight oil glands in its circumference. Flower buds grow on inflorescences located at the apex of branches.

Ant-exclusion experiment

The role of ant presence on the occurrence of lycaenids was examined in 30 individuals of B. malifolia, distributed evenly in approximately 10 ha within the study area. All shrubs were patrolled by C. blandus. A control and a treatment branch containing flower buds and young leaves with functional EFNs were tagged in each plant individual in late March. At the base of treatment branches (n = 30), a layer of atoxic wax (Tanglefoot® - Grand Rapids, MI, USA) was applied to prevent the access of ants to the plant structures (Nahas et al., 2012). The control branches (n = 30) were left unaltered, allowing the free access of ants to the plant parts. At this occasion, all B. malifolia shrubs were carefully examined and no immature lycaenids (egg or larva) were seen.

Sampling

Lycaenid sampling was performed once a week after wax application, from the first week of April until the end of May. On each occasion, buds, flowers, shoots, and both sides of leaves of the treatment and control branches were examined. Parrhasius polibetes larvae found in the field were then collected, individualized in plastic containers (250 ml), and reared in the laboratory until pupation. Other lycaenid larvae that were observed on the plant were also collected and reared in the laboratory. The comparison between the number of P. polibetes larvae found in the treatment and control branches was made with a Chi-squared test with Yates correction. The abundance of C. blandus and flower buds was also estimated at the beginning of the study. Flower bud counting was made in a randomly selected inflorescence within each plant. The number of ants foraging on each B. malifolia individual was counted once. The difference in the abundance of ants and flower buds in plants with and without P. polibetes was made with Student’s t tests (original data was log10 transformed to fit normal distribution).

Ant-lycaenid interactions

In the field, we performed 30 hours of observation (ad libitum) on the behavior of C. blandus towards P. polibetes larvae. Whenever C. blandus encountered a larva, we carefully observed whether ants attended the larvae or not. Ant attendance was characterized by quickly and alternate antennation on the dorsal nectary organ, coupled with walking back and forth over or near the larva (Ballmer & Pratt, 1991).
Ant hostility towards invaders

Camponotus blandus hostility towards invaders was examined by placing one live termite worker (Nasutitermes sp. - Termitidae) on inflorescences of B. malifolia (n = 21 individuals). Each plant received one termite. With this method, we intended to simulate possible wingless lycaenid natural enemies such as spiders, or other insects that might molest lycaenids or interfere with their feeding activity. Termite baits are usually used to investigate the behavior of patrolling ants towards plant invaders (Oliveira, 1997). The termites were followed for 10 minutes and interactions with ants were recorded.

Larval florivory

The estimation of the daily bud consumption by P. polibetes larvae was made under laboratory conditions. Five larvae were fed ad libitum with flower buds every 24h. Florivory estimation was conducted in fourth instar larvae only, as in this stage larvae can feed on several flower buds. Florivory rates were compared with the abundance of flower buds in the plants. All quantitative data is presented as mean ± standard error.

Results

We found eleven (0.37 ± 0.11; n = 30 plants examined) P. polibetes in B. malifolia and only one larva was found in an ant-excluded branch, while all the other larvae (n = 10) were found in branches with free ant-access (χ² = 5.82; df = 1; P < 0.05). Besides P. polibetes, other three lycaenid species were found in B. malifolia, but in low abundance: Rekoa sp. (0.1 ± 0.07; n = 3), R. marius (Lucas) (0.03 ± 0.03; n = 1), and Allosmaitia strophius (Godart) (0.03 ± 0.03; n = 1).

Each B. malifolia had on average 16.42 ± 1.91 C. blandus individuals. This plant also presented many flower buds per inflorescence (340.07 ± 35.77). Parrhasius polibetes larvae were found in nine B. malifolia and these plants had on average 10% more ants and 13% more flower buds. However, none of these variables was related to larvae occurrence (ants: t₉₈ = 0.5523; P > 0.05; flower buds: t₈ = 1.0968; P > 0.05; Fig. 1).

Camponotus blandus attacked all termite baits placed on B. malifolia inflorescences. The ants bit the termites several times, often throwing them away from the plant. Nevertheless, no lycaenid larva was preyed upon, attacked or molested by C. blandus. In fact ants attended P. polibetes larvae. The ants, alone or in groups of two to three individuals, walked over the larva and antennated the whole extension of the P. polibetes body, but the antennation was concentrated on the dorsal nectary organ (Fig. 2). In the meantime, the larva continued to feed on flower buds. The other lycaenids sampled in B. malifolia were not observed being attended by ants.

Parrhasius polibetes larvae fed on the whole content of buds, often leaving only an empty shell comprising the external surface of buds. Larvae consumed on average 17.0 ± 5.32 (range 5 – 35; n = 5 P. polibetes individuals) flower buds per day. This value refers to the feeding activity of the 4th instar larvae until pupation, which lasted on average seven days. Thus each 4th instar larva can damage on average 119 flower buds, which corresponds to approximately 35% of flower buds per inflorescence (flower bud abundance per inflorescence = 340.07 ± 35.77).

Figure 1. Relationship between the occurrence of Parrhasius polibetes according to: A - the abundance of Camponotus blandus; and B - the abundance of Banisteriopsis malifolia flower buds per inflorescence. Bars show the mean ± standard error.

Discussion

According to Price et al. (1995), cerrado vegetation supports a high diversity, but low abundance, of lepidopterans per species. Concerning lycaenids, Silva et al. (2011) showed that it is necessary large sampling effort to obtain a considerable abundance of larvae in different host plants; and despite considerable field sampling the authors found a low frequency of immature lycaenids in plants of common occurrence. This trend was also observed in our study, as we found a relative high community of lycaenids associated with B. malifolia, but the abundance of each species was low.

Parrhasius polibetes was the most abundant species (68.75% of individuals) while the other lycaenids were found in low numbers. The occurrence of P. polibetes in B. malifolia was observed to be mediated by the presence, but not the abundance, of C. blandus. Furthermore, P. polibetes presence was not related to the abundance of flower buds, but no larvae were found in the end of B. malifolia flowering season (May). Parrhasius polibetes was the only lycaenid species attended by C. blandus. Ant-mediated occurrence seems to be common in P. polibetes and Kaminski and Rodrigues (2011) showed that P. polibetes tended by Camponotus experienced increased performance and survivorship, as ants usually reduced the abundance of lycaenid natural
enemies (see also Kaminski et al., 2010a; Rodrigues et al., 2010). In our study, *C. blandus* was very aggressive towards invaders, as demonstrated by the termite bait exposure experiment, suggesting that ants can molest or displace wingless lycaenid natural enemies. *Banisteriopsis malifolia* supports a diverse community of arthropods, including herbivores and predators (Alves-Silva, 2011; Alves-Silva et al., 2012), but in the field no larvae was observed to be attacked or injured by natural enemies, such as spiders or parasitoids, or molested by any other insect.

In our study, despite the limitation of larvae reared in the laboratory, *P. polibetes* fed on approximately 35% of flower buds per inflorescence, but its effect on *B. malifolia* fitness was negligible. *Banisteriopsis malifolia* bears tens of inflorescences, which together may contain more than 5000 flower buds (pers. obs.). Therefore, the reduction of up to 35 flower buds per day (4th instar larvae feeding estimation) will account for only a small portion of flower buds produced by the plant. In this context, the occurrence of *P. polibetes* in *B. malifolia* is unlikely to affect plant fitness to any great extent.

Malpighiaceae are considered as important hosts for...


