Sociobiology
An international journal on social insects

RESEARCH ARTICLE - ANTS

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

Lycaenids are abundant and widely distributed in the Neotropics, accounting for 1200 species distributed in several biomes and vegetation types (Brown Jr., 1993; Robbins & Lamas, 2004). In Brazil, lycaenid records are based mostly on adult individuals, thus little is known about the larval host range (Emery et al., 2006; Francini et al., 2011). Lycaenid larvae generally feed on plant reproductive parts, such as flowers and flower buds (Robbins & Aiello, 1982). However, despite the vast diversity of flora in Brazilian biomes, only recently the interactions among lycaenid larvae, their host plants, and mutualistic ants have been considered in ecological studies (Kaminski & Freitas, 2010; Kaminski et al., 2010a; Silva et al., 2011; Bächtold & Alves-Silva, 2012).

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-

vorable plants, taking into account not only the presence of ants, but in the case of *P. polibetes*, females will also seek for high quality food (Rodrigues et al., 2010).

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) nectaried shrub *Banisteriopsis malifolia* (Nees & Martius) B. Gates (Malpighiaceae) is patrolled by a wide range of EFN feeding ants (Alves-Silva, 2011). *Camponotus blandus* (Smith), Formicinae, is one of the most abundant ant species in *B. malifolia*, being very aggressive towards other arthropods (Alves-Silva et al., 2012). Nonetheless, *C. blandus* has mutualistic relationships with membracids (Oliveira & Del-Claro, 2005). Facultative myrmecophilous lycaenids are found in *B. malifolia* feeding on flower buds and are susceptible to ant contact, but whether larvae are attended by *C. blandus* and the role of ant presence on larval occurrence have not previously been studied.

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 (18º59' S – 48º18' 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 (χ^2 = 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: t_{28} = 0.5523; P > 0.05; flower buds: t_{28} = 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).
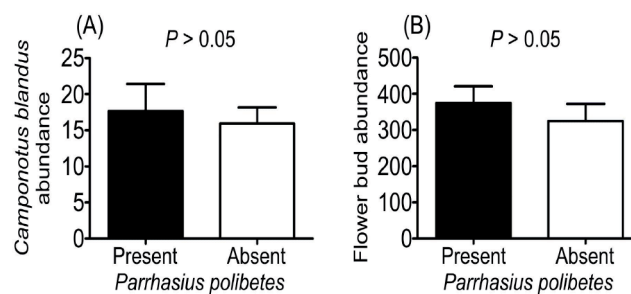

Figure 1. Relationship between the occurrence of *Parrhasius polibetes* according to: A - the abundance of *Camponotus blandus*; and B - the abundance of *Banisteriopsis malifolia* flower buds per inflorescence. Bars show the mean ± standard error.

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

Figure. 2. *Camponotus blandus* tending a *Parrhasius polibetes* larva in *Banisteriopsis malifolia*. The ant is drumming the last body segments of larva. Ant size - 5 mm.

Rekoa and *A. strophius* in the neotropics (Robbins, 1991; Monteiro, 2000; Kaminski & Freitas, 2010), but in our study the abundance of these lycaenids was low. Both *Rekoa* and *A. strophius* are polychromatic and this characteristic was observed in *B. malifolia*, where larvae turned pink. No *Rekoa* and *A. strophius* larvae were observed in contact with ants in *B. malifolia*, but both species have ant-organs and are facultative myrmecophilous (Robbins, 1991; Monteiro, 2000; Kaminski & Freitas, 2010).

The current knowledge about lycaenid-ant-host systems is based mostly on studies performed in the northern he-

misphere. In the neotropics, only recently the basic aspects of lycaenid life histories such as their distribution, host range, and ant-associations have received attention (Kaminski & Freitas, 2010; Rodrigues et al., 2010; Silva et al., 2011). Given the risk of extinction of some species (see Brown Jr., 1993; Kaminski et al., 2010b), lycaenid studies in the neotropics are imperative. Further studies will aim at unraveling the relationships between lycaenids and Malpighiaceae, as this seems to be an important host for lycaenid larval development (Robbins, 1991; Monteiro, 2000; Kaminski & Freitas, 2010; Bächtold et al., 2013).

Acknowledgements

References

Alves-Silva, E. (2011). Post fire resprouting of *Banisteriopsis malifolia* (Malpighiaceae) and the role of extrafloral nectaries on the associated ant fauna in a Brazilian Savanna. Sociobiology, 58(2): 327-340.

Alves-Silva, E., Barônio, G. J., Torezan-Silingardi, H. M. & Del-Claro, K. (2012). Foraging behavior of *Brachygastra lecheguana* (Hymenoptera: Vespidae) on *Banisteriopsis malifolia* (Malpighiaceae): Extrafloral nectar consumption and herbivore predation in a tending ant system. Entomol. Sci. 16(2):162-169. DOI: 10.1111/ens.12004.

Bächtold, A. & Alves-Silva, E. (2012). Behavioral strategy of a lycaenid (Lepidoptera) caterpillar against aggressive ants in a Brazilian savanna. Acta Ethol, in press, DOI: 10.1007/s10211-012-0140-2.

Bächtold, A., Alves-Silva, E., Del-Claro, K. (2013). Notes on Lycaenidae (Lepidoptera) in inflorescences of P*eixotoa parviflora* (Malpighiaceae): A new host plant in a Brazilian Atlantic forest. J. Lepidop. Soc. 67(1): 65-67.

Badenes-Pérez, F. R., Alfaro-Alpízar, M. A. & Johnson, M. T. (2010). Diversity, ecology and herbivory of hairstreak butterflies (Theclinae) associated with the velvet tree, *Miconia calvescens* in Costa Rica. J. Insect Sci. 10(209): 1-9.

Ballmer, R. G. & Pratt, G. F. (1991). Quantification of ant attendance (myrmecopily) of lycaenid larvae. J. Res. Lepidop., 30(1-2): 95-112.

Brown Jr, K. S. (1993). Neotropical Lycaenidae: an overview. In T. R. New (Ed.), *Conservation Biology of Lycaenidae* (Butterflies) (pp. 45-61). Gland, Switzerland: International Union for Conservation of Nature and Natural Resources.

Emery, E. O., Jr, K. S. B. & Pinheiro, C. E. G. (2006). As borboletas (Lepidoptera, Papilionoidea) do Distrito Federal, Brasil. Rev. Bras. Entomol. 50(1): 85-92.

Fiedler, K. (1991). Systematic, evolutionary, and ecological implications of myrmecophily within the Lycaenidae (Insecta: Lepidoptera: Papilionoidea). Bonner Zool. Monog, 31(1): 1-210.

Francini, R. B., Duarte, M., Mielke, O. H. H., Caldas, A. & Freitas, A. V. L. (2011). Butterflies (Lepidoptera, Papilionoidea and Hesperioidea) of the "Baixada Santista" region, coastal São Paulo, southeastern Brazil. Rev. Bras. Entomol. 55(1): 55-68.

Jordano, D., Haeger, J. F. & Rodríguez, J. (1990). The effect of seed predation by *Tomares ballus* (Lepidoptera: Lycaenidae) on *Astragalus lusitanicus* (Fabaceae): Determinants of differences among patches. Oikos, 57(2): 250-256.

Kaminski, L. A. & Freitas, A. V. L. (2010). Natural history and morphology of immature stages of the butterfly *Allosmaitia strophius* (Godart) (Lepidoptera: Lycaenidae) on flower buds of Malpighiaceae. Stud. on Neotrop. Fauna and Envir., 45(1): 11-19.

Kaminski, L. A., Freitas, A. V. L. & Oliveira, P. S. (2010a). Interaction between mutualisms: Ant-tended butterflies exploit enemy-free space provided by ant-treehopper associations. The Am. Nat. 176(3): 322-334.

Kaminski, L. A., Thiele, S. C., Iserhard, C. A., Romanowski, H. P. & Moser, A. (2010b). Natural history, new records, and notes on the conservation status of *Cyanophrys bertha* (Jones) (Lepidoptera: Lycaenidae). Proc. the Entomol. Soc. of Wash., 112(1): 54-60.

Kaminski, L. A. & Rodrigues, D. (2011). Species-specific levels of ant attendance mediate performance costs in a facultative myrmecophilous butterfly. Physiol. Entomol., 36(3): 208-214.

Malicky, H. (1970). New aspects of the association between lycaenid larvae (Lycaenidae) and ants (Formicidae, Hymenoptera). Journal of the Lepidop. Soc., 24(3): 190-202.

Monteiro, R. F. (2000). Coloração críptica e padrão de uso de plantas hospedeiras em larvas de duas espécies mirmecófilas de Rekoa Kaye (Lepidoptera, Lycaenidae). In R. P. Martins, T. M. Lewinsohn & M. S. Barbeitos (Eds.), Ecologia Comportamental de Insetos (pp. 259-280). Rio de Janeiro: PPGE-UFRJ.

Nahas, L., Gonzaga, M. O. & Del-Claro, K. (2012). Emergent impacts of ant and spider interactions: Herbivory reduction in a tropical savanna tree. Biotropica, 44(4), 498-505.

Oliveira, P. S. (1997). The ecological function of extrafloral nectaries: Herbivore deterrence by visiting ants and reproductive output in *Caryocar brasiliense* (Caryocaraceae).

Funct. Ecol., 11(3): 323-330.

Oliveira, P. S. & Del-Claro, K. (2005). Multitrophic interactions in a neotropical savanna: Ant-hemipteran systems, associated insect herbivores and a host plant. In: D. F. R. P. Burslem, M. A. Pinard & S. E. Hartley (Eds.), Biotic Interactions in the Tropics (pp. 414-438). Cambridge: Cambridge University Press.

Pierce, N. E., Braby, M. F., Heath, A., Lohman, D. J., Mathew, J., Rand, D. B., Travassos, M. A. (2002). The ecology and evolution of ant association in the Lycaenidae (Lepidoptera). Annu. Rev. Entomol., 47(1): 733-771.

Price, P. W., Diniz, I. R., Morais, H. C. & Marques, E. S. A. (1995). The abundance of insect herbivore species in the tropics: The high local richness of rare species. Biotropica, 27(4): 468-478.

Robbins, R. K. & Aiello, A. (1982). Foodplant and oviposition records for Panamanian Lycaenidae and Ridonidae. J. Lepidop. Soc., 36(2): 65-75.

Robbins, R. K. (1991). Evolution, comparative morphology, and identification of the eumaeine butterfly genus Rekoa Kaye (Lycaenidae: Theclinae). Smiths. Contrib. Zool., 498(1): 1-64.

Robbins R. K. & G. Lamas. (2004). Lycaenidae. Theclinae. Eumaeini. In G Lamas & J. B. Heppner. Checklist: Part 4A. Hesperioidea—Papilionoidea. Atlas of Neotropical Lepidoptera. (pp.118-137). Volume 5A. Gainesville (IL): Association for Tropical Lepidoptera,Scientific Publishers.

Rodrigues, D., Kaminski, L. A., Freitas, A. V. L. & Oliveira, P. S. (2010). Trade-offs underlying polyphagy in a facultative ant-tended florivorous butterfly: the role of host plant quality and enemy-free space. Oecologia, 163(1): 719-728.

Seufert, P. & Fiedler, K. (1996). The influence of ants on patterns of colonization and establishment within a set of coexisting lycaenid butterflies in a south-east Asian tropical rain forest. Oecologia, 106(1): 127-136.

Silva, N. A. P., Duarte, M., Diniz, I. R. & Morais, H. C. (2011). Host plants of Lycaenidae on inflorescences in the central Brazilian cerrado. J. Res. Lepidop., 44(1): 95-105.

Wagner, D. & Kurina, L. (1997). The influence of ants and water availability on oviposition behaviour and survivorship of a facultatively ant-tended herbivore. Ecol. Entomol., 22(1): 352-360.

Weeks, J. A. (2003). Parasitism and ant protection alter the survival of the lycaenid Hemiargus isola. Ecolog. Entomol., 28(1): 228-232.

Wynhoff, I., Grutters, M. & van Langevelde, F. (2008). Looking for the ants: selection of oviposition sites by two myrmecophilous butterfly species. An. Biol., 58(4): 371-388.