

Interaction between Mutualisms: Ant-Tended Butterflies Exploit Enemy-Free Space Provided by Ant-Treehopper Associations

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ABSTRACT: Although mutualisms have been intensively investigated, demonstration of indirect effects between co-occurring mutualistic systems is rare. For instance, the ecological consequences of co-occurrence of ant-tended insects on a plant have never been examined for survival effects on either trophobiont species. Here, we assess the selective pressures mediating co-occurrence of a facultative ant-tended butterfly (*Parrhasius polibetes*) with ant-tended treehoppers (*Guayaquila xiphias*) on *Schefflera vinosa* shrubs. We evaluated host plant selection and caterpillar survival in *P. polibetes* in the presence and absence of ant-treehopper associations. Paired trials revealed that butterflies preferably oviposit on branches hosting ant-tended treehoppers when they had a choice between those and branches without this interaction. Presence of ant-tended treehoppers on a branch reduced the abundance of *P. polibetes*' natural enemies and improved caterpillar survival in both premyrmecophylic and ant-tended phases. Thus ant-tended treehoppers create an enemy-free space on foliage that butterflies exploit to protect larval offspring. These findings connect two widely documented ant-trophobiont mutualisms and highlight the importance of considering multiple interactions for a proper understanding of ant-plant-herbivore systems. Detection of other ant-based mutualisms on oviposition to improve offspring survival may have represented an important evolutionary step in the process of host plant selection in facultative myrmecophilous butterflies.

Keywords: ant-plant-herbivore interactions, cerrado savanna, insect trophobionts, natural enemies, oviposition behavior, trophic and nontrophic indirect effects.

Introduction

The niche dimensions of a species are determined by many variables, including abiotic factors, the nature and rate of available food resources, interspecific competition for limiting resources such as food or space, and natural enemies

(Jeffries and Lawton 1984). For insect herbivores, natural enemies (predators and parasitoids) are recognized as one of the most important factors determining niche dimensions (see Price et al. 1980; Singer and Stireman 2005). Ants are extremely abundant on foliage and are considered major predators of insect herbivores in tropical habitats (Jeanne 1979; Floren et al. 2002). The main factor accounting for the remarkable dominance of ants on plant surface is the high occurrence of predictable liquid food sources such as extrafloral nectaries and honeydew-producing insects (Rico-Gray and Oliveira 2007). The frequent presence of liquid-feeding ants on foliage represents a constant threat to herbivore insects because exudate-fueled ant foragers of particularly dominant species complement their diets by actively preying on herbivores (Davidson et al. 2003). Thus, insect herbivores face a major problem in order to feed on plant tissue: they need to find a safe spot on foliage, that is, an "enemy-free space" (Price et al. 1980). In this scenario, the capacity to make appropriate colonization decisions in the process of host plant selection is an important behavioral trait in insect herbivores (Thompson and Pellmyr 1991). Hence information about predation risks can be critical, and natural selection may favor the ability of herbivores to detect and avoid predators before oviposition, especially if offspring mortality risk is high (Schmitz et al. 2004). This was recently demonstrated for a tropical butterfly that feeds on a risky ant-visited plant (Sendoya et al. 2009).

Some types of insect herbivores, however, not only circumvent ant predation but even attract them for their own benefit. Myrmecophily (i.e., life associated with ants) is widespread among numerous insect taxa, especially in the Hemiptera and Lepidoptera (Hölldobler and Wilson 1990). By producing liquid nutritional rewards, such insects attract aggressive ants that collect the exudate and in return act as bodyguards by warding off their natural enemies (a relationship known as trophobiosis; see Stadler

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and Dixon 2008). As a result of intense patrolling activity in the vicinity of their exudate-producing partners, aggressive ants create an enemy-free space around the trophobionts. Because of this important benefit, natural selection on trophobiont herbivores may favor behavioral abilities to detect mutualistic ants before oviposition and to select more protected (i.e., ant-occupied) foliage, which improve offspring survival. This behavioral pattern is the opposite of that recorded for nonmyrmecophilous herbivores (see, e.g., Sendoya et al. 2009).

In Lepidoptera, myrmecophily is widespread in two butterfly families (Lycaenidae and Riodinidae) whose larvae produce nutritional liquid rewards to tending ants (Fiedler 1991; Pierce et al. 2002). Butterfly-ant symbiosis probably arose on plants that commonly have liquid food sources for ants such as extrafloral nectaries or honeydew-producing hemipterans (DeVries 1991), and it is expected that these ant attractants should affect oviposition decisions and host plant use in myrmecophilous butterflies (Atsatt 1981*b*; Thompson and Pellmyr 1991). Indeed, species from different lineages of myrmecophilous butterflies exploit plants that are constantly visited by ants, because the plants have ant attractants and/or because they regularly house ant colonies (see, e.g., Cottrell 1984; Maschwitz et al. 1984; DeVries and Baker 1989).

Although ant-based mutualistic systems frequently include multiple participants (see Bronstein and Barbosa 2002), the range of indirect effects among interacting species remains poorly documented. For instance, the ecological consequences of co-occurrence with hemipteran trophobionts have been only marginally treated with respect to host plant selection by myrmecophilous butterflies (see Atsatt 1981*a*; Pierce and Elgar 1985) and have never been examined for effects on larval survival. Recently, Oliveira and Del-Claro (2005) found evidence of spatio-temporal co-occurrence between larvae of the facultative myrmecophilous butterfly *Parrhasius polibetes* (mentioned as *Panhiades polibetes*; Lycaenidae) and ant-tended treehopper aggregations (*Guayaquila xiphias*; Membracidae; figs. 1, 2*A*, 2*B*). This system offers an ideal opportunity to investigate how the presence of an ant-tended herbivore on a plant can affect colonization decisions by a myrmecophilous butterfly. Our hypothesis is that *P. polibetes* butterflies would prefer to lay eggs near honeydew-producing treehoppers because the enemy-free space generated by tending ants in the vicinity of such trophobionts significantly improves larval survival.

We conducted a series of field experiments to assess the selective pressures mediating the co-occurrence of *P. polibetes* larvae with ant-treehopper associations. Specifically, we addressed the following questions. (1) Do butterflies use ant-treehopper associations as a cue for host plant selection? (2) Does larval survival improve in the vicinity

of ant-treehopper associations? (3) Does the presence of ant-treehopper associations decrease the abundance of potential natural enemies on a plant, thus creating an enemy-free space for butterfly larvae? (4) Does co-occurrence with ant-tended treehoppers improve discovery of butterfly larvae by prospective tending ants? A full assessment of the reciprocal indirect interactions between the two coexisting trophobiont species is beyond the scope of this study, although the whole scenario is addressed in the "Discussion."

Methods

Study Site and System

The study was carried out in a site of cerrado savanna of the Laboratório Nacional de Luz Síncrotron (22°48'S, 47°03'W) in Campinas, southeast Brazil. The vegetation consisted of a dense scrubland of shrubs and trees, classified as cerrado sensu stricto (Oliveira-Filho and Ratter 2002). Experiments were performed in 2008 and 2009 during the dry season (May–July), when adult butterflies are abundant and larval host plants have plenty of inflorescences (Del-Claro and Oliveira 1999).

The study system includes the gregarious honeydew-producing treehopper *Guayaquila xiphias*, which commonly occurs on shrubs of *Schefflera vinosa* (= *Didymopanax vinosum*; Araliaceae) in cerrado areas of southeast Brazil (fig. 2*B*). The treehopper can be tended day and night by more than 20 species of honeydew-gathering ants, which climb onto host plants after finding scattered droplets of flicked honeydew on the ground (Del-Claro and Oliveira 1996, 1999). The aggressive behavior of ants near *G. xiphias* aggregations decreases the incidence of natural enemies (salticid spiders, syrphid flies, and mymarid parasitoid wasps) on the host plant and increases treehopper survival (Del-Claro and Oliveira 2000). Moreover, patrolling behavior by honeydew-gathering ants can reduce plant damage by other herbivores (Oliveira and Del-Claro 2005). Plants with *G. xiphias* aggregations, however, are more infested by *Parrhasius polibetes* butterflies, whose ant-tended larvae feed on reproductive plant tissue (buds and flowers; Oliveira and Del-Claro 2005). Female butterflies lay about three eggs on the inflorescences per oviposition event; the larvae are solitary and develop in four instars (Kaminski 2010). Early nonmyrmecophylic instars (first and second) present numerous morphological and behavioral defensive traits to appease and/or hide from ants (Malicky 1970). The dorsal nectar organ (DNO) becomes functional in the third instar and caterpillars can be facultatively tended by the same ants that attend *G. xiphias* aggregations on a plant (fig. 1). Immature stages of *P. polibetes* are attacked by a variety of natural enemies

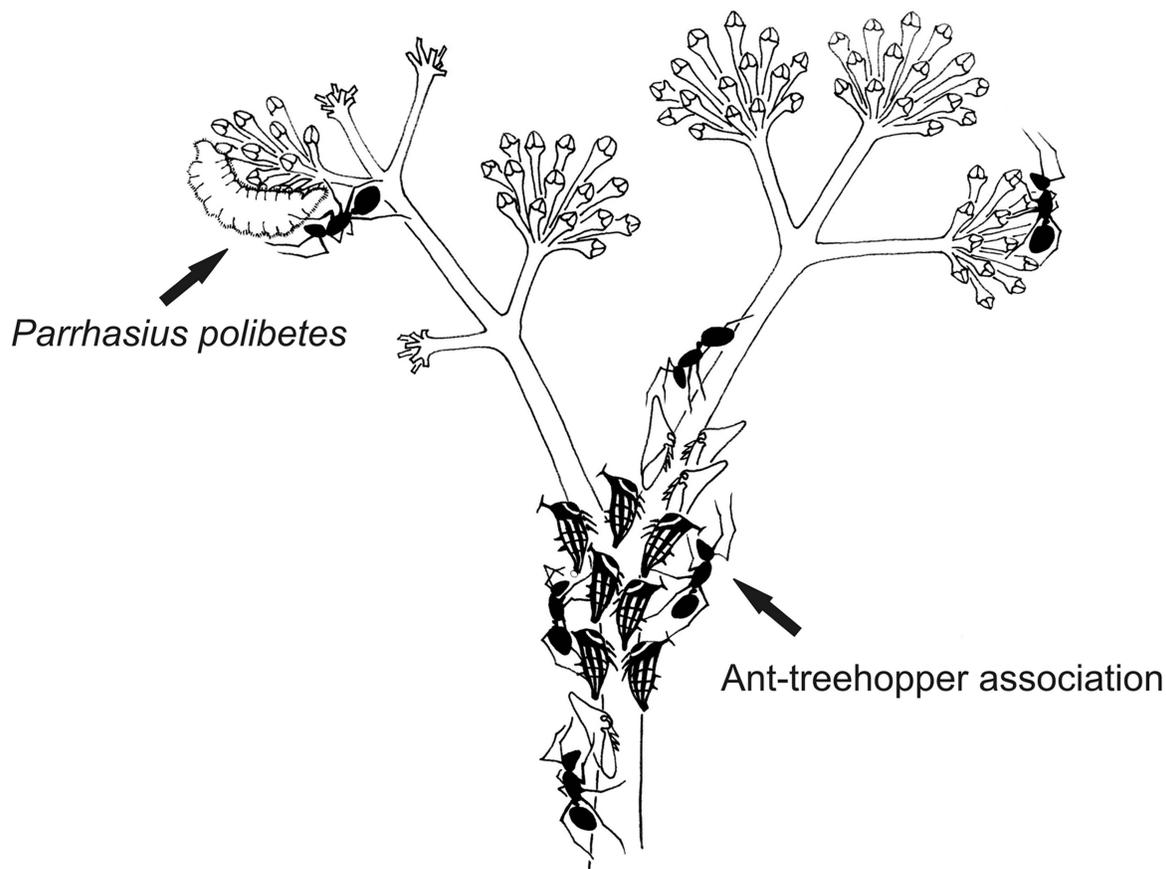


Figure 1: Schematic representation of the study system involving ant-tended *Guayaquila xiphias* treehoppers (adults and nymphs), myrmecophilous larvae of the butterfly *Parrhasius polibetes*, and the host plant *Schefflera vinosa*. Ants (*Camponotus rufipes* or *Camponotus renggeri*) from the same colony attend both trophobiont species on the inflorescence branch.

(fig. 4A, 4B), but larvae are mostly attacked by spiders (Araneidae, Thomisidae, and Salticidae) and parasitoid wasps (Braconidae, Chalcididae, and Ichneumonidae; Kaminski 2010).

The Impact of Ant-Treehopper Associations on Host Plant Selection by Parrhasius polibetes

To evaluate the role of ant-tended treehoppers as a cue used for host plant selection by *P. polibetes*, we carried out a series of paired oviposition trials in the field (see also Freitas and Oliveira 1996; Sendoya et al. 2009). For each tagged shrub of *S. vinosa*, we selected a pair of branches at approximately the same height (1–2 m) and with similar inflorescence size and number of leaves. The distance between branches of a pair ranged from 0.4 to 1 m. Each branch of a selected pair was designated as “occupied” by an ant-*Guayaquila* association or “unoccupied” by such

an association. Two groups of experimental host plants were set simultaneously for the oviposition trials. In one group of plants, we did not manipulate insect presence within paired branches: one branch was naturally occupied by ant-tended treehoppers and the other was unoccupied ($n = 20$ plants “without manipulation”). In a second group of plants, however, both paired branches were already occupied by ant-treehopper associations upon our arrival. We then manipulated the presence of ant-treehopper associations by manually removing them from one of the branches (assigned by the flip of a coin). Trials consisted of experimental pairs formed by a branch occupied by ant-tended treehoppers and a branch from which treehoppers had recently been manually removed ($n = 16$ plants “with manipulation”). We used only *G. xiphias* aggregations tended by *Camponotus rufipes* or *Camponotus renggeri* (Formicinae). These species are similar in size (~0.8 cm) and tending behavior, behave ag-

gressively toward intruders, and monopolize day and night the *G. xiphias* aggregations (Del-Claro and Oliveira 1999). A sticky barrier of Tanglefoot (Tanglefoot, Grand Rapids, MI) was applied at the base of unoccupied branches to prevent ant access. Occupied branches had resin applied on only one side so that ants could still reach the foliage. To control for unknown effects of common insect visitors other than ants and treehoppers on butterfly oviposition, we pinned one dried honeybee specimen (*Apis mellifera*, common flower visitor) next to the inflorescence of each experimental branch (for a similar method, see Sendoya et al. 2009). Vegetation bridges providing aerial ant access to experimental plants were removed. Nearby branches with inflorescences were clipped off so as to induce prospective ovipositing butterflies to choose between selected branches during oviposition experiments. Except for treehoppers and tending ants, all eggs and larvae of *P. polibetes* as well as all other arthropods were removed from the branches before trials (but see above trials “with manipulation”). Experimental branches were set up at 1430 hours and checked after 48 h. Only plants receiving at least one egg on a branch of a pair were considered for the analyses ($n = 36$). Whenever an oviposition event was seen, all behavioral aspects of host plant selection by female *P. polibetes* were reported (fig. 2A). Because experiments were performed during the period of highest butterfly abundance, oviposition decisions were assumed to be independent (i.e., made by different females).

Indirect Effects of Ant-Treehopper Associations on Larval Survival

The indirect effects of the presence of ant-treehopper associations on *P. polibetes* larvae were evaluated through two field experiments in which caterpillars were placed on *S. vinosa* host plants and regularly checked for survival in subsequent days. For both experiments, we selected one pair of similar-sized branches, in which one branch was naturally occupied by ant-tended treehoppers and one branch was naturally unoccupied by treehoppers. As with the oviposition experiment, we used only *G. xiphias* aggregations tended day and night by *C. rufipes* or *C. renggeri*.

Experiment I: Larval Survivorship. This 25-day experiment evaluated the indirect effect of ant-treehopper associations on larval survival in *P. polibetes* and on the abundance of its potential natural enemies on host plants ($n = 25$). The experiment included both the premyrmecophylic early larval phase (~12 days), as well as the third and fourth myrmecophylic instars. Branches occupied by ant-tended treehoppers received Tanglefoot resin on only one side so that ants could still reach the foliage, whereas unoccupied branches had resin applied at the base to prevent ant ac-

cess. Neighboring plant bridges were clipped to impede aerial ant access to plants. On each branch of a pair we placed one newly hatched *P. polibetes* larva (~0.2 cm) obtained from field-collected eggs. Larval survival on paired branches was checked daily for up to 5 min per plant (0900–1400 hours) over 25 days. Because the larvae pupate off the host plant, caterpillars were removed from experimental plants on the fifth day of the last instar. Missing larvae were considered dead, although we continued to check the experimental branches until the end of the experiment, when live caterpillars were collected for adult emergence in the laboratory. Potential natural enemies of *P. polibetes* larvae (spiders and parasitoid wasps) were checked every other day for up to 10 min per plant (0900–1400 hours).

Experiment II: Levels of Ant Tending. In this 10-day experiment, we assessed the indirect effects of ant-treehopper associations on larval survival in *P. polibetes* during the myrmecophylic period (third and fourth instars) in which caterpillars can potentially be tended by ants on host plants. In this experiment, however, ants had free access to both branches in a pair and thus were able to find *P. polibetes* larvae on either type of foliage: occupied or unoccupied by ant-tended treehoppers ($n = 25$ plants). One newly hatched third-instar larva (~0.8 cm; obtained from field-collected eggs) was placed on each branch of a pair. Larval survival on either branch was checked daily for up to 5 min per plant (0900–1400 hours) over 10 days; missing larvae were considered dead. We also recorded the number of scout ants walking on foliage or tending *P. polibetes* larvae on either type of branch, as well as the abundance of potential natural enemies (inspections of up to 10 min; 0900–1400 hours).

Statistical Analyses

For each series of oviposition trials (with and without manipulation), the proportion of experimental branches receiving eggs and the number of eggs oviposited on each branch category were analyzed with contingency G-tests and Wilcoxon signed-rank tests, respectively. Subsequently, to evaluate whether the experimental procedures (with and without manipulation) affected branch selection and number of eggs laid by females, we performed a contingency G-test and a two-way ANOVA, respectively. Survival curves of *P. polibetes* larvae were analyzed with log-rank (Mantel-Cox) tests, both for the premyrmecophylic larval phase in experiment I and for the entire extent of experiments I and II. Abundance data of natural enemies (spiders and parasitoid wasps) on branch pairs were analyzed with repeated measures ANOVAs, fitting paired experimental branches as a blocking factor and treatment

(presence or absence of ant-tended treehoppers) as a fixed effect. Separate ANOVAs were performed for the premyrmecophylic larval phase. We performed square-root transformations on the data to stabilize treatment variances for the statistical analyses. Mean numbers of ants on branches or tending experimental *P. polibetes* larvae (experiment II) were analyzed using Mann-Whitney *U*-tests.

Results

Presence of Ant-Tended Treehoppers and Host Plant Selection by Butterflies

Parrhasius polibetes females normally flutter around the host plant for 5–15 s ($n = 11$) before oviposition (pre-lighting phase). In the postlighting phase, however, the butterflies take 5–60 s ($n = 11$), and in this process they repeatedly touch the flower bud surface with the tip of the abdomen before ovipositing (fig. 2A). Direct contact of egg-laying females with foliage-dwelling ants was never observed. Paired oviposition experiments revealed an overall tendency of *P. polibetes* females to lay eggs on branches of *Schefflera vinosa* hosting ant-tended treehoppers compared to branches without this interaction (fig. 2C, 2E). This preference is significant for trials “with manipulation” (*G*-test, $G = 4.61$, $df = 1$, $P = .032$) and marginally significant for trials “without manipulation” of ant-tended treehoppers ($G = 3.66$, $df = 1$, $P = .056$). However, branch selection by females did not differ between experimental procedures ($G = .56$, $df = 1$, $P = .81$), and pooled data indicate that females do prefer to lay eggs on branches hosting ant-tended treehoppers ($G = 8.55$, $df = 1$, $P = .004$; fig. 2G). Similarly, butterflies laid more eggs on branches with ant-treehopper associations (fig. 2D, 2F). In this case, however, the tendency is significant for trials without manipulation (Wilcoxon signed-rank test, $Z = 48.00$, $P = .014$) and not significant for trials with manipulation of ant-tended treehoppers ($Z = 36.00$, $P = .164$). Again, the number of eggs laid did not differ between experimental procedures (two-way ANOVA, interaction treatment \times manipulation procedure mean square = .04, $F_{1,48} = .01$, $P = .92$), and pooled data indicate that females do lay increased number of eggs on branches with ant-treehopper associations ($Z = 163.00$, $P = .007$; fig. 2H).

Indirect Effects of Ant-Treehopper Associations on Larval Survival

Parrhasius polibetes larvae survive better when developing on branches of *S. vinosa* hosting ant-tended treehoppers than on branches without these associations (log-rank [Mantel-Cox] test, $\chi^2 = 4.54$, $P < .001$; fig. 3A). After 25

days, survivorship of butterfly larvae in the vicinity of ant-tended treehoppers was approximately sixfold higher than survivorship away from trophobionts. In addition, survival differences between paired branches were already significant in premyrmecophylic phase, when the dorsal nectar organs are nonfunctional (log-rank [Mantel-Cox] test, $\chi^2 = 4.02$, $P < .05$; fig. 3A). This early difference in larval survival may be related to the indirect effects of the presence of ant-treehopper associations on occupied branches, which reduced the abundance of natural enemies during the first 9 days when caterpillar are unattended by ants (table 1; fig. 4C, 4D). In the myrmecophylic phase, because of the combined ability to attract ants by treehoppers and larvae, survival differences between paired branches persisted consistently until the end of the experiment (fig. 3A). Indeed, predation by spiders and parasitism by wasps (fig. 4A, 4B) accounted, respectively, for 20.8% and 12.5% of the identifiable causes of death of *P. polibetes* larvae developing on branches without ant-treehopper associations (fig. 3A; total mortality of 96%). We were unable to detect mortality sources on branches occupied by ant-tended treehoppers (fig. 3A; total mortality of 68%).

Experiment II revealed that larval survival in the vicinity of ant-tended treehoppers is nearly threefold higher than away from such associations (log-rank [Mantel-Cox] test, $\chi^2 = 4.62$, $P = .05$; fig. 3B). As in experiment I, branches hosting ant-tended treehoppers had lower numbers of potential natural enemies of *P. polibetes* larvae than branches free from these associations (table 1; fig. 4E, 4F). Although ant access was allowed to either branch category, branches hosting ant-treehopper associations had increased numbers of ant foragers on leaves and inflorescences (Mann-Whitney test, $U = 0.00$, $df = 8$, $P < .001$; fig. 5A). Consequently, *P. polibetes* larvae growing on branches with ant-tended treehoppers had a higher probability of being discovered by prospective tending ants than those developing on plant locations visited only by occasional scout ants (Mann-Whitney test, $U = 14.00$, $df = 8$, $P < .05$; fig. 5B).

Discussion

Although mutualisms have been intensively investigated in the past decades, very few studies have focused on the interaction between co-occurring mutualistic systems despite their commonness in nature (Stanton 2003). Indeed, some mutualisms can only be understood within a broad context since pairwise interactions are relatively rare (Bronstein and Barbosa 2002; Holland et al. 2005). The current study is important because it unveils some of the selective pressures acting at the interface of two widely documented ant-based mutualisms that hitherto have been treated mostly as separate systems. We provide the first

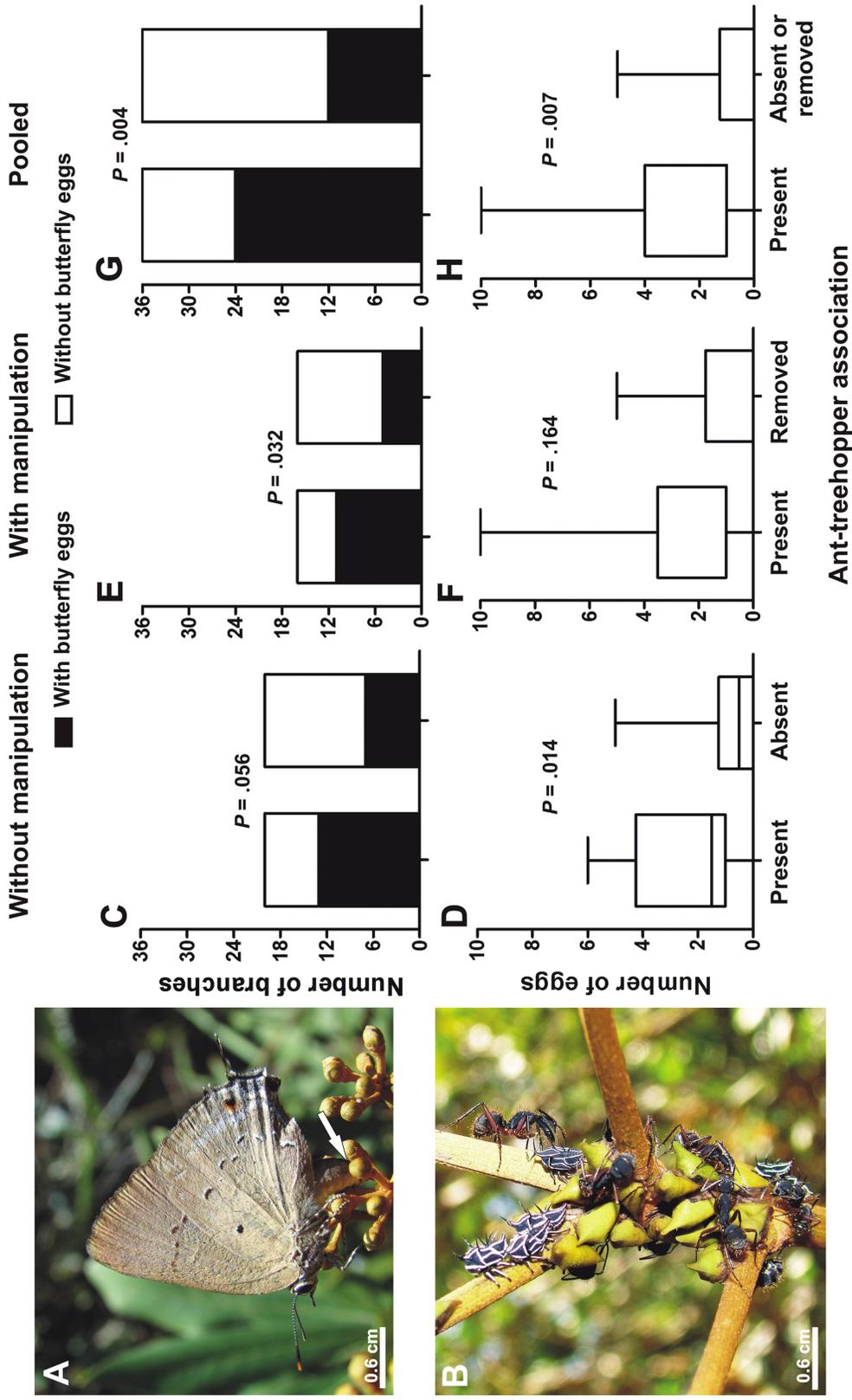


Figure 2: A, *Parrhasius polibetes* butterfly laying eggs on a *Schefflera vinosa* inflorescence; note abdomen tip curved (arrow). B, *Guayaquila xiphias* aggregation (adults and nymphs) tended by *Camponotus rufipes* ants on *S. vinosa*. C–H, Oviposition pattern by *P. polibetes* butterflies during choice experiments (48-h trials) using paired branches of *S. vinosa*. C, D, Branches were naturally occupied by ant-treeshopper associations or unoccupied (without manipulation). E, F, Both branches were occupied by ant-treeshopper associations before trials; after manipulation, the experimental pairs consisted of one branch occupied by ant-tended treeshoppers and one branch from which treeshoppers were manually removed (with manipulation). G, H, Pooled data of experiments without and with manipulation. C, E, G, Selection of plant location by egg-laying butterflies. D, F, H, Number of eggs laid per branch. Boxes show the lower and upper quartiles; whiskers show total range.

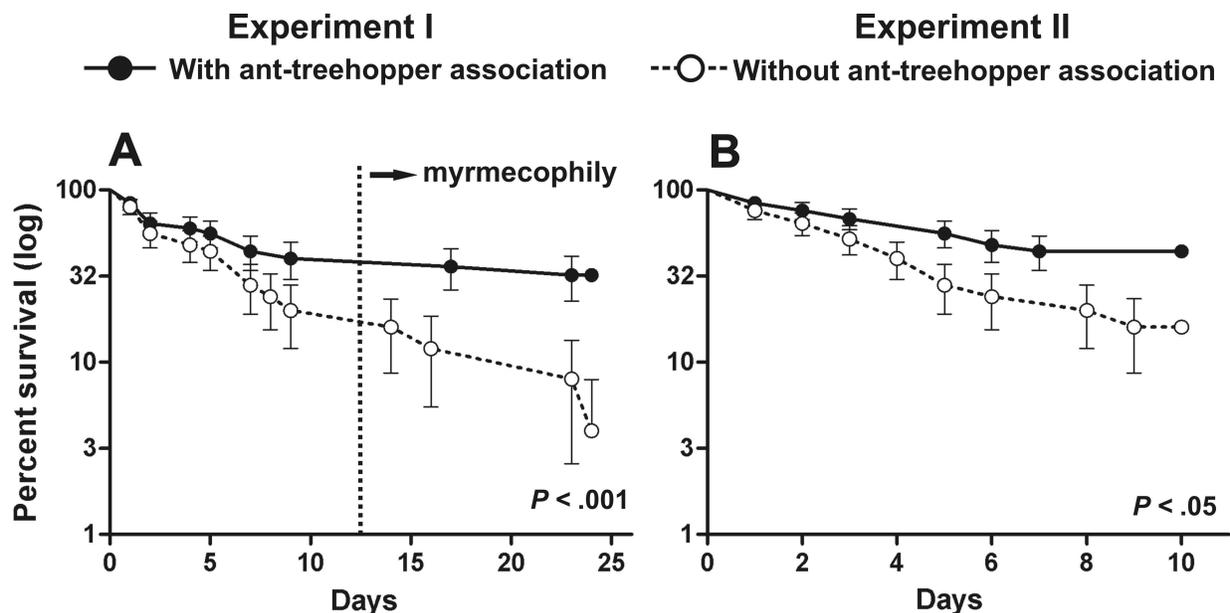


Figure 3: Survival curves of *Parrhasius polibetes* larvae on paired branches of *Schefflera vinosa* through time, as a function of the presence or absence of ant-treehopper associations. A, Experiment I included both the premyrmecophytic early larval phase (~12 days), as well as the third and fourth myrmecophytic instars (dashed line indicates when myrmecophily begins); occasional scout ants were excluded from unoccupied branches. B, Experiment II included only the myrmecophytic larval instars (third and fourth), and ants had free access to either branch category. Values are means \pm SE.

experimental evidence that an ant-treehopper mutualism can mediate behavioral decisions by a facultative myrmecophilous butterfly, with relevant fitness-related consequences for the latter.

Experimental results show that *Parrhasius polibetes* uses the presence of another ant-trophobiont interaction as an oviposition cue. We also demonstrate that butterfly larvae developing in the vicinity of ant-tended *Guayaquila xiphias* treehoppers survive better compared to those growing on plant locations free from these trophobionts (fig. 3A) and that mortality is greater where the butterfly larva relies solely on its own ability to attract ants rather than on the additional pulling power of the treehoppers (fig. 3B). Our data show that honeydew-gathering ants around treehopper aggregations create an “enemy-free space” (Price et al. 1980) for butterfly larvae in the more vulnerable premyrmecophytic phase. In addition to growing in a safer place due to the ants’ negative impact on natural enemies, caterpillars have an increased chance of being discovered by prospective tending ants if treehoppers are nearby. Thus the spatiotemporal co-occurrence between *P. polibetes* and ant-tended *G. xiphias* treehoppers previously reported by Oliveira and Del-Claro (2005) can be explained by both host plant selection by ovipositing females and increased larval survival near hemipteran trophobionts.

Ant-mediated host plant selection in myrmecophilous

butterflies has been suggested for many species, but so far it has only been demonstrated experimentally for a few obligate ant-tended species (see, e.g., Atsatt 1981a; Pierce and Elgar 1985). For facultative myrmecophilous species, there is only one study providing evidence of ant-mediated oviposition (Wagner and Kurina 1997), although the authors were unable to separate the effects of host plant quality and of nearby ant-tended trophobionts in the choice experiments (see also Oliveira and Del-Claro 2005; Collier 2007). Both these factors were controlled in our experiment by using paired branches of the same plant individual. Moreover, because oviposition responses did not differ between experimental procedures (with and without manipulation of ant-tended treehoppers; see fig. 2), we discarded the possibility that butterflies and treehoppers merely preferred branches with the same quality. We have not identified, however, what kind of signal (visual and/or chemical) and which component of the association (ants and/or treehoppers) are most critical in the selection process by egg-laying butterflies. Additional experiments using dried insect specimens should help clarify these issues (see Sendoya et al. 2009).

Host plant selection by phytophagous insects is carried out by the adult female and is often linked to components of immature performance (Price et al. 1980; Thompson and Pellmyr 1991). From this point of view, our results

Table 1: Repeated-measures ANOVAs performed on the number of natural enemies (spiders and parasitoid wasps) of *Parrhasius polibetes* larvae through time, per experimental plant hosting (occupied) or not hosting (unoccupied) an ant-treehopper association

Source	SS	df	MS	F	P
Experiment I (premyrmecophylic phase):					
Spiders:					
Ant-treehopper treatment	15.58	1	15.58	13.34	<.005
Plant	18.13	24	.75	.65	.854
Error 1	28.03	24	1.17		
Time	1.88	5	.38	2.89	.050
Interaction time × treatment	1.51	5	.22	1.72	.131
Error 2	92.09	240	.13		
Parasitoid wasps:					
Ant-treehopper treatment	.29	1	.29	7.54	<.005
Plant	1.13	24	.05	1.21	.854
Error 1	.94	24	.04		
Time	.31	5	.06	1.70	.134
Interaction time × treatment	.10	5	.02	.53	.750
Error 2	8.79	240	.04		
Experiment I (whole experiment):					
Spiders:					
Ant-treehopper treatment	9.43	1	9.43	15.58	<.001
Plant	14.29	24	.60	.98	.516
Error 1	14.53	24	.60		
Time	5.42	13	.48	11.45	<.001
Interaction time × treatment	.34	13	.03	.71	.756
Error 2	22.74	624	.04		
Parasitoid wasps: ^a					
Ant-treehopper treatment	.20	1	.20	6.41	<.050
Plant	.86	24	.04	1.15	.36
Error 1	.74	24	.04		
Time	.34	8	.48	1.24	.276
Interaction time × treatment	.24	8	.03	.86	.546
Error 2	13.11	384	.04		
Experiment II:					
Spiders:					
Ant-treehopper treatment	69.83	1	69.83	56.58	<.001
Plant	52.76	24	2.20	1.78	.822
Error 1	29.62	24	1.23		
Time	1.02	8	.13	.92	.500
Interaction time × treatment	1.59	8	.20	1.43	.183
Error 2	53.31	384	.14		
Parasitoid wasps: ^b					
Ant-treehopper treatment	.82	1	.82	4.10	.054
Plant	4.81	24	.02	1.00	.500
Error 1	4.81	24	.02		
Time	.29	8	.04	1.50	.154
Interaction time × treatment	.29	8	.04	1.50	.154
Error 2	9.16	384	.02		

Note: For experiment I, a separate analysis was performed for the premyrmecophylic larval phase. Calculations were performed on square-root-transformed data. Significant *P* values are in bold. See also figure 4.

^a Days in which parasitoid wasps were not recorded on either experimental branch were excluded from the analysis (see fig. 4D).

^b Because of the complete absence of parasitoid wasps on branches occupied by ant-treehopper associations (see fig. 4F), an appropriate statistical treatment with ANOVA was not possible. However, when considering the number of branches in each category with at least one wasp recorded during the whole experiment, a significant negative effect of ant-tended treehoppers on wasp occurrence is detected ($G = 12.62$, $df = 1$, $P < .001$).

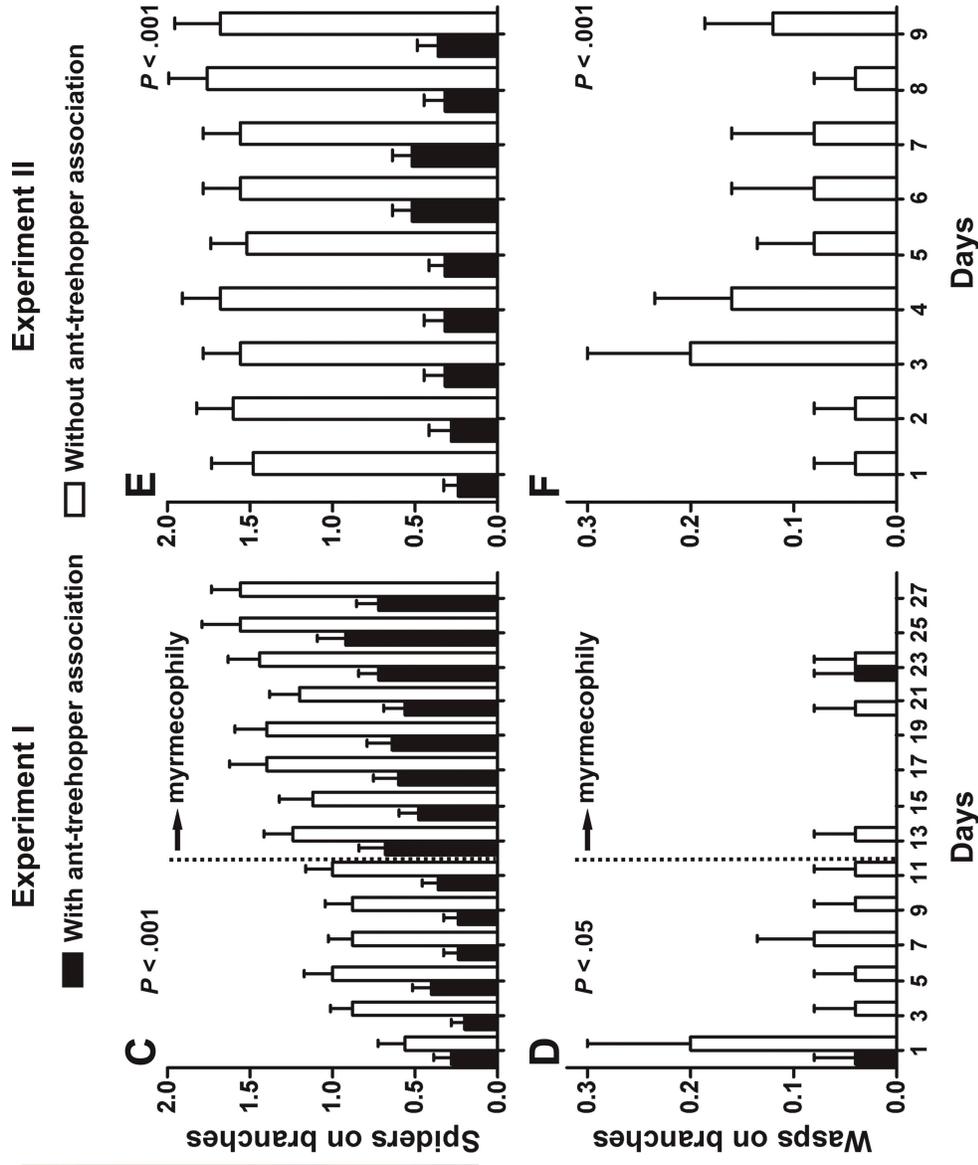


Figure 4: A, B, Natural enemies of *Parrhasius polibetes* butterflies. A, Remains of a caterpillar preyed on by an araneid spider. B, Wasp (*Conura* sp.; Chalcididae) parasitizing a caterpillar. C–F, Number of natural enemies (spiders and parasitoid wasps) of *P. polibetes* larvae on experimental branches of *Schefflera vinosa* through time, as a function of the presence or absence of ant-treehopper associations. C, D, Experiment I included the premyrmecophytic early larval phase (~12 days) as well as the third and fourth myrmecophytic instars (dashed lines indicate when myrmecophily begins); occasional scout ants were excluded from unoccupied branches. E, F, Experiment II included only the myrmecophytic larval instars (third and fourth), and ants had free access to either branch category. Values are means \pm SE. See also table 1.

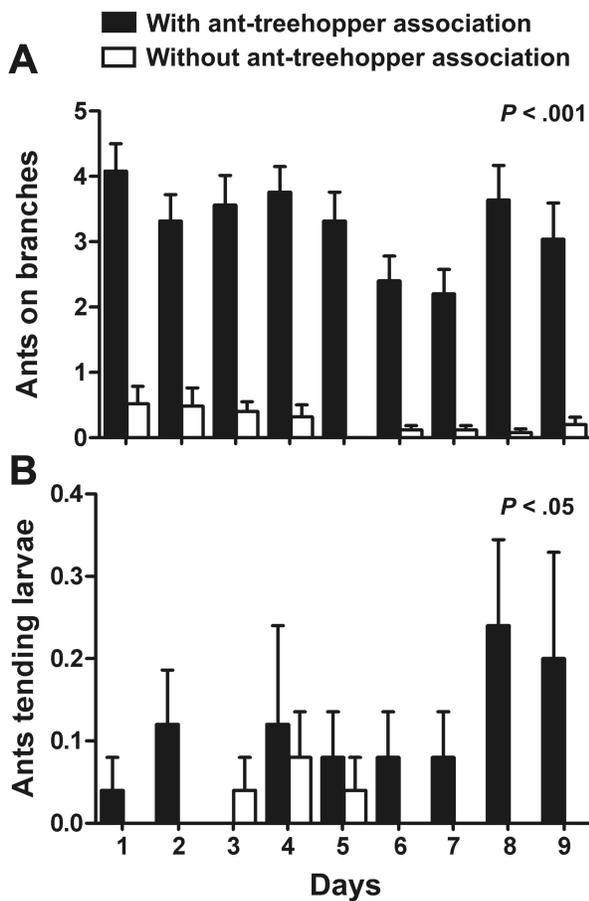


Figure 5: Ant foraging pattern on the host plant *Schefflera vinosa*. *A*, Number of ant foragers walking on experimental branches. *B*, Number of ants tending *Parrhasius polibetes* larvae (third and fourth instars) through time, as a function of the presence or absence of ant-treehopper associations. Values are means + SE.

for host plant selection can be explained by improved larval survival on plants offering enemy-free space. The positive effect by tending ants on larval survival through the provision of an enemy-free space on foliage has already been demonstrated for obligate myrmecophilous species (see, e.g., Pierce et al. 1987). For facultative ant-tended species such as *P. polibetes*, however, there is no consensus on the existence of such benefits (see Pierce and Easteal 1986; DeVries 1991; Peterson 1993; Wagner and Kurina 1997; Weeks 2003). The difficulty in detecting benefits in facultative ant-tended butterfly larvae is probably related to the usual conditionality of facultative mutualisms, since cost-benefit relationships vary over time and space by a number of factors (Bronstein and Barbosa 2002). However, since the association between *G. xiphias* treehoppers and tending ants is relatively stable in cerrado savanna (Del-Claro and Oliveira 1993, 1999), it should provide a fa-

vorable environment to maintain the benefits to a nearby ant-tended trophobiont. Indeed, Atsatt (1981b) has suggested that host plant traits such as the presence of honeydew-producing treehoppers may increase ant abundance and predictability and thus enhance the co-occurrence of ants with other insect trophobionts (such as lepidopteran larvae), which may promote myrmecophily.

The main benefit afforded by tending ants to myrmecophilous butterfly larvae is protection against natural enemies, including insect parasitoids, predatory wasps, and spiders (Pierce and Mead 1981; Pierce et al. 1987; DeVries 1991). Our results are meaningful because they show that protection to *P. polibetes* larvae from parasitoid wasps and spiders can also be indirectly provided by nearby ant-treehopper associations in the premyrmecophylic phase and persist in the late ant-tended instars. Because Tanglefoot resin also decreased the abundance of walking predators (see Dempster 1967), it is likely that protective effects from ants and differential larval survival were underestimated by our design (fig. 3A).

As suggested for other ant-hemipteran associations (see Styrsky and Eubanks 2007), the multitrophic system involving honeydew-producing *G. xiphias* on *Schefflera vinosa* shrubs should be seen as a “keystone interaction” and can be depicted under the perspective of a nontrophic, indirect interaction web (fig. 6; see also Ohgushi 2005, 2007). Ants not only benefit honeydew-producing treehoppers by reducing the abundance of their natural enemies on *S. vinosa* host plants but also deter nontrophobiont herbivores. Thus, the direct negative effect of sap-feeding treehoppers on the plant is counterbalanced by the indirect positive effect of herbivore deterrence by tending ants (fig. 6; Oliveira and Del-Claro 2005). The bud-destroying lycaenid *P. polibetes*, on the other hand, uses ant-tended treehoppers as a cue for host plant selection and improves larval survival by exploiting the ant-generated enemy-free space in their vicinity. Thus, ant-tended *P. polibetes* can ultimately be considered opportunistic exploiters of other ant-based mutualisms occurring on foliage. Previous data show that presence of a nearby liquid food source has no effect on ant attendance levels to *G. xiphias* treehoppers (Del-Claro and Oliveira 1993), suggesting that competition for ant mutualists may not be critical in the study system. Whether the arrival of butterfly larvae on the plant has any consequence (positive or negative) for resident treehoppers awaits further investigation.

It seems clear that the traditional pairwise approach commonly used in studies of ant-based mutualisms would not have allowed us to properly assess some of the selective pressures operating within our study system. Indeed, research on ant-plant-herbivore interactions in cerrado savanna shows that the frequent occurrence of plant and

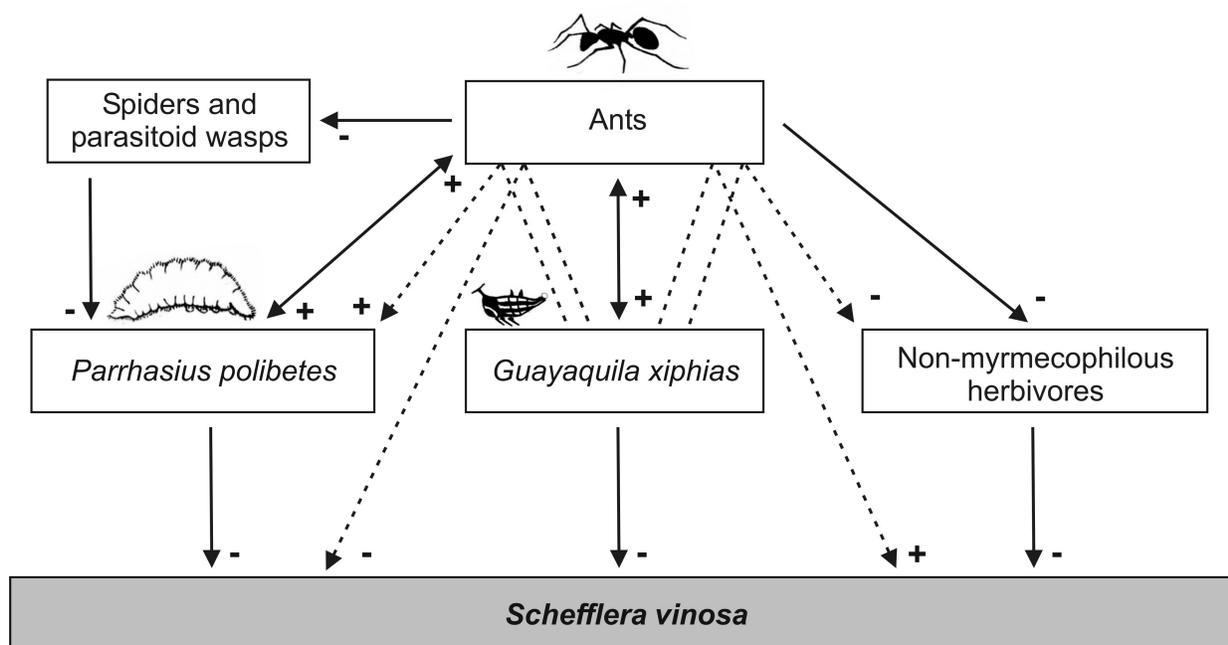


Figure 6: Indirect interaction web of the study system involving foliage-dwelling ants, herbivorous insects, and natural enemies on the host plant *Schefflera vinosa*. Solid and broken lines show direct and indirect effects, respectively. Plus and minus signs indicate positive and negative effects from an initiator to a receiver species, respectively. Depicted relationships are based on field experiments by Del-Claro and Oliveira (2000), Oliveira and Del-Claro (2005), and this study.

insect exudates on vegetation effectively promotes ant activity on foliage, which in turn produces a range of direct and indirect effects (positive and negative) among participant species from multiple trophic levels (Oliveira and Freitas 2004; Kaminski 2008; Sendoya et al. 2009; Silveira et al. 2010).

In conclusion, this study points out the importance of considering the multitude of interactions occurring on foliage for a proper understanding of the origin and maintenance of symbiotic associations between butterflies and ants. Although previously ignored, detection of other ant-based mutualisms on foliage to the benefit of larval offspring may have represented an important evolutionary step in the process of host plant selection in facultative myrmecophilous butterflies.

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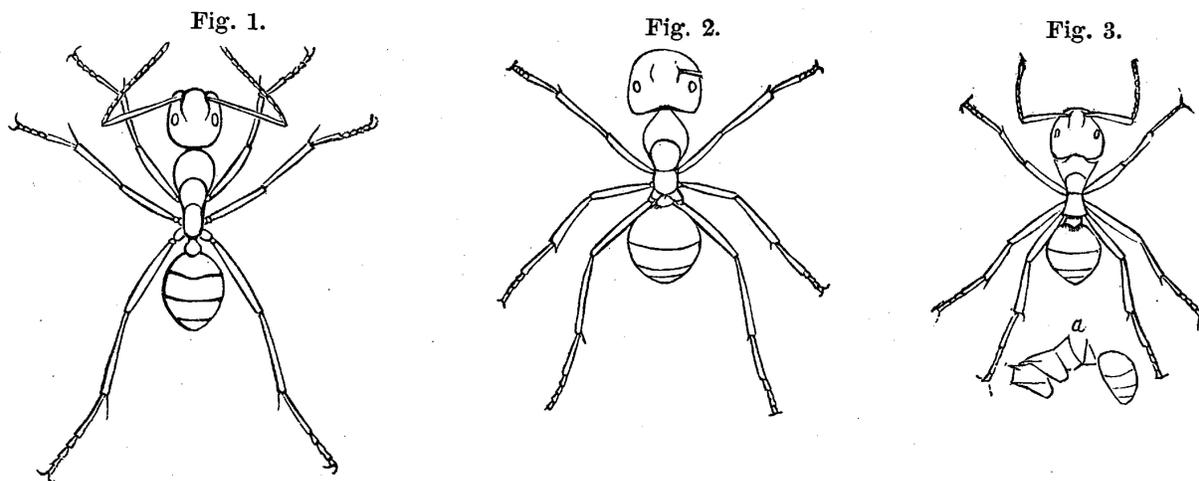


Fig. 1, *Formica fulvacea*, worker major, taken in Cordova. Fig. 2, *Tapinoma tomentosum*, worker (imperfect antennae), lives in little societies under stones. Fig. 3, *Polyrhachis arboricoa*, worker (with side view of thorax and abdomen), “It is quite vagabond in its habits, and one sees it running around on the trunks of all sorts of trees.” From “Notes on Mexican Ants” by Edward Norton (*American Naturalist*, 1868, 2:57–72).