

Ant–caterpillar antagonism at the community level: interhabitat variation of tritrophic interactions in a neotropical savanna

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Summary

1. Ant foraging on foliage can substantially affect how phytophagous insects use host plants and represents a high predation risk for caterpillars, which are important folivores. Ant–plant–herbivore interactions are especially pervasive in cerrado savanna due to continuous ant visitation to liquid food sources on foliage (extrafloral nectaries, insect honeydew). While searching for liquid rewards on plants, aggressive ants frequently attack or kill insect herbivores, decreasing their numbers.

2. Because ants vary in diet and aggressiveness, their effect on herbivores also varies. Additionally, the differential occurrence of ant attractants (plant and insect exudates) on foliage produces variable levels of ant foraging within local floras and among localities. Here, we investigate how variation of ant communities and of traits among host plant species (presence or absence of ant attractants) can change the effect of carnivores (predatory ants) on herbivore communities (caterpillars) in a cerrado savanna landscape.

3. We sampled caterpillars and foliage-foraging ants in four cerrado localities (70–460 km apart). We found that: (i) caterpillar infestation was negatively related with ant visitation to plants; (ii) this relationship depended on local ant abundance and species composition, and on local preference by ants for plants with liquid attractants; (iii) this was not related to local plant richness or plant size; (iv) the relationship between the presence of ant attractants and caterpillar abundance varied among sites from negative to neutral; and (v) caterpillars feeding on plants with ant attractants are more resistant to ant predation than those feeding on plants lacking attractants.

4. Liquid food on foliage mediates host plant quality for lepidopterans by promoting generalized ant–caterpillar antagonism. Our study in cerrado shows that the negative effects of generalist predatory ants on herbivores are detectable at a community level, affecting patterns of abundance and host plant use by lepidopterans.

5. The magnitude of ant-induced effects on caterpillar occurrence across the cerrado landscape may depend on how ants use plants locally and how they respond to liquid food on plants at different habitats. This study enhances the relevance of plant–ant and ant–herbivore interactions in cerrado and highlights the importance of a tritrophic perspective in this ant-rich environment.

Key-words: ant predation, ant–plant interaction, cerrado vegetation, extrafloral nectaries, herbivores, indirect effects, insect honeydew, Lepidoptera, trait-mediated interaction

Introduction

Many ant species in tropical ecosystems forage on foliage, and as a group ants comprise the majority of the

arthropods living in the canopy of tropical forests (Tobin 1995). Among other things, the dominance of ants in the canopy environment can be attributed to the existence of abundant predictable and renewable liquid food sources that are important to ant survival (Davidson *et al.* 2003). In addition to providing a foraging substrate, plants also provide nest sites for many ant species, which may use

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hollow stems left by boring insects or gall makers (Schoederer *et al.* 2010). As they search for sugary secretions on foliage, ant foragers attack or kill insect herbivores, frequently decreasing their numbers and associated levels of herbivory, which in turn may be translated into a fitness benefit to the plant (reviewed by Rico-Gray & Oliveira 2007).

At a local scale, ant presence on plants may vary in time and space due to the heterogeneous distribution of resources on foliage (e.g. variation in quantity and/or quality of extrafloral nectar (EFN); see Heil *et al.* 2000; Díaz-Castelazo *et al.* 2004; Rudgers 2004). At larger spatial scales, ant predation rates on leaf-feeding insects may be determined by the composition of the ant fauna at a given site or region, which in turn may depend on historic or biogeographic factors (Jeanne 1979). Foliage-foraging ants differ in their dietary requirements: whereas most of them depend on plant-derived food to fuel their foraging activities, some species are prodigious predators (Blüthgen, Gebauer & Fiedler 2003; Davidson *et al.* 2003). Dominant, exudate-feeding aggressive ants tend to be effective plant protectors by acting as herbivore deterrents (Mody & Linsenmair 2004; Sendoya, Freitas & Oliveira 2009), whereas opportunistic or timid ants may not provide benefits to the plant in terms of reduced herbivory or increased fitness (Tillberg 2004; Byk & Del-Claro 2010; but see Letourneau 1983). Thus, variation in attributes of the ant fauna (ant identity and abundance) may determine how ant presence on foliage will affect herbivore infestation on plants, which in turn will mediate potential ant-derived fitness benefits to plants (see Heil & McKey 2003; Rico-Gray & Oliveira 2007).

Ant–plant–herbivore interactions are especially pervasive in the cerrado savanna of Brazil due to continuous ant visitation to plants (Oliveira & Freitas 2004). The main food sources on cerrado foliage are EFNs which can be present in up to 25% of the woody plant species (Oliveira & Oliveira-Filho 1991), as well as exudates from sap-sucking hemipterans and myrmecophilous butterfly larvae (Del-Claro & Oliveira 1999; Kaminski, Freitas & Oliveira 2010). Ant visitation to these liquid food sources negatively affects associated herbivores. For instance, decreased herbivore infestation to *Caryocar brasiliense* (Caryocaraceae) may be caused by direct aggression by EFN-visiting ants to immature and adult herbivores (Oliveira 1997) or by visual avoidance of aggressive ants by ovipositing females (Sendoya, Freitas & Oliveira 2009).

Although lepidopterans are important herbivores in cerrado, the biology of most species is poorly known, especially the immature stages (Diniz & Morais 1997). Because vulnerability to natural enemies during feeding can be high, most morphological and behavioural characteristics of lepidopteran larvae are directed towards increasing feeding efficiency and at the same time reducing risks of predation and parasitism (Price *et al.* 1980; Bernays 1998). Foliage-foraging ants are one of the main guilds of predators of lepidopteran larvae, affecting their

survival and density on host plants (e.g. Floren, Biun & Linsenmair 2002). Hence, the continuous presence of ants on plants may represent a mortality risk factor that affects caterpillar development, as well as caterpillar communities, especially in ant-rich environments such as the cerrado savanna (Freitas & Oliveira 1992; Sendoya, Freitas & Oliveira 2009).

Price *et al.* (1980) emphasized the importance of tritrophic interactions for evolutionary ecology by stressing that variation in plant traits can influence top-down control of herbivore populations by predators and parasites, plant bottom-up effects on herbivore and their natural enemies, as well as selective pressure on traits of interacting species (see also Lill & Marquis 2001). However, most studies investigating the dynamics of tritrophic systems, including ant–plant–herbivore interactions, have focused on a single plant species and its few associated herbivores and predators (see Tscharrntke & Hawkins 2002; Letourneau, Dyer & Vega 2004; and included references). For instance, variation in EFN traits (volume, composition and volatiles), a key attribute promoting ant activity on cotton, has been shown to affect ant–herbivore interactions and herbivore infestation (Rudgers 2004). Studies on tritrophic interactions based on focal species usually evaluate indirect effects of ants on herbivores mediated by numerical (e.g. densities) or functional (e.g. behaviour) responses of ants to resources on plants (Heil & McKey 2003; Styrsky & Eubanks 2007). However, extrapolation to the web of species interactions at a larger community scale is uncertain. A recent study by Singer *et al.* (2012) has shown that variation in food quality among temperate forest tree species can alter avian suppression of an assemblage of dietary generalist caterpillars, which enhances the interrelatedness between plant–herbivore and herbivore–carnivore interactions, and thus the relevance of a tritrophic perspective at the community level. The degree to which such an effect on caterpillar communities can be detected in a tropical environment, having ants as carnivores, is unknown.

In this study, we investigate how variation of ant communities and of traits among host plant species can change the effect of carnivores (predatory ants) on herbivore communities (caterpillars) in a cerrado savanna landscape. We evaluate the importance of ant visitation to plants as determinants of caterpillar communities, specifically in relation with the presence and abundance of this herbivore guild on cerrado foliage. We expect ant occupancy on plants (and their predatory/aggressive behaviour while foraging) to reduce presence of lepidopteran larvae (Oliveira 1997; Sendoya, Freitas & Oliveira 2009). However, considering that ant visitation levels and ant community composition vary across space (as well as other aspects of ant–plant interactions), we also expect that ant deterring effects on caterpillars would vary spatially from negative to neutral (see Bronstein 1994, 1998). Consequently, benefits of ant visitation to plants offering food resources (EFNs, insect honeydew) should also vary in

space. Specifically, we addressed the following questions: (i) Does ant visitation to cerrado foliage mediate infestation by lepidopteran larvae on plants? (ii) Is there spatial variation of this effect among plant individuals (fine scale) and/or among localities (large scale)? (iii) If so, will local and geographic variation in ant-induced effects on caterpillar assemblages be mediated by the presence of ant attractants (EFNs or honeydew-producing hemipterans) on plants? (iv) Do common, foliage-foraging ants attack caterpillars on their host plants? (v) If so, are caterpillars feeding on host plants with ant attractants more resistant to attacks than those feeding on plants without liquid rewards?

Materials and methods

STUDY SITE

Field work was carried out during the wet season (February–April 2009), when most vegetative plant growth occurs in cerrado, at four areas in southeast Brazil (Fig. S1, Supporting information). In order to avoid effects of seasonal variation on ant and caterpillar communities, all localities were visited during the wet period, with minimum time difference (5–6 days) between samplings at different sites (climate regime is the same at all sites; INMET 2012). The vegetation physiognomy in all sampled sites consisted of a dense cover of trees and shrubs, which corresponds to the cerrado *sensu stricto* (Oliveira-Filho & Ratter 2002). The study was carried out in the following cerrado reserves: (i) Itirapina (22°15'S, 47°47'W), (ii) Mogi-Guaçu (22°15'S, 47°10'W), (iii) Assis (22°35'S, 50°21'55"W) and (iv) Uberlândia (18°59'S, 48°18'W; see Fig. S1, Supporting information).

PLANT, ANT AND LARVAL SAMPLINGS

At every locality, we sampled ants and lepidopteran larvae on vegetation along six transects 2 m wide and ~30 m long. The first transect was located 150 m from the cerrado border, after which the others were sequentially established in parallel towards the interior of the cerrado (100 m from one another). Along each transect, we extended a string line through the vegetation and selected all plants (0.6–3.0 m tall) within 1 m of the string until we completed 50 sampled plants. Plants were carefully inspected for the presence of liquid food rewards for ants – EFNs or honeydew-producing hemipterans (hereafter referred to as 'ant attractants'). Presence of EFNs was confirmed by direct observation of nectar-gathering ants and by comparison with available plant lists (Oliveira & Leitão-Filho 1987; Machado *et al.* 2008).

The foliage of each sampled plant was visually inspected for 10 min, searching for lepidopteran larvae. Plants hosting ant-tended butterfly larvae bearing specialized glands to attract ants were excluded from the analyses (families Lycaenidae and Riodinidae), since their interactions with ants are mutualistic rather than antagonistic (Hölldobler & Wilson 1990). All caterpillars were photographed, manually removed from the plant and taken to the laboratory for rearing. Since only ~45% of the caterpillars were successfully reared into adults, most individuals were sorted to morphospecies, and taxonomic identifications were performed up to the family level by comparing with available lists (e.g. Diniz *et al.* 2013). Because we wanted to directly relate

larval presence with ant visitation on plants, ants were surveyed 24 h after caterpillars had been collected from plants. Field observations indicate that collection of larvae had no effect on ant visitation levels after 24 h. Ants were sampled with pitfall traps adapted to arboreal sampling (modified from Ribas *et al.* 2003). We used two pitfall traps per plant and left them for 24 h: one baited with sardine (protein source) and another with honey (sugar source). Ant species were preserved in alcohol, separated and counted. Because ant aggressiveness is usually enhanced near the nest and attacks towards intruding insects may sometimes represent a defensive response rather than foraging behaviour (Hölldobler & Wilson 1990), plants with stem galleries hosting ant colonies were not included in the surveys.

ANT PRESENCE ON PLANTS: COMPARISON AMONG LOCALITIES

In order to determine if caterpillars were potentially exposed to different ant assemblages, we compared the ant species composition and visitation levels among plants in the four cerrado localities. The ant species composition was estimated quantitatively by counting the total number of plants on which each species was recorded in each transect. Considering the differences in sampling size between transects, the data were transformed to relative proportions according to the total presence of ants in each transect. We compared ant species composition among transects by constructing distance matrices with the Bray–Curtis index (Magurran 2004) and by using Non-metric Multi-Dimensional Scaling analysis (NMDS). These analyses were performed with the *vegan* package for R environment V.2.15.2 (R Development Core Team 2012). To find out if ant assemblages varied among localities, we used a one factor permutational multivariate analysis of variance (permutational MANOVA; Anderson 2001). We performed multiple comparisons among groups, and *P*-values were corrected by Bonferroni's method. Analyses were performed with software PAST V. 2.1 (Hammer, Harper & Ryan 2001).

The total abundance of ants in each site may be affected by the local abundance of plants with liquid food rewards. To account for this effect, we performed an ANCOVA to investigate how mean ant abundance per transect was affected by the proportion of plants with food rewards and to evaluate its interaction with locality. Ant visitation levels at the scale of individual plants were measured as the total number of ants observed per plant. To determine if ant visitation levels varied among localities and between plants with or without ant attractants, we constructed a generalized linear mixed model (GLMMs). This analysis allowed us to account for possible spatial dependence among plants in the same transect. In this model, sampling transect (nested within locality) was considered as a random variable which grouped the sampling units (plants). Sampling locality and presence of ant attractants on plants were used as fixed variables and the number of collected ants per plant as the response variable. Given that the response variable was a count, and to avoid overdispersion, we fitted models with a negative binomial distribution correcting the standard errors with the dispersion parameter. We used a Laplace approximation to estimate model parameters. The effects of the fixed variables and their interactions were evaluated by comparing concurrent models (models constructed by sequentially deleting the effect of interest) using likelihood ratio tests. This process was repeated until the minimal adequate model was obtained (see Bolker *et al.* 2009; Zuur *et al.*

2009). The model was constructed with the `glmmADMB` package for R environment V.2.15.2 2 (R R Development Core Team 2012).

ANALYSES OF CATERPILLAR OCCURRENCE ON PLANTS

To investigate if the proportion of plants with liquid food rewards and the mean abundance of ants per transect affect the local abundance of caterpillars (total number per transect), we performed an ANOVA including the interaction of both predictor variables with the sampling locality.

At the scale of individual plants, larval presence was treated as a binary variable, meaning that occurrence of several caterpillars of the same species on a plant was considered as a single presence of that species (i.e. the number of eggs on a plant may result from the reproductive strategy of the lepidopteran; see Thompson & Pellmyr 1991). We employed GLMMs (using a binomial distribution) to investigate which factors associated with ant visitation and plant traits could be affecting larval presence. Similarly to the ant visitation model previously described, the sampling transect nested in locality was considered as a random variable. To test if ant visitation could affect larval presence on a plant and if this effect varies spatially, the number of ant individuals and species per plant, locality (and their interaction) and the presence of ant attractants (and its interaction with locality) were included as fixed factors. Finally, to investigate if differences in plant size could affect the probability of finding a larva, we also included plant height as a fixed variable. Due to the high number of factors (five variables and five interactions), the model was refined using a simplification procedure as done with the ant visitation model. The models and model selection were performed with the `lme4`-package for R environment V.2.15.2 (R R Development Core Team 2012).

To investigate if the local composition of ant species was affecting the presence of caterpillars on plants in each locality, we used permutational MANOVAS, following the same procedure used to compare localities. In this case, we compared the species composition of ant assemblages on plants with or without caterpillars. Separated analyses were performed independently for each locality.

EXPERIMENTS ON ANT–CATERPILLAR INTERACTIONS

These experiments aimed to test the effectiveness of ants as predators of cerrado caterpillars, and the hypothesis that caterpillar species associated with plants possessing ant attractants show increased resistance to ant predation. Considering that caterpillars feeding on plants highly visited by ants would have to deal continuously with the threat of ant predation, we expected that caterpillars recorded on plants with ant attractants would be more resistant to ant attacks than those feeding on species without rewards. To test the potential of ants as predators of caterpillars living on plants with or without ant attractants, we collected 56 caterpillars (24 morphospecies) in the field to carry out laboratory experiments of ant–caterpillar behavioural interactions. Trials were made with each caterpillar species on its own host plant species, some of which bearing EFNs and others without such glands. Trials were performed with *Camponotus crassus* and *Camponotus renggeri*, two of the most frequent arboreal ant species in our surveys and in previous work in cerrado as well (Oliveira & Brandão 1991; Del-Claro & Oliveira 1999; Schoederer

et al. 2010). Interaction experiments were carried out using three captive colonies of each ant species. Ants were conditioned to search for food in a foraging arena containing artificial diet (Hölldobler & Wilson 1990). Ant–caterpillar encounters took place on a potted branch of each host plant placed in the arena, and containing one live resident caterpillar. Baits of sugar solution were added to foliage to promote ant visitation (and ant–caterpillar encounters) on potted branches of plant species in either group (i.e. bearing or not EFNs). All trials were thus performed on plants bearing an artificial ant attractant (given that EFNs of potted plants were non-functional). This allowed us to evaluate comparatively the vulnerability to ants by caterpillars naturally occurring on host plant species with or without EFNs. Ant attacks on caterpillars were recorded for 10 min after they encountered each other. We considered an attack when a worker bit any part of the caterpillar body, after which the trial was terminated. Interaction experiments were carried out using only caterpillar species seen in the field walking/feeding on open leaves (i.e. free-living caterpillars). We did not use shelter-building caterpillars in interaction experiments because they are well-protected against ant attacks inside their retreats (S.F. Sendoya & P.S. Oliveira, unpublished data).

ANT VISITATION TO HOST PLANTS AND COLONIZATION PATTERNS BY LEPIDOPTERANS

Ant presence on cerrado foliage can affect not only caterpillar survival (Freitas & Oliveira 1992, 1996), but host plant choice by ovipositing females as well (Sendoya, Freitas & Oliveira 2009). Thus, to investigate potential effects from foliage-foraging ants on colonization decisions by females at a short spatial scale, it is useful to consider ant visitation levels on all nearby plants the herbivore may actually use as a host. We then compared the level of ant visitation on the infested host plant (i.e. where the larva was actually found) with the level of visitation on the nearest uninfested host plant (i.e. of the same species where that caterpillar was found in the same transect). We used the same approach to investigate ant visitation levels by each ant subfamily. Comparisons were performed with a Wilcoxon paired test using the corresponding function in R environment V.2.15.2 2 (R R Development Core Team 2012).

Results

ANT PRESENCE ON PLANTS

The composition of the ant community visiting plants varied among the four cerrado localities (Fig. S2, Supporting information). Ant species composition in transects in the same locality were more similar than among localities (PERMANOVA test: $F_{(3)} = 4.63$, $P = 0.0001$). Multiple comparisons between localities indicated that there were no pairs of localities with the same ant species composition (Bonferroni-corrected $P < 0.03$). Mean ant abundance per transect was affected by the interaction between sampling locality and local proportion of plants with liquid food rewards ($F_{(3)} = 3.34$, $P = 0.0497$). The GLMM for ant numbers on plants indicated that locality was the most important predictor variable (Table 1). Plants were more visited by ants in Mogi-Guaçu and

Assis than in Uberlândia and Itirapina (Fig. 1a; Table 1; see also Table S1, Supporting information). The model also indicated an important effect of the interaction

Table 1. Results of the selected generalized mixed model (GLMM) investigating the effect of sampling locality, and presence of ant attractants on foliage, on ant visitation levels to plants in four cerrado localities. The intercept of the model is the abundance of ants at the locality Assis. The table shows the estimated coefficients for the linear model for each variable or category (\pm standard error) and the results of likelihood ratio test.

Random factor		Variance		
Transect		0.255		
Fixed factor (factor level)	Estimate	Standard error	χ^2	<i>P</i>
Intercept	3.1109	0.2272	–	–
Locality	–	–	33.66	<0.0001*
Itirapina	–0.7986	0.3204	–	–
Mogi-Guaçu	0.1839	0.3196	–	–
Uberlândia	–1.6568	0.3221	–	–
Presence of ant attractants	0.0129	0.1721	40.60	<0.0001*
Locality \times Ant attractants	–	–	76.01	<0.0001*
Itirapina	0.8103	0.2599	–	–
Mogi-Guaçu	–0.2049	0.2744	–	–
Uberlândia	0.7574	0.2548	–	–

*Significant effect of factor $P < 0.05$.

between presence of ant attractants (plant and insect exudates) on individual plants and sampling locality. Extrafloral nectaries and honeydew-producing hemipterans had a positive effect on ant visitation to individual plants in Uberlândia and Itirapina, but not in Mogi-Guaçu and Assis (Table S2, Supporting information; Fig. 1a).

CATERPILLAR OCCURRENCE ON PLANTS

Both the number of lepidopteran larvae and the proportion of infested plants varied among sampling localities, with more caterpillars found in Uberlândia and Itirapina than in Assis and Mogi-Guaçu (Fig. 1b; Table S3, Supporting information). Neither the abundance nor the richness of caterpillars per transect were associated to plant richness (Spearman correlation $\rho < -0.3$, $P > 0.05$, $r^2 < 0.12$), which indicates that this general property of plant communities is not determining larval presence at a community level. The total abundance of caterpillars varied with sampling locality ($F_{(3)} = 4.50$, $P = 0.025$), and with the locality \times mean ant abundance interaction ($F_{(3)} = 3.94$, $P = 0.035$). However, abundance of caterpillars per transect was not affected by the proportion of plants with liquid food rewards ($F_{(1)} = 1.70$, $P = 0.216$) or by its interaction with locality ($F_{(3)} = 0.36$, $P = 0.778$; see also Tables S4 and S5, Supporting information).

The final (selected) model retained sampling locality (Tables 2 and S3, Supporting information), which confirms that some localities (Itirapina and Uberlândia) had

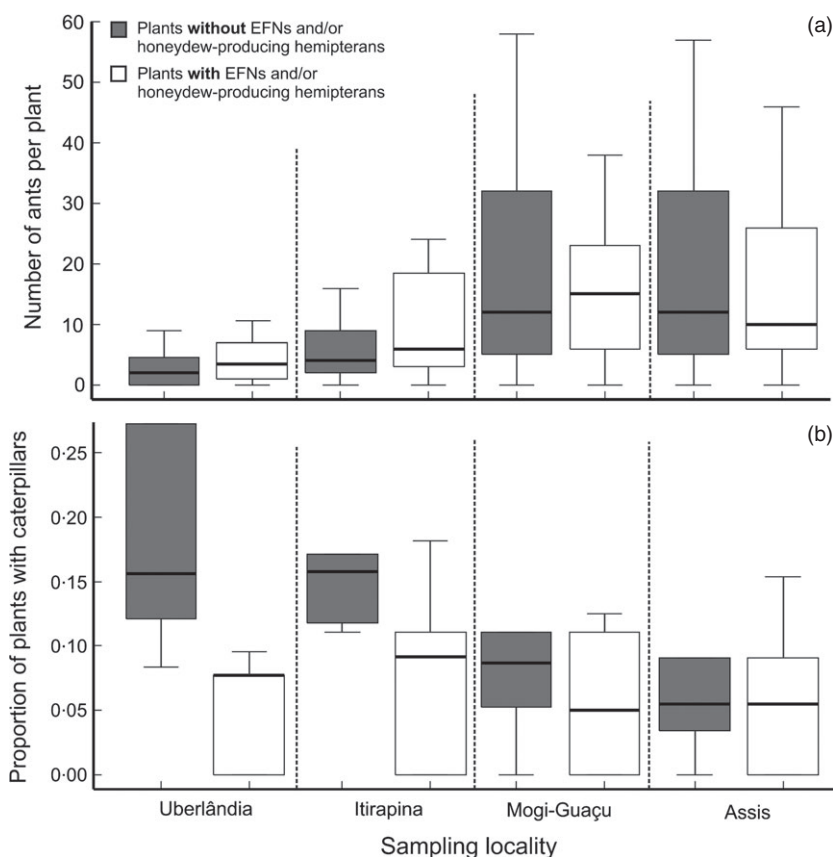


Fig. 1. Ant visitation patterns and presence of caterpillars on cerrado plants. Number of ants per plant (a) and proportion of plants with caterpillars per transect (b) in each cerrado locality, for plants with or without ant attractants (extrafloral nectaries or honeydew-producing hemipterans). Black horizontal lines represent the median, boxes designate the second and third quartiles, and vertical bars indicate the range of data without outliers. See also Tables 1 and 2.

significantly more caterpillars than others (Mogi-Guaçu and Assis). The model also retained a negative effect of ant visitation, as well as the interaction between locality and ant visitation (Table 2). The fitted values calculated from the model predicted a decreasing probability of having caterpillars on a plant as the number of visiting ants increased at Itirapina and Uberlândia, but this effect was weak at Assis and Mogi-Guaçu (Fig. 2). Additionally, the final model included the presence of ant attractants (Table 2); indicating that plant and insect exudates are negatively affecting larval presence on plants (Fig. 2). The ant species composition on plants with caterpillars was different than on plants without caterpillars in two localities (Itirapina: PERMANOVA test: $F_{(1)} = 4.93$, $P = 0.014$; Mogi-Guaçu: PERMANOVA test: $F_{(1)} = 1.92$, $P = 0.002$) and marginally different in one site (Uberlândia: PERMANOVA test: $F_{(1)} = 1.79$, $P = 0.051$). In one locality where the effect of ant abundance on caterpillars was low (Assis), the ant species composition did not differ between plants with or without caterpillars (PERMANOVA test: $F_{(1)} = 1.57$, $P = 0.109$).

ANT AGGRESSIVENESS TOWARDS CATERPILLARS

Free-living caterpillars were frequently attacked by the ants during behavioural trials in captivity on potted host

Table 2. Results of the selected generalized mixed model (GLMM) investigating different factors potentially affecting the presence of caterpillars on cerrado plants. The intercept of the model is the abundance of ants at the locality Assis. The table shows the estimated coefficients for the linear model for each variable or category (\pm standard error) and the results of likelihood ratio test. See also Fig. 3

Random factor		Variance		
Transect		0.130		
Fixed factor (factor level)	Estimate	Standard error	χ^2	P
Intercept	-2.484	0.345	-	-
Locality (L)	-	-	15.797	0.015*
Itirapina	1.185	0.442	-	-
Mogi-Guaçu	0.178	0.522	-	-
Uberlândia	1.241	0.418	-	-
Presence of ant attractants (AT)	-0.650	0.282	6.361	0.012*
Ant visitation (V)	-0.006	0.009	23855	<0.0001*
Locality \times Ant visitation	-	-	9.487	0.023*
Itirapina	-0.087	0.043	-	-
Mogi-Guaçu	-0.018	0.021	-	-
Uberlândia	-0.106	0.046	-	-
Ant species (AS)	-	-	0.119	0.731
Plant height (PH)	-	-	2.649	0.104
AV \times AT	-	-	0.007	0.933
L \times AT	-	-	0.639	0.887
L \times AS	-	-	0.307	0.959
PH \times AV	-	-	0.001	0.999

*Significant effect; $P < 0.05$.

plants baited with sugar solution (67.8% of tested individuals; Fig. 3). Caterpillars recorded in the field on host plants with EFNs were proportionally less attacked by ants once encountered than those feeding on plants without EFNs (G -test = 4.54, d.f. = 1; $P = 0.037$).

ANT VISITATION TO HOST PLANTS AND COLONIZATION BY LEPIDOPTERANS

Abundance of visiting ants varied strongly on plants where we did not find caterpillars, from a few plants without ants to several plants with hundreds of them. Comparisons between infested plants (with caterpillars) with the nearest uninfested host plants (without caterpillars) showed that ant visitation was lower on plants with larvae in all localities (Fig. 4a). Notably, an analysis by ant subfamily indicated that uninfested plants had greater levels of visitation by the Formicinae and Myrmicinae (Fig. 4b).

Discussion

We found a negative relationship between the presence of caterpillars and the level of ant visitation to plants in cerrado savanna. The effect of foliage-foraging ants on caterpillar infestation, however, varied among sites from negative to neutral (Figs 2 and 3). This result suggests a conditional effect of ant visitation to plants on caterpillar occurrence. The negative relationship between ant and larval presence may be also caused by abiotic or biotic factors (e.g. climate, historic factors) limiting lepidopteran presence regardless of ant distribution (or even affecting ants in an opposite way). Indeed, Brown & Gifford (2002) suggested that diversity patterns of cerrado lepidopterans may depend on vegetation physiognomy and climatic factors. Although plant species composition differed slightly among our study sites, all localities had similar vegetation physiognomies (cerrado *sensu stricto*; Oliveira-Filho & Ratter 2002) and climate regimes (INMET 2012), suggesting that abiotic factors do not differ markedly among localities. Moreover, characteristics of plant communities such as local patterns of plant richness and plant size did not predict caterpillar presence. Therefore, the current study indicates that biotic interactions with predators (antagonistic relationships with ants), especially at the scale of individual plants, can indeed account for patterns of caterpillar occurrence and host plant use by lepidopterans on cerrado foliage.

The interaction experiments showed that caterpillars found on highly ant-visited host plants (i.e. bearing ant attractants) seem more resistant to ant attacks than those feeding on plants where ants are occasional visitors only (Fig. 3). Our results also indicated that ant-plant interactions affect local patterns of host plant use by lepidopterans (i.e. which plants they are using in each transect or locality). As a result, caterpillar presence is negatively affected by the presence of plant traits promoting interaction with ants (e.g. EFNs, Fig. 1), and the use of

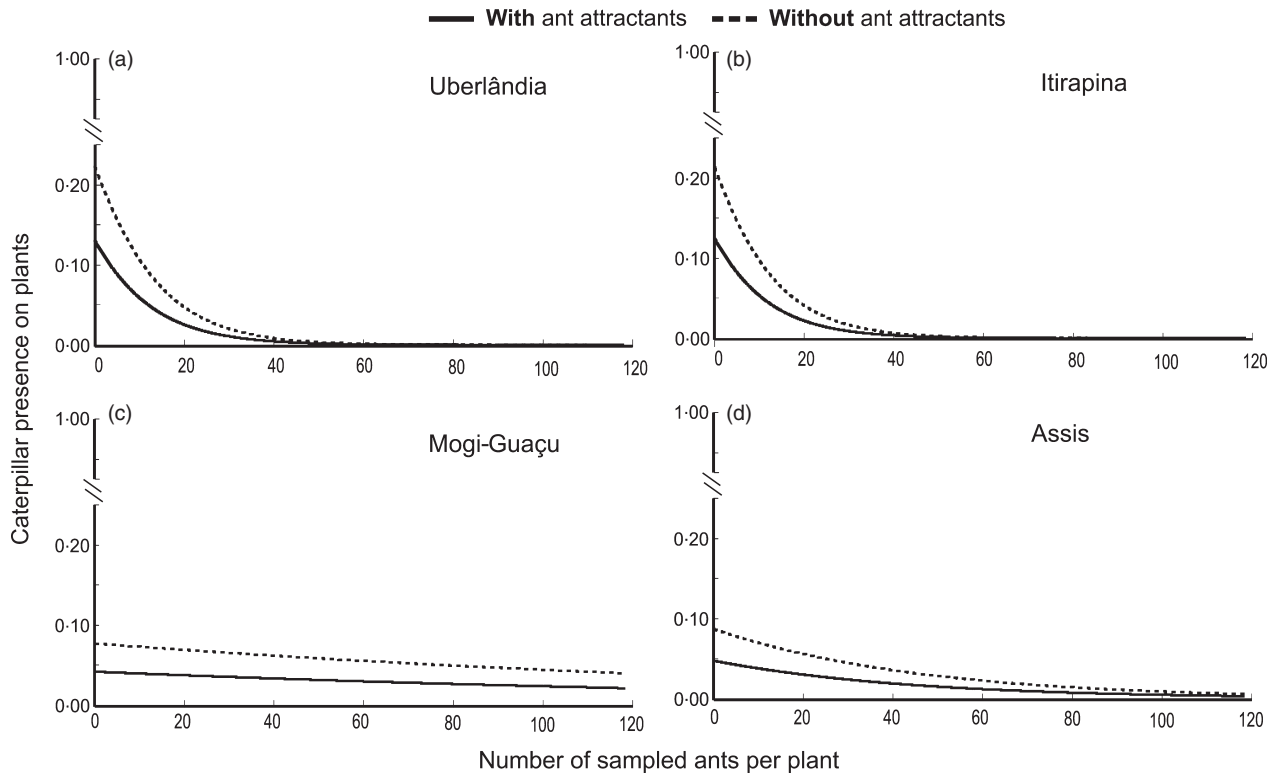


Fig. 2. Predictions of the selected generalized mixed model for larval presence (probability to have a caterpillar on a plant), relative to the number of ants visiting individual plants. Each graph represents the model prediction for each site: Uberlândia (a), Itirapina (b), Mogi-Guaçu (c), Assis (d). Continuous lines designate plants with ant attractants (extrafloral nectaries or honeydew-producing hemipterans), and dashed lines represent plants without ant attractants. See also Table 2.

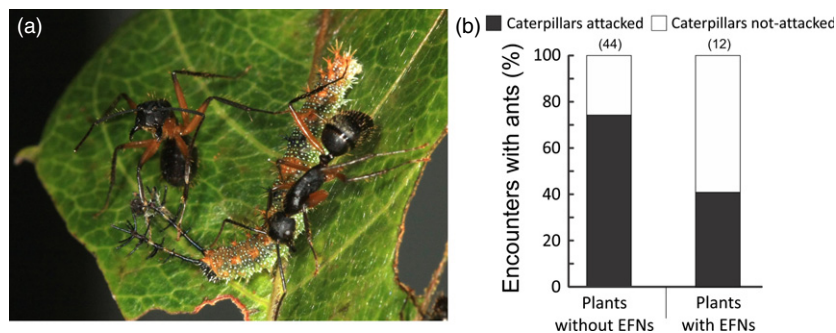


Fig. 3. *Camponotus* ants as potential predators of caterpillars feeding on host plant species with or without extrafloral nectaries in cerrado savanna. Interaction experiments aimed to test the effectiveness of ants as predators of caterpillars, and the hypothesis that caterpillar species associated with plants possessing ant attractants show increased resistance to ant predation. (a) Workers of *C. renggeri* attacking a caterpillar of *Callicore sorana* (Nymphalidae) on *Serjania lethalis* A.St.-Hil (Sapindaceae). (b) Percentage of caterpillars attacked by ants during interaction trials in the laboratory. Trials were performed on potted host plants containing one live caterpillar; baits of sugar solution promoted ant visitation to plant species in either group (extrafloral nectaries of potted plants were non-functional). Ant attacks to caterpillars were recorded for 10 min after they encountered each other. Values above bars indicate number of trials.

individual host plants at any given locality is mediated by the relative abundance of ants on foliage (Fig. 4a).

Two mechanisms may determine the negative effect of ants on caterpillars. First, ants may prey on (or removing) eggs and caterpillars on plants (Letourneau 1983; Freitas & Oliveira 1996; Floren, Biun & Linsenmair 2002). Secondly, predation risk on highly ant-visited plants may cause avoidance response by ovipositing lepidopteran females (Sendoya, Freitas & Oliveira 2009; De-Silva,

Vásquez & Mallet 2011). The relative importance of these mechanisms may vary among caterpillar species depending on their defensive strategies against ant predation.

SPATIAL VARIATION IN THE PRESENCE OF ANTS AND CATERpillARS ON LEAVES

Aggressive arboreal ant species are known to affect host plant use by lepidopterans through increased predation

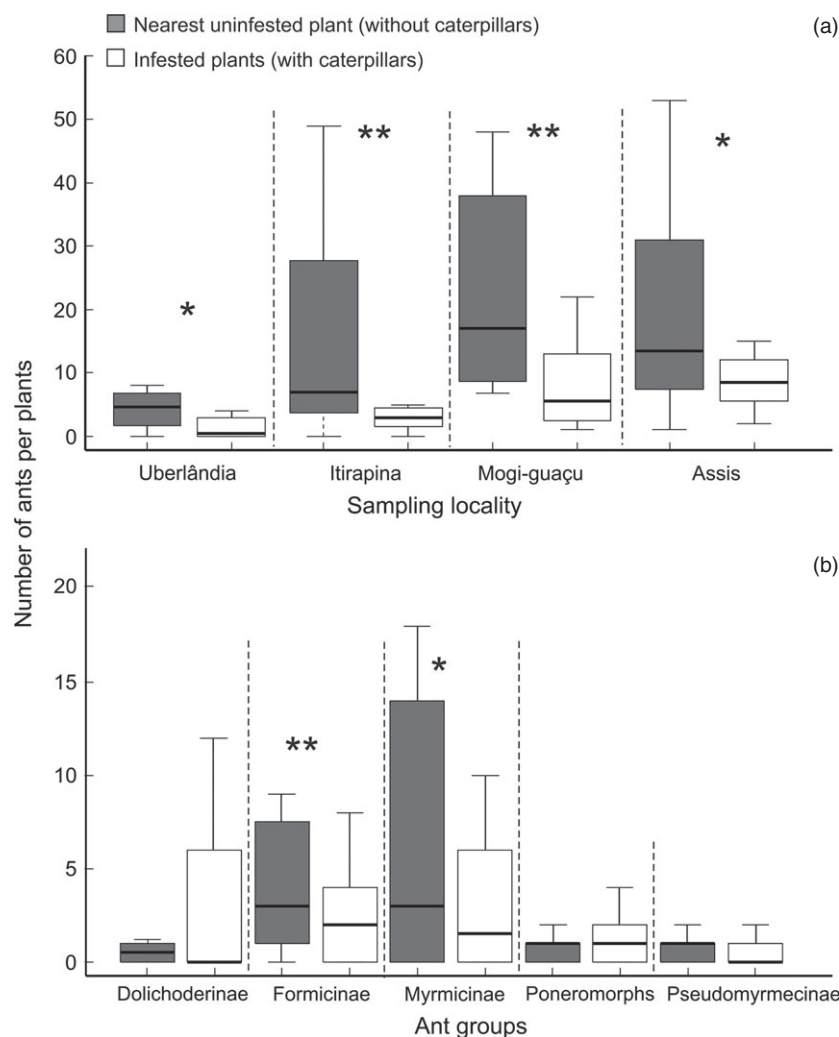


Fig. 4. Comparisons of ant visitation levels between infested plants (with caterpillars) and the nearest uninfested plant of the same species in the same transect (without caterpillars). Plant groups were compared separately for each sampling locality (a) and for each ant subfamily (b). Poneromorphs include subfamilies Ponerinae, Ectatomminae and Heteroponerinae. Black horizontal lines represent the median, boxes designate the second and third quartiles, and vertical bars indicate the range of data without outliers. Asterisks denote significance of Wilcoxon paired test (* $P < 0.05$; ** $P < 0.01$).

pressure on caterpillars (Floren, Biun & Linsenmair 2002; Mody & Linsenmair 2004). We showed that the composition of visiting ant species is relevant to understand how lepidopterans use host plants, since plants with caterpillars were visited by a different set of ants than plants without caterpillars. This effect, however, varies geographically and in one locality (Assis) caterpillars use plants regardless of ant species composition on leaves. Thus, differences in species composition of local ant faunas in cerrado, and the corresponding variation in ant visitation levels to plants, may explain why ant effects on caterpillars were not the same in all cerrado localities of this study. Ants in the subfamilies Formicinae and Myrmicinae seem to account for most of the negative effect detected on larval presence (Fig. 4b). Indeed, formicines include several aggressive species well adapted to arboreal foraging (Davidson *et al.* 2003). In particular, the ant groups which better respond to the presence of liquid food rewards on leaves (e.g. *Camponotus*) are also the ones mostly affecting caterpillar presence on plants (S. F. Sendoya & P.S. Oliveira, unpublished data). The myrmicines comprise a very diverse and heterogeneous ant group, both in ecology and behaviour (Hölldobler & Wil-

son 1990), and includes several highly abundant genera in cerrado vegetation (Oliveira & Brandão 1991; Schoederer *et al.* 2010). Whereas some of them (e.g. *Cephalotes*) may not deter herbivores (Sendoya, Freitas & Oliveira 2009; Byk & Del-Claro 2010), others (e.g. *Pheidole*, *Wasmannia*) are able to recruit large numbers of workers and threaten herbivores on foliage (Koptur 1984; Letourneau & Dyer 2005).

The strong selective pressure exerted by foliage-foraging ants may influence the composition of the herbivore fauna in tropical environments (Floren, Biun & Linsenmair 2002; Mody & Linsenmair 2004). Indeed, lepidopterans present different morphological or/and behavioural defensive larval traits that may allow some species to avoid or resist ant attack and thus exploit highly ant-visited plants, as suggested by our ant–caterpillar experiments (Fig. 3; see also Freitas & Oliveira 1992, 1996; Dyer 1997; Machado & Freitas 2001; Gentry & Dyer 2002; Sendoya, Freitas & Oliveira 2009). Not surprisingly, most caterpillars in the ant-rich cerrado are found inside shelters (72.7%; $n = 128$), where they enjoy safety against ant predation (S.F. Sendoya & P.S. Oliveira, unpublished data). The efficiency of larval defences varies with the identity of vis-

iting ant species, and this in turn affects caterpillar survival and host plant use in the cerrado savanna (S.F. Sendoya & P.S. Oliveira, unpublished data).

CONSIDERATIONS ON MULTITROPHIC EFFECTS

Our study provides a good example of a trait-mediated effect on trophic cascades (Schmitz, Krivan & Ovadia 2004; Mooney, Pratt & Singer 2012): the indirect effects of ant presence on plants depend not only on plant traits attractive to ants (EFNs), but also on behavioural and foraging traits of local ants (i.e. aggressiveness, use of plant-derived resources), as well as on the defensive traits of local caterpillars.

Liquid food sources such as EFNs and honeydew-producing hemipterans have repeatedly been shown to promote ant visitation to plants (Rico-Gray & Oliveira 2007). In this study, the relative abundance of these resources affected the local abundance of ants, although this effect varied with locality. We expected that plants with these ant attractants would exhibit higher levels of ant visitation compared to those lacking exudates, since they represent important foraging areas for cerrado ants (Oliveira & Freitas 2004; Schoederer *et al.* 2010). Although the local abundance of plants with ant attractants was not good predictor of caterpillar abundance, our data suggest that the presence of liquid rewards on individual plants in Itirapina and Uberlândia could increase predation risk for herbivorous insects (Fig. 1; Table 1). Continuous visitation to exudate sources by ants, as well as by other natural enemies such as parasitoids (Pemberton & Lee 1996), could thus reduce herbivore infestation levels. In two cerrado localities (Mogi-Guaçu and Assis), however, we did not find higher levels of ant visitation to plants with ant attractants compared to those without liquid rewards. Because these two areas had the highest levels of ant visitation to plants among the four sampled sites, the presence or absence of liquid rewards on plants apparently had no effect on the generalized high occurrence of ants on foliage (see Fig. 1a; Table 1). Moreover, Mogi-Guaçu and Assis also presented the lowest levels of caterpillar infestation, regardless of the presence of ant attractants on plants (Fig. 1b). Therefore, a differential effect of ant visitation on caterpillar infestation occurs only in places where the local ant community responds positively to the presence of plant and insect exudates on foliage, as shown for the cerrados at Itirapina and Uberlândia.

Based on our results, the outcomes from mutualistic interactions between plants and ants may depend on how ant visitation levels vary spatially in the cerrado landscape: whereas under some circumstances, ant-derived benefits may occur (Itirapina, Uberlândia), in others they may be neutral or reduced (Assis, Mogi-Guaçu). The variable nature of the plant–ant–caterpillar interactions shown here corroborates the predicted ‘geographic mosaic’ of interspecific interactions (Thompson 2005).

Accordingly, in order to understand the evolution of mutualistic interactions between plants and ants, including the traits that promote them (e.g. EFNs, or particular foraging and physiological adaptations to exudate-feeding by ants), it is necessary to consider how they vary geographically and which factors affect the interactions in space (see Bronstein 1994, 1998; Heil & McKey 2003; Rico-Gray & Oliveira 2007; Chamberlain & Holland 2008).

Although experimental studies have used ant-exclusion approaches to show that larval mortality in Lepidoptera increases on ant-visited plants compared to ant-free ones (e.g. Koptur 1984; Smiley 1985; Barton 1986; Dutra, Freitas & Oliveira 2006), few studies have addressed this effect at a community level. Floren, Biun & Linsenmair (2002) demonstrated an inverse relationship between ant presence on foliage and the lepidopteran larval community in a Malaysian tropical rain forest. The present study in cerrado savanna shows that the negative effects of generalist predatory ants on herbivores (caterpillars) are detectable at a community level, affecting patterns of abundance and host plant use by lepidopteran communities. The magnitude of ant-induced effects on caterpillar occurrence is apparently conditioned across the cerrado landscape by how ants use plants locally and how they respond to liquid food resources on plants at different habitats. Which biotic or abiotic factors are shaping this conditional effect is still unclear and awaits further investigation.

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Data accessibility

Data available from the Dryad Digital Repository: <http://doi.org/10.5061/dryad.gh81t> (Sendoya & Oliveira 2014).

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Table S1. Ant species sampled on plants at each cerrado locality.

Table S2. Complementary results of the generalized mixed models investigating potential factors affecting ant visitation levels on plants.

Table S3. Complementary results of the generalized mixed models investigating the factors affecting larval presence on plants.

Table S4. List of sampled plant species bearing extrafloral nectaries in each cerrado locality.

Table S5. List of sampled plant species lacking extrafloral nectaries in each cerrado locality.

Fig. S1. Distribution of study areas in southeastern Brazil, in the states of São Paulo (SP) and Minas Gerais (MG).

Fig. S2. MDS ordination analysis showing differences in ant composition among localities according to Bray–Curtis index distance matrix (Stress = 1.69).