Behavioural ecology of defence in a risky environment: caterpillars *versus* ants in a Neotropical savanna

S E B A S T I Á N F . S E N D O Y A* and P A U L O S . O L I V E I R A   Departamento de Biologia Animal, Universidade Estadual de Campinas, Campinas, Brazil

Abstract. 1. Predatory ants may reduce infestation by herbivorous insects, and slow-moving Lepidopteran larvae are often vulnerable on foliage. We investigate whether caterpillars with morphological or behavioural defences have decreased risk of falling prey to ants, and if defence traits mediate host plant use in ant-rich cerrado savanna.

2. Caterpillars were surveyed in four cerrado localities in southeast Brazil (70–460 km apart). The efficacy of caterpillar defensive traits against predation by two common ant species (*Camponotus crassus*, *C. renggeri*) was assessed through experimental trials using caterpillars of different species and captive ant colonies.

3. Although ant presence can reduce caterpillar infestation, the ants' predatory effects depend on caterpillar defence traits. Shelter construction and morphological defences can prevent ant attacks (primary defence), but once exposed or discovered by ants, caterpillars rely on their size and/or behaviour to survive (secondary defence).

4. Defence efficiency depends on ant identity: *C. renggeri* was more aggressive and lethal to caterpillars than *C. crassus*. Caterpillars without morphological defences or inside open shelters were found on plants with decreased ant numbers. No unsheltered caterpillar was found on plants with extrafloral nectaries (EFNs). Caterpillars using EFN-bearing plants lived in closed shelters or presented morphological defences (hairs, spines), and were less frequently attacked by ants during trials.

5. The efficiency of defences against ants is thus crucial for caterpillar survival and determines host plant use by lepidopterans in cerrado. Our study highlights the effect of EFN-mediated ant-plant interactions on host plant use by insect herbivores, emphasizing the importance of a tritrophic viewpoint in risky environments.

Key words. Ant predation, anti-predator defence, cerrado savanna, host plant use, indirect effects, tritrophic interaction.

Introduction

Top-down effects exerted by natural enemies are among the most important factors controlling populations and communities of insect herbivores (Price *et al.,* 1980; Tscharntke & Hawkins, 2002). Parasitoids and predators account in great part for mortality of insect herbivores, as well as their performance and use of host plants, especially in the tropics (Schoonhoven *et al.,* 2005). The effect of natural enemies is strong enough to produce a tritrophic evolutionary dynamic involving predators, herbivores, and host plants (Rudgers, 2004; Mooney *et al.,* 2012). Moreover, predators have driven the evolution of several herbivore traits (Nosil & Crespi, 2006), and an assessment of their variable capacities to find and subdue prey is important to understand the function and evolution of insect defences (Ruxton *et al.,* 2004).

Lepidoptera is the second most diverse insect order (Price *et al.,* 2011) but the biology of most species in tropical ecosystems is unknown (Diniz & Morais, 1997; Greeney *et al.,* 2012). Most caterpillars feed on foliage and their vulnerability to natural enemies while feeding is high (Bernays, 1997). Pathogens and parasitoids are important agents reducing larval survival, but predation is often the highest source of mortality

Correspondence: Paulo S. Oliveira, Departamento de Biologia Animal, Rua Monteiro Lobato 255, Universidade Estadual de Campinas, C.P. 6109, 13083-862 Campinas, São Paulo, Brazil. E-mail: pso@unicamp.br

*Current address: Programa de Pós-Graduação em Entomologia, Departamento de Zoologia, Av Cel Francisco Santos 100, Universidade Federal do Paraná, C.P. 19020, 81531-980 Curitiba, Brazil.

(Cornell & Hawkins, 1995). Therefore, caterpillars exhibit diverse defensive traits (chemical, morphological or behavioural) which vary in efficiency according with the natural enemy (Gentry & Dyer, 2002; reviewed by Greeney *et al.*, 2012).

Ants and birds are considered the main predators of lepidopteran larvae (Remmel *et al.*, 2011; Singer *et al.*, 2012; Sendoya & Oliveira, 2015), and their relative effects may vary with the type of habitat (Sam *et al.*, 2015). Most interactions between ants and caterpillars are antagonistic. Several studies have shown that ants may both depredate or deter caterpillars from plants (predatory effects), reducing infestation rates on host plants and negatively affecting larval survival during first developmental instars (e.g. Freitas & Oliveira, 1996; De-Silva *et al.*, 2011). Acting as generalist predators, ants may also exert strong pressure on patterns of host plant use and on defensive traits of phytophagous insects, including Lepidoptera (Bernays & Graham, 1988; Rico-Gray & Oliveira, 2007; Stamp, 2001; Sendoya *et al.*, 2009).

Anti-predator defences can be considered as primary defence (reduced encounter with predators) or secondary defence (increased prey survival after encounter with predator; Gross, 1993). Caterpillars exhibit several defensive strategies against predation, from constructing shelters to elaborate protective behaviours (Salazar & Whitman, 2001). Defences related to coloration (camouflage or aposematism) are primary defences against visually oriented predators such as birds, whereas morphological or behavioural defences are secondary defences that seem better suited against non-visually oriented enemies such as invertebrate predators (Greeney *et al.*, 2012; Lichter-Marck *et al.*, 2015). Some caterpillars may also present chemical signatures in the cuticle that allow them to camouflage against the host plant surface, which help them pass undetected by chemically oriented predators (Portugal & Trigo, 2005). Construction of protective shelters using plant tissue and faeces is a frequent habit in lepidopteran larvae (Eubanks *et al.*, 1997; Jones *et al.*, 2002; Mega & de Araujo, 2008; Moraes *et al.*, 2012). Indeed, frass chains constructed at leaf margins have been shown to reduce the probability of encounter with aggressive ants, thus decreasing larval predation (Freitas & Oliveira, 1992, 1996). Moreover, morphological traits such as hairs, spines or cephalic scoli have also been demonstrated to protect caterpillars against invertebrate predators (Dyer, 1997; Murphy *et al.*, 2010; Sugiura & Yamazaki, 2014), although the defence arsenal is typically complemented by specific behaviours (Castellanos *et al.*, 2011). Larval behavioural responses to encounters with ants may include biting, thrashing, twisting and regurgitating (Freitas & Oliveira, 1992; Greeney *et al.*, 2009; Greeney *et al.*, 2012), jumping off the host plant directly to the substrate (Castellanos *et al.*, 2011; Yamazaki, 2010), or hanging on a silken 'life-line' before climbing back to the leaf (Oliveira & Freitas, 2004; Sugiura & Yamazaki, 2006). In addition, caterpillar defences may depend on their size, and bigger (older) caterpillars may trigger defensive responses only when predation risk is very high (Thaler & Griffin, 2008).

There is evidence that the presence of ants and ant-attractive exudates on host plants, such as extrafloral nectaries and honeydew-producing insects, may have a significant effect on caterpillar assemblages in Neotropical cerrado savanna (Diniz *et al.*, 2012; Sendoya & Oliveira, 2015; Sendoya *et al.*, 2016). Ant visitation to plants increases predation risk for caterpillars and thus affects both caterpillar survival and host plant use by Lepidoptera (Sendoya *et al.*, 2009; Sendoya & Oliveira, 2015). However, predation risk associated with ant presence varies with the species of ant; while some ant species may reduce caterpillar infestation, others are innocuous (Sendoya *et al.*, 2009). Moreover, avoidance behaviour of adult butterflies in combination with defensive traits of caterpillars may determine how effective ants are at protecting plants against lepidopterans in cerrado (Freitas & Oliveira, 1996; Sendoya *et al.*, 2009). Studies assessing the specific effect of foliage-foraging ants on host plant use by non-myrmecophilous lepidopterans or on their defensive strategies are scarce (but see Dyer, 1995, 1997) or consider only single species (e.g. Sendoya *et al.*, 2009). Nonetheless, negative effects of generalist predatory ants on herbivores are detectable at a community level and have recently been shown to affect patterns of abundance and host plant use by lepidopterans in cerrado (Sendoya & Oliveira, 2015).

Here, we identify the most common morphological and behavioural defensive traits (strategies) in lepidopteran larvae using plants in cerrado vegetation. Additionally, we experimentally evaluate the efficiency of these defences against two of the most abundant ants on cerrado foliage. Considering the importance of ants as predators on cerrado foliage, we expected that morphological and behavioural traits previously reported as efficient against invertebrate predators (e.g. hairs, shelters, biting, dropping, regurgitating; see Dyer, 1997; Greeney *et al.*, 2012) would be very common in cerrado caterpillars, allowing them to survive better to ant encounters. We also expected that such defences would be especially common and efficient in caterpillars using plants highly visited by ants (e.g. plants bearing EFNs). Finally, considering that ants vary in size and aggressiveness, we expect that the efficiency of defences would be affected by ant identity. Specifically, we addressed the following questions: (i) Is predation risk caused by ants reduced for caterpillars with morphological (hairs, spines) or behavioural (shelter-building, biting, or thrashing) defences? (ii) Is predation risk affected by ant identity? (iii) Are larvae with the best defensive traits more frequently found on plants highly visited by ants (i.e. with plant or insect exudates)?

Materials and methods

Study site

Caterpillar surveys were performed in four cerrado localities with vegetation consisting of a dense scrub of shrubs and trees inside an herbaceous matrix, which corresponds to the physiognomy of cerrado *sensu stricto* (Oliveira-Filho & Rater, 2002). The study was carried out in southeast Brazil at the following cerrado reserves (70–460 km apart from one another): (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). Sampling was performed in the wet season (January–April of 2009) and presented similar climatic

regimes (INMET, 2012; see Sendoya & Oliveira, 2015 for further information on sampling areas).

Traits of cerrado caterpillars

In each locality, we sampled ants and lepidopteran larvae along six transects 2 m wide and ~30 m long. We extended a string line in each site and selected all plants (0.6–3.0 m tall) within 1 m of the string until we completed 50 sampled plants. The first transect was located 150 m from the cerrado edge, and others were sequentially established (100 m from one another) towards the interior (complete lists of sampled plant species can be found in Sendoya & Oliveira, 2015; Sendoya *et al.,* 2016). Plants were scrutinized for the presence of caterpillars as well as liquid food sources commonly used by ants such as extrafloral nectaries (EFNs) and honeydew-producing hemipterans (hereafter referred to as 'ant attractants'). Caterpillars were taken to the laboratory for rearing and taxonomic identification through comparison with available lists (e.g. Diniz *et al.,* 2013). Since only ~45% of the caterpillars were successfully reared into adults, most individuals were sorted to morphospecies and identified up to family level.

Initial observations on collected caterpillars allowed us to compile their morphological and behavioural defensive traits and sort them into general groups accordingly (hereafter 'sets of defensive traits'). This was complemented by observations during caterpillar rearing in the laboratory. Shelter-building caterpillars were grouped according to their level of exposure while inside the shelter and the presence/absence of faeces inside the shelter, since natural enemies may use excrements as cues to detect caterpillar presence (Weiss, 2003). Non-shelter-building caterpillars were grouped according with the type of morphological defence (i.e. hairs, spines or scoli), and solitary or gregarious habit (see details of each group in Table S1). We used a G test to compare the proportion of larvae occurring inside shelters between plant groups (with or without ant attractants). Tests were performed in R environment V.2.15.2 2 (R Development Core Team, 2015).

Host plant use by lepidopterans, larval defensive traits, and ant visitation to foliage

We recorded caterpillar and ant presence on individual plants in order to establish if caterpillars with certain defensive traits were more frequently found on plants with low ant abundance. On the day after larval collection, we registered ant visitation to individual plants (both plants with and without EFNs) using pitfall traps adapted to arboreal sampling (adapted from Ribas *et al.,* 2003). We used two pitfall traps per plant during 24 h; one baited with sardine and another with diluted honey. Field observations indicated that caterpillar collection did not affect ant visitation after 24 h. We preserved ant specimens in alcohol for counting and taxonomic identification.

The effect of foliage-foraging ants on lepidopteran colonization decisions (Sendoya *et al.,* 2009) was evaluated by recording ant visitation levels on the actual host plant (i.e. plant individual where the larva was actually found) in comparison with

visitation levels on the nearest host plant not infested by caterpillars (i.e. of the same species where that caterpillar was found in the same transect). The infested and the uninfested host plants were considered as a pair for the analysis. Plants infested by caterpillars but without nearby individuals of the same species were not included. Plants with two different caterpillar species were not considered. We pooled together plant pairs in which the infested plants hosted caterpillars presenting the same set of defensive traits. Caterpillars that walked with their shelters and gregarious caterpillars were not considered for this test because of their low frequencies. Comparisons were performed with a Wilcoxon paired test using the corresponding function in R environment V.2.15.2 2 (R Development Core Team, 2015).

Experimental tests on ant-caterpillar interactions

To test the effectiveness of the caterpillars' defensive traits against ants, we performed experimental encounters between ants and caterpillars in the laboratory. We carried out a new collection of caterpillars on plants only in the cerrado of Itirapina (N = 129 caterpillars from 45 morphospecies). For each larva, we registered in the field its morphological and behavioural defensive traits such as shelter-building and behavioural reactions to touch by thin forceps (following the behavioural categories in Table 3), as well as total body length. We brought caterpillars to the lab in plastic boxes containing a branch of the original host plant and, after 24 h, we performed behavioural trials of ant-caterpillar interactions. Trials involved foragers of two of the most common ant species found on cerrado leaves: *Camponotus crassus* Mayr (N = 64 trials) and *Camponotus renggeri* Emery (N = 61 trials) (see Oliveira & Brandão, 1991; Schoereder *et al.,* 2010). These two ants differ in size from ≈6–7 mm for *C. crassus* to ≈12–13 mm for *C. renggeri*. We used captive colonies (three from each species) cultured in 3–5 test tubes (2 × 20 cm) containing water trapped by a cotton plug and placed in plastic trays (70 × 40 cm), which were connected to a foraging arena (40 × 25 cm). Ants were conditioned to search for food (dead insects, sugar solution) in the foraging arena for 2 weeks before the interaction trials.

Experimental encounters between ants and caterpillars were performed on a potted branch of the host plant (at the centre of the arena) on which the caterpillar was observed in the field. Some of the host plant species used in the experiments had EFNs which, however, were non-functional in the potted branches used in trials. Baits of sugar solution were added to potted foliage (both on species with and without EFNs) to promote ant visitation and ant-caterpillar encounters during trials. Interaction trials were performed using one resident caterpillar on the potted branch. Experiments began when ants encountered the caterpillar on the plant, and behavioural interactions were recorded for 10 min (or until ants had killed the caterpillar). We registered if caterpillars were attacked or not by the ants, as well as the behavioural responses of caterpillars. We defined an attack as an instance when the foraging ant bit any part of the caterpillar body. Gregarious caterpillars were tested in their natural groups, and a bite by an ant to any member of the group was considered an attack. We also recorded caterpillars that were killed by ants on the potted host plant, or on the foraging arena (in case the

larva jumped off the branch), or which were carried by the ants to the nest. After each trial, we removed potted branches and cleaned experimental trays from remaining bodies or fluids from attacked larvae, and removed the ants that had interacted with caterpillars (this ensured that previous experience of individual ants with caterpillars did not affect subsequent trials). Trials using the same ant colony were carried out at least 2 h apart. The nutritional state of the colony can affect ant aggressiveness (Ness *et al.,* 2009) and this may mask the effectiveness of caterpillar defensive traits during our trials. Ants with low supplies of food in days before trials were more responsive to caterpillar presence (S. F. Sendoya, pers. obs.). Therefore, we kept colonies unfed for 72 h prior to the experiments. We divided ant-caterpillar interaction trials into three experimental categories: (A) trials using caterpillars found unsheltered in the field (i.e. free-living), and exposed unsheltered on leaves to ants (N = 52); (B) trials using caterpillars found inside any type of shelter in the field, and placed on experimental potted branches inside their natural shelter still attached to their original host plant to interact with ants (N = 71); (C) trials using caterpillars found inside any type of shelter in the field, but purposely exposed (shelter opened) on leaves to interact with ants (N = 71).

Construction of generalized linear models

To determine which condition better explained the probability of a caterpillar being attacked or killed by ants in the interaction experiments, we constructed generalized linear models. We constructed two sets of models: one for testing which factors affected the attack of ants on caterpillars (Attack Models) and a second for testing the factors affecting the death or survival of caterpillar (Caterpillar Death/Survival Models) after ant attack.

In the first set of models (Attack Models) the attack (or absence of attack) of ants on caterpillars was considered as the response variable. We also included five predictor variables: (i) species of ant used in the experiment (*As*); (ii) presence of morphological defence in caterpillar (e.g. glabrous larvae, with hairs or spines/scoli; *Mp*); (iii) if larvae were inside a shelter during experiments (*Sh*); (iv) caterpillar total length (*Sz*); (v) presence of EFNs in the original host plant species (*En*). We then separated the data in three different ways in order to construct three separated groups of models, as follows. In 'Attack Models I', we were interested in testing larval defences under the same condition they were found in the field; hence we included only data from trials with non-manipulated caterpillars (Experimental categories A and B; see above). Here, we tested if the effect of morphological defences, larval size, and possession of EFNs by the host plant on attacked larvae varied with the ant species used in the trial (thus the respective interactions between each variable and ant species were added as predictive factors). In 'Attack Models II', we considered that shelter-building behaviour may restrict other defensive traits of caterpillars (e.g., shelter-building caterpillars lack morphological defences). We were thus interested in testing our predictive variables for non-shelter-building caterpillars only (data from experimental category A). In 'Attack Models III', we tested our predictor variables for ant attacks on shelter-building caterpillars only when exposed (shelter opened; Experimental category C), and thus we excluded from the predictive factors both morphological defence (*Mp*) and construction of shelters (*Sh*).

For the second set of models (Caterpillar Death/Survival Models), the mortality or survival of larvae after trials was considered as the response variable. In these models, in addition to morphological defences we also considered the behavioural responses of caterpillars when facing attacks by ants. We categorized all observed caterpillar behaviours in nine groups: (i) fleeing to another part of the plant after initial encounter with ants (*Fl*); (ii) remaining motionless during experiment (*Rm*); (iii) curling its body to expose morphological defences (*Cb*); (iv) thrashing with the head or defensive structures to strike ants (*Tr*); (v) twisting, rolling or wriggling violently to avoid further contact with the ants (*Tw*); (vi) spinning of lines of silk around body parts or near plant structures to prevent contact with ants (*Ss*); (vii) dropping from host plant (*Dr*); (viii) dropping and spinning of a silk 'life-line' that allows the caterpillar to climb back up to the host-plant (*Ll*); (ix) regurgitating (*Rg*). Each caterpillar showed behaviours corresponding with one or more of these categories. We proceeded to construct two groups of models, as follows. In the 'Caterpillar Death/Survival Models I', we tested our predictive variables for the death/survival for non-shelter-building caterpillars only (Experimental category A). In the 'Caterpillar Death/Survival Model II', we tested our predictor variables for death/survival after the trial for shelter-building caterpillars when exposed (shelter opened; Experimental category C).

For both sets of models (Attack Models, and Caterpillar Death/Survival Models), response variables were considered binary, hence we assumed a binomial distribution. In all cases, models were constructed using the *glm* function on R language 2.15.2 (R Development Core Team, 2015). We fitted models with Logit function and using a Laplace approximation (Raudenbush *et al.,* 2000) to estimate model parameters. For each group of models we started with a saturated model (including all variables and interactions) and constructed all possible nested (simplified) models using the R package *Multi-model inference* (MuMin). We applied a model selection procedure based on corrected Akaike Information Criteria (AICc) calculated for each concurrent model, and selected more parsimonious models (with a smaller number of factors) between those with delta AICc values lower than 2 (Johnson & Omland, 2004; Bolker *et al.,* 2009).

Results

Traits of cerrado caterpillars

We observed a total of 58 caterpillar species and 155 individuals in the field and most of which (73.5%) were found inside some type of shelter or refuge (Table S2). The proportion of larvae with/without shelters did not differ between plants with or without ant attractants (G test$_{(d.f.:3)}$ = 2.053; P = 0.153). Most shelters (78%) were constructed with a mix of silk and faeces although they were highly variable in structure and complexity (Fig. 1a–c). Among unsheltered caterpillars, 56.1% presented some type of morphological defence such as hairs, spines or cephalic scoli (Fig. 1d–f). Only a small percentage of caterpillars (1.8%) were found in aggregations.
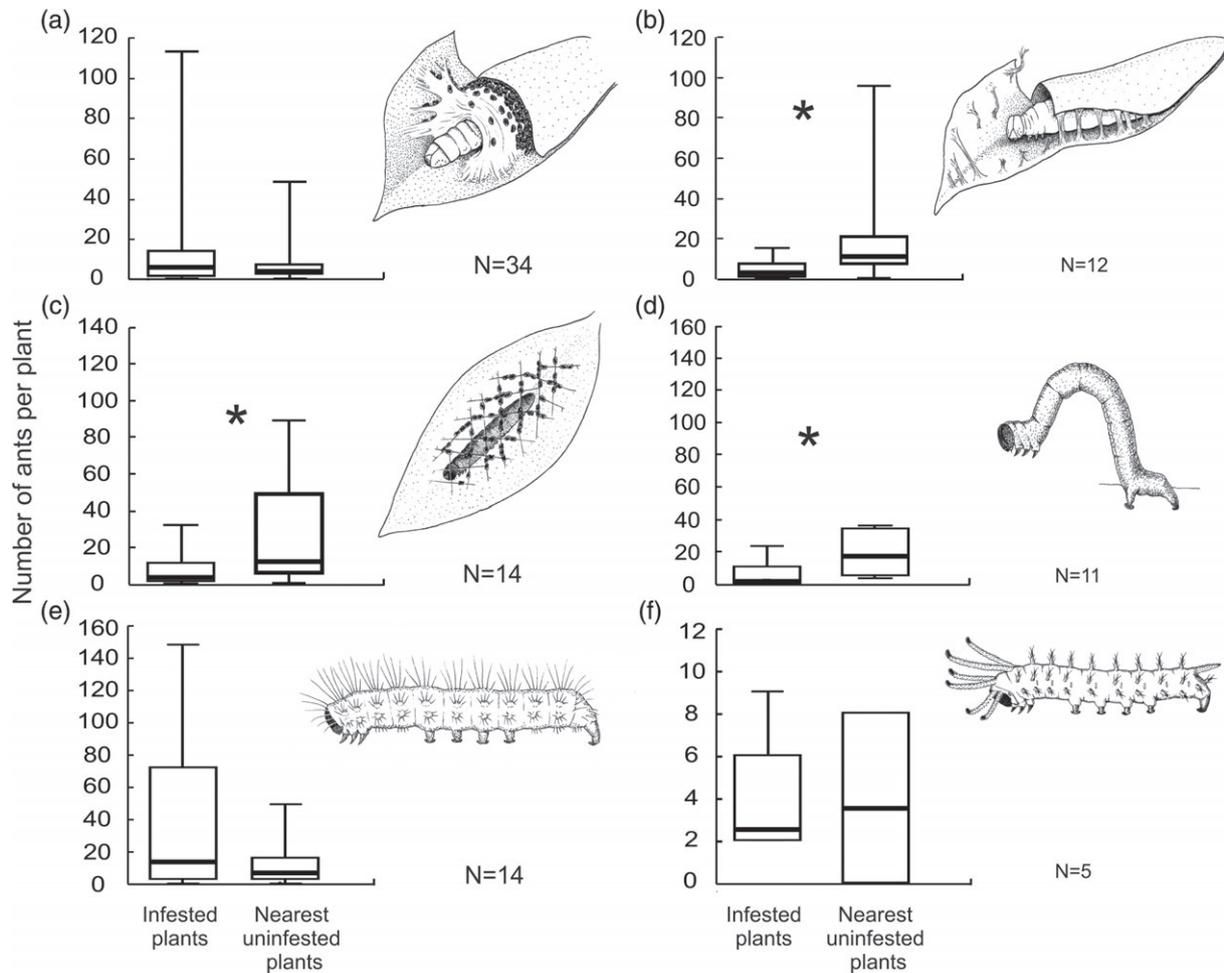
Fig. 1. Groups of morphological and behavioural defensive strategies observed in cerrado caterpillars (see detailed descriptions and examples in Table S1). For each group, the number of sampled ants on the plant with the caterpillar and on nearby unoccupied host plants is shown (boxes represent the second and third quartiles and whiskers the data range). Asterisks indicate significant difference (paired Wilcoxon P < 0.05), and values below caterpillars indicate the number of pairs. (a) Caterpillars in shelters with silk and frass; (b) Caterpillars in shelters with silk only; (c) Caterpillars in shelters not completely closed; (d) Caterpillars without shelters and glabrous bodies; (e) Caterpillars with hairs or small spines; (f) Caterpillars with big spines or scoli.

Host plant use by lepidopterans, larval defensive traits, and ant visitation to foliage

The abundance of ants on infested host plants (with caterpillars) was lower than on the nearest uninfested host plants (without caterpillars) in the same transect only for three larval sets of defensive traits: Caterpillars inside leaf shelters closed with silk (Fig. 1b), caterpillars inside shelters of silk or frass (no leaf cover) (Fig. 1c), and unsheltered caterpillars with smooth bodies (Fig. 1d).

Experimental tests on ant-caterpillar interactions: ant attacks

In the model selection for the 'Attack Model I' (data without manipulation of caterpillars), we found that the best model included: Shelter construction by caterpillar (*Sh*), ant species used in experiment (*As*), presence of extrafloral nectaries (EFNs)

in original host plant (*En*), size of caterpillar (*Sz*), the interaction between ant species and size of caterpillar (*As × Sz*), and the interaction between ant species and presence of EFNs (*As × En;* Table S3). Shelter construction was the most efficient defence against ant attack; only three (2.9%) shelter-building caterpillars were attacked by ants and none was killed (Fig. 2). In addition, the mean model coefficient associated with this variable (indicative of effect size) was the lowest of all (Table 1). Shelter-building caterpillars found on plants with EFNs were never attacked. Larval size was also important to predict ant attack, but differed between ant species: Negative for *Camponotus crassus* and positive for *C. renggeri* (Table 1; Fig. 3a,b).

In the 'Attack model II' (unsheltered caterpillars, Experimental category A only), the variables included in the selected model were: Presence of EFNs in original host plant (*En*), ant species used in experiment (*As*), morphological defence of caterpillar (*Mo*) and size of caterpillar (*Sz*). The interaction
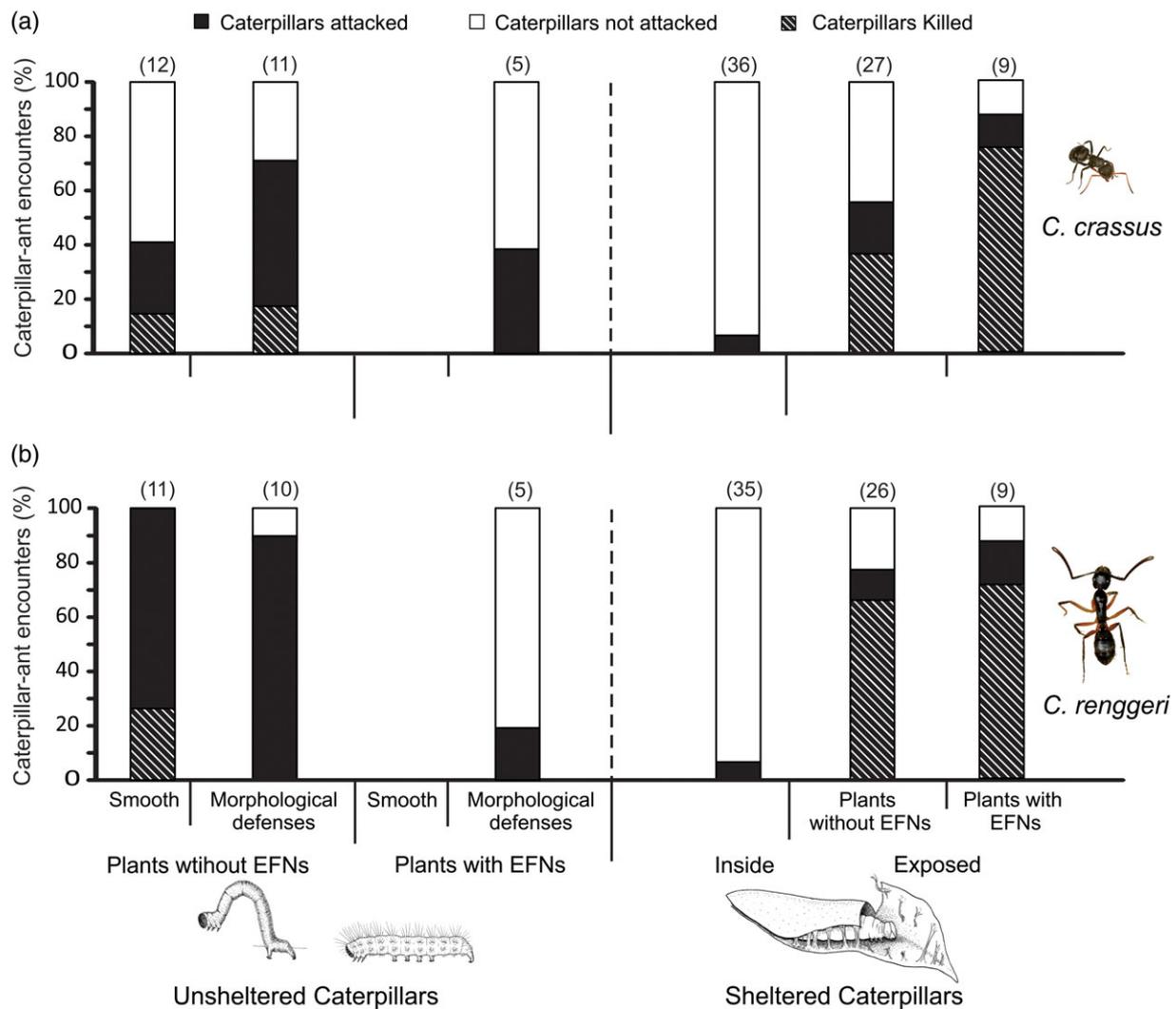
Fig. 2. Frequency of attacks (black bars) and kills (hatched bars) of caterpillars by ants during experimental trials on potted host plants. (a) Trials with *Camponotus crassus*. (b) Trials with *Camponotus renggeri* (see Table 1 for statistical analyses). On the left panel, results of ant-caterpillar encounters are shown for smooth caterpillars (no morphological defence) and for caterpillars bearing hairs or spines (morphological defence), and on the original host plant species of each tested caterpillar (with or without extrafloral nectaries, EFNs). On the right panel, behavioural results are shown for shelter-building caterpillars inside their refuge or purposely exposed on leaves, in accordance with the presence of EFNs in their original host plant. [Colour figure can be viewed at wileyonlinelibrary.com].

between morphology and size of caterpillar was included in the model with lowest AICc value ($Mo \times Sz$; Table S3). The proportion of unsheltered caterpillars attacked by ants was higher than for sheltered caterpillars (51.7% by *C. crassus*; 81.4% by *C. renggeri*). The presence of morphological defences affected the probability of being attacked, and this effect was dependent on the ant species in question. Attacks by *C. crassus* increased in the presence of morphological defences (from 33.3% of attacks to smooth caterpillars to 64.7% to haired caterpillars), and attacks by *C. renggeri* decreased in the presence of morphological defences (from 100% of attacks to smooth caterpillars to 68.7% to hairy caterpillars; Table 1, Fig. 2). Small and smooth caterpillars were highly attacked by ants, whereas hairy caterpillars were less frequently attacked irrespective of the size.

Caterpillars found in the field on host plants with EFNs were also less attacked, especially by *C. renggeri*. However, those two last effects (morphology and EFNs) are difficult to separate because all caterpillars found on plants with EFNs showed some type of morphological defence.

In the 'Attack Model III' we purposely exposed shelter-building caterpillars (by opening the shelter; Experimental category C), the most important variable for the models were ant species used in experiment (*As*) and size of caterpillar (*Sz*; Table 1, Table S2). Caterpillars were more frequently attacked by *C. renggeri* (74.2%) than by *C. crassus* (48.6%). However, while the probability of a caterpillar being attacked by *C. renggeri* increased quickly with larval size, it slowly decreased with *C. crassus* (Fig. 3c).

Table 1. Results of the model selection procedure among the possible predictors of ant attacks on caterpillars during ant-caterpillar interaction experiments (attack models).

Included variable	'Attack Model I': non-manipulated caterpillars (experimental categories A + B)		'Attack Model II': unsheltered caterpillars (experimental category A)		'Attack Model III': purposely-exposed caterpillars (experimental category C)	
	Estimated coefficient	Standard error	Estimated coefficient	Standard error	Estimated coefficient	Standard error
Intercept	1.788	0.89	7.120	2.924	4.136	1.492
Shelter (*Sh*)	−4.428	0.799	–	–	–	–
Ant species/*C. renggeri* (*As*)	−1.011	1.351	−6.284	3.378	−5.4	1.879
Extrafloral nectaries (*En*)	−0.486	0.997	−3.021	1.249	NS	NS
Morphological defence (*Mo*)	NS	NS	4.287	1.846	–	–
Size of caterpillar (*Sz*)	−0.815	0.403	−3.896	1.453	−2.917	1.161
As × *En*	−3.743	1.864	NS	NS	NS	NS
As × *Mo*	NS	NS	NS	NS	–	–
As × *Sz*	1.572	0.673	4.557	1.609	5.622	1.674
Mo × *Sz*	NS	NS	NS	NS	–	–

We present results for the best model (with the lowest AIC value) (see Table S3). NS indicates a factor excluded (not selected) in the selected model.

Experimental tests on ant-caterpillar interactions: caterpillar behaviour and mortality caused by ants

Although non-shelter-building caterpillars were frequently attacked by ants, mortality due to ant attacks was low (12.9% of encounters; Fig. 3). No unsheltered caterpillar was found in the field on a host plant bearing EFNs. The most common defensive behaviours showed by this group of caterpillars when encountering ants were (i) fleeing to another part of the plant (*Fl*), (ii) curling the body to expose morphological defences (*Cb*), and (iii) dropping from host plant (*Dr*; see Table 3). For smooth larvae, a very common behaviour was to maintain the anterior portion of body elevated from the plant surface and vigorously thrash to strike the ant with the head or scoli (*Tr*). Nevertheless, according to the model selection for 'Caterpillar Death/Survival Model I' (Table S4), none of the displayed behaviours was related to a reduction in the probability of the caterpillar surviving ant attacks. The variables negatively influencing the probability of being killed by ants (and maintained in the best/selected model; Table 2) were larval size and the presence of morphological defences. For instance, all caterpillars bearing morphological defences survived encounters with *C. renggeri* (Fig. 3).

Ants killed none of the shelter-building caterpillars in Experimental category B (Fig. 3). However, after opening the shelter to purposely expose the larvae (Experimental category 3), caterpillar mortality increased drastically (53.62%). Wriggling and twisting violently (*Tw*) and dropping from host plant (*Dr*) (sometimes spinning a silk dragline; *Li*) were among the most common defensive behaviours displayed by purposely unsheltered caterpillars (Table 3). Sometimes caterpillars began to reconstruct the shelter by spinning silk around their bodies (*Ss*), or just remained motionless until the end of the experiment (*Rm*). Regurgitation upon attacks was also common and disturbed the ants. Our model selection procedure indicated that the most efficient behaviour in response to ant attacks was dropping and spinning a dragline (Table S4). Our selected model for 'Caterpillar Death/Survival Model II' (with lower AICc value) also included the ant species used in the experiment (*As*). The model with the lowest AICc also included the behaviour of remaining motionless (*Rm*; Table 2, Table S3).

Discussion

We showed that ant foraging on plants promotes antagonistic encounters between ants and most lepidopteran larvae, but the outcome of this interaction depends on the specific set of defences exhibited by the caterpillar. Shelter construction and morphological defences (for unsheltered caterpillars) are important traits for caterpillars to avoid ant attacks on leaves, but once caterpillars are exposed or discovered by the ants they must rely on their size and/or behavioural responses to survive.

For caterpillars without morphological defences or that live inside shelters without a complete protection of leaves, ant presence on foliage may affect host plant use, which explains why they were found on plants with lower ant numbers. These two groups of caterpillars were indeed the most affected by ant presence, indicating that the ants' predatory effects on cerrado foliage depend on the life style of the caterpillars (see Ito & Higashi, 1991, on oak-feeding moth larvae in Japan). Ant-induced effects on host plant use by lepidopterans may result either from avoidance behaviour by ovipositing females of ant-occupied foliage (Sendoya *et al.,* 2009), or by reduced caterpillar survival on plants with increased numbers of foraging ants (Dutra *et al.,* 2006; Sendoya & Oliveira, 2015). Plant volatiles induced by herbivory may promote the presence of natural enemies (e.g. ants) of caterpillars (Brouat *et al.,* 2000), reducing caterpillar survival. Although caterpillars from all groups may trigger this effect, caterpillars without efficient defensive traits may be removed faster.

The role of EFNs as promoters of ant visitation to cerrado foliage and the resulting decrease of herbivore infestation, including lepidopteran larvae, is well documented (Oliveira &
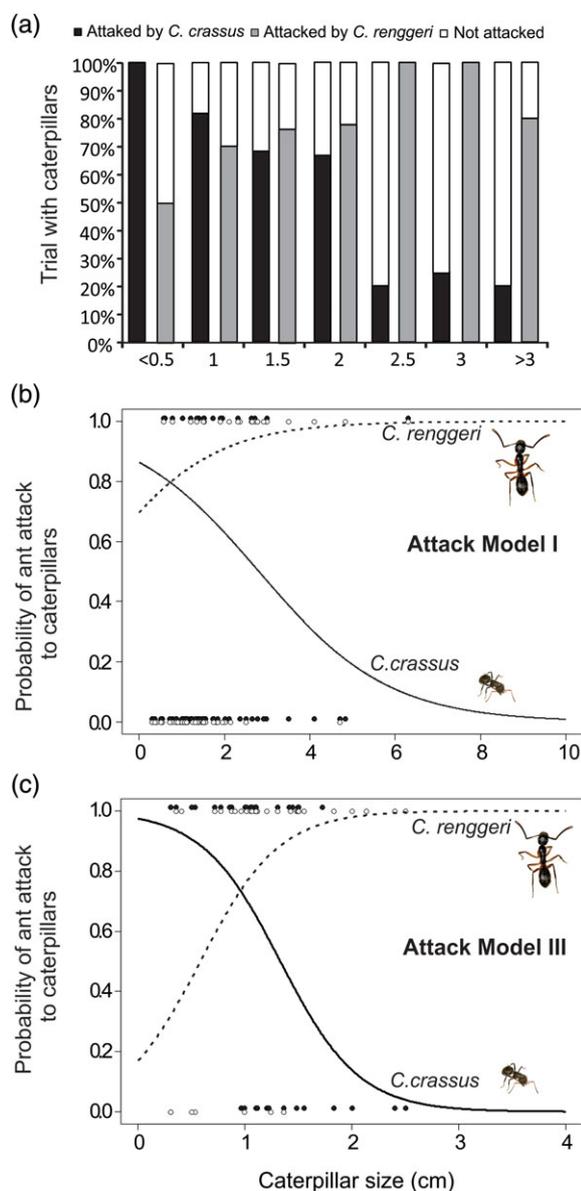
Fig. 3. Relationship between larval size and attacks by ants during experimental trials. (a) Percentage of caterpillars attacked by *C. crassus* (black bars) and *C. renggeri* (grey bars) for different size categories (results include data from experimental categories A and C, see Table 1). (b) Relationship between larval size and probability of being attacked by each ant species (black line for *C. crassus* and dash line for *C. renggeri*) according with the selected model for 'Attack Models I' and (c) for 'Attack Models III'. Black dots represent trials with *C. crassus* and white dots with *C. renggeri*. [Colour figure can be viewed at wileyonlinelibrary .com].

Brandão, 1991; Oliveira, 1997; Schoereder *et al.,* 2010). Diniz *et al.* (2012) found that shelter-building caterpillars were more abundant on EFN-bearing plants, suggesting a defensive role of shelters against ants and/or parasitoids. In order to use highly ant-visited foliage (i.e., with EFN), caterpillars must present efficient defensive traits. In fact, caterpillars that use

Table 2. Results of the model selection procedure among the possible predictors for the death of caterpillars by ant attacks during ant-caterpillar interaction experiments (caterpillar death/survival models).

Predictor variable	'Caterpillar Death/Survival Models I': unsheltered caterpillars (experimental category A)		'Caterpillar Death/Survival Models II': exposed larvae (experimental category C)	
	Estimated coefficient	Standard error	Estimated coefficient	Standard error
Intercept	5.416	2.313	−0.662	0.499
Size of caterpillar (*Sz*)	−3.770	1.447	NS	NS
Morphological defences (*Mp*)	−2.760	1.397	NS	NS
Fleeing (*Fl*)	NS	NS	NS	NS
Dropping (*Dr*)	NS	NS	1.726	0.702
Remaining motionless (*Rm*)	NS	NS	1.300	0.839
Thrashing (*Tr*)	NS	NS	NS	NS
Silk 'life-line' (*Li*)	NS	NS	−2.385	0.769
Curling (*Cb*)	NS	NS	NS	NS
Ant species/*C. renggeri* (*As*)	NS	NS	0.951	0.544

We present results for the best model (with the lowest AIC value). NS indicates a factor excluded (not selected) in the selected model.

plants highly visited by ants commonly present behavioural and morphological defences against ant predation (Freitas & Oliveira, 1996; Eubanks *et al.,* 1997; Bächtold *et al.,* 2012). This may also explain our finding that caterpillars using EFN-bearing host plants were less attacked by ants during interaction trials. Indeed, all caterpillars found on host plants with EFNs were in shelters or presented morphological defences. This suggests that the associated risk of predation by ants on such plants favours an efficient set of larval defences such as closed shelters (Jones *et al.,* 2002; Mega & de Araujo, 2008), hairs and spines (Dyer, 1997; Murphy *et al.,* 2010), or even chemical camouflage (Portugal & Trigo, 2005).

Shelter construction was the most efficient and most common defence against ant attacks, and the abundance of this defensive strategy in the current study was even higher than in previous records in cerrado (Diniz *et al.,* 2012). This behaviour provides several advantages to caterpillars, such as maintaining adequate micro-environmental conditions while having direct contact with the host plant (Loeffler, 1996), and mainly protecting the caterpillar against natural enemies (Jones *et al.,* 2002; Greeney *et al.,* 2012). When shelters are adequately closed, they confer the best chances of surviving ant attacks (Ito & Higashi, 1991). Not surprisingly, shelter building allows lepidopteran larvae to use myrmecophytic plants housing ant colonies (Eubanks *et al.,* 1997) or highly ant-visited, EFN-bearing plants (Bächtold *et al.,* 2012; Diniz *et al.,* 2012; Moraes *et al.,* 2012). Our experiments show that larvae living in leaf shelters not entirely closed can be negatively affected by ant presence; if taken out from shelters ant-induced mortality increases markedly, especially in ant-rich

Table 3. Number of caterpillars from eight defensive groups and their respective defensive behaviours exhibited during encounters with ants (*Camponotus crassus* and *C. renggeri*).

	Set of defensive traits							
	Sheltered caterpillars[†]			Unsheltered caterpillars			Total of trials	Total mortality
	Shelters with silk and frass	Shelters with silk	Shelters not completely closed	Glabrous	With big spines or scoli	With small hairs		
	Number of larval trials			Number of larval trials				
Defensive behavioural category of caterpillars	25	16	22	21	15	18	117	
Thrashing (*Tr*)	0	0	0	12 (57.1%)	2 (13.3%)	9 (50%)	23 (18.3 %)	4.3%
Curling (*Cb*)	0	0	0	0	5 (33.3%)	12 (66.7%)	17 (13.5%)	5.9%
Remaining motionless (*Rm*)	0	3 (18.8%)	7 (31.8%)	0	3 (20%)	4 (22.2%)	17 (13.5%)	47.1%
Fleeing (*Fl*)	6 (24%)	1 (6.3%)	4 (18.2%)	5 (23.8%)	10 (66.7%)	8 (44.4%)	33 (28.2%)	23.5%
Dropping (*Dr*)	13 (52%)	13 (81.3%)	16 (72.7%)	11 (52.4%)	5 (33.3%)	4 (22.2%)	65 (26.9%)	49.2%
Regurgitating (*Rg*)	4 (16%)	4 (25%)	4 (18%)	0	0	0	15 (26.9%)	46.7%
Twisting (*Tw*)	25 (100%)	14 (87.5%)	22 (100%)	1 (4.8%)	0	2 (11.1%)	64 (54.7%)	52.1%
Silk 'life-line' (*Li*)	6 (24%)	7 (43.7%)	10 (45.4%)	5 (23.8%)	0	2 (11.1%)	30 (26.9%)	36.7%
Spinning silk around (*Ss*)	7 (28%)	1 (6.3%)	9 (40.9%)	0	2 (13.3%)	0	19 (15.1%)	42.1%
Ant-caterpillar interaction trials[‡]								
Camponotus crassus								
Attacked	0 (72.7%)	0 (62.5%)	16.7% (66.7%)	27.3%	50%	77.8%	–	–
Killed	(63.6%)	(37.5%)	(41.7%)	0%	12.5%	11.1%	–	–
Camponotus. renggeri								
Attacked	0 (63.6%)	0 (100%)	9.1% (81.8%)	100%	57.1%	77.8%	–	–
Killed	(45.5%)	(77.7%)	(81.8%)	60%	0.0%	11.1%	–	–

†Behavioural data correspond to trials using caterpillars found inside any type of shelter in the field, but purposely exposed (shelter opened) on leaves to interact with ants (Experimental category C).

‡Values indicate the percentage of caterpillars attacked or killed in trials with ants. Values in parentheses indicate percentage of sheltered caterpillars attacked when exposed out of their shelters (Experimental category C).

The numbers inside parenthesis indicate the percentage of larvae showing the behaviour (one larva could show several behaviours). See details of groups in Tables S1 and S2. Last column summarizes the total mortality due to ant attacks for each group.

environments (Tvardikova & Novotny, 2012). Shelter-building caterpillars may be vulnerable to ant attacks especially during periods of shelter construction or repair (Freitas & Oliveira, 1996; Greeney *et al.,* 2012).

The efficiency of the caterpillars' defensive traits was dependent on ant identity, and foragers of *Camponotus renggeri* were more aggressive and lethal to caterpillars than those of *C. crassus*. However, larval defences may interact with ant species identity in different ways. For instance, whereas all larvae with morphological defences survived attacks from *C. renggeri*, some were killed in trials with *C. crassus*. Variation in the effects of each ant species on caterpillars may be related to differences in ant foraging strategies and ant size (≈6–7 mm for *C. crassus*; ≈12–13 mm for *C. renggeri*). Species-specific lethality from ant attacks helps explain why the composition of ant assemblages on leaves can determine caterpillar survivorship as well as herbivory and patterns of host plant use by Lepidoptera in variable habitats (e.g. Mody & Linsenmair, 2004; Sendoya *et al.,* 2009; Sendoya & Oliveira, 2015).

Chemical defence in caterpillars is known to play an important role against ant attacks (Dyer, 1995; Dyer & Bowers, 1996), especially when ants are very abundant (Inui *et al.,* 2015). It is thus possible that some type of chemical defence may have

accounted for the lack of attack by ants to some of the caterpillars used in our experiments. However, although chemical defence in Lepidoptera is related to the type of host plant, it is not necessarily associated with the existence of morphological or behavioural defence by the caterpillar (Greeney *et al.,* 2012). Notwithstanding, our results show that shelter-building, size of caterpillar, and morphological defence are relevant traits when larvae face aggressive ants on foliage.

Predation caused by arthropods is in most cases negatively affected by prey size, and as prey grows larger predation by vertebrates becomes more important (Remmel *et al.,* 2011). Predation by ants on caterpillars on host plants decreases as the larvae grow larger (Freitas & Oliveira, 1996; Lopez & Potter, 2000). Size by itself can be considered a defence against natural enemies that cannot handle big prey, and larvae without efficient morphological or behavioural defences may invest in growing fast during initial developmental stages to decrease exposure time to ants, or even invest in chemical defence (Akino, 2008). Dyer (1997) argues, however, that the recruiting ability of ants allows them to deal with large prey. Although the two *Camponotus* species used in our trials have high recruitment ability (Oliveira & Brandão, 1991), our data show that prey size matters but depends on the ant species. *Camponotus crassus*

workers are smaller in size, and more frequently attacked small caterpillars. Conversely, *C. renggeri* workers are larger and preferred to attack larger caterpillars. Size relationships between predators and prey can indeed affect the probability of finding prey by a predator (Sandre *et al.*, 2007), or how easily a predator deals with prey (Dyer, 1997; Remmel *et al.*, 2011). Bigger ants, thus, seem better adapted to find and kill large caterpillars and may ignore small ones, either because the latter do not represent a worthy energetic reward or because they are too small to be detected. Analogously, Schatz *et al.* (1997) showed that small ants are not only better suited to deal with small caterpillars, but may even be injured when dealing with large prey.

Our results show that caterpillars (especially unsheltered ones) may rely on morphological defences or size to avoid and survive ant attacks. On the other hand, when exposed from their retreat, behavioural defence may be the only alternative response of shelter-building caterpillars, which often lack morphological defences. Dropping from the host plant is one of the most common secondary defence strategies of insects (Gross, 1993). However, this strategy is most effective when larvae can maintain the connection with host plants, for example, by hanging on a silken dragline (Oliveira & Freitas, 2004; Sugiura & Yamazaki, 2006). In natural conditions, caterpillars dropping to the ground run a high risk of being found by other ground predators, including ants (Yamazaki, 2010). Indeed, during our trials, larvae dropping to the ground after ant attacks on leaves ended up killed by other ants on the foraging arena.

Our results show that different ants represent different risks to caterpillars. In order to understand how local ant assemblages affect local composition of lepidopterans, it would be necessary to use experimental manipulative approaches of ant composition and more detailed analyses including functional and phylogenetic composition of ant communities. Here, we presented experimental evidence of clear effects of ant-plant interactions (mediated by EFNs) on caterpillar presence at the scale of individual plants, and hence, on host plant use by lepidopterans according to their defensive traits. In ant-rich cerrado savanna, where plants bearing EFNs are so abundant and ant-plant-herbivore interactions so pervasive (Oliveira & Freitas, 2004), the defensive sets presented by lepidopteran larvae help us to understand which species would be better suited to survive and use plants highly visited by ants.

Acknowledgements

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/een.12416

Table S1. Characterization of larval groups according to defensive traits.

Table S2. Number of caterpillars and their main defence traits at each of the four localities, on plants with extrafloral nectaries.

Table S3. Results of the model selection procedure among the possible predictors of ant attacks on caterpillars during ant-caterpillar interaction experiments (Attack models).

Table S4. Results of the model selection procedure among the possible predictors for the death of caterpillars due to ant attacks during ant-caterpillar interaction experiments (Caterpillar Death/Survival models).

References

Akino, T. (2008) Chemical strategies to deal with ants: a review of mimicry, camouflage, propaganda, and phytomimesis by ants (Hymenoptera: Formicidae) and other arthropods. *Myrmecological News*, **11**, 173–181.

Bächtold, A., Del-Claro, K., Kaminski, L.A., Freitas, A.V.L. & Oliveira, P.S. (2012) Natural history of an ant–plant–butterfly interaction in a Neotropical savanna. *Journal of Natural History*, **46**, 943–954.

Bernays, E.A. (1997) Feeding by lepidopteran larvae is dangerous. *Ecological Entomology*, **22**, 121–123.

Bernays, E. & Graham, M. (1988) On the evolution of host specificity in phytophagous arthropods. *Ecology*, **69**, 886–892.

Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H.H. *et al.* (2009) Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in Ecology & Evolution*, **24**, 127–135.

Brouat, C., McKey, D., Bessiere, J.M., Pascal, L. & Hossaert-McKey, M. (2000) Leaf volatile compounds and the distribution of ant patrolling in an ant-plant protection mutualism: preliminary results on Leonardoxa (Fabaceae: Caesalpinioideae) and Petalomyrmex (Formicidae: Formicinae). *Acta Oecologica-International Journal of Ecology*, **21**, 349–357.

Castellanos, I., Barbosa, P., Zuria, I., Tammaru, T. & Christman, M.C. (2011) Contact with caterpillar hairs triggers predator-specific defensive responses. *Behavioural Ecology*, **25**, 975–983.

Cornell, H.V. & Hawkins, B.A. (1995) Survival patterns and mortality sources of herbivorous insects: some demographic trends. *The American Naturalist*, **145**, 563–593.

De-Silva, D.L., Vásquez, A.S. & Mallet, J. (2011) Selection for enemy-free space: eggs placed away from the host plant increase survival of a neotropical ithomiine butterfly. *Ecological Entomology*, **36**, 667–672.

Diniz, I. & Morais, H. (1997) Lepidopteran caterpillar fauna of cerrado host plants. *Biodiversity and Conservation*, **6**, 817–836.

Diniz, I.R., Hay, J., Ricogray, V., Greeney, H.F. & Morais, H.C. (2012) Shelter-building caterpillars in the cerrado: seasonal variation in relative abundance, parasitism, and the influence of extra-floral nectaries. *Arthropod-Plant Interactions*, **6**, 583–589.

Diniz, I.R., Braga, L., Lepesqueur, C., Silva, N. & Morais, H.C. (2013) *Lagartas do Cerrado – Guia de Campo*. Technical Books, Rio de Janeiro, Brazil.

Dutra, H.P., Freitas, A.V.L. & Oliveira, P.S. (2006) Dual ant attraction in the neotropical shrub Urera baccifera (Urticaceae): the role of ant visitation to pearl bodies and fruits in herbivore deterrence and leaf longevity. *Functional Ecology*, **20**, 252–260.

Dyer, L.A. (1995) Tasty generalists and nasty specialists – antipredator mechanisms in tropical lepidopteran larvae. *Ecology*, **76**, 1483–1496.

Dyer, L.A. (1997) Effectiveness of caterpillar defenses against three species of invertebrate predators. *Journal of Research on the Lepidoptera*, **34**, 48–68.

Dyer, L.A. & Bowers, M.D. (1996) The importance of sequestered iridoid glycosides as a defense against an ant predator. *Journal of Chemical Ecology*, **22**, 1527–1539.

Eubanks, M.D., Nesci, K.A., Petersen, M.K., Liu, Z. & Sanchez, A.B. (1997) The exploitation of an ant-defended host plant by a shelter-building herbivore. *Oecologia*, **109**, 454–460.

Freitas, A.V.L. & Oliveira, P.S. (1992) Biology and behavior of *Eunica bechina* (Lepidoptera: Nymphalidae) with special reference to larval defense against ant predation. *Journal of Research on the Lepidoptera*, **31**, 1–11.

Freitas, A.V.L. & Oliveira, P.S. (1996) Ants as selective agents on herbivore biology: effects on the behaviour of a non-myrmecophilous butterfly. *Journal of Animal Ecology*, **65**, 205–210.

Gentry, G.L. & Dyer, L.A. (2002) On the conditional nature of neotropical caterpillar defenses against their natural enemies. *Ecology*, **83**, 3108–3119.

Greeney, H.F., DeVries, P.J., Penz, C.M., Granizo, R.B., Connahs, H., Stireman, J.O. *et al.* (2009) The early stages and natural history of *Antirrhea adoptiva porphyrosticta* (Watkins, 1928) in eastern Ecuador (Lepidoptera: Nymphalidae: Morphinae). *Journal of Insect Science*, **9**, 30.

Greeney, H.F., Dyer, L.A. & Smilanich, A.M. (2012) Feeding by lepidopteran larvae is dangerous: a review of caterpillars' chemical physiological, morphological, and behavioral defenses against natural enemies. *Invertebrate Survival Journal*, **9**, 7–34.

Gross, P. (1993) Insect behavioral and morphological defenses against parasitoids. *Annual Review of Entomology*, **38**, 251–273.

INMET (2012) *Clima*. Instituto Nacional de Meteorologia [WWW document]. URL http://www.inmet.gov.br/portal/index.php?r=clima/prev_estocastica [accessed on January 2012].

Inui, Y., Shimizu-kaya, U., Okubo, T., Yamsaki, E. & Itioka, T. (2015) Various chemical strategies to deceive ants in three *Arhopala* species (Lepidoptera: Lycaenidae) exploiting *Macaranga* myrmecophytes. *PLoS ONE*, **10**, e0120652.

Ito, F. & Higashi, S. (1991) Variance of ant effects on the different life forms of moth caterpillars. *Journal of Animal Ecology*, **60**, 327–334.

Johnson, J.B. & Omland, K.S. (2004) Model selection in ecology and evolution. *Trends in Ecology & Evolution*, **19**, 101–108.

Jones, M.T., Castellanos, I. & Weiss, M.R. (2002) Do leaf shelters always protect caterpillars from invertebrate predators? *Ecological Entomology*, **27**, 753–757.

Lichter-Marck, I.H., Wylde, M., Aaron, E., Oliver, J.C. & Singer, M.S. (2015) The struggle for safety: effectiveness of caterpillar defenses against bird predation. *Oikos*, **124**, 525–533.

Loeffler, C.C. (1996) Adaptive trade-offs of leaf folding in *Dichomeris* caterpillars on goldenrods. *Ecological Entomology*, **21**, 34–40.

Lopez, R. & Potter, D.A. (2000) Ant predation on eggs and larvae of the black cutworm (Lepidoptera: Noctuidae) and Japanese beetle (Coleoptera: Scarabaeidae) in turfgrass. *Environmental Entomology*, **29**, 116–125.

Mega, N.O. & de Araujo, A.M. (2008) Do caterpillars of *Dryas iulia alcionea* (Lepidoptera, Nymphalidae) show evidence of adaptive behaviour to avoid predation by ants? *Journal of Natural History*, **42**, 129–137.

Mody, K. & Linsenmair, K.E. (2004) Plant-attracted ants affect arthropod community structure but not necessarily herbivory. *Ecological Entomology*, **29**, 217–225.

Mooney, K.A., Pratt, R.T. & Singer, M.S. (2012) The tri-trophic interactions hypothesis: interactive effects of host plant quality, diet breadth and natural enemies on herbivores. *PLoS ONE*, **7**, e34403.

Moraes, A.R., Greeney, H.F., Oliveira, P.S., Barbosa, E.P. & Freitas, A.V.L. (2012) Morphology and behavior of the early stages of the skipper, *Urbanus esmeraldus*, on *Urera baccifera*, an ant-visited host plant. *Journal of Insect Science*, **12**, 52.

Murphy, S.M., Leahy, S.M., Williams, L.S. & Lill, J.T. (2010) Stinging spines protect slug caterpillars (Limacodidae) from multiple generalist predators. *Behavioral Ecology*, **21**, 153–160.

Ness, J.H., Morris, W.F. & Bronstein, J.L. (2009) For ant-protected plants, the best defense is a hungry offense. *Ecology*, **90**, 2823–2831.

Nosil, P. & Crespi, B.J. (2006) Experimental evidence that predation promotes divergence in adaptive radiation. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 9090–9095.

Oliveira, P.S. (1997) The ecological function of extrafloral nectaries: herbivore deterrence by visiting ants and reproductive output in *Caryocar brasiliense* (Caryocaraceae). *Functional Ecology*, **11**, 323–330.

Oliveira, P.S. & Brandão, C.R.F. (1991) The ant community associated with extrafloral nectaries in Brazilian cerrados. *Ant-Plant Interactions* (ed. by D. F. Cutler and C. R. Huxley), pp. 198–212. Oxford University Press, Oxford, U.K.

Oliveira, P.S. & Freitas, A.V.L. (2004) Ant-plant-herbivore interactions in the neotropical cerrado savanna. *Naturwissenschaften*, **91**, 557–570.

Oliveira-Filho, A.T. & Rater, J.A. (2002) Vegetation physiognomies and woody flora of the Cerrado biome. *The Cerrados of Brazil: Ecology and Natural History of a Neotropical Savanna* (ed. by P. S. Oliveira and R. J. Marquis), pp. 91–120. Columbia University Press, New York, New York.

Portugal, A.H.A. & Trigo, J.R. (2005) Similarity of cuticular lipids between a caterpillar and its host plant: a way to make prey undetectable for predatory ants? *Journal of Chemical Ecology*, **31**, 2551–2561.

Price, P.W., Bouton, C.E., Gross, P., McPheron, B.A., Thompson, J.N. & Weis, A.E. (1980) Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. *Annual Review of Ecology and Systematics*, **11**, 41–65.

Price, P.W., Denno, R.F., Eubanks, M.D., Fink, D.L. & Kaplan, I. (2011) *Insect Ecology: Behavior, Populations and Communities*. Cambridge University Press, Cambridge, U.K.

Raudenbush, S.W., Yang, M. & Yosef, M. (2000) Maximum likelihood for generalized linear models with nested random effects via high-order, multivariate Laplace approximation. *Journal of Computational and Graphical Statistics*, **9**, 141–157.

R Development Core Team (2015) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Remmel, T., Davison, J. & Tammaru, T. (2011) Quantifying predation on folivorous insect larvae: the perspective of life-history evolution. *Biological Journal of the Linnean Society*, **104**, 1–18.

Ribas, C.R., Schoereder, J.H., Pic, M. & Soares, S.M. (2003) Tree heterogeneity, resource availability, and larger scale processes regulating arboreal ant species richness. *Austral Ecology*, **28**, 305–314.

Rico-Gray, V. & Oliveira, P.S. (2007) *The Ecology and Evolution of Ant-Plant Interactions*. University of Chicago Press, Chicago, Illinois.

Rudgers, J.A. (2004) Enemies of herbivores can shape plant traits: selection in a facultative ant-plant mutualism. *Ecology*, **85**, 192–205.

Ruxton, G.D., Sherratt, T.N. & Speed, M.P. (2004) *Avoiding Attack: The Evolutionary Ecology of Crypsis, Warning Signals, and Mimicry*. Oxford University Press, Oxford, U.K.; New York, New York.

Salazar, B.A. & Whitman, D.W. (2001) Defensive tactics of caterpillars against predators and parasitoids. *Insects and Plant Defences Dynamics* (ed. by T. N. Ananthakrishnan), pp. 161–207. Science Publishers, Inc., Plymouth, U.K.

Sam, K., Koane, B. & Novotny, V. (2015) Herbivore damage increases avian and ant predation of caterpillars on trees along a complete elevational forest gradient in Papua New Guinea. *Ecography*, **38**, 293–300.

Sandre, S.L., Tammaru, T. & Mänd, T. (2007) Size-dependent colouration in larvae of *Orgyia antiqua* (Lepidoptera: Lymantriidae): a trade-off between warning effect and detectability? *European Journal of Entomology*, **104**, 745–752.

Schatz, B., Lachaud, J.-P. & Beugnon, G. (1997) Graded recruitment and hunting strategies linked to prey weight and size in the ponerine ant *Ectatomma ruidum*. *Behavioral Ecology and Sociobiology*, **40**, 337–349.

Schoereder, J.H., Sobrinho, T.G., Madureira, M.S., Ribas, C.R. & Oliveira, P.S. (2010) The arboreal ant community visiting extrafloral nectaries in the Neotropical cerrado savanna. *Terrestrial Arthropod Reviews*, **3**, 3–27.

Schoonhoven, L.M., van Loon, J.A. & Dicke, M. (2005) *Insect-Plant Biology*. Oxford University Press, New York, New York.

Sendoya, S.F. & Oliveira, P.S. (2015) Ant-caterpillar antagonism at the community level: interhabitat variation of tritrophic interactions in a neotropical savanna. *Journal of Animal Ecology*, **84**, 442–452.

Sendoya, S.F., Freitas, A.V.L. & Oliveira, P.S. (2009) Egg-laying butterflies distinguish predaceous ants by sight. *American Naturalist*, **174**, 134–140.

Sendoya, S.F., Blüthgen, N., Fernandez, F., Tamashiro, J. & Oliveira, P.S. (2016) Foliage-dwelling ants in a neotropical savanna: effects of plant and insect exudates on ant communities. *Arthropod-Plant Interactions*, **10**, 183–195.

Singer, M.S., Farkas, T.E., Skorik, C.M. & Mooney, K.A. (2012) Tritrophic interactions at a community level: effects of host plant species quality on bird predation of caterpillars. *American Naturalist*, **179**, 363–374.

Stamp, N. (2001) Enemy-free space via host plant chemistry and dispersion: assessing the influence of tri-trophic interactions. *Oecologia*, **128**, 153–163.

Sugiura, S. & Yamazaki, K. (2006) The role of silk threads as lifelines for caterpillars: pattern and significance of lifeline-climbing behaviour. *Ecological Entomology*, **31**, 52–57.

Sugiura, S. & Yamazaki, K. (2014) Caterpillar hair as a physical barrier against invertebrate predators. *Behavioral Ecology*, **25**, 975–983.

Thaler, J.S. & Griffin, C.A.M. (2008) Relative importance of consumptive and non-consumptive effects of predators on prey and plant damage: the influence of herbivore ontogeny. *Entomologia Experimentalis et Applicata*, **128**, 34–40.

Tscharntke, T. & Hawkins, B.A. (2002) Multitrophic level interactions: an introduction. *Multitrophic Level Interactions* (ed. by T. Tscharntke and B. A. Hawkins), pp. 1–7. Cambridge University Press, Cambridge, U.K.

Tvardikova, K. & Novotny, V. (2012) Predation on exposed and leaf-rolling artificial caterpillars in tropical forests of Papua New Guinea. *Journal of Tropical Ecology*, **28**, 331–341.

Weiss, M.R. (2003) Good housekeeping: why do shelter-dwelling caterpillars fling their frass? *Ecology Letters*, **6**, 361–370.

Yamazaki, K. (2010) Parachuting behavior and predation by ants in the nettle caterpillar, *Scopelodes contracta*. *Journal of Insect Science*, **10**, 1–10.