

Protection from herbivores varies among ant genera for the myrmecophilic plant *Leea aculeata* in Malaysian Borneo

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ABSTRACT. Some plants use food bodies to attract ants that then provide protection from herbivory. A brief report from 1898 describes the myrmecophilic plant *Leea aculeata* Bl. as bearing food bodies on its young shoots, which accumulate when they are not harvested by ants. However, ant efficacy in deterring herbivores and consequences for herbivory rates remain unknown. Here we investigate (1) which ant taxa patrol these plants and whether they remove significant numbers of food bodies, (2) if these ants attack herbivores, and (3) if any anti-herbivore activity correlates negatively with herbivory. We found that a diverse community of ants patrolled young *L. aculeata* shoots and removed food bodies (1.2 food body per cm² per 24 h), with food bodies accumulating when ants are experimentally excluded. Attack rates on surrogate herbivores (termite baits) differed among ant genera, with *Crematogaster* and *Lophomyrmex* being most active. Although herbivory did not differ among ant genera, herbivory was greater when ants took a longer time to detect herbivores and recruit fellow ants, providing evidence for the mutualism of *L. aculeata* with ants. However, the variation in protection among ant genera raises questions regarding the stability of this mutualism in the face of exploitation by ants.

Keywords myrmecophily; mutualism; food bodies; herbivory; *Leea*

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INTRODUCTION

Interactions between ants and plants are common in many terrestrial habitats (Davidson & McKey 1993), particularly in tropical ecosystems where diversity of both plants and ants is higher than elsewhere (Hölldobler & Wilson 1990; Mayer *et al.* 2014). Such interactions range from obligate, when the ant partner inhabits the plant (myrmecophyte), to facultative and generalized, when the ant partner nests elsewhere (myrmecophile; Bronstein *et al.* 2006; Rico-Gray & Oliveira 2007). Over millions of years of co-evolution between these two groups, plants have developed food resources to specifically attract ants, including structures such as extrafloral nectaries (EFNs) and food bodies (O'Dowd 1982; Dutra *et al.* 2006; Ness *et al.* 2006). Both parties of this ant-plant interaction seem to benefit: Ants gain access to nutrients or plant-grown nesting sites (domatia), while plants obtain protection from herbivory as ants can drive away or predate leaf-eating arthropods (Janzen 1972; Rosumek *et al.* 2009) and defend against fungal pathogen infection (Letourneau 1998). Plants can experience additional benefits where ants prune encroaching vegetation (Federle *et al.* 2002), clear plants of microepiphylls (Miler *et al.* 2016), or provide nutrients to their plant partner (Chanam *et al.* 2014). The plant partner may even gain additional mineral nutrients indirectly, as ground-nesting ants that feed on EFNs tend to nest close to EFN-providing plants (Janzen 1972; Wagner & Nicklen 2010). Stability of these ant-plant mutualisms may vary, with stronger selective pressures in obligate interactions where partners are intimately dependent on each other than in facultative interactions, which tend to be more opportunistic in nature and include multiple ant partners (Bronstein *et al.* 2006; Kessler & Heil, 2011).

The interactions between ants and plants have been extensively studied, in particular those with myrmecophytes and those in which myrmecophiles attract ants using EFNs (Fiala & Linsenmair 1995; Mayer *et al.* 2014; Heil 2015; Nelsen *et al.* 2018). However, for many myrmecophilic species with ant-attracting structures, the benefits for plants do not seem clear. Food bodies are small epidermal structures contain-

ing highly nutritious substances, such as carbohydrates, proteins and lipids that are produced to attract foraging ants (Risch & Rickson 1981; Heil *et al.* 1998; Fischer *et al.* 2002; Heil *et al.* 2004a). Food body production has been reported in at least 50 genera of plants worldwide (Beattie & Hughes 2002). Although food bodies are known as ant attractants and have proven to be an important resource for resident ants of myrmecophytic plants (e.g. O'Dowd 1982; Heil *et al.* 1997; Dinda & Mondal 2004), collection by ants patrolling on myrmecophilic plants has only been directly confirmed in a relatively small number of cases (Risch & Rickson 1981; O'Dowd 1982; Beattie 1985; Fiala & Linsenmair 1995).

Numerous studies show that nutrients produced by plants often attract multiple ant species (Apple & Feener 2001) and in some cases, these species coexist on a single host plant, sharing available food resources (Blüthgen *et al.* 2000). Ant species can vary in their body size, colony size and their level of aggression towards potential herbivores (Djiéto-Lordon *et al.* 2004; Ness *et al.* 2006; Shimoji *et al.* 2014). Ants of the genera *Crematogaster*, *Pheidole*, *Pristomyrmex* and *Formica* are known to show aggressive behaviour against herbivores when they occur on their plant-partner (Yamawo *et al.* 2017). Other genera such as *Camponotus* and *Nylanderia* can be less effective in deterring herbivores (Yamawo *et al.* 2017). This behaviour might be caused by different foraging strategies as more aggressive ants forage in groups whereas less aggressive species tend to be solitary foragers (Yamawo *et al.* 2017). Therefore, ant species involved in symbiosis with a myrmecophilic plant may vary in the effectiveness of protection provided against herbivory (Ness *et al.* 2006; Xu & Chen 2010; Yamawo *et al.* 2017).

This study focuses on ant-plant interactions on the species *Leea aculeata* (Bl. ex. Spreng), a woody vine from the family Vitaceae (the plant list, 2013; APG IV, 2016), although recent research shows that the genus *Leea* may taxonomically be better placed in the separate family Leeaceae (Zhang *et al.* 2016; Wen *et al.* 2018; Ma *et al.* 2020). This species initially grows as a freestanding plant, and only later uses other woody stems as support. *Leea aculeata* is a

pioneer plant species, usually found in secondary regrowth and disturbed habitats of mixed dipterocarp forest, being most common on alluvial sites, along rivers and streams (Nakashima *et al.* 2010; Cleary 2017). The species is distributed throughout south-east Asia, in Sumatra, Java, Borneo, the Philippines, Moluccas, Celebes and New Guinea (Wen 2007). *Leea aculeata* produces food bodies on the leaves and stems of young shoots and EFNs on the stipules of young leaves that fall off as the shoot matures, although it lacks domatia (ant housing). Food bodies were first reported in the genus *Leea* in 1898 when Raciborski (1898) related the following:

“Closer observations have shown that most of *Leea* species provide food for ants in the form of food bodies. [...] During one of my visits to [the Bogor Botanical Gardens], as a pre-study on food bodies, I was able to inspect the *Leea* species that are cultivated in the botanical garden and was able to observe them on young shoots, petioles, stipules, and also on the underside of the leaves along the main veins for *Leea divaricata*, *L. sumatrana*, *L. aculeata*, while the spiny *Leea horrida* does not form any.” (Translated from the original: “Eine genauere Untersuchung hat gezeigt, dass die meisten Leeaarten den Ameisen Nahrung in der Form jener kleinen Ameisenbrödchen liefern. [...] Bei einem meiner Besuche in Buitenzorg konnte ich die in dem botanischen Garten daselbst cultivirten Leeaarten in Bezug auf Vorhandensein der Ameisenbrödchen untersuchen und habe dieselben an jungen Sprossen, Blattstielen, Nebenblättern, auch an der der Blätter längs der Hauptnerven noch bei *Leea divaricata* T. B., *L. sumatrana*, *L. aculeata* Bl. constatiren können, während die stachlige *Leea horrida* T. et B. gar keine bildet.”)

For *L. aequata* L., Raciborski described ants removing food bodies from the youngest shoots, and an increase of food body density when ants were excluded, indicating that substantial numbers of food bodies were removed by the ants. However, despite this intriguing finding, Raciborski did not report sample sizes nor effect sizes, nor whether the results differed between ant species.

Food body structures of myrmecophytes generally contain lipids, proteins and carbohydrates, while those produced by *Leea* species, like those of other myrmecophiles, tend to be rich in sugars and starches (O'Dowd 1982; Heil *et al.* 1998). Therefore, *Leea* food bodies are potentially effective attractants for a wide range of ant species. However, there has been no further research on ant-plant interactions in the genus *Leea* since this anecdotal report from the end of the 19th century. Whether the ants consistently remove food bodies, if they protect the plants from leaf-eating insects, and how ant taxa differ in the protection they provide remain unknown for this system. This study aims to understand the role of ants on *L. aculeata* as protection from herbivores and to explore the role of food bodies in this interaction. We address the following questions:

1. Do ants remove significant numbers of food bodies, and hence does experimental exclusion of ants cause food bodies to accumulate on young shoots of *L. aculeata*?
2. Do different genera of ants on young shoots of *L. aculeata* differ in their protective behaviours they display against herbivores?
3. Do any differences in protective behaviour translate into differences in amounts of herbivore damage on young shoots?

METHODS

Study area

Data collection took place from 19 to 24 October 2019 close to Danum Valley Field Centre, in the eastern part of Sabah, Malaysian Borneo (Fig. 1), with most sampling being conducted just outside of Danum Valley Conservation Area along the Segama River (Fig. 1). The study area consisted of primary lowland dipterocarp rainforest that is part of the Class I Ulu Segama Forest Reserve. The areas sampled were lightly disturbed, being moderately close to the area cleared for the field centre, and to the Segama River. The local aseasonal climate has an annual rainfall of approximately 2669 mm, mean humidity of 72%, and annual mean temperature of 26.7°C with a daily variation of 8.4°C (Walsh & Newbery 1999).

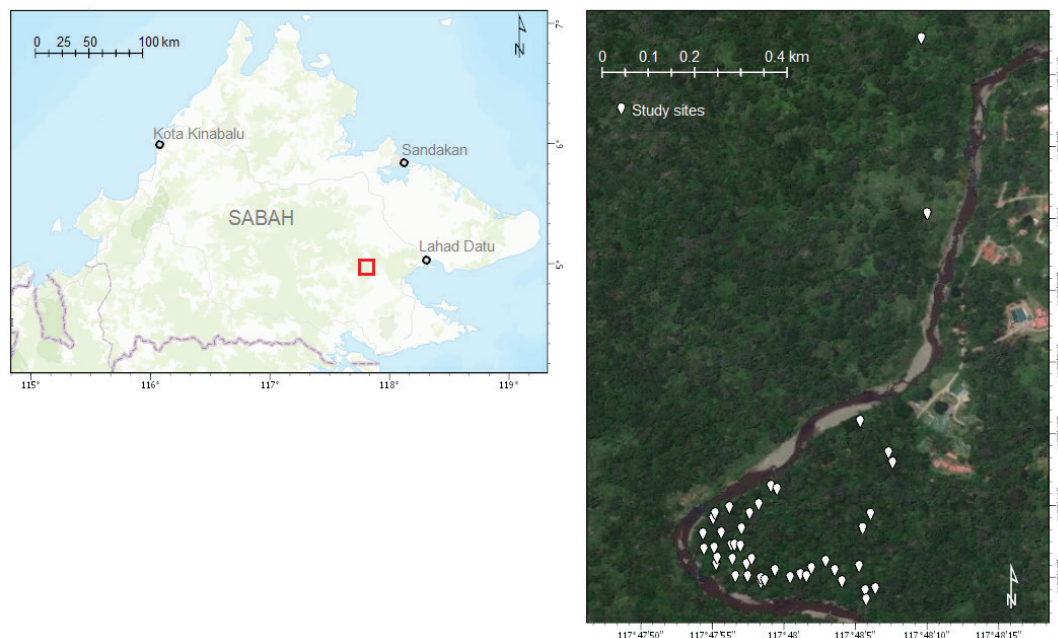


Fig. 1. Maps of the location of the study area at Danum Valley in East Sabah, Malaysia. (Left) The red square indicates the location of Danum Valley. (Right) Distribution of the sampled *Leea aculeata* individuals in the study area, (Maps made in ArcGIS Pro; Satellite image [B] from Basemap “World Imagery” from Esri; 2019)

Impacts of experimental ant exclusion on food body density: data collection

To test whether ants were removing significant numbers of food bodies from *L. aculeata*, and hence influencing food body accumulation, we experimentally excluded ants from young shoots. We defined a young shoot as the most recently developed shoot from an apical meristem bearing newly flushed leaves with red pigment. We chose 18 individuals of *L. aculeata* (Fig. 2) that each had two young shoots of similar age. For each plant, shoots were randomly assigned to either Control or Enclosure groups. Without damaging the shoots, all food bodies on the two shoots were gently removed with a soft brush to ensure that both shoots were free of food bodies at the start of the experiment. Next, a 0.5 mm mesh nylon bag was placed over the Enclosure shoot and secured with masking tape for a period of 24 hours. This was to prevent ants from accessing the shoot and removing food bodies. The unbagged shoot represents the Control in the experiment. After the 24-hour interval, food body density was estimated for shoots from each group. The number of food bodies was counted in a 1 cm × 1 cm square aperture in a piece of black plastic randomly placed abaxially on each of two

selected leaflets (of similar size) on both the Control and Enclosure shoot (four leaflets per plant in total). From these measurements on the two selected leaflets, a mean was calculated for each shoot. Our exclusion method could not distinguish increased production of food bodies in response to ant exclusion, as opposed to reduced removal by ants. However, the facts that we observed multiple cases of ants removing food bodies on controls, and that our exclusions were highly effective at preventing ant foraging (no ants were observed within the cloth bags), indicates that any changes are likely to be caused by reductions in ant removal rates.

Impacts of experimental ant exclusion on food body density: data analysis

All data analyses were performed in the program R (v. 4.0.2; RCoreTeam 2019) using the RStudio environment (RStudioTeam 2018). To test for accumulation of food bodies on leaflets when ants were excluded, a paired-sample t-test was performed, using the *t.test* function. The distribution of group differences was assessed and checked for outliers, independence of sampling, normality and homoscedasticity.

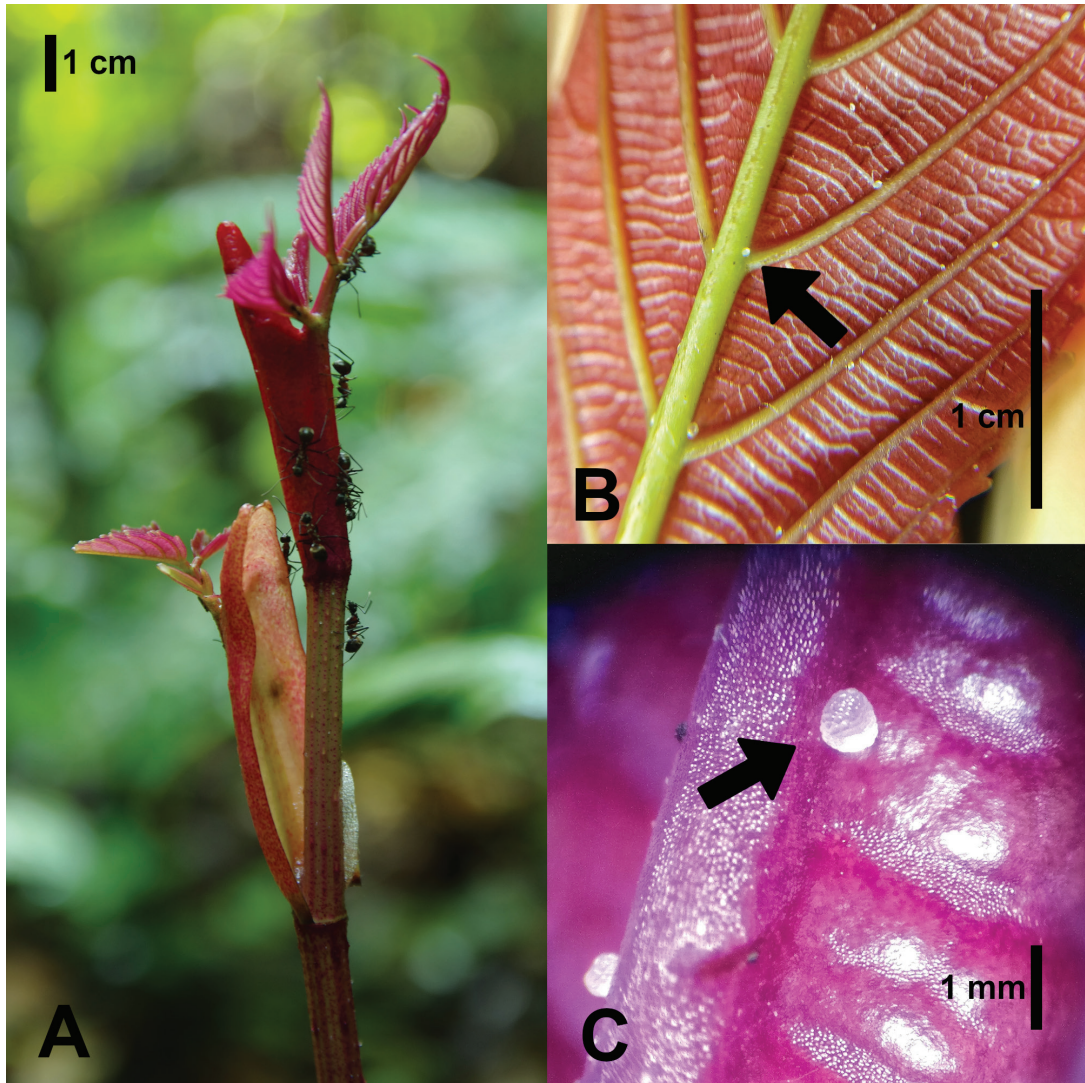


Fig. 2. Photographs of a young shoot of *Leea aculeata*. (A) A young shoot with ants (*Polyrhachis* sp.) foraging on food bodies and EFNs. (B) A young abaxial leaflet of *L. aculeata* covered with food bodies with a black arrow indicating a single food body. (C) Detail of a food body on an abaxial leaflet with a black arrow indicating the food body

Testing anti-herbivory defence by ants: data collection

An additional 56 *L. aculeata* individuals were identified on which herbivory damage and ant protection services on young shoots were measured. Herbivory was measured using two methods: (i) the proportion of leaflets damaged by herbivory out of the total number of leaflets per shoot (mean \pm SD = 8.2 ± 3.7), hereafter referred to as *leaflet-herbivory*; and (ii) a visually estimated percentage of leaf area lost to herbivory

for the shoot (precision to nearest 5% if greater than 10%, and to the nearest 1% if below 10%), hereafter referred to as *leaf-area-herbivory*. The aggression of ants defending plants was measured using termite baiting. Termites do not occur naturally on leaves and do not behave as typical free feeding herbivores. However, when pinned through a leaf, they can represent a suitable surrogate for herbivorous insects (Heil *et al.* 2004b). Furthermore, termite workers are easy to acquire in large numbers, providing an easily replicable

assay. This method is used to evaluate and compare aggression of ants towards potential herbivores and their protective behaviour regarding the plant, but the method does not yield information on effectiveness of ants in deterring particular herbivores (Bentley 1981; Barton 1986; Oliveira *et al.* 1987; Oliveira 1997; Apple & Feener 2001). We used *Macrotermes gilvus* and *Dicuspitermes spp.* worker termites as live pinned baits as surrogate herbivores since large numbers of these undefended insects were available in the study area. In the field, we observed that workers from both termite species did not show any defensive behaviour apart from occasional biting. A live termite and a paper control (5 mm × 5 mm) were pinned to a basal leaflet approximately 2 cm from the petiole and from each other. We used a paper control to enable us to test whether ant reactions were simply those expected towards a foreign object in the territory of the colony. During the ten-minute interval following bait placement, we recorded, for both the termite and the paper control following methods of Edwards *et al.* (2010) and Plowman *et al.* (2017):

- i. The time until first discovery by an ant, i.e. touching the bait/control with the ant's antennae.
- ii. The time until the arrival of the first recruited ant, defined as the second ant arriving.
- iii. Total period of attack by ant(s), defined as biting using mandibles.
- iv. The maximum number of ants involved in any attack event(s).

After the experiment, two to three ant individuals per species from each plant were collected and stored in 70% ethanol. Ant identification was then performed *ex situ* using an Olympus SZ51 (8X-40X magnification) dissecting microscope, following Fayle *et al.* (2014), with updates for recent taxonomic changes. Because we found a large number of ant species, we chose to test for differences in ant behaviour at the level of genus. Sample sizes for individual species would have been too small and would reduce statistical power.

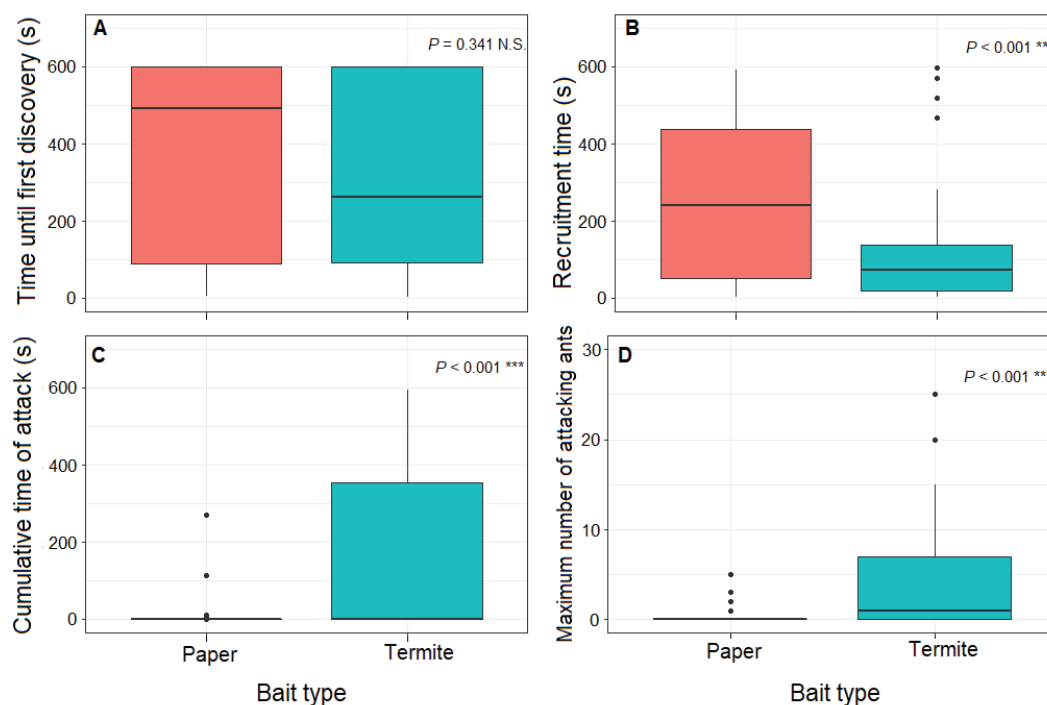


Fig. 3. Box plots showing the response differences between the two bait types, i.e. the paper control (in red) and the live termite bait (in blue), for (A) time until discovery, (B) recruitment time, (C) cumulative period of attack, and (D) the maximum number of attacking ants. Significance levels of are represented in the top right of each panel.

Testing anti-herbivory defence by ants: data analysis

Using four paired Wilcoxon signed-rank tests (function *wilcox.test*) we tested if ants reacted to the termite bait in a manner distinct from their reaction to the non-insect foreign object (the paper control). This was the case: Ant discovery of the two baits did not differ (Wilcox: $W = 430.5$; $P = 0.341$; Fig. 3a), but for the paper control, compared to the live termite bait, recruit arrival was later (Wilcox: $W = 1380$; $P < 0.001$; Fig. 3b), attack period shorter (Wilcox: $W = 400.5$; $P < 0.001$; Fig. 3c) and maximum number of attacking ants lower (Wilcox: $W = 544.5$; $P < 0.001$; Fig. 3d). Having determined that ants were actively responding to the termite bait in a manner differing from their response to a novel inanimate control, for all subsequent analyses we present results relating only to the termite bait, not the paper control.

Attack frequency was assigned to each ant genus based on the number of plants on which ants of that genus initiated an attack towards the termite bait. Difference in attack frequency on termite baits among genera was assessed using Fisher's exact-test (because some of the expected values were < 5 ; electronic supplementary material Table. S1; function *fisher.test*). A linear model and a generalized linear model (functions *lm* and *glm*) were used to test if there was a difference in leaf-area-herbivory and leaflet-herbivory, respectively, between plants where ants attacked the termite bait and plants where the bait was not attacked. We expected that plants where ants attacked the termite bait would be better protected from herbivores, and hence would have experienced less herbivory. The generalized linear model with leaflet-herbivory as the response was fitted to the binomial distribution of the data. Leaf-area-herbivory was transformed using the natural logarithm such that the model's residuals follow a normal distribution. Cook's distance and residual plots were inspected to check for outliers, independence of sampling, normality and homoscedasticity. From this it was apparent that after transformation the linear model's assumptions were met.

We tested for differences among ant genera in the time until discovery, recruitment time, maximum number of attacking ants, the

cumulative time of attack, leaf-area-herbivory and leaflet-herbivory. Only genera present on > 3 plants were included in analyses. Therefore, several ant genera were excluded from analyses (see electronic supplementary material, Table S2 for the genera included in analyses). A linear model was run for time until discovery as a response variable, using the *lm* function. Differences among genera were further assessed using Tukey's Honest Significant Difference post-hoc tests with the *glht* R function from the *multcomp* package (Hothorn *et al.* 2008). Assumptions of normality and homoscedasticity of the model's residuals were inspected using residual plots. Models with recruitment time, cumulative attack time, maximum number of attacking ants and leaf-area-herbivory as response deviated from these assumptions. These analyses were therefore performed using non-parametric models, i.e. using a Kruskal-Wallis analysis of variance for each (with the *kruskal.test*). We further analysed differences between genera using Dunn's tests of multiple comparisons (*dunn.test* in the *dunn.test* package; Dinno 2017). To test for a difference in leaflet-herbivory a generalized linear model (the *glm* function) was performed with binomial error distribution.

We hypothesised that plants on which ants more rapidly discovered herbivores, recruited more rapidly, attacked for longer, and attacked with greater numbers of workers would experience less herbivory. Hence, we performed multiple regression and multiple logistic regression, using the *lm* and *glm* functions (with a binomial distribution), with leaf-area-herbivory and leaflet-herbivory as the responses, respectively. Time until discovery, recruitment time, cumulative time of attack and the maximum number of attacking ants, as well as the interaction between time until discovery and recruitment time, were included as predictors. These analyses were performed only for those trials in which ants discovered the termite bait within the 10-minute observation interval. To improve the fit of the model, we applied model reduction: we sequentially excluded predictor variables that explained the least variation in the model, using a cut-off value of $P > 0.1$. For the logistic regression, we controlled for marginally non-significant overdispersion by recalculating standard errors with the function

anova.glm, as the model showed slight signs of overdispersion (dispersion parameter = 1.23; $P = 0.064$; as calculated with *testDispersion* from the *DHARMA* package; Hartig 2020). Variance inflation factor (VIF) values and a correlation matrix were inspected to corroborate the absence of collinearity between predictor terms (Fig. S1). For this, we used the functions *vif* and *rcorr* from the *car* (Fox & Weisberg, 2019) and *Hmisc* packages in R (Harrell *et al.* 2019), respectively.

RESULTS

Impacts of experimental ant exclusion on food body density

Leaflets from which ants were excluded had significantly more food bodies (FBs) 24 hours after clearing than control leaflets (t-test: $t = 4.02$; $df = 17$; $P < 0.001$). Control shoots had an average of 1.2 FB/cm² fewer than Exclosure shoots after 24 hours (95% CI = 0.6–1.8). The ant genus that caused the greatest difference between treatments was *Lophomyrmex*. When this genus was present, it reduced food body density by 3.0 FB/cm² (Fig. 4); On plants patrolled entirely by *Crematogaster*, the most abundant ant genus found on *L.*

aculeata (Fig. 5a), mean food body density was reduced by 1.0 FB/cm² on Exclosure shoots compared to Control shoots. The only genera of ants that did not have higher food body density on the Exclosure shoots were *Colobopsis*, *Polyrhachis* and *Paraparatrechina* (Fig. 4b).

Testing anti-herbivory defence by ants

L. aculeata shoots were patrolled by either one (89%) or two (11%) ant species. The total number of recorded ant morphospecies was 26, representing 12 genera and three subfamilies (electronic supplementary material, Tab. S3). Of the termite baits placed on the 56 sampled plants, 48.2% were attacked by ants. The observed ant genera differed significantly in attack frequency (Fisher's exact-test: $P < 0.006$; Fig. 5a). Being present on 25 of the sampled plants, *Crematogaster* was the most common ant genus. Of the ant genera encountered on five or more plants (four ant genera in total), *Crematogaster* was the only genus to attack termite baits on a majority of the plants on which it was present (18 of 25 plants; 72%; Fig. 5a). Other common ant genera attacked the termite bait less frequently: *Paraparatrechina* (1 of 5 plants), *Tetramorium* (1 of 8 plants), and *Polyrhachis* (2 of 9 plants).

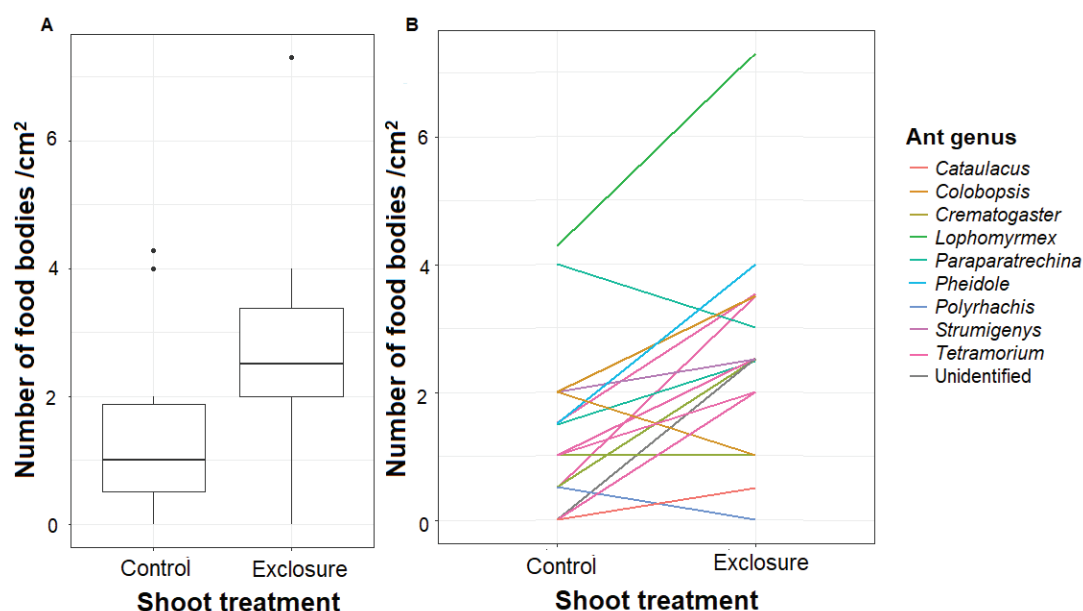


Fig. 4. Changes in accumulated food body density over a 24-hour period on young shoots of *L. aculeata*, for shoots from which ants had been experimentally excluded ('Exclosure') and ant-patrolled controls ('Control') plotted for (A) the different treatment groups and (B) the mean difference between treatments for each ant genus.

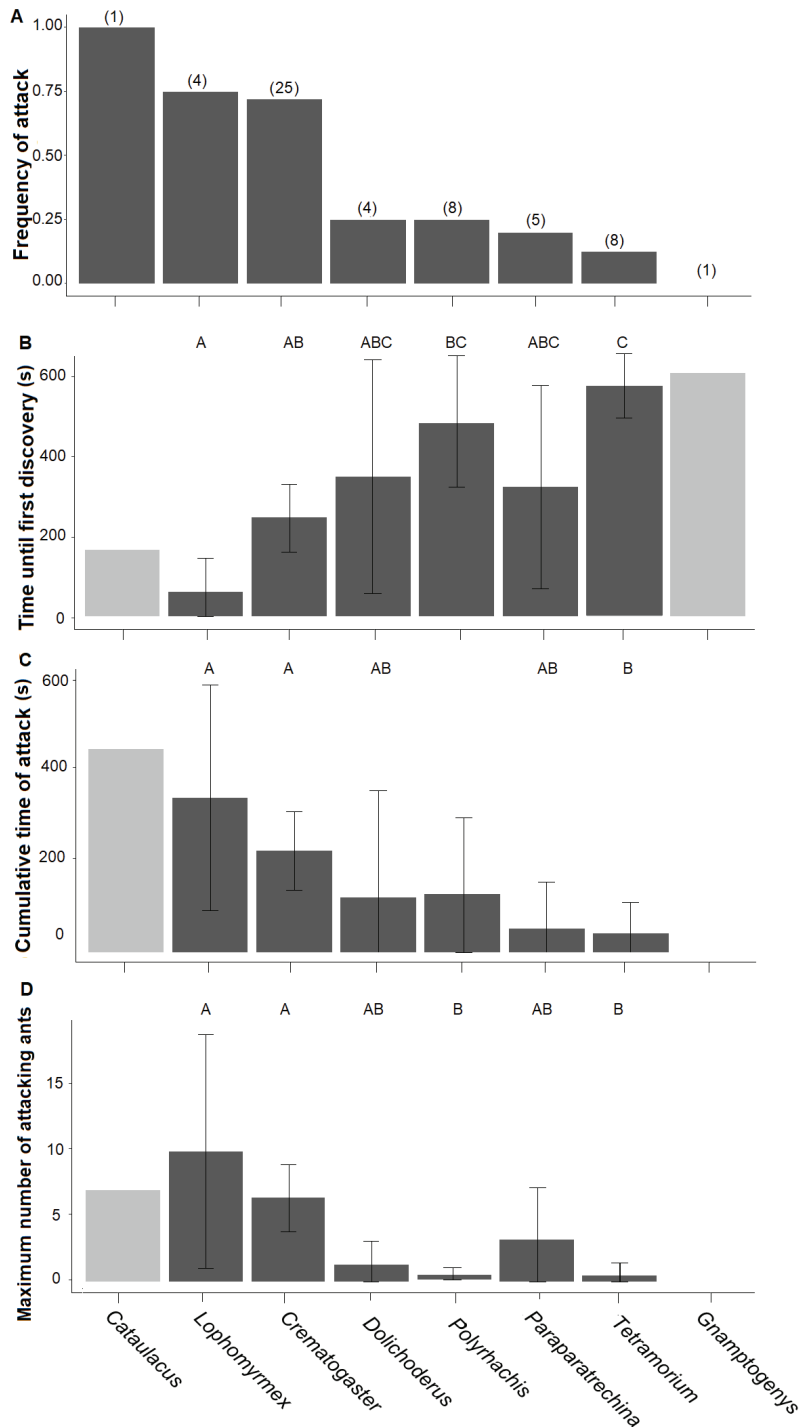


Fig. 5. Differences among ant genera in (A) the frequency of attack, (B) the time until first discovery, (C) the cumulative time of attack, and (D) the maximum number of ants involved in the attack. The total number of plants that the genera were observed on is given above the bars in (A). Bars in (B-D) present the mean for each genus (with error bars displaying 95% confidence intervals). Bars are light grey for those genera that were observed on less than three plants and consequently were not included in statistical analyses. Significant differences between genera are presented for (B-D) with letters for a level of statistical significance of $P < 0.05$.

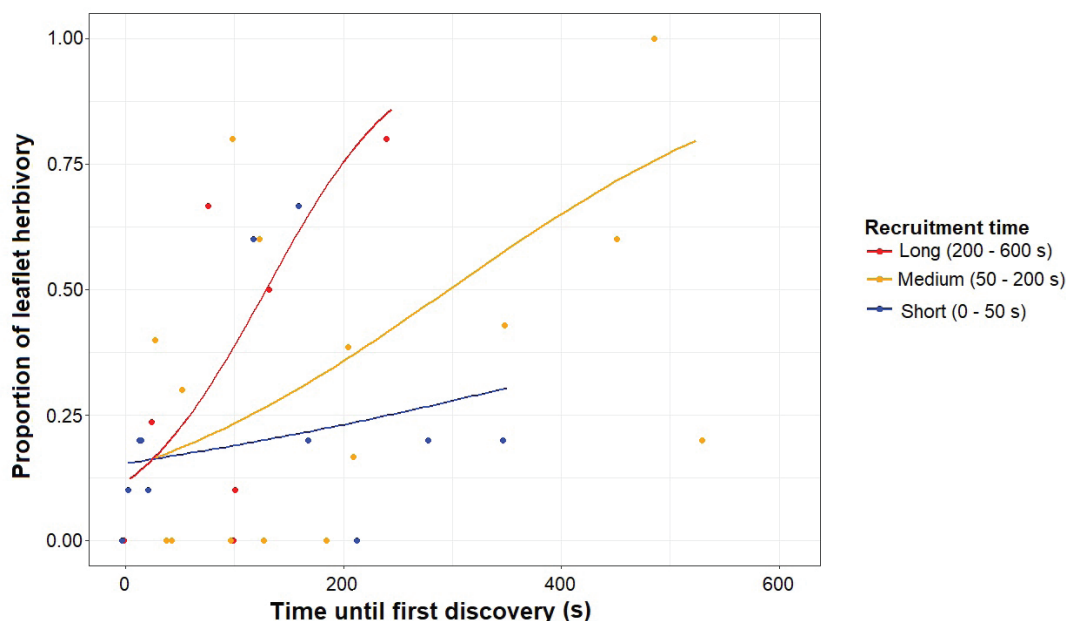


Fig. 6. The relationship between the proportion of leaflet-herbivory and time until discovery. This relationship is shown for a long (200 - 600 s), medium (50- 200 s), and short (0 - 50 s) recruitment time. Trend-lines (produced with the *predict* R-function) are calculated for a long (400 s), medium (125 s), and short (25 s) recruitment time.

Table 1. Regression statistics of the effects of the predictor variables describing ant aggressiveness on the proportion of herbivory damage across all *L. aculeata* individuals on which termite baiting experiments were executed. *Leea aculeata* plants patrolled by different ant species are pooled together here. The regression coefficient estimate (b), χ^2 -values, degrees of freedom (Df) and *P*-values for the logistic regression model are given for those predictors that were retained through backward model selection (using a cut-off *P*-value of 0.05). Significant *P*-values are presented in bold.

Predictors	Leaflet-herbivory			
	b	χ^2	Df	<i>P</i>
Time until discovery	$1.622 \cdot 10^{-3}$	0.981	31	0.322
Recruitment time	$-0.856 \cdot 10^{-3}$	0.440	30	0.507
Cumulative time of attack	-	-	-	-
Maximum number of attacking ants	-	-	-	-
Time until discovery * Recruitment time	$0.035 \cdot 10^{-3}$	6.741	29	0.022

Genera varied significantly in the time they took to discover the termite bait (One-Way ANOVA: $F_{5,48} = 4.784$; $P = 0.001$; Fig. 5b). Ant genera with a high attack frequency, such as *Lophomyrmex*, and *Crematogaster* (Fig. 5a), were faster at discovering the termite bait (Fig. 5b). Genera that were slower in discovering the herbivore were *Paraparatrechina*, *Polyrhachis*, and *Tetramorium* (Fig. 5a). Pairwise comparisons showed that both *Crematogaster* and *Lophomyr-*

mex were significantly faster than *Tetramorium* in discovering the termite bait (Tukey HSD: $t = 3.677$; $P = 0.007$; and $t = 3.844$; $P = 0.004$, respectively; Fig. 5b). *Polyrhachis* showed a similar trend to *Tetramorium*, being on average 235 and 418 seconds slower in discovery than *Crematogaster* (marginally non-significant) and *Lophomyrmex* (Tukey HSD: $t = 2.717$; $P = 0.085$; and $t = 3.208$; $P = 0.026$, respectively; Fig. 5b).

Ant genera did not differ in their mean recruitment time (electronic supplementary material, Fig. S2c; Kruskal-Wallis: $\chi^2_{2,25} = 0.569$; $P = 0.6102$) but did differ in their mean cumulative time of attack (Fig. 5c; Kruskal-Wallis: $\chi^2_{5,48} = 11.943$; $P = 0.036$) and the maximum number of ants involved in attacks (Fig. 5d; Kruskal-Wallis: $\chi^2_{5,48} = 16.592$; $P = 0.005$). *Crematogaster* and *Lophomyrmex* ants spent significantly more time attacking the bait than *Tetramorium* (Fig. 5c; Dunn's test, $\alpha = 0.025$: $Z = 2.59$; $P = 0.005$; and $Z = 2.21$; $P = 0.014$, respectively). *Paraparatrechina* ants showed a similar trend of spending less time in attack compared with *Crematogaster* and *Lophomyrmex* (Fig. 5c; Dunn's test, $\alpha = 0.025$: $Z = 1.98$; $P = 0.024$; and $Z = 1.90$; $P = 0.029$, respectively). Moreover, attacks by *Crematogaster* and *Lophomyrmex* involved a greater number of ants than attacks by *Polyrhachis* (Fig. 5d; Dunn's test, $\alpha = 0.025$: $Z = 2.77$; $P = 0.003$; and $Z = 2.33$; $P = 0.010$; respectively) or attacks by *Tetramorium* (Fig. 5d; Dunn's test, $\alpha = 0.025$: $Z = 3.04$; $P = 0.001$; and $Z = 2.50$; $P = 0.006$; respectively). We found no difference in the mean leaf-area-herbivory, nor in leaflet-herbivory, among different ant genera (electronic supplementary material, Fig. S2a-b; Kruskal-Wallis: $\chi^2_{5,48} = 8.730$; $P = 0.120$; and Logistic regression: $\chi^2_{5,48} = 11.26$; $P = 0.684$ respectively).

Neither leaf-area-herbivory, nor leaflet-herbivory, was affected by whether ants attacked the termite bait (electronic supplementary material, Fig. S3a-b; t-test: $t_{55} = -1.201$; $P = 0.235$; and logistic regression: $\chi^2_{3,59} = 7.623$; $P = 0.135$ respectively). However, on plants where the termite bait was discovered by ants, there was an interaction between the effects of time to discovery and recruitment time on leaflet-herbivory; The longer ants took to discover the bait, the stronger was the positive effect of recruitment time on leaflet-herbivory (i.e. where ants were slow to find the bait, herbivory was greater; Table 1; Fig. 6). The other behavioural descriptors (recruitment time, cumulative time of attack, and maximum number of attacking ants) were not significantly related to leaflet-herbivory (Table 1). Furthermore, leaf-area herbivory was not related to any of these factors.

DISCUSSION

We found that ants removed food bodies from young *L. aculeata* shoots, different ant genera varied in their plant protective behaviour, and that leaflet-herbivory of the plant was greater when ants were slow in discovering the herbivore and slow in recruiting fellow ants. These results confirm the mutualism of *L. aculeata* with ants and suggest that different ant genera could play different roles in this mutualism. Furthermore, by performing our ant-exclusion experiment, we successfully replicated the anecdotally reported study of Raciborski (1898) from over 100 years ago and verified his observation that food bodies accumulate when ants are unable to collect them.

Our study is the first to document ants patrolling on shoots of *L. aculeata* and shows that the 12 different ant genera vary in the protection they provide for the plant. Specifically, we observed differences among genera in attack frequency, time until discovery, cumulative time of attack and maximum number of attacking ants. These differences could be due to variation in aggressiveness and foraging behaviour (Yamawo *et al.* 2017). The genus *Crematogaster*, the most commonly found genus in our study, is known for its predatory and defensive behaviour (Richard *et al.* 2001; Yamawo *et al.* 2017). Most species in this genus are arboreal (Blaimer 2012) and often associate closely with plants, forming mutualisms (Eguchi *et al.* 2011). *Crematogaster* has also been shown to exhibit dietary preference for carbohydrate rich resources in Danum Valley by Menzel *et al.* (2012), which may explain the high abundance of *Crematogaster* on *L. aculeata* in our study area since it produces such resources. In our study, *Crematogaster* and *Lophomyrmex* showed the strongest defensive behaviour against herbivores, with high frequency of attack, short time until discovery of the termite bait, and a long cumulative time of attack, relative to other ant genera. Additionally, *Lophomyrmex* was found to attack with a significantly greater maximum number of ants compared to the other genera. This genus is seldom reported as a mutualist, with only a single study reporting protection of pollination in a species of *Ficus* (Lin *et al.* 2018). However, *Lophomyrmex* is potentially an important preda-

tor of termites in Sabah (Tuma 2020), and hence further testing using non-termite baits would be needed to show that this response does not relate uniquely to termites for this genus.

Interestingly, we also discovered that there were some ant genera patrolling *L. aculeata* while not showing effective protection of the shoots: *Tetramorium*, *Paraparatrechina*, and *Polyrhachis* showed a considerably lower attack frequency than did other ants. We noted one *L. aculeata* individual patrolled by *Tetramorium insolens* ants, a species not native to (Bolton 1977) and that did not attack the termite bait on this plant. However, a single individual is too small a sample size on which to base speculation about behaviour of non-native species in this mutualism. In addition, we observed *Tetramorium* and *Paraparatrechina* to attack for a significantly shorter time than other genera. *Tetramorium* and *Polyrhachis* also attacked with a smaller number of ants and were significantly slower than other ant genera in discovering the termite bait. Thus, these three genera seem to provide the plant with less effective protection against herbivores but still exploit food bodies (and EFN resources). This could well be due to natural differences among ant genera in their feeding ecology and competitive behaviour as some, such as *Crematogaster*, are naturally more predatory and aggressive while others, such as *Polyrhachis*, forage more on nectar and other plant resources rather than pursuing meat prey (Blüthgen *et al.* 2003). Exploitation by ants in ant-plant mutualisms has been observed in other studies (Kautz *et al.* 2009; Palfi *et al.* 2020) and can either result from a previously mutualistic ant partner that has recently developed to exploit their plant-partner or an outside party exploiting the mutualism (Bronstein 2001). Another explanation for the presence of exploiters on our myrmecophilic plant could be that such facultative relationships which attract ants in a more opportunistic way are more prone to fall prey to ants that are less active in defending plants. Which of these strategies applies to the mutualism between *L. aculeata* and its ants requires further study. Yet, such ants may still provide some indirect benefit to the plant compared to plants without any patrolling ants. Even if benefits provided by low-quality ant partners are low, if the corresponding costs to the plant are

also small, then the plant may still receive fitness benefits from ant presence (Stanton & Palmer 2011). An ant exclusion experiment where plant fitness is measured and compared between plants with and without ants (much like the experiment by Rudgers and Strauss; 2004) could be fruitful for future research on *L. aculeata*. This might shed light on the costs incurred and benefits received by the plant from ants that are not active in defending plants when even these ants are excluded. Finally, it is worth noting that plants hosting ants that are not active defenders may experience opportunity costs if presence of such ants prevents protection by more active ant species (Gaume & McKey 1999).

The variability in protection by different ants is not unique to *L. aculeata*. Fagundes *et al.* (2017) documented interactions between 23 ant species and 10 plant species, using termite baiting to simulate herbivore attack, and found that half of the ant species patrolled *Senna reniformis* (Fabaceae) plants, while the nine other plant species interacted only with five to seven ant species. The authors speculated that termite baiting might not realistically represent the herbivore threat, as the herbivore community is generally quite diverse, especially in tropical regions (Novotny *et al.* 2006; Alves-Silva *et al.* 2015). Different ant species can have different strategies when it comes to deterring herbivores and hence a greater diversity of ants patrolling could be more effective in reducing herbivory (Alves-Silva *et al.* 2015; Del-Claro & Marquis 2015). This could explain why the mutualism was able to persist even with such a large number of ant species patrolling *L. aculeata*.

We did not find significant differences in herbivory rates between plants patrolled by different ant genera. This might relate to the fact that measures of patrolling, while differing between genera, were quite variable within genera, and hence with our relatively small sample sizes, this variation meant that we failed to detect corresponding differences for herbivory. If this was the case, then we would expect measures of patrolling efficiency to correlate better with herbivory rates, and this was indeed the case. The synergistic positive effect of time until discovery and recruitment time on leaflet-herbivory suggests that a swift response of the ant partner to herbivores is effective in reducing herbivory to the plant part-

ner. Where time until first discovery was short, recruitment time did not affect herbivory, which remained low for all combinations of predictors, presumably because with short discovery times plants are always well protected. However, when discovery time was long, plants only remained well protected if recruitment time was short, and suffered more herbivory when recruitment was slower. The connection directly linking ant protective performance with the provision of resources (food body and EFN production) in this mutualistic interaction remains unstudied. We were unable to explore this as the *L. aculeata* individuals we sampled for the termite baiting and ant exclusion experiments did not overlap in sufficient numbers for statistical analyses. It would be worth exploring whether plants that provide greater numbers of food bodies are better protected from herbivory. Other studies do indicate such a link between ant protective performance and resource production: For example, greater production of EFN has been found to favour a greater richness of patrolling ants (Lange *et al.* 2017). Additionally, presence of EFN of better nutritional quality leads to a greater number of ants being recruited (Ness *et al.* 2006). Although this suggests that more investment in resource production by plants leads to better protection by ants, further studies are needed to test the generality of this pattern.

In any mutualism, each partner will try to maximise its benefits and minimise its costs. For the ant partner in our system, this means providing enough protection for the plant against herbivores such that the plant still produces food bodies and EFN. For the plant partner, this means providing enough food body and EFN rewards to attract protective ants. One challenge for the plant is to filter out any ants that do not provide effective protection. In ant-plant mutualisms where a myrmecophytic plant harbours a resident ant colony completely dependent on its resources, this can be achieved through host sanctions, with plants reducing growth and survival of ant housing when herbivory is greater (Edwards *et al.* 2006). Another strategy is for plants to provide nutrients that can only be utilised by the ants that protect them better. For example, the mutualist ant partner of *Acacia* myrmecophytes has been found to be digestively specialized to the plant

resource reward (Kautz *et al.* 2009). This is likely to be a successful strategy for preventing existing hosts from cheating, although it may not prevent parasitism of the mutualism by other species. However, methods to stabilize mutualisms are potentially more challenging when ants nest elsewhere, as in our system. Additionally, with the large number of unspecialised ant species involved in our study, the ability of the host plant to “capture” ant partners by way of digestive specialisation might be limited. Instead, our system seems to exhibit a more facultative interaction where ants residing nearby are opportunistically attracted to food bodies and EFN provided by the plant and deter herbivores while foraging on these resources. A study by Miller (2007) suggests that a generalised ant-plant mutualism with an EFN producing myrmecophile and multiple ant partners, much like ours, may have smaller benefits for the plant. Yet, such a facultative mutualism could potentially be stabilized by competition among ant species for the plant’s resources if the stronger competitor provides greater protection for the plant from herbivores (Miller 2007).

Generalisation of our findings for *L. aculeata* may be limited by the relatively small geographic range of our study. The abundance of ant genera patrolling *L. aculeata* might partly reflect the local ant relative abundance and composition. It would therefore be interesting to study *L. aculeata*’s interactions with ants in other areas to test for similar trends in patrolling between ant genera and their varying protective behaviour.

Our study is the first to quantitatively document the interaction between *L. aculeata* and its ant partners. With an ant-exclusion experiment, we confirmed that ants do in fact forage on the plant’s food bodies and, hence, that the ants benefit from the mutualism. Individuals of *L. aculeata* on which ants were both slow in discovering the termite bait and in recruiting more ants experienced greater leaflet-herbivory, thus providing evidence for benefits to plant partner in this interaction. We observed a relatively high diversity of ants patrolling the plant, with ant genera showing differences in protective performance. Our study suggests a non-specialised, opportunistic interaction between ants and *L. aculeata* and raises questions regarding the stability of this facultative mutualism.

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