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Small but spicy? Small ants repel herbivores fast of a Mexican endemic shrub

Nora Villamil[1] · **Ornela De Gasperin**[2] · **Karina Boege**[3]

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Abstract

Ant–plant mutualisms are widespread, with over 4000 angiosperm species worldwide bearing extra-floral nectaries where sugar is secreted in exchange for protection against herbivores. *Turnera velutina*, an endemic Mexican shrub, has facultative mutualist interactions with different ant species, and these interactions have variable effects on its fitness. Specifically, the presence of *Camponotus* ants have been linked to the highest *T. velutina's* fruit production, in comparison with the presence of other patrolling ant species. Whether this outcome is because *Camponotus* ants are the most aggressive, the largest, the fastest at locating herbivores, and/or more gregarious with many workers patrolling/attacking herbivores, has not yet been formally investigated. In this study, we assessed the defensive behavior and repelling capacity of six ant species against larvae of *Euptoieta hegesia* (Lepidoptera), the main herbivore of *T. velutina* in the studied population. Contrary to our expectations, *Camponotus* ants were not the most effective defenders, and they were only better than *Cephalotes* ants, a genus that has been found to be opportunistic in other plant systems. Surprisingly, the two smallest ants, *Dorymyrmex bicolor* and *Brachymyrmex* sp., were the best defenders and the fastest at repelling herbivores, partly because many workers simultaneously attacked the larvae. We discuss how different ants may impact various aspects of a species' fitness, highlighting the dynamic and complex outcomes of ant–plant interactions, as a function of species identity and their impacts on herbivores.

Keywords Aggressiveness · Ant–plant interactions · Extra-floral nectaries · Defense · Herbivory · Mutualism

Introduction

Mutualisms typically have net benefits for both parties (Herre et al. 1996), yet these benefits can fluctuate across time and space (Bronstein 1994), depending on the ecological context and the interacting individuals (Bronstein 1994). Ant–plant mutualisms are widespread, with over 4000 angiosperm species (flower-producing plants) worldwide bearing extra-floral nectaries (EFNs), which are non-reproductive structures that provide sugary rewards for mutualists, mainly ants (Keeler et al. 2008; Calixto et al. 2018; Souza et al. 2024; do Rosario Nogueira 2025). In return, ants protect plants from herbivores (Keeler et al. 2008; Calixto et al. 2018; Souza et al. 2024; do Rosario Nogueira 2025). EFNs are easily accessible and can thus be exploited by multiple ant species (Rico-Gray and Oliveira 2008; Rosumek et al. 2009; Palmer et al. 2010) and by other organisms, which may or may not benefit the plant. As with other facultative interactions (where each party can survive independently), ant–plant facultative mutualisms are often more variable in the net benefits for each interacting party, in comparison to obligate mutualisms, in which both parties depend on each other for survival and/or for successful reproduction (Itioka et al. 2000; Apple and Feener 2001; Ness 2006; Fagundes et al. 2017). Correspondingly, ant species within facultative

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Nora Villamil and Ornela De Gasperin are co-first authors.

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✉ Karina Boege
kboege@unam.mx

[1] Lothian Analytical Services, NHS Lothian, National Health Service Scotland, NHS Lothian- Mainpoint, 102 West Port, Edinburgh EH3 9DN, Scotland

[2] Red de Ecoetología, Instituto de Ecología A.C., 91073 Xalapa, Veracruz, Mexico

[3] Instituto de Ecología, Universidad Nacional Autónoma de México, 04510 Mexico City, Mexico

mutualisms tend to be less aggressive than those in obligate mutualisms (Rosumek et al. 2009).

Ant species engaging in mutualisms with EFN-bearing plants vary in their capacity to protect plants from herbivores, an effect that is influenced by the ant's aggressive behavior. Ant aggression assays towards insects have been carried out on over 35 different plant species, with over 30 ant genera tested (Souza et al. 2024). These studies have found *Camponotus* and *Crematogaster* ant species to be the best defenders, i.e., having the highest percentage of successful attacks, whereas *Brachymyrmex* species have the lowest percentage of successful attacks (Souza et al. 2024). Ant–plant mutualisms in tropical ecosystems seem to be especially common, as approximately one third of woody dicots, herbs, and vines produce ant-luring structures (Davidson et al. 2008), and ants represent half of arthropod species (Davidson et al. 2008). Nevertheless, the outcomes of ant–plant interactions for plant fitness are variable and depend on the identity and aggressiveness of the ant species involved (Palmer et al. 2010). In this study, we quantified and compared the behavior of six different ant species, and their ability at repelling a naturally occurring herbivore of the myrmecophile plant *Turnera velutina* (Cuautle and Rico-Gray 2003).

Turnera velutina (Passifloraceae) (Ochoa-López et al. 2015) is an endemic Mexican perennial shrub that grows in lowland coastal environments (Arbo 1997). *Turnera velutina* establishes facultative mutualisms with up to 13 different ant species, by providing ants with extrafloral nectar in paired cup-shaped glands located at the bottom of the leaf blade or on the leaf petiole (Cuautle et al. 2005; Zedillo-Avelleyra 2017). Ants interacting with *T. velutina* can influence plant fitness by reducing levels of herbivore leaf damage (Torres-Hernández et al. 2000; Cuautle and Rico-Gray 2003). Nevertheless, not all ant species benefit *T. velutina* equally. Experimental manipulations of ant species' patrolling *T. velutina* have shown that the presence of *Camponotus abdominalis* is linked to greater fruit production, in comparison to the presence of other ant species (Torres-Hernández et al. 2000). Differences in the efficiency of ants to protect *T. velutina* from herbivores may be related to their ability to detect herbivores, the speed at which fellow workers arrive, or to their aggressiveness. Some or all these factors may underlie the different outcomes linked to having some ant species patrolling a plant over others, but this has not yet been formally tested.

To understand how the identity of ants can modify the outcomes of plant–ant interactions in *T. velutina*, we evaluated and compared the behavior of six ant species naturally associated with *T. velutina*, all of which consume nectar from EFNs of this species. Unlike most studies that damage the host plant artificially or that use generalist non-native herbivores (e.g., Christianini and Machado 2004; Romero and Koricheva 2011; Zedillo-Avelleyra 2017), we used an in situ experimental approach with a live herbivore naturally occurring in the studied population. Using natural herbivores is important, because ants may be better adapted to their behavior than to herbivore species rarely or never encountered by the host plants. This is likely the case for the herbivore *Euptoieta hegesia* (Nymphalidae), an oligophagous herbivore specialized on Passifloraceae species, with specific adaptations to overcome *Turnera* species' toxins (Schappert and Shore 1999), and the main and dominant herbivore in the studied population (Cuautle and Rico-Gray 2003; Villamil-Buenrostro 2012; Ochoa-López et al. 2018). We hypothesized that different ant species would differ in their behavior and ability to repel herbivores, as has been shown in other systems (Apple and Feener 2001; Raine et al. 2004; Ness 2006; Heil et al. 2009; Del-Claro and Marquis 2015; Fagundes et al. 2017; Souza et al. 2024). We also predicted that the most aggressive ants with the highest herbivore repelling rate (e.g., *Camponotus* ants) would be the best defenders, as they have been associated with higher fruit production in *T. velutina* (Torres-Hernández et al. 2000), and according with previous reports on their defensive abilities (Souza et al. 2024).

Methods

Experimental design: ant aggressiveness assays

This experiment was conducted between October and November 2016 at La Mancha, Veracruz, within the CICOLMA field station, located on the coast of the Gulf of Mexico (19° 36′ N, 96° 22′ W, elevation < 30 masl). We carried out all trials on sunny days without rain, on the 22nd, 23rd and 24th October, and on the 4th of November, between 9 am and 17 h. We included replicates with different ant species in the same day. Larvae of *E. hegesia* were obtained from an in situ-reared colony, maintained in a shade house with mesh exclusions. Larvae were kept in 0.5 L plastic containers with a tulle mesh window lid for ventilation. Containers were lined with wet filter paper and fresh *T. velutina* foliage was provided for food and shelter. We measured the frontal width of the head capsule of each larva, and only large larvae (2.77 ± 0.019 mm) were used in assays to homogenize herbivore size in the experiment. We used a total of 40 healthy, large reproductive plants, defined as shrubs with six or more branches bearing flowers, buds, or fruits, and a foliage crown > 40 cm in diameter. Plants were randomly selected across a 6-ha portion of the sand dune, and distance between plants within a patch was > 5 m. For practical reasons, we selected plants that we could access and observe all around, excluding those amongst dense vegetation patches. After verifying the presence of patrolling ants (i.e., with at least one individual), we identified their species identity on specific branches (hereafter focal branches, which were selected independently of the branch position in the plant). We used the same focal plant for a maximum of six trials, and plant ID was controlled for in the statistical analyses. For each trial, we placed one *E. hegesia* larva on a randomly chosen leaf of the focal branch with at least one patrolling ant, avoiding leaves with reproductive structures. We recorded the number and identity of the ant species on the focal branch before and after placing the larva, as well as their behavior towards the herbivore. If more ants entered the leaf, we counted them using a counter (although we did not follow ants individually, thus some ants may have left and re-entered the leaf). To be able to carry out as many replicates per day as possible, all behavioral observations lasted for maximum 10 min. If the larva relocated or dropped itself off the leaf, we stopped the trial before this period.

We documented if ants (1) ignored the larva, either because they did not reach the leaf with the larva or found the larva but did not interact with it, (2) patrolled (walked around), or (3) attacked (with bites, stinging, or spraying the larvae through the acidopore) the larvae. We also counted how many new ants from the same species reached the leaf, and the time it took the first additional ant from the same species to arrive to the leaf. We recorded the ants' ability to protect the plant by assessing if they succeeded at repelling the larva (if the larvae left the plant/branch by dropping off or moving away from the leaf), and the time it took the ants to repel it. Unharmed larvae were used for a maximum of four assays in a single day, as we did not have enough large larvae to use a unique individual per trial. Harmed larvae, however, were not reused. After a trial, larvae were placed back into their individual containers and were left to rest for at least 2 h before being reused again. Larval containers were washed, the wet lining was changed every night, and foliage was replenished as required. We carried out a total of 91 trials using 40 focal plants, including 14 trials with *Brachymyrmex* sp., 21 with *Camponotus planatus*, 16 with *Cephalotes minutus*, 11 with *Crematogaster* sp., 14 with *Dorymyrmex bicolor*, and 15 with *Paratrechina longicornis.*

Statistical analyses: general procedures

Analyses were conducted in R (R Core Team 2014), using 'glmer', 'lmer', and 'glm' functions from the 'lme4' R-package (Bates et al. 2014), unless otherwise stated. Model estimates, standard errors (SE), and *p* values were obtained using the 'summary' or 'Anova' functions with type II sum of squares (Team 2014) (models did not have interactions). We evaluated model residuals following Zuur et al. (2009). *Post-hoc* comparisons were carried out using false discovery rate (FDR) correction.

Behavior and protective abilities of different ant species associated to *T. velutina*

First, we tested if each ant species had the same number of workers patrolling the leaves before and after placing the larvae, by fitting a general linear mixed effects model using a normal error distribution, with the response variable log-transformed to ensure normality of residuals (model 1). We included as fixed effect ant species (with six levels), and as random effect the plant identity (to control for the use of different leaves of the same plant in different assays).

We compared the propensity of each ant species to ignore (model 2); attack (model 3); have at least one extra worker arriving at the leaf (model 4); and repel (model 5) the larva using generalized linear mixed effects models with binomial error distribution. We included as fixed effect the ant species (with six levels), and as random effect the plant identity. In all models we found complete separation between the response and the species' identity (e.g., no *C. minutus* trial resulted in repellence, and no *D. bicolor* trial ignored the larvae), which leads to very high standard errors. To overcome these problems, we ran Firth's bias-reduced logistic regressions; using the 'logistf' package (Firth 1993), with the species that showed complete separation as a reference to contrast whether other species differed from it (models type 'a'). Then, we excluded that species and carried out generalized linear mixed effects models (models type 'b'). We repeated model 5 (probability to repel), replacing the ant species with the number of ants that arrived during the trial, as a continuous variable (model 5c).

We compared the time (in seconds) that took the first workers to arrive to the leaf (model 6), as well as the number of workers that arrived during the assay (model 7), with general linear mixed effects models, using a normal error distribution, with the response variable log-transformed to ensure normality of residuals. In both models, we included only assays in which at least one more worker from the same species arrived at the leaf.

Finally, because the time it takes the ants to repel the herbivore can lead to higher or lower leaf damage, we compared the time (in seconds) it took each species to repel the larvae, excluding trials where larvae were not repelled (thus all *C. minutus* trials). We fitted a generalized linear mixed effects models with normal error distribution, with the response variable log-transformed to ensure normality of residuals (model 8).

Table 1 Ant species associated with *T. velutina* at CICOLMA field station, Veracruz, Mexico

Family	Sting	Acidopore	Species
Dolichoderinae	Vestigial	Present (slit)	**_Dorymyrmex bicolor_*(2–3 mm)**
Formicinae	Absent	Present (circular)	**_Camponotus planatus_*(5–6 mm)**
			Camponotus claviscapus
			Camponotus mucronatus
			Camponotus novogranadensis
			_Brachymyrmex_sp.* (2–3 mm)
			_Paratrechina longicornis_* (2–3 mm)
Myrmicinae	Present but retracted	Present	*Myrmicocrypta* sp.
			Monomorium ebenimum
			_Cephalotes minutus_*(4–6 mm)
			_Crematogaster_sp.* (4.2–5 mm)
Pseudomyrmeciinae	Present	Absent	*Pseudomyrmex gracilis*
			Pseudomyrmex sp.*1*
			Pseudomyrmex sp.*2*

Ant species studied in this work are highlighted in bold and marked with an asterisk (*). Information from each species was taken from (Zedillo-Avelleyra 2017) and from Jorge Valenzuela. Body length refers to the worker's total body length, extracted from Antwiki, and from Zedillo-Avelleyra (2017)

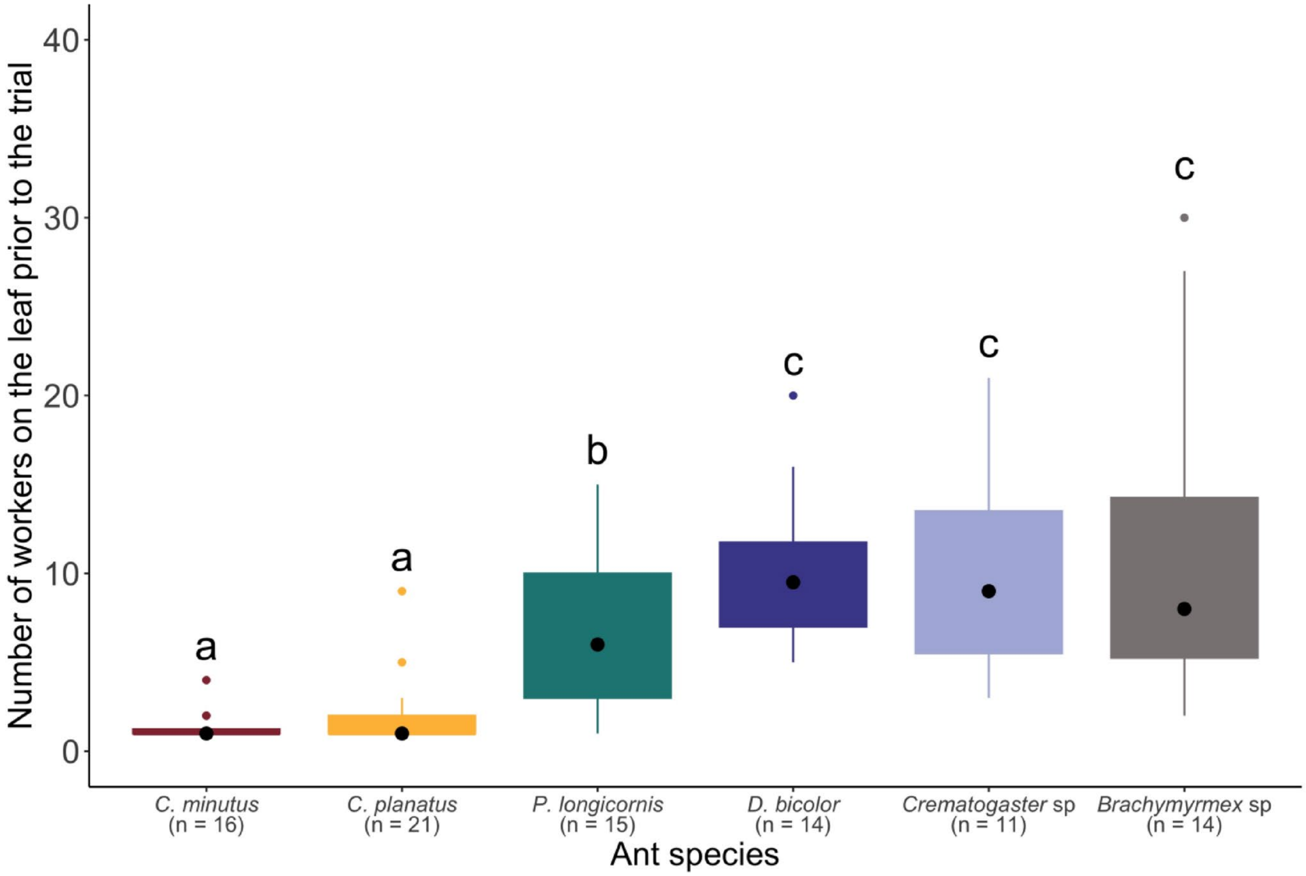

Fig. 1 Ant species differed in their gregariousness. Number of ants present on a leaf prior to starting the trial. Different letters indicate significant differences between species ($p < 0.05$) after FDR-corrected *post-hoc* tests. The box shows the interquartile range per species, and whiskers represent 1.5× the inter-quartile range. Black dots represent median values per ant species

Results

Ant species associated with *T. velutina*

We observed ten different ant species from four families interacting with *T. velutina* (Table 1) and quantified the aggressiveness of the six most frequent ant species actively patrolling the plants (Fig. 1). We did not observe *C. abdominalis*, the ant species reported to be linked to higher fruit rate production in previous years (Torres-Hernández et al. 2000), and some ants could only be identified up to morphospecies.

Ant species' behavior towards herbivores

Ant species differed in their gregariousness (i.e., in the number of workers already present on a leaf at the start of the trial, Table 2a; Fig. 1), and in their behavior towards *E. hegesia* larvae (Table 2b–m; Fig. 2). *Cephalotes minutus* never attacked nor repelled a single larva (Table 2d, f; Fig. 2c, d), and they ignored (or did not find) the larvae in half the assays (Table 2b, c; Fig. 2a). *Dorymyrmex bicolor* and *Brachymyrmex* sp., in contrast, never ignored a larva, had more workers arriving in all trials (Fig. 2c), and successfully repelled the larvae in most trials (Fig. 2d).

Ant species differed in their capacity to protect the plant from herbivores (Table 2h, i; Fig. 2d). Workers from different ant species differed in the propensity to arrive where the larva was placed, and in the quantity of ant workers that arrived during the trial (Table 2j; Fig. 3a, b). *Brachymyrmex* sp. and *D. bicolor* were the fastest to arrive, and arrived with the most workers to the leaf during the trial (Fig. 3a, b). These two species were also the fastest to repel the herbivore (Table 2m; Fig. 4b), both succeeding in 75% of the assays. The likelihood of successfully repelling the larvae was positively associated with the number of ants arriving to the leaf (Table 2j).

Ant species differed in the speed at which more workers arrived during the trial (Table 2; Fig. 3a), and in the number of workers that arrived (Table 2; Fig. 3b). Ant species also differed in their speed to repel the herbivore (Table 2; Fig. 4).

Discussion

Mutualisms can have dynamic outcomes as a function of the specific ecological context, the species identity, plant ontogeny (Ochoa-López et al. 2020) and of specific individual traits (Bronstein 1994). For example, *Ficus* plants abort seeds not pollinated by cheating wasps (Herre et al. 1996; Herre and West 1997), and *Acacia* plants reduce EFN rewards when colonized by commensal or parasitic ants (Heil et al. 2009). Furthermore, species may interact with multiple mutualists, which also vary in time and space, and in their effect on the other species' fitness and traits. Our results show that the Mexican endemic shrub, *T. velutina*, has a mix of associated ants with different protective abilities, from likely opportunistic species to efficient defenders that succeed at repelling herbivores, highlighting the dynamic outcomes of this ant–plant facultative mutualism.

Ant species associated with *T. velutina* differed in how many workers were present on leaves prior to a trial and in their behavior towards *E. hegesia* larvae, specifically in the time it took more workers of the same species to arrive, in the number of workers that arrived, and in their ability (or tendency) to repel the herbivore. However, for some species we had a low sample size. For instance, only 11 replicates were achieved with the ant species *Crematogaster* sp., a caveat to be considered.

Small (Fiala et al. 1989; Cuautle and Rico-Gray 2003; Cuautle et al. 2005) and stinging (instead of biting) ants (Davidson et al. 2008) are often reported as poor defenders (Torres-Hernández et al. 2000). However, in our study, the ants with the smallest body sizes (*D. bicolor* and *Brachymyrmex* sp.) and with biting habits were better defenders than those with spraying behaviors (such as *Crematogaster* or *Camponotus*). Specifically, even though all species except for *C. minutus* had the same probability of repelling the herbivore, *D. bicolor* and *Brachymyrmex* sp. were faster than other species at successfully promoting the herbivore to drop off the leaf or relocate. If the longer an herbivore stays on the plant translates into greater consumption or plant damage, repellence speed is probably important for plant's fitness (but plant fitness was not assessed in our study). Noteworthy, ant species differed in how many workers where on the leaf, arrived at the leaf where the larva was placed, in their arrival speed, which all together increased the likelihood of herbivores being repelled. The differences in the number of ants arriving to the leaf revealed by our study may reflect behavioral differences in patrolling habits amongst ant taxa, and/or differences in chemical signaling, aspects that may be more important than individual body size.

Camponotus ants have been found to be the best defenders on a recent review (Souza et al. 2024) and *C. abdominalis'* presence has been linked to the highest fruit production in *T. velutina* (Torres-Hernández et al. 2000). However, we did not detect *C. abdominalis* in our study. Temporal differences between studies in the sampling month and/or year may underlie the differences in ant composition between studies. Specifically, Torres-Hernández et al. (2000) carried out their sampling in August 1992, and we carried out our sampling in late October, 2016. Yet, the *Camponotus* genus is common within the study site, as Torres-Hernandez (2000) found *C. abdominalis* in August and we detected *C. planatus* in October. This suggests that different species of this genus are consistently foraging on *T. velutina* throughout the year and their continuous presence might imply continuous protection. It is also possible that *Camponotus* sp. are better at attacking generalist herbivores or termites (both commonly used in experimental trials or) than they are at attacking more specialised herbivores like the one we used in this study. Differences in the efficiency at repelling generalist as opposed to specialist herbivores by *Camponotus* ants could explain why our results contrast those of Souza et al. (2024). The overall efficiency of *Camponotus* ants (and

Table 2 Results from the models predicting ant behavior before and after placing larvae of *E. hegesia* on leaves of *T. velutina* plants

(a) Model 1: Number of ant workers per species prior to placing the larva

Explanatory variable	χ^2	d.f.	*p*
Ant species	143.71	5	**<0.00001**

(b) Model 2a: Proportion of replicates that did not find/ignore (using *D. bicolor* as reference)

Ant species	Estimate	SE (coef)	Lower 0.95 CI	Upper 0.95 CI	χ^2	*p*
C. planatus	2.49	1.51	0.22	7.40	4.80	**0.02**
P. longicornis	2.09	1.56	−0.37	7.03	2.64	0.10
Crematogaster sp.	2.03	1.60	−0.61	7.00	2.17	0.14
Brachymyrmex sp.	0.00	2.03	−5.25	0.00	0.00	1.00
C. minutus	3.36	1.51	1.08	8.27	10.07	**0.001**

(c) Model 2b: Proportion of replicates that did not find/ignore (excluding *D. bicolor* and Brachymyrmex)

Explanatory variable	χ^2	d.f.	*p*
Ant species	4.25	3	0.23

(d) Model 3a: Proportion of replicates that attacked the larvae (using *C. minutus* as reference)

Ant species	Estimate	SE (coef)	Lower 0.95 CI	Upper 0.95 CI	χ^2	*p*
C. planatus	3.22	1.49	1.00	8.11	9.80	**0.001**
P. longicornis	3.62	1.52	1.33	8.53	12.04	**0.0005**
Crematogaster sp.	4.38	1.57	1.97	9.33	16.92	**<0.0001**
Brachymyrmex sp.	4.68	1.56	1.98	9.33	21.53	**0.0001**
D. bicolor	5.69	1.67	3.10	10.74	29.59	**<0.0001**

(e) Model 3b: Proportion of replicates that attacked the larvae (excluding *C. minutus*)

Explanatory variable	χ^2	d.f.	*p*
Ant species	5.77	4	0.21

(f) Model 4a: Proportion of replicates in which more workers arrived during the trial (using D. bicolor as reference)

Ant species	Estimate	SE (coef)	Lower 0.95 CI	Upper 0.95 CI	χ^2	*p*
C. planatus	−1.70	1.55	−6.63	0.74	1.68	0.19
P. longicornis	−1.68	1.59	−6.63	0.94	1.45	0.22
Crematogaster sp.	−2.03	1.60	−7.00	0.61	2.17	0.14
Brachymyrmex sp.	0.00	2.03	−5.25	5.25	0.00	1.00
C. minutus	−3.13	1.52	−8.04	−0.84	8.27	**0.004**

(g) Model 4b: Proportion of replicates in which more workers arrived during the trial (excluding *D. bicolor* and Brachymyrmex)

Explanatory variable	χ^2	d.f.	*p*
Ant species	5.34	3	0.14

(h) Model 5a: Proportion of replicates that repelled the larvae (using *C. minutus* as reference)

Ant species	Estimate	SE (coef)	Lower 0.95 CI	Upper 0.95 CI	χ^2	*p*
C. planatus	3.22	1.49	1.00	8.11	9.80	**0.001**
P. longicornis	2.55	1.53	0.17	7.48	4.54	**0.03**
Crematogaster sp.	3.66	1.54	1.29	8.59	10.99	**0.0009**
Brachymyrmex sp.	3.76	1.52	1.46	8.68	12.98	**0.0003**
D. bicolor	4.86	1.55	2.31	9.63	21.53	**<0.0001**

(i) Model 5b: Proportion of replicates that repelled the larvae (excluding *C. minutus*)

Explanatory variable	χ^2	d.f.	*p*
Ant species	4.64	4	0.32

(j) Model 5c: Proportion of replicates that repelled the larvae (excluding *C. minutus*)

Explanatory variable	χ^2	d.f.	*p*
Arriving ants	8.24	1	**0.004**

(k) Model 6: Time it took new workers of the same species to arrive to the leaf

Explanatory variable	χ^2	d.f.	*p*
Ant species	19.57	5	**0.0001**

(l) Model 7: Number of new workers of the same species that arrived at the leaf

Explanatory variable	χ^2	d.f	*p*
Ant species	38.69	5	**<0.0001**

(m) Model 8: Time to repel the larva

Explanatory variable	χ^2	d.f.	*p*
Ant species	25.82	4	**<0.0001**

Significant differences are indicated in bold *p* values

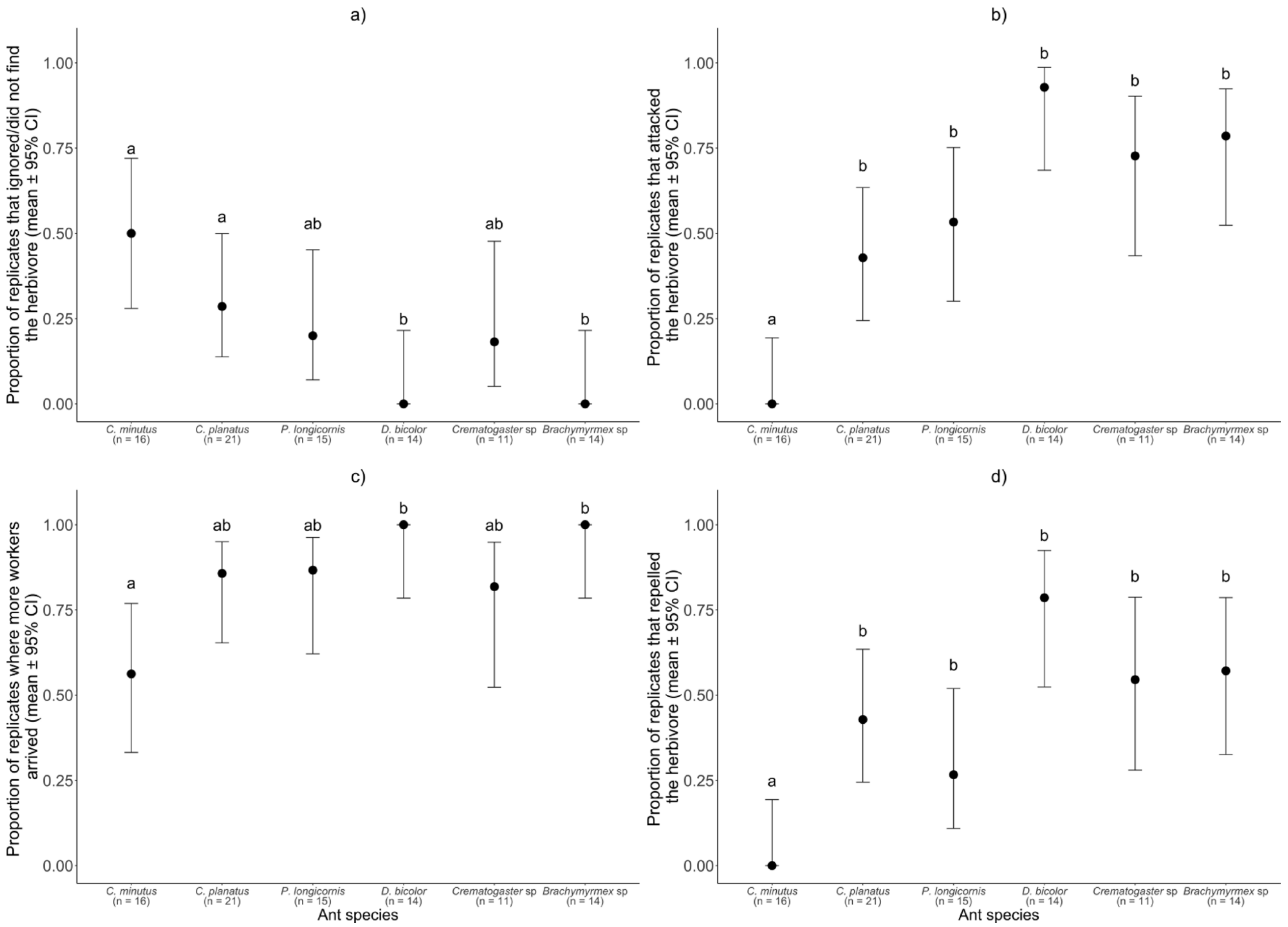

Fig. 2 Ant species differed in their behavior towards the herbivore. Propensity to ignore (**a**), to attack (**b**), of arrival from at least one extra worker (**c**), and to repel (**d**) a natural herbivore. Different letters indicate significant differences between species ($p<0.05$) after FDR-corrected *post-hoc* tests

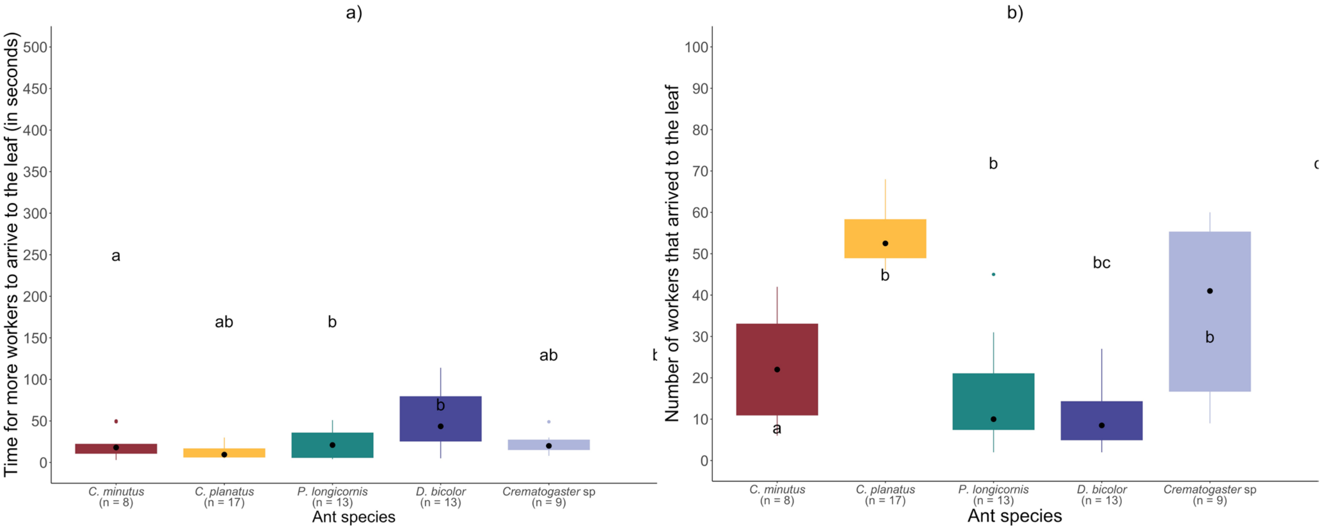

Fig. 3 Ant species differed in the speed at which more workers arrived during the trial (**a**), and in the number of workers that arrived (**b**). Different letters indicate significant differences between species ($p<0.05$) after FDR-corrected *post-hoc* tests. The box shows the interquartile range per species, and whiskers represent 1.5× the inter-quartile range. Black dots represent median values per ant species

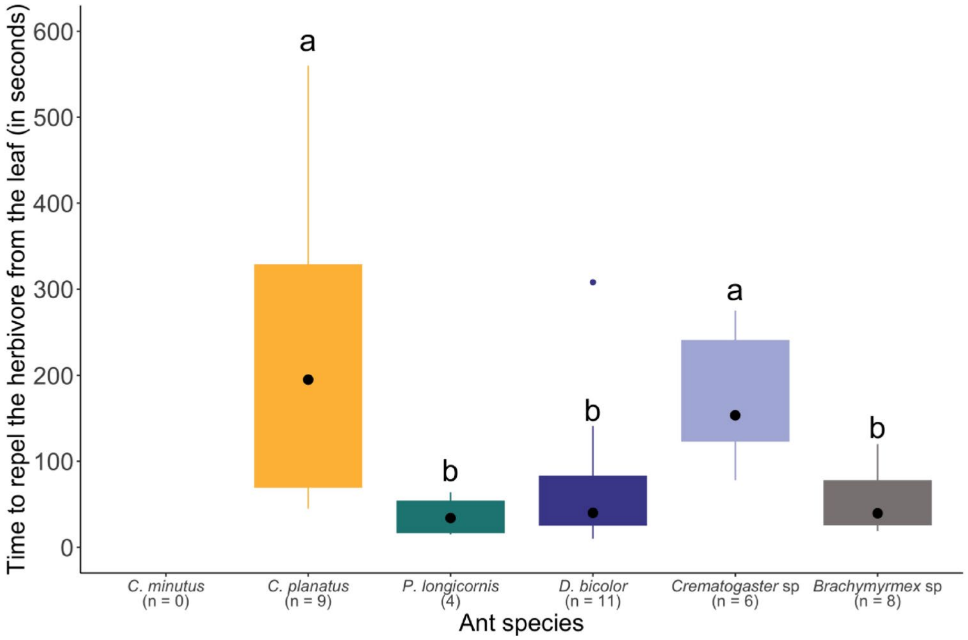

Fig. 4 Ant species differed in their speed to repel the herbivore. Different letters indicate significant differences between species ($p < 0.05$) after FDR-corrected *post-hoc* tests. The box shows the interquartile range per species, and whiskers represent 1.5× the interquartile range. Black dots represent median values per ant species. Only replicates that resulted in repellence were included. N represent the number of trials where a larva was successfully repelled

of other ant species) on *T. velutina* may depend on its habits, the abundance of different herbivores, the amount of nectar consumed by ants (as more ants may consume more nectar from EFNs and this could influence the net costs and benefits of patrolling ants (e.g., Heil et al. 2000), and the potential effects that the ants may have on pollinators' behavior (see below).

Cephalotes minutus ants may be an opportunistic species of *T. velutina.* Other studies have found *Cephalotes* species to be opportunistic species as well. For instance, *Cephalotes* sp. does not defend *Ouratea spectabilis* Engl. (Ochnaceae) (Byk and Del-Claro 2010) nor *Banisteriopsis malifolia* (Alves-Silva et al. 2015) in Brazil (Fagundes et al. 2017), and neither does *Cephalotes pusillus* which consumes pollen and nectar without defending plants (Byk and Del-Claro 2010). For *T. velutina*, the presence of *C. minutus* could increase ant-pollinator conflicts by eating the pollinator's food (Villamil et al. 2018), and it may create ant–plant conflicts by eating nectar intended for real defenders. Future studies should assess overall effects of *Cephalotes* sp. ants on plant fitness, and on other ant mutualist's fitness such as pollinators.

Consequences of having multiple partners for plant fitness can co-vary with plant age or other traits. For instance, in *Acacia drepanolobium*, fitness is enhanced by partnering with different sets of ant mutualists throughout its lifetime, including species which could be deemed as 'parasites' when assessing their effect over the trees' lifespan (Palmer et al. 2010). Conversely, in the pioneer plant *Mallotus japonicus*, ant species diversity decreased the effectiveness of ant defenses (Yamawo et al. 2021). Furthermore, specific ant species can vary in their ability to repel herbivores depending on their colony size, colony age, foraging strategies, diurnal-nocturnal activity, and on other life-history traits such as the timing of sexual reproduction of the colony (Souza et al. 2024; do Rosario Nogueira 2025). Similarly, plants may be threatened by different herbivores which vary in size and behavior, and different ants may be better at protecting plants from different herbivores (Souza et al. 2024; do Rosario Nogueira 2025). Ants are generally more effective against small, less sclerotized, herbivores, such as caterpillars (e.g., Fiala et al. 1989). Thus, an apparently redundant array of patrolling ants may be beneficial (e.g., Cuautle and Rico-Gray 2003; Palmer et al. 2010).

Protective ants can also shape other ecological interactions such as pollinator visitation, and thus ultimately influence plant fitness. Consquently, ant aggresivity against pollinators could explain differences in *T. velutina* fruit production associated with different patrolling ants. For example, in contrast with *Camponotus*, *D. bicolor* tends to be more aggressive towards pollinators, reducing visit duration (Villamil et al. 2018) and number of pollen grains exported per flower, thereby reducing male fitness (Villamil et al. 2022). Thus, although *D. bicolor* may be faster at repelling herbivores, likely increasing plant fitness by reducing leaf damage, these effects may be offset if pollinators are deterred and flowers are visited less. Therefore, the net fitness effects obtained by *T. velutina* plants from different mutualists is likely the result of a complex and dynamic process that varies in space and time. Measuring leaf herbivore damage in addition to pollination and fruit production when *T. velutina* is occupied by these different ant species, in different times of the day, and months of the year, many help disentangle these impacts.

Overall, our results show that *T. velutina* has a mix of associated ants with different protective abilities, from opportunistic to highly efficient defenders against a naturally occurring herbivore, highlighting the dynamic outcomes of ant–plant facultative mutualisms. Understanding ant behavior and ant-life history traits, and how these traits co-vary with the plant's own life history changes and needs, can help to unveil the complex array of mutualistic interactions *T. velutina* has, and their non-additive effects on plant fitness (Martínez-Bauer et al. 2015; Santos and Leal 2019; Nogueira et al. 2021). This opens new research questions regarding the evolution of mutualist management strategies as a function of the community composition of ants visiting host plants (Junker et al. 2007) across different geographic areas and habitats.

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Author contributions NV & KB. conceived the study, NV & KB. carried out the research, NV & ODG analysed the data, NV, ODG & KB. discussed the results and wrote the manuscript.

Data availability Data and code are available at: https://figshare.com/s/44963a0bcb464489c497.

Declarations

Conflict of interest We declare we have no conflict of interest.

References

Alves-Silva E, Bächtold A, Barônio GJ, Torezan-Silingardi HM, Del-Claro K (2015) Ant–herbivore interactions in an extrafloral nectaried plant: Are ants good plant guards against curculionid beetles? J Nat Hist 49:841–851

Apple J, Feener D (2001) Ant visitation of extrafloral nectaries of Passiflora: the effects of nectary attributes and ant behavior on patterns in facultative ant–plant mutualisms. Oecologia 127:409–416

Arbo MM (1997) Estudios sistemáticos en *Turnera* (Turneraceae) I Series Salicifoliae y Stenodictyae. Bonplandia 9:151–208

Bates D, Mächler M, Bolker B, Walker S (2014) Fitting linear mixed-effects models using lme4. arXiv preprint arXiv: https://arxiv.org/abs/14065823

Byk J, Del-Claro K (2010) Nectar-and pollen-gathering *Cephalotes* ants provide no protection against herbivory: a new manipulative experiment to test ant protective capabilities. Acta Ethol 13:33–38

Calixto ES, Lange D, Del-Claro K (2018) Protection mutualism: an overview of ant–plant interactions mediated by extrafloral nectaries. Oecol Aust 22:410–425

Christianini AV, Machado G (2004) Induced biotic responses to herbivory and associated cues in the Amazonian ant–plant *Maieta poeppigii*. Entomol Exp Appl 112:81–88

Cuautle M, Rico-Gray V (2003) The effect of wasps and ants on the reproductive success of the extrafloral nectaried plant *Turnera ulmifolia* (Turneraceae). Funct Ecol 17:417–423

Cuautle M, Rico-Gray V, Diaz-Castelazo C (2005) Effects of ant behaviour and presence of extrafloral nectaries on seed dispersal of the Neotropical myrmecochore *Turnera ulmifolia* L.(Turneraceae). Biol J Linn Soc 86:67–77

Davidson DW, Cook SC, Carson W, Schnitzer S (2008) Tropical arboreal ants: linking nutrition to roles in rainforest ecosystems. Tropical Forest Community Ecology Wiley-Blackwell, UK, pp 334–348

Del-Claro K, Marquis RJ (2015) Ant species identity has a greater effect than fire on the outcome of an ant protection system in Brazilian Cerrado. Biotropica 47:459–467

do Rosario Nogueira R (2025) Mutualistic interactions between plants with extrafloral nectaries and ants: ecological impacts, ant sharing among neighboring plants, and the effects of environmental conditions. Community Ecol 25:1–11

Fagundes R, Dáttilo W, Ribeiro S, Rico-Gray V, Jordano P, Del-Claro K (2017) Differences among ant species in plant protection are related to production of extrafloral nectar and degree of leaf herbivory. Biol J Linn Soc 122:71–83

Fiala B, Maschwitz U, Pong TY, Helbig AJ (1989) Studies of a South East Asian ant-plant association: protection of Macaranga trees by *Crematogaster borneensis*. Oecologia 79:463–470

Firth D (1993) Bias reduction of maximum likelihood estimates. Biometrika 80:27–38

Heil M, Fiala B, Baumann B, Linsenmair KE (2000) Temporal, spatial and biotic variations in extrafloral nectar secretion by *Macaranga tanarius*. Funct Ecol 1:749–757

Heil M, González-Teuber M, Clement LW, Kautz S, Verhaagh M, Bueno JCS (2009) Divergent investment strategies of *Acacia myrmecophytes* and the coexistence of mutualists and exploiters. Proc Natl Acad Sci 106:18091–18096

Herre EA, West SA (1997) Conflict of interest in a mutualism: documenting the elusive fig wasp–seed trade–off. Proc R Soc Lond Ser B Biol Sci 264:1501–1507

Herre EA, Machado C, Bermingham E, Nason J, Windsor D, McCafferty S, Houten WV, Bachmann K (1996) Molecular phylogenies of figs and their pollinator wasps. J Biogeogr 23:521–530

Itioka T, Nomura M, Inui Y, Itino T, Inoue T (2000) Difference in intensity of ant defense among three species of Macaranga

Myrmecophytes in a Southeast Asian dipterocarp forest 1. Biotropica 32:318–326

Junker R, Chung AY, Blüthgen N (2007) Interaction between flowers, ants and pollinators: additional evidence for floral repellence against ants. Ecol Res 22:665–670

Keeler K, Porturas L, Weber M (2008) World list of plants with extrafloral nectaries. Available at: https://bsweb.unl.edu/Emeriti/keeler/extra-floral/Cover.htm

Martínez-Bauer AE, Martínez GC, Murphy DJ, Burd M (2015) Multitasking in a plant–ant interaction: How does *Acacia myrtifolia* manage both ants and pollinators? Oecologia 178:461–471

Ness JH (2006) A mutualism's indirect costs: the most aggressive plant bodyguards also deter pollinators. Oikos 113:506–514

Nogueira RR, Santos DFB, Calixto ES, Torezan-Silingardi HM, Del-Claro K (2021) Negative effects of ant–plant interaction on pollination: costs of a mutualism. Sociobiology 68:e7259–e7259

Ochoa-López S, Villamil N, Zedillo-Avelleyra P, Boege K (2015) Plant defence as a complex and changing phenotype throughout ontogeny. Ann Bot 116:797–806

Ochoa-López S, Rebollo R, Barton KE, Fornoni J, Boege K (2018) Risk of herbivore attack and heritability of ontogenetic trajectories in plant defense. Oecologia 187:413–426

Ochoa-López S, Damián X, Rebollo R, Fornoni J, Domínguez CA, Boege K (2020) Ontogenetic changes in the targets of natural selection in three plant defenses. New Phytol 226:1480–1491

Palmer TM, Doak DF, Stanton ML, Bronstein JL, Kiers ET, Young TP, Goheen JR, Pringle RM (2010) Synergy of multiple partners, including freeloaders, increases host fitness in a multispecies mutualism. Proc Natl Acad Sci 107:17234–17239

R Development Core Team (2014) R: A language and environment for statistical computing Vienna. R Foundation for Statistical Computing, Austria

Raine NE, Gammans N, Macfadyen IJ, Scrivner GK, Stone GN (2004) Guards and thieves: antagonistic interactions between two ant species coexisting on the same ant–plant. Ecol Entomol 29:345–352

Rico-Gray V, Oliveira PS (2008) The ecology and evolution of ant–plant interactions. University of Chicago Press, Chicago

Romero GQ, Koricheva J (2011) Contrasting cascade effects of carnivores on plant fitness: a meta-analysis. J Anim Ecol 80:696–704

Rosumek FB, Silveira FA, de Neves S, de Barbosa F, Diniz NP, Oki L, Pezzini Y, Fernandes F, Cornelissen GW T (2009) Ants on plants: a meta-analysis of the role of ants as plant biotic defenses. Oecologia 160:537–549

Santos ATF, Leal LC (2019) My plant, my rules: bodyguard ants of plants with extrafloral nectaries affect patterns of pollinator visits but not pollination success. Biol J Linn Soc 126:158–167

Schappert PJ, Shore JS (1999) Effectsof cyanogenesis polymorphism in *Turnera ulmifolia* on *Euptoieta hegesia* and potential *Anolis* predators. J Chem Ecol 25:1455–1479

Souza L, Calixto E, Domingos S, Bächtold A, Alves-Silva E (2024) Ant protection effectiveness in myrmecophytes and extrafloral nectary plants. J Zool 322:329–339

Torres-Hernández L, Rico-Gray V, Castillo-Guevara C, Vergara JA (2000) Effect of nectar-foraging ants and wasps on the reproductive fitness of *Turnera ulmifolia* (Turneraceae) in a coastal sand dune in Mexico. Acta Zool Mex 81:13–21

Villamil N, Boege K, Stone GN (2018) Ant-pollinator conflict results in pollinator deterrence but no nectar trade-offs. Front Plant Sci 9:1093

Villamil N, Boege K, Stone GN (2022) Defensive mutualists affect outcross pollen transfer and male fitness in their host plant. Oikos 202:8

Villamil-Buenrostro N (2012) Caracterización de las trayectorias ontogenéticas de la defensa contra herbívoros en *Turnera ulmifolia*. Bachelor in Biology Dissertation. Facultad de Ciencias, Instituto de Ecología, Universidad Nacional Autónoma de México, México, p 103

Yamawo A, Suzuki N, Tagawa J (2021) Species diversity and biological trait function: effectiveness of ant–plant mutualism decreases as ant species diversity increases. Funct Ecol 35:2012–2025

Zedillo-Avelleyra P (2017) Variación genética en la expresión de trayectorias ontogenéticas de la defensa de *Turnera velutina*. Posgrado en Ciencias Biológicas, Instituto de Ecología Universidad Nacional Autónoma de México Ciudad de México, p 72

Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM (2009) Mixed effects models and extensions in ecology with R, vol 574. Springer, New York

Bronstein JL (1994) Conditional outcomes in mutualistic interactions. Trends Ecol Evol 9(6):214–217