Ecological Entomology

Ecological Entomology (2020), DOI: 10.1111/een.12904

Defence variation within a guild of aphid-tending ants explains aphid population growth

ANDRÉS M. DEVEGILI,[1] MARÍA N. LESCANO,[1]
ERNESTO GIANOLI[2,3]
and ALEJANDRO G. FARJI-BRENER[1] [1]Laboratorio de Investigaciones en Hormigas (LIHO), Laboratorio Ecotono, INIBIOMA (CONICET-UNComa), Bariloche, Argentina, [2]Departamento de Biología, Universidad de La Serena, La Serena, Chile and [3]Departamento de Botánica, Universidad de Concepción, Concepción, Chile

Abstract. 1. Mutualism studies often focus on the service provided by single species, while variation in benefits provided by multiple partners is less understood. Ant-aphid food-for-protection mutualisms are suitable to study the implications of intra-guild service variation because they often involve several ant species with varying levels of aggressiveness.

2. We studied an aphid species and its associated ant guild to address whether intra-guild defence variation against aphid natural enemies explains aphid performance on plants (thistles). We surveyed plants with natural abundances of aphids associated with different ant species and estimated aphid population growth. We conducted confrontation experiments between ant species and aphid natural enemies (ladybugs and hoverfly larvae). In plants patrolled by the most aggressive ant species, we determined the ant's probability of expelling aphid natural enemies and tested whether ant exclusion affects the abundance of aphids and their natural enemies.

3. The ant *Dorymyrmex tener* was the most abundant and frequent species on plants and the most aggressive against aphid natural enemies. Aphid populations grew faster on plants patrolled by *D. tener* compared to plants patrolled by *Camponotus distinguendus* or *D. richteri*. Field experiments confirmed that *D. tener* effectively expels aphid natural enemies from plants. When *D. tener* was excluded, the density of aphids decreased, while the abundance of aphid natural enemies increased.

4. The disruption of aphid predation by aggressive and numerically dominant ant species is a determinant of aphid population dynamics. This study illustrates the importance of considering intra-guild service variation to better understand multi-partner mutualisms.

Key words. Aphids, aphid natural enemies, aphid-tending ants, mutualism, population growth, protection.

Introduction

Mutualisms almost always include a diversity of partners that benefit a shared mutualist with services that vary in quality and/or quantity (Stanton, 2003; Palmer *et al.*, 2015). For example, service variation has been described in mutualisms, including pollination (Herrera, 1987, 1989; Morris, 2003; Madjidian *et al.*, 2008), seed dispersal (Schupp, 1993; Ness *et al.*, 2006; Brodie *et al.*, 2009), and protection against natural enemies (Rico-Gray & Thien, 1989; Del-Claro & Oliveira, 2000; Stanton, 2003; Clark & Singer, 2018). Variation in service quality and/or quantity may result in variation in the shared mutualist fitness (Palmer *et al.*, 2010; Ford *et al.*, 2015). Therefore, to accurately determine the impact of mutualistic interactions on partner population dynamics, it is necessary to identify all service providers within the community and quantify their effects on mutualistic partners. Furthermore, to fully understand the ecological significance of these multi-partner mutualisms

Correspondence: Andrés M. Devegili, Laboratorio Ecotono, CONICET-UNComa, Pasaje Gutiérrez 1125, C.P: 8400, S.C. de Bariloche, Río Negro, Argentina.
Email: andresdevegili@comahue-conicet.gob.ar

in nature, it is necessary to integrate several approaches. Thus, behavioural assays may contribute information on the quality of the services provided by mutualist partners, while field observations and experiments may allow the estimation of quantitative features of the interactions as well as their impact on the mutualistic partners' fitness.

Protective associations are suitable systems to study the effect of variation in service quality and/or quantity on mutualism. First, these relationships are often facultative, resulting in numerous species engaged in the interaction over time and space (Pringle & Gordon, 2013; Clark & Singer, 2018). Second, defence quality and/or quantity often vary within the guild of protectors (Ness *et al.*, 2006; Miller, 2007; Clark & Singer, 2018). Third, characteristics associated with protection quality and quantity (e.g. aggressiveness, abundance, and frequency) are easy to measure and feasible to manipulate in experiments (Ness *et al.*, 2006; Detrain *et al.*, 2017). Fourth, protected species are often sensitive to defence service variation (Ness *et al.*, 2006; Palmer *et al.*, 2010).

Ant-aphid food-for-protection mutualisms are widespread interactions that can be 'keystone interactions' with significant consequences on ecological communities and potential economic impact on crops (Styrsky & Eubanks, 2007; Zvereva *et al.*, 2010; Clark *et al.*, 2019). Aphids, which are phloem-feeders, excrete honeydew that is consumed by ants, thus providing carbohydrates that support ant activity and colony growth (Shik *et al.*, 2014; Wills *et al.*, 2015; Wittman *et al.*, 2018). During aphid-tending, ant workers remain near aphid colonies guarding them against aphid natural enemies (Stadler & Dixon, 2008). These associations often involve multiple species of protective ants, which may vary in their defensive capabilities both qualitatively and quantitatively (Ness *et al.*, 2006; Mooney & Mandal, 2010; Senft *et al.*, 2017). If a single ant species in the guild is highly efficient in protecting aphids, then it may reduce the abundance of natural enemies and thus change the composition of the arthropod community (Chamberlain & Holland, 2009; Clark & Singer, 2018). Usually, highly efficient aphid protectors tend to monopolise aphid honeydew and are frequently the behaviourally and numerically dominant species in the ant assemblage (Kaplan & Eubanks, 2005; Grover *et al.*, 2007).

In northwestern Patagonia (Argentina), the exotic aphid *Brachycaudus cardui* (L.) (Aphididae: Macrosiphini; Figure S1B, C) feeds on exotic thistles (*Carduus thoermeri* Weinm., Asteraceae; Figure S1A) where it is tended by native aphid-tending ants (Lescano & Farji-Brener, 2011). The relationship between *B. cardui* and its associated ants is facultative and generalised, which allowed us to find several ant species engaged in aphid-tending (Lescano & Farji-Brener, 2011). These ant species are readily distinguishable in the field (Figure S1B-D) and show differences in aggressiveness towards other insects on thistles (A. M. Devegili, pers. observation). Usually, only one ant species visits a plant at a time (Lescano *et al.*, 2014). Given that aphid natural enemies are abundant, diverse (ladybugs, hoverfly larvae, and parasitoids), and co-occur with ant species in thistle patches (A. M. Devegili, pers. observation; Figure S1E-I), the dynamics of aphid populations may depend on the particular tending ant species present on the plant. We took advantage of this ecological scenario and performed a comparative analysis along with field and behavioural experiments that allowed us to evaluate comprehensively whether variation in defence services provided by ant species affects *B. cardui* populations. We hypothesised that protection by tending ants is a major factor determining aphid population growth on thistles. In particular, we expected that aphid populations on thistles patrolled by the most aggressive and efficient ant species would show the highest rates of population growth.

Materials and methods

Study site and species

We carried out the study in northwestern Patagonia (Argentina, 41.12°S; 71.22°W), in an area comprising herbaceous and shrub-steppe vegetation (Figure S1A). Climate is dry and cold, with a mean annual temperature of 8 °C and mean annual precipitation of 600 mm falling mostly during winter (Dimitri, 1962).

Brachycaudus cardui is a small (1.9–2.3 mm long) green aphid native to Europe, Asia, North Africa, and North America, that occurs in dense colonies on stems and leaves of many Asteraceae species (Blackman & Eastop, 2006). In northwestern Patagonia, *B. cardui* feeds on the exotic and invasive *C. thoermeri*, reducing thistle seed output (Chalcoff *et al.*, 2019). Colonies of *B. cardui* are tended by different ant species, which usually monopolise aphid-infested plants (Lescano and Farji-Brener, 2011). In the study area, there were three ant species tending *B. cardui* on thistles: *Dorymyrmer tener* (Mayr, 1868) (Dolichoderinae; Figure S1B), *Camponotus distinguendus* (Spinola, 1851) (Formicinae; Figure S1C), and *D. richteri* (Forel, 1911) (Dolichoderinae; Figure S1D). These ant species nest in the soil and/or under stones, but never on thistles (A. M. Devegili, pers. obs.). The *Dorymyrmex* genus comprises the numerically dominant ant species of the Patagonian steppe (Farji-Brener *et al.*, 2002; Sackmann & Farji-Brener, 2006). Particularly, *D. tener* is the most abundant ant species in the area and it is highly abundant in disturbed sites and roadsides, where *C. thoermeri* also thrives (Farji-Brener *et al.*, 2002). *Dorymyrmex richteri* is the second most abundant ant species in the area, but it is less frequent in disturbed areas compared to *D. tener* (Farji-Brener *et al.*, 2002). *C. distinguendus* is mainly found in scrublands preying on insects on trees or shrubs (Farji-Brener *et al.*, 2002), but this opportunistic species is also found tending aphids on the steppe vegetation (A. M. Devegili, pers. obs.). The defensive roles of these three ant species against aphid natural enemies are currently unknown. The natural enemies of *B. cardui* in thistle patches include ladybugs, such as *Hippodamia variegata* (Goeze, 1777), *Cycloneda* sp., *Eriopis* sp., and *Harmonia axyridis* (Pallas, 1772) (Figure S1E-H), as well as hoverfly larvae: *Allograpta* sp. and *Syrphus* sp. (Figure S1I). Since identifying hoverfly species at the egg and larval stages was difficult, we did not distinguish hoverfly species in the analyses.

Field pattern: Ant identity and abundance versus. aphid population growth

To determine the effect of aphid-tending ants on aphid population growth, in a thistle patch of 200 × 4 m we marked plants and characterised the ant and aphid assemblage every 15 days during 3 months (i.e. six surveys). We sampled plants during late spring and early summer (November–January), the seasons when the insect species in the study system are fairly abundant. To estimate aphid abundance on thistles, we counted the aphid groups on a plant and multiplied this number by the average number of individuals per group. To estimate the average number of aphids in a group, we took photographs of different aphid groups ($n = 60$) and counted individuals with ImageJ software (Schneider *et al.*, 2012). We estimated aphid population growth with the intrinsic rate of increase (r),

$$r = \frac{\ln(\text{aphid abundance}_{t_f}) - \ln(\text{aphid abundance}_{t_i})}{(\mathrm{t}_f - t_i)},$$

where t_f and t_i are the final and initial time of the sampling period, respectively. Values of $r > 0$, $r = 0$, and $r < 0$ indicate that aphid population is increasing, stable or decreasing, respectively (Begon *et al.*, 2006). The intrinsic rate of increase follows an exponential growth model, which assumes that resources are unlimited for aphid populations. Several observations support that this assumption is met in our system. First, thistles are abundant in the study area, representing a large source of food and space for aphids (Figure S1A). Second, thistles are available during the whole life cycle of *B. cardui*. Third, thistles showing high levels of aphid infestation, i.e. plants with all stems covered with aphids, were uncommon (<3% of the plants surveyed).

In all the thistle surveys, we determined whether plants were occupied by ants, identified ant species, and counted ant workers. Aphid-infested plants that were found to host a single ant species in ≥50% of the surveys, and otherwise were ant-free, were categorised as occupied by this particular ant species (*D. tener*: $n = 41$, *C. distinguendus*: $n = 25$, and *D. richteri*: $n = 24$). Thistles with aphids but lacking ants during the whole survey were categorised as ‘aphid-infested plants without ants’ ($n = 34$; see Supporting Information for more details on how we determined the ant-guarding state). To estimate the abundance of ants on thistles, we averaged the six ant counts coming from the six plant surveys. Surveyed thistles were (i) close to each other, (ii) naturally interspersed in the area, and (iii) similar in size and other characteristics (Table S1); therefore, it can be safely assumed that plant quality was similar in all groups. As a consequence, we assumed that the initial conditions of aphid-infested thistles were similar for all ant species and that variation in aphid population growth rates depended on the differences in defence services provided by each ant species. To examine the relationship between ant identity and aphid population intrinsic rate of increase (r), we performed a one-way ANOVA including aphid-infested plant status as a four-level factor: (i) without ants, (ii) occupied by *D. tener*, (iii) occupied by *C. distinguendus*, or (iv) occupied by *D. richteri*. To examine the relationship between ant number and r, we used correlation tests for each species (Pearson’s product–moment correlation; Puth *et al.*, 2014). In this regard, we further fitted linear and nonlinear regression models (*glm* and *drm* functions from *stats* and *drc* packages; Ritz *et al.*, 2016), and searched for possible threshold values (*davies. test* function from *segmented* package; Muggeo, 2008). Among the nonlinear models, we particularly tested saturation functions, which have proven effective in describing the relationship between defence quality and quantity (Ness *et al.*, 2006). To choose the best regression model, we used the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) (Kuha, 2004). Analyses were done in R (R Core Team, 2016).

Ant aggressiveness in the lab: Pairwise encounters with aphid natural enemies

To quantify the agonistic behaviours displayed by aphid-tending ants, we observed pairwise interactions between ants and aphid natural enemies (adult ladybugs and hoverfly larvae). In a steppe area next to the study site, we collected ant workers directly from thistles using an insect aspirator (~200 ants per species). To account for colony variation in ant aggressiveness, we collected ants from thistles that were at least 30 m apart (5–8 thistles per ant species). This distance was sufficient to assume that ants occupying thistles came from different colonies (see Supporting Information for more details). We took ants to the laboratory and housed them in plastic containers (10 × 8 × 5 cm). We separated ants by species and colony (i.e. the thistle from, which they were collected). Ants had free access to water, sugary water, and were fed one *Tenebrio molitor* larva daily. Likewise, we collected from thistles adult ladybugs (*Hippodamia variegata*: $n = 23$, *Cycloneda* sp.: $n = 29$, and *Eriopis* sp.: $n = 34$) and hoverfly larvae ($n = 91$), and took them to the laboratory where they were kept in plastic containers (10 × 8 × 5 cm). Ladybugs and hoverfly larvae had free access to water (moistened cotton) and were fed aphids (*B. cardui*) *ad libitum*. We housed ladybugs according to species identity. The confrontation arena was a 3 cm-diameter container with a mobile septum that divided the arena into two compartments, thus isolating the ant and the aphid natural enemy before the encounter. To further elicit ant defensive behaviour, we fitted at the base of the arena a filter paper marked with the odour of the tested ant species. The filter paper was placed 24 h before the encounter inside the target colony container together with a piece of thistle stem infested with *B. cardui*. Each confrontation test lasted 4 min ($n = 27–31$). Following a habituation time of 1 min, we removed the septum and video-recorded (×20 magnification) the confrontation for the remaining 3 min. To characterise ant defence efficiency, we measured the time to first contact with the aphid natural enemy (hereafter, encounter time). We identified six ant behaviours from the recordings: (i) ‘escape’, following contact the ant flees in opposite direction; (ii) ‘antennation’, antennae placed onto the aphid enemy for more than 2 s; (iii) ‘threat’, ant standing still with mandibles open and antennae slightly extended towards the aphid enemy; (iv) ‘short bites’, sudden attack with brief bites (less than 2 s); (v) ‘long bites’, persistent biting (more than 2 s) of any part of the aphid enemy body; and (vi) ‘gaster flexing’, ant bends its gaster towards the aphid enemy, releasing a repellent substance

(Video S1). Then, we scored ant aggressiveness using the following index:

$$\text{Aggressiveness index} = \frac{\sum_{i}^{6} \text{frequency}_i \times \text{behaviour}_i}{\sum_{i}^{6} \text{frequency}_i},$$

in which 'frequency' is the occurrence of each interaction in a trial and 'behaviour' is a constant that weights the aggression intensity ('escape' = −1, 'antennation' = 0, 'threat' = 1, 'short bites' = 2, 'long bites' = 3, and 'gaster flexing' = 3). A negative, zero, or positive aggression index means that ants exhibit (on average) elusive, neutral, or aggressive behaviours towards aphid natural enemies, respectively. To increase sample size, we did not distinguish ladybug species and hoverfly sizes in the analyses. To analyse the encounter time of ant species with ladybugs or hoverfly larvae, we used a generalised linear model with a negative binomial distribution (function *glm.nb* from R package 'Mass'; Ripley *et al.*, 2013). To compare the aggressiveness indices between the three ant species, we used a linear mixed effect model (LME) with ant species as a fixed factor and thistle plant (i.e. colony identity) as a random factor. To build LME we used function *lmer* from package 'lme4' (Bates *et al.*, 2015). Analyses were done in R.

Ant defence service in the field: Interactions between ants, aphids, and their enemies

We used the ant species with the highest aggressiveness index (*D. tener*, see Section 3) to test in natural conditions whether ants deter aphid enemies and affect aphid populations on plants. We selected thistles ($n = 30$) patrolled by *D. tener* and infested with *B. cardui*. In each plant, we selected two similar stems: in one stem ants were left undisturbed (control) and in the other stem ants were excluded (ant exclusion). To exclude ants, we wrapped the base of the stems with duct tape (Ductac®), gently coated the tape with lithium grease (Lubrigras®), and removed the remaining ants with an insect aspirator. In each stem, we selected an aphid group and, with a permanent marker, drew a spot on the stem at 2 cm distance from the aphid group that served as reference. We then gently placed on the spot the ladybugs or hoverfly larvae, which were transferred singly using soft tweezers (Video S2). Each experiment lasted 5 min, and the aphid natural enemy was considered successfully deterred when it was ejected from the plant or killed by ants. Conversely, we considered an ejection unsuccessful when the aphid enemy stayed at the plant during the whole trial. We used four ladybug species, *Eriopis* sp. (ants present: $n = 23$, ants excluded: $n = 16$), *H. variegata* (ants present: $n = 23$, ants excluded: $n = 14$), *Cycloneda* sp. (ants present: $n = 20$, ants excluded: $n = 15$), and *H. axyridis* (ants present: $n = 20$, ants excluded: $n = 11$), and the larvae of two hoverfly species (ants present: $n = 30$, ants excluded: $n = 19$). Because the aggressiveness of ants towards hoverfly larvae depends on the larval size (Detrain *et al.*, 2017), we categorised hoverfly larvae into three size groups: (i) small: <4 mm long, (ii) medium: 4–10 mm, and (iii) large: >10 mm, and performed analyses accordingly. Ladybug species differed in size (Figure S1E-H), but we did not consider size as a covariate in the analysis because it showed negligible variation at the within-species level. To analyse the probability for aphid enemies of being ejected from the plant, we used a generalized linear model (GLM) with binomial error distribution.

To evaluate the defence service provided by the ant *D. tener* to the aphid *B. cardui*, we excluded ants from stems, surveyed aphid populations, and counted aphid enemies. We identified thistle plants ($n = 30$) infested with *B. cardui* and patrolled by *D. tener*. In each plant, we selected two similar stems and randomly assigned one of them to the ant exclusion treatment (ants were excluded as described above), and the other stem served as control (free access to ants). In each stem, we marked one aphid group and took photographs of the aphids (from all cardinal points) every 5 days during 20 days; then we counted individuals with ImageJ software (Schneider *et al.*, 2012) and calculated aphid density dividing the number of individuals by the area occupied by the aphid group (stem surface with aphids: $2\pi \times \textit{stem radius} \times \textit{stem height}$). We also counted the number of (i) adult ladybugs, (ii) hoverfly larvae, and (iii) hoverfly eggs. Finally, to assure that the effects of the ant exclusion treatment on aphids and aphid natural enemies were actually caused by the lack of ants, on the 15th day we allowed ant access to half of the ant-excluded stems ($n = 15$; hereafter, 'ant exclusion reversion' treatment). Using sisal yarn, we made artificial bridges that connected the ant-excluded stem and the adjacent stem with ants; this procedure was proven effective earlier (Lescano *et al.*, 2015). To analyse the effects of ant exclusions on aphid density and abundance of ladybugs, hoverfly larvae, and hoverfly eggs, we fitted generalised linear mixed models (GLMM, function *glmmadmb* from R package 'glmmADMB'; Skaug *et al.*, 2014) with stem treatment as a fixed factor (levels: control and ant exclusion) and plant identity as a random factor; we used negative binomial error distribution for aphid density and Poisson error distribution for the abundance of ladybugs, hoverfly larvae, and hoverfly eggs. In the 20th day, we compared ant exclusion and 'ant exclusion reversion' treatments with a zero-inflated regression model (function *zeroinfl* from R package 'pscl'; Jackman *et al.*, 2017). All the analyses were done in R.

Results

Field pattern: Ant identity and abundance versus. aphid population growth

Among aphid-tending ants, *D. tener* occupied and patrolled thistles with higher frequency and abundance than *C. distinguendus* and *D. richteri* (Table 1). Ant identity affected the intrinsic rate of increase of aphid populations (r) (ANOVA, $F_{3,123} = 11.5$, $P < 0.001$), with aphid populations increasing at a higher rate on plants hosting *D. tener* ($r = 0.06 \pm 0.004\ \text{day}^{-1}$, mean ± SE) than on plants hosting *C. distinguendus* (0.03 ± 0.006), *D. richteri* (0.03 ± 0.005) or lacking ants (0.003 ± 0.007) (Fig. 1a). The relation between the number of *D. tener* ants and r was statistically significant, positive (Pearson correlation test, $t = 3.2$, $P = 0.002$, $r = 0.47$; GLM, $t = 3.2$, $P = 0.003$), and linear (Fig. 1b; Table S2); no threshold points were found for the linear relationship. Neither *C. distinguendus* nor *D. richteri* ant numbers showed an association with r (Fig. 1b).

Table 1. Comparison of defence features of the aphid-tending ant species *Dorymyrmex tener*, *Camponotus distinguendus*, and *D. richteri*. Within rows, values that share the same lowercase letter are not significantly different (Tukey test, following GLMs and linear mixed effects).

	D. tener (mean ± SE)	*C. distinguendus* (mean ± SE)	*D. richteri* (mean ± SE)	*P*
Defence quantity				
Occupancy percentage*	83 ± 3a	60 ± 4b	55 ± 4b	0.03
Abundance†	20.3 ± 2.7a	5.4 ± 0.9b	2.3 ± 0.3c	<0.001
Defence quality				
Aggressiveness index‡	1.8 ± 0.1a	0.2 ± 0.1b	−0.3 ± 0.1c	<0.001

*The same plant in six surveys.

†Average number of ants in six plant surveys.

‡Average aggressiveness towards ladybugs or hoverfly larvae; values range from −1 (avoidance behaviour) to +3 (aggressive behaviour)

Ant aggressiveness in the lab: Pairwise encounters with aphid natural enemies

Ant species differed in their encounter time with aphid natural enemies, with *D. tener* contacting ladybugs (GLM, $\chi^2 = 34.01$, $P < 0.001$) or hoverfly larvae ($\chi^2 = 28.81$, $P < 0.001$) faster than *C. distinguendus* and *D. richteri* (Fig. 2a, d). *Dorymyrmex tener* showed a high frequency of aggressive behaviours, such as 'threat', 'short bites', 'long bites', and 'gaster flexing', while *C. distinguendus* and *D. richteri* showed a high frequency of passive behaviours, such as 'escape' and 'antennation' (Fig. 2b, e). Accordingly, ant species differed in their aggressiveness index (Table 1), with *D. tener* being the most aggressive ant towards ladybugs (LME, $\chi^2 = 88.8$, $P < 0.001$; Fig. 2c) and hoverfly larvae (LME, $\chi^2 = 348.0$, $P < 0.001$; Fig. 2f).

Ant defence service in the field: Interactions between ants, aphids, and their enemies

The number of *D. tener* was positively associated with ejection probability for ladybugs in three of the four ladybug species (GLM, *Eriopis* sp.: $z = 2.84$, $P = 0.004$; *H. variegata*: $z = 3.09$, $P = 0.002$; *H. axyridis*: $z = 3.02$, $P = 0.003$; and *Cycloneda* sp.: $z = 1.72$, $P = 0.08$) (Fig. 3a). The number of *D. tener* was positively associated with ejection probability for hoverfly larvae in two of the three size categories (GLM, small size: $z = 2.11$, $P = 0.03$; medium size: $z = 2.26$, $P = 0.02$; and large size: $z = 1.60$, $P = 0.10$) (Fig. 3b). Neither adult ladybugs nor hoverfly larvae dropped from thistles when placed on ant-excluded stems.

Aphid density was higher on stems with the ant *D. tener* than on stems with ant exclusion (GLMM, LR$\chi^2 = 108.66$, $P < 0.001$) (Fig. 4a). The average number of ladybugs and hoverfly larvae was lower on stems with the ant *D. tener* than on stems with ant exclusion (GLMM, ladybugs: LR$\chi^2 = 10.70$, $P = 0.0011$, hoverfly larvae: LR$\chi^2 = 15.48$, $P < 0.001$) (Fig. 4b, c). The 'ant exclusion reversion' caused an increase in aphid density (Zero-inflated, $z = 2.462$, $P = 0.014$) (Fig. 4a), a decrease in the number of hoverfly larvae ($z = 2.562$, $P = 0.024$) (Fig. 4c), and despite the abundance of ladybugs did not change ($z = 0.081$, $P = 0.94$), it also showed a tendency to decrease (Fig. 4b). Likewise, the number of hoverfly eggs was lower

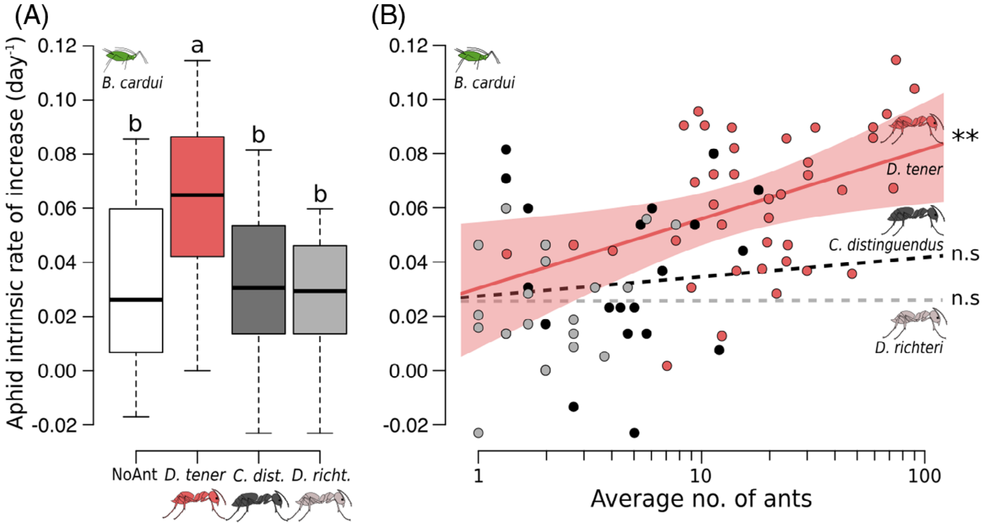

Fig. 1. Field pattern: ant identity and abundance versus aphid population growth. (a) Boxplots of aphid population intrinsic rate of increase (r, day^{-1}) on plants occupied by different ant species. Aphid-infested plants without ants are labelled as 'NoAnt' (white box). Aphid-infested plants with ants are labelled with the ant species name: *Dorymyrmex tener* (*D. tener*, red box), *Camponotus distinguendus* (*C. dist.*, black box); *D. richteri* (*D. richt.*, grey box). Boxplots sharing lowercase letters above error bars are not significantly different ($P > 0.05$, Tukey tests following One-way ANOVA). (b) Relationship between ant abundance (average number of ants in six plant surveys) and aphid population intrinsic rate of increase (r, day^{-1}) for the three aphid-tending ant species. The shaded area is the standard error of linear regression. **$P < 0.01$; n.s.: not significant. [Colour figure can be viewed at wileyonlinelibrary.com].

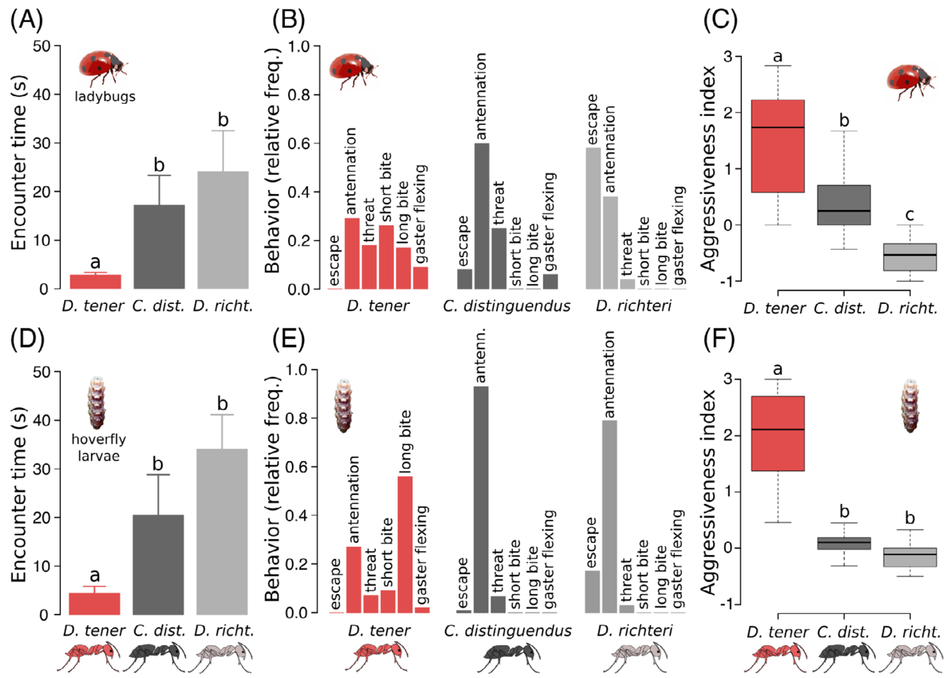

Fig. 2. Pairwise encounters between aphid-tending ants and aphid natural enemies. Encounter time of ant species with ladybugs (a) and hoverfly larvae (d). Relative frequency of each ant behaviour towards ladybugs (b) and hoverfly larvae (e); ant behaviours increase in aggressiveness from left to right. Aggressiveness indices of ant species towards ladybugs (c) or hoverfly larvae (f). Dots and whiskers represent Mean ± SE. Lowercase letters on error bars depict differences between ant species (Post-Hoc Tukey tests). *Dorymyrmex tener* (red) = *Dorymyrmex tener*; *C. dist.* (black) = *Camponotus distinguendus*; *D. richt.* (grey) = *D. richteri*. [Colour figure can be viewed at wileyonlinelibrary.com].

on stems with the ant *D. tener* than on stems with ant exclusion (GLMM, LR$\chi^2 = 78.56$, $P < 0.001$), and the number of hoverfly eggs decreased after the 'ant exclusion reversion' (Zero-inflated, $z = 2.346$, $P = 0.019$).

Discussion

Within mutualist guilds, service providers of different quality and quantity are expected to be found exerting differential effects on shared mutualists. Understanding this variation may yield insights into the ecological dynamics of mutualisms (Stanton, 2003; Palmer *et al.*, 2015). In the studied aphid-tending ant guild, both defence quality and quantity were important to explain the aphid population growth rate. Concerning defence quality, a particular ant species, *D. tener*, was by far the most aggressive species towards aphid natural enemies in laboratory confrontation experiments and in the field, where *D. tener* efficiently expelled aphid natural enemies from thistle host plants; the other ant species, *C. distinguendus* and *D. richteri* exhibited little aggressiveness. Likewise, quantitative aspects of defence were clearly different among ant species, with the aggressive *D. tener* being more abundant and frequent on thistles than the other ant species. Furthermore, the experimental exclusion of *D. tener* caused an increase in aphid density and a decrease in the abundances of aphid natural enemies, evidencing that defence provided by *D. tener* is the main cause of increased aphid population growth rate. Collectively, results support the notion that evaluations of defence quality and quantity can accurately predict, which ant species is the best aphid protector in the community. Moreover, this study illustrates the importance of integrating behavioural assays with ecological analyses to better understand the impact of multi-partner associations in nature.

Defence quality has been shown to be important in other food-for-protection associations between ants, plants, and homopterans, in which usually the best protectors were competitively dominant ant species that exhibited aggressive behaviours towards other arthropods in the community (Grover *et al.*, 2007; Fagundes *et al.*, 2017; Clark & Singer, 2018). Variation in service quantity, which stems from differences in abundances and frequencies of the mutualist partners, may also be important in determining the population dynamics of mutualists (Palmer *et al.*, 2010). We showed that *D. tener* is the most abundant and frequent ant visitor of thistle plants and that its numerical dominance played an important role in the mutualist interactions studied. Thus, *D. tener* abundance was associated with both the probability of expelling aphid natural enemies from plants and aphid population growth, which supports the idea that – together with service quality – service quantity may determine the outcome of aphid protection against natural enemies. Aphid natural enemies are abundant, diverse (e.g. ladybugs, hoverfly larvae, and parasitoids), and with different attack strategies. A high density of attending ants may increase the chances of killing or expelling aphid enemies, even if they are initially resistant to ants, and may also decrease the encounter time of aphid enemies. The latter is particularly important with

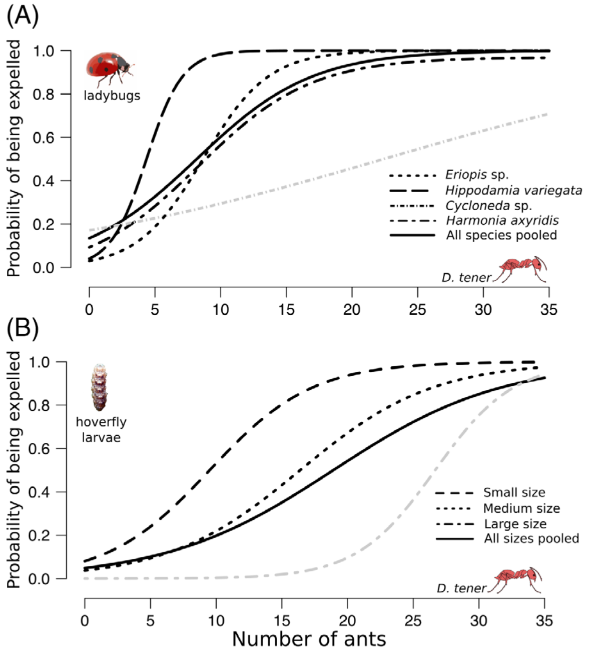

Fig. 3. Ant number and expulsion of aphid natural enemies. Relationship between the number of *Dorymyrmex tener* ants tending aphids and the probability for aphid natural enemies (a, Ladybugs; b, Hoverfly larvae) of being expelled from thistle stems. Relationships were tested with univariate logistic regressions. Black curves = significant regression; grey curves = non-significant regression. [Colour figure can be viewed at wileyonlinelibrary.com].

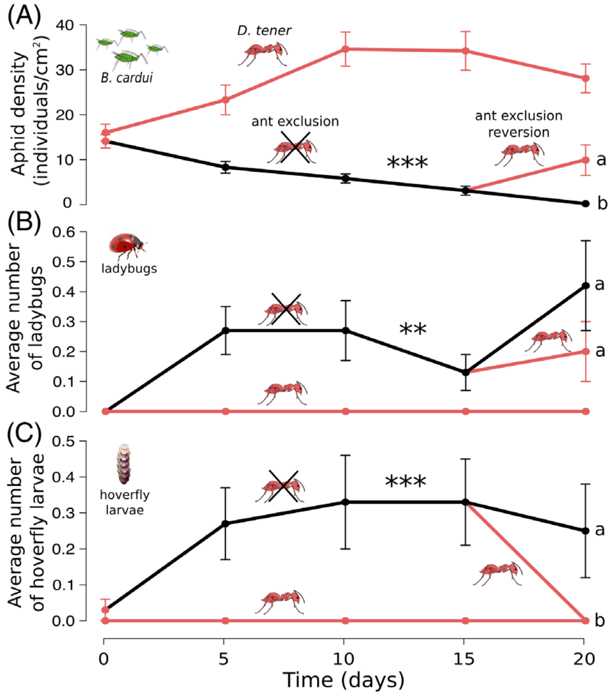

Fig. 4. Ant exclusion versus aphid density and aphid enemy abundances. Effects of excluding the aggressive ant *Dorymyrmex tener* from thistle stems on aphid density (a) and the average number of ladybugs (b) and hoverfly larvae (c). Red curves represent thistle stems with *D. tener* ($n = 30$) and black curves represent thistle stems in which *D. tener* was excluded ($n = 30$). On the 15th day, ants were allowed access to half of the ant-excluded stems using artificial bridges of sisal yarn (ant exclusion reversion). Asterisks depict significant differences between stems with and without ant exclusion. Different lowercase letters indicate significant differences between ant exclusion and 'ant exclusion reversion' treatments. Dots and whiskers represent mean ± SE. $^{**}P < 0.01$; $^{***}P < 0.001$. [Colour figure can be viewed at wileyonlinelibrary.com].

ladybugs, which are active and voracious predators that can decrease aphid populations in a short time (Beltrà *et al.*, 2018). Although increased ant number may be positively associated with aphid protection, a high number of attending ants could also be detrimental if increased tending intensity imposes a cost on aphids (Yoo & Holway, 2011). Interestingly, we found that *D. tener* abundance was positively associated with aphid population growth in a linear fashion, suggesting that a high level of ant attendance is not costly for aphids and that defence quantity provided by ants may not necessarily compromise defence quality.

Although mutualisms are usually considered pairwise beneficial associations, a more realistic approach considers them as beneficial interactions between guilds of multiple partner species (Stanton, 2003; Palmer *et al.*, 2015). Interestingly, here the aphid-tending ant assemblage did not behave as a mutualist guild. Thus, only the aggressive and abundant ant species increased aphid fitness, i.e. acted as a "true" mutualist, while the subordinate ant species did not affect aphid fitness, i.e. behaved as commensalists (we verified that all three ant species consume aphid honeydew). Losing control of aphids and plants from rival ants can be costly to the ant colony, even more so than the cost associated with aphid predation by natural enemies (Phillips & Willis, 2005). Therefore, in multi-partner associations, dominant ant species are often found monopolising the mutualistic interaction with aphids and decreasing the abundance of subordinate ant species (Blüthgen & Fiedler, 2004; Grover *et al.*, 2007; Wilder *et al.*, 2013). In fact, previous studies in the study system showed that aphid-tending ants usually monopolise aphid-infested thistles and exhibit nocturnal defensive behaviours to secure thistles and aphid honeydew (Lescano *et al.*, 2014, 2015), suggesting that interspecific competition within the aphid-tending guild is significant.

Although the most aggressive and abundant ant species acted as the only mutualist in the aphid-tending ant guild, this interaction might shift to either commensalism, parasitism, or predation. For example, if the abundance of aphid enemies decreases considerably, the best aphid protector would continue to benefit from sugary rewards while aphids obtain negligible benefits. This scenario could result in commensalism if ants do not disturb aphids, or shift to parasitism if honeydew consumption imposes a cost on aphids (Cushman & Whitham, 1989; Stadler & Dixon, 1998; Vantaux *et al.*, 2015). Ant-aphid mutualisms may even shift to predation if aphid abundance increases dramatically, and thus aphid honeydew provision exceeds ant requirements; under this scenario, ants may start preying on aphids to maintain the sugar: protein balance in the diet (Sakata, 1994). Finally, reduced interspecific competition within the aphid-tending ant guild, for example in plant patches lacking the aggressive (and mutualistic) ant species, could cause behaviourally passive (and so far commensalistic) ant species to increase in abundance and probably start providing actual protection to aphids, despite fitted lines in Fig. 1b would suggest otherwise. This could occur since an increase in ant number can enhance ant aggressiveness towards other insects (Tanner, 2006, 2008) and improve the detection of aphid natural enemies. In summary, the ecological scenario may influence the actual role of aphid-tending ants as aphid mutualists in the community.

By combining behavioural assays with field experiments and observations, we showed that defence quality and quantity vary within an aphid-tending ant guild and that this variation is associated with aphid population growth. We found that only the most aggressive and abundant ant species increased aphid fitness, thus behaving like a true mutualist, while subordinate, behaviourally passive ant species had no effect on aphid fitness and thus behaved as commensalists. Knowledge of which ant species actually behave as aphid mutualists are critical since ant-hemiptera associations are often ‘keystone interactions’ (Styrsky & Eubanks, 2007; Zhang *et al.*, 2012) with far-reaching consequences for the communities in which they are embedded (Kaplan & Eubanks, 2005; Clark *et al.*, 2019). In this regards the present study contributes to our understanding of the effects of defence service variation on aphid populations, which will enhance our ability to predict the ecological consequences of ant-aphid mutualisms.

Acknowledgements

We thank Agustín Lovrich, Tomás González, and Daniela González for field assistance and Florencia Baudino and Guillermo González for help with the identification of ladybug species. We thank Marina Alma, Victoria Werenkraut, Micaela Buteler, Daniela Ortiz, and members of the ECOLFUN laboratory for helpful suggestions during the course of the research. We thank two anonymous reviewers and the editors for helpful recommendations on the manuscript. Andrés Matías Devegili is supported by a doctoral fellowship from the Consejo Nacional de Investigaciones Científicas y Técnicas of Argentina.

Conflict of interest

The authors declare that they have no conflict of interest.

Author Contributions

All the authors conceived the study and designed the methodology, AMD collected the data, AMD analysed the data, and AMD and AGFB led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Data availability statement

The data that support the findings of this study are openly available in the Figshare Repository at https://doi.org/10.6084/m9.figshare.12196719.v3.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1: Supporting information.

Fig. S1. Study system. Photographs of a *Carduus thoermeri* patch and thistle plants with aphid-tending ants (*Dorymyrmex tener*, *Camponotus distinguendus,* or *D. richteri*), aphids (*Brachycaudus cardui*), or aphid natural enemies (ladybugs and hoverfly larvae).

Table S1. Comparison of *Carduus thoermeri* traits (height, stem number, and inflorescence number) and aphid initial abundance under two natural conditions: i) ant-guarded thistles (*Dorymyrmex tener*, *Camponotus distinguendus,* or *D. richteri*) and ii) unguarded thistles (without aphid-tending ants).

Table S2. Rankings and goodness of fit of null, linear, and nonlinear models linking *Dorymyrmex tener* abundance and aphid population growth rate.

Video S1. Ant behaviours used to characterise the aggressiveness of ant species towards aphid natural enemies.

Video S2. Response of the most aggressive ant species to the intrusion of a ladybug into an aphid-infested thistle stem.

References

Bates, D., Maechler, M., Bolker, B., Walker, S., Christensen, R.H.B., Singmann, H. … & Fox, J. (2015) *Package ‘lme4’. R package version 1.1–23*. URL https://cran.r-project.org/web/packages/lme4/index.html [accessed on 1 April 2020].

Begon, M., Townsend, C.R. & Harper, J.L. (2006) *Ecology: From Individuals to Ecosystems*, 4th edn. Blackwell Publishing, Malden.

Beltrà, A., Wäckers, F.L., Nedvěd, O. & Pekas, A. (2018) Predation rate and performance of three ladybirds against the green peach aphid *Myzus persicae* in sweet pepper. *Entomologia Experimentalis et Applicata*, **166**, 491–499.

Blackman, R.L. & Eastop, V.F. (2006) *Aphids on the World’s Herbaceous Plants and Shrubs*. John Wiley & Sons, Chichester.

Blüthgen, N. & Fiedler, K. (2004) Competition for composition: lessons from nectar-feeding ant communities. *Ecology*, **85**, 1479–1485.

Brodie, J.F., Helmy, O.E., Brockelman, W.Y. & Maron, J.L. (2009) Functional differences within a guild of tropical mammalian frugivores. *Ecology*, **90**, 688–698.

Chalcoff, V.R., Lescano, M.N. & Devegili, A.M. (2019) Do novel interactions with local fauna have reproductive consequences for exotic plants? A case study with thistles, ants, aphids, and pollinators. *Plant Ecology*, **220**, 125–134.

Chamberlain, S.A. & Holland, J.N. (2009) Quantitative synthesis of context dependency in ant–plant protection mutualisms. *Ecology*, **90**, 2384–2392.

Clark, R.E., Illan, J.G., Comerford, M.S. & Singer, M.S. (2019) Keystone mutualism influences forest tree growth at a landscape scale. *Ecology Letters*, **22**, 1599–1607.

Clark, R.E. & Singer, M.S. (2018) Differences in aggressive behaviors between two ant species determine the ecological consequences of a facultative food-for-protection mutualism. *Journal of Insect Behavior*, **31**, 510–522.

Cushman, J. & Whitham, T.G. (1989) Conditional mutualism in a membracid-ant association: temporal, age-specific, and density-dependent effects. *Ecology*, **70**, 1040–1047.

Del-Claro, K. & Oliveira, P.S. (2000) Conditional outcomes in a neotropical treehopper-ant association: temporal and species-specific variation in ant protection and homopteran fecundity. *Oecologia*, **124**, 156–165.

Detrain, C., Fichaux, M. & Verheggen, F. (2017) Tuned protection of aphids by ants against a predatory hoverfly. *Ecological Entomology*, **42**, 235–244.

Dimitri, M.J. (1962) La Flora Andino-Patagónica. *Anales de Parques Nacionales*, pp. 1–115. SAGN, Direccion Nacional de PN, Buenos Aires.

Fagundes, R., Dáttilo, W., Ribeiro, S.P., 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. *Biological Journal of the Linnean Society*, **122**, 71–83. https://doi.org/10.1093/biolinnean/blx059.

Farji-Brener, A.G., Corley, J.C. & Bettinelli, J. (2002) The effects of fire on ant communities in North-Western Patagonia: the importance of habitat structure and regional context. *Diversity and Distributions*, **8**, 235–243.

Ford, K.R., Ness, J.H., Bronstein, J.L. & Morris, W.F. (2015) The demographic consequences of mutualism: ants increase host-plant fruit production but not population growth. *Oecologia*, **179**, 435–446.

Grover, C.D., Kay, A.D., Monson, J.A., Marsh, T.C. & Holway, D.A. (2007) Linking nutrition and behavioural dominance: carbohydrate scarcity limits aggression and activity in argentine ants. *Proceedings of the Royal Society B: Biological Sciences*, **274**, 2951–2957.

Herrera, C.M. (1987) Components of pollinator "quality": comparative analysis of a diverse insect assemblage. *Oikos*, **50**, 79–90.

Herrera, C.M. (1989) Pollinator abundance, morphology, and flower visitation rate: analysis of the "quantity" component in a plant-pollinator system. *Oecologia*, **80**, 241–248.

Jackman, S., Tahk, A., Zeileis, A., Maimone, C., Fearon, J. & Meers, Z. (2017) *Package 'pscl'. R package version 1.5.2*. URL https://cran.r-project.org/web/packages/pscl/index.html [accessed on 15 February 2019].

Kaplan, I. & Eubanks, M.D. (2005) Aphids alter the community-wide impact of fire ants. *Ecology*, **86**, 1640–1649.

Kuha, J. (2004) AIC and BIC: comparisons of assumptions and performance. *Sociological Methods & Research*, **33**, 188–229.

Lescano, M.N. & Farji-Brener, A.G. (2011) Exotic thistles increase native ant abundance through the maintenance of enhanced aphid populations. *Ecological Research*, **26**, 827–834.

Lescano, M.N., Farji-Brener, A.G. & Gianoli, E. (2014) Nocturnal resource defence in aphid-tending ants of northern Patagonia. *Ecological Entomology*, **39**, 203–209.

Lescano, M.N., Farji-Brener, A.G. & Gianoli, E. (2015) Outcomes of competitive interactions after a natural increment of resources: the assemblage of aphid-tending ants in northern Patagonia. *Insectes Sociaux*, **62**, 199–205.

Madjidian, J.A., Morales, C.L. & Smith, H.G. (2008) Displacement of a native by an alien bumblebee: lower pollinator efficiency overcome by overwhelmingly higher visitation frequency. *Oecologia*, **156**, 835–845.

Miller, T.E.X. (2007) Does having multiple partners weaken the benefits of facultative mutualism? A test with cacti and cactus-tending ants. *Oikos*, **116**, 500–512.

Mooney, K.A. & Mandal, K. (2010) Competition hierarchies among ants and predation by birds jointly determine the strength of multi-species ant–aphid mutualisms. *Oikos*, **119**, 874–882.

Morris, W.F. (2003) Which mutualists are most essential? Buffering of plant reproduction against the extinction of pollinators. *The Importance of Species: Perspectives on Expendability and Triage, 1st Edn* (ed. by P. Kareiva and S. A. Levin), pp. 260–280. Princeton University Press, Princeton, New Jersey.

Muggeo, V.M.R. (2008) *Package 'segmented'. R package version 1.1–0*. URL https://cran.r-project.org/web/packages/segmented/index.html [accessed on 1 April 2020].

Ness, J.H., Morris, W.F. & Bronstein, J.L. (2006) Integrating quality and quantity of mutualistic service to contrast ant species protecting *Ferocactus wislizeni*. *Ecology*, **87**, 912–921.

Palmer, T.M., Doak, D.F., Stanton, M.L., Bronstein, J.L., Kiers, E.T., Young, T.P. *et al.* (2010) Synergy of multiple partners, including freeloaders, increases host fitness in a multispecies mutualism. *Proceedings of the National Academy of Sciences*, **107**, 17234–17239.

Palmer, T.M., Pringle, E.G., Stier, A. & Holt, R.D. (2015) Mutualism in a community context. *Mutualism*, 1st edn (ed. by J. L. Bronstein), pp. 159–180. Oxford University Press, New York, New York.

Phillips, I.D. & Willis, C.K. (2005) Defensive behavior of ants in a mutualistic relationship with aphids. *Behavioral Ecology and Sociobiology*, **59**, 321–325.

Pringle, E.G. & Gordon, D.M. (2013) Protection mutualisms and the community: geographic variation in an ant-plant symbiosis and the consequences for herbivores. *Sociobiology*, **60**, 242–251.

Puth, M.T., Neuhäuser, M. & Ruxton, G.D. (2014) Effective use of Pearson's product–moment correlation coefficient. *Animal Behavior*, **93**, 183–189.

R Core Team (2016) *R: A Language and Environment for Statistical Computing*.

Rico-Gray, V. & Thien, L.B. (1989) Effect of different ant species on reproductive fitness of *Schomburgkia tibicinis* (Orchidaceae). *Oecologia*, **81**, 487–489.

Ripley, B., Venables, B., Bates, D.M., Hornik, K., Gebhardt, A., Firth, D. & Ripley, B. (2013) *Package 'Mass'. R package version 7.3–51.4*. URL https://cran.r-project.org/web/packages/MASS/index.html [accessed on 15 February 2019].

Ritz, C., Strebig, J.C. & Ritz, M.C. (2016) *Package 'drc'. R package version 3.0–1*. URL https://cran.r-project.org/web/packages/drc/index.html [accessed on 5 April 2020].

Sackmann, P. & Farji-Brener, A.G. (2006) Effect of Fire on Ground Beetles and Ant Assemblages along an Environmental Gradient in NW Patagonia: Does Habitat Type Matter? *Ecoscience* **13**, 360–371.

Sakata, H. (1994) How an ant decides to prey on or to attend aphids. *Population Ecology*, **36**, 45–51.

Schneider, C.A., Rasband, W.S. & Eliceiri, K.W. (2012) NIH image to ImageJ: 25 years of image analysis. *Nature Methods*, **9**, 671–675.

Schupp, E.W. (1993) Quantity, quality and the effectiveness of seed dispersal by animals. *Vegetatio*, **107**, 15–29.

Senft, M., Weisser, W.W. & Zytynska, S.E. (2017) Habitat variation, mutualism and predation shape the spatio-temporal dynamics of tansy aphids. *Ecological Entomology*, **42**, 389–401.

Shik, J.Z., Kay, A.D. & Silverman, J. (2014) Aphid honeydew provides a nutritionally balanced resource for incipient argentine ant mutualists. *Animal Behaviour*, **95**, 33–39.

Skaug, H., Fournier, D., Nielsen, A., Magnusson, A & Bolker, B. (2014) *Package 'glmmADMB'. R package version 0.8.3.4*. URL http://glmmadmb.r-forge.r-project.org [accessed on 30 January 2019].

Stadler, B. & Dixon, A.F.G. (1998) Costs of ant attendance for aphids. *Journal of Animal Ecology*, **67**, 454–459.

Stadler, B. & Dixon, A.F.G. (2008) *Mutualism: Ants and their Insect Partners*. Cambridge university press, Cambridge.

Stanton, M.L. (2003) Interacting guilds: moving beyond the pairwise perspective on mutualisms. *The American Naturalist*, **162**, S10–S23.

Styrsky, J.D. & Eubanks, M.D. (2007) Ecological consequences of interactions between ants and honeydew-producing insects. *Proceedings of the Royal Society B: Biological Sciences*, **274**, 151–164.

Tanner, C.J. (2006) Numerical assessment affects aggression and competitive ability: a team-fighting strategy for the ant *Formica xerophila*. *Proceedings of the Royal Society B: Biological Sciences*, **273**, 2737–2742.

Tanner, C.J. (2008) Aggressive group behaviour in the ant *Formica xerophila* is coordinated by direct nestmate contact. *Animal Behaviour*, **76**, 1335–1341.

Vantaux, A., Schillewaert, S., Parmentier, T., Van Den Ende, W.I.M., Billen, J. & Wenseleers, T.O.M. (2015) The cost of ant attendance and melezitose secretion in the black bean aphid *Aphis fabae*. *Ecological Entomology*, **40**, 511–517.

Wilder, S.M., Barnum, T.R., Holway, D.A., Suarez, A.V. & Eubanks, M.D. (2013) Introduced fire ants can exclude native ants from critical mutualist-provided resources. *Oecologia*, **172**, 197–205.

Wills, B.D., Chong, C.D., Wilder, S.M., Eubanks, M.D., Holway, D.A. & Suarez, A.V. (2015) Effect of carbohydrate supplementation on investment into offspring number, size, and condition in a social insect. *PLoS One*, **10**, e0132440.

Wittman, S.E., O'Dowd, D.J. & Green, P.T. (2018) Carbohydrate supply drives colony size, aggression, and impacts of an invasive ant. *Ecosphere*, **9**, e02403.

Yoo, H.J.S. & Holway, D.A. (2011) Context-dependence in an ant–aphid mutualism: direct effects of tending intensity on aphid performance. *Ecological Entomology*, **36**, 450–458.

Zhang, S., Zhang, Y. & Ma, K. (2012) The ecological effects of the ant–hemipteran mutualism: a meta-analysis. *Basic and Applied Ecology*, **13**, 116–124.

Zvereva, E.L., Lanta, V. & Kozlov, M.K. (2010) Effects of sap-feeding insect herbivores on growth and reproduction of woody plants: a meta-analysis of experimental studies. *Oecologia*, **163**, 949–960.

Accepted 29 May 2020
Associate Editor: Joshua King