The indirect consequences of a mutualism: comparing positive and negative components of the net interaction between honeydew-tending ants and host plants

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Summary

1. In ecological webs, net indirect interactions between species are composed of interactions that vary in sign and magnitude. Most studies have focused on negative component interactions (e.g. predation, herbivory) without considering the relative importance of positive interactions (e.g. mutualism, facilitation) for determining net indirect effects.

2. In plant/arthropod communities, ants have multiple top-down effects via mutualisms with honeydew-producing herbivores and harassment of and predation on other herbivores; these ant effects provide opportunities for testing the relative importance of positive and negative interspecific interactions. We manipulated the presence of ants, honeydew-producing membracids and leaf-chewing beetles on perennial host plants in field experiments in Colorado to quantify the relative strength of these different types of interactions and their impact on the ant's net indirect effect on plants.

3. In 2007, we demonstrated that ants simultaneously had a positive effect on membracids and a negative effect on beetles, resulting in less beetle damage on plants hosting the mutualism.

4. In 2008, we used structural equation modelling to describe interaction strengths through the entire insect herbivore community on plants with and without ants. The ant's mutualism with membracids was the sole strong interaction contributing to the net indirect effect of ants on plants. Predation, herbivory and facilitation were weak, and the net effect of ants reduced plant reproduction. This net indirect effect was also partially because of behavioural changes of herbivores in the presence of ants. An additional membracid manipulation showed that the membracid's effect on ant activity was largely responsible for the ant's net effect on plants; ant workers were nearly ten times as abundant on plants with mutualists, and effects on other herbivores were similar to those in the ant manipulation experiment.

5. These results demonstrate that mutualisms can be strong relative to negative direct interspecific interactions and that positive interactions deserve attention as important components of ecological webs.

Keywords: ant–membracid interactions, context dependence, *Formica obscuripes*, honeydew, indirect mutualism, interaction strength, net effects, *Publilia modesta*, trait-mediated interactions

Introduction

Within ecological webs, net indirect interactions between species result from component interactions that can be strong or weak, positive or negative, direct and/or indirect and density-mediated and/or trait-mediated (Miller 1994; Wootton 1994, 2002; Werner & Peacor 2003; Ohgushi 2008). Previous studies that have looked at multiple component pathways within a net indirect interaction have largely focused on chains of negative direct interactions, such as predation and herbivory (e.g. Wootton 1994, 2002; Werner & Peacor 2003; Schmitz 2008; but see e.g. Goudard & Loreau 2008; Ohgushi 2008). Predator–prey interactions have received particular attention because they are often particularly strong direct interactions, and ecological webs are thought to be composed of few strong and many weak interactions (e.g. Paine 1992; Wootton & Emmerson 2005). However, mounting evidence suggests that positive direct interactions (e.g. mutualism, facilitation) are common and have the potential for community-wide consequences within ecological webs (e.g. Messina

1981; Wimp & Whitham 2001; Styrsky & Eubanks 2007; Rudgers & Clay 2008). To date, few studies have quantified both positive and negative direct effects in the same interaction web (but see Goudard & Loreau 2008; Ohgushi 2008); this hinders our understanding of how the net outcome of indirect interactions is governed by the relative strength of positive and negative component interactions.

Terrestrial plant/arthropod communities that include ants are good systems in which to investigate the relative strengths of different types of interspecific interactions because ants perform multiple top-down roles. Ants harass and prey upon many herbivorous insects, while simultaneously engaging in mutualisms with honeydew-producing hemipterans. In these mutualisms, hemipterans exchange food for ant protection against predators and competing herbivores (Way 1963; Messina 1981). Honeydew-producer performance often improves under ant protection, causing concomitant increases in damage to host plants; yet, a majority of studies have shown that honeydew-tending ants actually have a net positive effect on host plants mediated through the ant's deterrence of other herbivores, particularly chewing beetles (Messina 1981; reviewed in Styrsky & Eubanks 2007). The ant's protection services can also benefit herbivores (or herbivore life stages) that do not provide ants with food. For example, Fritz (1983) found that ants provided honeydew-producing membracids with protection from predatory arthropods and benefited the early life stages of a leaf-mining beetle but were detrimental to defoliating adult beetles. This implies that the net effect of ants on the host plant is influenced by multiple component effects of opposite signs, some stronger than others, but this net indirect effect has not yet been decomposed into its component interaction strengths. Furthermore, few studies (4 of 30 reviewed by Styrsky & Eubanks 2007) have rigorously tested the net effect of honeydew mutualisms on plants by considering how the effects of ants on other herbivores are contingent upon the presence of the honeydew producers.

In this study, we sought to understand the net effect of honeydew-tending ants on host plants by identifying positive and negative pathways linking ants to plants, decomposing the net indirect interaction into component interaction strengths and evaluating how the effects of ants on the plant/arthropod community depend on the presence of mutualist honeydew producers. We focused on interactions between three insect species, an ant, a honeydew-producing membracid the ant tends and a defoliating beetle, and the effects on their perennial host plant.

We conducted three field experiments. In the first, we determined whether there are simultaneous positive and negative effects of ants on plants by crossing manipulations of ants and defoliating adult beetles and measuring the responses of membracids and plants (Ant × beetle experiment). We then broadened our scope to consider the effects of ants on plants through the entire insect herbivore community. We asked whether the net indirect effect of ants on plants is dominated by positive or negative component inter-

actions by manipulating ants and using structural equation models (SEM) to quantify the interaction strengths composing the top-down net effect of ants on plants (Ant-effect experiment). Finally, we tested whether the ant's effects on non-honeydew-producing herbivores and plants are contingent upon the presence of mutualist membracids (i.e. the membracid's bottom-up effect on ant activity is largely responsible for the ant's top-down effects on plants) by manipulating membracids and quantifying interactions between membracids and other species via ants (Membracid-effect experiment). Together, these investigations demonstrate how interaction chains composed of positive and negative direct effects combine to control the net outcome of a common indirect interspecific interaction.

Materials and methods

EXPERIMENTAL SYSTEM

Our study focused on the ant *Formica obscuripes* Forel (Hymenoptera: Formicidae), the mutualist honeydew-producing membracid *Publilia modesta* Uhler (Hemiptera: Membracidae), the defoliating beetle *Monoxia schyzonycha* Blake (Coleoptera: Chrysomelidae) and the woody perennial *Chrysothamnus viscidiflorus* Nuttall (Asteraceae) in a meadow near Almont, Colorado (experimental meadow = 100 m × 200 m, elevation = 2769 m, latitude = 38·719, longitude = −106·816) dominated by *Artemisia tridentata* Nuttall (Asteraceae) and *C. viscidiflorus*. *Formica obscuripes* and *P. modesta* are abundant on *C. viscidiflorus*, which is the superior host plant for this generalist membracid at this site (Reithel & Campbell 2008). We used a plant-centred approach and defined the community as the arthropods occurring on individual *C. viscidiflorus* (Ohgushi 2008).

Chrysothamnus viscidiflorus is found throughout the western USA and at our site begins flowering in late July and matures wind-blown seeds in September. Study plants were 20–47 cm tall and had two ramets on average. On this host, *P. modesta* forms aggregations of up to several hundred newly emerged nymphs in late June and July; nymphs develop through five instars until adulthood in August and September. *Formica obscuripes* employs a highly organized honeydew harvest system (McIver & Yandell 1998) and is the numerically and behaviourally dominant ant tending *P. modesta*. Several guilds of herbivores are present, which we represent with synthetic variables. The green aphids *Pleotrichophorus utensis* Pack & Knowlton and *Uroleucon escalantii* Knowlton (Hemiptera: Aphidae) are both tended and eaten by *F. obscuripes* at this site (Billick *et al.* 2007) and together with several cicadellid species (Hemiptera: Cicadellidae) are collectively referred to as 'other phloem feeders'; these species all preferentially feed on inflorescence phloem. 'Larval lepidopterans' are represented by tent caterpillars and casebearing Coleophoridae. 'Leaf-miners' include black blotch fly larvae (Diptera: Agromyzidae) and larvae of the beetle *M. schyzonycha*, which in its adult form is a skeletonizing herbivore feeding alone or in aggregations of up to 15 individuals (Grinath personal observation). Adult *M. schyzonycha* were considered independently in our first experiment. We tracked the adult beetles' damage to plants in both years of study; damage by larval *M. schyzonycha* was considered in our later experiments. Total damage by both stages of the beetle's development was used as a synthetic variable in statistical analyses.

2007 ANT × BEETLE EXPERIMENT

In our first experiment, we focused on ant effects on two important herbivores, testing whether ant effects on membracids and herbivorous beetles were negative or positive and whether these effects altered host plant damage and growth. In a 2 × 3 factorial manipulation, we crossed ant presence/absence with three *Monoxia schyzonycha* beetle density treatments (reduced/ambient/added) on isolated *C. viscidiflorus* randomly selected within 3 m of ant mounds that were randomly chosen from within the meadow. Ninety *C. viscidiflorus* in groups of six plants near each of 15 replicate ant mounds (blocks) were randomly assigned to the six treatments, and all plants were initially supplied with 20 membracid nymphs transferred from an alternative host species. Ant presence was manipulated by applying sticky Tanglefoot (Tanglefoot Company, Grand Rapids, MI, USA) to the base of plant stems; bridging vegetation was trimmed, but winged and jumping arthropods could access the plant. Beetle treatments were maintained by manual removal in weekly surveys and by adding a total of six beetles to plants in three applications between July 19 and August 2. All treatment levels reflect the natural variation on this host in the field, representing common herbivore aggregation sizes and the insects' patchy distributions. Abundances of ants, beetles, membracid adults and nymphs, and per cent of leaves damaged by beetles were quantified in five weekly surveys of each plant from July 10 to August 7. Adult *M. schyzonycha* damage was estimated as the proportion of skeletonized leaves of the 50 topmost leaves on a randomly selected stem. Plant height and circumference (surface area covered) were recorded on July 10. Plant volume (cm^3) was calculated using these two plant measurements and the equation for a cone. This approach provides a non-destructive estimate of plant size and approximates the growth form of this plant, a small bush growing from a few closely spaced ramets. Between-year volume growth was estimated as the difference between the plant's size on 10 July 2007 and 20 June 2008.

We analysed the ant's effects on membracid nymphs and per cent damage with repeated-measures analysis of variance (RM ANOVA) using the PROC MIXED procedure in SAS 9.1 (SAS 2008) with unstructured Var-Cov, because these data were collected in sequential surveys and were approximately normally distributed following $ln(+1)$ transformations. Abundances of other insects were low and were thus analysed as cumulative abundances over all censuses. For these data and for plant variables that were only measured once, ANOVAS were performed in R 2.8.1 (R Development Core Team 2009) using the Drop-1 procedure to calculate results based on Type III Sums of Squares. To test for the effects of ants on total beetle abundance, we fitted a Quasipoisson-distributed generalized linear model (GLM) using cumulative abundance in R. Because there were many highly correlated membracid variables, we also performed a MANOVA in R on cumulative abundances of all the membracid variables to test for significant effects while accounting for these correlations.

2008 ANT-EFFECT EXPERIMENT

Next, we sought to determine whether the net indirect effect of ants on *C. viscidiflorus* was dominated by positive or negative component interactions and to analyse the distribution of interaction strengths composing this net effect. We combined experimental ant manipulations with SEM (Grace 2006) to quantify the top-down effects of ants on plants mediated through the entire insect herbivore community. Of the 90 plants studied in the 2007 Ant × beetle experiment, 75 survived and were reused (keeping the same ant treatment) in 2008 to track plant growth across years. In 2008, we manipulated the presence of ants while providing all plants with aggregations of membracid nymphs as in the 2007 experiment (20–30 nymphs transferred between July 12 and 15). Ant treatments were reapplied in June 2008, and individual plants received the same treatments in both years. The abundances of all insects were surveyed on July 16, July 29 and August 13. Surveys quantifying per cent of leaves damaged by both adult and larval *M. schyzonycha* beetles were conducted on July 8, July 23 and August 6. Plant volume (cm^3) was calculated using data recorded on June 20 and August 18. Volume growth was estimated as the difference between plant size early and late in the season. Seed production (mg) was quantified by weighing seeds from inflorescences bagged with fine mesh on August 19 and harvested on September 16; wind-blown pollen could still fertilize flowers in the bags. Seed production was standardized by each plant's initial number of flower buds (surveyed July 23, prior to flowering) in the statistical analyses.

The component per capita effect sizes estimated by SEM were used to determine the importance of positive and negative components, and the distribution of interaction strengths within the net indirect effect. Data from the 2008 Ant-effect experiment were used to fit the structural equation models. We performed a confirmatory (i.e. of path models specified prior to data analysis) nested analysis (Ant-effect SEM, Fig. 1a) that quantified the ant's direct effects on change in membracid abundance, leaf-chewing by adult beetles, other phloem feeders, leaf-miners and larval lepidopterans, and these herbivores' direct effects on plant seed production (mg). Because membracid abundances were initially manipulated, we used the dependent variable 'change in membracids': the number of membracids in the last survey minus that in the first survey. Plant damage caused by leaf-chewing adult beetles was used as a proxy for beetle abundance, because too few adult beetles were observed in 2008 to include this variable in the models. Cumulative abundance data were used for the other insect variables. We included flower bud abundance, indicating plant condition early in the season when plants are colonized by membracid nymphs and have not initiated flowering, as an independent variable to account for variation because of bottom-up effects. We accounted for additional unresolved variation by including correlation terms between herbivores (double-headed arrows in Fig. 1). Because a goal was to find the best-fitting model to describe important component interactions between ants and host plants, we employed a model pruning strategy, where paths (labelled A–P in Fig. 1a) were sequentially deleted and evaluated for their contribution to model fit with chi-square lack-of-fit tests, Akaike's information criteria (AIC), the root mean error of approximation (RMSEA), and the expected cross-validation index (ECVI). Paths were retained in subsequent models if they were significant and/or caused greater lack of fit (i.e. significant chi-square probability or increased index scores) when deleted. The best model was selected based on the lowest AIC, RMSEA and ECVI scores (Grace 2006).

To understand how interactions between herbivores and plants depend on the presence of the mutualism, we followed the nested SEM analysis with an exploratory (i.e. of path models specified after analysing the initial SEMs) analysis (multi-group SEM) comparing interaction strengths in webs with and without ants. This analysis used the 2008 Ant-effect data split into groups with and without ants and compared with the same causal model (see Grace 2006; Hillebrand *et al.* 2009). Containing a subset of the interactions in the SEM described above (Fig. 1a), this model included interactions between flower buds, seed production and all herbivores and the unresolved correlation between leaf-miners and beetle chewing. The interaction strengths in the Ants Present group were compared to the Ants Absent group for changes in magnitude and sign. All SEM analyses were performed using maximum likelihood estimation in AMOS
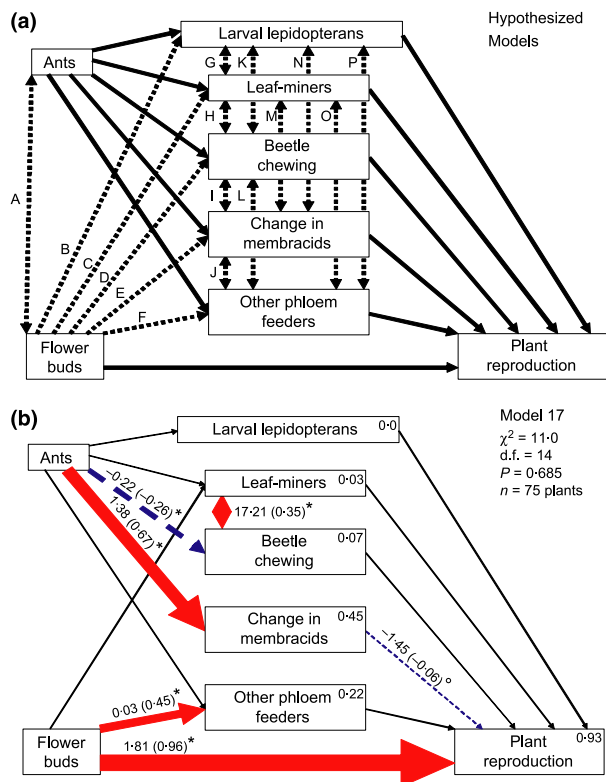
Fig. 1. Nested Ant-effect experiment structural equation models. (a) Seventeen models were considered in a stepwise model pruning procedure to eliminate paths that did not contribute to model fit (dotted paths labelled 'A' through 'P'; see Table 1). Each single-headed arrow represents a direct effect, whereas double-headed arrows are unresolved covariances/correlations. (b) Model 17 had the most acceptable model-fit scores and was selected as the best model, shown with unstandardized, per capita interaction strengths above each significant path (as well as interaction strengths standardized by their standard deviations in parentheses). Significant effects are shown as coloured arrows (red, solid = positive; blue, dashed = negative) with thickness representing the magnitude of the standardized interaction and interaction strength estimates denoted by (*) for $P < 0.05$ and (°) for $P < 0.10$. Non-significant effects are shown as skinny black arrows. Endogenous (dependent) variables are boxes with R^2 values in the top right, whereas exogenous (independent) variables lack this term.

5.0.1 (Arbuckle 2003) with non-transformed data. Models were accepted as a good fit to the data if the chi-square lack-of-fit test exceeded $P = 0.05$.

Total, absolute effects of ants on herbivores and plants were analysed with MANOVA and univariate statistics, which provide complementary perspectives for the same relationships analysed as per capita and net effects with SEM. First, we used MANOVA to test the significance of ant effects on synthetic variables (i.e. herbivore guilds and total beetle damage) in our data set. We performed MANOVAs in *R* for the variables composing these synthetic variables to test for significant effects while taking into account correlations that may exist among the component dependent variables. We conducted univariate analyses determined by the type of data and the distribution of residuals for the variable under consideration, which in some cases differed between variables within a MANOVA. Membracid nymphs, other phloem feeders and per cent beetle damage were analysed through time with RM ANOVAs in SAS because these data were collected in sequential surveys and were approximately normally

distributed following ln(+1) transformations. Other insects were analysed as cumulative abundances over all censuses because their abundances in individual surveys were low; we performed GLMs in R for these data and for plant variables that were only measured once. Gaussian-distributed GLMs (equivalent to ANOVAs but limited to analysing only two levels per factor) were performed for variables meeting normality assumptions, and Poisson-distributed GLMs were used for variables with non-normal distributions of residuals; a Quasipoisson distribution was fit to models when the deviance exceeded the degrees of freedom in the Poisson-distributed model.

2008 MEMBRACID-EFFECT EXPERIMENT

Lastly, we tested whether the membracid's bottom-up effect on ants was largely responsible for the ant's top-down effects on herbivores and plants. We manipulated membracids to analyse their effect on the abundance of foraging ants on host plants, their indirect effects on other herbivores and their direct effect on host plants. Similarities between the results from manipulating membracids and manipulating ants would suggest that membracids are the main driver of the ant's net effect on plants. We used 80 randomly selected plants in groups of four, within 3 m of an *F. obscuripes* mound. Membracid presence or absence was randomly assigned within 20 replicate ant mounds (blocks) that were randomly chosen in the experimental meadow. Membracid presence was manipulated by supplying initial populations of nymphs, and membracid absence was maintained by weekly manual removal. Although ant barriers were not used for this experiment, bridging vegetation was trimmed for consistency with the other two experiments. We recorded the same response variables for this experiment as for the 2008 Ant-effect experiment, and the ant mounds for the two experiments were spatially intermixed within the same meadow.

To test for similarities between results from membracid and ant manipulations, the statistical significance of membracid effects on each dependent variable was determined using the same MANOVA and univariate analyses that were performed for the Ant-effect experiment. Considering the same dependent variable in both experiments, qualitatively similar results would indicate that membracid presence was largely responsible for the effect on that variable. Ant abundance on host plants was the sole dependent variable analysed in this experiment that was not assessed in the Ant-effect experiment; the effect of membracids on ants was analysed with a RM ANOVA in SAS. To further quantify the membracid's effects on other herbivores via ants, we also performed a nested SEM analysis with the Membracid-effect experimental data (similar to the Ant-effect SEM), which can be found in the supporting information (Figs S3 and S4, Tables S5–S7).

Results

ANT × BEETLE EXPERIMENT

In 2007, we wanted to know whether ants simultaneously had positive effects on membracids and negative effects on beetles and whether these herbivores had negative effects on plants. Ants reduced adult beetle abundances (GLM: Quasipoisson, $t = 2.31$, $P = 0.025$; Fig. 2a), resulting in less beetle damage in the presence of ants (across all beetle treatments: RM ANOVA, $F_{6,78} = 8.12$, $P = 0.006$; Fig. 2b; analysing only the ambient beetle treatment data: RM ANOVA, $F_{3,25} = 3.80$, $P = 0.062$). Ants had significant positive effects on their membracid mutualists, an effect seen for all
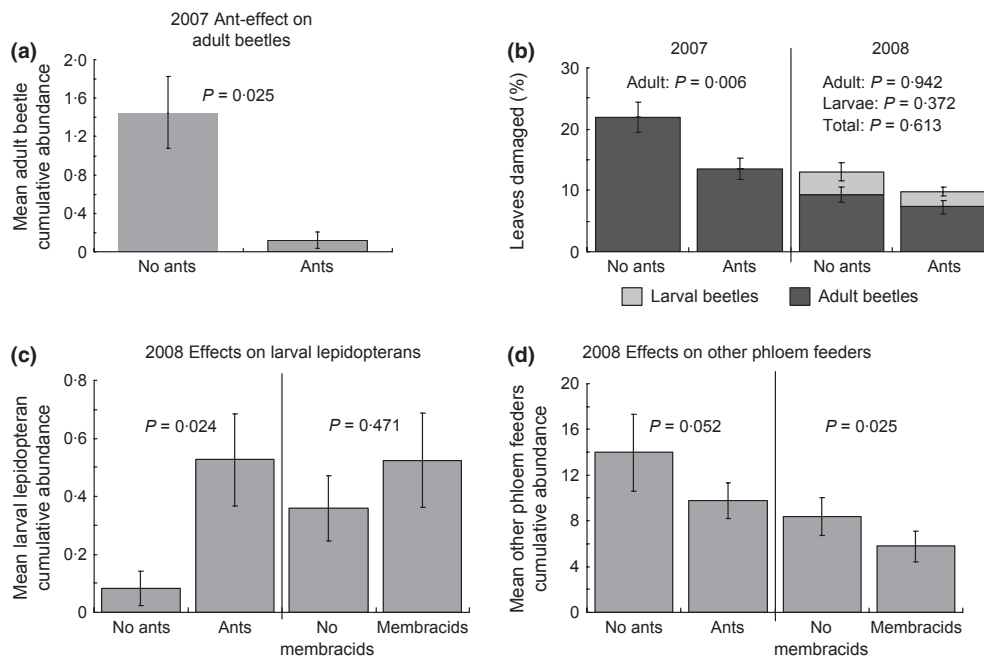
Fig. 2. Univariate results for effects on insects and plant damage from both years of study. Error bars are ±1 SE. (a) Ant effect on adult *M. schyzonycha* abundance (Ant × beetle experiment, generalized linear model (GLM): Quasipoisson). (b) Ant effect on per cent damage by *M. schyzonycha* adults in 2007 (Ant × beetle experiment, repeated-measures (RM) ANOVA), and in 2008 for damage by adults, larvae and both age classes combined (Ant-effect experiment, RM ANOVA). (c) Ant and membracid effects on larval lepidopterans (Ant-effect and Membracid-effect experiments, GLM: Quasipoisson). (d) Ant and membracid effects on phloem feeders other than membracids (Ant-effect and Membracid-effect experiments, RM ANOVA).

developmental stages and through time (Table S1). Ants did not affect plant growth between 2007 and early 2008 (ANOVA, $F_{2,69} = 1·26$, $P = 0·266$; Fig. 3a, Table S1). The adult *M. schyzonycha* beetle treatments did not significantly alter beetle abundances on plants (GLM: Quasipoisson, $t = −0·60$, $P = 0·554$; mean cumulative abundance was 0·56, 0·59 and 1·17 for beetles reduced, ambient and added, respectively), and beetle treatments did not affect membracids or the plant (Table S1). No significant statistical interactions between ant and beetle treatments were found in any of the analyses (Table S1).

ANT-EFFECT EXPERIMENT

Ant-effect SEM

In 2008, we asked whether the net effect of ants on plants was dominated by positive or negative component interactions and whether this net effect resulted from a few strong interactions or many interactions of similar magnitude (i.e. what was the distribution of interaction strengths). The Ant-effect structural equation models (SEM) depicted in Fig. 1a measure ant effects on plants mediated through all insect herbivores. Using our model pruning strategy, we chose the model including only paths C, F and H (model 17) as the best model because it had the lowest AIC, RMSEA and ECVI model-fit index scores (Table 1). Figure 1b depicts model 17 with unstandardized, per capita interaction strengths and interaction strengths standardized by their standard deviations (in

parentheses) to obtain relative effect sizes. The unstandardized interaction strengths, standard errors and their probability values are provided in Table S3, and bivariate scatter plots for the model's variables are in Fig. S1.

One of the component interactions between ants and plants was relatively strong, and all others were relatively weak. Ants had a significant strong positive effect on membracid survival, and membracids had a marginally significant weak negative effect on seed production. Ants significantly reduced beetle chewing damage; this effect was of intermediate strength and was less than half the relative strength of the positive effect on membracids. All other component direct interactions were weak and non-significant. There was one significant unresolved correlation, which was of intermediate strength and occurred between leaf-miners and adult beetle damage to leaves. The strengths of the component indirect interactions between ants and plant variables were calculated by multiplying the coefficients along the individual paths mediated by particular herbivores. The net indirect interaction is the sum of all possible component indirect interactions. Ants had a negative net effect on plant reproduction, an effect that was dominated by the indirect path through membracids (Fig. 1b, Table S4).

Ant-effect multi-group SEM

To explore how interactions between insect herbivores and their host plants depended on the presence of the mutualism, we performed the multi-group SEM analysis comparing
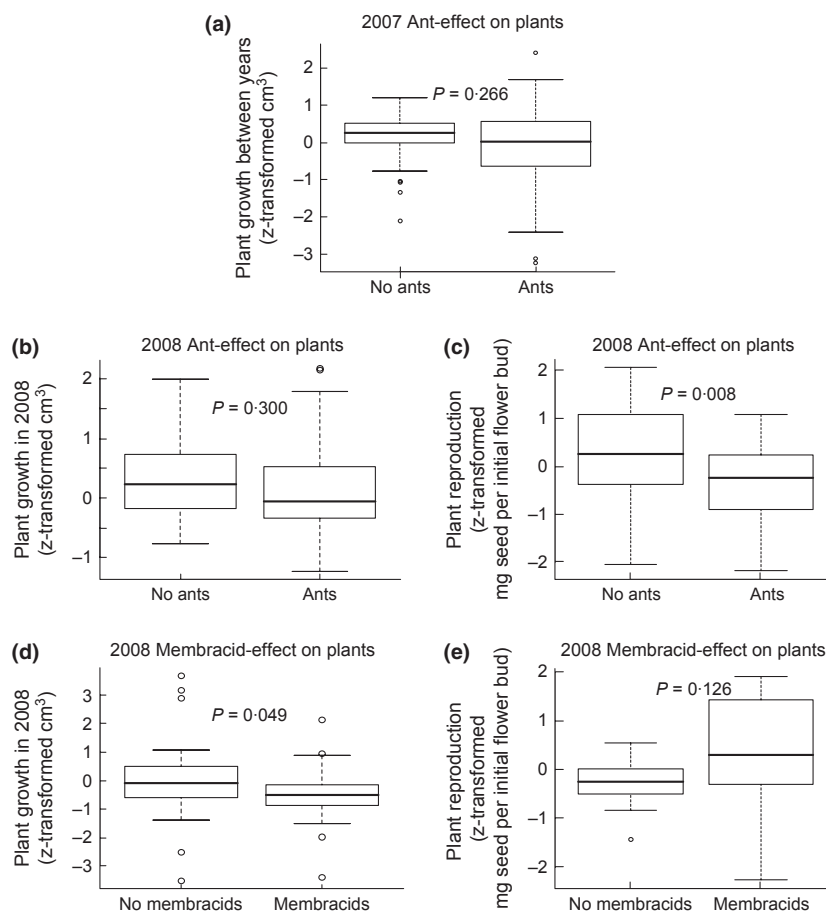
Fig. 3. Univariate results for effects on plant growth and reproduction from both years of study. (a) Ant effect on plant growth between 2007 and early 2008 (Ant × beetle experiment, ANOVA, ant × beetle interaction term was non-significant). (b) Ant effect on plant growth in 2008 (Ant-effect experiment, generalized linear model (GLM): Gaussian). (c) Ant effect on plant seed production in 2008 (Ant-effect experiment, GLM: Gaussian). (d) Membracid effect on plant growth in 2008 (Membracid-effect experiment, GLM: Gaussian). (e) Membracid effect on plant seed production in 2008 (Membracid-effect experiment, GLM: Gaussian).

Table 1. Ant-effect SEM nested model selection.

Model	Path deleted	Path retained	df	χ^2	P	AIC	RMSEA	ECVI
1	None		1	0·1	0·748	86·1	0	0·563
2	A		2	1·7	0·414	85·8	0	0·561
3	B		3	2·6	0·450	84·6	0	0·553
4	C	+	4	5·2	0·264	85·2	0·045	0·557
5	D		4	2·7	0·604	82·7	0	0·541
6	E		5	4·3	0·502	82·3	0	0·538
7	F	+	6	20·7	0·002	96·7	0·127	0·632
8	G		6	5·5	0·478	81·5	0	0·533
9	H	+	7	16·2	0·023	90·2	0·093	0·590
10	I		7	5·8	0·558	79·8	0	0·522
11	J		8	6·4	0·601	78·4	0	0·513
12	K		9	8·3	0·501	78·3	0	0·512
13	L		10	9·5	0·483	77·5	0	0·507
14	M		11	10·0	0·527	76·0	0	0·497
15	N		12	10·1	0·609	74·1	0	0·484
16	O		13	10·3	0·673	72·3	0	0·472
17	P		14	11·0	0·685	71·0	0	0·464

A best model was selected using a model pruning strategy, where paths (labelled A–P in Fig. 1a) were sequentially deleted and evaluated for their contribution to model fit with AIC, RMSEA and ECVI indices. Paths were retained in subsequent models if they were significant and/or caused increased index scores when deleted. Model 17 was selected as the best model, with an acceptable χ^2 probability and the lowest AIC, RMSEA and ECVI scores of all models considered.

communities with and without ants (Figs 4 and S2, Tables S3 and S4). The lack-of-fit test indicates that this causal model is acceptable for both groups (ants present: $\chi^2 = 8\cdot3$, d.f. = 9, $P = 0\cdot500$; ants absent: $\chi^2 = 2\cdot2$, d.f. = 9, $P = 0\cdot988$).

Notably, flower buds had a significant positive effect on the change in membracid abundance in the presence of ants, but not when ants were absent. Similarly, beetle chewing damage had a marginally significant positive effect on plant
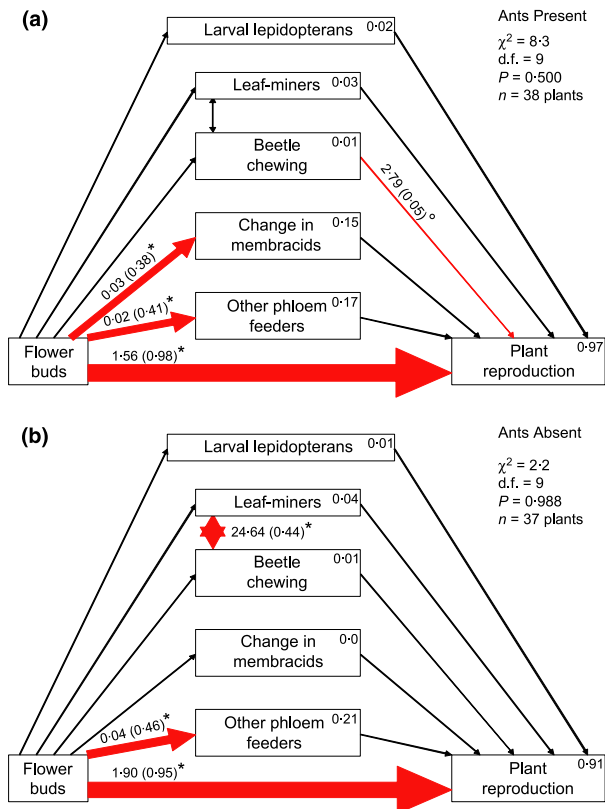
(a)

Larval lepidopterans 0·02

Leaf-miners 0·03

Beetle chewing 0·01

Change in membracids 0·15

Other phloem feeders 0·17

Flower buds

Plant reproduction 0·97

Ants Present
$\chi^2 = 8\cdot3$
d.f. = 9
$P = 0\cdot500$
$n = 38$ plants

2·79 (0·05) °

0·03 (0·38)*

0·02 (0·41)*

1·56 (0·98)*

(b)

Larval lepidopterans 0·01

Leaf-miners 0·04

Beetle chewing 0·01

Change in membracids 0·0

Other phloem feeders 0·21

Flower buds

Plant reproduction 0·91

Ants Absent
$\chi^2 = 2\cdot2$
d.f. = 9
$P = 0\cdot988$
$n = 37$ plants

24·64 (0·44)*

0·04 (0·46)*

1·90 (0·95)*

Fig. 4. Multi-group structural equation models for plants with ants (a) present and (b) absent. Unstandardized, per capita interaction strengths (as well as interaction coefficients standardized by their standard deviations in parentheses) are given above their respective significant paths. Significant effects are shown as coloured arrows (red = positive) with thickness representing the magnitude of the standardized interaction and interaction strength estimates denoted by (*) for $P < 0\cdot05$ and (°) for $P < 0\cdot10$. Non-significant effects are shown as skinny black arrows; unresolved covariances/correlations are indicated by double-headed arrows. R^2 values are in the top right of boxes with endogenous (dependent) variables; exogenous (independent) variables lack this term.

reproduction only in the presence of ants. Conversely, leaf-miners significantly covaried with beetle chewing damage to plants only when ants were absent. All other paths between herbivores and plant variables were non-significant in the SEMs.

Ant-effect complementary analyses

We performed MANOVA and univariate analyses on the absolute responses to ant manipulation (Table S2) to provide complimentary perspectives for the same relationships analysed with per capita and net effects using SEM. In 2008, ants had a positive effect on larval lepidopterans (GLM: Quasi-poisson, $t = -2\cdot30$, $P = 0\cdot024$; Fig. 2c), a negative effect on other phloem feeders (RM ANOVA, $F_{2,73} = 3\cdot09$, $P = 0\cdot052$, significant ant × time interaction reported; Fig. 2d) and no effect on leaf-miners, including larval *M. schyzonycha* beetles (GLM: Quasipoisson, $t = 1\cdot61$, $P = 0\cdot113$; Table S2). Although the presence of ants negatively influenced adult

beetle damage in 2007, neither this effect (RM ANOVA, $F_{2,73} = 0\cdot01$, $P = 0\cdot942$) nor an effect on larval damage (RM ANOVA, $F_{2,73} = 0\cdot81$, $P = 0\cdot372$) was evident in 2008 (Fig. 2b, Table S2), when adult beetles were nearly absent. Ant manipulations did not significantly affect plant growth (GLM: Gaussian, $t = 1\cdot04$, $P = 0\cdot300$; Fig. 3b), but had a significant negative effect on seed production when standardized by the initial number of flower buds (GLM: Gaussian, $t = 2\cdot74$, $P = 0\cdot008$; Fig. 3c, Table S2). In the absence of ants, mean seed production was 349·8 mg (1·60 mg seed per initial flower bud); in the presence of ants, mean seed production was 200·2 mg (1·18 mg seed per initial flower bud).

MEMBRACID-EFFECT EXPERIMENT

We also wanted to know whether membracids were the main driver of the ant's effects on other herbivores and plants through their influence on ant activity. Concurrent with the Ant-effect experiment in 2008, we manipulated membracid presence to investigate their effect on foraging ant abundance and to compare the effects on other herbivores and host plants with results from the ant manipulation. Ant worker abundance was nearly ten times greater when membracids were present on plants (RM ANOVA, $F_{2,77} = 11\cdot60$, $P < 0\cdot001$; ant mean cumulative abundance was 6·45 with membracids and 0·69 without membracids). As in the Ant-effect experiment, significantly fewer other phloem feeders were found when membracids were present (RM ANOVA, $F_{2,77} = 3\cdot87$, $P = 0\cdot025$, significant ant × time interaction reported; Fig. 2d). Although non-significant, larval lepidopteran abundance responded similarly to membracid manipulation as to ant manipulation (GLM: Quasipoisson, $t = -0\cdot72$, $P = 0\cdot471$; Fig. 2c). There was no effect on leaf-miners (GLM: Gaussian, $t = -0\cdot32$, $P = 0\cdot750$; Table S2) in either experiment. We also considered the membracid's direct effects on host plants; membracid presence significantly negatively affected plant growth (GLM: Gaussian, $t = 2\cdot00$, $P = 0\cdot049$; Fig. 3d) but not seed production (GLM: Gaussian, $t = -1\cdot59$, $P = 0\cdot126$; Fig. 3e, Table S2), although sample size was small for the latter test. The Membracid-effect SEM analysis corroborates these results and can be found in the supporting information (Figs S3 and S4, Tables S5–S7). The similarities between the effects found in this and the Ant-effect experiment suggest that membracid presence was responsible for the effect of ants on herbivores and plants.

Discussion

We found that a positive interspecific interaction is important for mediating a net indirect effect, which is similar to other studies showing that mutualisms can have community-wide impacts (e.g. Messina 1981; Wimp & Whitham 2001; Styrsky & Eubanks 2007; Rudgers & Clay 2008). Our results indicate that the honeydew-tending ants in this system had a negative net indirect effect on host plants, which was mediated

by component effects of opposite signs and driven by the membracid's positive effect on ant activity. As in other studies, we found that ants indirectly benefit plants by deterring chewing herbivores (Messina 1981; reviewed in Styrsky & Eubanks 2007), but unlike other studies, we found that ants were more costly than beneficial for plants. Similar to the distribution of a few strong and many weak interactions found in other ecological webs (Paine 1992; Wootton & Emmerson 2005), the ant–membracid mutualism was the sole strong component interaction found in our SEMs. This strong mutualism overwhelmed other component interactions to result in the negative net indirect effect of ants on plants. Also, this negative net effect was reinforced by ants providing benefits to larval lepidopteran herbivores, which is analogous to the positive 'spillover' protection effects of honeydew-tending ants on non-honeydew-producing herbivores found in other studies (Fritz 1983; Wimp & Whitham 2001).

Differences in ecological context between years or sites can cause conditional outcomes in food webs (Bronstein 1994), and the net indirect effect of ants on plants in our study is no exception. During this study, adult *M. schyzonycha* beetles were abundant in 2007 but nearly absent in 2008. Although beetles were manipulated in 2007, this treatment did not have significant effects; thus, we argue that the influence of ants on beetle abundance and plant damage can be compared across years. Based on the result that ants had significant effects on leaf-chewing beetle damage in 2007 but not in 2008 (RM ANOVA), opposing influences through positive and negative component interactions may have been more balanced in 2007 when beetles were abundant, thereby causing inter-annual variation in the magnitude of the ant's net indirect effect on plants. This net interaction will also be influenced by variation in the abundance of membracid predators, which largely determines the degree of benefit membracids receive from ants (Cushman & Whitham 1989; Bronstein 1994). A series of previous studies at this field site (Billick & Tonkel 2003; Reithel & Billick 2006; Abbot *et al.* 2008; Reithel & Campbell 2008) suggests that the ant–membracid mutualism is a perennially important interaction on *C. viscidiflorus*, but longer-term studies are required to discern the amount of variation in the strength of this mutualism, the effect of ants on beetles and the long-term net effect of ants on plants.

The strength of a net indirect effect will also depend on changes in behaviour by component species (trait-mediated interactions; reviewed in Werner & Peacor 2003). Our SEMs indicate that the effect of flower buds on membracids was strong only when ants were present; we interpret this to mean that membracids took advantage of preferred foraging sites in the presence of ants. Ant protection may allow greater membracid foraging on plant parts that are more nutritious for the herbivores, such as flower buds and fast-growing stems. In contrast, leaf-miners and beetle chewing strongly covaried in the absence, and not the presence, of ants. Because leaf-miners include larval *M. schyzonycha* beetles, this pattern

may result if new adult beetles remain to forage on the plants on which they have developed, and adult beetles migrate away from plants on which they are harassed by ants. The same covariance could potentially arise if adult beetles preferentially migrate to and forage on plants with many leaf-miners when ants are absent, but further study is required to determine causality in this relationship. Additionally, although we expected beetles to be detrimental to plants, the SEMs indicate that leaf damage by chewing beetles increased plant reproduction in the presence of ants. This surprising result may be due to overcompensation by the plant (e.g. Hawkes & Sullivan 2001), or it may be an artefact of the SEM analysis. Because SEMs use correlational data to provide relative effect sizes, causality within an SEM often cannot be determined and can create counterintuitive results. Experimentally manipulating focal species may be necessary to establish trustworthy causal pathways in SEMs; paths that occur further from the manipulated species will be estimated using less reliable observational data.

We found that SEM was helpful for two reasons. First, SEM allowed us to decompose a net indirect effect into component direct and indirect effects, as opposed to more conventional types of analyses (e.g. ANOVA) that assume all effects are direct. This advantage was Wright's motivation for developing the first simple path analyses as an alternative to ANOVA (Wright 1920). Second, SEM allowed us to determine the distribution of interaction strengths and the relative strength of both negative and positive types of interactions within the net indirect effect of ants on plants. SEM and similar statistical tools have been criticized for requiring large sample sizes (e.g. Petraitis, Dunham & Niewiarowski 1996; Grace 2006). However, combining SEM with univariate and other statistical approaches (e.g. MANOVA) can provide complementary support for conclusions. Although care must be taken when combining experimental evidence with SEM (Grace 2006), we are confident that our manipulations reflect the natural variation at the field site based upon years of experience in this system (e.g. Billick *et al.* 2007; Abbot *et al.* 2008); this has allowed us to conclude that ants had a negative net indirect effect on plants as a result of their mutualism with membracids.

Community ecology's focus on competition and predation (e.g. Wootton 1994, 2002; Werner & Peacor 2003; Wootton & Emmerson 2005; Schmitz 2008) may have led us to ignore important positive interactions in ecological webs (Ohgushi 2008). Some have argued that ant/honeydew mutualisms are important enough to constitute keystone interactions (Styrsky & Eubanks 2007). Because the mutualist species in this study was numerically dominant despite relatively small experimental membracid aggregation sizes (Reithel & Campbell 2008), we argue that this interaction is more accurately described as a strong, dominant mutualism. We expect that other mutualisms between relatively abundant species will also play important roles in their local communities. Our findings suggest that we should pay greater attention to positive interactions in studies of ecological webs.

Acknowledgements

References

Abbot, P., Grinath, J., Brown, J., Peeden, E., Erickson, D. & Billick, I. (2008) Insect herbivore stoichiometry: the relative importance of host plants and ant mutualists. *Ecological Entomology*, **33**, 497–502.

Arbuckle, J.L. (2003) Amos. *Amos Development Corporation*, Spring House, PA.

Billick, I. & Tonkel, K. (2003) The relative importance of spatial vs. temporal variability in generating a conditional mutualism. *Ecology*, **84**, 289–295.

Billick, I., Hammer, S., Reithel, J.S. & Abbot, P. (2007) Ant-aphid interactions: are ants friends, enemies, or both? *Annals of the Entomological Society of America*, **100**, 887–892.

Bronstein, J.L. (1994) Conditional outcomes in mutualistic interactions. *Trends in Ecology and Evolution*, **9**, 214–217.

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

Fritz, R.S. (1983) Ant protection of a host plant's defoliator: consequence of an ant-membracid mutualism. *Ecology*, **64**, 789–797.

Goudard, A. & Loreau, M. (2008) Nontrophic interactions, biodiversity, and ecosystem functioning: an interaction web model. *The American Naturalist*, **171**, 91–106.

Grace, J.B. (2006) *Structural Equation Modelling and Natural Systems*, Cambridge University Press, New York.

Hawkes, C.V. & Sullivan, J.J. (2001) The impact of herbivory on plants in different resource conditions: a meta-analysis. *Ecology*, **82**, 2045–2058.

Hillebrand, H., Borer, E.T., Bracken, M.E.S., Cardinale, B.J., Cebrian, J., Cleland, E.E., Elser, J.J., Gruner, D.S., Harpole, W.S., Ngai, J.T., Sandin, S., Seabloom, E.W., Shurin, J.B., Smith, J.E. & Smith, M.D. (2009) Herbivore metabolism and stoichiometry each constrain herbivory at different organizational scales across ecosystems. *Ecology Letters*, **12**, 516–527.

McIver, J.D. & Yandell, K. (1998) Honeydew harvest in the western thatching ant (Hymenoptera: Formicidae). *American Entomologist*, **44**, 30–35.

Messina, F.J. (1981) Plant protection as a consequence of an ant-membracid mutualism: interactions on goldenrod (*Solidago* sp.). *Ecology*, **62**, 1433–1440.

Miller, T.E. (1994) Direct and indirect species interactions in an early old-field plant community. *The American Naturalist*, **143**, 1007–1025.

Ohgushi, T. (2008) Herbivore-induced indirect interaction webs on terrestrial plants: the importance of non-trophic, indirect, and facilitative interactions. *Entomologia Experimentalis et Applicata*, **128**, 217–229.

Paine, R.T. (1992) Food-web analysis through field measurement of per capita interaction strength. *Nature*, **355**, 73–75.

Petraitis, P.S., Dunham, A.E. & Niewiarowski, P.H. (1996) Inferring multiple causality: the limitations of path analysis. *Functional Ecology*, **10**, 421–431.

R Development Core Team. (2009) *R: A Language and Environment for Statistical Computing*, R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Reithel, J. & Billick, I. (2006) Bottom-up mediation of an ant-membracid mutualism: effects from different host plants. *Evolutionary Ecology*, **20**, 27–38.

Reithel, J.S. & Campbell, D.R. (2008) Effects of aggregation size and host plant on the survival of an ant-tended membracid (Hemiptera: Membracidae): potential roles in selecting for generalized host plant use. *Annals of the Entomological Society of America*, **101**, 70–78.

Rudgers, J.A. & Clay, K. (2008) An invasive plant-fungal mutualism reduces arthropod diversity. *Ecology Letters*, **11**, 831–840.

SAS. (2008) *Statistical Analysis Software*. SAS Institute Inc, Cary, NC.

Schmitz, O.J. (2008) Effects of predator hunting mode on grassland ecosystem function. *Science*, **319**, 952–954.

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

Way, M.J. (1963) Mutualism between ants and honeydew-producing Homoptera. *Annual Reviews in Entomology*, **37**, 479–503.

Werner, E.E. & Peacor, S.D. (2003) A review of trait-mediated indirect interactions in ecological communities. *Ecology*, **84**, 1083–1100.

Wimp, G.M. & Whitham, T.G. (2001) Biodiversity consequences of predation and host plant hybridization on an aphid-ant mutualism. *Ecology*, **82**, 440–452.

Wootton, J.T. (1994) Predicting direct and indirect effects: an integrated approach using experiments and path analysis. *Ecology*, **71**, 151–165.

Wootton, J.T. (2002) Indirect effects in complex ecosystems: recent progress and future challenges. *Journal of Sea Research*, **48**, 157–172.

Wootton, J.T. & Emmerson, M. (2005) Measurement of interaction strength in nature. *Annual Review of Ecology, Evolution and Systematics*, **36**, 419–444.

Wright, S. (1920) The relative importance of heredity and environment in determining the piebald pattern of guinea-pigs. *Proceedings of the National Academy of Sciences of the United States of America*, **6**, 320–332.

Supporting Information

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

Table S1. 2007 Ant × beetle experiment univariate and MANOVA results.

Table S2. 2008 Ant-effect experiment and Membracid-effect experiment univariate and MANOVA results, as well as results summarizing the effects of the mutualism across both experiments.

Table S3. Structural equation model (SEM) estimates of unstandardized direct interaction strengths measured as per capita (partial covariance) effects for the Ant-effect SEM and Multi-group SEM analyses.

Table S4. Matrices for total net effects standardized by their standard deviations in the (a) Ant-effect SEM and the (b) Multi-group SEM.

Table S5. Membracid-effect SEM nested model selection.

Table S6. Structural equation model estimates of unstandardized direct interaction strengths measured as per capita (partial covariance) effects for the Membracid-effect SEM analysis.

Table S7. Matrix for total net effects standardized by their standard deviations in the Membracid-effect SEM.

Figure S1. Bivariate scatter plots for variables in the Ant-effect SEM analysis.

Figure S2. Bivariate scatter plots for variables in the Multi-Group SEM analysis, with ants (a) present and (b) absent.

Figure S3. Membracid-effect SEM: (a) the full nested model, and (b) the best-fitting model.

Figure S4. Bivariate scatter plots for variables in the Membracid-effect SEM analysis.

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