COMMUNITY ECOLOGY

Mutualism in a community context: the positive feedback between an ant–aphid mutualism and a gall-making midge

Amy M. Savage · Merrill A. Peterson

Abstract Although mutualisms are widespread and often described in natural history accounts, their ecological influences on other community members remain largely unexplored. Many of these influences are likely a result of indirect effects. In this field study, we investigated the indirect effects of an ant–aphid mutualism on the abundance, survival rates and parasitism rates of a co-occurring herbivore. *Rabdophaga salicisbrassicoides* (Diptera: Cecidomyiidae) induces rosette galls on the developing shoots of *Salix exigua* trees, and populations can reach outbreak densities (up to 1,000 galls/stem) in central Washington State (USA). Ant-tended aphids feed on these same stems and often feed on gall tissue. In this study we used a combination of manipulative experiments and observational surveys to test the hypothesis that the abundances of aphids, ants, and galls have positive and reciprocal effects on one another, in a manner that would create a positive feedback loop in population growth. In addition, we examined whether the combined presence of ants and aphids reduces parasitism rates for the gallers. In support of the positive feedback loop hypothesis, aphids enjoyed higher population growth rates in the presence of ants and galls, the presence of ants and aphids resulted in increased abundance of galls, and the abundances of ants, aphids and galls were all positively correlated with one another. However, the mechanism underlying the positive effect of ants and aphids on galler density remains unknown, as the mutualism did not affect parasitism rates. More broadly, this study demonstrates that mutualisms can have significant and complex indirect effects on community and population ecology.

Keywords Ant–aphid mutualism · Galls · Community ecology · Direct and indirect effects · Positive feedback

Introduction

Indirect effects are widespread in ecological communities and can exert strong influences on population and community dynamics. For example, predators can indirectly benefit plants by consuming herbivores, causing shifts in plant biomass and plant composition (Moran et al. 1996; Letourneau and Dyer 1998). Therefore, understanding the dynamics of indirect effects, which occur only in the presence of three or more species, is critical to advancing the study of community ecology (Wootton 1994; Fox and Olsen 2000; Peacor and Werner 2001; Webster and Almany 2002; Pearson and Callaway 2003). Until recently, most studies of indirect effects have focused primarily on the indirect effects associated with antagonistic interactions, such as competition (Sih et al. 1985; Denno et al. 1995; Abrams 2003), predation (Sih et al. 1985; Schmitz et al. 2000; Bernot and Turner 2001), herbivory (Ohgushi 2005 and references therein) and parasitism (Price et al. 1986; Bonsall and Hassell 1999). However, given the ubiquity of positive

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interactions in ecological communities (Bronstein 1994; Pemberton 1998; Stachowicz 2001; Langley and Hungate 2003; Hay et al. 2004), indirect effects associated with mutualism and facilitation may have profound ecological importance. Therefore, it is important that we broaden our view of indirect effects in communities to encompass both antagonistic and beneficial interactions.

Although still the least studied aspect of mutualism, recent investigations have documented the importance of mutualisms to community structure and function (Kearns et al. 1998; Clay and Holah 1999; Christian 2001; Schmitt and Holbrook 2003; Strauss and Irwin 2004; Hay et al. 2004). Furthermore, growing evidence suggests that much of the influence of mutualisms on ecological communities occurs through indirect effects (Martinez del Rio et al. 1996; Cushman et al. 1998; Kearns et al. 1998; Marler et al. 1999; Langley and Hungate 2003; Callaway et al. 2004). However, more research is needed, particularly spanning a greater diversity of ecosystems and types of mutualistic interactions. Additionally, to adequately understand the community level dynamics of mutualisms, we need studies that investigate not only how mutualisms affect other community members, but also whether and how mutualist-mediated effects on the community feed back to influence the dynamics of the mutualistic partners.

A widespread category of mutualisms with potential for strong indirect effects involves organisms that are protected by mutualistic ants. Ant-protective mutualisms are widespread in nature and can be very common (Bronstein 1998). Furthermore, they inherently include the potential for indirect effects because one of the fundamental aspects of ant-protective mutualisms is that they involve at least three species: the ant, the protected organism, and the threatening species. Ants in these mutualisms attack herbivorous and/or predacious insects, trim vines from partner plants, and chase away parasitoids of herbivores (Heil and McKey 2003; Stadler and Dixon 2005). Because these mutualisms intrinsically include a minimum of three species, it stands to reason that ant protective mutualisms would have strong indirect effects on community dynamics. Recent evidence supports this prediction (Davidson 1997; Bluthgen 2002). For example, Wimp and Whitham (2001) found that aphids feeding on *Populus* indirectly influenced the rest of the arthropod community because their ant guards altered the composition and reduced abundance of other herbivores and their natural enemies.

Although such negative outcomes are generally predicted by competition theory, the indirect effects of ant protective mutualisms on other community members can also be positive. For example, some studies have shown that ant tended homopterans can benefit both figs and their specialized pollinators through indirect pathways (Compton and Robertson 1988, 1991; Cushman et al. 1998). Internally feeding herbivores are more likely to benefit from ant protective mutualisms, since ants are more successful when attacking externally feeding herbivores (Mody and Linsenmair 2004). Another important factor in determining a positive or negative outcome is timing of oviposition. For example, if an internally feeding herbivore oviposits before ants reach high abundances but the parasitoids/predators of the herbivore attack after ants are abundant, then it is likely that the herbivore would benefit from the presence of an ant protective mutualism.

We explored the reciprocal effects of an ant–aphid mutualism on a gall-making fly sharing the same host plant as the aphids and parasitoid wasps that attack the gall. In this ant protective mutualism, ants (*Formica neoclara*) tend and defend aphids (*Chaitophorus utahensis*), and may indirectly reduce mortality rates of gall larvae (*Rabdophaga salicisbrassicoides*), perhaps by chasing away parasitoids. Alternatively, galler populations may be greater on plants with ants and aphids if aphid-induced changes in plant quality benefit gallers or if gallers preferentially oviposit on plants bearing ants and aphids. Thus, there are several mechanisms by which the ant–aphid mutualism might facilitate the outbreak densities of *R. salicisbrassicoides* galls that can be observed in central Washington State (USA) where these species co-occur (M. A. Peterson, unpublished data). A positive feedback loop (Fig. 1) could result if aphid populations are higher in the presence of *R. salicisbrassicoides* galls, due to enhanced nutrient levels on galled tissue, access to microhabitats in which desiccation is reduced, and/or decreased exposure to predators.

In this study, we addressed this positive feedback loop hypothesis by testing three predictions: (1) the abundances of ants, aphids and galls per plant are positively correlated with each other, (2) aphid population growth is highest in the presence of both ant guards and *R. salicisbrassicoides* galls, and (3) gall density is higher in the presence of both ants and aphids than in the absence of one or both. In addition, to examine one of the possible mechanisms underlying such dynamics, we assessed whether: (4) galler parasitism rates are lower in the presence of the ant–aphid mutualism, leading to higher survival rates of midge larvae.
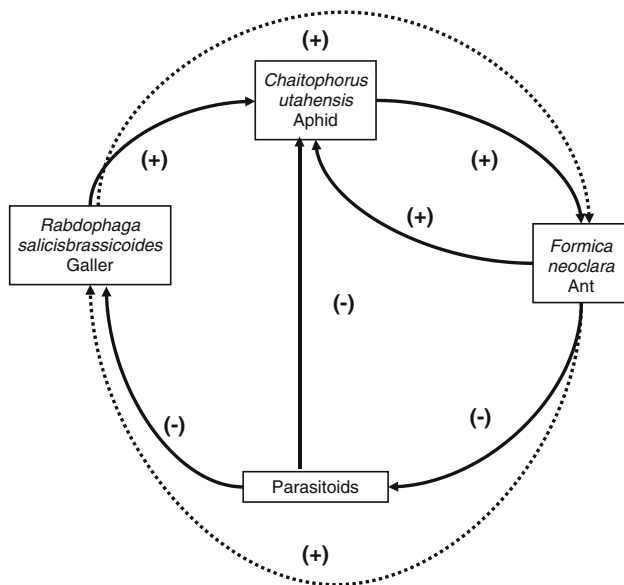
Fig. 1 Proposed positive feedback loop of aphids, ants, and galls produced by *Rabdophaga salicisbrassicoides* (Diptera: Cecidomyiidae) on *Salix exigua* in Central Washington State, USA. *Solid lines* represent direct interactions and *dashed lines* represent indirect interactions. Individual beneficial (+) and antagonistic (−) interactions explained in text

Materials and methods

Study system

In this study, the hypothesis of a positive feedback loop between an ant–aphid mutualism and a gall-making midge (Fig. 1) was tested in patches of sandbar willow, *Salix exigua*, in central Washington State (USA). *S. exigua*, the host plant of both the galler, *R. salicisbrassicoides* and the aphid, *C. utahensis*, grows in isolated patches along the Columbia River. Individual stems, which normally reach a height of 2–3 m, are typically clustered in groups of clones (Price 1989). In central Washington, *Rabdophaga* galls are usually present at low densities in most *S. exigua* patches and most years. However, occasionally they reach exceedingly high densities, in which the trees become deformed (>1,000 galls/stem, M. A. Peterson unpublished data).

While there are no published accounts of the natural history of *R. salicisbrassicoides*, we provide a description for its congener, *R. strobiloides*, which also feeds on willows, and has a very similar life history (A. M. Savage and M. A. Peterson, personal observation). Morphologically, the two species are so similar that the major distinguishing feature is that *R. salicisbrassicoides* galls are borne in clusters, while those of *R. strobiloides* are borne singly (R. Gagne, personal communication). *R. strobiloides* females lay eggs on

the tips of developing shoots in late April. As the leaves begin to unfurl, larvae emerge from their eggs and bore into the center of the shoot, inducing rosette galls (Walsh and Riley 1869). Galls reach their maximum size (22 × 19 mm) in late July. Over the winter, galls persist on willow shoots, although both the plants and larvae are dormant. Adults mate soon after emergence in mid to late April, and within 3–4 days, females lay eggs on new hosts (Wilson 1968; Weis 1984; Weis and Kapelinski 1984; DeClerck-Floate and Price 1994).

Ant-tended aphids (*C. utahensis*) are often found feeding on the same plants that are heavily infested with *R. salicisbrassicoides* galls. *Chaitophorus* species feed exclusively on *Salix* and *Populus* spp. (Richards 1972). Additionally, aphids within this genus are often (but not always) found in association with mutualistic ants, and myrmecophily has evolved multiple times in *Chaitophorus* (Shingleton and Stern 2003).

In this study system, *Formica neoclara* workers tend *C. utahensis* colonies. *F. neoclara* is a widespread ant species that is commonly found in warm habitats and among the roots of pioneer tree species. This species is common in central Washington (J. Longino, personal communication) and frequently tends homopterans in the region. *F. neoclara* workers are generalists, and have been found tending 42 different aphid species, from 15 genera on 29 different species of host plants (Francoeur 1973).

Prediction 1: the abundances of ants, aphids and galls are positively correlated

If the ant–aphid mutualism facilitates outbreak densities of *R. salicisbrassicoides* galls via a positive feedback loop, then we expect positive correlations among the abundances of ants, aphids, and galls (Fig. 1) It is important to note that such correlations could also occur either if the ant–aphid mutualism facilitates increased galler abundance or if gall midges enhance population growth of ants and aphids. Thus, establishing such pairwise correlations does not rule out non-reciprocal positive effects. Nonetheless, because these correlations are an important component of the positive feedback loop hypothesis, it is necessary to test the prediction of pairwise correlations between all three organisms. To test this prediction, we conducted a survey of 95 naturally occurring *S. exigua* stems along the western bank of the Columbia River, 5.5 km from Vantage, Washington (46°56′ N, 119°59′ W) during 26–27 August 2003. Survey stems were haphazardly chosen and classified based upon gall density class [0 galls (A); 1–10 (B); 11–50 (C); 51–100 (D); and 101+ (E)] with 20 stems per class. However, only 15 stems were selected

from density class D, because there were not enough stems in this density class to use 20. For each stem, the total number of galls, ants and aphids were counted. We applied partial correlation analysis (Statistix version 8, analytical software 2003) to determine pairwise relationships among galls, ants, and aphids.

Prediction 2: aphid population growth is highest in the presence of both ants and galls

To test this prediction, we excluded both ants and *R. salicibrassicoides* galls from willows in a 2 × 2 factorial design at a study site along the eastern bank of the Columbia River near George, Washington (47.08° N, 119.85° W) and ~10 km from the site used for the within-site survey. On 11 September 2003, when fully developed *R. salicisbrassicoides* galls were present on *S. exigua* stems, 20 blocks of four *S. exigua* stems were haphazardly selected. In each block, we trimmed vegetation to eliminate vegetation bridges for ants.

Treatment application

We applied treatments to stems at random within each block in a 2 × 2 design with an ant treatment (excluded or ambient) and a gall treatment (eliminated or ambient). To exclude ants, we placed foam banding (Tanglefoot, Grand Rapids, Mich.) around the bases of the stems. Next, we applied Tree Tanglefoot Pest Barrier over the foam banding, which minimized the risk of damage to *S. exigua* from the Tanglefoot. In the companion ambient ants treatment, foam banding was applied as a control without addition of Tanglefoot. To eliminate galls, we removed galls from all stems on plants, using shears to clip the stem immediately below each gall or gall cluster. In the ambient gall treatment, a similar amount of non-galled stem tissue was clipped at the same time, to control for the effects of clipping. Finally, we manually removed all aphids from treatment stems using a small brush.

Aphid response

One week after treatment application, we used a small brush to remove the few aphids that had migrated to treatment stems. Next, we inoculated all 80 stems with ~100 aphids. To do this, we attached a non-experimental branch bearing ~100 apterous aphids to each treatment stem, ensuring that all stems began the experiment with aphids that had the same population size and establishment history. Aphid population size was recorded on all treatment stems 1, 4, 7, and 12 days after inoculation with aphids. In addition, on each of

these dates as well as on the date of aphid inoculation, we trimmed vegetation away from treatment stems, cleared any debris that had blown into the treatment stems, scraped Tree Tanglefoot Pest Barrier off stems and applied a fresh coat. This maintenance ensured the integrity of the treatments for the duration of the experiment.

Data analysis

The influence of both ants and *R. salicisbrassicoides* galls on aphid population growth trajectories was analyzed using a factorial repeated measures ANOVA (ANOVAR). Because all stems began the experiment with ~100 aphids, day 0 aphid population densities were not included in the analysis. The independent factors in the model included the ant treatment, the gall treatment, their interaction, and the random effect of block. A priori least square differences contrasts were used to make multiple comparisons among treatments.

Prediction 3: gall density is highest in the presence of both ants and aphids

We tested this prediction using a 2 × 2 factorial experiment manipulating ants (excluded/ambient) and aphids (eliminated/ambient) at the same site at which we conducted the survey (above). There were approximately 300 *S. exigua* stems present in patches of probable clones at this site. We established the experiment on 12 April 2003, when there was no evidence of *R. salicisbrassicoides* adults or eggs from 2003, although galls from the previous year were present throughout the patch in varying densities. Bud burst of *S. exigua* stems had just begun. Aphids were present in low densities (<20/plant), feeding on *S. exigua* stems and tended by a small number of ants.

Treatment application

We haphazardly selected 20 blocks of four *S. exigua* stems, with all stems within a block occurring within 0.5 m of each other. In most cases, the stems within each block were either individual stems from a large plant or different stems that were likely clones of one another (although no genetic analyses were conducted for confirmation). In each block, we isolated stems by clearing the surrounding vegetation, ensuring that there were no vegetation bridges between them. Next, one stem per block was randomly assigned to each of the treatments.

For this two-way factorial experiment, ant and aphid removals were achieved as in the aphid survival

experiment. We initially removed aphids from all stems, regardless of treatment. Then, we subsequently inoculated stems in the ambient aphid treatments by attaching to each a small *S. exigua* branch bearing 30–40 aphids.

Treatment maintenance

We maintained all four treatments weekly until 10 October 2003. During this weekly maintenance, we surveyed all treatment stems to confirm that the treatments were effective. We used the following scores to assess ant abundance on treatment stems: $0 = 0$, $1 = 1$–5, $2 = 6$–20, $3 = 21$–50, $4 = 51$–100, and $5 = $ over 100 ants per stem. Aphids were also surveyed using a scoring system, but with slightly different values: $0 = 0$, $1 = 1$–10, $2 = 11$–50, $3 = 51$–100, $4 = 101$–500, and $5 = $ over 500 aphids per stem. We also trimmed all of the vegetation around the ant exclusion stems and on rare occasion, any branches that were growing into nearby trees were clipped. Next, the Tanglefoot Pest Barrier was scraped off the foam banding of ant exclusion stems and a fresh coat was applied. We removed any ants that had circumvented the barrier. Also, we manually brushed off any aphids that had colonized stems in the aphid-eliminated treatments. Finally, to maintain the presence of aphids on all stems to which aphids had been originally inoculated, ~30–40 aphids from non-treatment stems were transplanted to any stems in the aphid ambient treatments that had no aphids. For the most part, the need for these aphid supplementations was restricted to the treatment with ants excluded and ambient aphids.

Galls are not well developed until early summer, so galls were censused on all treatment stems on 1 July 2003 to determine the density of galls as soon as possible after successful gall formation was obvious. Gall density was again assessed on 10 October 2003, when all of the galls from the treatment plants were collected.

Data analysis

Because gall density was assessed on the same stems on two dates, ANOVAR was used to determine the effect of ants and aphids on gall density. The independent factors in the model included the ant treatment, the aphid treatment, their interaction, and the random effect of block. A priori LSD was used to make multiple comparisons among treatments (Fig. 1). ANOVAR were also used to assess the efficacy of ant and aphid treatments. In these ANOVARs, the independent factors were ant treatment, aphid treatment and their interaction and a random error term. A. priori LSD

was used for multiple comparisons between treatments.

Prediction 4: parasitism rates are lowest in the presence of the ant–aphid mutualism, leading to increased midge larval survival rates

If the positive feedback loop operates via aggressive behaviors of aphid-tending ants towards galler parasitoids, then we expect decreased parasitism rates and, conversely, increased midge larval survival rates in the presence of ants and aphids. To test this prediction, we examined gall contents at the end of the field season. Upon collection, galls were stored in a $-80°C$ freezer. Galls were dissected in the laboratory, with the contents of the galls sorted broadly into five categories: *R. salicis-brassicoides* larvae, parasitoids, moldy larvae, desiccated larvae, and other unidentifiable material (such as fragments of insect bodies). All insects were preserved in 70–95% ethanol and stored in the laboratory.

Host plant mortality

Sixteen trees (19%) died during the course of the experiment, apparently in response to strong winds, and galls from those stems were not collected. Of these stems, one (6%) was from the treatment class with ants permitted and aphids present, five (31%) were from the treatment class with both ants and aphids excluded, six (38%) were from the treatment class with aphids excluded and ants present, and four (25%) were from the treatment class with ants excluded and aphids present. These stems were not available for the following analyses.

Data analysis

Parasitism and midge larval survival rates on each stem were calculated to determine the effects of ants and aphids. Gallers that died of unknown causes were omitted from analyses of parasitism rates, but were included in analyses of survival. It is noteworthy that results from analyses that included moldy larvae as "not parasitized" were qualitatively similar to analyses that excluded moldy larvae. Nonetheless, since it is possible that parasitized larvae were among the moldy larvae, the values reported here for parasitism rates are conservative. To correct for non-normality of proportional data, both survival and parasitism rates were subjected to angular transformation (Sokal and Rohlf 1995). These transformed values were used in the statistical tests, but actual proportions are presented in the figures, for clarity.

The influence of ants and aphids on the proportion of larvae parasitized and the proportion of larvae surviving was assessed using ANOVA, with the independent effects of ant treatment, aphid treatment, their interaction and a random error term. Blocks were removed from analyses because the data included significant interactions, but were not replicated within blocks. Once blocks were removed from the analyses, the data included sufficient replication that no ANOVA assumptions were violated. To determine the influence of ants and aphids on the mean number of larvae per plant at the end of the growing season (October), we conducted a maximum likelihood analysis using the PROCGEN-MOD procedure in SAS 9.1.3 (SAS Institute 2004), assuming a Poisson distribution for the number of larvae per plant. This analysis was used because the count data violated the ANOVA assumption of normality.

Results

Prediction 1: ants, aphids and galls are positively correlated within a site

The abundances of all three species: ants, aphids, and galls, were significantly positively correlated (Fig. 2). Although these positive correlations were consistent with the positive feedback loop hypothesis (Fig. 1), the same patterns could also result from both aphids and gall makers responding to differences in plant quality/palatability or environmental variability in choosing stems for feeding. Experimental manipulations, described below, were needed to distinguish among these alternative hypotheses.

Prediction 2: aphid population growth is highest in the presence of both ants and galls

Overall, the presence of both ants ($F_{1, 57} = 228$, $P < 0.001$) and galls ($F_{1, 2} = 83.50$, $P < 0.001$) had a significant positive influence on aphid population density. Importantly, there was a positive synergistic effect of the interaction between ants and galls on aphid density ($F_{1, 37} = 18$, $P < 0.001$), in that aphid populations only grew when both ants and galls were present (Fig. 3). However, there was no significant effect of time and no two- or three-way interactions between time, ants and galls (Table 1). It is important to note that throughout the experiment, nearly all aphids on the experimental plants were apterous. Although we did not survey predators of aphids during the course of this experiment, we did observe coccinellid beetles foraging and lacewing adults ovipositing near treatment stems.
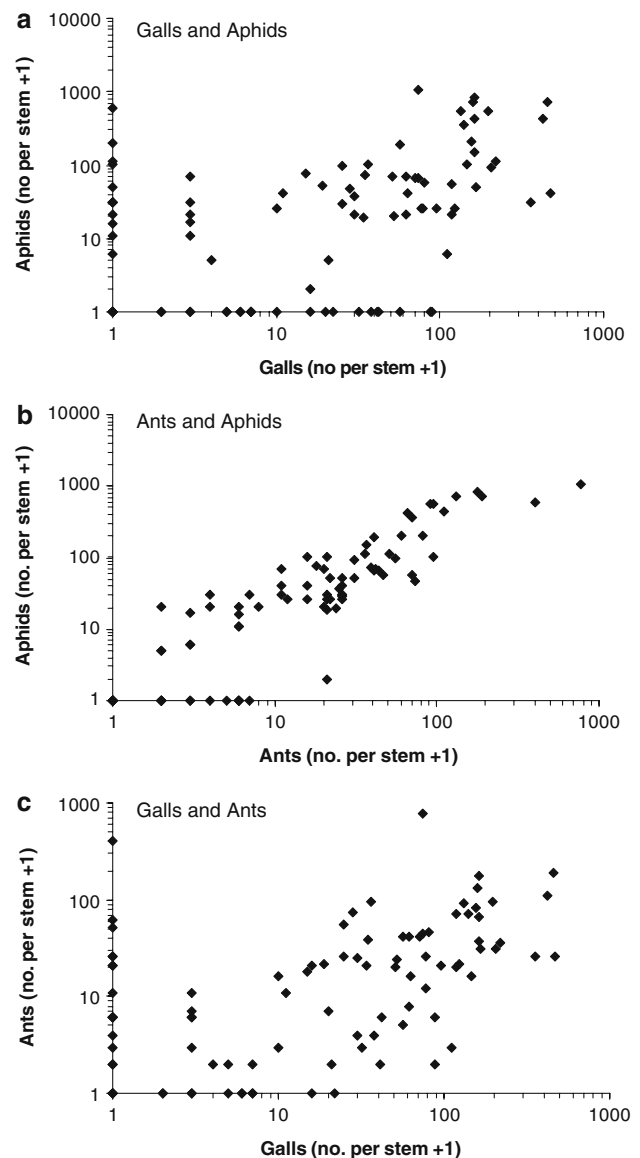

Fig. 2 Pairwise correlations between the density of **a** galls and aphids, **b** ants and aphids, and **c** galls and ants per stem from the survey conducted on 26–27 August 2003. All pairwise interactions were analyzed using partial correlation analysis (**a** $P < 0.001$, **b** $P < 0.0001$, **c** $P < 0.01$). Note log scale on both axes

Prediction 3: gall density is highest in the presence of both ants and aphids

Treatment effectiveness

Surveys of ant abundance on treatment stems over the course of the field season confirmed that ant exclusions effectively reduced ant abundances (ANOVAR, $F_{1, 55} = 111.6$, $P < 0.0001$). There was also a significant effect of aphids on ant abundances ($F_{1, 55} = 31.57$, $P < 0.0001$), with a significant interaction between ant and aphid
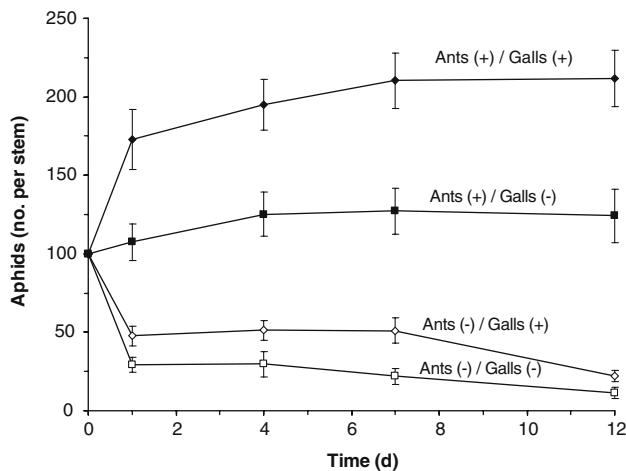
Fig. 3 The effect of the presence (+) or absence (−) of ants and galls on aphid population growth over 12 days. Aphid population growth is indicated by the mean number (±1 SE) of aphids per *S. exigua* stem (*n* = 20 stems per treatment). *Solid symbols* indicate ant presence, *open symbols* indicate ant absence, *diamonds* indicate gall presence and *squares* indicate gall absence

Table 1 Results from factorial repeated measures ANOVA (ANOVAR) conducted on aphid population growth data[a]. One hundred *Chaitophorus utahensis* aphids were transplanted to *Salix exigua* stems with no aphids present and stems were organized in blocks using different levels (present/absent) of *Rabdophaga salicisbrassicoides* galls and *Formica neoclara* ants

Source	df	SS	MS	F	P
Time	2	555	277	0.06	0.944
Time × Block	57	2,72,196	4,775		
Ants	1	7,76,556	7,76,512	228.00	<0.001
Galls	1	1,45,616	1,45,616	83.50	<0.001
Time × Ants	2	435	218	0.06	0.938
Time × Galls	2	1,080	1,080	0.31	0.735
Ants × Galls	1	38,410	38,410	17.90	<0.001
Time × Ants × Galls	2	181	91	0.04	0.959
Time × Block × Ants × Galls	37	79,333	2,144		
Error	20	28,504	1,425		
Total	239				

[a] Aphid populations were recorded 1, 4, 7 and 12 days after set-up

treatments ($F_{1, 55} = 31.62$, $P < 0.0001$, Fig. 4a). Aphid populations were also effectively reduced by aphid removals (ANOVAR, $F_{1, 55} = 50.31$, $P < 0.0001$), ant exclusion ($F_{1, 55} = 79.6$, $P < 0.0001$) and the interaction between ant and aphids treatments ($F_{1, 55} = 30.15$, $P < 0.0001$, Fig. 4b).

Gall density

The ant- and aphid-removal treatments influenced gall density in a manner consistent with the positive feedback loop hypothesis (Fig. 1). Specifically, in both July and October, ants and aphids had a significant positive
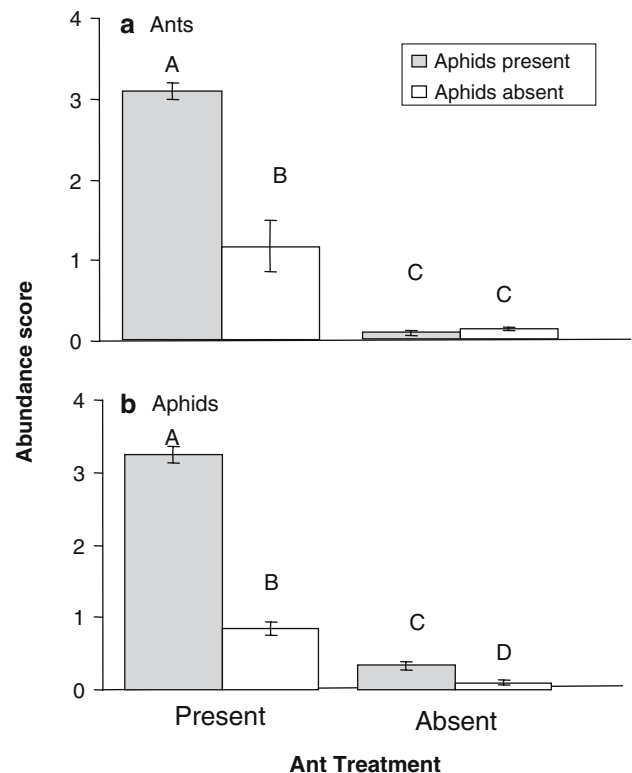

Fig. 4 Effectiveness of treatment maintenance on the abundance of **a** ants [none (*0*), 1–5 ants (*1*), 6–20 ants (*2*), 21–50 ants (*3*), 51–100 ants (*4*)] and **b** aphids [none (*0*), 1–10 aphids (*1*), 11–50 aphids (*2*), 51–100 aphids (*3*), 101–500 aphids (*4*)] based on weekly counts from July to October 2003 (means ± 1SE). *Different letters* indicate significant differences in treatment means (LSD, *P* < 0.05)

effect on gall density per stem. There was also a significant interaction between ants and aphids, with the greatest gall densities occurring in the presence of both ants and aphids (Table 2; Fig. 5). Differences in gall density among treatments did not change significantly between the July survey and the October survey (Fig. 5).

Table 2 Factorial ANOVAR of gall densities[a] in July and October

Source	df	SS	MS	F	P
Time	7	18.4	2.6		
Ants	1	538.0	538.0	1,133.4	<0.001
Aphids	1	118.0	118.0	125.8	<0.001
Time × Ants	7	3.3	0.48		
Time × Aphids	7	6.6	0.94		
Ants × Aphids	1	130.9	130.90	120.0	<0.001
Time × Ants × Aphids	7	7.6.0	1.09		
Error	509	404	0.79		
Total	540				

[a] All galls were surveyed on *S. exigua* stems with experimentally manipulated combinations (present/absent) of *F. neoclara* ants and *C. utahensis* aphids which were maintained from April 2003 to October 2003
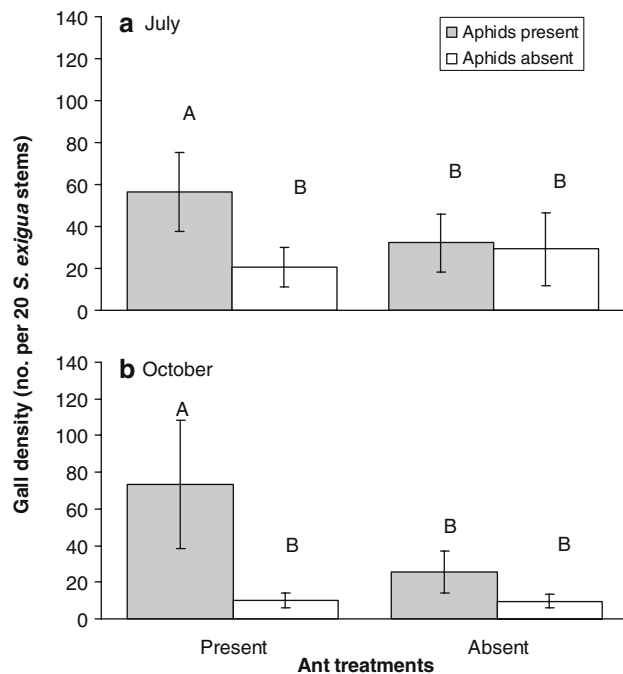
Fig. 5 *R. salicisbrassicoides* gall density (mean ± 1 SE) in **a** July and **b** October 2003. Each combination of ants and aphids was applied to 20 *Salix exigua* stems in early April 2003. For each date, *different letters* indicate significant differences in treatment means (factorial repeated measures ANOVA, $P = 0.0005$; LSD $P < 0.01$)

Prediction 4: parasitism rates are lowest in the presence of the ant–aphid mutualism, leading to increased midge larval survival rates

Galler parasitism

Three morphospecies of parasitoid wasps were extracted from the *R. salicisbrassicoides* galls that were collected in mid October, including two Platygasteridae and one Braconidae morphospecies. Ninety-eight percent of all parasitoids inside *R. salicisbrassicoides* galls were platygastrid wasps. Within the platygastrids collected, 99.5% were one morphospecies. The average overall rate of gall parasitism was 46.7% (SE = 4%). In contrast to the influence on gall abundance and overall galler density, parasitism rates did not vary significantly in response to either ant ($F_{1, 57} = 0.88$, $P = 0.35$) or aphid ($F_{1, 57} = 2.28$, $P = 0.14$) presence (Fig. 6a).

Other gall contents

There were also galls that contained neither parasitoids nor intact *R. salicisbrassicoides* larvae. Larvae covered in mold were present in 8.5% of the collected galls, 0.55% of the galls were empty and 5.4% contained desiccated larvae.
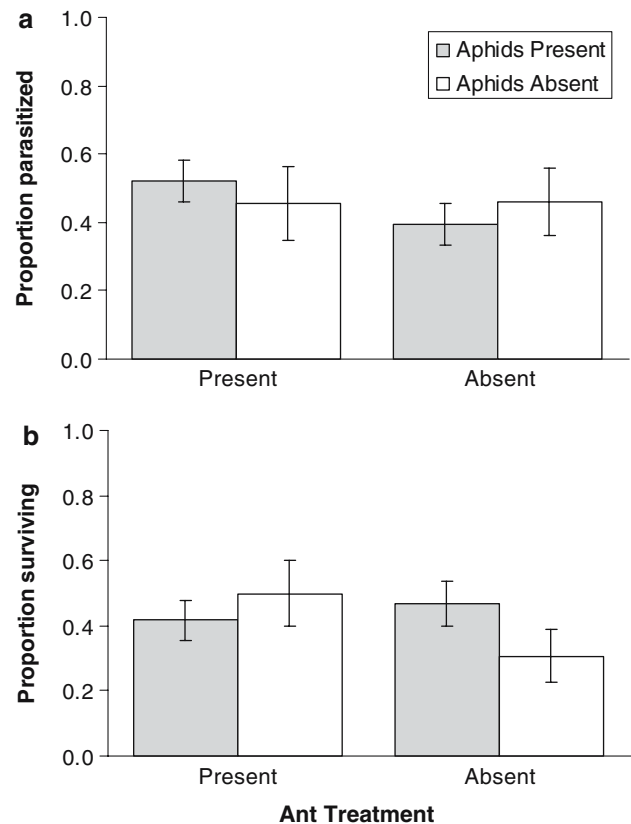

Fig. 6 **a** Gall parasitism and **b** gall maker survival for galls collected in October 2003 (mean ± 1 SE). The proportion surviving was defined as the number of gall makers per total number of galls collected. There were no significant effects of the presence/absence of either ants or aphids on gall maker survival or frequency of parasitism, and moldy larvae are not included in this figure (see text)

Midge larva survival

The positive feedback loop hypothesis predicts highest gall larval survival in the presence of both ants and aphids (Fig. 1). In support of this prediction, the average number of *Rabdophaga* larvae in galls at the end of the field season was highest in those stems with both ants and aphids present and lowest in treatment stems that excluded ants and aphids (Fig. 7). However, this pattern was not due to a higher proportion of galler larvae surviving in that treatment. Survival rates did not differ significantly among treatments, in response to the presence of ants ($F_{1, 57} = 0.88$, $P = 0.38$) or aphids ($F_{1, 57} = 2.28$, $P = 0.21$) (Fig. 6b).

Discussion

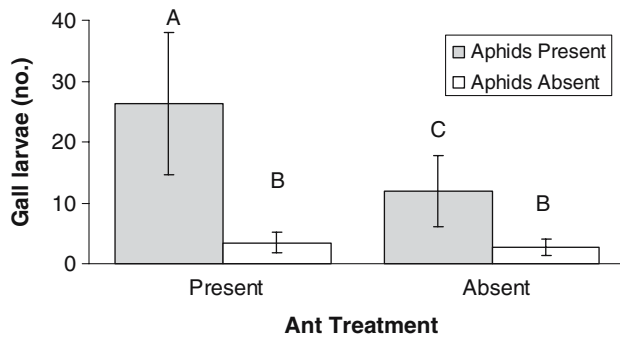According to the positive feedback loop hypothesis, ant-tended aphids are expected to enjoy the highest

Fig. 7 Gall larvae per stem (mean ± 1 SE) from galls collected at the end of the season in October 2003. *Different letters* indicate significant differences in treatment means (maximum likelihood analysis, $P < 0.0001$) (see text)

population growth rates on galled willows. Furthermore, the presence of aphids and their attendant ants should promote increased gall population densities, whether by creating enemy-free space, inducing changes in host plant quality, or stimulating ant-dependent oviposition by female gallers. In turn, such increases in gall population densities would reciprocally increase the abundance of ants and aphids (Fig. 1). To determine the validity of this hypothesis, we assessed whether gallers, ants, and aphids enhance each other's densities. In addition, we examined whether the presence of ants and aphids does create enemy-free space, reducing parasitism rates as a result.

The findings of this study supported the three general predictions of the positive feedback loop hypothesis (Fig. 1). First, naturally occurring densities of ants, aphids, and *R. salicisbrassicoides* galls were all positively correlated with each other (Fig. 2). Second, aphid populations were highest in the combined presence of ants and *R. salicisbrassicoides* galls (Fig. 3). In fact, this was the only combination of ants and galls that promoted aphid population growth. Third, gall density and gall larvae per stem were highest on treatment stems with both ants and aphids present (Figs. 5, 7). Interestingly, this effect of ants and aphids on galler abundance was apparently not due to the creation of enemy-free space by ants, as neither parasitism rates nor galler survival were influenced by the presence of aphids and/or ants (Fig. 6). Thus, although we have established that ants, aphids, and gallers positively influence each other's abundance on willows, at least some of the underlying mechanisms remain unresolved.

The finding that aphid and ant abundances are positively correlated is to be expected. Ants frequently tend aphids, and without their ant guards, aphid populations often experience dramatic crashes (Yao et al. 2000; Katayama and Suzuki 2003), an effect most

directly attributable to the fact that ants defend aphids from predators/parasitoids and decrease competition from other herbivorous insects (Banks 1962; Fowler and Macgarvin 1985; Buckley 1987). Although we observed predators of aphids on treatment stems during our experiment, we did not quantify either their abundance or rates of consumption. Aphids may also benefit indirectly from tending by ants, whether due to an increase in the ratio of reproductives to sterile adults (and thus increasing fecundity), decreased development time, or maintenance of optimum population densities (Huxley and Cutler 1991; Larsen et al. 2001). These indirect effects can be dramatic; daily offspring production, which ranges between 0.35 and 3.3 nymphs/aphid/day in untended aphids (Kersting et al. 1999; Hentz and Nuessly 2004; McCornack et al. 2004; Diaz and Fereres 2004), can be nearly 2 times greater for ant-tended aphids than for aphids denied access to ants (Flatt and Weisser 2000). Thus, the rapid response of aphid populations to ant exclusion in our experiment was probably due to differential reproduction and/or predation.

More novel was our discovery that galls increase the abundance of aphids and their attendant ants. Various mechanisms could be responsible for this effect. Morphologically, galls may shield aphids from desiccation or provide more complex architecture for predator avoidance. Additionally, ants have been shown to be more effective at attacking aphid parasitoids on highly sculptured substrates than smooth surfaces (Völkl and Kroupa 1997). Therefore, ants may be able to provide aphids with more effective anti-parasitoid defense when aphids feed on gall tissue. Alternatively, *R. salicisbrassicoides* galls may function as sinks for water and plant nutrients. Recently, Nakamura et al. (2003) found that *Rabdophaga rigidae* galls on *Salix eriocarpa* trees had higher levels of both water and plant nutrients than non-galled plant tissue, leading to relatively higher levels of aphid feeding on galled tissue (Nakamura et al. 2003). In other systems, galls have been shown to contain higher levels of carbon, nitrogen and water than non-galled plant material, while often containing decreased levels of plant phenolics (Larson and Whitham 1991; Harris and Shorthouse 1996; Nyman and Julkunen-Tiitto 2000; Raman et al. 2006). If present, either a mechanical or chemical mechanism would exaggerate any anti-predator or reproductive benefits that aphids might gain from being tended by ants.

The combined presence of ants and aphids has a reciprocal positive effect on gall density, an effect with several potential underlying causes. First, aphid feeding often elevates nutrient concentrations near the feeding sites (Girousse et al. 2003; 2005). Therefore,

aphid feeding may promote higher gall densities, if gall abortion rates, larval survival rates, or female oviposition preferences are influenced by plant nutrition. Alternatively, *R. salicisbrassicoides* females may oviposit preferentially on *S. exigua* stems with abundant ants, as has been shown for both ant-tended homopterans (Morales 2002) and lycaenid butterflies (Pierce and Elgar 1985; Fraser et al. 2002).

Another possibility is that ants tending aphids either on or near galls create enemy-free space for those galls, as is the case for nectar-excreting galls (Fernandes et al. 1999; Inouye and Agrawal 2004). An alternate scenario may be that the ant–aphid mutualism indirectly supports population growth of the primary parasitoids of the galler. In studies on the dynamics of aphid parasitoids, ants have been shown to decrease populations of hyperparasitoids, leading to increased abundances of primary parasitoids, to the detriment of their aphid partners (Völkl 1992; Sullivan and Völkl 1999). In this system, such dynamics may well be occurring, since 98% of the parasitoids collected in galls were primary endoparasitoids (Hymenoptera: Platygasteridae). These findings contrast starkly with findings for other *Rabdophaga* gallers (Van Hezewijk and Roland 2003) and preliminary surveys of this system (M. A.Peterson, unpublished data), in which parasitoids from the facultatively hyperparasitic family, Torymidae, were the most abundant galler parasitoids. Torymids oviposit later in the season, when parasitoids are more likely to encounter aggressive ant guards because aphid populations are denser.

However, given that we found no effect of the presence of ants on either the parasitism rates or survival of galler larvae, it is unlikely that enemy-free space explains the positive effect of aphids and ants on galler abundance. We cannot completely reject the enemy-free space hypothesis based on our results, because among-treatment differences in parasitism rates may have been masked by other factors. For example, parasitism rates of cecid fly galls are influenced by site of induction (Tscharntke 1992), gall diameter (Van Hezewijk and Roland 2003), number of galls per cluster (Tscharntke 1992), proximity to neighboring plants (Briggs and Latto 2000), and synchrony between gall induction and parasitoid oviposition (Clancy and Price 1986). If any such factors varied consistently among treatments, a situation we view as unlikely, they could have countered any effects of ants on parasitism rates. Nonetheless, given that the effects of ants and aphids on gall density occurred within a single cohort of galls, such effects are almost certainly due to some factor other than enemy-free space, such as ant-dependent oviposition or differential gall abortion.

Clearly, further study is needed to determine the complete array of mechanisms underlying the positive reciprocal effects between aphids, ants, and galls on *S. exigua*. Nonetheless, our findings demonstrate that these effects are substantial and consistent with the hypothesis of a positive feedback loop connecting the dynamics of *R. salicisbrassicoides* with the ant–aphid mutualism.

More broadly, because of their sustained and cascading influences, indirect effects often have a strong influence on community structure (Wootton 1994; Fox and Olsen 2000; Peacor and Werner 2001; Webster and Almany 2002; Pearson and Calloway 2003). For example, as our study demonstrates, herbivores feeding on the same host plant need not always have detrimental effects on each other through antagonistic interactions such as competition, but may facilitate each other's population growth through indirect pathways. Other species, in turn, may suffer through such indirect effects. For example, ants may drive off external feeders on willows, such as chrysomelid beetles and saturniid moth larvae, more than they affect internal feeders, such as gallers or leaf-rollers (Mody and Linsenmair 2004). Similar ant-driven shifts in community structure have been found in insects on *Populus* (Wimp and Whitham 2001). Such community-wide studies, taken together with our evidence for positive feedback between ants, aphids, and galls, suggest a need for more effort in population and community ecology to extend studies of mutualism beyond simple analyses of pair-wise interactions. Indeed, studies that examine the indirect effects of generalized and facultative as well as specialized and obligate mutualisms will both broaden and solidify our understanding of the ecological influence of mutualisms on communities. Ant-protective mutualisms occur at both ends of this continuum, are found throughout the world, and are well described. Therefore, the study of these mutualisms could greatly advance our understanding of the ecological consequences of mutualisms.

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