

What is the nature of the ant-aphid relationship?

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Abstract

Many aphid species, on a wide variety of plants, are tended by ants. We tested whether the relationship between the ant species *Formica obscuripes* and an aphid species on rabbitbrush (*Chrysothamnus viscidiflorus*) was a mutualism and, if so, whether it was obligate or facultative. Using a fully factorial design, we measured the response of aphid numbers to the presence and absence of ants and predators, resulting in four treatments. We found that the aphid numbers were lower in the presence of ants and about equal regardless of the presence or absence of predators. For two out of the three surveys, aphid numbers were highest in the absence of both ants and predators. In the absence of ants and the presence of predators, aphid numbers were intermediate. We conclude that there is no ant-aphid mutualism in this system.

Introduction

Mutualisms exist along a continuum of relationships that vary from facultative to obligate (Margulis 1993). In a facultative relationship, organisms benefit from the interaction, but do not require the presence of a partner to survive. As organisms become more adapted to attract a specific partner, the increasing costs can evolve to the point where the organism needs the benefit of its partner to outweigh the costs in order to survive. At this point, the mutualism has become obligate. Depending on conditions, a particular mutualistic relationship can shift somewhat towards a more facultative or obligate relationship (Stadler and Dixon 1998). External factors, such as food availability or presence of predators can cause this shift.

Understanding the costs as well as the benefits of a mutualism is important to fully understand how the relationship is maintained over ecological and evolutionary time. One or both partners typically expend additional energy to offer a reward that will attract the other partner (Addicott 1978a; Cushman and Addicott 1989). The costs and benefits of mutualisms vary within and between different partner sets, and may even affect certain stages of the life history negatively while having an overall positive affect (Yao et al. 2000).

Ants tending insects have been good model systems for studying this range of mutualisms. A variety of insects are partners to ants, including lycaenid butterfly larvae (Baylis and Pierce 1992), aphids (Addicott 1979), and membracids (Morales 2000). Most of these insects, especially aphids, have short generation times, resulting in relatively rapid feedback between the partners (Addicott 1978a). Ants and insects are also rather easy to manipulate and count. The variety of types of mutualisms that result from the different species combinations in different areas makes them a rich resource for study.

We tested whether an ant-aphid relationship is a facultative or obligate mutualism. We manipulated the presence and absence of predators and ants. If the mutualism is obligate, we would expect to see a positive ant effect on aphids in both the presence and absence of predators. Whereas, if the relationship is facultative, there should be a cost to the aphids in attracting ants to tend them, that would be expressed as either no ant effect or a negative effect in the absence of predators. If there is a negative effect of ant-tending on aphids in the presence and absence of predators, there relationship may not be a mutualism.

Methods and Materials

The study was conducted in the Almont Triangle, a management area of the Gunnison National Forest about 25 km north of Gunnison, Gunnison County, Colorado. The site is relatively arid and the dominant plants are the woody perennials *Artemisia tridentata* and *Chrysothamnus viscidiflorus* (Asteraceae), as well as grasses and herbaceous perennials (Billick and Tonkel 2003).

We know that there is one aphid species at the site, but no not yet know what it is. We have identified seven possible species that are found on the host plant (Palmer 1952). They are not often seen on *Chrysothamnus viscidiflorus*, but appear on the plants in high

numbers when predators are excluded (M. Relyea, *personal observation*). Several alates (winged adults) and several other forms have been collected and will be sent to an expert in the area to identify the aphid species at the study site which was used in the experiment.

In this study, we focused on the ant-aphid mutualism. To test the costs and benefits of ants to aphids in the absence of predators, we set up a two-way randomized-block ANOVA design that excluded or allowed predators and ants. Treatments excluded or allowed ants, and excluded or allowed predators, resulting in four treatments each with 30 plants: + ants/+ predators, + ants/- predators, - ants/+ predators, and - ants/- predators. An fifth group of plants was left unmanipulated to serve as a reference to monitoring natural aphid population dynamics. We chose 150 *C. viscidiflorus* as aphid host plants and blocked them into groups of five. In the group, each plant received a different one of the five treatments. We chose plants with membracids, since study on the ant-membracid mutualism was conducted simultaneously. Plants were chosen with intermediate-sized membracid aggregations, such that numbers would not become too large to count accurately, but aggregations were not likely to go extinct.

We erected 10" tall aluminum cylinders approximately 7' in diameter around each individual host plant and nailed them into the ground. In the center of each was a 103 cm wooden stake. We attached bridal veil (25 holes/cm²) to the cylinders with Velcro (1" wide) and gathered and tied it at the stake to complete the cage. The predator exclusion was completely covered with bridal veil. Where predators were allowed access, we covered half the cylinder on the south side to control for any cage effects. We applied Tanglefoot™ (Tanglefoot Company, Grand Rapids, Michigan, USA) to the inside top of all cylinders. On the half-cages, Tanglefoot was also applied to the outside at the top to

keep ants either in or out of the cages. We manipulated ant presence and absence by creating artificial colonies in Rubbermaid tubs and placing these in the appropriate cylinders. As a control, we placed a tub filled with dirt in treatments without ants.

We counted aphids during the weeks of July 12, July 26, August 9. When aggregations were very large, we estimated aphid number by counting a plant bud that appeared to have an average number of aphids on it, and then multiplied this number by the number of buds with aphids present. In the final survey, we also counted aphid alates (winged adults) and parasitized aphids. In addition to the aphid surveys, we made weekly measurements of other variables that may affect aphid performance, including membracid nymph and adult numbers, ant numbers, and predator numbers.

Because the data were strongly nonrandom and could not be normalized with a transformation, we used a randomization program written in True Basic. We calculated the F-value associated with the main effects and the interactions, then randomized the data and calculated F-values 1000 times in order to generate probability data. We also graphed frequency distribution of aphid numbers in the different treatments and used a contingency table to analyze the effect of the treatments on the presence of parasitized aphids. Because the data contained a large number of zeros, we used the mean number of aphids per plant to compare treatments instead of the median.

Results

In weeks one and five, there was no significant difference between the open treatment and the + ant/ + predator treatment (Mann-Whitney U; week 1: $U = 440.5$, $p = 0.8623$; week 5: $U = 381.5$, $p = 0.182$). Aphid numbers increased overall during the course of the experiment, with treatments causing similar effects relative to each other each

week (Figure 1). For all weeks, there were significant ant effects, predator effects, and ant x predator effects.

In the first week, aphids numbers were highest in the absence of both predators and ants; the mean number per plant was 5.87. But, in the presence of predators and the absence of ants, the mean number was 2.53 aphids/plant, about half as many as with predators. Aphid numbers were lowest in the presence of ants, about the same with (2.00 aphids/plant) or without predators (1.97 aphids/plant).

In the fifth week, aphid numbers were fairly consistent with the first week. Aphid numbers were highest in the absence of both ants and predators (138 aphids/plant). When plants were categorized into groups by aphid numbers of 1-24, 25-99, 100-499 and >500, this treatment had a high number (9) in the lower category, but had several in each of the other categories as well, with fewer in each as numbers increased. Aphid numbers were lowest and approximately equal in the presence of ants, with (15.2 aphids/plant) or without (16.0 aphids/plant) predators. These two treatments had similar frequency distributions of aphid numbers, with most of the plant with aphids (7-8) being in the lower two categories, and 1 plant in the next to highest category, and no plants with more than 500 aphids. The – ant/ + predator treatment had a higher number of aphids (30.8 per plant) than the ant treatments, but a quarter the number of the no ant, no predator treatment. It had the most plant in the lowest category, declining from there, but with one plant that had greater than 500 aphids.

During the fifth week, there were significant ant, predator, and ant x predator interaction effects on aphid alate number (Table 1). Alate numbers were highest in the absence of predators and ants (5.03 alates/plant). They were a third of that in the presence

of predators and the absence of ants (1.37 alates/plant). They were lowest and similar in the presence of ants, with (0.100 alates/plant) and without predators (0.067 alates/plant).

The presence or absence of ants caused a significant difference in the number of plants with parasitized aphids (chi-squared p-value: 0.0006). In the absence of ants there were nearly six times as many plants with parasitized aphids (17), than in the presence of ants (3) (Table 2).

Discussion

Our findings show that the ant effect is much stronger than the predator effect on aphid numbers. In the presence of ants, only the negative ant effect could be seen, but when ants were removed, the predator effect became evident. We observed this pattern most strongly in the last week, when without ants, aphid numbers were four times higher without predators than with them. The ants have the more important effect; the predator effect can only be seen when ants were removed. The higher alatae numbers and increased presence of parasitized aphids in the absence of ants probably only reflects the higher aphid numbers in general in those treatments, rather than being a separate effect.

The negative effect of ants regardless of the presence of predators indicates that the ant-aphid relationship is not either of the hypothesized obligate or facultative mutualisms. Therefore, we must accept the final hypothesis that if there is no effect or a negative one, as was found, there is no mutualism in this system.

There are several possible explanations for our results. One alternative is that the ant-aphid relationship could be on the very facultative end of the mutualism range, and conditions were such this year that the relationship was not a mutualism. Also, the ants might be getting enough sugar from the membracids that the ants are eating the aphids for protein. However, it is possible the aphids and membracids are competing for services

provided by the ants or plant resources. Finally, the ants could be causing a behavioral change in the aphids. Sometimes aphids may respond to predators by falling off a plant. If the aphids cannot tell the difference between an ant and a predator, the high numbers of ants on the experimental plants could have caused low aphid numbers.

It is noteworthy that there have been few reports in the literature of a negative effect of ants on aphids in the field. Yao et al. (2000) found a negative effect of ants on aphid growth and reproduction in the field, but no effect on aphid numbers because they regulated the densities. Addicott (1978b) also found that ants could have a negative effect on aphids when densities were extremely high, but he postulated that this benefited aphids in the long term because it prevented host-plant damage. He also found a negative effect of ants on the species *Macrosiphum valerianae*, which is not ant-tended (1978b). In the laboratory, Stadler and Dixon (1998) found a negative effect of ants on aphid performance, though it is difficult to evaluate the overall effect because the experiment was done in the laboratory and only examined indirect measures of fitness.

Our results demonstrate that ants can have a negative effect on aphid numbers. This possibly has interesting implications for the evolution of ant-aphid mutualisms. Most people work from the assumption that the ant-aphid relationship started out as a commensalism, and then aphids began trying to attract ants. These results suggest that instead the relationship could have started differently, with ants preying on aphids, who began producing honeydew as an attempt to appease the ants. This possibility should be investigated further.

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Tables and Figures

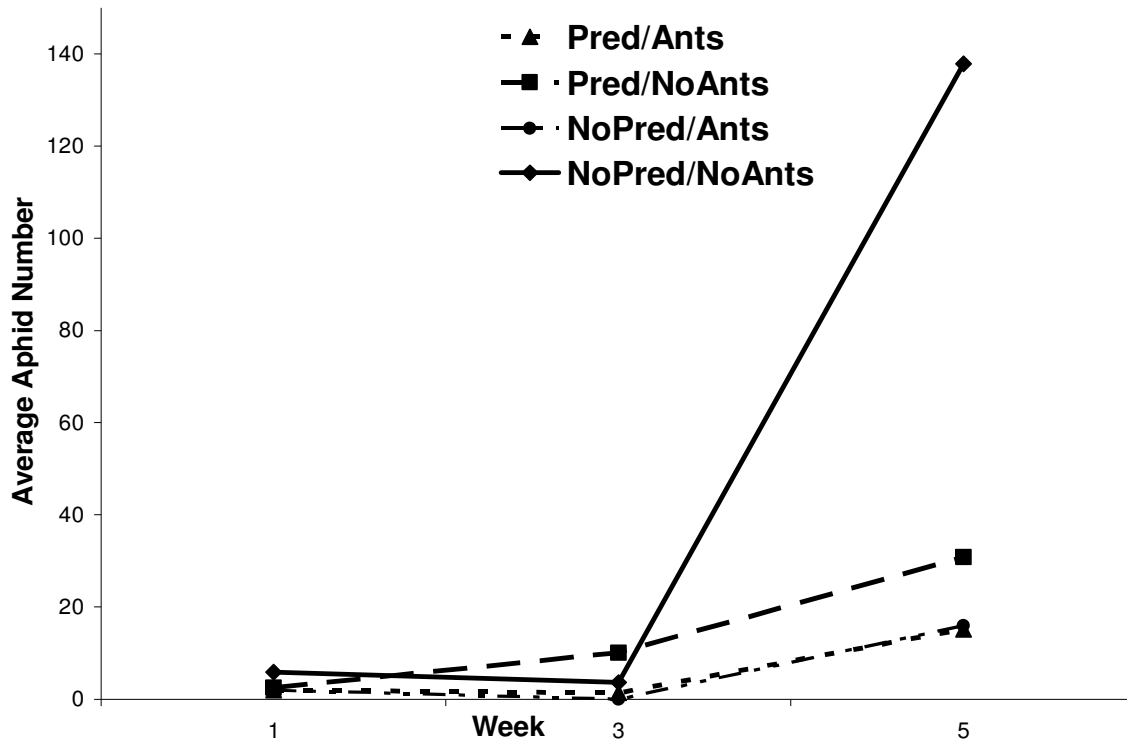
Table 1. ANOVA table for week five alates.

Source of Variation	df	SS	MS	F _x	p-value
Predator	1	99.0	99.0	0.555	0.004
Ant	1	291	291	1.63	0.001
Interaction	1	103	103	0.575	0.002
Error		233745	178		

Table 2. Contingency table for week 5 presence/absence of parasitized aphids (df: 1, chi-squared: 11.76, chi-squared p-value: 0.0006).

	Y	N	
Ants	3	57	60
No Ants	17	43	60
	20	100	

Figure 1. Average aphid numbers in each treatment over the five weeks of the experiment. In week one, there was a significant ant effect ($F_{1,116}=1.09$, $p<0.001$), a significant predator effect ($F_{1,116}=0.606$, $p=0.004$), and a significant ant x predator interaction ($F_{1,116}=0.631$, $p=0.002$). In week three, there was a significant ant effect ($F_{1,116}=2.42$, $p<0.001$), a significant predator effect ($F_{1,116}=1.00$, $p=0.001$), and a significant ant x predator interaction ($F_{1,116}=0.419$, $p<0.001$). In week five, there was a significant ant effect ($F_{1,116}=2.58$, $p<0.001$), a significant predator effect ($F_{1,116}=1.59$, $p=0.001$), and a significant ant x predator interaction ($F_{1,116}=1.54$, $p<0.001$).



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