

Role of floral nectaries and plant sex in mediating ant-aphid interactions
on *Valeriana edulis*

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Abstract

Multi-species interactions profoundly affect community composition and function of ecosystems. Foraging behavior and resource allocation are two key factors that determine how species interact. This study investigates the role of floral nectaries and plant sex in mediating ant-aphid interactions on the dioecious plant *Valeriana edulis*. Male and female plants were selected in a paired experiment, with one control and one “exclusion” flowering stalk per plant. Open flowers of exclusion stalks were glued closed to deny ants’ access to floral nectar as a food resource. No effect of nectary exclusion was found, but plant sex and aphid abundance were important factors in determining ant visitation to experimental plants. Consistent with previous studies on this system, ant visitation was greater on female plants. Despite lower ant visitation on males, a trend suggested higher aphid-tending by ants on male plants. Both results elicit further investigation into resource quality and quantity as mediators of this ant-aphid interaction.

Key Words

ants, aphids, floral nectar, species interactions, *Valeriana edulis*

Introduction

Living organisms must intake nutrients to survive and, in doing so, affect the physical environment and other living organisms in a complex web of energy exchange. Relationships that develop over time between organisms, especially related to direct energy exchange through foraging behavior, are an important facet of ecological dynamics. Relationships exist across all trophic levels and can involve more than pairwise interactions. Understanding the tradeoffs, causes, and consequences of interactions between food resources and foragers lends insight into the evolutionary history and complexity of energy flow through an ecosystem.

Ant-aphid interactions

The ubiquity of ant-hemipteran interactions on plants allows for investigation from a spectrum of viewpoints. At the interspecific level, aphid-produced honeydew, a carbohydrate-rich reward for ants which “tend” aphids, is the foundation of the ant-aphid mutualism; the composition of sugars in honeydew has been found to directly affect ant foraging behavior (DeTrain et al 2010). However, ant-aphid interactions are better described as a continuum, from mutualism to antagonism, based on the context within which the interactions evolved (Stadler and Dixon 2005).

Stadler and Dixon (2005) describe the benefits and costs in the following, simplified, terms. Aphids release excess honeydew due to their high rate of phloem sap ingestion; and, aphids have been known to increase honeydew production in the presence of tending ants, perhaps breaching an optimum nitrogen assimilation rate. Since honeydew is low in amino nitrogen content, it is unlikely to be a nitrogen source for ant colonies. However, it is a sugar-rich fuel for foraging ants. Eventually, ants may become dependent upon aphids for this resource and must adapt to transfer the energy to other nest mates. This dependence may restrict the distribution of tended aphid colonies. However, because the aggregation of tended aphid colonies is attractive to predators, aphids benefit from the protection ants provide against potential predators. Given these tradeoffs, the context in which ant-aphid interactions develop in turn affects higher-level interactions.

At the community level, hemipteran tending by ants is described by Styrsky and Eubanks (2007) to sometimes be a “keystone interaction,” affecting the structure of the arthropod and plant community of a system. The consequences of this interaction for host plants depends conditionally on the initial structure of the community; because hemipteran-tending ants prey upon other untended herbivores, high herbivory by other herbivores makes the mutualism more beneficial for the host plant. Alternatively, low herbivory can make the interaction costly due to the negative effects of sap-feeding hemipterans.

Nectaries affect foraging behavior

Aside from honeydew production, the abundance and composition of alternative resources may influence an ant’s interaction with hemipterans. Nectaries, either floral or extra-floral, are a potentially complementary carbohydrate-rich resource for foraging ants (Ness 2006; Petry and Mooney 2009). From the hemipteran’s perspective, an alternative resource such as nectar could have a positive effect

by recruiting more tending ants to the host plant or a negative effect through competition for ant attendance. Del-Claro and Oliviera (1993) suggest that nectaries may benefit hemipterans by attracting more potential ant caretakers, thereby increasing hemipteran fecundity.

Alternatively, extra-floral nectaries have been posited as a defense mechanism to distract ants from tending honeydew-producing hemipterans, thus decreasing damage by these sap-feeding herbivores (Becerra and Venable 1989). While ants may benefit a host plant by reducing the number of untended herbivores that damage it, ants can potentially decrease plant fitness, as in deterring pollinators (Ness 2006). Further investigation into these complementary and competing strategies will aid in the comprehension of the conditions that underlie the evolution of plant-insect relations.

Sex-based differentiation in floral rewards

Allocation of nutrients and investment in additional resources, such as floral nectar, may differ between sexes of dioecious plants. A meta-analysis by Cornelissen and Stiling (2005) suggests that male plants are typically attacked and damaged more frequently by herbivores than female plants. Females are expected to harbor more secondary compounds, which may serve to deter herbivores. Given the energy demand required to produce seeds, female plants should intuitively be better defended against herbivory.

Current studies

Several aspects of ant-aphid interactions on *Valeriana edulis* have already been described in a long-term community study at the Rocky Mountain Biological Laboratory. Petry and Mooney (2009) found that the ant species *Formica podzolica* tended aphids when fed a tuna diet, but ignored aphids when prescribed a honey diet. Here, the availability of an alternative carbohydrate resource provides evidence for the hypothesis that nectar may act as a form of defense against ant-tended hemipterans. Other studies found that ants tended aphids more frequently on female plants of *V.edulis* than on male plants and that aphid densities were higher on female plants (Perry and Mooney 2009; Fremgen and Mooney 2010). These patterns are likely due to multiple factors, such as plant sex-based variation in phloem sap, which in turn affect honeydew quality and quantity. Nectar quality and quantity may also be sex-based, affecting host plant choice by foragers. However, higher herbivory by aphids on female plants contrasts with Cornelissen and Stiling's (2005) findings. Their analysis only investigated studies on

chewing herbivores; generalities about effects of sap-feeding insects cannot therefore be inferred due to the lack of literature on the subject. Future studies of this system will address resource quantity and quality to determine if these are significant factors in host plant selection by foragers.

In this study, the plant *Valeriana edulis* (Valerianaceae), ants (Hymenoptera: Formicidae), and aphids (Hemiptera: Aphididae) which utilize resources of *V. edulis* are viewed as a model system of food-forager interactions at the community level. The goal of this study is to contribute details to form a sketch of the community dynamics in this system. Since ants can have broad effects on an ecosystem, effects of resource availability on ants' foraging behavior can indirectly affect community structure. We addressed two questions with this study: (1) Does the availability of floral nectar affect tending of aphids by ants? and (2) Do the effects of floral nectar on ant-aphid interactions differ between male and female plants?

Methods

Study site

This study was conducted in an open meadow near the Rocky Mountain Biological Laboratory in Gothic, Gunnison County, Colorado (approximately 38° 57' 33" N, 106° 59' 21" W, 2895 m). The meadow is located adjacent to the Judd Falls parking lot south of CO County Road 317. The site has been recently utilized for studies involving this plant-insect system. The most common aphid-tending ant species observed on experimental plants were *Tapinoma sessile* Wheeler and *Formica spp.* (neonagates group); the aphid species has been described as *Aphis valerianae* Cowen (Petty and Mooney 2009).

Experimental design

To describe ant behavior related to resource selection, two experimental treatments were prescribed in a fully factorial design: access to nectaries (open or blocked) and plant sex (male or female). We measured ant visitation and behavior, aphid abundance, and the number of open flowers on each experimental flowering stalk as response variables.

Ten experimental blocks were manipulated for a total of 20 experimental plants (10 male, 10 female). Two flowering stalks of each plant each received one of the two nectary treatments. Bamboo stakes were placed in the soil next to the plants and metal wire was wrapped around the experimental stalks to prevent damage by deer.

To block ant access to nectaries, Aleene's OK to Wash-It fabric glue (Duncan Enterprises, Fresno, CA) was applied with a paintbrush to every open flower of exclusion stalks, glue used previously by Rudgers (2004). Five drops of yellow food coloring (McCormick, Hunt Valley, MD) were added to each bottle of glue to make it visible upon drying. A line of glue approximately three centimeters long was applied to the leaves subtending the inflorescences and to one side of the stalk on control plants. Between zero and four pairs of inflorescences were removed from each stalk to reduce the number of flowers to be observed and maintained over the study period. Open flowers on exclusion stalks were re-glued at the beginning of each day that observations were made.

Aphids were collected from the study site to establish experimental colonies on each flowering stalk used in the study. Between 15 and 20 aphids were originally transferred; following the first day, additional aphids were added if a colony of five or more aphids had not been established. Aphid abundance was periodically recorded. If a colony went extinct, 15 more aphids were added to the stalk.

Ant visitation to each plant was recorded over three weeks to determine an approximate rate of visitation. Snapshot counts of ant activity were taken one to four times per day during favorable weather conditions for ant foraging between 19 July 2011 and 8 August 2011, during the hours between 9:00 a.m. and 7:00 p.m. Counts were conducted at least one hour apart to avoid repeat counts of individual foraging ants.

Categories used to describe ant behavior include: scouting, tending, and collecting floral nectar. "Scouting" ants were observed walking on the stalk or inflorescence, but not appearing to be collecting nectar or attending to aphids. "Tending" ants appeared in proximity to aphids and were observed stroking aphids with their antennae or collecting honeydew. "Nectar" ants were observed collecting nectar from open flowers. Ant species and additional notes, such as the time of senescence, were also recorded.

Following the conclusion of data collection on ant visitation, the stalks of five experimental blocks were covered with breathable mesh fabric (to prevent predation and ant tending) to determine whether glue alone affected aphid performance. Initial colonies of 25 to 75 aphids per stalk were allowed to live beneath the bags for ten days; a final aphid count was recorded following this period.

Statistical analysis

The software program JMP (V9.0.2, SAS Institute, Cary, NC) was used to perform multi-factor ANOVA tests on ant visitation data. Aphid abundance, plant sex, experimental treatment, block number, and interactions between these were independent variables. Mean number of ants per stalk, ratio of ants tending aphids to ants not tending, and aphid abundance were dependent variables. The mean of all snapshot counts for each experimental stalk was used in analysis for each dependent variable. An unpaired t-test was performed online (QuickCalcs, GraphPad Software, 2002-2005) on data from the bagged experimental stalks to analyze the effect of glue on aphid performance using initial and final counts of aphids.

Results

Glue treatment

Repeatedly gluing open flowers throughout the experiment effectively reduced the number of open flowers on exclusion stalks. The average number of open flowers on female stalks was reduced from 258 for the control to 29 for the exclusion treatment; the average number of open flowers on male stalks was reduced from 98 for the control to 26 for the exclusion treatment.

Aphid abundance

Aphid abundance data were approximately normally distributed. Ratios of final aphid counts (mean = 173.4 ± 21.9) to initial aphid counts (mean = 49.4 ± 4.3) were compared for the nectary treatments, assuming no difference between the sexes. No significant effect of the glue treatment on aphid abundance was detected for the five blocks tested ($df = 18$, $t = 0.2358$, $p = 0.8163$). There was no effect of plant sex, treatment, or the interaction between sex and treatment on mean aphid abundance per flowering stalk (Fig 1; Table 1).

Ant visitation

Ant visitation data were right-skewed; a logarithmic transformation was performed to normalize the data for analysis. Ant visitation was 180% higher on female plants versus male plants, while the blocking of nectaries had no effect (Fig 2A; Table 2).

When this analysis was repeated using aphid abundance as a covariate, both aphid abundance and plant sex were significant factors. However, the interactions between aphid abundance and plant sex, and aphid abundance and treatment, were not significant (Table 3).

Tending ratios

Tending data were right-skewed; a logarithmic transformation was performed to normalize the data for analysis. The ratio of ants observed tending aphids versus not (i.e. scouting and collecting nectar) was not significantly affected by blocking the nectaries, but there was a suggestion ($df = 1,12$, $F = 3.8137$, $p = 0.0613$) for an effect of plant sex, with 13% higher tending ratios on male plants versus female plants (Fig 2B, Table 2).

When this analysis was repeated with aphid abundance as a covariate, tending behavior was significantly affected by both aphid abundance and plant sex, with tending ratios typically increasing with aphid abundance. As with ant visitation, the interactions between aphid abundance and plant sex, and aphid abundance and treatment, were not significant (Table 3).

Discussion

These results suggest that floral nectaries do not mediate ant-aphid interactions on *Valeriana edulis*. Excluding floral resources had no effect on aphid abundance, ant visitation, or the ratio of ants tending aphids to scouting or collecting nectar. However, aphid abundance had a significant effect on ant visitation and tending behavior. This suggests that the size of aphid colonies differentially recruit foraging ants.

Consistent with previous studies of this system, plant sex was an important factor in ant visitation, with more ants found on female plants. This suggests that females supply more attractive resources to foraging ants. Female plants were even more attractive with blocked nectaries, so the honeydew produced by aphids feeding on females seems more attractive than honeydew produced by aphids feeding on males. Studies of plant chemistry may reveal reduced herbivore defenses, enhanced nectar resources, or both, in female plants. Honeydew composition may also differ between the sexes. Subsequent studies should be conducted to determine if these factors are important in ant foraging behavior.

Previous work found that plant quality between the sexes of *V. edulis* did not significantly affect per capita growth of aphids; and, that aphid colonies occurred more frequently and were more often tended by ants on female plants (Perry and Mooney 2009). This study suggested a trend toward higher tending on male plants. Given this confounding evidence, the strength of plant sex in relation to aphid-tending behavior is still unclear.

While this experiment did not reveal any effect of floral nectar on ant-aphid interactions, it does deepen questions about differences in sex-based resources and herbivory. Becerra and Venable (1989) suggest that extra-floral nectaries may serve as a distraction for hemipteran-tending ants. Floral nectaries could plausibly serve the same purpose while concurrently attracting potential pollinators. This study denies the distraction hypothesis since the treatment effect of blocking floral nectaries did not affect ant visitation or tending rates. However, floral nectaries may have effects on insect-to-insect relations in other systems with different evolutionary histories.

Literature on sex-based herbivory mostly compares pairwise (plant-herbivore) interactions, missing complex indirect effects of sex-based insect such as ant-aphid interactions. This study demonstrates that plant sex is a major factor in ant tending behavior; looking at either plant-insect or insect-insect interactions separately too far simplifies the system. Both beneficial mutualisms and competition between natural enemies can have broad effects at the community level, so it is critical to integrate pairwise interactions into a more unified picture of complex plant-insect interactions in an ecosystem.

While floral resources may not influence insect interactions on *Valeriana edulis*, this study reveals that host plant selection by sap-feeding insects can have community-level consequences. Differences in plant traits can affect species interactions and therefore affect the structure of an ecosystem. Given the complexity of the cascading effects of food-forager relationships, multiple scales of investigation are required to better understand such ecosystem dynamics.

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Figures & Tables

Fig 1. Test for aphid performance between control and exclusion treatments. Glue repeatedly applied to open flowers on exclusion stalks; glue applied once on stem of control stalks. Aggregated means across blocks (N = 10) represent mean number of aphids recorded per experimental stalk. No effect was found between treatments.

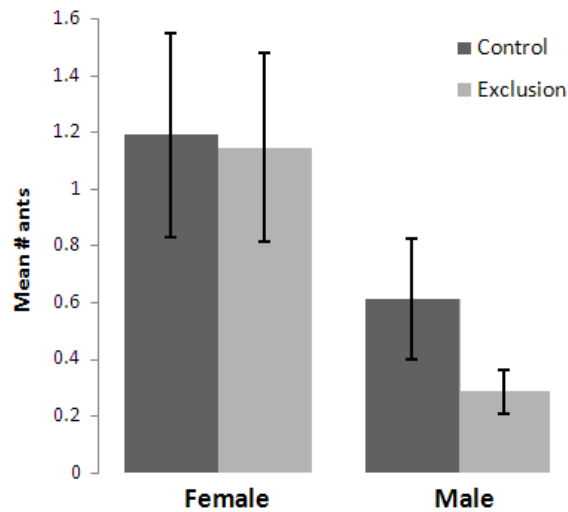


Fig 2. Ant visitation per flowering stalk by plant sex and treatment (A) and ratio of ants tending to ants not tending aphids (B). Control stalks had open access to nectaries; exclusion stalks had blocked nectaries. Aggregated means across blocks (N=10) represent mean number of ants recorded per experimental stalk per snapshot count. Plant sex was a significant factor for both ant visitation and tending behavior when controlling for aphid abundance.

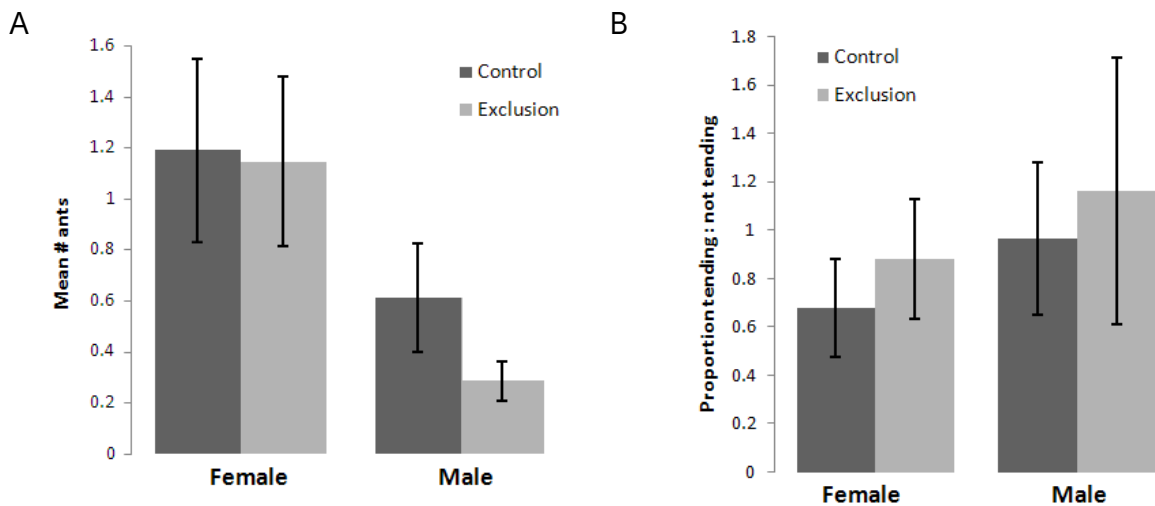


Table 1. Test for effect of plant sex, treatment, sex and treatment interaction, and block number on mean aphid abundance on each experimental flowering stalk. Data approximately normally distributed.

Mean aphid abundance			
Effect	DF	F	P
Plant sex	1,12	1.5847	0.2189
Treatment	1,12	0.0364	0.8500
Sex * Treatment	1,12	0.2587	0.6152
Block	9,12	1.5627	0.1770

Table 2. Test for effect of plant sex, treatment, sex and treatment interaction, and block number on mean ant visitation and tending ratios on each experimental flowering stalk. Dependent variables log-transformed. Significant values marked with an asterisk (*).

Ant total				Ratio ants tending : not tending		
Effect	DF	F	P	DF	F	P
Plant sex	1,12	19.6005	0.0001*	1,12	3.8137	0.0613
Treatment	1,12	0.0538	0.8183	1,12	0.2237	0.6401
Sex * Treatment	1,12	0.0077	0.9308	1,12	1.1074	0.3020
Block	9,12	5.4971	0.0003*	9,12	1.06409	0.4191

Table 3. Test for effect of mean aphid abundance, plant sex, treatment, and effect interactions on mean ant visitation and tending ratios on each experimental flowering stalk. Dependent variables log-transformed. Significant values marked with an asterisk (*).

Ant total				Ratio ants tending : not tending		
Effect	DF	F	P	DF	F	P
Aphid abundance	1,6	25.9887	<0.0001*	1,6	9.1778	0.0047*
Plant sex	1,6	7.2685	0.0110*	1,6	7.5284	0.0097*
Treatment	1,6	0.0119	0.9139	1,6	0.5505	0.4633
Sex * Treatment	1,6	0.2566	0.6158	1,6	2.1179	0.1550
Aphid * Sex	1,6	0.6056	0.4420	1,6	2.1740	0.1498
Aphid * Treatment	1,6	0.9842	0.3284	1,6	0.0070	0.9337