

VARIATION IN HOST PLANT SEX MEDIATES ANT-APHID INTERACTIONS

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Abstract

Sex-biased herbivory towards male plants has been shown to be common in many dioecious species. *Valeriana edulis* is a perennial, dioecious dicot that is the plant host to an ant-aphid mutualism. We investigated the interaction between ants and aphids to determine if it differs based on the sex of a plant. In order to address the question of whether variation in host plant sex mediates this mutualistic relationship, experiments, on host plant quality, ant recruitment, and the effects of predators on aphids, were conducted, in addition to collecting observational data. Results showed no difference in the host plant quality of *V. edulis* for aphid population growth. However, ant recruitment data suggested that ants tend aphids significantly more often on female *V. edulis* than on male plants ($p = 0.0513$). Predators reduced aphid abundance, but the strength of these effects was the same on male and female plants. In addition, our study of the natural distribution of aphid populations found them to be more common on female plants ($p = 0.025$). In contrast to past studies, we thus found female-biased herbivory that appears to be mediated not by host plant quality, but rather by variation in ant tending between male and female plants.

Introduction

Mutualisms are interactions that occur in nature between two species in which both species benefit from the relationship. However, there are both benefits and costs that are associated with every mutualistic relationship (Stadler and Dixon 2005). Typically the outcome of a mutualism is context dependent, which is affected by variations within the environment, both biotic and abiotic (Breton and Addicott 1992b; Billick et al. 2007). Because of this, interactions are not always mutualistic and the outcome of the relationship can range from beneficial (+,+; these two symbols indicating the sign of the effect for each species) to neutral (0,0) or antagonistic (+,-).

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Ant-aphid mutualisms have been well studied, but many aspects of this relationship are not fully understood yet. In this mutualistic relationship, aphids (Hemiptera: Aphidae) can be directly stimulated by ants (Hymenoptera: Formicidae) to produce honeydew, which is a sugary excretion derived from phloem sap that contains carbohydrates, protein and water (Way 1963). This process is known as tending, which is usually defined as ants collecting honeydew in exchange for the protection from predators and not preying upon aphids themselves (Way 1963). Depending upon the ecological setting of the interaction, ants can have either positive or negative effects upon the aphids. Ants may treat the aphids in a variety of ways, which include tending (+), ignoring (0), and preying upon them (-) (Billick et al. 2007). There are several factors that could cause ant-aphid interactions to vary from a mutualism to a neutral relationship or parasitism. Some of these factors include plant genotype, host plant quality, ant recruitment, and predator recruitment, each of which is described below. It is important to note that these three factors may act independently of one another or they may all be inter-related to cause a shift in the mutualistic relationship.

One major factor that can influence this relationship shift is plant genotype. Variation in plant genotype is increasingly being recognized to be an important factor for structuring arthropod communities and host plant fitness (Crutsinger et al. 2006; Crutsinger et al. 2007; Mooney and Agrawal 2008; Johnson and Agrawal 2005; Whitham et al. 2006). Genetic variation in host plants has been shown to affect community dynamics, greatly influencing species interactions as well as the abundance, diversity, and distribution of herbivore populations (Johnson 2008; Johnson and Agrawal 2005). The direct and indirect effects on herbivore populations, which include population dynamics, degree of host plant resistance, predator occurrence, and host plant resource quality, can be governed by this variation in plant genotype,

influencing the preference, performance, and survival of herbivores (Johnson 2008; Maddox and Root 1990; Mooney and Agrawal 2008; Wimp and Whitham 2001). Because of these possible effects, variation in plant genotype may have profound implications for mutualistic relationships, specifically ant-aphid interactions (Johnson 2008).

In previous research, genetic variation in host plants has been shown to mediate ant-aphid interactions. Mooney and Agrawal (2008) found that ant-aphid interactions were either mutualistic or antagonistic depending on the genetic family of milkweed (*Asclepias syriaca*). In other words, the genotype of the plant affected whether the relationship was positive or negative (Mooney and Agrawal 2008). Studies have suggested that plant height in fireweed (*Epilobium angustifolium*) may influence different aspects of aphid ecology, including intraspecific competition, ant recruitment, and predator impacts (Breton and Addicott 1992a). Plant genotype of the common evening primrose (*Oenothera biennis*) was found to strongly effect aphid population growth, ant abundance, and generalist predator diversity (Johnson 2008).

Depending upon the presence of ants and the species of aphid, host plant quality has been found to affect aphid abundance, which may be due to the composition of the phloem sap upon which the aphids feed (Mooney and Agrawal 2008). Host plant quality may be one of the most influential factors in host selection for aphids (Wimp and Whitham 2001). Because the composition of phloem sap could potentially determine honeydew quality, host plant quality was found to determine whether the ant-aphid interaction was mutualistic (+,+) or parasitic (+,-). Ant recruitment and tending greatly relies on the quality and quantity of the resources produced by aphids (Mooney and Agrawal 2008).

The main question we were interested in is how variation within plant genotypes mediates ant-aphid interactions. More specifically, does the mutualistic relationship between

ants and aphids differ based on the sex of a plant. Previous research has documented sex-biased herbivory toward male plants in dioecious species, suggestive of sex-biased variation in host plant quality. However, sap-feeding hemipterans have not been studied in this regard (Cornelissen and Stiling 2005), and the implications for sex-biased variation in plant quality for ant-hemipteran interactions are similarly unclear. This experiment addressed three specific questions: (1) Does host plant quality differ between male and female plants of the same species, causing aphids to perform better on one sex versus the other? (2) Does ant recruitment differ within a dioecious system, causing aphids to be tended more often on one sex versus the other? (3) Are aphids more susceptible to predators on male or female plants of the same species? In addressing these questions, these studies provide a mechanistic understanding for how plant sex may influence ant-aphid interactions.

Methods

Study System

This study was conducted in a meadow located in the Gunnison National Forest, Gunnison County, Colorado, USA approximately one kilometer north of the Rocky Mountain Biological Laboratory (RMBL). Valerian (*Valeriana edulis*) is a perennial, polygamo-dioecious dicot in the family Valerianaceae that occurs in montane and subalpine meadows. The identity of the aphid species occurring on Valeriana is likely to be *Aphis valerianae* (Cowen) (Palmer 1952), but will be sent to an expert to identify. The *V. edulis* ant-aphid system has been previously studied by an REU student here at the Rocky Mountain Biological Laboratory. This work showed that the *V. edulis* ant-aphid system did not effect resource allocation within the plant and thus, the presence of ants and/or aphids did not alter plant growth or reproduction (Dupuy, *unpublished manuscript*). Natural ant colonies occurring in the field where this study

was conducted were used for this experiment. The most common aphid-tending ant species were *Tapinoma sessile* (Say) and *Formica podzolica* (Francoeur). Commonly found in disturbed or urban areas, *T. sessile* is a small black ant in the subfamily Dolichoderinae that builds nests under rocks. This ant is a scavenger that often abandons current nests and relocates weekly. *Formica podzolica* is an omnivorous, mound-building ant that often has polygynous colonies. Both ants *T. sessile* and *F. podzolica* are common throughout the area around the Rocky Mountain Biological Laboratory.

Observational Study

The occurrence of aphids and ants on *Valeriana edulis* was observed in a natural setting to determine if colonization and recruitment differed between plant sexes. In the summer of 2009, a total of 97 *V. edulis* plants were tagged and observed throughout the month of July. Several traits were documented for each plant, including sex, height, the number of stems, inflorescence volume, the number of aphids present, the number of mummies present, the ant species and number of ants found on the plant, the number of caterpillars present, and the number of stems consumed by deer on July. Observational data was collected for these plants three times: July 1-7, July 9, and July 15-16.

Experiment #1: Host Plant Quality

In order to test if host plant quality differed between plant sexes, groups of *V. edulis* plants (n=60; 30 female and 30 male) were enclosed using mesh material and twist-ties with varying numbers of initial aphids both adult and late instar nymphs (1-15 aphids). These aphids were collected from the field site and manually introduced to aphid-free *V. edulis* inflorescences with a paintbrush to test for and compare the population growth rate and the rate of intraspecific competition in aphid colonies feeding on male and female *V. edulis*. All aphids were counted on

July 10, July 17, and July 22. This experiment allowed for a simple test of host plant quality between male and female *V. edulis*. The enclosed aphid populations were protected from predators as well as the effects of ant tending in order to compare population growth between plant sexes in the absence of other interactions, and whether the degree of density dependence to population growth varied as well.

Experiment #2: Ant Recruitment

To test if ant recruitment differed between male and female *V. edulis*, slits were cut into the mesh enclosures (n=60; 30 male and 30 female) previously mentioned after 17 days to allow for ant and predator recruitment to the aphid colonies. These mesh enclosures were left on the plants to act as a deterrent to deer. The occurrence of ants and predators were recorded for all plants (n=60) on several dates in July (7-8, 10, 17, 22, 24, 27, 30) and on August 3 between the hours of 9:00-11:30am and 4:00-5:30pm along with the continued monitoring of aphid populations to compare ant abundance between male and female *V. edulis*.

Experiment #3: Predator Exclusion

In order to test for differences in predator recruitment between plant sexes, a predator exclusion experiment was conducted using *V. edulis* plants (n=30; 15 male and 15 female). Two stems were selected randomly from each plant to function as a control and an experimental stem. A total of 10 aphids were collected from the site and manually introduced to each stem with a paintbrush. The control stems were covered and sealed with mesh enclosures. Slits were cut into the 15 mesh enclosures over the experimental stems to allow for predator access. However, experimental stem enclosures were left uncut for approximately one day after aphids were introduced to ensure their colonization. Tanglefoot (Grand Rapids, MI) was applied to the base

of both the control and experimental stems to exclude ants. Aphid and predator counts were conducted on August 3, 5, 10, and 13.

Results

Observational Study

Observational data collected on the natural distribution of ants and aphids showed female sex-biased herbivory in *Valeriana edulis*. Aphid colonies were found significantly ($\chi^2 = 5.75$; $p = 0.025$) more often on female *V. edulis* than on males (Fig. 1 a). There was no significant difference ($\chi^2 = 0.276316$; $p = 0.5991$) between the occurrence of tending ants on female and male *Valeriana edulis* (Fig 1 b). However, it is important to note, only two male plants surveyed were found to have aphids in these surveys of unmanipulated plants. Of those two male plants, only one aphid colony had ants tending, which results in a 1:1 ratio of tending ants (Fig. 1 b).

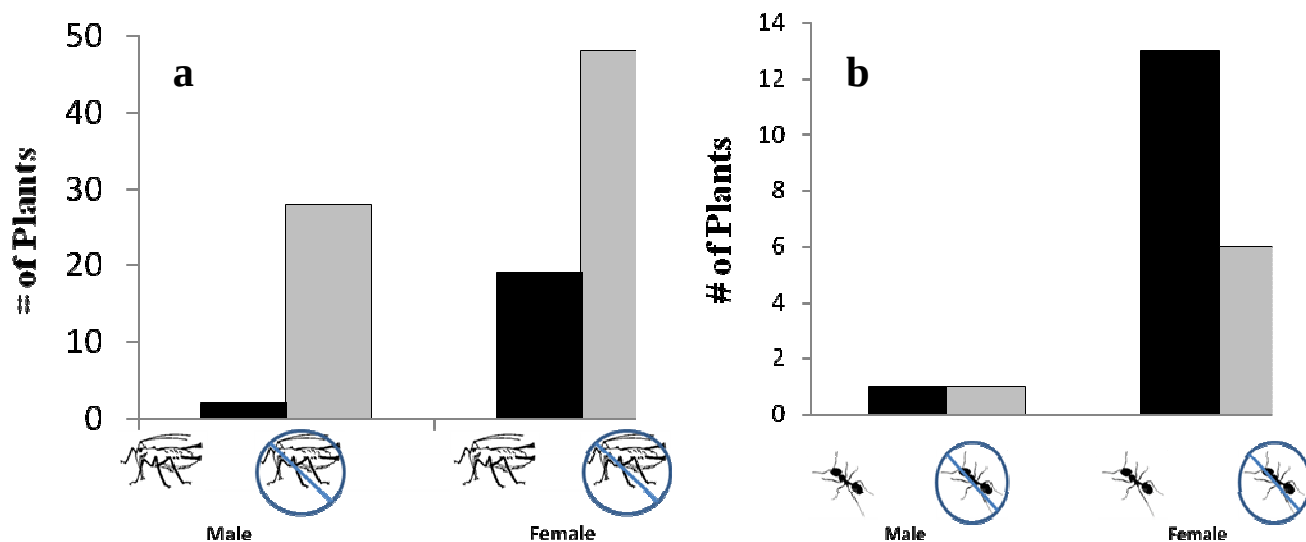


Figure 1. Natural Distributions of Aphids and Ants on *Valeriana edulis*. Observational data for the natural distribution of aphids (a) and ants (b) was collected for *Valeriana edulis* (n=97) on July 1-7, July 9, and July 15-16. The aphid occurrence significantly differed by plant sex (a), but the rate of ant-tending did not (b). Only the plants with aphid colonies are shown in Fig 1. b.

Experiment #1: Host Plant Quality

Host plant quality was found to not differ between male and female *Valeriana edulis*. The average per capita growth of aphids was calculated for both male and female plants (Fig 2). There was no significant difference between the growth of aphids on either male or female plants ($p = 0.5345$). There was also no effect of initial aphid population size on per capita growth (analyses not shown).

Experiment #2: Ant Recruitment

Ant tending was significantly more common on female *Valeriana edulis* than on males (Fig. 3). Aphid colonies occurring on female plants were found to be tended by ants more often than the aphid colonies on male plants ($\chi^2 = 3.8$; $p = 0.0513$). When exposed to predators and ants, aphid colony survival was not found to differ between male and female *V. edulis* ($\chi^2 = 0.36$; $p = 0.55$).



Figure 2. Host Plant Quality of Male and Female *Valeriana edulis*. Host plant quality was found to not be significantly different between male and female *Valeriana edulis* because the per capita growth of aphids did not differ between sexes. This figure shows the average number of nymphs produced per aphid on 60 total plants (30 male and 30 female).



Figure 3. Ant Recruitment for Male and Female *Valeriana edulis*. Ants were found to tend aphids on female *Valeriana edulis* significantly more often than on males. This figure shows the number of colonies (out of 60 plants; 30 male and 30 female) tended for male and female *V. edulis*.

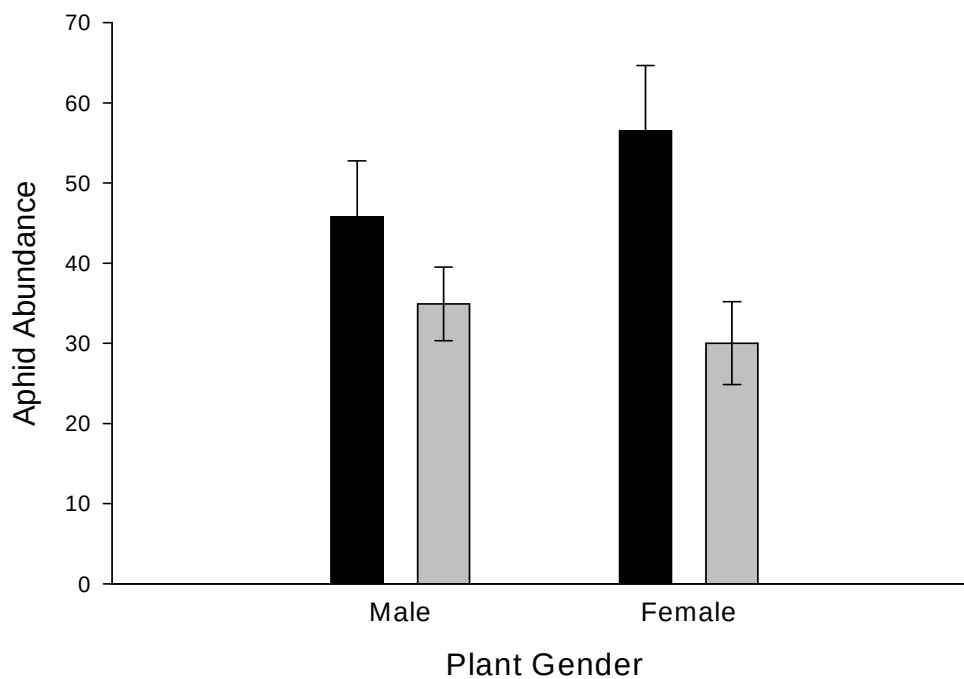


Figure 4. Predator Effects for Male and Female *Valeriana edulis*. In the presence and absence of predators, plant sex did not significantly affect aphid abundance. This figure shows the average number of aphids on male and female *Valeriana edulis* (n=30; 15 male and 15 female) when exposed and protected from predators. Aphid counts were conducted on August 3, 5, 10, and 13.

Experiment #3: Predator Exclusion

Differences in the effects of predators based on plant sex were determined by aphid abundance (Fig 4). Predators were found to have a significant effect on aphid abundance ($p=0.005$), but this effect was similar for aphid populations on both male and female plants as indicated by the fact that there was no interaction for plant sex and predator effects ($p=0.2247$). In addition, when comparing male and female *V. edulis*, no significant difference was found for aphid abundance ($p=0.6475$). In other words, there was no effect of plant sex.

Discussion

These data suggest that the ant-aphid mutualism occurring on *Valeriana edulis* does indeed differ depending upon host plant sex. The natural distribution of aphids shows that this herbivore seems to occur on female plants more often than male plants. When aphid behavior was controlled by manually introducing aphids to both male and female plants, ant recruitment to aphid colonies was found to differ based on the sex of *V. edulis*. The occurrence of ant-tending was significantly greater for aphid colonies on female plants.

Although predators reduced aphid abundance, their effect was found to not differ between male and female *Valeriana edulis*. Aphid colony survival did not differ between male and female plants when exposed to ants and predators despite the fact that ants tended significantly more on female plants. This result suggests ants do not benefit aphids in the form of predator protection. This result may be true for this system or possibly this time of the season. When aphids first disperse and colonize *V. edulis*, ants may benefit aphids by protecting them from predators at this time, but this positive effect may be density-dependent.

Host plant quality was not found to differ between male and female *V. edulis* as aphid population growth was consistent between plant sexes. Although host plant quality did not effect

aphid growth, it is possible that the phloem sap may still be mediating the ant-aphid mutualism. One hypothesis is that the phloem composition may differ between male and female *V. edulis* in the form of secondary metabolites or other compounds, resulting in the subsequent honeydew produced to be similar in composition to the plant phloem. This difference in honeydew composition may explain the difference in ant recruitment shown between male and female plants. Ants may recruit more often to aphids on female plants because they prefer the honeydew produced from the female phloem sap.

Another hypothesis as to why ants would recruit more to aphids colonizing female plants is that the flower nectar composition and/or volume may differ between male and female plants. It is possible that the sugar concentrations within the nectar differ between male and female *V. edulis*, causing ants to recruit to the plant with the sugar content they most prefer, which in this case would be the female plant. Differences in nectar volume produced by male and female plants may also cause ants to recruit to flowers with greater amounts of nectar or, in other words, a greater food source. Ants were often seen drinking the nectar from female flowers, but they were rarely seen at male flowers. Ants may recruit to female plants because they prefer the resources available there, such as better tasting honeydew, greater quantities of nectar, or higher sugar concentrations within the nectar. Aphids may then colonize female plants more often because of the greater probability of being tended by ants.

Our results show that sex-biased herbivory occurs towards female *Valeriana edulis* within this ant-aphid system. This female-biased herbivory differs from the male-biased herbivory trend from previous research on this topic. A meta-analysis by Cornelissen and Stiling (2005) suggests sex-biased herbivory occurs in dioecious plants, but towards male plants. However, this review article only included herbivore guilds such as gall-makers, folivores,

browsers, flower predators, miners/borers, and pathogens. Sap-feeding insects were not included in this meta-analysis and may not follow this trend (Cornelissen and Stiling 2005).

This study suggests that host plant sex of *Valeriana edulis* does indeed mediate ant-aphid interactions by influencing the behaviors of the aphids and the ants. In these other studies, host plant quality was most likely the driving force in sex-biased herbivory, and our results suggest that in ant-tended hemipterans herbivores more complex dynamics can lead to sex-biased herbivory in the absence of variation in host plant quality. It appears that aphids naturally choose to colonize female plants more often in comparison to male plants. These choices (a) are not due to variation in host plant quality or (b) the effects of predators. It may be that aphids choose to colonize plants where they are tended because ants appear to recruit to and tend aphids colonizing female *V. edulis* more often than males.

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