Genetic basis of plant-insect interactions: insect recruitment to *Boechera stricta*

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Abstract

Mutualistic interactions are ubiquitous in nature. These type of interactions generally provide benefits to interacting species. Insects and plants are commonly involved in diverse forms of mutualism, however little is known if genetic variation among plants allows for a positive symbiosis with arthropods. In this study, we investigated whether plant traits in *Boechera stricta* (Brassicaceae) play a role in arthropod recruitment, and if so, whether the genetic basis of the plant contribute to this interaction. We also addressed whether visitation by ants and other insects minimized herbivory. We established a common garden experiment in which insect visitation to *B. stricta* was observed. We assessed insect visitation and herbivory on >400 known genotypes, as well as other plant traits. Within plant characteristics (number of flowers and fruits), plant height had a predominantly positive effect on the abundance of arthropods. Overall, plant height led to higher insect visitation, but the abundance of ants and other insects had no effect on herbivory due low insect observations and insufficient statistical power. Furthermore, we found a significance in the genetic basis of ant recruitment. These results indicate ant visitation can be influenced by plant traits, yet further studies are needed to determine what specific genes underlie in these traits.

Introduction

Numerous organisms engage in symbiotic interactions, ranging from those which are deleterious to those which are beneficial. If both organisms benefit from the association, then it is known as a mutualism (Jackson 2008). The highly diversified and abundant forms of mutualisms play a vital part in structuring ecosystems. Protection mutualisms, for example, commonly occur among ants and extrafloral nectar-secreting plants. This form of mutualism is a defined relationship in which an organism offers resource rewards to a protector species in return for removal of natural enemies from the host species (Bronstein and Barbosa 2002).

Common mutualistic insect-plant interaction includes four main mutualisms in which insects (1) protect plants from herbivores, (2) provide plants necessary nutrients, (3) disperse seeds and fruit, and/or (4) pollinate (Blatrix 2009). Services by mutualistic partners are provided in exchange for food rewards or nesting sites provided by the plants (Blatrix 2009). However, the net costs and benefits to plants of engaging in the interaction with insects can rely on factors such as the identity of the colonizing arthropods, which can vary in their effectiveness of protection (Feldhaar 2003). If the costs are much more substantial than the benefits for either individual, then this can result in the breakdown of the mutualism, with one species becoming parasitic (Sachs 2006). A major gap in our knowledge in association with plant-insect symbiosis is whether arthropod services positively affect plant growth, survivorship, and plant fitness or if this type of interaction is solely favorable to one species.

Nectar-secreting organs known as extra-floral nectaries (EFNs) occur in over 2,000 plant species. EFN glands are generally located on either leaf laminae, petioles, rachis, bracts, stipules, pedicels, or fruit **(Fig. 1)** (Bentley 1977). The constitution of the gland secretions is primarily mono- and disaccharides (fructose, glucose and sucrose) and amino acids (Koptur 1994). Reports have also shown the presence of fatty acids and phospholipids in a few species (Stone et al. 1985). Content, flow patterns, and location of EFNs vary amongst different taxa, and may contribute to frequency and abundance of visitation by ants and other arthropods (Pemberton and Lee 1996).

EFNs may function as a form of plant defense against herbivory by means of recruitment of ants, parasitoids, and other predatory insects (Heil 2008). The provided protection is exchanged for nutritional or resource rewards supplied by the plants (Bronsted 2006). Nectaries, however, can also be susceptible to exploitation by arthropods, including herbivores that feed on the plants (Heil et al. 2004). For instance, various studies failed to find protective effects of EFN-consuming ants (Freitas et al. 2000, Mackay & Whalen 1998, O'Dowd & Catchpole 1983, Rashbrook et al.1992, Tempel 1983). This can be largely due to factors such as ant abundance, ant species, and competition between other arthropods and ants for the extrafloral nectar (Heil et al 2003, O' Dowd 1979).

Although many protection mutualisms are well studied, the genetic basis of traits that are essential to this interaction have received little examination. Connecting a plant's genetic makeup to its ability to form a positive symbiosis is the next step towards understanding whether and how the genetic basis of plants shapes ecological variation in mutualisms within the flora.

In this study, we addressed (1) whether there is a relationship between plant traits and the ability to recruit ants and other insects (2) whether arthropod visitation reduces herbivory (3) if the genetic basis of *B. stricta* contributes to visitation by arthropods.

Methods

Study Site and Species

Our study was conducted in the Mitchell-Olds lab's experimental garden in the vicinity of Rocky Mountain Biological Laboratory in Gunnison County, Colorado, USA. The site is directly located in Schofield Park approximately 8 miles north of Gothic, CO **(Supplementary fig. 1)**.

The species examined included *Boechera stricta*, the ant species *Formicinae fusca,* and other arthropods that interacted with *B. stricta*. *Boechera stricta* (Brassicaceae, previously Arabis drummondii) is a perennial forb found across a broad geographic distribution in western North America (Rushworth et al 2011). *B. stricta* is predominantly diploid, asexual, and highly self-

fertilizing (Dobeš et al. 2004). It is one of the most morphologically and molecularly well-defined *Boechera* species (Rushworth et al. 2011). *Boechera* species' inbreeding model system is ideal for experimental use (Rushworth et al. 2011). Because of the high recombination rate per kilobase, it is possible to map key polymorphisms controlling traits of ecological and evolutionary interest (Song et al. 2009, Rushworth et al. 2011).This set system can assist in differentiating between environmental and genetic factors that may play a key role in determining arthropod recruitment.

In this study, we utilized a panel of *B. stricta* genotypes that the Mitchell-Olds lab group is using to conduct genome-wide association studies (GWAS). This technique allows for the discovery of single-nucleotide polymorphisms (SNPs) that are correlated with phenotypes of interest (Rushworth et al. 2011), as well as broader analyses regarding the genetic variation underlying traits.

Formica fusca (Formicidae: Formicinae) was the only abundant ant at our study site. *F. fusca* regularly partakes in extensive ranges of mutualistic interactions with several different organisms including plants, insects, and fungi (Holldobler and Wilson 1990). A classic example is that of the ant-homopterans relationship in which ants receive continual food sources while homopterans acquire protection from predation or parasitism, transportation, maintenance of host plant quality, and heightened survival (Way 1963, Burns 1973, Wood 1977). Additionally, *F. fusca* is known to have a mutualistic relationship with *Solidago altissima* (Canada goldenrod). The beetles commonly attracted to goldenrod are deterred by the *Formica* ants, which have shown to experience significantly less defoliation on stems with *F. fusca* than those without ants (Messina 1981).

Experimental Design

In this study we used fully sequenced accessions of *B. stricta* genotypes originating from seeds collected from populations across the species range. Since *Boechera* experience selection by herbivores in natural populations, the common garden experimental design is setup to allow native herbivores (and ants) to freely access the plants. In 2015, the Mitchell-Olds lab planted a common garden at the Schofield site consisting of 7 randomized complete blocks each containing a single individual of 347 genotypes into the Schofield common garden (total N = 2,877 plants). Each block is in turn divided into sub-blocks of 50 plants. In 2016, the same design was repeated with 411 genotypes.

Visitation by ants and other arthropods

To evaluate arthropod visitation we observed each sub-block for 2 minutes, every other day, from July 14 to July 27 and recorded the plant ID of individual plants in which ants or others insect were seen at any part of the plant.

Covariates

To test if plant attributes contribute to insect visitation, we worked with the Mitchell-Olds lab group to determine plant height, number of flowers and number of fruits for each plant in the two GWAS cohorts. We also scored all plants for herbivore damage by visually estimating leaf area removal.

Data Analysis

We tested whether ant abundance and other arthropod abundance were dependent upon (1) plant height and (2) the number of flowers and fruits (summed together). This analysis was performed as a mixed model ANOVA with block included as a random effect. Next, we tested whether there was genetic variation in ant abundance and other arthropod abundance. This analysis was also performed as a mixed model ANOVA with both genotype and block as random effects. Finally, we tested whether leaf damage was dependant upon the abundance of ants and other arthropods. This analyses was also performed as a mixed model ANOVA with block as a random effect. All analysis were performed using JMP V. 13 (SAS Institute 2016).

Results

Final plant census for the 2016 GWAS cohort are ongoing, so we were only able to analyze results for the 2015 cohort of plants at this time. Visitation rates of ants (*P = 0.01; N = 1132*; **Fig. 2**) and other arthropods (*P < 0.0001; N= 1140*; **Fig. 3**) were positively affected by plant height, but neither ants (*P = 0.94; N = 1132*) nor other arthropods (*P = 0.76; N= 1140*) were affected by the number flowers and fruits per plant. Leaf damage was unaffected by ant abundance (*P = 0.82; N = 1142*) or other arthropod abundance (*P = 0.86; N = 1142*). There was significant genetic variation in ant abundance (*P = 0.02; N = 2329*), but not for other arthropods (*P = 0.83; N = 2350*). Genetic variation in ant abundance had a broad-sense heritability of H = 2.93% and genotype means for ant abundance ranged from a low of 0.004 ants per plant to a high of 0.079 ants per plant.

Discussion

Plant traits and insect recruitment:

We found that both ant and arthropod abundance was affected by plant traits, with height proving to be the best predictor of insect visitation. We tested the effect of flowers + fruits on visitation as indicators of EFN availability to determine if nectar was one of the primary drivers of insect visitation.

The positive relationship between arthropods, including ants, and plant height may be due to the fact that taller plants are highly conspicuous. Large plants could be particularly attractive to organisms as they may offer a greater quantity and variety of resources (Feeny 1976). In turn,this

enhances number and size of populations of certain herbivorous species, thereby offering a greater range of partner interactions.

Testing for associational defense:
We predicted that ant abundance would be high with herbivore damage because *B. stricta* is a potential source of nutrient resources for ants and other arthropod abundance would be low with herbivore damage because ants were anticipated to partake in a mutualistic relationship with *B. stricta*. However, neither ant abundance nor arthropod abundance affected leaf damage. There are several possible explanations for why we observed no relationship between ant visitation and herbivory on *B. stricta*. First, this may be due to an inadequate abundance of mutualistic partners. One potential factor that could account for a lower population size is elevation. Since composition of plant and animal communities change with altitude, partner availability may be altered. The reduction of partner availability at higher elevations can result in a less specialized mutualistic network, meaning that even for ant-inhabited plants, benefits can be diminished. Second, observations of potential mutualistic partners was low. While the plant sample size was significant, we did not observe a notable amount of insects on *B. stricta.* Therefore, all ants and other arthropods were summed together for analysis. This can imply that a mutualistic relationship may exist between certain insects and *B. stricta*. Or in contrary, there may not be a true biological effect but limiting factors in this study did not fully support either.

The genetics of ant-plant interactions:
While there was no apparent protective benefit of ant visitation for plant defense, we found significant heritable genetic variation for ant recruitment.

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Tables and Figures

Figure 1. Extrafloral nectaries (EFNs) in *Boechera stricta*. Photographs, K. Mooney.

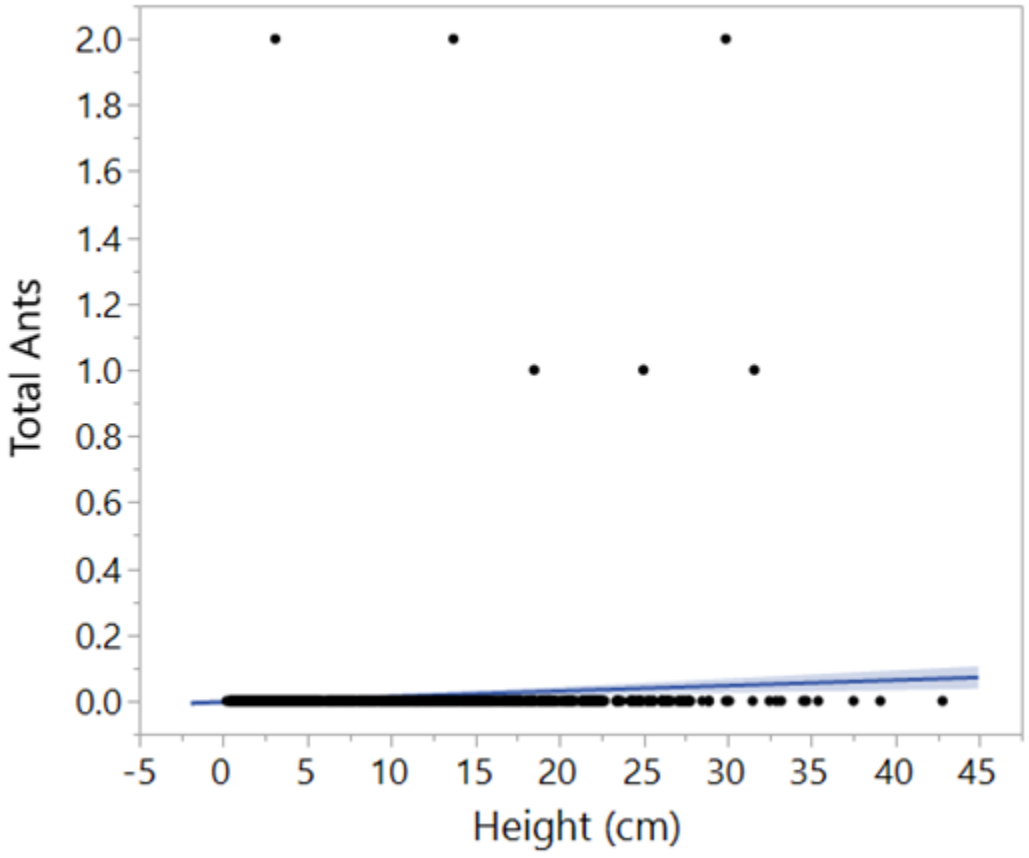

Figure 2. The main plant trait we observed to drive ant visitation was height. Ant abundance was positively correlated with plant height.

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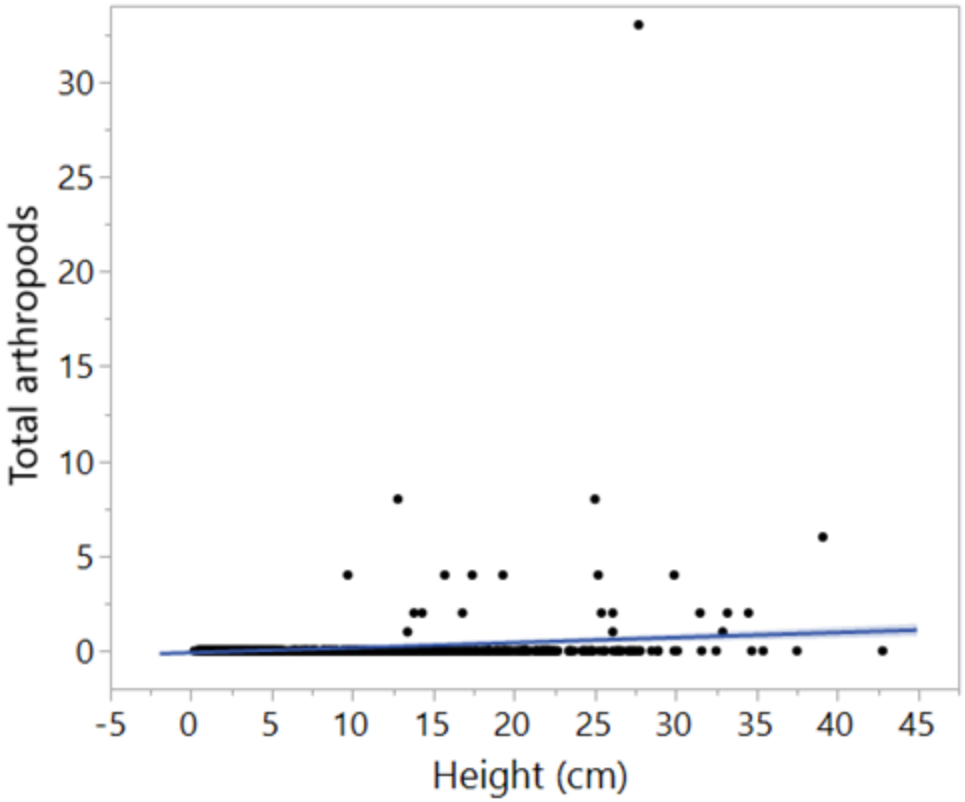


Figure 3. The main plant trait we observed to drive arthropod visitation was height. Arthropod abundance was positively correlated with plant height.

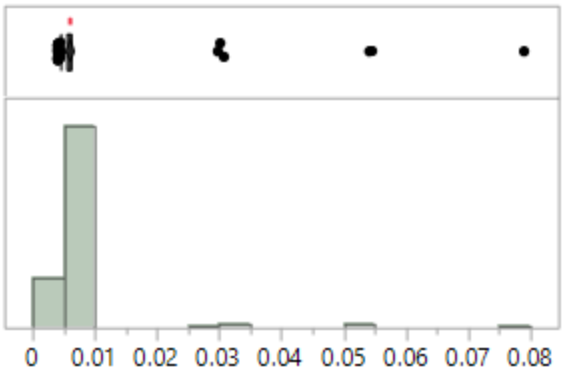
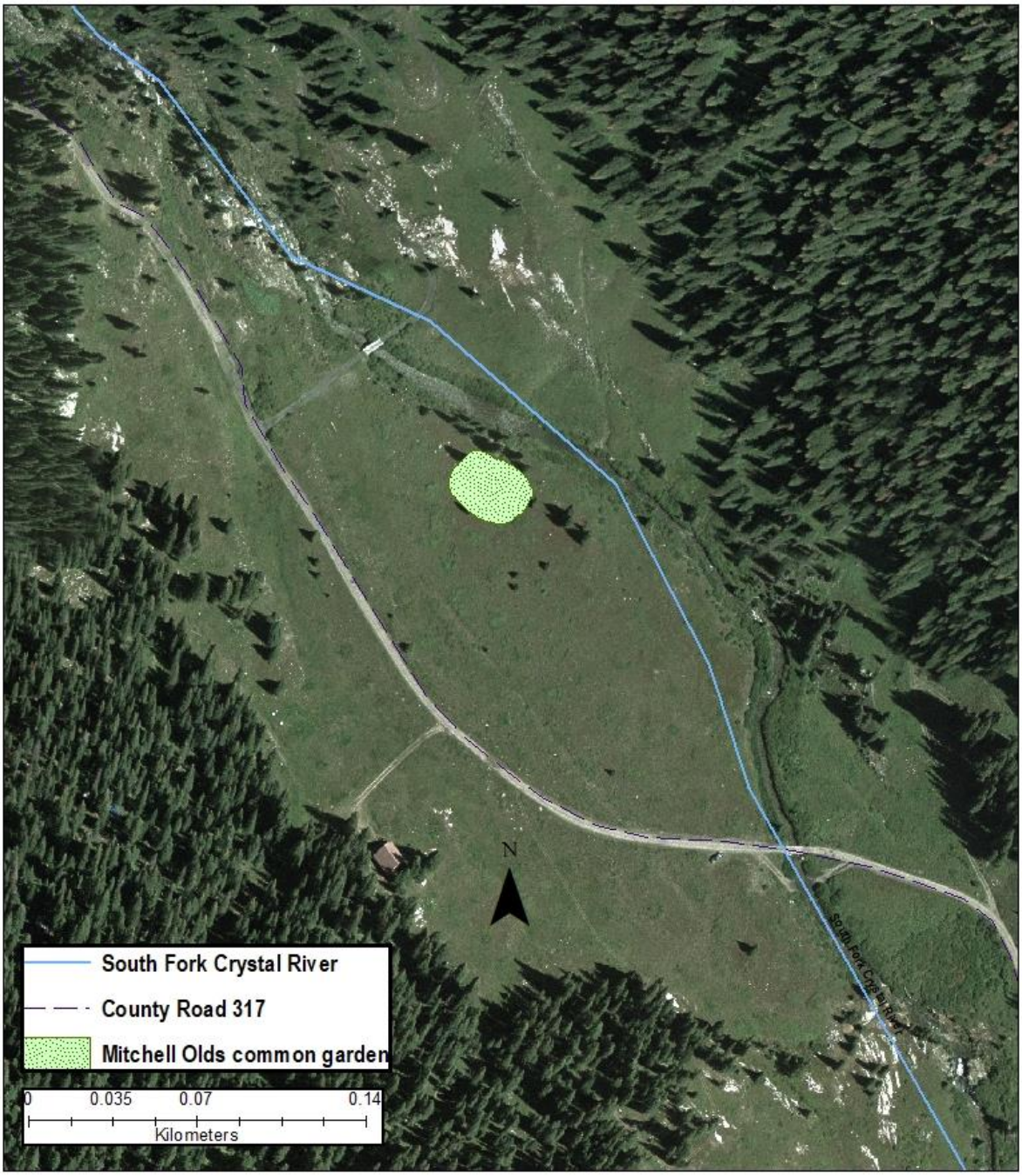


Figure 4. Frequencies of genotypic averages.



Supplementary figure 1. Mitchell-Olds lab's experimental garden. Located in Schofield Park approximately 8 miles north of Gothic, CO.