

Investigating Herbivore Relationships to *Boechera stricta* in a Climate Change
Context

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Abstract

Boechera stricta is a mustard that grows naturally throughout a wide range of altitudes in the Colorado Rocky Mountains. *B. stricta* has a variety of natural herbivores, which makes it an interesting system in which to study how plant-herbivore relationships vary over an elevational gradient. Through this study we begin to characterize the relationships between *Boechera stricta* and its surrounding insect herbivore community. We sampled the insect community surrounding *B. stricta* in a collection of quadrats, and analyzed how the diversity and abundance of these insects varied across an elevational gradient. We analyzed the insect community as a whole, and conducted with more pointed analyses focusing on insects in taxonomical groupings known to herbivore plants. Other variables affecting insect diversity were also taken into account such as the density of plant cover at each site. We found that insect diversity decreases as elevation increases, and that insect diversity increases as plant cover density increases as well. This study has laid important groundwork for future studies on herbivore-*B. stricta* relationships in the context of climate change.

Introduction

Plant traits vary with altitude because of biotic and abiotic differences in the environment, including climate and rates of herbivory (Rassman et al., 2014). Plants at higher elevations often develop rapidly to complete reproductive cycles in short growing seasons (Anderson and Gezon, 2015). Traits that enable rapid development in higher elevation environments, such as high foliar N and thin leaves (high specific leaf area), likely make these plants more susceptible to herbivory (Daws and Anderson, in preparation). In the mustard *Boechera stricta* (Brassicaceae), vulnerability to herbivory increases with source elevation of maternal families when planted into 5 common gardens, suggesting a tradeoff between rapid development and defense against herbivory (Anderson et al., 2015). These defenses include trichomes, small leaf hairs that provide physical protection against herbivores, and glucosinolates, chemicals produced by plants to help deter consumption by herbivorous insects (Mauricio and Rausher, 1997). *B. stricta* genotypes from higher elevations have lower trichome densities than low elevation genotypes when exposed to common garden environments (Anderson and Soto, unpubl.).

We predict that herbivory decreases with elevation, matching the levels of herbivory resistance *Boechera stricta* exhibits at each elevation (Anderson et al., 2015). Insects are ectotherms and are sensitive to extreme environmental conditions, making it difficult for them to live at higher elevations where the climate is cold and the growing season is short (Bale et al., 2002). Climate change could disrupt plant-insect relationships (Rassman et al., 2014). As the climate warms, insect herbivore populations could move upslope and begin to consume plants at higher elevations that lack herbivore defense traits. To predict how herbivore-plant relationships are affected in the context of climate change, we first had to investigate how insect communities varied with elevation. We quantified changes in insect herbivore diversity and abundance in relation to *B. stricta* over an elevational gradient, and produced preliminary data to model changing plant-herbivore interactions in relation to climate change.

We tested the following prediction:

- (1) Species richness and abundance of insects decrease as elevation increases.

Methods

Study System

Boechea stricta is a small perennial forb in the Brassicaceae family. It is native to North America and grows naturally throughout different altitudes in the Colorado Rocky Mountains (Anderson et al., 2012). *Boechea stricta*'s broad habitat makes it an excellent system in which to study how plant-insect interactions vary with elevation. *B. stricta* has a variety of natural herbivores that feed on its leaves and stems, as evidenced by different patterns of damage (Anderson and Mitchell-Olds, personal communication). Insects found on *B. stricta* include members of Coleoptera, Homoptera, Hymenoptera, Diptera, Lepidoptera, Hemiptera, and others (Anderson and Burns, unpubl.), although species- and genus-level identifications were rare prior to the current study.

Study Sites

A total of five locations were surveyed in this study in the area surrounding the Rocky Mountain Biological Laboratory in Gothic, CO (38°57' N, 106°59' W). The five experimental gardens are each located at a different elevation that spans a gradient of 2540-3430m. The sites are Estess Ranch (2540 m elevation), Peanut Mine (2710m), Gothic (2890m), Schofield (3133m) and North Pole (3430m) (see appendix 1 for a map). Within each location we laid five one meter squared quadrats within natural populations of *B. stricta*, five sites within Anderson's experimental gardens, and five control sites containing no *B. stricta* plants. At the lowest elevation garden (Estess), *B. stricta* individuals are not abundant because there were no adjacent natural populations *B. stricta*; therefore, we did not sample natural populations in that location. We sampled a total of 70 quadrats. The control quadrats and quadrats containing natural *B. stricta* were all directly adjacent to each experimental garden. The variation in quadrat type controlled for any differences in and out of the experimental gardens, and will eventually allow us to determine if there are insects that specialize on *B. stricta* by comparing the insect communities of plots with and without *B. stricta* plants. The GPS coordinates of each quadrat were recorded to be mapped and to mark quadrat locations for future use. We chose sites specifically to capture a diversity of herbivore communities at a wide range of elevations throughout our study area, which enabled us to do regression-based analyses of the collected data.

Data Collection

Within each quadrat, we used a vacuum aspirator to collect the insect community surrounding *Boechea stricta*. To minimize insect escape after sampling and prevent samples from consuming or harming each other, we immediately transferred the insects collected from each quadrat to a kill jar made from a five gallon bucket, craft foam, and 100% acetone. We placed collected insects in plastic bags and stored them in a cooler on ice until they were taken back to the lab freezers to keep them fresh for analysis. Each quadrat was vacuumed only once, for one minute at maximum power in a uniform sweeping pattern. We determined our sample dates based on order of snowmelt, beginning at the lowest elevation site and moving upward. It

would have been ideal to sample at uniform lengths of time after snowmelt at each site, however that sampling scheme was not feasible this summer. Estess, Peanut Mine and Gothic sites twelve weeks past snowmelt, and the Schofield and North Pole sites six weeks past snowmelt. We only sampled insects if cloud-cover was less than fifty percent and if it had not rained in the preceding 12 hours. The vacuum aspirated did not damage plants within each quadrat.

Insect Community Analysis

Once the herbivore specimens had been collected, insects were sorted, counted, and identified using a dissecting microscope in the lab at RMBL. Full identification to species was determined when possible. When this is not feasible, specimens were identified to morphospecies and preserved in 100% ethanol for genetic analysis (DNA barcoding) to be done in the fall by the Mitchell-Olds lab at Duke with the help of the Whiteman lab at UC Berkeley. At least one specimen from each species or morphospecies was preserved in this way to ensure the accuracy of identifications. From this combination of visual and genetic identification, the composition of the herbivore community in each quadrat at each study site will be determined.

Data Analysis

Variation in herbivore community abundance and diversity was quantified on a quadrat-to-quadrat (α -diversity) and site-to-site (β -diversity) basis as a function of elevation. Species diversity was determined using two indices, the Shannon-Wiener index and the Simpson index. The Shannon-Wiener index is more sensitive to rare species, while the Simpson index determines diversity based on the most abundant species. Using a combination of these indices allowed us to investigate both how common and rare insect species vary over elevation. We calculated diversity indices excluding any taxonomical groups that are not known to herbivore plants, such as insects in orders Hymenoptera and Diptera. The diversity indices of the remaining insects (orders Hemiptera, Homoptera, Lepidoptera and Coleoptera) were then regressed with elevation, with random effects for quadrat nested within site, to determine community changes over the gradient. In the field, we observed that we collected more insects at sites with particularly high plant densities. I conducted analyses to detect whether plant density affected insect diversity. We analyzed data from experimental and natural populations in a multiple regression, which examined the effects of plant cover and elevation on insect diversity, and included a fixed effect for plot category (the experimental plots and plots containing naturally occurring *B. stricta*) as well as interactions between category and elevation and plant cover. These models incorporated site as a random effect to account for non-independence of plots within a site. Statistical analyses were performed using SAS (ver. 9.3), and figures were generated using ggplot2 in R Studio.

Results

In the regression of Shannon-Wiener diversity excluding insects in orders Hymenoptera and Diptera by elevation in the natural and experimental plots, we found that diversity decreases as elevation increases in both plot categories ($F_{1,34} = 5.32$, $p = 0.0273$, Fig. 1). In the regression of Simpson diversity excluding insects in orders Hymenoptera and Diptera by elevation in the natural and experimental plots, we found slightly different patterns. For the Simpson's diversity index, we found significant interactions between plot category and: linear ($F_{1,33} = 4.73$, $p = 0.037$) and quadratic ($F_{1,33} = 4.6$, $p = 0.0395$) effects of elevation. In the natural plots Simpson diversity decreased linearly with elevation ($F_{1,15} = 5.15$, $p = 0.038$); however, in the experimental plots, Simpson diversity peaked at mid-elevation ($F_{1,18} = 8.44$, $p = 0.0094$) (Fig. 2 and 3). Additionally, as plant cover increases, insect diversity increases (Fig. 5 and 6). Insect abundance was also regressed on elevation, however no significant relationships between abundance and elevation were found.

Figure 1:

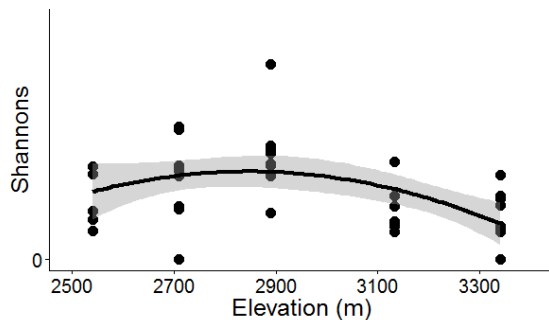


Figure 1 shows how Shannon-Weiner diversity excluding orders Hymenoptera and Diptera varies over an elevational gradient in the natural and experimental plots. Although it seems there is a quadratic relationship in the graph, we did not find significant results that this is the case. According to our analyses Shannon-Weiner diversity decreases as elevation increases ($F_{1,34} = 5.32$, $p = 0.0273$)

Figure 2:

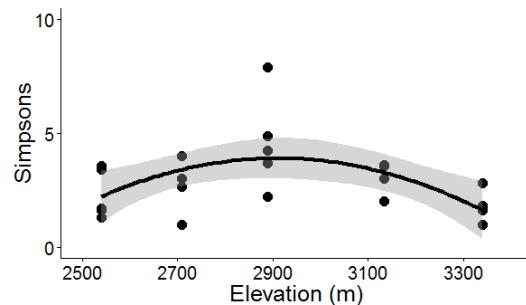


Figure 2 shows how Simpson diversity excluding orders Hymenoptera and Diptera varies over an elevational gradient in the natural plots. Although it seems there is a quadratic relationship in the graph, we did not find significant results that this is the case. According to our analyses, Simpson diversity decreases as elevation increases ($F_{1,33} = 4.73$, $p = 0.037$).

Figure 3:

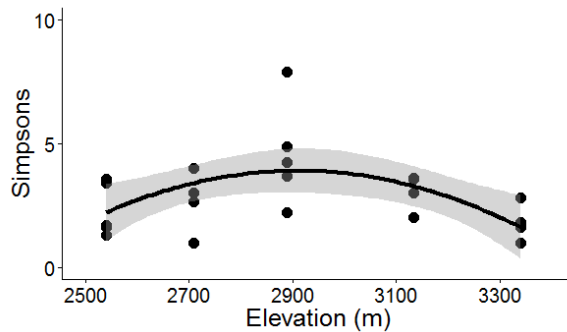
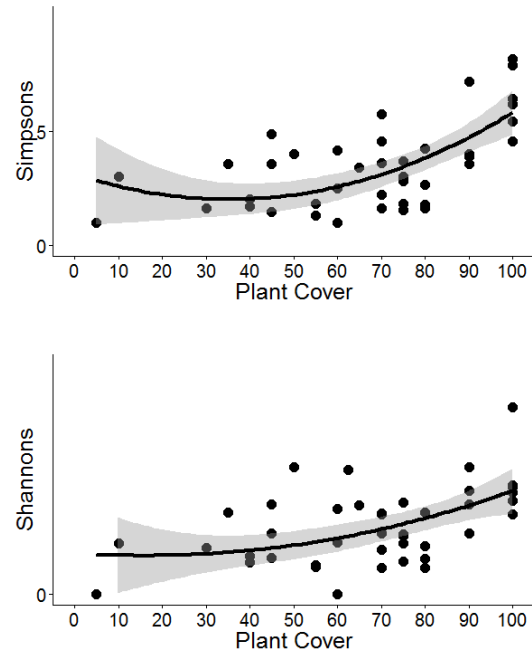


Figure 3 shows how Simpson's insect diversity excluding Hymenoptera and Diptera varies over an elevational gradient in the experimental plots. We see a significant quadratic relationship that shows that insect diversity peaks at mid-elevation ($F_{1,33}=4.6$, $p=0.0395$).

Figure 4:



The two graphs in Figure 5 show the relationship between plant cover density and the two diversity indices excluding orders Hymenoptera and Diptera in the experimental plots. As plant density increases, both Simpson ($F_{1,33}=5.92$, $p=0.0206$) and Shannon-Weiner diversity increase as well

Discussion

In this study we sampled the insect community in plots from three categories of plot type: natural populations, experimental populations and control (no *B. stricta*). By using this method of sampling, we collected insects interacting with *B. stricta* in each of our plots, and we sampled many insects that had no relationship with *B. stricta*, but were present at the moment we were sampling. We accounted for this in our study by excluding taxonomic groups that are not known to herbivorize plants, specifically insects in the orders Hymenoptera and Diptera. For the purpose of this paper we decided to focus on all insects we identified in the orders Hemiptera, Homoptera, Lepidoptera and Coleoptera. In the fall, after insects have been identified to species, we will revisit these analyses. It may be the case, for example that some of the larvae of the insects identified in the order Diptera may herbivorize plants, and we will include them in our study.

We found that insect diversity does decrease as elevation increases, except for in one instance. In the natural plots both Simpson and Shannon-Weiner diversity decrease as elevation increases. In the experimental plots within the gardens, Shannon-Weiner diversity decreases as elevation increases, however Simpson diversity peaks at the mid-elevation sites. This result could be due to an increase in the density of plant cover in the plots at our mid-elevation site.

We found a positive correlation showing that Simpson diversity increases as plant density increases, which could have caused the peak in insect diversity at the mid-elevation site. Our overall result that insect diversity decreases as elevation increases makes sense ecologically because insects are ectotherms and could have trouble living in the colder temperatures that are characteristic of higher elevation sites.

We have begun to identify the insect community surrounding *Boechera stricta*. Future analyses will determine which insects herbivore *B. stricta*. We are currently conducting a principle component analysis to assess which insect species drive diversity in each community, and how this diversity varies over an elevational gradient. We will also do a targeted species analysis by looking at species that have actually been seen in the field herbivore *B. stricta*, and examining how the abundance of these species varies. To understand how herbivore-plant relationships in *B. stricta* are affected by climate change, we must first characterize plant-herbivore relationships in the *B. stricta* study system. This study has been an important first step in this project, and has laid groundwork for many future studies on herbivore-*B. stricta* relationships in the context of climate change to come.

Literature Cited

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Appendix 1

