

The Effects of Elevation on Herbivorous Insect Communities and the Host Plant *Boechera stricta*

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ABSTRACT

Changes in elevation have strong influences on the taxonomic richness and population densities of terrestrial ecosystems (Hawkins *et. al*, 2013, McCain and Grytnes 2010, Rahbek 1995). These gradients are caused by changes in precipitation, temperature, and several other key environmental factors associated with altitude (Hawkins *et. al*, 2013, McCain and Grytnes 2010). Floral and faunal species exhibit divergence in traits across their elevational ranges, adapting to different selective pressures and environments (Hodkinson 2005, Prasad *et. al*, 2012). One such species is the mustard plant *Boechera Stricta* (Brassicaceae), whose low source elevation genotypes exhibit greater resistance to herbivores than their higher elevation counterparts (Anderson, in preparation, Prasad *et. al*, 2012). In this study, we examined the effects of altitude on insect communities and their modulation with the host plant *Boechera stricta* (Brassicaceae) across an elevational gradient. We hypothesized decreases in insect biodiversity and density with increased altitude. To test this, we sampled insect communities in common gardens of *B. Stricta* at varying altitudes. Presently, we have not yet completed our statistical analyses, largely due to a lack of statistical power. We discuss methods to be used in future studies of this nature which will produce more robust data sets.

INTRODUCTION

Elevation plays a key role in shaping the biotic and abiotic factors that characterize an ecosystem, and the environmental changes associated with elevation strongly influence the distribution and abundance of plants and animals (McCain and Grytnes 2010, Caldwell et al. 1998, Hodkinson 2005). These effects can be particularly intense for insects due to their sensitivity to temperature as ectotherms (Hodkinson 2005). In this study, we hoped to identify changes in diversity and abundance within herbivorous insect communities as a result of elevation.

Herbivorous insects are a crucial and ubiquitous component of terrestrial ecosystems. Herbivorous insects also have a very strong and direct relationship with the plant species they consume (Harms and Mattson 1992). These relationships with plant communities link insect herbivores directly to the foundations of the ecosystems they inhabit (Mattson and Addy 1975). Phytophagous insects fill an essential and formative role in many trophic systems. Therefore, understanding them, and their interactions with plants, can broaden our understanding of terrestrial systems as a whole.

As climate change increases global temperatures, it is predicted that many species will expand into higher elevations and latitudes as they become less limited by temperature tolerances. This response has been already been widely documented across a variety of plants and animals (Parmesan 2006). The effect of temperature change on species' distributions is especially great for ectotherms, whose survivability and reproductive success is highly dependent on ambient temperatures (Hodkinson 2005, Raffa et. al 2013). Climate-driven range shifts and the resulting migrations may lead to many new insect

and host plant associations. In alpine systems, temperature changes will likely lead to asynchrony between the phenologies of plants and herbivores as well. Historical data has shown the beginning of the growing season in these areas remaining relatively constant (due to snowpack), but Spring temperatures rising (Inouye et. al 2000, Raffa et al. 2013). This may lead to greater herbivore pressure as insects become active earlier and for a longer period of the growing season (Parmesan 2006). We hypothesize that these migrations and phenologic shifts will have dramatic effects on plant and insect communities at high elevations.

In this study, we analyzed the changes in diversity and abundance of herbivorous insect communities affecting the host plant *Boechea stricta* (Brassicaceae), which grows across a broad elevational gradient (approximately 1500 m – 3700 m) (Anderson *et al.*, 2011). Using the insect communities within common gardens of *B. stricta* as our model, we hoped to elucidate the effects elevation has on insect biodiversity. Along with changes in insect populations across elevation, this study also tested hypotheses about elevation specific plant-herbivore interactions in *B. stricta*, which can help predict the potential results of climate change on this system.

Insect herbivores exert significant selection on *B. stricta* populations, resulting in phenotypic divergence in plant defensive compounds (glucosinolates) across the range of the species (Prasad *et al.*, 2012). In two common gardens (2890m vs. 3133m in elevation) around the Rocky Mountain Biological Laboratory, *B. stricta* experimental transplants show a significant genetic cline in resistance to insect herbivores: Regardless of the garden elevation, low elevation genotypes are more resistant to herbivores than high elevation genotypes (Anderson, in preparation). Traits that confer insect resistance require resource investment that may not be adaptive for higher elevation genotypes with shorter growing seasons (Anderson, personal communication, Herms and Mattson 1992). This trade-off between growth and differentiation drives much of the phenotypic variation in *B. stricta* across its elevational range. At lower elevations, resistance to herbivory is a greater selective force than rapid growth. Yet, we do not know if these herbivore populations vary with altitude.

This study tested whether or not insect herbivores were more abundant or diverse at low elevations, which could impose greater selection there for resistance and other defensive traits in *B. stricta*. Alternatively, insect herbivores could simply be active for a longer proportion of the growing season in low vs. high elevations. We also tested whether reciprocally transplanted *B. stricta* from higher altitudes attract greater rates of herbivory and herbivore visitation.

METHODS

Field Sites

In September-October 2013, Dr. Jill Anderson and her lab planted a total of ~8200 experimental *B. stricta* juveniles into five experimental gardens. The study includes full siblings of >100 genotypes that were originally collected across a broad elevational gradient (2693m-3690m). In April-May 2014, Bashira Chowdhury conducted a snow removal manipulation in all gardens, such that half of the siblings of each

maternal family are exposed to contemporary conditions and half are exposed to snow removal. This manipulation simulates precipitation patterns predicted to arise with future climate change: earlier snowmelt and decreased snowpack. These five gardens are spread across Gunnison County, CO (Figure 1.1) at elevations ranging from 2,530 to 3,430 meters above sea level.

These five gardens were used as the observational sites for this study. These sites were chosen because of their high density of *B. stricta*, and the various data sets and phenological information available for the experimental plants, which provided a better experimental environment than naturally occurring vegetation.

Table 1.1. Common gardens used as field sites, with elevation and latitude/longitude

Garden	Estess	Peanut Mine	Gothic Valley	Schofield Meadow	North Pole Basin
Elevation	2530m	2710m	2890m	3133m	3430m
Lat./Long.	38°43'40.62 " N, 106°52' 20.00" W	38°53'07.85 " N, 106°59' 52.18" W	38°57'05.18 " N, 106°59' 27.87" W	39°02.346 " N, 107°03.818 " W	39°01'53.328 " N, 107°04' 42.770" W

Pitfall Trapping

Measurements of insect community density, species composition and richness were taken using pitfall traps. Ten traps were set at each garden, which remained on-site for 72 hours before being serviced. Traps were placed at each site for three separate 72-hour periods of collection throughout the study.

Each trap consisted of a 50 CC centrifuge tube filled with 30ml of a 1:3 solution of propylene glycol (EasyGoing Antifreeze, Camco Manufacturing Inc., Greensboro NC) and water, as well as a “box tent” (a small three-pronged plastic ‘table’ used in pizza delivery) protecting the open trap from debris and vertebrates. Each trap was buried with its top level with the soil and marked with a pin flag. These traps were dispersed randomly throughout the common gardens near plots of the experimental *B. stricta*.

The solution of propylene glycol was used for its qualities as a killing agent, preservative, and its slow rate of evaporation (Thomas 2008). These qualities all give this solution advantages in trapping over the common mixture of water and surfactant (dish soap). Unlike the ethylene glycol found in automotive anti-freeze, which shares many of the same properties and has been used in pitfall trapping in the past, propylene glycol is non-toxic, and therefore does not present a hazard to mammals or other vertebrates which may come in contact with the traps. Propylene glycol is rated GRAS, “Generally Regarded As Safe” by the U.S FDA and EPA (Thomas 2008).

To service the sites, traps were removed and capped to be stored in a refrigerator for later processing. To prepare sample for processing and identification, they were removed from the trap solution and placed in 70% ethyl alcohol.

Surveys

Herbivore surveys were conducted within the gardens to identify what insects were consuming *B. stricta*, as well as any differential rates of herbivory between genotypes. We surveyed each garden a total of three times from early to late July. Most specimens were collected by hand, but a vacuum aspirator was occasionally used to facilitate collection of flying or hopping insects. Surveying was done in the late morning (~0900-1100) when temperatures were ideal for insect activity (Dumas *et. al* 1967). Each survey covered 7 plots of *B. stricta*, in which each plot (≤ 120 plants) was given approximately 10 minutes of observation time. Time spent collecting specimens or making notes of collection was not counted towards observation time. Herbivores found on *Boechera stricta* during the surveys were captured and placed in vials labeled with the plant number they were collect from, and frozen for later identification.

Statistical Analyses

We ran several preliminary analyses looking for observable trends in our data sets, however, no significant patterns have been found. This is due to a lack of statistical power in the data collected. Our ability to differentiate statistical values was limited by the number of data points collected and the short sampling time available for this study. Uneven sampling effort also restricted our data set, making it difficult to analyze effectively.

Further attempts at analyses for this data set may look for trends in time of sampling and abundance, or plant elevational genotype and herbivore visitation. Species turnover between sites may be analyzed through the use of Jaccard's dissimilarity indices. Unfortunately, our data set was not robust enough to support our planned analysis of abundance and diversity across elevation.

RESULTS

Various analyses of our data yielded no observable patterns. We were unable to assess the existence of patterns because of weaknesses in the data. Namely, an uneven sampling effort in the insect surveys, and the small number of data points from our pitfall traps. Due to these restrictions in our methodology, the data sets drawn from this study were not statistically powerful enough to make analyses of patterns with confidence.

DISCUSSION

This study was conducted in the hopes of contributing to the body of knowledge on plant and herbivore responses to elevation, as well as genotype level changes in *B. stricta*'s resistance to and subsequent damage from herbivores. Through examining the diversity and abundance of herbivores at varying altitudes, we hoped to investigate how elevation was impacting these insect species, and how they were in turn influencing their associated plant communities. However, due to poor statistical weight and methodological issues, patterns of this nature were difficult to analyze. We believe that there are infact

trends to be observed within these systems, however, changes in methods and analyses must be made for a robust and workable data set to be collected in the future.

Field Sites

One of the restraints of this study was the sole use of the common gardens as sites for sampling. Intended for modeling climate manipulation for *B. Stricta* at different elevations, variables influencing herbivore communities in particular may not be standard across sites. Temperature, precipitation and vegetative growth will change with elevation (McCain and Grytnes 2010), but their effects on insects vary topographically and between microclimates (Hodkinson 2005). This microclimatic variation can override more general effects of altitude, and have greater influence on insect diversity and abundance than elevation alone (Weiss, Murphy and White 1988). There is also the issue of varying predator communities across gardens. Predator density can affect abundance and distribution of insect species (Klemola *et. al*, 2002), and again, may be subject to microclimate and habitat variation.

For example, one site, Peanut Mine (Figure 1.1), was located within a wild bird sanctuary. If this site had disproportionately high insectivorous bird populations, this may have led to greater insect predation within the garden. Indirect factors like these should be taken into account when planning future studies and the location of trapping and surveying. Concessions to these variables can be made by extending surveying and trapping across a broader area, rather than limiting them to within the confines of the common gardens.

Pitfall Trapping

The use of pitfall traps, such as in this study, is a common practice for measuring invertebrate abundance in ecological research. However, there are limitations to this method which must be taken into account when planning a sampling regimen. A variety of factors besides abundance can affect pitfall trap catch, such as relative activity, location of traps, and time of trapping (Topping and Sunderland 1992). Varying levels of activity between species will change the likelihood of them being caught in pitfalls, and therefore impact representations of their abundance within the samples. Activity also becomes a factor over time, as different species vary in phenology, and certain stages in their life cycles lead to lesser or greater levels of activity (Topping and Sunderland 1992). The vegetation surrounding pitfalls can also impact catch density and composition (Topping and Sunderland 1992). The design of the trap itself may also be a biasing factor (Greenslade 1973). The diameter of the trap opening can effect catch rate of individuals of different body sizes (Greenslade 1973). Also, trap solutions using propylene glycol may have attractive properties specific to certain taxa (Southwood 2005, Thomas 2008). All of these variables should be considered when using pitfall trapping to measure abundance. Because of these limitations, and for the purpose of extensive studies such as this, measurements of abundance using pitfalls should be viewed as relative between sites, rather than absolute. Also, a greater sampling effort over a longer time frame will mitigate these biases in results (Gotelli and Colwell 2001). Provided these extraneous phenomenon effecting pitfall trap catch are correlated with density, it can still be a viable means of quantifying communities of invertebrates.

To mitigate the biasing factors associated with pitfall trapping, their implications should be taken into account when developing sampling protocols. For instance, traps should be distributed across a variety of vegetation densities and types within the sample area. Pitfall trapping should also be conducted throughout the extent of an entire season, therefore reducing the effects of different phenologies and developmental stages on catch rate. Though it may not be as accurate as some other observational methods, careful use of pitfall trapping can still be an effective means of sampling invertebrate communities, especially on broad ecological scales such as in this study.

Herbivore Migration into Higher Altitudes

With increases in temperature being a global result of climate change, the geographic distribution of species has and will continue to change, along with their ecological interactions with one another (Parmesan 2006, Raffa et. al 2013, Inouye et. al 2000). Recent studies in paleobotany have also shown that historically analogous increases in temperatures and CO₂ levels are strongly correlated with increases in insect herbivory (Currano et. al 2008). We may predict, therefore, that greater herbivory at higher elevation will be a long term consequence of anthropogenic climate change (Currano et. al 2008). In the shorter term, it is also likely that insect herbivores' movement to higher altitudes will outpace that of lower elevation plant communities. These changes in biogeography may lead to problems for both native and migrant species. For species who lack strong herbivore defenses at high altitudes, such as *Boechera stricta* (Anderson, in preparation, Prasad et. al 2012), this could be especially harmful. This may be further compounded by earlier emergence of herbivores after warmer winters, another consequence of climate change (Parmesan 2006). As insect species migrate to higher elevations, high altitude plant communities may be ill-prepared for the new herbivore pressure, and population crashes or extinctions could result.

Predation, Herbivore Load, and Elevational Range Expansion

Differences in physiology, life history strategies, and dispersion explain the varying geographic responses of species to climate change (Parmesan 2006). Therefore, even as the edges of what might be habitable zones for many species shift, their integration into these new ecosystems will occur at different rates. This may cause imbalances in these ecosystems as certain niches are filled before others. For example, if herbivorous insects migrate faster than their predators, their populations will be less limited, and rates of herbivory would greatly increase due to unbridled population growth. Mismatches such as these could present serious problems for high elevation ecosystems.

In the communities within this study, ants, aphids, and *B. stricta* may be vulnerable to such a mismatch. In the common gardens, we found indications of complex and intriguing interactions between *B. stricta* and associated ant communities. Like many plant species, *B. stricta* produces extrafloral nectar for the purpose of recruiting non-herbivorous insects for defense. Within our common gardens, we observed ants tending the plants of herbivores in this manner, and retrieving nectar rewards in the process. We also found the bolts of many of the experimental plants chewed off. It was later hypothesized that while the ants were engaged in this mutualistic nectar-bearing relationship with the plants, they were also

responsible for the bolt chewing observed within the gardens. This is speculative, however, and further research is needed to investigate this relationship.

Aphids are a common herbivore of *B. Stricta*, and were found in 3 out of 5 of our sites (Gothic, Schofield, North Pole). As part of their mutualistic behavior, ants will remove aphids from *Boechera* to protect them from herbivore damage. If these two species integrate into higher elevations at different rates, levels of herbivory may increase as a result. Many aphids will have wings at some point in their life cycle, and can be carried great distances by wind, giving them greater means of dispersal than ants. Ants, on the other hand, are highly eusocial, and their colony structure limits their ability to migrate quickly and recruit into new elevations. Similar mismatches could potentially lead to population explosions as herbivores outpace their predators in moving into newly habitable altitudes.

Future Research

Understanding changes in plant-herbivore interactions associated with climate change can better prepare us for the consequences in management, breakout, invasion, and agriculture. Though this study gave insight into community changes with elevation, it did not analyze the underlying factors responsible. Future research into these types of gradients including water and temperature analysis could help associate these variables with changes in communities. Further studies into potential mismatches as species expand their ranges to higher altitudes would also contribute greatly to our understanding of the consequences of climate change.

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FIGURES

Fig 1.1. Map of common gardens in Gunnison County, CO, and their elevation above sea level

