

Environmental and Genetic Effects of Elevation on Plant Defense

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ABSTRACT

Plant communities are shaped by their interactions with biotic and abiotic factors in their environment, including herbivores. In response to pressures from herbivores, plants develop a variety of physical and chemical defenses to protect themselves. The investment that plants make in these defenses varies with the environment. Using fully reciprocal turf transplants at three different elevations and a variety of focal plant species, we tested patterns of plant defense against herbivory along elevational gradients and examined whether any intra-specific variation in plant defense is due to genetic variation among populations and/or are changed by the environment of the plant. After collecting leaves in the field and conducting grasshopper feeding bioassays, our experiment did not find any significant effect of treatments in either the environmental or the genetic tests. This result contradicts previous work done at RMBL and in other study systems. One possible explanation for this is that our elevational gradient was not large enough to see noticeable differences in plant defense. Another possible explanation is that it was a very low year for grasshopper abundance and it's possible they had a disease or parasite that was causing changes to their eating patterns. Further experiments are required to draw conclusions about genetic and environmental effects on plant defense.

INTRODUCTION

Insect herbivory is a major part of most ecosystems. By eating plants, the herbivores decrease plant fitness and reproductive potential. In response, plants will develop different types of chemical defenses to make their leaves less palatable to potential herbivores and decrease how much of their mass is eaten. Patterns of herbivory vary depending on biotic and abiotic factors in

the environment.^{1,2} We know these variations exist but the mechanisms that drive them are not well understood.

Plants have limited resources and energy so they must prioritize what traits they invest in, and defense is predicted to trade-off with growth rate.¹ The traits that they choose are heavily impacted by the abiotic environment that they are in. For example, the Resource Availability Hypothesis (RAH) predicts that fast-growing plants in high resource environments can easily regrow their leaves after being eaten and therefore invest less in defense.³ Another example of plants being impacted by their abiotic environment is growing season length, which varies depending on the environment. With a shorter growing season, plants must put more energy into fast growth and reproduction and therefore less into defense. The plants simply do not have resources to grow fast, make lots of seeds, and be strongly defended so they must “choose” based on what is most beneficial and necessary for the environment that they are in.

Gradients in elevation represent variation in both the biotic and abiotic environment that may affect plant investment in herbivore defense. At higher elevations there is more moisture and a shorter growing season. Both of these factors mean that high elevation plants have faster instantaneous growth rates than plants at low elevations sites, which are drier and have longer growing seasons (although plants may grow more overall at lower elevations because of the longer growing season). There is also evidence that there are fewer herbivores at high elevations, suggesting that plants can put more of their energy into growing fast instead of creating defenses against herbivores.^{4,6} Growing fast is beneficial at high elevations since the growing season is short, so it benefits the plant to trade off herbivore defense for growth rate.

Past work in our study system has shown that plant defenses against herbivory are lower at high elevations for multiple plant species⁴ and our experiment sought to test whether such variation is due to genetic differences or due to differences in the high and low elevation environments where the plants were growing.

METHODS

Study sites

In order to determine whether plant defense is driven by environmental or genetic conditions, we utilized the Enquist Lab turf transplants. Replicate 0.25 m² plots were excavated from sites at three different elevations in 2017. The lowest site, "Upper-montane", is 2895 m

above sea level. The mid-range site, "Pfeiler", is at 3155 m elevation. The highest site, "Monument" is at 3290 m of elevation. All of these sites are found along Washington Gulch, located outside of Crested Butte, Colorado (Figure 1). Each site received five replicate turfs from each elevation, including local turfs that were excavated and replanted at the same site, i.e. 15 turfs per site (5 local, 5 transplanted from each of the other two non-local sites) for a fully reciprocal transplant design. These plots are tented to prevent gene flow from the origin to the destination site. These tents prevent flying pollinators but do not prevent herbivorous insects from moving in and out. These plots have now been in place for five years with detailed data being collected about species composition and reproduction at the various elevations.⁵

Experimental design

Few plant species were shared in the turfs between the highest and lowest elevation sites, but there was substantial overlap between the low- and mid-elevation sites and the mid- and high-elevation sites. For the low/mid experiment, "low" refers to Upper Montane and "high" refers to Pfeiler. For the mid/high experiment, "low" refers to Pfeiler and "high" refers to Monument. Accordingly, we conducted two experiments comparing these pairs of sites separately. We studied all of the plant species that occurred in both sites in each experiment. In order to avoid plants that may have invaded the turfs since the transplant, we restricted the data to species that were present in a given turf in 2018 (immediately after transplant), and also present during the time of our work (2022).

Within each experiment, there were two factors: the origin of the plant (where the turf was from; high vs. low) and the destination of the plant where it was currently growing (in the high vs. low site). The combination of these two factors resulted in four treatments for each plant, high or low origin site and high or low destination site. Tests for genetic effects sought to determine whether the investments that plants make in defense has a genetic component. Genetic tests for a species consisted of bioassays comparing defense between plants of two origins (high and low) growing with a single destination site. Tests for environmental effects sought to determine whether the investment that plants make in defense has an environmental component. Environmental tests for a species consisted of bioassays comparing defense between plants of a single origin growing in two destination sites. In each bioassay, leaves were selected from two plants (of the same species) paired to make a test for a genetic or environmental effect. The sample size for each genetic or environmental effect ranged from 3-5 bioassays per species.

Each bioassay consisted of leaves from separate individuals collected from between 1-5 different plots. (Figure 2). Across all plant species and experiments we conducted a total of 198 total bioassays. (Figure 3) Depending on the leaf shape we either cut them into discs or placed whole leaves in the arena. For both methods we ensured that there were equal surface areas of leaf material from each treatment. When we used whole leaves, we took pictures before starting the trial and calculated the surface area. Arenas were left to run either in the Gothic Research Center or in a Weather-Port. We collected grasshoppers from a variety of elevations (Copper creek trail - meadow 2, Judd falls parking lot, Gothic research center, Upper montane site - low, Pfeiler site - mid, and between the low and mid sites) and randomly assigned them to arenas. We chose grasshoppers within the subfamilies of Oedipodinae and Melanoplinae. We took note of the sex, length, developmental stage, and taxonomic group of all the grasshoppers. (Figure 4) Each individual grasshopper was put in an arena with leaves of the same species from two different treatments (eg: high local vs low transplant.) The arenas were left to run until approximately 50% of the leaf matter had been eaten. The time this took varied from 30 minutes to 14 hours. We took pictures of the leaves in each arena after they finished and calculated the surface area using ImageJ. We calculated herbivory by computing the difference between initial surface area and final surface area.

Statistical analysis

We computed an effect size for each bioassay arena as the log response ratio for the effect of elevation with respect to genetic or environmental effects. For genetic effects, effect sizes were calculated as $\ln(\text{leaf area consumed high-origin leaf} / \text{leaf area consumed low-origin leaf})$. For environmental effects, effect sizes were calculated as $\ln(\text{leaf area consumed leaf growing in high elevation site} / \text{leaf area consumed leaf growing in low elevation site})$. This process thus generated a data set of effect sizes ($n = 3-5$) for genetic and environmental effects for each species. We then calculated overall mean effect sizes for genetic and environmental effects across all species, as well as the 95% confidence intervals. With this approach, a significant genetic or environmental effect is assessed according to whether the 95% confidence intervals include 0, i.e., no effect.

RESULTS

Our test for genetic and environmental treatments did not get significant results in the low/mid or mid/high experiments. Our test for an effect of plant species on feeding effect size was not significant for either environmental or genetic analyses, indicating that our results were consistent across all of the species we used. Across all species there were not significant effects of environmental or genetics for either the low/mid or the mid/high experiments. (Figure 5)

DISCUSSION

Using fully reciprocal turf transplant sites established in 2017, we tested for genetic and environmental effects on plant defense along elevational gradients. Using feeding bioassays, we offered sets of two leaves from the same species but different environmental or genetic treatments to grasshoppers and recorded the relative herbivory on the pairs of leaves. With this approach, we did not detect significant genetic or environmental effects of elevation on plant defense. This result is at odds with previous studies done on herbivory and elevation. While it is possible that our results are correct, it is also possible there is another explanation.

There have been numerous previous studies looking at the environmental component of plant defense. These studies have found that plants at higher elevations have more herbivory because they produce less defenses.^{4,7,8} Last summer, Gretchen Klens worked at RMBL and conducted bioassays very similar to ours. Her study found a significant difference in the plants that the grasshoppers preferred. They ate more of the high elevation leaves than the low elevation leaves. In our study, we were not able to replicate this result. One possible explanation for this is that Gretchen's elevational gradient was 488 m while ours was only 200 m for each comparison. We were limited by the placement of the existing turf transplant sites and it is possible that the gradient we used was too small to notice elevational changes in plant defense.

Genetic effects on plant defense are much less studied than environmental effects. This is in large part because it requires a multi-year study and moving plants around to different elevations before the defenses can be tested. Our study did not find evidence that there is a genetic component to plant defense. We tested across many species and failed to find a genetic component in any of the species we tested. The individual plants in the turfs were not marked and we had to assume that they were original to the turf. It is possible that this was not always a correct assumption and may have impacted our results.

In the summer of 2021 when Gretchen Klens was conducting her feeding trials, grasshoppers were exceptionally abundant in and around the Gothic valley. When we conducted our experiment the following summer, they were exceptionally rare. It is possible there was a density-dependent factor, like disease or parasites, that caused these fluctuations in the population and that these may have impacted our experiment. If present, these density-dependent factors may have changed the behavior and eating patterns of our grasshoppers and changed our results. We observed a small number of grasshoppers with *Trombidiidae*, red velvet mites, in our feeding trials.

If this experiment were to be done again in the future, one potential change to make would be to do it along a larger elevational gradient. This would require setting up reciprocal transplants similar to the ones used in our study along a larger gradient for multiple years and then testing the effects on plant defense. Another change that would avoid fluctuations in grasshopper populations and health would be to use mail-order Spodoptera instead, or in addition to, native herbivores. Using a generalist herbivore like Spodoptera would remove the potential effects of grasshopper variation but would also introduce new effects that come with using a non-native subject. Our experiment failed to find significant results for genetic or environmental effects but with adjustments to the procedure, it is possible future studies could generate significant results.

As we work to model how plant communities might be altered with climate change, determining information about plant defense and their plasticity is important. If plants are able to change their defense traits with the environment, they will be able to better keep up with the changing climate and potentially changing distributions of herbivores. If defense traits are not plastic and require long-term evolutionary processes, plant communities may not be able to adapt as quickly to a changing environment. More research is required to determine whether plants are able to change their defense investment based on a changing environment.

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TABLES AND FIGURES

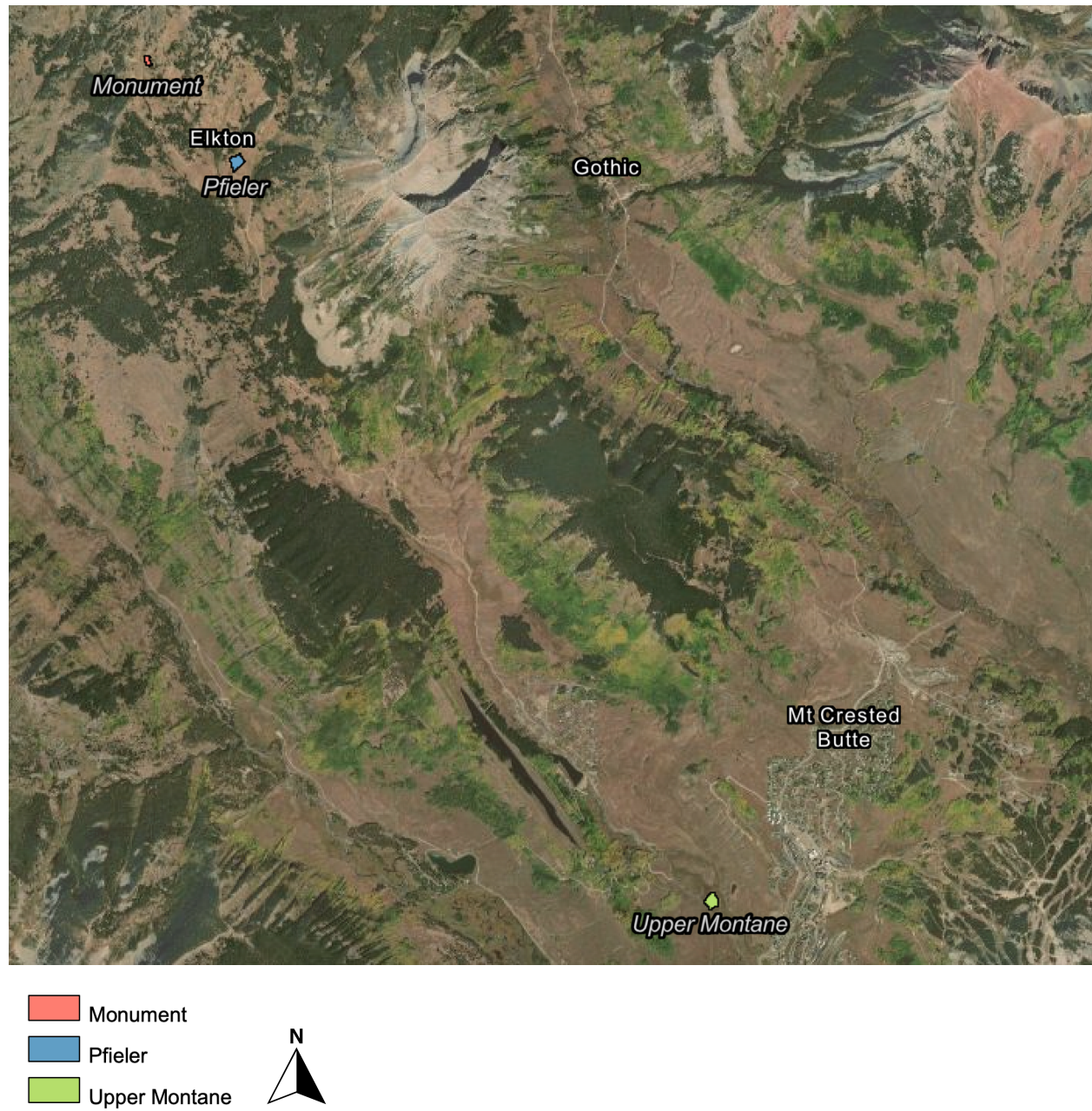


Figure 1. Map of turf transplant sites in Washington Gulch

Plant Species	Low-Mid	Mid-High
<i>Potentilla pulcherrima</i>	x	x
<i>Heliomeris multiflora</i>	x	x
<i>Helianthella quinquenervis</i>	x	x
<i>Hymenoxys hoopesii</i>	x	x
<i>Ligusticum porteri</i>	x	x
<i>Senecio crassulus</i>	x	x
<i>Lathyrus lanswertii</i>	x	
<i>Achillea millefolium</i>	x	
<i>Castilleja sulphurea</i>		x
<i>Fragaria virginiana</i>		x
<i>Heterotheca pumila</i>		x
<i>Sedum integrifolium</i>		x
	8 total species	10 total species

Figure 2. Plant species used for feeding bioassays in the two experiments

		Origin		
		High	Low	
Site	High	1	2	Genetic (a)
	Low	3	4	Genetic (b)
		Environmental (a)	Environmental (b)	

Figure 3. Pairing of treatments for the environmental and genetic tests

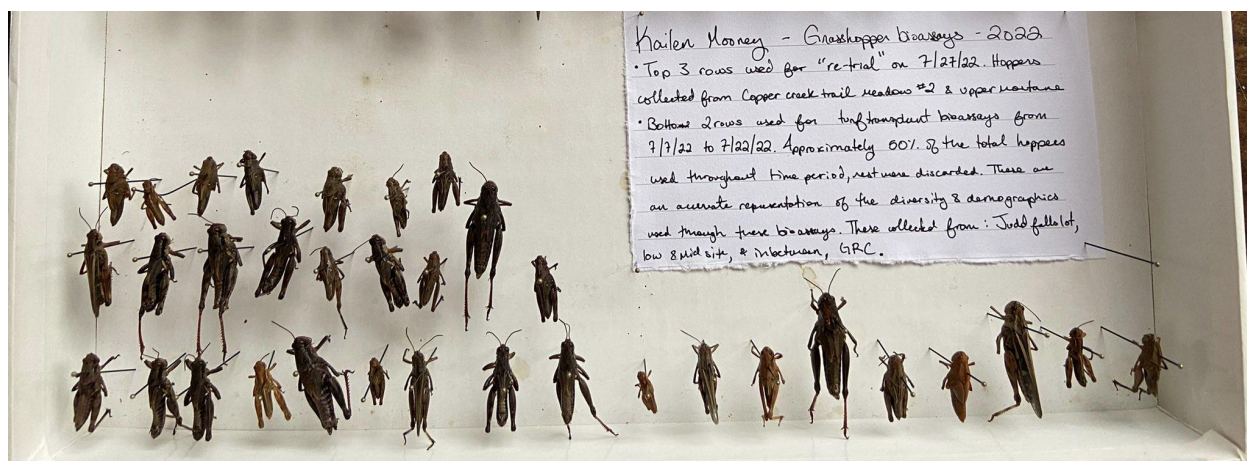


Figure 4. Pinned grasshoppers representing approximately 50% of total individuals used in bioassay arenas, representative of demographics of individuals used

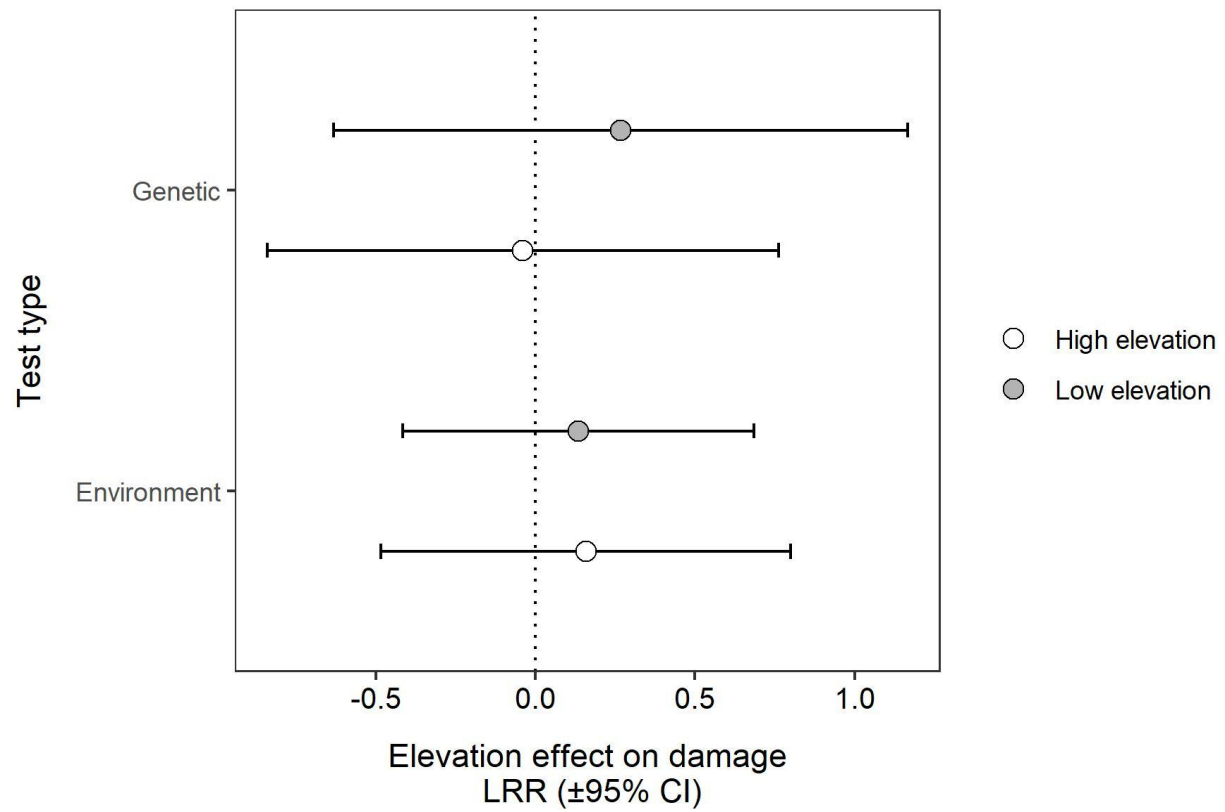


Figure 5. Log response ratios with a 95% confidence interval showing no significant effect of treatment in the environmental or genetic tests