

Combined Effects of Aridity and Herbivory on Plant Performance

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Project summary/abstract:

Plants adaptation to water availability and herbivory are intertwined as the plant traits that defend against herbivory may also increase plant tolerance for aridity. Aridity and herbivory vary predictably along elevation gradients, with both declining with increasing elevation. *Boechera stricta* is locally adapted to origin elevation but whether this was driven by variation in aridity and herbivory was unknown. This project measured performance of *B. stricta*, a self-pollinating forb, in a multifactorial study manipulating both water availability and grasshopper herbivory for plants sourced from (and locally adapted to) 36 different elevation origins where aridity and herbivory declined with increasing elevation. To determine the combined effects of aridity and herbivory on plant performance, plants received one of four combinations of herbivory (with grasshoppers vs. no grasshoppers) and watering treatments (arid vs. mesic). The treatments were not found to significantly affect the binary response of whether or not plants reproduced. However, we found a significant three-way interaction between elevation of origin, aridity, and herbivory in terms of the fecundity of plants that flowered: In one of the four aridity x herbivory treatment combinations - arid with herbivory - there was a significant effect of plant source elevation. This treatment combination simulated the low elevation environment of high aridity and herbivory. In this treatment (alone), plants from low elevations produced the highest numbers of fruits, and fruit number declined with increasing elevation. Accordingly, our results suggest that local adaptation to the elevational cline is driven by the combined effects of aridity and herbivory.

Introduction

Plants must simultaneously cope with aspects of both the abiotic and biotic environment (Moreira, 2018) and plant adaptations to aridity and herbivory may be especially important in this regard (Gutbrodt, 2011 ;Burkepile, 2017). The availability of water resources is a limiting factor for plant performance, viability, and success. Plants have evolved a variety of morphological, physiological, and biochemical adaptations to allow them to survive in environments with limited water and high aridity. These adaptations include stomatal regulation, high water use efficiency and low specific leaf area (Ordoñez et al., 2009; Passioura, 1996; Reich, 2014; Wright et al., 2005, 2001), which have been found to minimize water loss in arid conditions (Reich, 2014; Wright et al., 2005, 2001). Plants must adapt to herbivory as herbivores remove a substantial percentage of plant

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biomass, averaging 5.3% across all ecosystems (Turcotte, 2014). As a result, plants have evolved a variety of physical and chemical defenses (Gong, 2014). Physical defenses, such as leaf toughness and trichomes (hair-like outgrowths), and chemical defenses, including carbon and nitrogen-based compounds that function as toxins and reduce digestibility, reduce the likelihood of consumption (Descombes, 2017).

Plants always simultaneously cope with both herbivory and available resources. Herbivory and aridity are intertwined - a plant's resources affect its ability to defend against herbivory (Gutbrodt et al. 2011) and many of the same traits underlie both resistance and aridity tolerance (Agrawal 2009; Blumenthal 2020). There are two ways that aridity and herbivory may interact, having a combined effect greater (or less) than the sum of the individual effects. The same traits that protect a plant from herbivores may also help it cope with drought conditions (Agrawal 2009; Blumenthal 2020). The Resource Availability Hypothesis predicts a tradeoff between growth rate and plant defense, with a faster growing plant with access to more abundant resources being more poorly defended (Coley 1987, 1985). Water availability may thus impact herbivore defense (Reich 2014).

Aridity and herbivory vary in predictable ways across elevation gradients; plants at lower elevations experience a more arid environment (in the Rocky Mountains - our study system) and higher levels of herbivory (Anderson, et al. 2015; McDonald 1989; Hodkinson 2005; Moreira 2018). Herbivore defenses, which can be expensive for plants (Koricheva, 2002), also vary with elevation as plants at higher elevations tend to experience less herbivory (Descombes, 2017), and, therefore, susceptibility to insect herbivory increases with elevation (Anderson 2015, Descombes 2020). Thus, the effects of aridity and herbivory may not only affect each other, but they may also depend on the environment to which the plant is adapted, in this case the elevation to which it is adapted. Based on these clinal variations, we would predict plants from low elevations, with more aridity and herbivory, should maintain fitness when confronted with aridity and herbivory as compared to plants from high elevations.

Reciprocal transplant experiments have found *Boechera stricta*, our focal organism, to have been locally adapted to origin elevation (Anderson 2020). There are many studies that show local adaptation with reciprocal transplant approaches (Cheplick 2015; Johnson, et al. 2022). However, such studies do not show why species are locally adapted. The best evidence for the causative agents of selection and local adaptation is to manipulate an aspect of the biotic and/or abiotic environment that is believed to drive local adaptation and to see local adaptation is detectable in the presence, but not absence of the proposed agent of selection. In the studies that have done environmental manipulations to study the mechanisms underlying local adaption, few if any have looked at how multiple aspects of the

environment may drive local adaptation in combination. Understanding how plants have adapted to complex environments is especially important in the context of global change where historic correlations between aspects of a species environment may become decoupled as species find themselves in novel environments (Parmesan, 2006).

In this study we tested whether clinal variation in herbivory and aridity are important aspects of the environment that drive the observed patterns of clinal local adaptation in *Boechera*. The goal of this project is to test whether aridity and herbivory separately or together drive local adaptation. We used a common garden approach to manipulate grasshopper herbivory and aridity for plants sourced from along an elevation gradient that itself varies in aridity and herbivory. In so doing, this multi-factorial study tested for the individual and combined effects of aridity and herbivory on plant performance and whether these effects depended on the environment from which the plant is sourced.

Methods:

1. Study system

Boechera stricta is a perennial forb (flowering herbaceous plant) native to the Rocky Mountains. Its range stretches from Utah to Alaska, and it is distributed along an elevation gradient ranging from 700 m to 3900 m (Anderson, 2020). Herbivory and aridity co-vary along this elevation gradient, with high (vs. low) elevation being more moist and lower herbivory. *B. stricta* is self-pollinating, which allowed us to more easily distinguish between genotypic and environmental factors. Populations of *B. stricta* along the gradient are locally adapted (Anderson, 2020). This study was conducted near Gothic, Colorado on the property of the Rocky Biological Laboratory in a dry rocky meadow at 2890 m elevation, latitude and longitude (38° 99.1366" N and 106°59.4645"W).

In June 2021 and 2022, we set up with six grasshopper 2.5m x 2.5m x 2m cages the garden site. We placed cage frames constructed of PVC pipes, and a custom-made mesh with flaps along the bottom edges and zippers at each corner placed over the pre-planted plots in early to mid-June (before high numbers of grasshoppers were noted). In three cages, grasshoppers were removed (low herbivory) and in the other three grasshoppers were added to meet or exceed numbers outside the cages (high herbivory). Each week grasshoppers were removed from low herbivory cages and added to high herbivory cages to be equal to (or exceed) ambient densities. Densities within and outside of cages were assessed and compared weekly based upon 2 min sweeps with a net. Late in the summer of 2022, due to much lower numbers of grasshoppers both within treatment cages and outside the cages, a new method of comparison was instituted. Grasshoppers seen within a 3 min interval while disturbing vegetation with a stick were counted within each cage and in the areas surrounding the cages.

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Four 0.75m x 0.75m sub-plots were established within each cage. Two plots received supplemental water (2L per sub-plot per week; mesic treatment) while the other two sub-plots had rainout shelters deployed over the other plants during rain events to exclude precipitation (arid treatment) once the monsoons hit.

Multiple siblings from each of 36 genotypes (source elevations ranging from 2519 - 3673 m above sea level) were transplanted. *B. stricta* is a self-pollinating plant with limited within-population genetic variation. We maximized genetic variation within this experiment by transplanting only one genotype per source population. Each plot contained approximately 30 *B. stricta* sourced from across the local elevational gradient, with approximately 62% of plants surviving to 2022. The same experimental manipulations initiated in 2021 were maintained throughout the 2022 study. Soil moisture, volumetric water content of the soil, were tested once using Hydrosense to assess the efficacy of the aridity manipulations.

Investigators collected data in twice weekly censuses from late May to late July. They noted the status (alive/dead) of each plant. To provide phenology data, reproductive stage (bolting, flowering only, flowering and siliques, siliques only) for each plant was noted. Also included in census data were counts of the numbers of flowers and numbers of siliques of each plant. Investigators measured the size of the largest silique in mm with calipers and the heights of all stems (measured in cm with measuring tape from soil or top of roots to apical meristem) to estimate aboveground biomass.

In addition, surveys of percent leaf damage were conducted on a weekly basis to assess levels of herbivory. These surveys counted total number of leaves, total number of herbivore-damaged leaves, and

cumulative proportion of herbivore-removal. Leaf area removed (LAR) was obtained by summing the proportion of damage to each leaf divided by the total number of leaves. This provided the proportion leaf damage for each plant.

3. Data analysis:

We tested for the main and interactive effects of plant source elevation, watering treatment and herbivory treatment on proportion leaf damage by herbivores ("leaf damage"). Effects on herbivory were tested with a generalized linear model, including the random effects of block and source plant population.

We tested for the main and interactive effects of plant source elevation, watering treatment and herbivory treatment on plant fitness. We used a two-step approach, first testing for effects on whether a plant reproduced ("reproduction"), and second testing for effects on fruit number for those plants that did reproduce ("fecundity"). Effects on reproduction were tested with a logistic regression, including the random effects of block and source plant population as well as the fixed effect of plant initial size at the time of planting. Effects on fecundity were tested based on a generalized linear model with a gamma distribution, again including the random effects of block and source plant population as well as the fixed effect of plant initial size at the time of planting.

Results:

There were no significant main effects of watering treatment on proportion leaf damage by herbivores ("leaf damage") nor an interaction between watering and source plant elevation or herbivory treatment (Table 1, Fig. 3). While there were no main effects of plant source elevation and herbivory treatment on leaf damage, there was a significant herbivory-by-elevation two-way interaction (Table 1; Fig. 4). In this two-way interaction leaf damage increased with plant source elevation with grasshoppers present (but not when grasshoppers were excluded).

Table 1: Statistical results for the effect of watering treatment, herbivory treatment and plant source elevation on leaf area removed by herbivory. Statistically significant results indicated by an "**".

	Chisq	Df	Pr(>Chisq)
(Intercept)	0.6846	1	0.40801
Watering	0.0205	1	0.88619
Herbivory	2.0449	1	0.15272
Elevation	1.7378	1	0.18742
Watering*Herbivory	0.0379	1	0.84563
Watering*Elevation	0.0052	1	0.94257

Herbivory*Elevation	4.7964	1	0.02852 *
Watering*Herbivory*Elevation	0.1414	1	0.70688

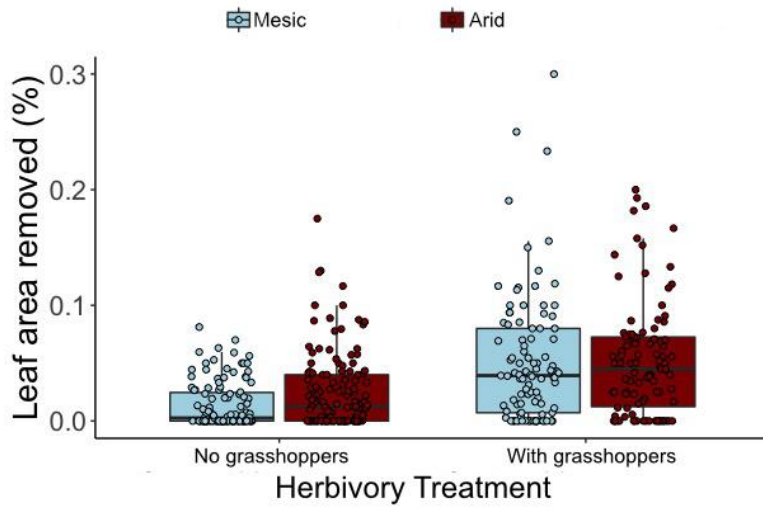


Fig. 3. Proportion leaf area removed by herbivory, “leaf damage,” with two levels of herbivory treatment (no grasshoppers, with grasshoppers) and with two levels of watering treatment (mesic, arid).

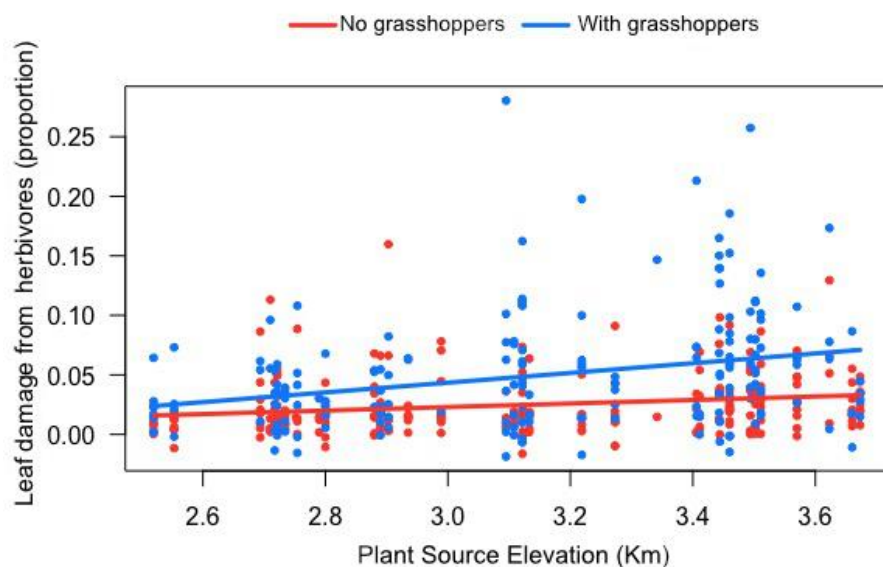


Fig. 4. Percentage leaf damage from herbivores with grasshoppers and no grasshoppers in mesic and arid treatments. Plants originate from a range of source elevations.

There were no significant main or interactive effects of plant source elevation, watering treatment and herbivory treatment on whether or not a plant reproduced ("reproduction"), although the water treatment and elevation-by-water treatment interaction were marginally significant ($0.10 > P > 0.05$; Table 1).

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Table 2. Statistical results for the effects plant source elevation, watering treatment and herbivory treatment on whether or not a plant reproduced. Statistically significant results indicated by an "*".

Effect	Chisq	Df	Pr(>Chisq)
Intercept	0.0694	1	0.79220
Initial plant size	32.5441	1	1.165e-08 *
Elevation	1.2888	1	0.25627

Herbivory	0.0253	1	0.87370
Watering	3.6785	1	0.05512
Elevation*Herbivory	0.0000	1	0.99697
Elevation*Watering	3.6923	1	0.05466
Herbivory*Watering	0.2723	1	0.60180
Elevation*Herbivory*Watering	0.2433	1	0.62185

There were no significant main effects of plant source elevation, watering treatment and herbivory treatment on the number of fruits (siliques) produced ("fecundity"), although there was a significant herbivory-by-water treatment two-way interaction and a significant herbivory-by-water-by-elevation three-way interaction (Table 2; Fig. 5). This three-way interaction occurred because there was only an association between elevation and fecundity in the arid treatment with grasshoppers. In this treatment, fecundity was highest for plants sourced from low elevation, and declined with increasing plant source elevation.

Table 3: Statistical results for the effects plant source elevation, watering treatment and herbivory treatment on fecundity as measured by number of fruits (siliques). Statistically significant results indicated by an "*".

	Chisq	Df	Pr(>Chisq)
(Intercept)	3.0812	1	0.07920 .
Initial plant size	0.8861	1	0.34652
Elevation	0.0950	1	0.75789
Herbivory	0.7541	1	0.38519
Watering	0.8924	1	0.34482
Elevation*Herbivory	0.5292	1	0.46696
Elevation*Watering	1.7742	1	0.18287
Herbivory*Watering	4.9988	1	0.02536 *
Elevation:Herbivory:Watering	4.4634	1	0.03463 *

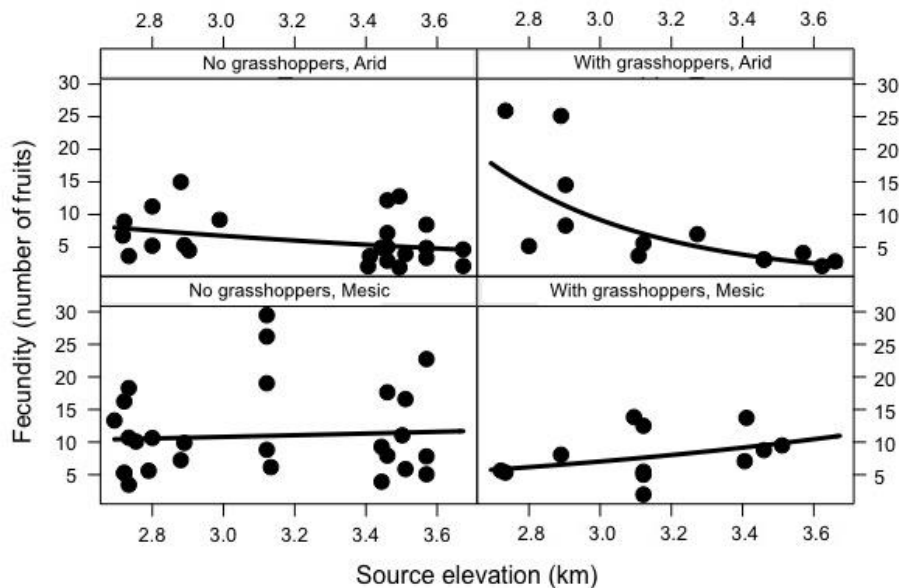


Fig. 5. Fecundity as measured by number of fruits(siliques) across plant source elevations for each of the four treatment combinations (no grasshoppers, arid; with grasshoppers, arid; no grasshoppers, mesic; with grasshoppers, mesic).

Discussion:

The goal of our research was to discover the combined effects of aridity and herbivory on the performance of plants sourced from (and adapted to) different elevations, and to test whether aridity and herbivory separately or together drive local adaptation. We found that only in the arid with herbivory treatment was there a significant effect of plant source elevation. This treatment combination simulated the low elevation environment of high aridity and herbivory and under these conditions plants from low elevations produced the highest numbers of fruits, and fruit number declined with increasing elevation. Accordingly, our results suggest that local adaptation to the elevational cline is driven by the combined effects of aridity and herbivory.

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Looking first at the analysis of leaf area removed by herbivory, we found that herbivory and aridity did not interact in affecting herbivore resistance; the aridity treatment did not change the amount that herbivores fed. Aridity could have affected plant fitness either directly or indirectly by mediating the amount of herbivore damage. So, this result showed that one potential mechanism by which aridity and herbivory could interact did not occur. Therefore, wetter or dryer environments will not likely affect the amount of herbivore damage experienced by *B. stricta* plants.

As predicted, there was a significant two-way interaction between herbivory and plant source elevation, with plants from higher elevations of origin receiving greater leaf damage. This replicates previous work (Anderson 2015, Descombes 2020) that showed there was an elevation gradient for plant resistance to herbivory with greater amounts of herbivore damage at higher elevations. This supports our prediction that lower herbivory at higher elevations would lead to less investment in plant defenses against herbivory by plants locally adapted to higher elevations. Therefore, as herbivores move up the elevation gradient as increasing temperatures make previously inhospitable environments increasingly accessible to herbivores (Descombes 2020) higher elevation plants that are not locally adapted to high levels of herbivory will likely experience greater levels of herbivore damage.

Moving on to examine plant fitness, the significant results that we found can provide a clue to how climate change may impact *B. stricta* populations through the drivers of local adaptations to combined aridity and high herbivory. While the treatments were not found to significantly affect the binary response of whether (or not) a plant reproduced, we did find a significant three-way interaction between elevation of origin, aridity, and herbivory in terms of fecundity of the plants that flowered: in one of the four treatment combinations - arid with grasshoppers - there was a significant effect of plant source elevation. This treatment combination simulated the low elevation environment of high aridity and herbivory. In this treatment (alone), plants from low elevations produced the highest numbers of fruits (siliques), and fruit number declined with increasing elevation. Accordingly, our results suggest that local adaptation to the elevational cline is driven by the combined effects of aridity and herbivory. Therefore, if a combination of aridity and herbivory move up the elevation gradient together, *B. stricta* plants adapted to higher elevations will likely have lower levels of fecundity than they have historically. However, herbivory and aridity may not necessarily move together in predictable ways. Herbivores may move upslope into more mesic rather than more arid environments thus making predicting the potential future for *B. stricta* and other plants more complicated.

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Understanding how plants have adapted to complex environments is especially important in the context of global change where historic correlations between aspects of a species environment may become decoupled as species find themselves in novel environments (Moreira 2018; Parmesan 2006). With climate change, herbivores have begun to move upslope as changes in temperature make higher elevations newly accessible. The consequences of this change can be unforeseen and catastrophic. Bark beetles moving into higher elevations resulted in devastating infestations, contributing to record-breaking forest fires throughout the western United States (Davis 2022; Schoennagel 2012; Bentz 2010). It is, therefore, essential, that we continue to investigate plant local adaptations to multiple biotic and abiotic factors as plants may be adapted to combinations of these factors rather than each of these factors individually as shown in our study.

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