

**Herbivory and water availability interact to shape the adaptive landscape in the perennial forb, *Boechera stricta***

M. Inam Jameel<sup>1</sup>, Lisa Duncan<sup>2</sup>, Kailen Mooney<sup>2,3</sup>, and Jill T. Anderson<sup>1,3-5</sup>

<sup>1</sup> Department of Genetics, University of Georgia, Athens, GA 30602 USA

<sup>2</sup> Department of Ecology and Evolutionary Biology, University of California, Irvine, CA 92697

<sup>3</sup> Rocky Mountain Biological Laboratory, Gothic, CO 81224 USA

<sup>4</sup> Odum School of Ecology, University of Georgia, Athens, GA 30602 USA

<sup>5</sup> Corresponding author: jta24@uga.edu

- **Data availability:** The data and code underlying this article are available in figshare <https://doi.org/10.6084/m9.figshare.25952857.v4>

- **Author contributions:** JA, KM, and MIJ conceived of the project. All authors established the first experiment, conducted manipulations of grasshoppers and water, and collected data. MIJ established the second experiment, did all pesticide applications, and collected data with the assistance of JA. JA and MIJ analyzed the data and wrote the first draft of the manuscript, which KM reviewed and revised.

- **Funding.** Funding for this study came from the National Science Foundation (DEB-1553408 to J.T.A), the National Institute of General Medical Sciences of the National Institutes of Health (T32GM007103 to M.I.J), the Jan and Kirby Alton Graduate Fellowship through the Department of Genetics at the University of Georgia (to M.I.J.), the Hunter Endowment and Snyder award through the Graduate Fellowship Committee of the Rocky Mountain Biological Laboratory (to M.I.J.).

- **Conflict of interest statement:** The authors declare no conflicts of interest.

- **Acknowledgments:** We thank Mike Boyd and Derek Denney for assistance in growing plants in the greenhouse and Samantha Day, Elena Hamann, Annette Marks, Emma Fetterly, Bronwyn Taylor, Racheal Eggleston and Nora Oviatt for aiding with data collection and manipulations in the field. Additionally, we would like to thank Megan DeMarche, Bob Schmitz, Andrea Sweigart, John Wares, Derek Denney, Mia Rochford, Kelly McCrum, Ephie Magige, Elizabeth Thomas, and Lillie Pennington for discussions and comments on a previous draft.

## Abstract

Abiotic and biotic factors interact to influence phenotypic evolution; however, identifying the causal agents of selection that drive the evolution and expression of traits remains challenging. In a field common garden, we manipulated water availability and herbivore abundance across three years, and evaluated clinal variation in functional traits and phenology, plasticity, local adaptation, and selection using diverse accessions of the perennial forb, *Boechera stricta*. Consistent with expectations, drought stress exacerbated damage from herbivores. Foliar traits exhibited greater plasticity than phenological traits, which displayed more consistent genetic clines. Water availability and herbivory interacted to exert selection, even on traits like flowering duration, which showed no clinal variation and limited plasticity. Furthermore, the direction of selection on specific leaf area in response to water availability mirrored the genetic cline and plasticity, suggesting that variation in water levels across the landscape influences the evolution of this trait. Finally, both herbivory and water availability likely contribute to local adaptation. This work emphasizes the additive and synergistic roles of abiotic and biotic factors in shaping phenotypic variation across environmental gradients.

**Keywords:** agent of selection, common garden, natural selection, cline, plasticity, phenology, herbivore resistance, water availability

Accepted

## Introduction

Species evolve in response to complex suites of interacting abiotic and biotic conditions. Phenotypic variation within and across natural populations arises from adaptation to past episodes of selection, plastic responses to the current environment, genotype by environment interactions, and neutral processes such as genetic drift (Trussell 2000; Keller et al. 2009; Kooyers et al. 2015). For species distributed along environmental gradients, adaptation to abiotic and biotic factors that vary continuously across the landscape can generate clinal variation in ecologically-relevant phenotypes (Huxley 1938; Kremer et al. 2014; Kooyers et al. 2017; O'Brien et al. 2019), which can arise from plasticity (Via and Lande 1985) or from evolutionary responses to variable selection (Kooyers et al. 2015). As agents of selection can co-vary across gradients, it is often difficult to disentangle the contributions of different abiotic and biotic factors to the evolution and expression of traits (Wade and Kalisz 1990; Pellissier et al. 2016; Sandel et al. 2021). Identifying which environmental drivers underlie plasticity, clinal variation and local adaptation can illuminate the processes that have contributed to the contemporary and historical evolutionary trajectories of populations (MacColl 2011), and generate more reliable predictions about trait expression and fitness in future climates (Wilczek et al. 2014; Hamann et al. 2021b; Gorton et al. 2022; Zettlemyer 2023).

The spatial and temporal grain of environmental variation can strongly influence the evolution of plasticity and local adaptation. In some habitats, individuals experience multiple conditions over their lifetimes or their progeny disperse into habitat types different from those of the parents; plasticity can be adaptive under this fine-grained temporal or spatial variation, as individuals can shift their phenotypes to match the environment (Baythavong 2011; Anderson et al. 2021). Plasticity does not always confer higher fitness; in some cases, plasticity is neutral or even represents a maladaptive response to stress (e.g., Van Kleunen et al. 2007; Baythavong 2011; Becker et al. 2022). One mechanism for examining the adaptive nature of plasticity is to test whether the direction of plasticity aligns with the direction of selection under specific conditions (Navarro et al. 2022). Furthermore, experimental manipulations can identify the specific abiotic and biotic factors that induce plasticity.

Under coarse-grained environmental variation, specialization to local environments can be advantageous. In this case, trait clines can evolve through a genetic response to divergent selection operating in different portions of the range (Montesinos-Navarro et al. 2011; Kooyers et al. 2014; Kremer et al. 2014; Anstett et al. 2015), which can generate local adaptation (Leimu and Fischer 2008; Gonzalo-Turpin and Hazard 2009). To examine whether phenotypic clines have a genetic basis, researchers grow accessions sourced from diverse populations in a single common environment, whether in the lab or the field (Stinchcombe et al. 2004; Woods et al. 2012; Kremer et al. 2014; Kooyers et al. 2015). However, the magnitude - or even the directionality - of these clines can vary

across test environments (Woods et al. 2012; Wadgyamar et al. 2017; De Kort et al. 2020). Some clines may not be present in benign laboratory conditions (Kellermann et al. 2015; Wadgyamar et al. 2017). Indeed, plants grown in the greenhouse often express trait values distinct from those in the field (Poorter et al. 2016). Thus, field studies are crucial for examining the eco-evolution of trait variation under natural conditions.

This study seeks to dissect the contributions of plasticity and local adaptation to phenotypic variation by manipulating two factors that shape trait expression and impose strong selection: water availability and herbivore abundance (Dorey and Schiestl 2022; Navarro et al. 2022). These two agents of selection vary across space and time (Kooyers et al. 2015, Nelson et al. 2019), which can lead to local adaptation (Garrido et al. 2012; Blumenthal et al. 2021) and plasticity in ecologically-relevant traits (Jordan et al. 2015, Descombes et al. 2020a, Lorts and Lasky 2020). For example, plants adapted to arid conditions coordinate leaf economic traits to pursue conservative strategies (Wright et al. 2004). At the same time, herbivores consume 5.3% of plant biomass annually averaged across ecosystems (Turcotte et al. 2014). In response to this ancient interaction (Labandeira and Currano 2013), plants have evolved defenses (e.g., Gong and Zhang 2014), which often come at the expense of investment in other life history functions, such as drought tolerance (e.g., Yin et al. 2023). Most scholarship has considered plant adaptation to aridity and herbivory separately, yet they are deeply intertwined (Lin et al. 2023). For example, many plant traits, such as specific leaf area, mediate responses to aridity and herbivores (Hauser 2014; Blumenthal et al. 2020). Furthermore, plant responses to drought directly affect herbivory. Herbivores can benefit from drought-stressed plants that allocate resources to maintain osmotic potential and survival instead of to anti-herbivore defenses (Gutbrodt et al. 2011; Bauerfeind and Fischer 2013). Thus, the degree and fitness consequences of herbivory can depend on the abiotic context (Rasmann et al. 2014; Moreira et al. 2018; Descombes et al. 2020b).

We evaluated the effects of these environmental factors using the forb, *Boechera stricta* [(Graham) Al-Shehbaz, Brassicaceae]. This species is native to western North America, where herbivore abundance (Nelson et al. 2019a,b) and aridity (Dunne et al. 2003; Körner 2007; Anderson and Wadgyamar 2020) both decline with elevation. The covariation of water availability and herbivore abundance across elevational gradients obscures their relative contributions to trait expression and evolution, necessitating multifactorial manipulations. In a multi-year field experiment, we tested three hypotheses (Table 1): 1) plasticity aligns with clinal trait variation; 2) water availability and herbivore abundance exert natural selection on ecologically-relevant traits; and 3) populations are locally adapted to water and herbivore pressure, such that plants perform best under conditions most similar to those experienced in their home sites. If the direction of a genetic cline (Fig. 1A) aligns with the direction of plasticity (Fig. 1B), plasticity could be adaptive (Eckhart et al. 2004; Gonzalo-Turpin and Hazard 2009; Ensing and Eckert 2019). Furthermore, if the direction of selection under a specific

manipulation accords with the direction of the genetic cline or plasticity, that agent of selection likely contributes to trait evolution (Fig. 1C). These hypotheses, in conjunction with elevational gradients in water availability and herbivore pressures, led to the specific predictions outlined in Table 1. For example, if drought limits the resources plants can allocate to defense, we expect damage from herbivores to be higher under water restriction (Gutbrodt et al. 2011; Jactel et al. 2012; Hamann et al. 2021a). In addition, climatic factors vary inter-annually in natural environments, which could generate different patterns of trait expression across years (Ensing and Eckert 2019; Ramirez-Parada et al. 2024). Therefore, we predict that **years with lower precipitation and higher herbivore abundance will induce trait values typically expressed in low elevation environments.** This experiment created environments that are rare in nature (e.g., mesic + high herbivory), enabling us to dissect the independent and joint effects of these agents of selection on phenotypic differentiation and local adaptation.

## Methods

### Study system:

***Boechera stricta* is a self-pollinating perennial forb, with populations distributed across broad elevational and latitudinal gradients in western North American mountains** (Lee and Mitchell-Olds 2013; Rushworth et al. 2022). This species displays clinal variation in defense against herbivores, such that plants sourced from high elevations incur more foliar damage than lower elevation genotypes in common gardens (Anderson et al. 2015). Furthermore, *B. stricta* exhibits adaptive genetic clines in flowering phenology, size at flowering, specific leaf area and water use efficiency (Wadgymar et al. 2017), with plants from high elevation environments flowering earlier at a shorter stature and maintaining higher specific leaf area and lower water-use efficiency. **Common garden experiments have documented co-gradient plasticity, with high elevation environments inducing shorter height at flowering, greater specific leaf area and reduced water-use efficiency** (Anderson and Gezon 2015, Wadgymar et al. 2017). **The relative contributions of herbivory and water availability to genetic clines and plasticity in traits remain unresolved.**

### Manipulation of water availability and herbivore abundance:

**To investigate plasticity, clinal variation, and selection, we manipulated water availability and herbivore abundance factorially in a common garden over three years (2021-2023).** We established the garden (38° 57.1203' N, 106° 59.4903' W) in a meadow at 2895 meters above sea level, near the Rocky Mountain Biological Laboratory (Colorado). Prior to the experiment, we collected seeds from naturally recruiting individuals in 36 populations (elevations: 2519 - 3673 m a.s.l., Fig. S1). To

homogenize maternal effects and create full sibling families of this inbred species, we grew field-collected seeds for at least one generation in the greenhouse. To maximize genetic and phenotypic diversity (Goudet and Büchi 2006; Blanquart et al. 2013), we included only one accession from each source population.

In January 2021, we sowed ~20 seeds of each accession in the lab, stratified them for two weeks in the dark, transplanted germinants into 3.8 cm diameter Stuewe and Sons Ray - Leach Cone- tainer (Tangent, OR, USA), and grew the plants in the greenhouse for four months. In June 2021, we transplanted the plants into the existing vegetation in the garden in randomized positions within experimental blocks (mean  $\pm$  standard deviation:  $4.8 \pm 2.5$  full siblings from the 36 accessions into each of the four treatment levels, N=658 plants total; Table S1). We measured the diameter of each individual prior to planting to use as a covariate in analyses (see also Table S1c and Fig. S4).

#### *Experimental manipulations:*

Immediately after transplanting in 2021, we installed six 1.8 m  $\times$  1.8 m  $\times$  1.8 m cages within the fenced-in garden, with 1-m-wide rows between the cages (Fig. S2). The cages were constructed with PVC pipe covered by Lumite mesh cloth with 8 x 8 strands/cm, zippered openings, and 30 cm buried flaps; these materials prevented insects from entering or exiting the cages. We inspected the integrity of the cages weekly, removed the cages in September of each year, and re-installed them in the following spring to prevent winter damage to the structures. In fall 2021 after removing the cages, we sunk metal flashing into 30 cm deep trenches around the perimeter of each cage to reduce the risk of gopher damage.

We focused our herbivore manipulation on grasshoppers (*Melanoplus* spp.), a dominant generalist herbivore in montane meadows (Descombes et al. 2017), and assigned each cage to either grasshopper removal or addition. We captured grasshoppers in the removal cages and in the meadow adjacent to the garden via sweep netting and released them into the addition cages (Table S2). Any other insects that were inadvertently captured during the sweep netting were released outside of the cages. This manipulation did not eliminate herbivory in the removal cages because we specifically targeted grasshoppers and did not reduce the abundance of other herbivores. In the addition cages, we added grasshoppers once per week in 2021 and 2022 and three times per week in 2023 which elevated grasshopper density above ambient levels (Table S2). Natural grasshopper density in and outside of the cages was lower in 2022 than the other years (Table S2).

We subdivided the cages into four blocks, with two blocks assigned to each water level: supplemental or restricted (Fig. S2A). For the supplemental water blocks, we supplied 1.5L of water



twice a week with a watering can early in the season (June – mid-July) when rain was infrequent. Once the monsoons began in mid- to late July, we ceased applications of supplemental water. Immediately before forecasted rain, in the restricted water blocks, we deployed rain out shelters constructed of polycarbonate sheets and angled so rain would runoff to the edge of the cage, away from experimental plants (Fig. S2C). We monitored soil moisture in all 24 blocks twice a week using a Hydrosense (Campbell Scientific, Logan, UT, USA); volumetric water content was significantly greater under supplemental watering than restricted watering across all three years ( $\chi^2=9.4$ , d.f= 1,  $p=0.0022$ , Fig. S3, Table S3).

#### *Fitness components and functional traits:*

During the growing season (June to September) from 2021 – 2023, we collected data on two fitness components: the probability of producing mature fruits and the total length of mature fruits (which is highly correlated with the number of viable seeds, Wadgyamar et al. 2017). *Boechera stricta* typically requires vernalization (exposure to non-freezing winter temperatures) to flower (Anderson et al. 2010); thus, it is not surprising that only three individuals flowered in 2021, as these transplants had not yet experienced vernalization. In all years, we measured foliar damage from arthropod herbivores. In 2022 and 2023, we also measured specific leaf area, leaf succulence, day of first flowering, flowering duration, and height at flowering. Natural populations of *B. stricta* exhibit phenotypic clines in these traits across elevation (Anderson and Gezon 2015; Anderson et al. 2015; Wadgyamar et al. 2017). Furthermore, herbivory (Descombes et al. 2020a; Dorey and Schiestl 2022) and water availability (Jactel et al. 2012; Lorts and Lasky 2020; Navarro et al. 2022; Kamps and Poelman 2024) can induce plasticity in these traits and shape their evolution.

We censused each plant for herbivory 2-3 times per growing season by first counting the total number of leaves, then counting the number of damaged leaves and estimating the amount of per-leaf damage visually. Only the first author and senior author quantified leaf damage, and we compared our estimates to ensure that they were similar. Experienced researchers assessing damage visually generate accurate and precise estimates of damage from herbivores (Johnson et al. 2016). We calculated the proportion of leaf area consumed by herbivores by multiplying the number of damaged leaves by the average per-leaf damage and dividing by the total number of leaves (Anderson et al. 2015). We sampled leaves for foliar traits in late July 2022 and 2023, which is approximately one month after first flowering and a point at which most leaves for the season have been produced. We collected 3-4 leaves on ice, scanned for leaf area on the same day, and weighed for fresh and dry biomass. We calculated specific leaf area by dividing leaf area (calculated using ilastik, Berg et al. 2019, and a custom python script) by dry leaf weight. We calculated succulence as: fresh leaf weight - dry leaf weight/ leaf area (Reimann and Breckle 1995).



Like many species, *B. stricta* produces basal rosette leaves and bolt leaves on reproductive stalks. When possible, we collected both types of leaves, but many plants did not bolt, and others senesced their rosette leaves after bolting. In our experiment, 68 plants had collections for bolt leaves only whereas 581 plants had data on rosette leaves. We calculated presumptive rosette leaf trait values for the 68 plants with only bolt leaves by regressing rosette specific leaf area and succulence on bolt leaf traits for the 42 plants for which we had both bolt and rosette collections (rosette specific leaf area =  $71.82 + 0.73 \times$  bolt specific leaf area,  $F_{1,40}=9.09$ ,  $p=0.0045$ ; rosette succulence =  $0.0024 + 0.56 \times$  bolt specific leaf area,  $F_{1,40}=67.15$ ,  $p<0.0001$ ). Models excluding these 68 plants produced nearly identical results.

We visited each plant 2-3 times a week to record the number of flowers and fruits, length of the longest fruit, and the height of bolts. For plants that flowered between censuses, we determined the day of first flowering from the fruit elongation rate (Table S4; see also Wadgymar et al. 2017). Models using the raw flowering data yielded quantitatively similar results. We calculated the duration of flowering by subtracting the first day of flowering from the day on which the plant ceased flowering. We measured plant height from the base of the plant to the apical meristem. In our analyses, we used the height of the tallest bolt at flowering, which is a reliable indicator of the tradeoff between size and timing of flowering (Wadgymar et al. 2017).

## Statistical Analyses

We employed a mixed effects repeated measures framework for all statistical models to analyze data collected across years. To evaluate the extent to which these traits can be treated independently, we examined correlations between traits (Table S5), and we assessed multicollinearity in our selection analyses. For all models, we examined the statistical significance of main effects using the Anova function of the R package car ver. 3.1-2 (Fox and Weisburg 2019) and we assessed normality and homoscedasticity of residuals using the simulateResiduals function of the R package DHARMa ver. 0.4.6 (Hartig and Lohse 2022). We visualized the regression results with the ggeffects R package ver. 1.3.2 (Lüdtke, 2018), extracted estimated marginal means using the emmeans function and regression coefficients and confidence intervals using the emtrends function from emmeans R package ver. 1.8.8 (Lenth et al. 2023).

### *Hypothesis 1: Concordance of plasticity and clinal trait variation:*

To test the hypothesis that plasticity aligns with clinal trait variation, we analyzed six traits as a function of source elevation, grasshopper treatment, and watering treatment: damage from herbivores, specific leaf area, foliar succulence, day of first flowering, flowering duration, and height at flowering. These analyses simultaneously modeled plasticity (trait response to treatment and temporal variation) and clines (trait response to source elevation). If plasticity aligns with clinal

variation (Table 1), our analyses would reveal significant effects of source elevation on trait expression, in the same direction as effects of water availability and herbivore abundance. **Significant interactions between treatments or year and source elevation would demonstrate that the magnitude or directionality of the cline itself depends on environmental conditions.**

Given that water availability (Fig. S3, Table S3) and herbivore abundance (Table S2) differed across years of this study, we first conducted generalized linear mixed models with main effects of water treatment, grasshopper treatment, source elevation, and year, along with all 2-, 3, and 4-way interactions, and random effects for plant identity, accession, and block nested within cage. However, the four-way interaction was never statistically significant. We then evaluated two reduced models for each trait, the first of which included main effects and 2-way interactions and the second also examined 3-way interactions. We used the `model.sel` function of the MuMIn R package ver. 1.48.4 (Bartón 2022) to contrast models using the corrected Akaike Information Criterion (AICc). When these two models generated similar results, we retained the model with 3-way interactions. **For the final models, we assessed statistical significance using an adjusted alpha of 0.0083 ( $=0.05/6$  traits) to account for the separate analyses from the same study.** We could not conduct one multivariate model of all traits, as the traits required different statistical distributions.

We analyzed specific leaf area, day of first flowering, flowering duration, and height of tallest stem at first flowering in generalized linear mixed models using a lognormal distribution with a log link, and leaf succulence using beta distribution with a logit link in the R package `glmmTMB` ver. 1.1.8 (Brooks et al. 2017). The proportion of leaf area removed by herbivores is the opposite of resistance to herbivory (herbivore resistance = 1 - leaf damage), as individuals that are well-defended and highly herbivore resistant will experience lower damage than their less resistant counterparts. We analyzed herbivore damage in a repeated measures generalized linear mixed model with a zero-inflated beta distribution and logit link (R package `glmmTMB` ver. 1.1.4 (Brooks et al. 2017). We included census nested within year as a random effect to account for repeated sampling of herbivore damage within and across years. As foliar damage is the only trait we measured in all years, this analysis included data from 2021-2023.

#### *Hypothesis 2: Water availability and herbivory impose selection:*

To examine how herbivore abundance and water conditions interact to impose selection, we conducted a two step hurdle approach first, analyzing **(1) how foliar traits (leaf damage averaged across all censuses within a year, specific leaf area, leaf succulence) influence the probability of reproduction (binomial distribution with logit link) and then analyzing (2) how foliar traits and reproductive phenology influence seed set (gamma distribution with log link).** Of the 358 plants that

died in 2022 and 2023, we only have a complete suite of foliar trait data for 21 individuals (one of which died after reproducing and is included with a value of 1 in our model of the probability of reproduction); thus, mortality preceded leaf collection for 94.1% of individuals, which restricts our ability to evaluate viability selection separately. Our analysis of the probability of reproduction modeled the 20 plants that died and the 526 records of living plants that failed to reproduce with values of 0, and the 101 records of successful reproduction with values of 1.

We assessed statistical significance using an adjusted alpha of 0.025 ( $=0.05/2$ ) to account for the two selection analyses. We used generalized linear mixed models in the R package glmmTMB ver. 1.1.4 (Brooks et al. 2017) to model fitness as a function of traits and all three-way interactions of each trait with herbivore and water treatments. We included initial size at planting and year as covariates, and modeled random effects for plant identity, accession, and block nested within cage. If plots of residual vs. predicted values suggested non-linear selection, we tested for stabilizing or disruptive selection by including a quadratic term for the relevant trait in the multivariate model. To evaluate potential multicollinearity of traits, we inspected variance inflation factors (VIF) using the check\_collinearity function of the R package performance ver. 0.12.2 (Lüdtke et al. 2021).

If water availability and herbivory exert selection (Table 1), analyses would show an interaction between manipulations and specific traits. Furthermore, if plasticity is adaptive, we predict that the direction of selection would match the direction of plasticity.

### *Hypothesis 3: Water availability and herbivory shape local adaptation:*

To evaluate agents of local adaptation, we analyzed the probability of reproduction and seed set jointly as a function of the three-way interactions of source elevation, herbivore abundance, and water availability, with a covariate for initial size at planting and random effects for plant identity, accession and block nested within cage. We focused on data from 2022 and 2023 here, as there was minimal variation in fitness in the first year of the study (2021) when survival was 98.7% (650 survived/658 planted) and only three plants reproduced. Over the course of the experiment, only 108 individuals flowered, leading to highly zero-inflated fitness values, as is often the case in field studies. Therefore, we employed a hurdle model using the zero-inflated gamma family (ziGamma) in the R package glmmTMB ver. 1.1.4 (Brooks et al. 2017). This framework simultaneously uses a binary distribution with a logit link to model the probability of reproduction and a Gamma regression with a log link to model seed set of individuals that successfully reproduced. If water availability and herbivore abundance contribute to local adaptation across the elevational gradient, analyses would uncover an interaction between the treatments and source elevation. Specifically, we predicted that

high elevation populations would have greater fitness under well-watered and grasshopper removal conditions, which reflects conditions in their home sites.

## Results

### Hypothesis 1: Concordance of plasticity and clinal trait variation:

*Herbivory:* The slope of the cline in foliar damage from herbivores varied in direction and magnitude in response to the grasshopper treatment across years (source elevation  $\times$  herbivore abundance  $\times$  year:  $\chi^2=26.4$ , d.f.=2,  $p<0.0001$ , Table S6, Fig. 2A). Foliar damage declined with source elevation in the herbivore addition treatment in 2021, which was contrary to predictions. As expected (Table 1), however, damage increased with source elevation in all four treatment combinations in 2022 and 2023, and the slope of this cline was significantly greater under grasshopper addition than removal (Table S6, Fig. 2A). Furthermore, foliar damage was significantly higher under restricted water than supplemental water conditions across all years and herbivore treatment levels (odds ratio  $\pm$  SE:  $1.19 \pm 0.62$ ;  $\chi^2=7.51$ , d.f.=1,  $p=0.0061$ , Fig. 2B).

*Specific leaf area:* Clinal variation in specific leaf area was apparent through the interaction of source elevation and year ( $\chi^2=12.48$ , d.f.= 1,  $p = 0.00041$ , Fig. 3A, Table S7). In 2022, specific leaf area increased by 27% for every 1-kilometer increase in source elevation (95% confidence interval: 16.28% to 37.31%, Table S7b), as expected (Table 1). No cline emerged in 2023. Supplemental watering induced higher specific leaf area ( $\chi^2=14.1$ , d.f.= 1,  $p = 0.00017$ , Fig. 3B); thus, the direction of plasticity was concordant with the cline and with predictions.

*Leaf succulence:* Discordant with predictions, we found no clinal variation in leaf succulence, and inconsistent plasticity (herbivore treatment  $\times$  year:  $\chi^2=34.48$ , d.f.= 1,  $p < 0.0001$ , Fig. 3F). Herbivore addition induced higher succulence in 2022 (z ratio = 2.84,  $p = 0.023$ ); however, this pattern reversed in 2023 (z ratio = -5.4,  $p < 0.0001$ ).

*Flowering phenology:* Source elevation interacted with water availability and herbivore abundance to influence the day of first flowering ( $\chi^2=7.3$ , d.f.= 1,  $p = 0.0069$ , Fig. 4A, Table S9). As expected (Table 1), flowering time decreased with source elevation under all treatment combinations except herbivore addition and restricted watering, for which we found no cline (Fig. 4A, Table S9b). Additionally, herbivore treatment interacted with year to shape the expression of flowering phenology ( $\chi^2=10.93$ , d.f.=1,  $p=0.00094$ ), with removal inducing earlier flowering in 2022 (z-ratio=3.58,  $p=0.002$ ) but not in 2023 (z-ratio = -1.6,  $p=0.38$ ).

*Flowering Duration:* We found significant spatio-temporal plasticity in flowering duration (Fig. 4D, 4E, Table S10), which varied as a function of interactions of water availability and herbivore abundance ( $\chi^2=7.49$ , d.f.=1,  $p=0.0062$ ) and water availability and year ( $\chi^2=7.99$ , d.f.=1,  $p=0.0047$ ). Restricted water shortened the duration of flowering under herbivore removal (Fig. 4E) and in 2023 (Table S10).

*Tallest stem at flowering:* For every 1-kilometer increase in source elevation, height at flowering decreased by 59.7% (95% confidence interval: 38.7%, 78.9%;  $\chi^2=10.77$ , d.f.= 1,  $p = 0.0001$ , Fig. 4G, Table S11). Water availability and grasshopper abundance did not induce plasticity in height (Fig. 4H). However, height at flowering was greater in 2023 than 2022 ( $\chi^2=16.87$ , d.f.= 1,  $p < 0.0001$ )

## Hypothesis 2: Water availability and herbivory impose selection on functional traits

*Selection on foliar traits via the probability of reproduction:* Stabilizing selection favored intermediate specific leaf area values across all four treatment combinations (quadratic effect of specific leaf area:  $\chi^2=6.09$ , d.f.= 3,  $p = 0.0136$ , Fig. 3C, Table S12). Context-dependent stabilizing selection operated on leaf succulence (Fig. 3G, Table S12b). Concordant with expectations, selection favored greater succulence in grasshopper addition than in removal cages (herbivore abundance  $\times$  succulence  $\chi^2=6.41$ , d.f.= 3,  $p = 0.011$ ; quadratic effect of succulence:  $\chi^2=19.68$ , d.f.= 3,  $p < 0.0001$ , Fig. 3G, Table S12). We found no evidence for selection via the probability of reproduction on leaf damage from herbivores (Table S12).

*Selection on six traits via seed set:* Context dependent selection emerged for leaf succulence in response to herbivore manipulation ( $\chi^2=5.26$ , d.f.= 1,  $p=0.0218$ , Fig. 3H, Table S13) and three other traits in response to water availability and herbivore abundance: specific leaf area (water availability  $\times$  herbivore abundance  $\times$  quadratic effect of specific leaf area;  $\chi^2=12.98$ , d.f.= 1,  $p = 0.0003$ , Fig. 3D); leaf damage from arthropod herbivores (water availability  $\times$  herbivore abundance  $\times$  quadratic effect of leaf damage:  $\chi^2=11.66$ , d.f.= 1,  $p=0.0006$ ; Fig. 2C); and flowering duration (water availability  $\times$  herbivore abundance  $\times$  flowering duration:  $\chi^2=24.61$ , d.f.= 1,  $p < 0.0001$ , water availability  $\times$  quadratic effect of flowering duration:  $\chi^2=17.62$ , d.f.= 1,  $p < 0.0001$ , and herbivore abundance  $\times$  quadratic effect of flowering duration:  $\chi^2=7.55$ , d.f.= 1,  $p = 0.0060$ ; Fig. 4F).

Directional selection under grasshopper addition favored increased succulence, as seed set increased by 40% for every 1 S.D. change in leaf succulence (95% CI: 20% to 66%; Fig. 3H), but there was no evidence for selection on succulence under grasshopper removal. Selection on specific leaf area generally accorded with predictions, as lower values were favored in dry conditions. For example, in the grasshopper addition, stabilizing selection favored low specific leaf area under

restricted water (optimum: 167.68 cm<sup>2</sup>/g), but directional selection favored increased trait values in supplemental water (seed set increased by 49.9% for every 1 SD unit change in specific leaf area; 95% CI: 31.6% to 63.4%). Under grasshopper removal, directional selection favored reduced specific leaf area in restricted water (seed set declined by 35.8% for every 1 S.D. change in the trait; 95% CI: 4.9%, 36.8%), but there was no evidence for selection under supplemental watering and grasshopper removal. We detected stabilizing selection on foliar damage from arthropod herbivores in one treatment only: supplemental water and grasshopper addition, in which seed set peaked at an average leaf damage of 7.9% (Fig. 2C, Table S13b).

We found complex patterns of selection on the duration of flowering. In herbivore addition, stabilizing selection favored an intermediate duration of flowering in the restricted water treatment (optimal duration: 25.9 days; Fig. 4F, Table S13b). In herbivore removal, stabilizing selection favored longer flowering duration under supplemental water (optimal duration: 26.3 days) and a shorter duration under restricted watering (optimal duration: 14 days).

Finally, across all treatment levels, directional selection favored both increased height at flowering ( $\chi^2=6.40$ , d.f= 1,  $p = 0.0114$ ), with seed set increasing by 41.4% for every 1 standard deviation increase in height (95% CI: 20.2%, 66.3%; Fig. 4I, Table S13b) and earlier flowering ( $\chi^2=7.10$ , d.f= 1,  $p = 0.0077$ ) with seed set decreasing by 42.1% for every 1 standard deviation increase in height (95% CI: 29.7%, 51.8%; Fig. 4I, Table S13b).

### Hypothesis 3: Water availability and grasshopper herbivory shape local adaptation

Seed set amongst individuals that successfully reproduced was strongly driven by interactions of water availability treatment and the quadratic effect of source elevation ( $\chi^2=5.71$ , d.f= 1,  $p =0.0169$ , Table S14). Consistent with local adaptation, high elevation elevated fitness under supplemental water, wherein the source elevation with optimal seed set occurs at 3242.76 m (Fig. 5). We found no evidence that the grasshopper treatment affected fitness. Initial plant size was the primary factor controlling the probability of reproduction ( $\chi^2=35.81$ , d.f= 1,  $p < 0.0001$ , Table S14).

## Discussion

Abiotic and biotic conditions co-vary across environmental gradients and contribute to the evolution and the maintenance of phenotypic variation (e.g., Kooyers et al. 2015). In our multifactorial field manipulation, we found evidence that clines depend upon the environmental context, plasticity can align with these clines, and water availability and the presence of a dominant generalist herbivore can impose selection on foliar traits and reproductive phenology. The concordance between the direction of clines, plastic shifts in trait expression, and context-dependent



patterns of selection for traits like specific leaf area suggest that (1) genetic clines likely evolved in response to variable selection across the landscape, and that (2) plasticity could be an adaptive response to finer grained temporal and spatial variation in conditions. For other trait, such as the height at first flowering, we found no evidence of plasticity but clear genetic clines and strong selection, which suggests that other agents of selection operating across the elevational gradient may drive the evolution of this trait. Studies that evaluate phenotypic clines should be careful to consider the environmental context, as the strength, direction and presence of clines can be influenced by the environment. Our investigations into the environmental drivers of phenotypic variation also captured local adaptation in response to water availability.

#### Concordance of clines, plasticity, and selection for foliar traits

The direction of plasticity can match the direction of clinal variation in a trait (i.e., co-gradient plasticity, Eckhart et al. 2004; Ensing and Eckert 2019), enabling researchers to identify environmental factors that influence both trait expression and evolution (Wade and Kalisz 1990). For example, accessions of *Artemisia californica* (Asteraceae) sourced from populations distributed across a precipitation gradient in California, USA, exhibit genetic clines in functional traits (Pratt and Mooney 2013). Additionally, supplemental watering, which resembles the climate of northern latitudes, induced greater foliar nitrogen, recapitulating the trait cline and demonstrating that water availability influences the evolution of both genetic clines and plasticity (Pratt and Mooney 2013). Likewise, in our study, plasticity in specific leaf area aligned with the elevational cline in this trait, as supplemental watering induced higher specific leaf area values (Fig. 3B, Table 1), reflecting the trait values of accessions from high elevation locations, which are more mesic than low elevation sites in this region (Anderson and Wadgymar, 2020).

High specific leaf area often evolves in wetter habitats, likely owing to greater photosynthetic and respiration rates (Wright et al. 2004; Poorter et al. 2009, 2019). In our study, the cline in specific leaf area only emerged in 2022 (Fig. 3A), which could have resulted from higher overall soil moisture content in that year compared to 2023 (Figs. 3A and S3). Previous studies of specific leaf area in *B. stricta* have leveraged temporal variation to reveal genetic clines in this trait only during benign years with higher snowfall (Wadgymar et al. 2017). In the Western U.S., decreasing snowpack and increasing growing season aridification (Pederson et al. 2011; Fyfe et al. 2017) could constrain the continued expression of clinal variation in traits such as specific leaf area.

Variation in abiotic and biotic conditions along environmental gradients likely impose complex patterns of selection on natural populations (Keller et al. 2009; Campitelli and Stinchcombe 2013; Kooyers and Olsen 2013; Muir and Angert 2017). In our study, selection on specific leaf area



via seed set aligned with expectations based on the leaf economic spectrum (Wright et al. 2004; Onoda et al. 2017), and with the cline (Fig. 3A) and the direction of plasticity (Fig. 3B). Selection via seed set for lower specific leaf area was driven more by restricted watering than grasshopper addition, even though lower specific leaf area is associated with greater leaf thickness and toughness (Wright and Cannon 2001; Cingolani et al. 2005), which may be adaptive in environments where herbivory is high (Zhu et al. 2024). In contrast, the analysis of the probability of reproduction revealed stabilizing selection. Indeed, the intermediate trait values favored via the probability of reproduction were higher than those favored in analyses of seed set for the restricted watering condition and lower than those favored under supplemental watering in herbivore addition. Thus, stabilizing selection via probability of reproduction could dampen divergent selection across the lifespan, leading to more similar optimal trait values across watering treatments.

Leaf succulence was subject to strong divergent selection. We expected selection to favor higher succulence in herbivore enriched environments, as leaves with higher succulence are difficult for insects to consume (Pérez-Harguindeguy et al. 2003; Moles et al. 2013). Concordant with predictions (Table 1), selection via the probability of reproduction favored increased succulence under herbivore addition relative to removal (Fig. 3G). Similarly, directional selection via seed set favored higher succulence in herbivore addition, despite the lack of seed set selection on this trait under herbivore removal (Fig. 3H). Selection via seed set could amplify the differences in optimal trait values across herbivore environments across the lifespan. The strength of divergent selection on this trait is in stark contrast with the lack of elevational clines (Fig. 3E) and lack of consistent patterns of plasticity (Fig. 3F). These discrepancies could suggest that other environmental factors control the evolution and expression of leaf succulence, or that genetic correlations across traits could constrain the adaptive response to selection (Etterson and Shaw 2001).

#### The evolution and expression of herbivore resistance

In this system, herbivore loads decline with elevation (Nelson et al. 2019b); thus, we predicted that low elevation accessions would experience the lowest foliar damage from insect herbivores across all treatments, owing to strong selection for anti-herbivore defenses in their home sites (Table 1). As expected, and concordant with a previous common garden study (Anderson et al. 2015), we found that damage from arthropod herbivores (the opposite of resistance to herbivory) increased with source elevation in all treatments (Fig. 2A), except in the year with the greatest water availability (2021). We propose that the observed cline in herbivore resistance reflects an evolutionary response to variable herbivore abundance across this elevational gradient. Furthermore, this cline was the most pronounced under grasshopper addition in 2023, the driest year of the study.

We expected plants under restricted watering to be poorly defended against herbivores (Table 1, Endara and Coley 2011; Jactel et al. 2012). Indeed, drought stress exacerbated the extent of foliar herbivory in our study (Fig. 2B). Similarly, in a reciprocal transplant experiment in Panama, seedlings of 13 tree species experienced greater herbivory in drier vs. wetter sites (Muehleisen et al. 2020). This increase in damage under restricted water availability could arise through reduced expression of anti-herbivore defenses during drought (Gutbrodt et al. 2011; Gely et al. 2020), perhaps owing to limited resources available for the production of defenses (Bauerfeind and Fischer 2013, Hamann et al. 2021a). With climate change, herbivores could track their climatic niches at a faster rate than plants through migration up mountain slopes (Schweiger et al. 2008; Rasmann et al. 2014; Becklin et al. 2016), which could heighten the extent of herbivore damage experienced by poorly-defended high elevation accessions. Increasing aridity with climate change (Pederson et al. 2011; Rangwala et al. 2012; Fyfe et al. 2017) could further augment herbivore damage (see also Hamann et al. 2021a), especially for high elevation accessions (Fig. 2A).

#### Reproductive phenology: Genetic clines, plasticity and selection

In line with predictions (Table 1), we observed a cline in flowering phenology, with low elevation accessions flowering later than high elevation accessions under supplemental watering (Fig. 4A). This result corresponds with other systems along elevational and latitudinal gradients (Montague et al. 2008; Monty and Mahy 2009; Kawakami et al. 2011; Ensing and Eckert 2019), but strikingly not with latitudinal clines in *Arabidopsis thaliana* (Stinchcombe et al. 2004). In other systems, flowering early can facilitate escape from drought stress (Franks et al. 2007; Rauschkolb et al. 2022) or herbivores (Sletvold et al. 2015). In our study, the magnitude of the cline varied across treatment combinations, as it was the steepest under herbivore addition and supplemental watering, equivalent across the two water availability treatment levels in herbivore removal, and non-existent under restricted watering and herbivore addition. That final treatment combination could reflect future conditions in this area under climate change, suggesting that climate change could reshape the evolution of this critical phenological trait. In one year of the study, herbivore addition delayed the timing of flowering, which could suggest that herbivorized plants have fewer resources available for rapid reproduction. Across all treatment combinations, selection favored earlier flowering, which is consistent with previous analyses of *Boechera stricta* (Wadgymar et al., 2017) and many other species (Austen et al. 2017). In sum, these results suggest that coarse-grained variation in water availability and herbivore abundance both contribute to the evolution of flowering time clines in this system, and that fine-grained variation in herbivory within a site can shape plasticity in this trait.

Height at flowering decreased with source elevation consistently across treatments (Fig. 4G), and directional selection favored taller plants at flowering, but herbivore abundance and water

availability did not influence the expression of this trait. Other factors, such as photoperiod and temperature, also control reproductive phenology (Rathcke and Lacey 1985; Li et al. 2018). In this system, complex seasonal dynamics, such as the extent of the snowpack and the timing of snowmelt, may be the primary selective agents driving the evolution of reproductive phenology (Wadgymar et al. 2018) and the size at first flowering.

Both water availability and herbivore abundance influenced the strength and direction of selection on flowering duration (Fig. 4F). Grasshopper removal favored shorter durations of flowering compared to grasshopper addition, in line with our predictions. The exact mechanism underlying herbivore-mediated selection on flowering duration remains unresolved. In some systems, herbivory can prolong flowering. For example, in a common garden, *Brassica rapa* (Brassicaceae) plants with the highest level of herbivory also had the longest duration of flowering (Austen and Weis 2015). However, that does not appear to be the case in this system, as plants under supplemental water in the herbivore removal flowered for the same duration as those in herbivore addition. Nevertheless, plasticity accorded with selection, as restricted watering under herbivore removal induced the shortest duration of flowering, and this treatment combination also had the shortest optimal flowering duration in the selection analyses. Thus, plasticity in the duration of flowering in response to local variation in water availability could confer a fitness advantage in this system. Furthermore, restricted water conditions favored shorter duration of flowering, even under grasshopper addition (Fig. 4F). Increased duration of flowering could expose sensitive floral and fruit tissue to prolonged drought, and reduced flowering duration could serve as a form of drought escape. Earlier snowmelt timing under climate change could increase exposure to drought stress (Blankinship et al. 2014; Sloat et al. 2015) and amplify herbivory, especially for high elevation populations (Rasmann et al. 2014). These shifts could result in novel patterns of selection on flowering duration across the range of *B. stricta* as climate change progresses.

We did not observe a cline in the duration of flowering (Fig. 4D), though a previous common garden experiment demonstrated that the duration of flowering decreased with source elevation in *B. stricta* (Anderson and Gezon 2015). If mesic conditions at high elevation underlay this cline and resulted in the shorter duration of flowering exhibited by high elevation accessions (Anderson and Gezon 2015), we would have expected supplemental water to favor shorter flowering duration. Instead, supplemental watering unmistakably favored longer flowering periods in both grasshopper addition and removal. We propose that limited growing season length at high elevations could control any reduction in flowering duration there.

Water availability and herbivore load contribute to local adaptation

Environmental differences across space can favor the evolution of local adaptation, especially when gene flow is limited (Kawecki and Ebert 2004; Leimu and Fischer 2008; Hereford 2009), but we rarely know which agents of selection local adaptation (Wade and Kalisz 1990; Wadgymar et al. 2022). If climatic factors drive adaptive population differentiation, rapid global change could disrupt local adaptation (Wilczek et al. 2014; Kooyers et al. 2019; Anderson and Wadgymar 2020). For example, during an atypically warm growing season, local accessions of *Erythranthe guttata* (Phrymaceae) were at a fitness disadvantage compared to those from lower latitudes where temperatures were historically higher (Kooyers et al. 2019). Previous experiments with *B. stricta* have revealed local adaptation to historical snowpack in the field (Anderson and Wadgymar 2020), to drought stress in the greenhouse (MacTavish and Anderson 2020), and to temperature and carbon dioxide concentration in growth chambers (Denney et al. 2024). However, the role of growing season water availability in local adaptation has remained difficult to isolate in the absence of manipulative field experiments. We found that supplemental watering augmented seed set for high elevation accessions (Fig. 5), which is consistent with expectations given that water availability increases with elevation (Nelson et al. 2019b; Anderson and Wadgymar 2020). Local populations that are adapted to historical soil moisture levels may lack the within population quantitative genetic variation necessary to respond rapidly to shifts in water availability under climate change (Hoffmann and Sgrò 2011; Derry et al. 2019; Christie et al. 2022).

Grasshopper herbivory did not modify fitness in a manner that reflects local adaptation, but the cline in foliar damage from herbivores (Fig. 2A) could result from local adaptation to differences in herbivore abundance across elevations. Similarly, Garrido et al. (2012) found that the annual plant *Datura stramonium* (Solanaceae) displayed patterns consistent with local adaptation to native vs. foreign accessions of a key herbivore (*Lema trilineata*, Chrysomelidae) in herbivore resistance, but they found no clear pattern of adaptation to local herbivores when considering plant fitness. We hypothesize that the growth-defense trade-off (e.g., Fine et al. 2006; Mooney et al. 2010; Hahn et al. 2019) drives the joint evolution of reduced herbivore resistance and early reproduction in high elevation populations. In contrast, high herbivore abundance at low elevations could increase allocation to defenses while delaying reproduction in the longer growing seasons of those sites. Finally, local adaptation emerged for seed set but not for the probability of reproduction, consistent with Hereford's (2009) finding that the extent of local adaptation depends upon the component of fitness that is measured in a study.

## Conclusion:

By manipulating two agents of selection that co-vary across elevational gradients, we found that genetic clines in traits can differ across environments, demonstrating that the abiotic and biotic context can shape the expression and magnitude of clines. We observed variable levels of plasticity across traits, highlighting that targets of selection often have different responses to the same environment conditions. Our selection analyses revealed the dual influence of water availability and herbivore abundance in exerting selection. We also detected signatures of local adaptation, implicating growing season water availability as an agent of local adaptation. Only a single trait, specific leaf area, showed concordance across clinal variation, the direction of plasticity, and the direction of selection (and only for one fitness component). Such alignment may arise when selective agents, like water availability, vary at a broad spatial scale across the gradient as well as temporally at a local scale across years. Plasticity could facilitate population persistence under climate change in the short term (Nicotra et al. 2010; Walter et al. 2023) if individuals can rapidly shift their phenotypes when exposed to novel abiotic and biotic factors. This region is predicted to experience increasing aridification under climate change (Talsma et al. 2022) and our results suggest that *B. stricta* individuals could respond via plastic shifts in some traits. Genetic clines in functional traits suggests that *B. stricta* could maintain the genetic variation necessary to confront novel climates, but that this variation likely does not exist within each local population. Those populations may face challenges in adapting to rapidly changing environments unless gene flow is high or assisted gene flow programs are implemented (Hufbauer et al. 2015; Hargreaves and Eckert 2018).

## Figure legends

**Figure 1: Conceptual diagram.** Hypothesis 1: Plastic responses to treatment align with clinal variation. A) We predict that variable selection across the landscape has driven the evolution of clinal variation in traits, which could be tested in a common garden environment using accessions sourced from populations inhabiting xeric and herbivore rich to mesic and herbivore sparse environments along a climatic gradient. B) Plasticity is concordant with phenotypic clines if traits shift in response to a specific manipulation or interannual variation to match the direction of the cline. Hypothesis 2: Water availability and herbivory impose selection on foliar and reproductive phenological traits. C) If the direction of selection matches the direction of clines and plasticity under experimental manipulation or interannual variation, then that agent of selection could underlie adaptive clinal variation and phenotypic plasticity.

**Figure 2: Clines and plasticity in foliar damage from herbivory, which is the opposite of herbivore resistance.** A) Herbivory increased with source elevation in all treatment combinations in 2022 and 2023, with the most pronounced cline under grasshopper addition. Contrary to predictions, foliar damage declined with source elevation under restricted watering and herbivore addition in the first year of the study (2021). Raw data points and significant regression lines are plotted for restricted watering (pink) and supplemental watering (blue) across herbivore treatment levels for each year. B) Leaf damage was significantly greater in the herbivore addition than herbivore removal and restricted watering increased damage in all years. Boxplots display data from restricted watering (pink) and supplemental watering (blue). Letters indicate significant pairwise differences across treatment levels after correction for multiple comparisons. C) Stabilizing selection via seed set favored low herbivore damage under herbivore addition and supplemental watering, but we detected no selection on this trait in other treatment combinations. Stars on the x axis represent averaged trait values for local accessions (collected <15 m from common garden site) in each treatment level.

**Figure 3: Clines, plasticity, and natural selection in specific leaf area and leaf succulence.** All panels show raw data points and significant regression lines and boxplots display data from all treatments (black), restricted watering (pink) and supplemental watering (blue) treatments or herbivore addition (dark purple) or herbivore removal (green) treatments. Letters indicate significant pairwise differences across treatments adjusted for multiple comparisons. The stars on the X axis indicate mean trait values for local accessions (originating from elevations of  $2891\text{m} \pm 15\text{m}$ ) under all treatments (gray), restricted watering (pink) and supplemental watering (blue) or herbivore addition (dark purple) or herbivore removal (green) treatments and are bracketed by  $2 \times$  the standard error. When there was no significant interaction with treatment, we plotted the raw data points and the regression line across all treatments in grayscale. **A)** Specific leaf area increased with source elevation, as predicted, in 2022 but not 2023. **B)** The direction of plasticity in specific leaf area was concordant with the cline, as supplemental watering induced greater trait values. **C)** Stabilizing selection via the probability of reproduction favored intermediate specific leaf area across environments. **D)** Directional selection via seed set favored reduced specific leaf area in restricted water conditions and greater values in supplemental watering under grasshopper addition. **E)** No cline emerged for leaf succulence. **F)** Succulence was significantly greater under herbivore addition than herbivore removal in 2022, but this trend switched in 2023. **G)** Stabilizing selection via the probability of reproduction favored higher values of leaf succulence under grasshopper addition than grasshopper removal. **H)** Directional selection via seed set for increased leaf succulence in grasshopper addition, but we did not detect selection in grasshopper removal.



**Figure 4: Clines, plasticity, and selection for reproductive phenology.** Across panels, we have plotted raw data points, significant regression lines, and box plots from all treatments (grayscale) restricted watering (pink), and supplemental watering (blue), with stars on the X axes of fitness landscapes representing the mean trait values for local accessions (source elevation:  $2891\text{m} \pm 15\text{m}$ ), bracketed by  $2 \times$  the standard error. When there was no significant interaction with treatment, we plotted the raw data points and the regression line across all treatments in grayscale. **A)** Flowering phenology declined with source elevation across three of the four treatment combinations. **B)** Herbivore removal induced earlier flowering in 2022. **C)** Selection via seed set favored earlier flowering under all treatment levels. **D)** No clinal variation emerged in flowering duration, yet **E)** restricted watering shortened the duration of flowering under herbivore removal. Similarly, **F)** stabilizing selection favored shorter flowering duration under restricted water conditions. **G)** Height of the tallest stem at flowering decreased with source elevation. **H)** Water availability and herbivore abundance did not influence the plastic expression of height of the tallest stem at flowering. **I)** Selection via seed set favored taller plants at flowering across all treatment levels.

**Figure 5: Fitness varies across water availability and source elevation.** Consistent with local adaptation, under supplemental water conditions, fecundity (total fruit length, which is a proxy for seed set) peaked for accessions from 3242.76 m, which is 352m above the elevation of the common garden. Shown are regression lines and raw data points from the conditional component of the hurdle model (i.e., seed set amongst individuals that successfully reproduced).



Table 1: Predictions and results for hypotheses 1 (clines and plasticity) and 2 (selection). We predict that the direction of plasticity will accord with genetic clines in traits, and that selection will favor suites of trait values that typically co-occur in low vs. high elevation ecotypes. For the reproductive phenology traits, the results for hypothesis 2 come from the analysis of selection via seed set.

	<i>H1: Concordance of plasticity and clinal trait variation</i>		<i>H2: Water availability and herbivory impose selection</i>	
Trait	Predictions	Results	Predictions	Results
Herbivore resistance	<u>Plasticity</u> : Lower under dry conditions with high herbivory (Gutbrodt et al. 2011; Jactel et al. 2012; Hamann et al. 2021a).	Matched predictions (Fig. 2B, Table S6b)	Dry conditions with high herbivory favor higher resistance against herbivory (Gutbrodt et al. 2011)	Probability of reproduction: No evidence of selection on herbivore damage (Table S12)
	<u>Genetic Cline</u> : Decreases with source elevation (Anderson et al. 2015) in dry environments with high herbivory	Decreased under herbivore addition, especially in 2023. No effect of water availability (Fig. 2A, Table S6)		Seed set: Directional selection for increased resistance under supplemental watering and herbivore addition (Fig. 2C, Table S13)
Specific Leaf Area	<u>Plasticity</u> : Lower under dry conditions (Onoda et al. 2017) with high herbivory (Cingolani et al. 2005).	Decreased under restricted watering, no effect of herbivore treatment (Fig. 3B, Table S7)	Dry conditions (Onoda et al. 2017) and high herbivory (Cingolani et al. 2005) favor lower specific leaf area	Probability of reproduction: Stabilizing selection for intermediate specific leaf area across all treatment levels (Fig. 3C, Table S12b)
	<u>Genetic Cline</u> : Increases with source elevation (Wadgymar et al 2017) when the conditions are dry with high herbivory or wet with low herbivory	Increased with source elevation in 2022, no shifts due to treatment (Fig. 3A, Table S7b)		Seed set: Accords with predictions (Fig. 3D, Table 13b)
Succulence	<u>Plasticity</u> : Lower under dry conditions (Kooyers et al. 2015) with high herbivory (Moles et al. 2013)	Increased under herbivore addition in 2022, but decreased in 2023, no effect of water availability (Fig. 3F, Table S8).	Dry conditions (Kooyers et al. 2015) and high	Probability of reproduction: Accords with predictions (Fig. 3G, Table S12b)

	<u>Genetic Cline</u> : Decreases with source elevation when the environment is dry with high herbivory or wet with low herbivory (Kooyers et al. 2015).	No cline detected (Fig. 3E, Table S8)	herbivory (Moles et al. 2013) favor increased succulence.	Seed set: Accords with predictions (Fig. 3H, Table S13b)
Flowering phenology	<u>Plasticity</u> : Will be delayed under dry conditions with high herbivory (Jordan et al. 2015)	Delayed flowering in 2022 under herbivore addition, no effect of water availability (Fig. 4B, Table S9)	Dry conditions and high herbivory favor delayed flowering (Jordan et al. 2015)	Directional selection for earlier flowering across all treatments (Fig. 4C, Table S13b)
	<u>Genetic Cline</u> : Declines with source elevation (Wadgyrmar et al. 2017) when the conditions are dry with high herbivory or wet with low herbivory	Declined with source elevation under all treatments except restricted watering and herbivore additions (Fig. 4A, Table S9b)		
Duration of flowering	<u>Plasticity</u> : Longer under conditions with high herbivory (Austen and Weis 2015)	Shorter duration of flowering under herbivore removal and water restriction (Fig. 4E, Table S10)	Dry conditions and high herbivory favor longer duration of flowering (Austen and Weis 2015)	Complex patterns of stabilizing selection. Stabilizing selection for intermediate duration under restricted watering and herbivore addition. Under herbivore removal, stabilizing selection for longer duration under supplemental watering and restricted watering favors shorter duration (Fig. 4F, Table S13b)
	<u>Genetic Cline</u> : Declines with source elevation (Anderson and Gezon 2015) when conditions are dry with high herbivory or wet with low herbivory	No cline detected (Fig. 4D, Table S10)		
Height at flowering	<u>Plasticity</u> : Shorter under dry conditions with high herbivory (Jordan et al. 2015)	No plasticity detected (Fig. 4G, Table S11)	Dry conditions and high herbivory favor shorter stature at flowering (Jordan et al. 2015)	Selection for increased height at flowering under all treatments (Fig. 4I, Table S13b)
	<u>Genetic Cline</u> : Declines with source elevation (Wadgyrmar et al. 2017) when conditions are	Declined with source elevation across all		

	either dry with high herbivory or wet with low herbivory	treatments (Fig. 4H, Table S11)		
--	--	---------------------------------	--	--

## References

- Anderson, J. T., and Z. J. Gezon. 2015. Plasticity in functional traits in the context of climate change: a case study of the subalpine forb *Boechera stricta* (Brassicaceae). *Glob Chang Biol* 21:1689–1703.
- Anderson, J. T., M. I. Jameel, and M. A. Geber. 2021. Selection favors adaptive plasticity in a long-term reciprocal transplant experiment. *Evolution* 75:1711–1726. Wiley.
- Anderson, J. T., N. Perera, B. Chowdhury, and T. Mitchell-Olds. 2015. Microgeographic Patterns of Genetic Divergence and Adaptation across Environmental Gradients in *Boechera stricta* (Brassicaceae). *Am Nat* 186 Suppl 1:S60-73.
- Anderson, J. T., and S. M. Wadgymar. 2020. Climate change disrupts local adaptation and favours upslope migration. *Ecol Lett* 23:181–192.
- Anstett, D. N., J. R. Ahern, J. Glinos, N. Nawar, J.-P. Salminen, and M. T. J. Johnson. 2015. Can genetically based clines in plant defence explain greater herbivory at higher latitudes? *Ecol. Lett.* 18:1376–1386.
- Austen, E. J., and A. E. Weis. 2015. What drives selection on flowering time? An experimental manipulation of the inherent correlation between genotype and environment. *Evolution* 69:2018–2033.
- Austen, E. J., L. Rowe, J. R. Stinchcombe, and J. R. K. Forrester. 2017. Explaining the apparent paradox of persistent selection for early flowering. *New Phytol.* 215:929–934.
- Bartón, K. 2022. MuMIn: Multi-Model Inference. R package.
- Bauerfeind, S. S., and K. Fischer. 2013. Testing the plant stress hypothesis: stressed plants offer better food to an insect herbivore. *Entomol. Exp. Appl.* 149:148–158.

- Baythavong, B. S. 2011. Linking the spatial scale of environmental variation and the evolution of phenotypic plasticity: selection favors adaptive plasticity in fine-grained environments. *Am Nat* 178:75–87.
- Becker, D., K. Barnard-Kubow, R. Porter, A. Edwards, E. Voss, A. P. Beckerman, and A. O. Bergland. 2022. Adaptive phenotypic plasticity is under stabilizing selection in *Daphnia*. *Nat Ecol Evol* 1–9. Nature Publishing Group.
- Becklin, K. M., J. T. Anderson, L. M. Gerhart, S. M. Wadgymar, C. A. Wessinger, and J. K. Ward. 2016. Examining Plant Physiological Responses to Climate Change through an Evolutionary Lens. *Plant Physiol* 172:635–649. American Society of Plant Biologists.
- Berg, S., D. Kutra, T. Kroeger, C. N. Straehle, B. X. Kausler, C. Haubold, M. Schiegg, J. Ales, T. Beier, M. Rudy, K. Eren, J. I. Cervantes, B. Xu, F. Beuttenmueller, A. Wolny, C. Zhang, U. Koethe, F. A. Hamprecht, and A. Kreshuk. 2019. ilastik: interactive machine learning for (bio)image analysis. *Nat. Methods* 16:1226–1232. Nature Publishing Group.
- Blankinship, J. C., M. W. Meadows, R. G. Lucas, and S. C. Hart. 2014. Snowmelt timing alters shallow but not deep soil moisture in the Sierra Nevada. *Water Resour. Res.* 50:1448–1456.
- Blanquart, F., O. Kaltz, S. L. Nuismer, and S. Gandon. 2013. A practical guide to measuring local adaptation. *Ecol Lett* 16:1195–1205.
- Blumenthal, D. M., K. E. Mueller, J. A. Kray, T. W. Ocheltree, D. J. Augustine, and K. R. Wilcox. 2020. Traits link drought resistance with herbivore defence and plant economics in semi-arid grasslands: The central roles of phenology and leaf dry matter content. *J. Ecol.* 108:2336–2351.
- Blumenthal, D. M., D. R. LeCain, L. M. Porensky, E. A. Leger, R. Gaffney, T. W. Ocheltree, and A. M. Pilmanis. 2021. Local adaptation to precipitation in the perennial grass *Elymus elymoides*: Trade-offs between growth and drought resistance traits. *Evol. Appl.* 14:524–535.
- Breheny, P., and W. Burchett. 2017. Visualization of Regression Models Using visreg. *R J.* 9:56–71.

- Campitelli, B. E., and J. R. Stinchcombe. 2013. Testing potential selective agents acting on leaf shape in *Ipomoea hederacea*: predictions based on an adaptive leaf shape cline. *Ecol. Evol.* 3:2409–2423.
- Christie, K., N. R. Pierson, D. B. Lowry, and L. M. Holeski. 2022. Local adaptation of seed and seedling traits along a natural aridity gradient may both predict and constrain adaptive responses to climate change. *Am J Bot*, doi: 10.1002/ajb2.16070. Wiley.
- Cingolani, A. M., G. Posse, and M. B. Collantes. 2005. Plant functional traits, herbivore selectivity and response to sheep grazing in Patagonian steppe grasslands. *J. Appl. Ecol.* 42:50–59.
- Cogni, R., and D. J. Futuyma. 2009. Local adaptation in a plant herbivore interaction depends on the spatial scale. *Biol. J. Linn. Soc.* 97:494–502.
- De Kort, H., B. Panis, K. Helsen, R. Douzet, S. B. Janssens, and O. Honnay. 2020. Pre-adaptation to climate change through topography-driven phenotypic plasticity. *J. Ecol.* 108:1465–1474.
- Denney, D., P. Patel, and J. T. Anderson. 2024. Elevated [CO<sub>2</sub>] and temperature augment gas exchange and shift the fitness landscape a montane forb. *New Phytol.*, doi: 10.1111/nph.19765.
- Derry, A. M., D. J. Fraser, S. P. Brady, L. Astorg, E. R. Lawrence, G. K. Martin, J. Matte, J. O. Negrín Dastis, A. Paccard, R. D. H. Barrett, L. J. Chapman, J. E. Lane, C. G. Ballas, M. Close, and E. Crispo. 2019. Conservation through the lens of (mal)adaptation: Concepts and meta- analysis. *Evol. Appl.* 12:1287–1304.
- Descombes, P., A. Kergunteuil, G. Glauser, S. Rasmann, and L. Pellissier. 2020a. Plant physical and chemical traits associated with herbivory in situ and under a warming treatment. *J. Ecol.* 108:733–749.

- Descombes, P., J. Marchon, J.-N. Pradervand, J. Bilat, A. Guisan, S. Rasmann, and L. Pellissier. 2017. Community-level plant palatability increases with elevation as insect herbivore abundance declines. *J. Ecol.* 105:142–151.
- Descombes, P., C. Pitteloud, G. Glauser, E. Defosse, A. Kergunteuil, P.-M. Allard, S. Rasmann, and L. Pellissier. 2020b. Novel trophic interactions under climate change promote alpine plant coexistence. *Science* 370:1469–1473.
- Dorey, T., and F. P. Schiestl. 2022. Plant phenotypic plasticity changes pollinator-mediated selection. *Evolution* 76:2930–2944. Wiley.
- Dunne, J. A., J. Harte, and K. J. Taylor. 2003. Subalpine Meadow Flowering Phenology Responses to Climate Change: Integrating Experimental and Gradient Methods. *Ecol. Monogr.* 73:69–86. Ecological Society of America.
- Eckhart, V. M., M. A. Geber, and C. M. McGuire. 2004. Experimental studies of adaptation in *Clarkia xantiana*. I. Sources of trait variation across a subspecies border. *Evol. Int. J. Org. Evol.* 58:59–70.
- Endara, M.-J., and P. D. Coley. 2011. The resource availability hypothesis revisited: a meta-analysis. *Funct. Ecol.* 25:389–398.
- Ensing, D. J., and C. G. Eckert. 2019. Interannual variation in season length is linked to strong co-gradient plasticity of phenology in a montane annual plant. *New Phytol* 224:1184–1200.
- Etterson, J. R., and R. G. Shaw. 2001. Constraint to adaptive evolution in response to global warming. *Science* 294:151–154.



- Fine, P. V. A., Z. J. Miller, I. Mesones, S. Irazuzta, H. M. Appel, M. H. H. Stevens, I. Sääksjärvi, J. C. Schultz, and P. D. Coley. 2006. The Growth–Defense Trade-Off and Habitat Specialization by Plants in Amazonian Forests. *Ecology* 87:S150–S162.
- Fox and Weisburg. 2019. *An R Companion to Applied Regression*, Third Edition. Sage, Thousand Oaks CA.
- Franks, S. J., S. Sim, and A. E. Weis. 2007. Rapid evolution of flowering time by an annual plant in response to a climate fluctuation. *Proc Natl Acad Sci U A* 104:1278–1282.
- Fyfe, J. C., C. Derksen, L. Mudryk, G. M. Flato, B. D. Santer, N. C. Swart, N. P. Molotch, X. Zhang, H. Wan, V. K. Arora, J. Scinocca, and Y. Jiao. 2017. Large near-term projected snowpack loss over the western United States. *Nat Commun* 8:14996.
- Garrido, E., G. Andraca-Gómez, and J. Fornoni. 2012. Local adaptation: simultaneously considering herbivores and their host plants. *New Phytol* 193:445–453.
- Gely, C., S. G. W. Laurance, and N. E. Stork. 2020. How do herbivorous insects respond to drought stress in trees? *Biol. Rev.* 95:434–448.
- Gong, B., and G. Zhang. 2014. Interactions between plants and herbivores: A review of plant defense. *Acta Ecol. Sin.* 34:325–336.
- Gonzalo-Turpin, H., and L. Hazard. 2009. Local adaptation occurs along altitudinal gradient despite the existence of gene flow in the alpine plant species *Festuca eskia*. *J. Ecol.* 97:742–751.
- Gorton, A. J., J. W. Benning, P. Tiffin, and D. A. Moeller. 2022. The spatial scale of adaptation in a native annual plant and its implications for responses to climate change. *Evolution*, doi: 10.1111/evo.14583.
- Goudet, J., and L. Büchi. 2006. The effects of dominance, regular inbreeding and sampling design on  $Q(ST)$ , an estimator of population differentiation for quantitative traits. *Genetics* 172:1337–1347.

- Gutbrodt, B., K. Mody, and S. Dorn. 2011. Drought changes plant chemistry and causes contrasting responses in lepidopteran herbivores. *Oikos* 120:1732–1740.
- Hahn, P. G., A. A. Agrawal, K. I. Sussman, and J. L. Maron. 2019. Population Variation, Environmental Gradients, and the Evolutionary Ecology of Plant Defense against Herbivory. *Am. Nat.* 193:20–34. The University of Chicago Press.
- Hamann, E., C. Blevins, S. J. Franks, M. I. Jameel, and J. T. Anderson. 2021a. Climate change alters plant-herbivore interactions. *New Phytol* 229:1894–1910.
- Hamann, E., D. Denney, S. Day, E. Lombardi, M. I. Jameel, R. MacTavish, and J. T. Anderson. 2021b. Review: Plant eco-evolutionary responses to climate change: Emerging directions. *Plant Sci* 304:110737. Elsevier.
- Hargreaves, A. L., and C. G. Eckert. 2018. Local adaptation primes cold-edge populations for range expansion but not warming-induced range shifts. *Ecol Lett*, doi: 10.1111/ele.13169.
- Hartig, F., and L. Lohse. 2022. DHARMA: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models.
- Hauser, M.-T. 2014. Molecular basis of natural variation and environmental control of trichome patterning. *Front. Plant Sci.* 5. Frontiers.
- Hereford, J. 2009. A quantitative survey of local adaptation and fitness trade-offs. *Am Nat* 173:579–588.
- Hoffmann, A. A., and C. M. Sgrò. 2011. Climate change and evolutionary adaptation. *Nature* 470:479–485. Nature Publishing Group.
- Hufbauer, R. A., M. Szűcs, E. Kasyon, C. Youngberg, M. J. Koontz, C. Richards, T. Tuff, and B. A. Melbourne. 2015. Three types of rescue can avert extinction in a changing environment. *Proc. Natl. Acad. Sci.* 112:10557–10562. Proceedings of the National Academy of Sciences.

Huxley, J. S. 1938. Clines: An Auxiliary Method in Taxonomy. *Bijdr. Tot Dierkd.* 27:491–520. Brill.

Jactel, H., J. Petit, M.-L. Desprez-Loustau, S. Delzon, D. Piou, A. Battisti, and J. Koricheva. 2012.

Drought effects on damage by forest insects and pathogens: a meta-analysis. *Glob. Change Biol.* 18:267–276.

Johnson, M. T. J., J. A. Bertrand, and M. M. Turcotte. 2016. Precision and accuracy in quantifying herbivory. *Ecol Entomol* 41:112–121. John Wiley & Sons, Ltd (10.1111).

Jordan, C. Y., D. Ally, and K. A. Hodgins. 2015. When can stress facilitate divergence by altering time to flowering? *Ecol. Evol.* 5:5962–5973.

Kamps, B. B. J., and E. H. Poelman. 2024. Adaptations to water gradient in three *Rorippa* plant species correspond with plant resistance against insect herbivory under drought and waterlogged conditions. *Ecol. Entomol.* 49:1–9.

Kawakami, T., T. J. Morgan, J. B. Nippert, T. W. Ocheltree, R. Keith, P. Dhakal, and M. C. Ungerer. 2011. Natural selection drives clinal life history patterns in the perennial sunflower species, *Helianthus maximiliani*. *Mol. Ecol.* 20:2318–2328.

Kawecki, T. J., and D. Ebert. 2004. Conceptual issues in local adaptation. *Ecol Lett* 7:1225–1241.

Keller, S. R., D. R. Sowell, M. Neiman, L. M. Wolfe, and D. R. Taylor. 2009. Adaptation and colonization history affect the evolution of clines in two introduced species. *New Phytol.* 183:678–690.

Kellermann, V., A. A. Hoffmann, T. N. Kristensen, N. N. Moghadam, and V. Loeschke. 2015.

Experimental Evolution under Fluctuating Thermal Conditions Does Not Reproduce Patterns of Adaptive Clinal Differentiation in *Drosophila melanogaster*. *Am. Nat.* 186:582–593. The University of Chicago Press.

- Kooyers, N. J., B. K. Blackman, and L. M. Holeski. 2017. Optimal defense theory explains deviations from latitudinal herbivory defense hypothesis. *Ecology* 98:1036–1048.
- Kooyers, N. J., J. M. Colicchio, A. B. Greenlee, E. Patterson, N. T. Handloser, and B. K. Blackman. 2019. Lagging Adaptation to Climate Supersedes Local Adaptation to Herbivory in an Annual Monkeyflower. *Am Nat* 000–000. The University of Chicago Press.
- Kooyers, N. J., L. R. Gage, A. Al-Lozi, and K. M. Olsen. 2014. Aridity shapes cyanogenesis cline evolution in white clover (*Trifolium repens* L.). *Mol. Ecol.* 23:1053–1070.
- Kooyers, N. J., A. B. Greenlee, J. M. Colicchio, M. Oh, and B. K. Blackman. 2015. Replicate altitudinal clines reveal that evolutionary flexibility underlies adaptation to drought stress in annual *Mimulus guttatus*. *New Phytol.* 206:152–165.
- Kooyers, N. J., and K. M. Olsen. 2013. Searching for the bull’s eye: agents and targets of selection vary among geographically disparate cyanogenesis clines in white clover (*Trifolium repens* L.). *Heredity* 111:495–504. Nature Publishing Group.
- Koricheva, J. 2002. Meta-Analysis of Sources of Variation in Fitness Costs of Plant Antiherbivore Defenses. *Ecology* 83:176–190.
- Körner, C. 2007. The use of ‘altitude’ in ecological research. *Trends Ecol. Evol.* 22:569–574.
- Kremer, A., B. M. Potts, and S. Delzon. 2014. Genetic divergence in forest trees: understanding the consequences of climate change. *Funct. Ecol.* 28:22–36.
- Labandeira, C. C., and E. D. Currano. 2013. The Fossil Record of Plant-Insect Dynamics. *Annu Rev Earth Planet Sci* 41:287–311. Annual Reviews.
- Lee, C.-R., and T. Mitchell-Olds. 2013. Complex trait divergence contributes to environmental niche differentiation in ecological speciation of *Boechera stricta*. *Mol Ecol* 22:2204–2217. Wiley.

- Leimu, R., and M. Fischer. 2008. A meta-analysis of local adaptation in plants. *PLoS One* 3:e4010.
- Li, X., T. Guo, Q. Mu, X. Li, and J. Yu. 2018. Genomic and environmental determinants and their interplay underlying phenotypic plasticity. *Proc Natl Acad Sci U S A* 115:6679–6684.
- Lin, P.-A., J. Kansman, W.-P. Chuang, C. Robert, M. Erb, and G. W. Felton. 2023. Water availability and plant–herbivore interactions. *J. Exp. Bot.* 74:2811–2828.
- Lorts, C. M., and J. R. Lasky. 2020. Competition  $\times$  drought interactions change phenotypic plasticity and the direction of selection on *Arabidopsis* traits. *New Phytol.* 227:1060–1072.
- Lüdecke, D., M. Ben-Shachar, I. Patil, P. Waggoner, and D. Makowski. 2021. performance: An R Package for Assessment, Comparison and Testing of Statistical Models. *J. Open Source Softw.* 6:3139.
- MacColl, A. D. C. 2011. The ecological causes of evolution. *Trends Ecol. Evol.* 26:514–522. Elsevier.
- MacTavish, R., and J. T. Anderson. 2020. Resource availability alters fitness trade-offs: implications for evolution in stressful environments. *Am J Bot* 107:308–318.
- Moles, A. T., B. Peco, I. R. Wallis, W. J. Foley, A. G. B. Poore, E. W. Seabloom, P. A. Vesk, A. J. Bisigato, L. Cella-Pizarro, C. J. Clark, P. S. Cohen, W. K. Cornwell, W. Edwards, R. Ejrnæs, T. Gonzales-Ojeda, B. J. Graae, G. Hay, F. C. Lumbwe, B. Magaña-Rodríguez, B. D. Moore, P. L. Peri, J. R. Poulsen, J. C. Stegen, R. Veldtman, H. von Zeipel, N. R. Andrew, S. L. Boulter, E. T. Borer, J. H. C. Cornelissen, A. G. Farji-Brener, J. L. DeGabriel, E. Jurado, L. A. Kyhn, B. Low, C. P. H. Mulder, K. Reardon-Smith, J. Rodríguez-Velázquez, A. De Fortier, Z. Zheng, P. G. Blendinger, B. J. Enquist, J. M. Facelli, T. Knight, J. D. Majer, M. Martínez-Ramos, P. McQuillan, and F. K. C. Hui. 2013. Correlations between physical and chemical defences in plants: tradeoffs, syndromes, or just many different ways to skin a herbivorous cat? *New Phytol.* 198:252–263.

- Montague, J. L., S. C. H. Barrett, and C. G. Eckert. 2008. Re- establishment of clinal variation in flowering time among introduced populations of purple loosestrife (*Lythrum salicaria*, Lythraceae). *J. Evol. Biol.* 21:234–245.
- Montesinos-Navarro, A., J. Wig, F. Xavier Pico, and S. J. Tonsor. 2011. *Arabidopsis thaliana* populations show clinal variation in a climatic gradient associated with altitude. *New Phytol.* 189:282–294.
- Monty, A., and G. Mahy. 2009. Clinal differentiation during invasion: *Senecio inaequidens* (Asteraceae) along altitudinal gradients in Europe. *Oecologia* 159:305–315.
- Mooney, K. A., R. Halitschke, A. Kessler, and A. A. Agrawal. 2010. Evolutionary Trade-Offs in Plants Mediate the Strength of Trophic Cascades. *Science* 327:1642–1644. American Association for the Advancement of Science.
- Moreira, X., W. K. Petry, K. A. Mooney, S. Rasmann, and L. Abdala-Roberts. 2018. Elevational gradients in plant defences and insect herbivory: recent advances in the field and prospects for future research. *Ecography* 41:1485–1496.
- Muehleisen, A. J., B. M. J. Engelbrecht, F. A. Jones, E. Manzané-Pinzón, and L. S. Comita. 2020. Local adaptation to herbivory within tropical tree species along a rainfall gradient. *Ecology* 101:e03151.
- Muir, C. D., and A. L. Angert. 2017. Grow with the flow: a latitudinal cline in physiology is associated with more variable precipitation in *Erythranthe cardinalis*. *J. Evol. Biol.* 30:2189–2203.
- Navarro, J., J. M. Powers, A. Paul, and D. R. Campbell. 2022. Phenotypic plasticity and selection on leaf traits in response to snowmelt timing and summer precipitation. *New Phytol.* doi:10.1111/nph.18084. Wiley.

- Nelson, A. S., R. T. Pratt, J. D. Pratt, R. A. Smith, C. T. Symanski, C. Prenot, and K. A. Mooney. 2019a. Progressive sensitivity of trophic levels to warming underlies an elevational gradient in ant–aphid mutualism strength. *Oikos* 128:540–550.
- Nelson, A. S., C. T. Symanski, M. J. Hecking, and K. A. Mooney. 2019b. Elevational cline in herbivore abundance driven by a monotonic increase in trophic-level sensitivity to aridity. *J Anim Ecol* 88:1406–1416.
- Nicotra, A. B., O. K. Atkin, S. P. Bonser, A. M. Davidson, E. J. Finnegan, U. Mathesius, P. Poot, M. D. Purugganan, C. L. Richards, F. Valladares, and M. van Kleunen. 2010. Plant phenotypic plasticity in a changing climate. *Trends Plant Sci* 15:684–692. Elsevier Ltd.
- O’Brien, A. M., R. J. H. Sawers, S. Y. Strauss, and J. Ross-Ibarra. 2019. Adaptive phenotypic divergence in an annual grass differs across biotic contexts\*. *Evolution* 73:2230–2246.
- Onoda, Y., I. J. Wright, J. R. Evans, K. Hikosaka, K. Kitajima, Ü. Niinemets, H. Poorter, T. Tosens, and M. Westoby. 2017. Physiological and structural tradeoffs underlying the leaf economics spectrum. *New Phytol.* 214:1447–1463.
- Pederson, G. T., S. T. Gray, C. A. Woodhouse, J. L. Betancourt, D. B. Fagre, J. S. Littell, E. Watson, B. H. Luckman, and L. J. Graumlich. 2011. The unusual nature of recent snowpack declines in the North American cordillera. *Science* 333:332–335.
- Pellissier, L., X. Moreira, H. Danner, M. Serrano, N. Salamin, N. M. van Dam, and S. Rasmann. 2016. The simultaneous inducibility of phytochemicals related to plant direct and indirect defences against herbivores is stronger at low elevation. *J. Ecol.* 104:1116–1125.
- Pérez-Harguindeguy, N., S. Díaz, F. Vendramini, J. H. C. Cornelissen, D. E. Gurvich, and M. Cabido. 2003. Leaf traits and herbivore selection in the field and in cafeteria experiments. *Austral Ecol.* 28:642–650.



- Poorter, H., F. Fiorani, R. Pieruschka, T. Wojciechowski, W. H. van der Putten, M. Kleyer, U. Schurr, and J. Postma. 2016. Pampered inside, pestered outside? Differences and similarities between plants growing in controlled conditions and in the field. *New Phytol.* 212:838–855.
- Poorter, H., Ü. Niinemets, N. Ntagkas, A. Siebenkäs, M. Mäenpää, S. Matsubara, and Thijs L. Pons. 2019. A meta-analysis of plant responses to light intensity for 70 traits ranging from molecules to whole plant performance. *New Phytol.* 223:1073–1105.
- Poorter, H., Ü. Niinemets, L. Poorter, I. J. Wright, and R. Villar. 2009. Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. *New Phytol.* 182:565–588.
- Pratt, J. D., and K. A. Mooney. 2013. Clinal adaptation and adaptive plasticity in *Artemisia californica*: implications for the response of a foundation species to predicted climate change. *Glob Chang Biol* 19:2454–2466.
- R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Ramirez-Parada, T. H., I. W. Park, S. Record, C. C. Davis, A. M. Ellison, and S. J. Mazer. 2024. Plasticity and not adaptation is the primary source of temperature-mediated variation in flowering phenology in North America. *Nat. Ecol. Evol.* 8:467–476.
- Rangwala, I., J. Barsugli, K. Cozzetto, J. Neff, and J. Prairie. 2012. Mid-21st century projections in temperature extremes in the southern Colorado Rocky Mountains from regional climate models. *Clim. Dyn.* 39:1823–1840.
- Rasman, S., L. Pellissier, E. Defossez, H. Jactel, and G. Kunstler. 2014. Climate-driven change in plant-insect interactions along elevation gradients. *Funct Ecol* 28:46–54.
- Rathcke, B., and E. P. Lacey. 1985. Phenological Patterns of Terrestrial Plants. *Annu. Rev. Ecol. Evol. Syst.* 16:179–214. Annual Reviews.

- Rauschkolb, R., Z. Li, S. Godefroid, L. Dixon, W. Durka, M. Májeková, O. Bossdorf, A. Ensslin, and J. F. Scheepens. 2022. Evolution of plant drought strategies and herbivore tolerance after two decades of climate change. *New Phytol*, doi: 10.1111/nph.18125. Wiley.
- Reimann, C., and S.-W. Breckle. 1995. Salt tolerance and ion relations of *Salsola kali* L.: differences between ssp. *tragus* (L.) Nyman and ssp. *ruthenica* (Iljin) Soó. *New Phytol.* 130:37–45.
- Rushworth, C. A., M. R. Wagner, T. Mitchell-Olds, and J. T. Anderson. 2022. The *Boechera* model system for evolutionary ecology. *Am J Bot* 109:1939–1961.
- Sandel, B., C. Pavelka, T. Hayashi, L. Charles, J. Funk, F. W. Halliday, G. S. Kandlikar, A. R. Kleinhesselink, N. J. B. Kraft, L. Larios, T. Madsen-McQueen, and M. J. Spasojevic. 2021. Predicting intraspecific trait variation among California's grasses. *J. Ecol.* 109:2662–2677.
- Schweiger, O., J. Settele, O. Kudrna, S. Klotz, and I. Kühn. 2008. Climate change can cause spatial mismatch of trophically interacting species. *Ecology* 89:3472–3479.
- Sletvold, N., K. K. Moritz, and J. Ågren. 2015. Additive effects of pollinators and herbivores result in both conflicting and reinforcing selection on floral traits. *Ecology* 96:214–221.
- Sloat, L. L., A. N. Henderson, C. Lamanna, and B. J. Enquist. 2015. The Effect of the Foresummer Drought on Carbon Exchange in Subalpine Meadows. *Ecosystems* 18:533–545.
- Stinchcombe, J. R., C. Weinig, M. Ungerer, K. M. Olsen, C. Mays, S. S. Halldorsdottir, M. D. Purugganan, and J. Schmitt. 2004. A latitudinal cline in flowering time in *Arabidopsis thaliana* modulated by the flowering time gene *FRIGIDA*. *Proc Natl Acad Sci U A* 101:4712–4717.
- Talsma, C. J., K. E. Bennett, and V. V. Vesselinov. 2022. Characterizing Drought Behavior in the Colorado River Basin Using Unsupervised Machine Learning. *Earth Space Sci.* 9:e2021EA002086.
- Trussell, G. C. 2000. Phenotypic clines, plasticity, and morphological trade-offs in an intertidal snail. *Evolution* 54:151–166.

- Turcotte, M. M., T. J. Davies, C. J. M. Thomsen, and M. T. J. Johnson. 2014. Macroecological and macroevolutionary patterns of leaf herbivory across vascular plants. *Proc. R. Soc. B Biol. Sci.* 281:20140555. Royal Society.
- Van Kleunen, M., J. P. M. Lenssen, M. Fischer, and H. De Kroon. 2007. Selection on phenotypic plasticity of morphological traits in response to flooding and competition in the clonal shore plant *Ranunculus reptans*. *J. Evol. Biol.* 20:2126–2137.
- Via, S., and R. Lande. 1985. Genotype-environment interaction and the evolution of phenotypic plasticity. *Evolution* 39:505–522.
- Wade, M. J., and S. Kalisz. 1990. THE CAUSES OF NATURAL SELECTION. *Evolution* 44:1947–1955.
- Wadgyman, S. M., S. C. Daws, and J. T. Anderson. 2017. Integrating viability and fecundity selection to illuminate the adaptive nature of genetic clines: Stabilizing selection on functional traits. *Evol. Lett.* 1:26–39.
- Wadgyman, S. M., M. L. DeMarche, E. B. Josephs, S. N. Sheth, and J. T. Anderson. 2022. Local Adaptation: Causal Agents of Selection and Adaptive Trait Divergence. *Annu. Rev. Ecol. Evol. Syst.* 53:87–111. Annual Reviews.
- Wadgyman, S. M., J. E. Ogilvie, D. W. Inouye, A. E. Weis, and J. T. Anderson. 2018. Phenological responses to multiple environmental drivers under climate change: insights from a long-term observational study and a manipulative field experiment. *New Phytol* 218:517–529.
- Walter, G. M., J. Clark, D. Terranova, S. Cozzolino, A. Cristaudo, S. J. Hiscock, and J. Bridle. 2023. Hidden genetic variation in plasticity provides the potential for rapid adaptation to novel environments. *New Phytol*, doi: 10.1111/nph.18744. Wiley.
- Wickham, H. 2016. *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York.

- Wilczek, A. M., M. D. Cooper, T. M. Korves, and J. Schmitt. 2014. Lagging adaptation to warming climate in *Arabidopsis thaliana*. *Proc Natl Acad Sci U S A* 111:7906–7913.
- Woods, E. C., A. P. Hastings, N. E. Turley, S. B. Heard, and A. A. Agrawal. 2012. Adaptive geographical clines in the growth and defense of a native plant. *Ecol. Monogr.* 82:149–168.
- Wright, I. J., and K. Cannon. 2001. Relationships between leaf lifespan and structural defences in a low-nutrient, sclerophyll flora. *Funct. Ecol.* 15:351–359.
- Wright, I. J., P. B. Reich, M. Westoby, D. D. Ackerly, Z. Baruch, F. Bongers, J. Cavender-Bares, T. Chapin, J. H. C. Cornelissen, M. Diemer, J. Flexas, E. Garnier, P. K. Groom, J. Gulias, K. Hikosaka, B. B. Lamont, T. Lee, W. Lee, C. Lusk, J. J. Midgley, M.-L. Navas, U. Niinemets, J. Oleksyn, N. Osada, H. Poorter, P. Poot, L. Prior, V. I. Pyankov, C. Roumet, S. C. Thomas, M. G. Tjoelker, E. J. Veneklaas, and R. Villar. 2004. The worldwide leaf economics spectrum. *Nature* 428:821–827.
- Yin, W., L. Zhou, K. Yang, J. Fang, A. Biere, R. M. Callaway, M. Wu, H. Yu, Y. Shi, and J. Ding. 2023. Rapid evolutionary trade-offs between resistance to herbivory and tolerance to abiotic stress in an invasive plant. *Ecol. Lett.* 26:942–954.
- Zettlemoyer, M. A. 2023. A demographic approach for predicting population responses to multifactorial stressors. *AoB PLANTS* 15:plad023.
- Zhu, J., L. Sun, C. He, Q. Cai, J. Zhu, and C. Ji. 2024. Large and thin leaves are compromised more by chewers. *Ecosphere* 15:e4748.

Figure 1

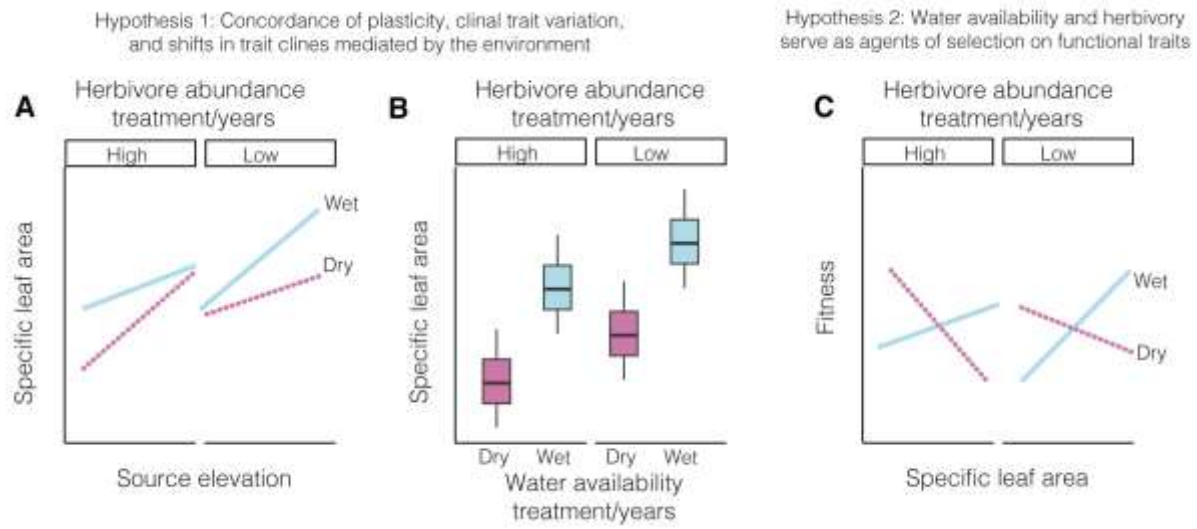


Figure 2

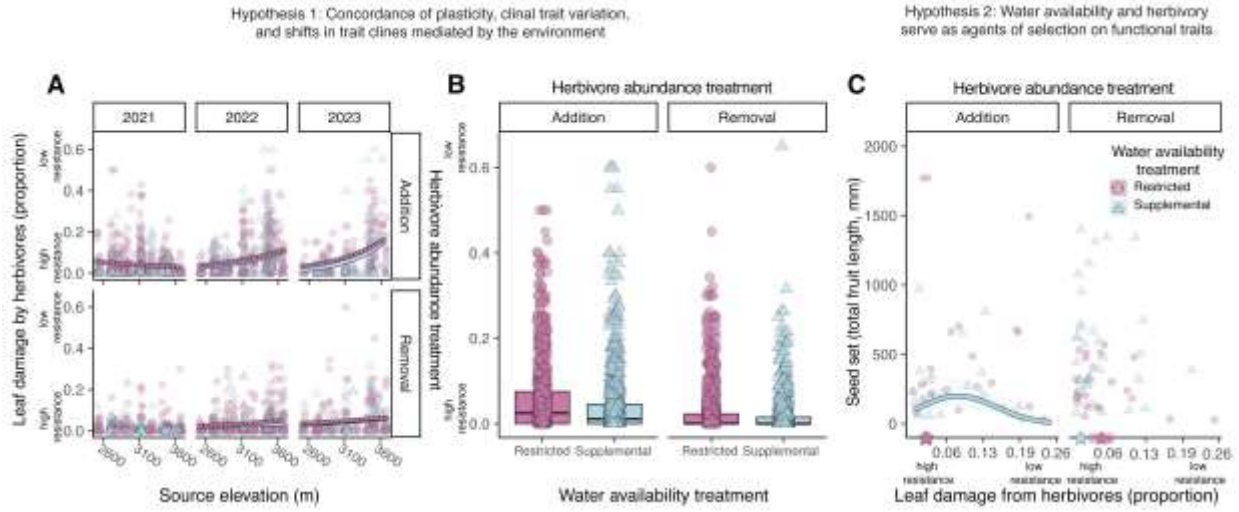


Figure 3

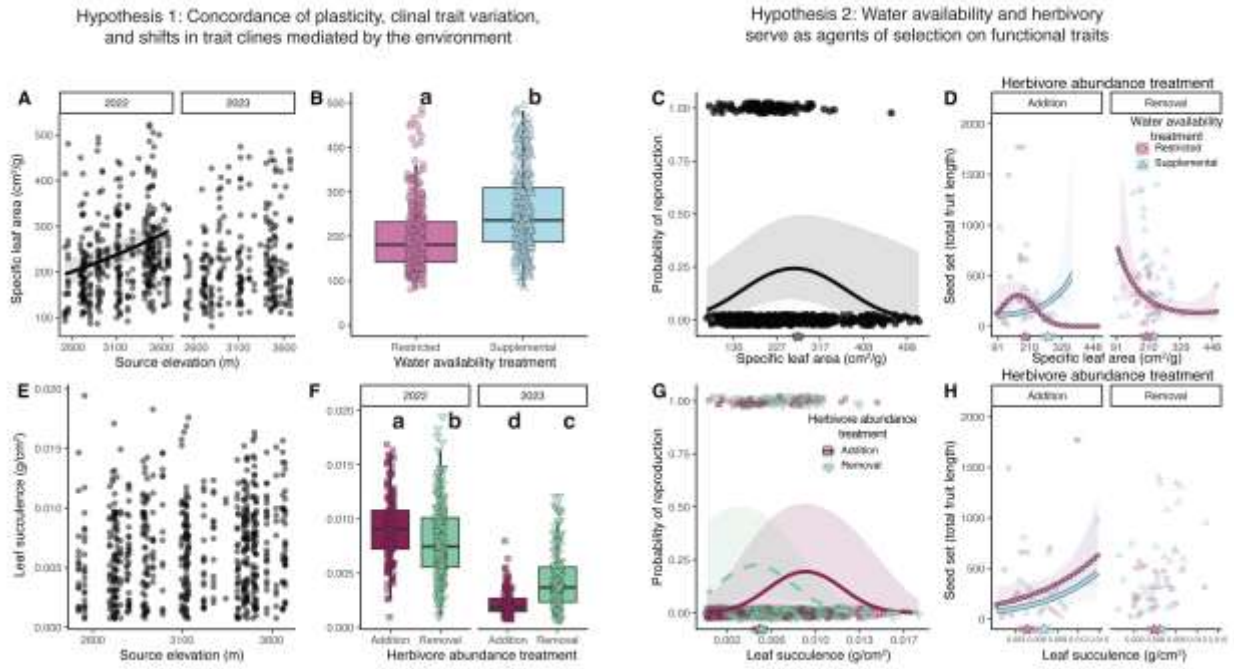




Figure 4

Hypothesis 1: Concordance of plasticity, clinal trait variation, and shifts in trait clines mediated by the environment

Hypothesis 2: Water availability and herbivory serve as agents of selection on functional traits

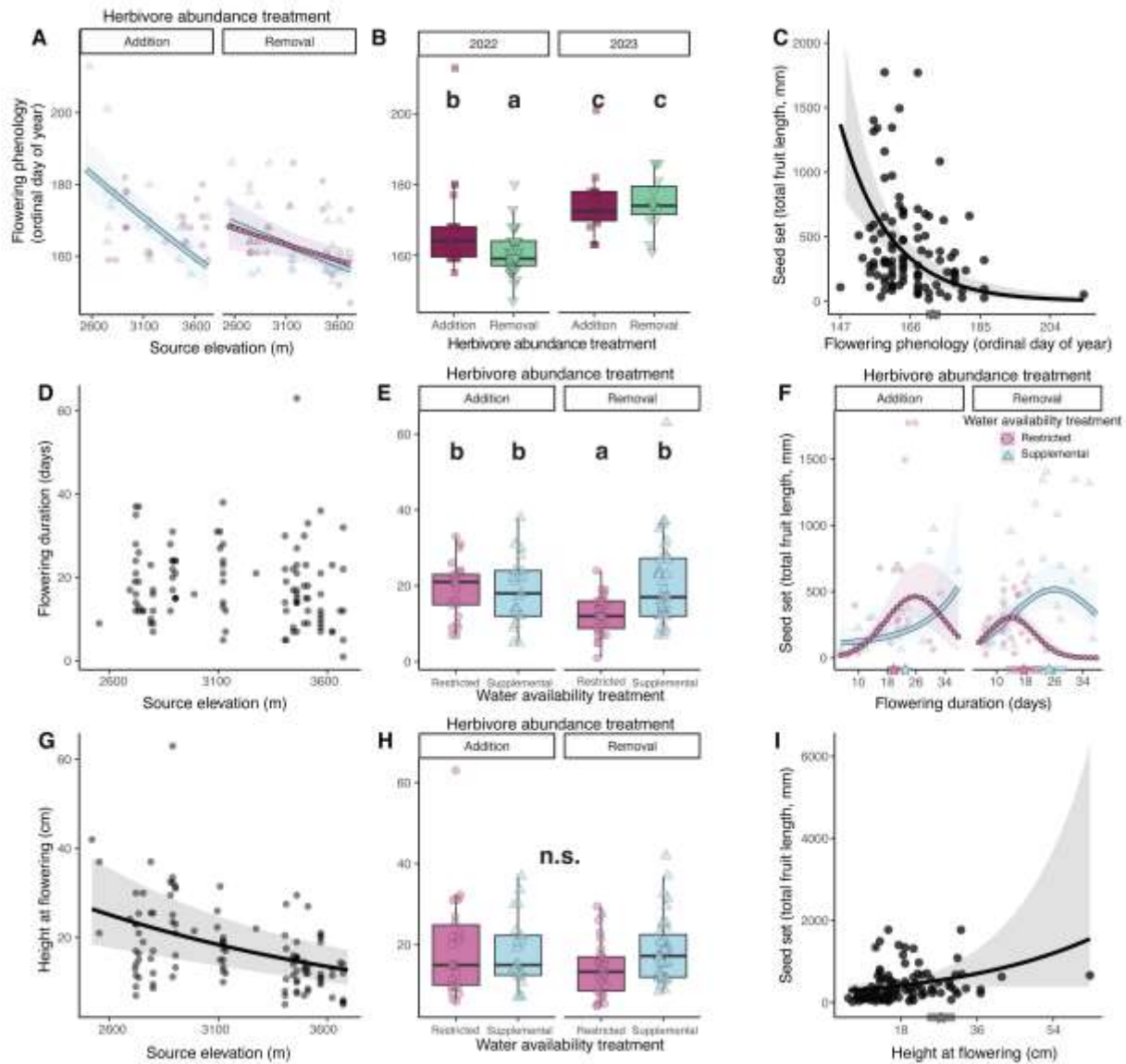


Figure 5

