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Original Article

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

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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 3 years, and evaluated clinal variation in functional traits and phenology, phenotypic plasticity, local adaptation, and selection using diverse accessions of the perennial forb, *Boechera stricta*. Consistent with expectations, drought stress exacerbated damage from herbivores. We found significant plasticity and genetic clines in foliar and phenological traits. Water availability and herbivory interacted to exert selection, even on traits like flowering duration, which showed no clinal variation. 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, cline, plasticity, herbivore resistance, water availability

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 (Keller et al., 2009; Kooyers et al., 2015; Trussell, 2000). 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; Kooyers et al., 2017; Kremer et al., 2014; O'Brien et al., 2019), which can arise from plasticity (Via & Lande, 1985) and 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 (Pellissier et al., 2016; Sandel et al., 2021; Wade & Kalisz, 1990). 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 (Gorton et al., 2022; Hamann et al., 2021b; Wilczek et al., 2014; Zettlemoyer, 2023).

The spatial and temporal grain of environmental variation strongly influences 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 (Anderson et al., 2021; Baythavong, 2011). However, plasticity does not always confer higher fitness; in some cases, plasticity is neutral or even represents a maladaptive response to stress (e.g., Baythavong, 2011; Becker et al., 2022; Van Kleunen et al., 2007). One mechanism for examining the adaptive nature of plasticity is to test whether the direction of plasticity aligns with the direction of selection (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 (Anstett et al., 2015; Kooyers et al., 2014; Kremer et al., 2014; Montesinos-Navarro et al., 2011), which can generate local adaptation (Gonzalo-Turpin & Hazard, 2009; Leimu & Fischer, 2008). To examine whether phenotypic clines have a genetic basis, researchers grow accessions sourced

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from diverse populations in a single common environment, whether in the lab or the field (Kooyers et al., 2015; Kremer et al., 2014; Stinchcombe et al., 2004; Woods et al., 2012). However, the magnitude—or even the directionality—of these clines can vary across test environments (De Kort et al., 2020; Wadgymar et al., 2017; Woods et al., 2012). Some clines may not be present in benign laboratory conditions (Kellermann et al., 2015; Wadgymar 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 & Schiestl, 2022; Navarro et al., 2022). These two agents of selection vary across space and time (Kooyers et al., 2015; Nelson et al., 2019a, b), which can lead to local adaptation (Blumenthal et al., 2021; Garrido et al., 2012) and plasticity in ecologically-relevant traits (Descombes et al., 2020a; Jordan et al., 2015; Lorts & 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 & Currano, 2013), plants have evolved defenses (e.g., Gong & 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 areas, mediate responses to aridity and herbivory (Blumenthal et al., 2020; Hauser, 2014). Furthermore, plant responses to drought directly affect herbivory. Herbivores can benefit from poorly-defended drought-stressed plants that allocate resources to maintain osmotic potential and survival instead of anti-herbivore defenses (Bauerfeind & Fischer, 2013; Gutbrodt et al., 2011). Thus, the degree and fitness consequences of herbivory can depend on the abiotic context (Descombes et al., 2020b; Moreira et al., 2018; Rasmann et al., 2014).

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 (Anderson & Wadgymar, 2020; Dunne et al., 2003; Körner, 2007) 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: (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 (Figure 1A) aligns with the direction of plasticity (Figure 1B), plasticity could be adaptive (Eckhart et al., 2004; Ensing & Eckert, 2019; Gonzalo-Turpin & Hazard, 2009). 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 (Figure 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; Hamann et al., 2021a; Jactel et al., 2012). In addition, climatic factors vary inter-annually in natural environments, which could generate different patterns of trait expression across years (Ensing & 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 & 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 & 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 3 years (2021–2023). We established the garden (38° 57.1203′ N, 106° 59.4903′ W) in a meadow at 2,895 m 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: 2,519–3,673 m a.s.l., Supplementary Figure 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 at the University of Georgia. To maximize genetic and phenotypic diversity (Blanquart et al., 2013; Goudet & Büchi, 2006), 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 2 weeks in the dark, transplanted germinants into 3.8 cm diameter Stuewe and Sons Ray—Leach

Table 1. Predictions and results for hypotheses 1 (clines and plasticity) and 2 (selection).

	H1: Concordance of plasticity and clinal trait variation		H2: Water availability and herbivory impose selection	
Trait	Predictions	Results	Predictions	Results
Herbivore resistance	Plasticity: Lower under dry conditions with high herbivory (Gutbrodt et al., 2011; Hamann et al., 2021a; Jactel et al., 2012).	Matched predictions (Figure 2B, Supplementary 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 resistance (Supplementary Table S12) Seed set: Directional selection for increased resistance under supplemental watering and herbivore addition (Figure 2C, Supplementary Table S13)
	Genetic cline: 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 (Figure 2A, Supplementary Table S6)		
Specific leaf area	Plasticity: Lower under dry conditions (Onoda et al., 2017) with high herbivory (Cingolani et al., 2005).	Decreased under restricted watering, no effect of herbivore treatment (Figure 3B, Supplementary 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 (Figure 3C, Supplementary Table S12b) Seed set: Concordant with predictions (Figure 3D, Supplementary Table S13b)
	Genetic cline: Increases with source elevation (Wadgymar et al, 2017)	Increased with source elevation in 2022 (Figure 3A, Supplementary Table S7b)		
Succulence	Plasticity: 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 (Figure 3F, Supplementary Table S8).	Dry conditions (Kooyers et al., 2015) and high herbivory (Moles et al., 2013) favor increased succulence	Probability of reproduction: Concordant with predictions (Figure 3G, Supplementary Table S12b) Seed set: Concordant with predictions (Figure 3H, Supplementary Table S13b)
	Genetic cline: Decreases with source elevation (Kooyers et al., 2015).	No cline detected (Figure 3E, Supplementary Table S8)		
Flowering phenology	Plasticity: Delayed under dry conditions with high herbivory (Jordan et al., 2015)	Delayed flowering in 2022 under herbivore addition, no effect of water availability (Figure 4B, Supplementary Table S9)	Dry conditions and high herbivory favor delayed flowering (Jordan et al., 2015)	Directional selection via seed set for earlier flowering across all treatments(Figure 4C, Supplementary Table S13b)
	Genetic cline: Declines with source elevation (Wadgymar et al., 2017)	Declined with source elevation under all treatments except restricted watering and herbivore additions (Figure 4A, Supplementary Table S9b)		
Duration of flowering	Plasticity: Longer flowering duration under high herbivory (Austen & Weis, 2015)	Shorter duration of flowering under herbivore removal and water restriction (Figure 4E, Supplementary Table S10)	Dry conditions and high herbivory favor longer duration of flowering (Austen & Weis, 2015)	Selection favored shorter flowering under restricted than supplemental watering in both herbivore addition and removal treatments. Selection favored shorter duration of flowering in herbivore removal relative to herbivore addition within each watering treatment level (Figure 4F, Supplementary Table S13b)
	Genetic cline: Declines with source elevation (Anderson & Gezon, 2015)	No cline detected (Figure 4D, Supplementary Table S10)		
Height at flowering	Plasticity: Shorter under dry conditions with high herbivory (Jordan et al., 2015)	No plasticity detected (Figure 4G, Supplementary 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 (Figure 4I, Supplementary Table S13b)
	Genetic cline: Declines with source elevation (Wadgymar et al., 2017)	Declined with source elevation across all treatments (Figure 4H, Supplementary Table S11)		

Note. 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.

Cone-tainer (Tangent, OR, USA), and grew the plants in the greenhouse for 4 months before transporting them to the field site. In June 2021, we transplanted the plants into the existing vegetation in the garden in randomized positions within experimental blocks (mean ± standard deviation: 4.8 ± 2.5 full siblings from the 36 accessions into each of the four treatment levels, $N = 658$ plants total; Supplementary Table S1). We measured the diameter of each individual prior to planting to use as a covariate in analyses (see also Supplementary Table S1c and Supplementary Figure S4).

Experimental manipulations

Immediately after transplanting in spring 2021, we installed six 1.8 m × 1.8 m × 1.8 m cages within the fenced-in garden, with 1-m-wide rows between the cages (Supplementary Figure S2). The cages were constructed with polyvinyl chloride (PVC) pipe covered by Lumite mesh cloth with 8 × 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 during the winter.

We focused our herbivore manipulation on grasshoppers (*Melanoplus* spp.), a dominant generalist herbivore in montane meadows (Descombes et al., 2017). We 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 (Supplementary 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 (Supplementary Table S2). Natural grasshopper density in and outside of the cages was lower in 2022 than in the other years (Supplementary Table S2).

We subdivided the cages into four blocks, with two blocks assigned to each water level: supplemental or restricted (Supplementary Figure S2A). For the supplemental water blocks, we supplied 1.5 L 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 (Supplementary Figure 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, $p = .0022$, Supplementary Figure S3, Supplementary Table S3).

Fitness components and functional traits

During the growing season (June to September) from 2021 to 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, Wadgymar et al., 2017). *Boechera stricta* typically requires vernalization (exposure to nonfreezing winter temperatures) to flower (Anderson et al., 2011); 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 elevations (Anderson & Gezon, 2015; Anderson et al., 2015; Wadgymar et al., 2017). Furthermore, herbivory (Descombes et al., 2020a; Dorey & Schiestl, 2022) and water availability (Jactel et al., 2012; Kamps & Poelman, 2024; Lorts & Lasky, 2020; Navarro et al., 2022) can induce plasticity in these traits and shape their evolution.

We censused each plant for herbivory 2–3 times per growing season by counting the total number of leaves and the number of damaged leaves, and then estimating the amount of per-leaf damage visually. Only the first senior authors (Jameel and Anderson) 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 the 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 & Breckle, 1995).

Like many species, *B. stricta* produces basal rosette leaves on vegetative plants 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 × bolt specific leaf area, $F_{1,40} = 9.09$, $p = .0045$; rosette succulence = 0.0024 + 0.56 × bolt specific leaf area, $F_{1,40} = 67.15$, $p < .0001$). 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, the 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 (Supplementary 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 trade-off between size and timing of flowering (Wadgymar et al., 2017).

Statistical analyses

We conducted all analyses in R (R Core Team., 2021). 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 (Supplementary Table S5), and we assessed multicollinearity in our selection analyses. For all models, we examined the statistical significance of the main effects using the Anova function of the R package car ver. 3.1-2 (Fox & Weisburg, 2019) and we assessed the normality and homoscedasticity of residuals using the simulateResiduals function of the R package DHARMa ver. 0.4.6 (Hartig & Lohse, 2022). We visualized the regression results with the ggeffects R package ver. 1.3.2 (Lüdecke, 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, 2024).

Hypothesis 1: concordance of plasticity and clinal trait variation

To test the hypothesis that plasticity aligns with clinal trait variation, we analyzed the following 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 (Supplementary Figure S3, Supplementary Table S3) and herbivore abundance (Supplementary Table S2) differed across the 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 two-, three-, and four-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 two-way interactions and the second also examined three-way interactions. We used the model.sel function of the MuMIn R package ver. 1.48.4 (Bartón, 2024) to contrast models using the corrected Akaike Information Criterion. When these two models generated similar results, we retained the model with three-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 etal., 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 a 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 to 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

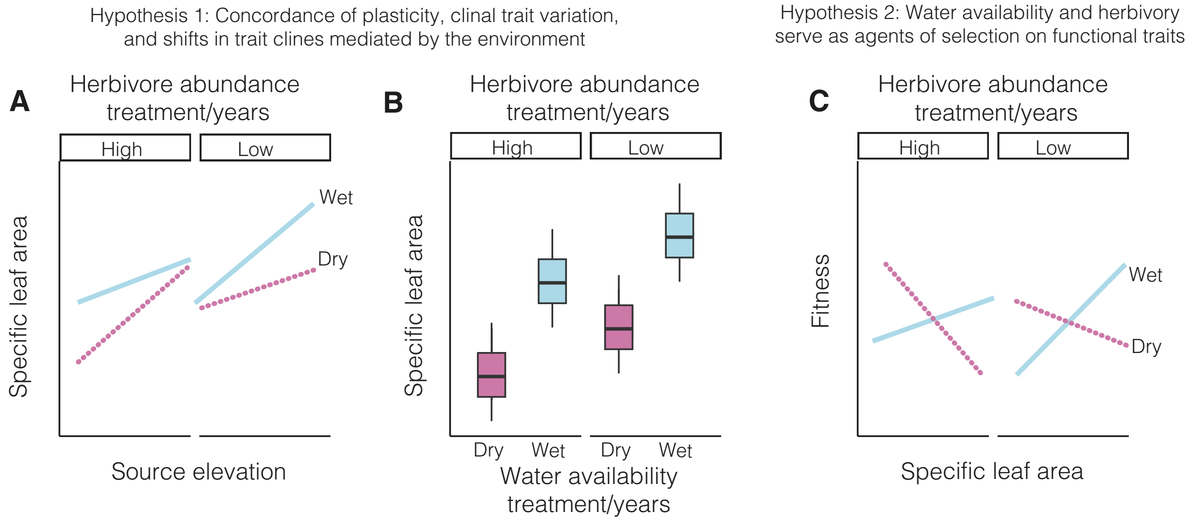

Figure 1. Conceptual diagram. Hypothesis 1: Plastic responses to treatment align with clinal variation. (A) We predict that variable selection across the landscape has driven the evolution of clinal variation in traits, which could be tested in a common garden environment using accessions sourced from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

model, 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 the 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 the potential multicollinearity of traits, we inspected variance inflation factors using the check_collinearity function of the R package performance ver. 0.12.2 (Lüdecke 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 (Wadgymar et al., 2022), 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 the cage. We focused on data from 2022 and 2023, 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 a 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 × herbivore abundance × year: $\chi^2 = 26.4$, d.f. = 2, $p < .0001$, Supplementary Table S6, Figure 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 (Supplementary Table S6, Figure 2A). Furthermore, foliar damage was significantly higher under restricted water than supplemental water conditions across all years and herbivore treatment levels [odds ratio ± standard error (SE): 1.19 ± 0.62; $\chi^2 = 7.51$, d.f. = 1, $p = .0061$, Figure 2B].

Specific leaf area

Clinal variation in specific leaf areas was apparent through the interaction of source elevation and year ($\chi^2 = 12.48$, d.f = 1, $p = .00041$, Figure 3A, Supplementary Table S7). In 2022, specific leaf area increased by 27% for every 1-km increase in source elevation (95% confidence interval: 16.28% to 37.31%, Supplementary 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, $p = .00017$, Figure 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 × year: $\chi^2 = 34.48$, d.f = 1, $p < .0001$, Figure 3F). Herbivore addition induced higher succulence in 2022 (z ratio = 2.84, $p = .023$); however, this pattern reversed in 2023 (z ratio = −5.4, $p < .0001$).

Flowering phenology

Source elevation interacted with water availability and herbivore abundance to influence the timing of first flowering ($\chi^2 = 7.3$, d.f = 1, $p = .0069$, Figure 4A, Supplementary Table S9). As expected (Table 1), flowering time decline with source elevation under all treatment combinations except herbivore addition and restricted watering, for which we found no cline (Figure 4A, Supplementary Table S9b). Additionally, herbivore treatment interacted with year to shape the expression of flowering phenology ($\chi^2 = 10.93$, d.f.=1, $p = .00094$), with removal inducing earlier flowering in 2022 (z ratio = 3.58, $p = .002$) but not in 2023 (z ratio = −1.6, $p = .38$)

Flowering duration

We found significant spatio-temporal plasticity in flowering duration (Figure 4D and E, Supplementary Table S10), which varied as a function of interactions of water availability and herbivore abundance ($\chi^2 = 7.49$, d.f. = 1, $p = .0062$) and water availability and year ($\chi^2 = 7.99$, d.f. = 1, $p = .0047$). Restricted water shortened the duration of flowering under herbivore removal (Figure 4E) and in 2023 (Supplementary Table S10).

Tallest stem at flowering

For every 1-km increase in source elevation, height at flowering decreased by 59.7% [95% confidence interval (CI): 38.7%, 78.9%; $\chi^2 = 10.77$, d.f = 1, $p = .0001$, Figure 4G, Supplementary Table S11]. Water availability and grasshopper abundance did not induce plasticity in height (Figure 4H). However, height at flowering was greater in 2023 than in 2022 ($\chi^2 = 16.87$, d.f = 1, $p < .0001$).

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, $p = .0136$, Figure 3C, Supplementary Table S12). Context-dependent stabilizing selection operated on leaf succulence (Figure 3G, Supplementary Table S12b). Concordant with expectations, selection favored greater succulence in grasshopper addition than in removal cages (herbivore abundance × succulence $\chi^2 = 6.41$, d.f = 3, $p = .011$; quadratic effect of succulence: $\chi^2 = 19.68$, d.f = 3, $p < .0001$, Figure 3G, Supplementary Table S12). We found no evidence for selection via the probability of reproduction on leaf damage from herbivores (Supplementary Table S12).

Selection via seed set

Context-dependent selection emerged for leaf succulence in response to herbivore manipulation ($\chi^2 = 5.26$, d.f = 1, $p = .0218$, Figure 3H, Supplementary Table S13) and three other traits in response to water availability and herbivore abundance: specific leaf area (water availability × herbivore abundance × quadratic effect of specific leaf area; $\chi^2 = 12.98$, d.f = 1, $p = .0003$, Figure 3D); leaf damage from arthropod herbivores (water availability × herbivore abundance × quadratic effect of leaf damage: $\chi^2 = 11.66$, d.f = 1, $p = .0006$; Figure 2C); and flowering duration (water availability × herbivore abundance × flowering duration: $\chi^2 = 24.61$, d.f = 1, $p < .0001$, water availability × quadratic effect of flowering duration: $\chi^2 = 17.62$, d.f = 1, $p < .0001$, and herbivore abundance × quadratic effect of flowering duration: $\chi^2 = 7.55$, d.f = 1, $p = .0060$; Figure 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%; Figure 3H), but there was no evidence for selection on succulence under grasshopper removal. Selection on specific leaf areas generally accorded with predictions, as dry conditions favored lower values. For example, in the grasshopper addition, stabilizing selection favored low specific leaf area under restricted water (optimum: 167.68 cm^2/g), but directional selection favored increased trait values in supplemental water [seed set increased by 49.9% for every 1 standard deviation (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 the seed set peaked at an average leaf damage of 7.9%, suggesting strong selection for herbivore resistance in this treatment combination (Figure 2C, Supplementary 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

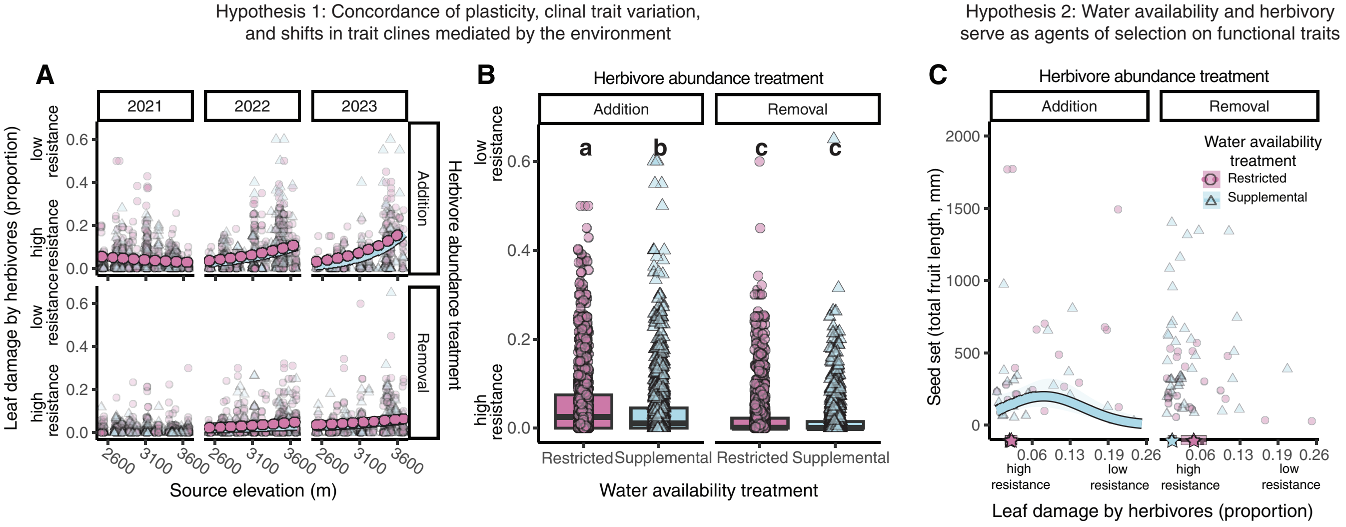

Figure 2. Clines and plasticity in foliar damage from herbivory (=1-herbivore resistance). (A) Herbivory increased with source elevation in all treatment combinations in 2022 and 2023, with the most pronounced cline under grasshopper addition. Contrary to predictions, foliar damage declined with source 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 circles) and supplemental watering (blue triangles) 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 circles) and supplemental watering (blue triangles). 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.

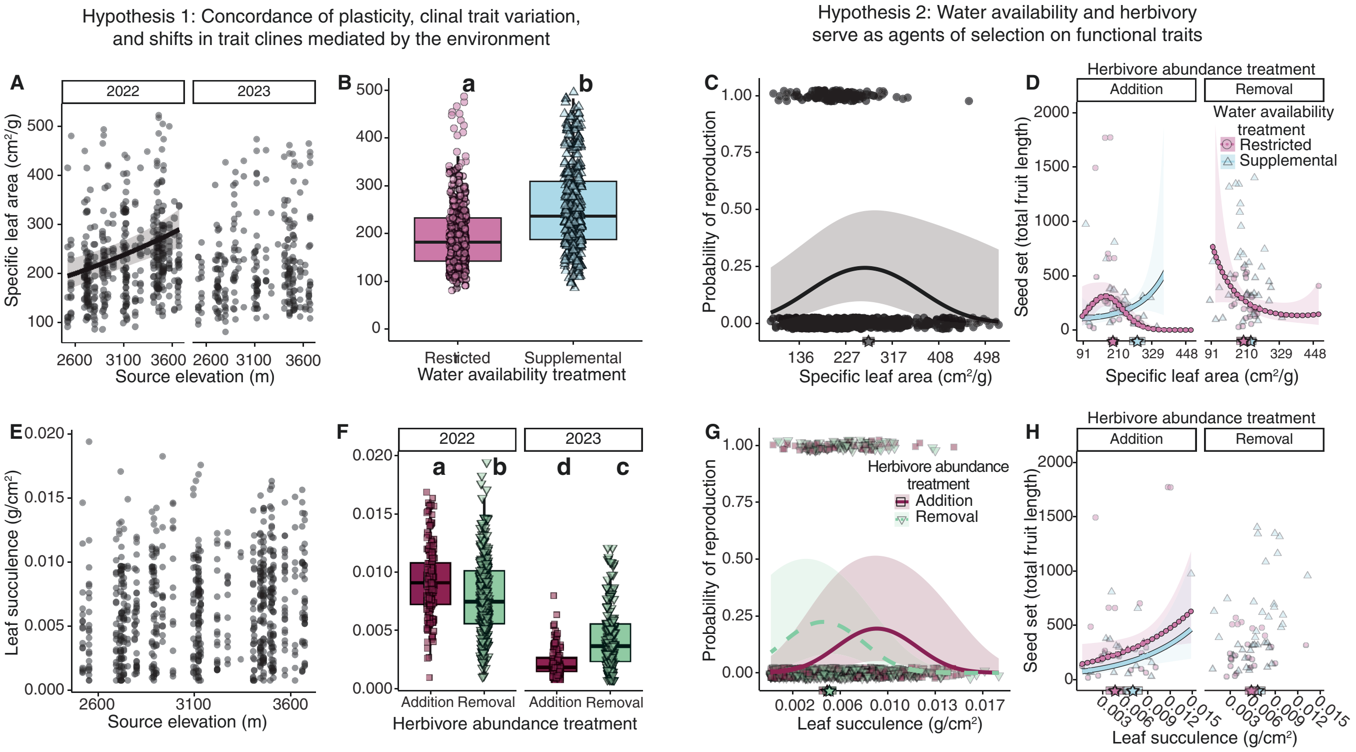

Figure 3. Clines, plasticity, and natural selection in specific leaf area and leaf succulence. All panels show raw data points and significant regression lines and boxplots display data from all treatments (black), restricted watering (pink circles) and supplemental watering (blue triangles) treatments or herbivore addition (dark purple squares) or herbivore removal (green circles) 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 m ± 15 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 × 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.

treatment (optimal duration: 25.9 days) and directional selection for increased flowering duration in the supplemental watering treatment (seed set increased by 37.7% for every 1 S.D. change in flowering duration; CI: 0.6%, 88.4%; Figure 4F, Supplementary Table S13b). In herbivore removal, stabilizing selection favored a 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, $p = .0114$), with seed set increasing by 41.4% for every one standard deviation increase in height (95% CI: 20.2%, 66.3%; Figure 4I, Supplementary Table S13b) and earlier flowering ($\chi^2 = 7.10$, d.f = 1, $p = .0077$) with seed set decreasing by 42.1% for every one standard deviation increase in height (95% CI: 29.7%, 51.8%; Figure 4I, Supplementary 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, $p = .0169$, Supplementary Table S14). Consistent with local adaptation and the increase in water availability with elevation, high elevation ecotypes showed elevated fitness under supplemental water, as the source elevation with optimal seed set occured at 3,242.76 m (Figure 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, $p < .0001$, Supplementary Table S14). We highlight that there was no relationship between initial size and source elevation in this experiment (Supplementary Figure S4).

Discussion

Abiotic and biotic conditions co-vary across environmental gradients and contribute to the evolution and 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

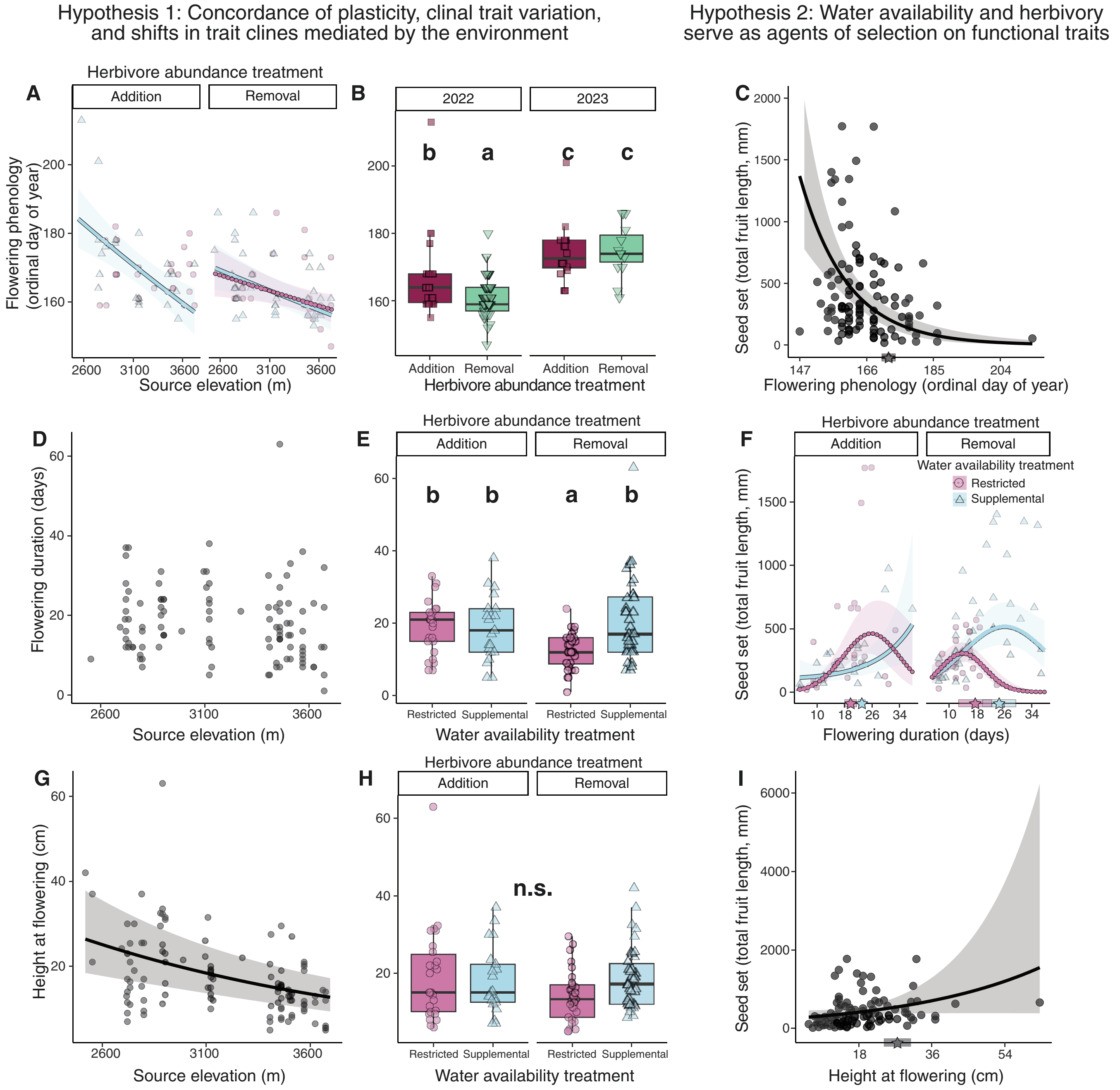

Figure 4. Clines, plasticity, and selection for reproductive phenology. Across panels, we have plotted raw data points, significant regression lines, and box plots from restricted watering (pink circles), and supplemental watering (blue triangles) treatments, with stars on the x axes of fitness landscapes representing the mean trait values for local accessions (source elevation: 2891 m ± 15 m), bracketed by 2 × 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 relative to supplemental watering. (G) The height of the tallest stem at flowering decreased with source elevation, but (H) we found no plasticity in this trait. (I) Selection via seed set favored taller plants at flowering across all treatment levels.

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 traits, 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 evolutionary dynamics. 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 and herbivory.

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 & Eckert, 2019), enabling researchers to identify environmental factors that influence both trait expression and evolution (Wade & 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 & Mooney, 2013). Additionally, supplemental watering, which mimics 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 & Mooney, 2013). Likewise, in our study, plasticity in specific leaf areas aligned with the elevational cline in this trait, as supplemental watering induced higher specific leaf area values (Figure 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 & Wadgymar, 2020).

High specific leaf area often evolves in wetter habitats, likely owing to greater photosynthetic and respiration rates (Poorter et al., 2009, 2019; Wright et al., 2004). In our study, the cline in specific leaf areas only emerged in 2022 (Figure 3A), which could have resulted from higher overall soil moisture content in that year compared to 2023 (Figure 3A, Supplementary Figure S3). Previous studies of specific leaf areas 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 US, decreasing snowpack and increasing growing season aridification (Fyfe et al., 2017; Pederson et al., 2011) could constrain the continued expression of clinal variation in traits such as specific leaf areas.

Variation in abiotic and biotic conditions along environmental gradients likely impose complex patterns of selection on natural populations (Campitelli & Stinchcombe, 2013; Keller et al., 2009; Kooyers & Olsen, 2013; Muir & Angert, 2017). In our study, selection on specific leaf area via seed set aligned with expectations based on the leaf economic spectrum (Onoda et al., 2017; Wright et al., 2004), and with the cline (Figure 3A) and the direction of plasticity (Figure 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 (Cingolani et al., 2005; Wright & Cannon, 2001), 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 across all environments, which 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 (Moles et al., 2013; Pérez-Harguindeguy et al., 2003). Concordant with predictions (Table 1), selection via the probability of reproduction favored increased succulence under herbivore addition relative to removal (Figure 3G). Similarly, directional selection via seed set favored higher succulence in herbivore addition, despite the lack of selection via seed set on this trait under herbivore removal (Figure 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 (Figure 3E) and lack of consistent patterns of plasticity (Figure 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 & 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 (Figure 2A), except in the year with the greatest water availability (2021). Furthermore, this cline was the most pronounced under grasshopper addition in 2023, the driest year of the study. We propose that the observed cline in herbivore resistance reflects an evolutionary response to variable herbivore abundance across this elevational gradient.

Consistent with our expectation that plants under restricted watering would be poorly defended against herbivores (Table 1, Endara & Coley, 2011; Jactel et al., 2012), we found that drought stress exacerbated the extent of foliar herbivory (Figure 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 (Gely et al., 2020; Gutbrodt et al., 2011), perhaps owing to limited resources available for the production of defenses (Bauerfeind & 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 (Becklin et al., 2016; Rasmann et al., 2014; Schweiger et al., 2008), which could heighten the extent of herbivore damage experienced by poorly defended high elevation accessions. Increasing aridity with climate change (Fyfe et al., 2017; Pederson et al., 2011; Rangwala et al., 2012) could further augment herbivore damage (see also Hamann et al., 2021a), especially for high elevation accessions (Figure 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 (Figure 4A). This result corresponds with other systems along elevational and latitudinal gradients (Ensing & Eckert, 2019; Kawakami et al., 2011; Montague et al., 2008; Monty & Mahy, 2009), 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

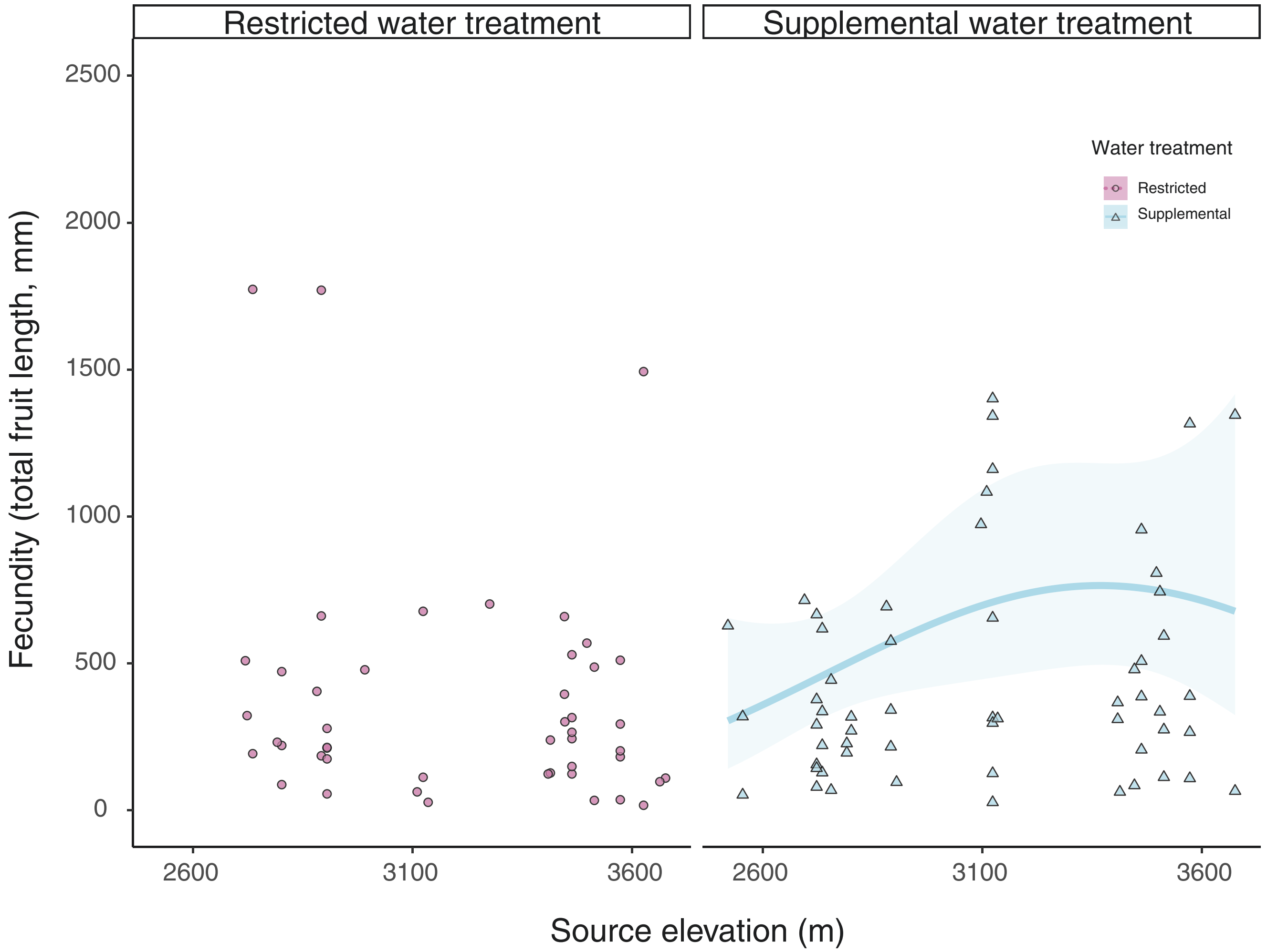

Figure 5. Fitness varies with water availability and source elevation. Consistent with local adaptation, under supplemental water, fecundity (total fruit length, which is a proxy for seed set) peaked for accessions from 3,242.76 m, which is 352 m above the elevation of the common garden. Shown are regression lines and raw data points from the conditional component of the hurdle model (i.e., seed set amongst individuals that successfully reproduced).

(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 (2022), 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 (Figure 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 (Li et al., 2018; Rathcke & Lacey, 1985). 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 (Figure 4F). In line with our predictions, grasshopper removal favored shorter durations of flowering compared to grasshopper addition within each watering treatment level 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 & 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 a shorter duration of flowering, even under grasshopper addition (Figure 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 (Figure 4D), though a previous common garden experiment demonstrated that the duration of flowering decreased with source elevation in *B. stricta* (Anderson & 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 & 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 (Hereford, 2009; Kawecki & Ebert, 2004; Leimu & Fischer, 2008), but we rarely know which agents of selection underlie local adaptation (Wade & Kalisz, 1990; Wadgymar et al., 2022). If climatic factors drive adaptive population differentiation, rapid global change could disrupt local adaptation (Anderson & Wadgymar, 2020; Kooyers et al., 2019; Wilczek et al., 2014). 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 & Wadgymar, 2020), to drought stress in the greenhouse (MacTavish & 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 (Figure 5), which is consistent with expectations, given that water availability increases with elevation (Anderson & Wadgymar, 2020; Nelson et al., 2019b). 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 (Christie et al., 2022; Derry et al., 2019; Hoffmann & Sgrò, 2011).

Grasshopper herbivory did not modify fitness in a manner that reflects local adaptation, but the cline in foliar damage from herbivores (Figure 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; Hahn et al., 2019; Mooney et al., 2010) 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 environmental 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 suggest 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 (Hargreaves & Eckert, 2018; Hufbauer et al., 2015).

Supplementary material

Supplementary material is available online at *Evolution*.

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 acquired funding for this study. JA, KM, and MIJ designed the experiment. All authors established the experiment, conducted manipulations of grasshoppers and water, and collected data. JA and MIJ analyzed the data and wrote the first draft of the manuscript, which KM reviewed and revised.

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Conflict of interest: The authors declare no conflicts of interest.

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References

Anderson, J. T., & Gezon, Z. J. (2015). Plasticity in functional traits in the context of climate change: A case study of the subalpine forb *Boechera stricta* (Brassicaceae). *Global Change Biology*, *21*(4), 1689–1703. https://doi.org/10.1111/gcb.12770

Anderson, J. T., Jameel, M. I., & Geber, M. A. (2021). Selection favors adaptive plasticity in a long-term reciprocal transplant experiment. *Evolution*, *75*(7), 1711–1726. https://doi.org/10.1111/evo.14280. Wiley.

Anderson, J. T., Lee, C.-R., & Mitchell-Olds, T. (2011). Life-history QTLs and natural selection on flowering time in Boechera stricta, a perennial relative of Arabidopsis. *Evolution*, *65*(3), 771–787. https://doi.org/10.1111/j.1558-5646.2010.01175.x

Anderson, J. T., Perera, N., Chowdhury, B., & Mitchell-Olds, T. (2015). Microgeographic patterns of genetic divergence and adaptation across environmental gradients in *Boechera stricta* (Brassicaceae). *American Naturalist*, *186*(Suppl 1), S60–S73. https://doi.org/10.1086/682404

Anderson, J. T., & Wadgymar, S. M. (2020). Climate change disrupts local adaptation and favours upslope migration. *Ecology Letters*, *23*(1), 181–192. https://doi.org/10.1111/ele.13427

Anstett, D. N., Ahern, J. R., Glinos, J., Nawar, N., Salminen, J. -P., & Johnson, M. T. J. (2015). Can genetically based clines in plant defence explain greater herbivory at higher latitudes? *Ecology Letters*, *18*, 1376–1386.

Austen, E. J., Rowe, L., Stinchcombe, J. R., & Forrest, J. R. K. (2017). Explaining the apparent paradox of persistent selection for early flowering. *New Phytologist*, *215*(3), 929–934. https://doi.org/10.1111/nph.14580

Austen, E. J., & Weis, A. E. (2015). What drives selection on flowering time? An experimental manipulation of the inherent correlation between genotype and environment. *Evolution*, *69*(8), 2018–2033. https://doi.org/10.1111/evo.12709

Bartón, K. (2024). *MuMIn: Multi-model inference. R package version 1.48.4*. https://doi.org/10.32614/CRAN.package.MuMIn.

Bauerfeind, S. S., & Fischer, K. (2013). Testing the plant stress hypothesis: Stressed plants offer better food to an insect herbivore. *Entomologia Experimentalis et Applicata*, *149*(2), 148–158. https://doi.org/10.1111/eea.12118

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. *American Naturalist*, *178*(1), 75–87. https://doi.org/10.1086/660281

Becker, D., Barnard-Kubow, K., Porter, R., Edwards, A., Voss, E., Beckerman, A. P., & Bergland, A. O. (2022). Adaptive phenotypic plasticity is under stabilizing selection in Daphnia. *Nature Ecology and Evolution*, *6*(10), 1449–1457. https://doi.org/10.1038/s41559-022-01837-5. Nature Publishing Group.

Becklin, K. M., Anderson, J. T., Gerhart, L. M., Wadgymar, S. M., Wessinger, C. A., & Ward, J. K. (2016). Examining plant physiological responses to climate change through an evolutionary lens. *Plant Physiology*, *172*(2), 635–649. https://doi.org/10.1104/pp.16.00793. American Society of Plant Biologists.

Berg, S., Kutra, D., Kroeger, T., Straehle, C. N., Kausler, B. X., Haubold, C., Schiegg, M., Ales, J., Beier, T., Rudy, M., Eren, K., Cervantes, J. I., Xu, B., Beuttenmueller, F., Wolny, A., Zhang, C., Koethe, U., Hamprecht, F. A., & Kreshuk, A. (2019). ilastik: Interactive machine learning for (bio)image analysis. *Nature Methods*, *16*(12), 1226–1232. https://doi.org/10.1038/s41592-019-0582-9. Nature Publishing Group.

Blankinship, J. C., Meadows, M. W., Lucas, R. G., & Hart, S. C. (2014). Snowmelt timing alters shallow but not deep soil moisture in the Sierra Nevada. *Water Resources Research*, *50*(2), 1448–1456. https://doi.org/10.1002/2013wr014541

Blanquart, F., Kaltz, O., Nuismer, S. L., & Gandon, S. (2013). A practical guide to measuring local adaptation. *Ecology Letters*, *16*(9), 1195–1205. https://doi.org/10.1111/ele.12150

Blumenthal, D. M., LeCain, D. R., Porensky, L. M., Leger, E. A., Gaffney, R., Ocheltree, T. W., & Pilmanis, A. M. (2021). Local adaptation to precipitation in the perennial grass *Elymus elymoides*: Trade-offs between growth and drought resistance traits. *Evolutionary Applications*, *14*(2), 524–535. https://doi.org/10.1111/eva.13137

Blumenthal, D. M., Mueller, K. E., Kray, J. A., Ocheltree, T. W., Augustine, D. J., & Wilcox, K. R. (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. *Journal of Ecology*, *108*(6), 2336–2351. https://doi.org/10.1111/1365-2745.13454

Brooks, M., Kristensen, K., Benthem, K., Magnusson, A., Berg, C., Nielsen, A., Skaug, H., Mächler, M., & Bolker, B. (2019). glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R Journal*, *9*(2), 378. https://doi.org/10.32614/RJ-2017-066

Campitelli, B. E., & Stinchcombe, J. R. (2013). Testing potential selective agents acting on leaf shape in *Ipomoea hederacea*: Predictions based on an adaptive leaf shape cline. *Ecology and Evolution*, *3*(8), 2409–2423. https://doi.org/10.1002/ece3.641

Christie, K., Pierson, N. R., Lowry, D. B., & Holeski, L. M. (2022). Local adaptation of seed and seedling traits along a natural aridity gradient may both predict and constrain adaptive responses to climate change. *American Journal of Botany*, *109*(10), 1529–1544. https://doi.org/10.1002/ajb2.16070

Cingolani, A. M., Posse, G., & Collantes, M. B. (2005). Plant functional traits, herbivore selectivity and response to sheep grazing in Patagonian steppe grasslands. *Journal of Applied Ecology*, *42*(1), 50–59. https://doi.org/10.1111/j.1365-2664.2004.00978.x

De Kort, H., Panis, B., Helsen, K., Douzet, R., Janssens, S. B., & Honnay, O. (2020). Pre-adaptation to climate change through topography-driven phenotypic plasticity. *Journal of Ecology*, *108*(4), 1465–1474. https://doi.org/10.1111/1365-2745.13365

Denney, D., Patel, P., & Anderson, J. T. (2024). Elevated [CO2] and temperature augment gas exchange and shift the fitness landscape a montane forb. *New Phytologist*, *243*(1), 58–71. https://doi.org/10.1111/nph.19765

Derry, A. M., Fraser, D. J., Brady, S. P., Astorg, L., Lawrence, E. R., Martin, G. K., Matte, J., Negrín Dastis, J. O., Paccard, A., Barrett, R. D. H., Chapman, L. J., Lane, J. E., Ballas, C. G., Close, M., & Crispo, E. (2019). Conservation through the lens of (mal)adaptation: Concepts and meta-analysis. *Evolutionary Applications*, *12*(7), 1287–1304. https://doi.org/10.1111/eva.12791

Descombes, P., Kergunteuil, A., Glauser, G., Rasmann, S., & Pellissier, L. (2020a). Plant physical and chemical traits associated with herbivory in situ and under a warming treatment. *Journal of Ecology*, *108*(2), 733–749. https://doi.org/10.1111/1365-2745.13286

Descombes, P., Marchon, J., Pradervand, J. -N., Bilat, J., Guisan, A., Rasmann, S., & Pellissier, L. (2017). Community-level plant palatability increases with elevation as insect herbivore abundance declines. *Journal of Ecology*, *105*, 142–151.

Descombes, P., Pitteloud, C., Glauser, G., Defossez, E., Kergunteuil, A., Allard, P. -M., Rasmann, S., & Pellissier, L. (2020b). Novel trophic interactions under climate change promote alpine plant coexistence. *Science*, *370*(6523), 1469–1473. https://doi.org/10.1126/science.abd7015

Dorey, T., & Schiestl, F. P. (2022). Plant phenotypic plasticity changes pollinator-mediated selection. *Evolution*, *76*(12), 2930–2944. https://doi.org/10.1111/evo.14634. Wiley.

Dunne, J. A., Harte, J., & Taylor, K. J. (2003). Subalpine meadow flowering phenology responses to climate change: Integrating experimental and gradient methods. *Ecological Monographs*, *73*(1), 69–86. https://doi.org/10.1890/0012-9615(2003)073[0069:smfprt]2.0.co;2. Ecological Society of America.

Eckhart, V. M., Geber, M. A., & McGuire, C. M. (2004). Experimental studies of adaptation in *Clarkia xantiana*. I. Sources of trait variation across a subspecies border. *Evolution; International Journal of Organic Evolution*, *58*(1), 59–70. https://doi.org/10.1111/j.0014-3820.2004.tb01573.x

Endara, M. -J., & Coley, P. D. (2011). The resource availability hypothesis revisited: A meta-analysis. *Functional Ecology*, *25*(2), 389–398. https://doi.org/10.1111/j.1365-2435.2010.01803.x

Ensing, D. J., & Eckert, C. G. (2019). Interannual variation in season length is linked to strong co-gradient plasticity of phenology in a montane annual plant. *New Phytologist*, *224*(3), 1184–1200. https://doi.org/10.1111/nph.16009

Etterson, J. R., & Shaw, R. G. (2001). Constraint to adaptive evolution in response to global warming. *Science*, *294*(5540), 151–154. https://doi.org/10.1126/science.1063656

Fine, P. V. A., Miller, Z. J., Mesones, I., Irazuzta, S., Appel, H. M., Stevens, M. H. H., Sääksjärvi, I., Schultz, J. C., & Coley, P. D. (2006). The growth–defense trade-off and habitat specialization by plants in Amazonian forests. *Ecology*, *87*(sp7), S150–S162. https://doi.org/10.1890/0012-9658(2006)87[150:tgtahs]2.0.co;2

Fox and Weisburg. (2019). *An R companion to applied regression*. 3rd ed. Sage.

Franks, S. J., Sim, S., & Weis, A. E. (2007). Rapid evolution of flowering time by an annual plant in response to a climate fluctuation. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(4), 1278–1282. https://doi.org/10.1073/pnas.0608379104

Fyfe, J. C., Derksen, C., Mudryk, L., Flato, G. M., Santer, B. D., Swart, N. C., Molotch, N. P., Zhang, X., Wan, H., Arora, V. K., Scinocca, J., & Jiao, Y. (2017). Large near-term projected snowpack loss over the western United States. *Nature Communications*, *8*, 14996. https://doi.org/10.1038/ncomms14996

Garrido, E., Andraca-Gómez, G., & Fornoni, J. (2012). Local adaptation: Simultaneously considering herbivores and their host plants. *New Phytologist*, *193*(2), 445–453. https://doi.org/10.1111/j.1469-8137.2011.03923.x

Gely, C., Laurance, S. G. W., & Stork, N. E. (2020). How do herbivorous insects respond to drought stress in trees? *Biological Reviews of the Cambridge Philosophical Society*, *95*(2), 434–448. https://doi.org/10.1111/brv.12571

Gong, B., & Zhang, G. (2014). Interactions between plants and herbivores: A review of plant defense. *Acta Ecologica Sinica*, *34*(6), 325–336. https://doi.org/10.1016/j.chnaes.2013.07.010

Gonzalo-Turpin, H., & Hazard, L. (2009). Local adaptation occurs along altitudinal gradient despite the existence of gene flow in the alpine plant species *Festuca eskia*. *Journal of Ecology*, *97*, 742–751.

Gorton, A. J., Benning, J. W., Tiffin, P., & Moeller, D. A. (2022). The spatial scale of adaptation in a native annual plant and its implications for responses to climate change. *Evolution*, *76*(12), 2916–2929. https://doi.org/10.1111/evo.14583

Goudet, J., & Büchi, L. (2006). The effects of dominance, regular inbreeding and sampling design on Q(ST), an estimator of population differentiation for quantitative traits. *Genetics*, *172*(2), 1337–1347. https://doi.org/10.1534/genetics.105.050583

Gutbrodt, B., Mody, K., & Dorn, S. (2011). Drought changes plant chemistry and causes contrasting responses in lepidopteran herbivores. *Oikos*, *120*(11), 1732–1740. https://doi.org/10.1111/j.1600-0706.2011.19558.x

Hahn, P. G., Agrawal, A. A., Sussman, K. I., & Maron, J. L. (2019). Population variation, environmental gradients, and the evolutionary ecology of plant defense against herbivory. *American Naturalist*, *193*(1), 20–34. https://doi.org/10.1086/700838. The University of Chicago Press.

Hamann, E., Blevins, C., Franks, S. J., Jameel, M. I., & Anderson, J. T. (2021a). Climate change alters plant-herbivore interactions. *New Phytologist*, *229*(4), 1894–1910. https://doi.org/10.1111/nph.17036

Hamann, E., Denney, D., Day, S., Lombardi, E., Jameel, M. I., MacTavish, R., & Anderson, J. T. (2021b). Review: Plant eco-evolutionary responses to climate change: Emerging directions. *Plant Science*, *304*, 110737. https://doi.org/10.1016/j.plantsci.2020.110737. Elsevier.

Hargreaves, A. L., & Eckert, C. G. (2018). Local adaptation primes cold-edge populations for range expansion but not warming-induced range shifts. *Ecology Letters*, *22*(1), 78–88. https://doi.org/10.1111/ele.13169

Hartig, F., & Lohse, L. (2022). *DHARMa: Residual diagnostics for hierarchical (multi-level / mixed) regression models. R package version 0.4.7*. https://github.com/florianhartig/dharma.

Hauser, M. -T. (2014). Molecular basis of natural variation and environmental control of trichome patterning. *Frontiers in Plant Science*, *5*, 320. https://doi.org/10.3389/fpls.2014.00320. Frontiers.

Hereford, J. (2009). A quantitative survey of local adaptation and fitness trade-offs. *American Naturalist*, *173*(5), 579–588. https://doi.org/10.1086/597611

Hoffmann, A. A., & Sgrò, C. M. (2011). Climate change and evolutionary adaptation. *Nature*, *470*(7335), 479–485. https://doi.org/10.1038/nature09670. Nature Publishing Group.

Hufbauer, R. A., Szűcs, M., Kasyon, E., Youngberg, C., Koontz, M. J., Richards, C., Tuff, T., & Melbourne, B. A. (2015). Three types of rescue can avert extinction in a changing environment. *Proceedings of the National Academy of Sciences*, *112*(33), 10557–10562. https://doi.org/10.1073/pnas.1504732112

Huxley, J. S. (1938). Clines: An auxiliary method in taxonomy. *Bijdragen tot de Dierkunde*, *27*(1), 491–520. https://doi.org/10.1163/26660644-02701023. Brill.

Jactel, H., Petit, J., Desprez-Loustau, M. -L., Delzon, S., Piou, D., Battisti, A., & Koricheva, J. (2012). Drought effects on damage by forest insects and pathogens: A meta-analysis. *Global Change Biology*, *18*, 267–276.

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

Jordan, C. Y., Ally, D., & Hodgins, K. A. (2015). When can stress facilitate divergence by altering time to flowering? *Ecology and Evolution*, *5*(24), 5962–5973. https://doi.org/10.1002/ece3.1821

Kamps, B. B. J., & Poelman, E. H. (2024). Adaptations to water gradient in three Rorippa plant species correspond with plant resistance against insect herbivory under drought and waterlogged conditions. *Ecological Entomology*, *49*(1), 1–9. https://doi.org/10.1111/een.13273

Kawakami, T., Morgan, T. J., Nippert, J. B., Ocheltree, T. W., Keith, R., Dhakal, P., & Ungerer, M. C. (2011). Natural selection drives clinal life history patterns in the perennial sunflower species, *Helianthus maximiliani*. *Molecular Ecology*, *20*(11), 2318–2328. https://doi.org/10.1111/j.1365-294X.2011.05105.x

Kawecki, T. J., & Ebert, D. (2004). Conceptual issues in local adaptation. *Ecology Letters*, *7*(12), 1225–1241. https://doi.org/10.1111/j.1461-0248.2004.00684.x

Keller, S. R., Sowell, D. R., Neiman, M., Wolfe, L. M., & Taylor, D. R. (2009). Adaptation and colonization history affect the evolution of clines in two introduced species. *New Phytologist*, *183*(3), 678–690. https://doi.org/10.1111/j.1469-8137.2009.02892.x

Kellermann, V., Hoffmann, A. A., Kristensen, T. N., Moghadam, N. N., & Loeschcke, V. (2015). Experimental evolution under fluctuating thermal conditions does not reproduce patterns of adaptive clinal differentiation in *Drosophila melanogaster*. *American Naturalist*, *186*(5), 582–593. https://doi.org/10.1086/683252. The University of Chicago Press.

Kooyers, N. J., Blackman, B. K., & Holeski, L. M. (2017). Optimal defense theory explains deviations from latitudinal herbivory defense hypothesis. *Ecology*, *98*(4), 1036–1048. https://doi.org/10.1002/ecy.1731

Kooyers, N. J., Colicchio, J. M., Greenlee, A. B., Patterson, E., Handloser, N. T., & Blackman, B. K. (2019). Lagging adaptation to climate supersedes local adaptation to herbivory in an annual monkeyflower. *American Naturalist*, *194*(4), 541–557. https://doi.org/10.1086/702312. The University of Chicago Press

Kooyers, N. J., Gage, L. R., Al-Lozi, A., & Olsen, K. M. (2014). Aridity shapes cyanogenesis cline evolution in white clover (*Trifolium repens* L.). *Molecular Ecology*, *23*(5), 1053–1070. https://doi.org/10.1111/mec.12666

Kooyers, N. J., Greenlee, A. B., Colicchio, J. M., Oh, M., & Blackman, B. K. (2015). Replicate altitudinal clines reveal that evolutionary flexibility underlies adaptation to drought stress in annual *Mimulus guttatus*. *New Phytologist*, *206*(1), 152–165. https://doi.org/10.1111/nph.13153

Kooyers, N. J., & Olsen, K. M. (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*(6), 495–504. https://doi.org/10.1038/hdy.2013.71. Nature Publishing Group.

Körner, C. (2007). The use of "altitude" in ecological research. *Trends in Ecology and Evolution*, *22*(11), 569–574. https://doi.org/10.1016/j.tree.2007.09.006

Kremer, A., Potts, B. M., & Delzon, S. (2014). Genetic divergence in forest trees: Understanding the consequences of climate change. *Functional Ecology*, *28*(1), 22–36. https://doi.org/10.1111/1365-2435.12169

Labandeira, C. C., & Currano, E. D. (2013). The fossil record of plant-insect dynamics. *Annual Review of Earth and Planetary Sciences*, *41*(1), 287–311. https://doi.org/10.1146/annurev-earth-050212-124139. Annual Reviews.

Lee, C. -R., & Mitchell-Olds, T. (2013). Complex trait divergence contributes to environmental niche differentiation in ecological speciation of *Boechera stricta*. *Molecular Ecology*, *22*(8), 2204–2217. https://doi.org/10.1111/mec.12250. Wiley.

Leimu, R., & Fischer, M. (2008). A meta-analysis of local adaptation in plants. *PLoS One*, *3*(12), e4010. https://doi.org/10.1371/journal.pone.0004010

Lenth, R. (2024). *emmeans: estimated marginal means, aka least-squares means. R package version 1.10.6-090002*, https://rvlenth.github.io/emmeans/, https://rvlenth.github.io/emmeans/.

Li, X., Guo, T., Mu, Q., Li, X., & Yu, J. (2018). Genomic and environmental determinants and their interplay underlying phenotypic plasticity. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(26), 6679–6684. https://doi.org/10.1073/pnas.1718326115

Lin, P. -A., Kansman, J., Chuang, W. -P., Robert, C., Erb, M., & Felton, G. W. (2023). Water availability and plant–herbivore interactions. *Journal of Experimental Botany*, *74*(9), 2811–2828. https://doi.org/10.1093/jxb/erac481

Lorts, C. M., & Lasky, J. R. (2020). Competition × drought interactions change phenotypic plasticity and the direction of selection on Arabidopsis traits. *New Phytologist*, *227*(4), 1060–1072. https://doi.org/10.1111/nph.16593

Lüdecke, D. (2018). ggeffects: tidy data frames of marginal effects from regression models. *Journal of Open Source Software*, *3*(26), 772.

Lüdecke, D., Ben-Shachar, M., Patil, I., Waggoner, P., & Makowski, D. (2021). Performance: An R package for assessment, comparison and testing of statistical models. *Journal of Open Source Software*, *6*(60), 3139. https://doi.org/10.21105/joss.03139

MacColl, A. D. C. (2011). The ecological causes of evolution. *Trends in Ecology and Evolution*, *26*, 514–522. Elsevier.

MacTavish, R., & Anderson, J. T. (2020). Resource availability alters fitness trade-offs: Implications for evolution in stressful environments. *American Journal of Botany*, *107*(2), 308–318. https://doi.org/10.1002/ajb2.1417

Moles, A. T., Peco, B., Wallis, I. R., Foley, W. J., Poore, A. G. B., Seabloom, E. W., Vesk, P. A., Bisigato, A. J., Cella-Pizarro, L., Clark, C. J., Cohen, P. S., Cornwell, W. K., Edwards, W., Ejrnæs, R., Gonzales-Ojeda, T., Graae, B. J., Hay, G., Lumbwe, F. C., Magaña-Rodríguez, B., … Hui, F. K. C. (2013). Correlations between physical and chemical defences in plants: Tradeoffs, syndromes, or just many different ways to skin a herbivorous cat? *New Phytologist*, *198*, 252–263.

Montague, J. L., Barrett, S. C. H., & Eckert, C. G. (2008). Re-establishment of clinal variation in flowering time among introduced populations of purple loosestrife (*Lythrum salicaria*, Lythraceae). *Journal of Evolutionary Biology*, *21*(1), 234–245. https://doi.org/10.1111/j.1420-9101.2007.01456.x

Montesinos-Navarro, A., Wig, J., Xavier Pico, F., & Tonsor, S. J. (2011). *Arabidopsis thaliana* populations show clinal variation in a climatic gradient associated with altitude. *New Phytologist*, *189*, 282–294.

Monty, A., & Mahy, G. (2009). Clinal differentiation during invasion: *Senecio inaequidens* (Asteraceae) along altitudinal gradients in Europe. *Oecologia*, *159*(2), 305–315. https://doi.org/10.1007/s00442-008-1228-2

Mooney, K. A., Halitschke, R., Kessler, A., & Agrawal, A. A. (2010). Evolutionary trade-offs in plants mediate the strength of trophic cascades. *Science*, *327*(5973), 1642–1644. https://doi.org/10.1126/science.1184814. American Association for the Advancement of Science.

Moreira, X., Petry, W. K., Mooney, K. A., Rasmann, S., & Abdala-Roberts, L. (2018). Elevational gradients in plant defences and insect herbivory: Recent advances in the field and prospects for future research. *Ecography*, *41*(9), 1485–1496. https://doi.org/10.1111/ecog.03184

Muehleisen, A. J., Engelbrecht, B. M. J., Jones, F. A., Manzané-Pinzón, E., & Comita, L. S. (2020). Local adaptation to herbivory within tropical tree species along a rainfall gradient. *Ecology*, *101*(11), e03151. https://doi.org/10.1002/ecy.3151

Muir, C. D., & Angert, A. L. (2017). Grow with the flow: A latitudinal cline in physiology is associated with more variable precipitation in *Erythranthe cardinalis*. *Journal of Evolutionary Biology*, *30*(12), 2189–2203. https://doi.org/10.1111/jeb.13184

Navarro, J., Powers, J. M., Paul, A., & Campbell, D. R. (2022). Phenotypic plasticity and selection on leaf traits in response to snowmelt timing and summer precipitation. *New Phytologist*, *234*(4), 1477–1490. https://doi.org/10.1111/nph.18084

Nelson, A. S., Pratt, R. T., Pratt, J. D., Smith, R. A., Symanski, C. T., Prenot, C., & Mooney, K. A. (2019a). Progressive sensitivity of trophic levels to warming underlies an elevational gradient in ant–aphid mutualism strength. *Oikos*, *128*(4), 540–550. https://doi.org/10.1111/oik.05650

Nelson, A. S., Symanski, C. T., Hecking, M. J., & Mooney, K. A. (2019b). Elevational cline in herbivore abundance driven by a monotonic increase in trophic-level sensitivity to aridity. *Journal of Animal Ecology*, *88*(9), 1406–1416. https://doi.org/10.1111/1365-2656.13034

Nicotra, A. B., Atkin, O. K., Bonser, S. P., Davidson, A. M., Finnegan, E. J., Mathesius, U., Poot, P., Purugganan, M. D., Richards, C. L., Valladares, F., & Kleunen, M. van (2010). Plant phenotypic plasticity in a changing climate. *Trends in Plant Science*, *15*, 684–692. Elsevier Ltd.

O'Brien, A. M., Sawers, R. J. H., Strauss, S. Y., & Ross-Ibarra, J. (2019). Adaptive phenotypic divergence in an annual grass differs across biotic contexts*. *Evolution*, *73*(11), 2230–2246. https://doi.org/10.1111/evo.13818

Onoda, Y., Wright, I. J., Evans, J. R., Hikosaka, K., Kitajima, K., Niinemets, U., Poorter, H., Tosens, T., & Westoby, M. (2017). Physiological and structural tradeoffs underlying the leaf economics spectrum. *New Phytologist*, *214*(4), 1447–1463. https://doi.org/10.1111/nph.14496

Pederson, G. T., Gray, S. T., Woodhouse, C. A., Betancourt, J. L., Fagre, D. B., Littell, J. S., Watson, E., Luckman, B. H., & Graumlich, L. J. (2011). The unusual nature of recent snowpack declines in the North American cordillera. *Science*, *333*(6040), 332–335. https://doi.org/10.1126/science.1201570

Pellissier, L., Moreira, X., Danner, H., Serrano, M., Salamin, N., van Dam, N. M., & Rasmann, S. (2016). The simultaneous inducibility of phytochemicals related to plant direct and indirect defences against herbivores is stronger at low elevation. *Journal of Ecology*, *104*(4), 1116–1125. https://doi.org/10.1111/1365-2745.12580

Pérez-Harguindeguy, N., Díaz, S., Vendramini, F., Cornelissen, J. H. C., Gurvich, D. E., & Cabido, M. (2003). Leaf traits and herbivore selection in the field and in cafeteria experiments. *Austral Ecology*, *28*, 642–650.

Poorter, H., Fiorani, F., Pieruschka, R., Wojciechowski, T., van der Putten, W. H., Kleyer, M., Schurr, U., & Postma, J. (2016). Pampered inside, pestered outside? Differences and similarities between plants growing in controlled conditions and in the field. *New Phytologist*, *212*(4), 838–855. https://doi.org/10.1111/nph.14243

Poorter, H., Niinemets, U., Ntagkas, N., Siebenkäs, A., Mäenpää, M., Matsubara, S., & Pons, T. L. (2019). A meta-analysis of plant responses to light intensity for 70 traits ranging from molecules to whole plant performance. *New Phytologist*, *223*(3), 1073–1105. https://doi.org/10.1111/nph.15754

Poorter, H., Niinemets, U., Poorter, L., Wright, I. J., & Villar, R. (2009). Causes and consequences of variation in leaf mass per area (LMA): A meta-analysis. *New Phytologist*, *182*(3), 565–588. https://doi.org/10.1111/j.1469-8137.2009.02830.x

Pratt, J. D., & Mooney, K. A. (2013). Clinal adaptation and adaptive plasticity in *Artemisia californica*: Implications for the response of a foundation species to predicted climate change. *Global Change Biology*, *19*(8), 2454–2466. https://doi.org/10.1111/gcb.12199

R Core Team. (2021). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Ramirez-Parada, T. H., Park, I. W., Record, S., Davis, C. C., Ellison, A. M., & Mazer, S. J. (2024). Plasticity and not adaptation is the primary source of temperature-mediated variation in flowering phenology in North America. *Nature Ecology and Evolution*, *8*(3), 467–476. https://doi.org/10.1038/s41559-023-02304-5

Rangwala, I., Barsugli, J., Cozzetto, K., Neff, J., & Prairie, J. (2012). Mid-21st century projections in temperature extremes in the southern Colorado Rocky Mountains from regional climate models. *Climate Dynamics*, *39*(7-8), 1823–1840. https://doi.org/10.1007/s00382-011-1282-z

Rasmann, S., Pellissier, L., Defossez, E., Jactel, H., & Kunstler, G. (2014). Climate-driven change in plant-insect interactions along elevation gradients. *Functional Ecology*, *28*, 46–54.

Rathcke, B., & Lacey, E. P. (1985). Phenological patterns of terrestrial plants. *Annual Review of Ecology and Systematics*, *16*(1), 179–214. https://doi.org/10.1146/annurev.es.16.110185.001143. Annual Reviews.

Rauschkolb, R., Li, Z., Godefroid, S., Dixon, L., Durka, W., Májeková, M., Bossdorf, O., Ensslin, A., & Scheepens, J. F. (2022). Evolution of plant drought strategies and herbivore tolerance after two decades of climate change. *New Phytologist*, *235*(2), 773–785. https://doi.org/10.1111/nph.18125

Reimann, C., & Breckle, S. -W. (1995). Salt tolerance and ion relations of *Salsola kali* L.: differences between ssp. tragus (L.) Nyman and ssp. ruthenica (Iljin) Soó. *New Phytologist*, *130*(1), 37–45. https://doi.org/10.1111/j.1469-8137.1995.tb01812.x

Rushworth, C. A., Wagner, M. R., Mitchell-Olds, T., & Anderson, J. T. (2022). The Boechera model system for evolutionary ecology. *American Journal of Botany*, *109*(11), 1939–1961. https://doi.org/10.1002/ajb2.16090

Sandel, B., Pavelka, C., Hayashi, T., Charles, L., Funk, J., Halliday, F. W., Kandlikar, G. S., Kleinhesselink, A. R., Kraft, N. J. B., Larios, L., Madsen-McQueen, T., & Spasojevic, M. J. (2021). Predicting intraspecific trait variation among California's grasses. *Journal of Ecology*, *109*, 2662–2677.

Schweiger, O., Settele, J., Kudrna, O., Klotz, S., & Kühn, I. (2008). Climate change can cause spatial mismatch of trophically interacting species. *Ecology*, *89*(12), 3472–3479. https://doi.org/10.1890/07-1748.1

Sletvold, N., Moritz, K. K., & Ågren, J. (2015). Additive effects of pollinators and herbivores result in both conflicting and reinforcing selection on floral traits. *Ecology*, *96*(1), 214–221. https://doi.org/10.1890/14-0119.1

Sloat, L. L., Henderson, A. N., Lamanna, C., & Enquist, B. J. (2015). The effect of the foresummer drought on carbon exchange in subalpine meadows. *Ecosystems*, *18*(3), 533–545. https://doi.org/10.1007/s10021-015-9845-1

Stinchcombe, J. R., Weinig, C., Ungerer, M., Olsen, K. M., Mays, C., Halldorsdottir, S. S., Purugganan, M. D., & Schmitt, J. (2004). A latitudinal cline in flowering time in Arabidopsis thaliana modulated by the flowering time gene FRIGIDA. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(13), 4712–4717. https://doi.org/10.1073/pnas.0306401101

Talsma, C. J., Bennett, K. E., & Vesselinov, V. V. (2022). Characterizing drought behavior in the Colorado river basin using unsupervised machine learning. *Earth and Space Science*, *9*(5), e2021EA–e002086.

Trussell, G. C. (2000). Phenotypic clines, plasticity, and morphological trade-offs in an intertidal snail. *Evolution*, *54*(1), 151–166. https://doi.org/10.1111/j.0014-3820.2000.tb00016.x

Turcotte, M. M., Davies, T. J., Thomsen, C. J. M., & Johnson, M. T. J. (2014). Macroecological and macroevolutionary patterns of leaf herbivory across vascular plants. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1787), 20140555. https://doi.org/10.1098/rspb.2014.0555. Royal Society.

Van Kleunen, M., Lenssen, J. P. M., Fischer, M., & De Kroon, H. (2007). Selection on phenotypic plasticity of morphological traits in response to flooding and competition in the clonal shore plant *Ranunculus reptans*. *Journal of Evolutionary Biology*, *20*(6), 2126–2137. https://doi.org/10.1111/j.1420-9101.2007.01431.x

Via, S., & Lande, R. (1985). Genotype-environment interaction and the evolution of phenotypic plasticity. *Evolution*, *39*(3), 505–522. https://doi.org/10.1111/j.1558-5646.1985.tb00391.x

Wade, M. J., & Kalisz, S. (1990). The causes of natural selection. *Evolution*, *44*(8), 1947–1955. https://doi.org/10.1111/j.1558-5646.1990.tb04301.x

Wadgymar, S. M., Daws, S. C., & Anderson, J. T. (2017). Integrating viability and fecundity selection to illuminate the adaptive nature of genetic clines: Stabilizing selection on functional traits. *Evolution Letters*, *1*(1), 26–39. https://doi.org/10.1002/evl3.3

Wadgymar, S. M., DeMarche, M. L., Josephs, E. B., Sheth, S. N., & Anderson, J. T. (2022). Local adaptation: Causal agents of selection and adaptive trait divergence. *Annual Review of Ecology, Evolution, and Systematics*, *53*(1), 87–111. https://doi.org/10.1146/annurev-ecolsys-012722-035231. Annual Reviews.

Wadgymar, S. M., Ogilvie, J. E., Inouye, D. W., Weis, A. E., & Anderson, J. T. (2018). Phenological responses to multiple environmental drivers under climate change: Insights from a long-term observational study and a manipulative field experiment. *New Phytologist*, *218*(2), 517–529. https://doi.org/10.1111/nph.15029

Walter, G. M., Clark, J., Terranova, D., Cozzolino, S., Cristaudo, A., Hiscock, S. J., & Bridle, J. (2023). Hidden genetic variation in plasticity provides the potential for rapid adaptation to novel environments. *New Phytologist*, *239*(1), 374–387. https://doi.org/10.1111/nph.18744

Wilczek, A. M., Cooper, M. D., Korves, T. M., & Schmitt, J. (2014). Lagging adaptation to warming climate in Arabidopsis thaliana. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(22), 7906–7913. https://doi.org/10.1073/pnas.1406314111

Woods, E. C., Hastings, A. P., Turley, N. E., Heard, S. B., & Agrawal, A. A. (2012). Adaptive geographical clines in the growth and defense of a native plant. *Ecological Monographs*, *82*(2), 149–168. https://doi.org/10.1890/11-1446.1

Wright, I. J., & Cannon, K. (2001). Relationships between leaf lifespan and structural defences in a low-nutrient, sclerophyll flora. *Functional Ecology*, *15*(3), 351–359. https://doi.org/10.1046/j.1365-2435.2001.00522.x

Wright, I. J., Reich, P. B., Westoby, M., Ackerly, D. D., Baruch, Z., Bongers, F., Cavender-Bares, J., Chapin, T., Cornelissen, J. H. C., Diemer, M., Flexas, J., Garnier, E., Groom, P. K., Gulias, J., Hikosaka, K., Lamont, B. B., Lee, T., Lee, W., Lusk, C., … Villar, R. (2004). The worldwide leaf economics spectrum. *Nature*, *428*(6985), 821–827. https://doi.org/10.1038/nature02403

Yin, W., Zhou, L., Yang, K., Fang, J., Biere, A., Callaway, R. M., Wu, M., Yu, H., Shi, Y., & Ding, J. (2023). Rapid evolutionary trade-offs between resistance to herbivory and tolerance to abiotic stress in an invasive plant. *Ecology Letters*, *26*(6), 942–954. https://doi.org/10.1111/ele.14221

Zettlemoyer, M. A. (2023). A demographic approach for predicting population responses to multifactorial stressors. *AoB Plants*, *15*(3), plad023. https://doi.org/10.1093/aobpla/plad023

Zhu, J., Sun, L., He, C., Cai, Q., Zhu, J., & Ji, C. (2024). Large and thin leaves are compromised more by chewers. *Ecosphere*, *15*(1), e4748.