VARIATION IN SOIL WATER AND NUTRIENT AVAILABILITY ALTER LIFE HISTORY EVOLUTION OF *BOECHERA STRICTA* (BRASSICACEAE): IMPLICATIONS FOR ECO-EVOLUTIONARY RESPONSES TO CLIMATE CHANGE

by

Rachel M. MacTavish

(Under the Direction of Jill T. Anderson)

ABSTRACT

Life history theory seeks to explain how natural selection shapes organisms' cycles of birth, maturation, and death to optimize lifetime fitness. Local environmental conditions often exert strong selection on life history traits like reproductive phenology. Climate change directly and indirectly shapes local environments through increased global temperatures and shifts in regional precipitation patterns, altering soil water and nutrient resource availability. Climate change imposes novel selection on natural plant populations and influences eco-evolutionary dynamics. Here, I examine fitness trade-offs across and within environments, patterns of selection, and plasticity in reproductive phenology in the context of climate change using the perennial forb, *Boechera stricta* (Brassicaceae). I exposed 35 accessions originating from natural populations across a 1000 m elevational gradient to a large-scale multi-factorial greenhouse manipulation of three soil water and two nutrient resource availabilities. I quantified fitness (seed count) and four life history traits, which are often genetically-correlated: the timing of first flowering, the duration of flowering, and height and leaf number at flowering. I found clear evidence for local adaptation to drought stress. In addition, drought intensified the trade-off between reproduction and growth, suggesting drought conditions constrain adaptation despite the high heritability of these fitness-related components (chapter 2). Water and nutrient availability imposed selection on all traits. This research expands on field studies, finding persistent selection for early flowering could represent selection for a longer flowering duration (chapter 3).

Additionally, I found strong adaptive plasticity in flowering time response to nutrient manipulations, and plasticity in flowering time increased with elevation. This adaptive plasticity could enhance short-term population persistence in response to climate change in high elevation populations. My results bolster our ability to predict evolutionary responses to shifting resource availabilities under future climate change conditions.

INDEX WORDS: life history trade-offs, genetic covariance, soil resource availability, abiotic stress, drought, plant fitness, selection, local adaptation, water and nutrient availability, climate change, multi-factorial, greenhouse, G matrix, phenotypic plasticity, natural populations

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by

Rachel M. MacTavish

B.S., Kennesaw State University, 2011

M.S., Georgia Southern University, 2014

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by

RACHEL M. MACTAVISH

Major Professor:	Jill T. Anderson
Committee:	Kelly Dyer Andrea Sweigert Nina Wurzberger Lisa Donovan

Electronic Version Approved:

Ron Walcott
Vice Provost for Graduate Education and Dean of the Graduate School
The University of Georgia
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DEDICATION

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

Life history evolution seeks to explain life history strategies employed by organisms to optimize lifetime fitness (Stearns, 1992). Life history events such as birth, growth, reproduction, and senescence have evolved across species. In addition, local environmental conditions often exert strong selection on the timing of transitions between these events. For example, reproducing too early or too late could increase the risk of mortality and reduce reproductive success. Life history traits are often phenotypically plastic and heritable, leading to rapid evolutionary responses to strong selection (Roff, 2002).

Climate change is directly and indirectly altering environmental conditions by increasing global annual temperatures, disrupting precipitation regimes, and increasing the frequency of extreme weather events (Allan et al., 2021). These factors alter soil moisture and nutrient availability (Shaw and Harte, 2001). Climatic changes likely impose novel selection on natural populations, and a burgeoning body of literature has begun to quantify the extent to which climate change alters the direction or magnitude of selection (Jamieson et al., 2012) and organismal evolution (Shaw and Etterson, 2012), and life history traits (Wagner et al., 2016). The availability of soil, water, and nutrients shape life history evolution, yet we know very little about the eco-evolutionary consequences of changes in these conditions. Mountain regions are excellent study systems for examining the effects of climate change. Hot and arid conditions of low elevation environments contrast with cool and moist conditions at higher elevation sites. Additionally, available soil nitrate concentrations decrease with elevation.

Predicting the evolutionary trajectories of natural plant populations as climate change advances requires multifactorial experiments that examine the responses to suites of genetically-correlated traits to multiple changing conditions (Wadgymar, Lowry, et al., 2017). Small shifts in life histories can alter population growth and persistence as global change precedes (Williams et al., 2015). Abiotic conditions impose strong selection on resource acquisition and allocation traits, yet genetic trade-offs between life history traits can constrain adaptive responses to selection (Stearns, 2000). Genetic trade-offs and phenotypic plasticity can also maintain genetic variation which may facilitate or constrain evolution by natural selection (Houle, 1992). Plasticity can facilitate population persistence under rapidly changing environments if populations have evolved plasticity in response to shifting conditions (Zettlemoyer and Peterson, 2021). In my dissertation, I investigated life history responses to soil nutrient and water availability in a large-scale multifactorial greenhouse experiment.

My overarching objective was to evaluate the evolutionary consequences of altered water and nutrient resource availability on reproductive phenology and fitness using *Boechera stricta* (Brassicaceae), an emerging ecological model plant species. In chapter 2, I examine fitness trade-offs across and within environments to address whether: 1) resource supply alters fitness trade-offs across environments (local adaptation) 2) novel resource environments induce trade-offs between key fitness components, and 3) genetic correlations constrain adaptation. In chapter 3, I investigate patterns of selection imposed by novel water and nutrients on life history traits. I hypothesize that: 1) soil resources impose selection on reproductive phenology, and 2) patterns of selection reflect local adaptation to soil water and nutrient availability. Finally, chapter 4 explores the extent of plasticity in life history trait evolution, asking if: 1) resource availability induces plasticity, 2) plasticity shows clinal variation in response to elevation, and 3) plasticity in life history traits is adaptive.

I exposed accessions from natural populations of *B. stricta* to a large-scale multifactorial greenhouse manipulation of soil water and nutrient resource availability. *Boechera stricta* is a perennial forb distributed along a wide elevational gradient in the Rocky Mountains of North America from Utah to Alaska (Rushworth et al., 2011). This selfing species has seeds dispersed by gravity and wind, resulting in high genetic differentiation between populations. Populations in this system are locally adapted to latitudinal, elevational, and soil moisture gradients (Anderson et al., 2013; Lee and Mitchell-Olds, 2013; Anderson and Gezon, 2015; Anderson and Wadgymar, 2020). I selected 35 maternal lines from natural populations separated by ~30 meters along a 1000 m elevational gradient in sub-alpine meadows, which had been grown for one generation in greenhouse conditions to homogenize maternal effects and generate full-sibling families. I quantified fitness (seed count) and four life history traits, which are often genetically-correlated: the timing of first flowering, the duration of flowering, and height and leaf number at flowering. Through a series of generalized linear regression mixed models, I tested genetic trade-offs across and within environments, novel patterns of selection, and phenotypic plasticity in response to soil resource combinations.

I found clear evidence for local adaptation to drought stress. In addition, drought intensified the trade-off between reproduction and growth, suggesting drought conditions constrain adaptation despite the high heritability of these fitness-related components. Additionally, water and nutrient availability altered trade-offs between fitness components (chapter 2). Water and nutrient availability imposed selection on all traits. Longitudinal field studies have documented strong selection for early flowering in *B. stricta* (Anderson, Panetta, et al., 2012; Wadgymar, Lowry, et al., 2017). Additionally, strong selection for early flowering in *Arabidopsis thaliana* was found in Mediterranean climates of Italy compared to weak selection in Sweden (Ågren et al., 2016) which may be due to soil moisture (Kenney et al., 2014). However, very few studies have investigated selection on the full flowering schedule including flowering duration (but see: Austen and Weis, 2015, 2016; Austen et al., 2017). It is possible that apparent selection for earlier flowering could, in fact, represent selection for a longer duration of flowering, if individuals that flower early also flower for more extended time periods (Austen et al., 2017). In my study, selection favored both early flowering and increased duration of flowering, suggesting that both traits are targets of selection (chapter 3). These results have implications for biological responses to global change, as climate change has induced earlier flowering in many systems (Anderson, Panetta, et al., 2012), with unknown consequences for the length of the flowering period.

In my final chapter (4), I found strong adaptive plasticity in flowering time response to nutrient treatment. Additionally, plasticity in flowering time increased with source elevation, suggesting that plasticity could enable rapid responses to climate change in high elevation populations. Water and nutrient treatments influenced plant size at flowering, as measured through the number of leaves at first flower. Stabilizing selection favored intermediate values of plasticity in flowering duration.

My results enhance our ability to predict evolutionary responses to shifting resource availability under future climate change conditions. Soil water and nutrient availability influence fitness trade-offs in stressful environments and patterns of selection on critical life history traits. Furthermore, plasticity may enhance short-term population persistence under climate change, especially in high elevation populations. However, I acknowledge that genetic correlations, heritability, and selection estimates can vary between greenhouse and field (Campbell, 1996; Conner et al., 2003; Poorter et al., 2016) due to additional abiotic and biotic factors stresses in natural settings. I highlight the need to conduct multifactorial field studies to enhance models predicting evolutionary trajectories in response to rapid climate change.

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CHAPTER 2

RESOURCE AVAILABLITY ALTERS FITNESS TRADE-OFFS: IMPLICATIONS FOR EVOLUTION IN STRESSFUL ENVIRONMENTS[1]

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Abstract

Climate change has elevated temperatures and caused novel precipitation patterns, altering soil moisture and nutrient availability. Predicting evolutionary responses to climate change requires information on the agents of selection that drive local adaptation and influence resource acquisition and allocation. Here, we examined the contribution of nutrient and drought stress to local adaptation, and we tested whether trade-offs across fitness components constrain or facilitate adaptation under resource stress. We exposed 35 families of *Boechera stricta* (Brassicaceae) to three levels of water and two levels of nutrient supply in a factorial design in the greenhouse. We sourced maternal families from a broad elevational gradient (2499-3530m), representing disparate soil moisture and nutrient availability. Concordant with local adaptation, maternal families from arid low elevation populations had enhanced fecundity under severe drought over those from more mesic high elevation sites. Furthermore, fitness trade-offs between growth and reproductive success depended on the environmental context. Under high, but not low, nutrient levels, we found a negative phenotypic relationship between the probability of reproduction and growth rate. Similarly, a negative phenotypic association only emerged between fecundity and growth under severe drought stress, not the benign water treatment levels, indicating that stressful resource environments alter the direction of trait correlations. Genetic covariances were broadly concordant with these phenotypic patterns. Despite high heritabilities in all fitness components across treatments, trade-offs between growth and reproduction could constrain adaptation to increasing drought stress and novel nutrient levels.

Introduction

Natural populations evolve in response to abiotic and biotic agents of selection, which shape fitness trade-offs across environments (local adaptation) and across life history stages (life history evolution) (Roff, 2002). Global climate change is directly and indirectly introducing novel environmental conditions through increasing temperatures, disrupted precipitation regimes, and increased frequency of extreme weather events (IPCC 2014). Climate change also directly influences the soil environment, elevating soil temperature, and lowering moisture through increased evapotranspiration rates (Anderson, 1992; Rosenzweig and Hillel, 2000). Long periods of drought followed by heavy precipitation have resulted in extreme soil moisture variability (Sheffield and Wood, 2008). Climate change will likely decouple plant-soil interactions; however, we know very little about the evolutionary consequences of this decoupling for plants (Bardgett et al., 2013; Classen et al., 2015; Yuan and Chen, 2015). Here, we examine the extent to which abiotic stresses associated with climate change influence local adaptation and life history trade-offs.

Variation in climatic, edaphic, and abiotic agents of selection across space can favor the evolution of local adaptation, such that ecotypes have enhanced performance in their home sites and reduced fitness in contrasting environments (Kawecki and Ebert, 2004). Disentangling the causal agents of local adaptation remains challenging because multiple interacting factors can contribute to fitness trade-offs across environments (Lowry et al., 2008; Wadgymar et al., 2017b). In some systems, variation in climatic factors has been directly linked to local adaptation (Liancourt et al., 2013). In other systems, variation in competitive interactions strongly influence local adaptation (Sambatti and Rice, 2006).

Additionally, both climatic factors and soil environment can drive local adaptation for some species (Macel et al., 2007). Only through manipulative experiments will we gain insight into the causal agents of local adaptation, which is critical for predicting population persistence as advancing climate change may disrupt local adaptation, favoring foreign ecotypes that evolved under warmer and more arid conditions (Wang et al., 2010; Wilczek et al., 2014; Kooyers et al., 2019).

Abiotic and biotic conditions not only influence local adaptation, but they can also alter trade-offs between fitness components. Correlations across fitness components arise through variation in resource acquisition or allocation (Riska, 1986; Van Noordwijk and de Jong, 1986; Roff, 2002; Villellas and García, 2018). That is, individuals can vary in how they allocate limited resources to various critical functions like growth or reproduction; for example, high growth rates could come at a cost to reproduction (Stearns, 1989; Reznick et al., 2000; Roff, 2002; Villellas and García, 2018). Alternatively, individuals can vary in their capacity to acquire resources, such that some individuals have more resources to allocate to multiple functions, which would generate positive relationships between fitness components like growth and survival (Van Noordwijk and de Jong, 1986; Zera and Harshman, 2001). Trade-offs have emerged in response to altered conditions associated with climate change, such as new soil moisture levels (Rosenzweig and Hillel, 2000; Sheffield and Wood 2008) and nutrient availability (Kuzyakov, 2010; Siemens et al., 2010). Drought stress has altered both the sign and magnitude of phenotypic and genetic correlations associated with fitness in barley, *Hordeum vulgare* (Shakhatreh et al., 2001). Additionally, water deficiency exposed a trade-off between stress tolerance (measured via growth rate) and herbivory defense (glucosinolate production) in natural populations of *Boechera stricta* (Siemens et al., 2012).

Physiological or phenotypic trade-offs could be highly plastic and evolutionarily labile when plants confront novel environments. In contrast, genetic trade-offs could impose more severe evolutionary constraints. Even when genetic variation exists in fitness components, trade-offs across traits can restrict adaptive evolution if genetic correlations across life history traits are antagonistic to the joint vector of selection; in contrast, genetic correlations can facilitate adaptation when selection operates in the same direction as the genetic correlation (Etterson and Shaw, 2001). Novel resource environments can alter genetic correlations of life history traits by inducing trade-offs under stress (Giesel, 1988; Krebs and Loeschcke, 1999; Ackermann et al., 2001; Sgro and Hoffmann, 2004). For example, the sign and magnitude of genetic correlations among traits of *Impatiens capensis* changed depending on intraspecific plant density and light environments (Donohue et al., 2000). Additionally, herbivore removals altered genetic correlations of herbivory tolerance and fitness in *Ipomoea herderacea* (Stinchcombe, 2002). Examining fitness trade-offs at the physiological and genetic levels will reveal how novel resource stress may alter evolutionary potential as climate change continues.

Natural selection operates strongly on fitness components (Price and Schluter, 1991), rapidly reducing genetic variation in survival, fecundity, and other traits that are tightly linked to lifetime fitness. Therefore, fitness components typically have reduced heritability values relative to morphology or other traits that are more distantly related to fitness (Mousseau and Roff, 1987a). Genetic variation in fitness is crucial for natural populations to adapt to current environmental conditions, as Fisher's Fundamental theorem states that the adaptive potential of populations is proportional to genetic variation in lifetime fitness (Sheth et al., 2018). Novel conditions can induce the expression of cryptic genetic variation, thereby enhancing the capacity of populations to adapt to environmental change (Hoffmann and Merilä, 1999; McGuigan et al., 2011; Paaby and Rockman, 2014). Alternatively, new stressful conditions could depress fitness and genetic variation in fitness (Hoffmann and Merilä, 1999), restricting adaptive potential. Evaluating the extent of heritability in fitness components under novel environments will enable robust predictions about the potential for populations to adapt to climate change factors (Sheth et al., 2018).

This study investigates the effects of resource availability in the context of climate change on fitness trade-offs and heritabilities. We evaluated local adaptation to drought and nutrient stress using, *Boechera stricta* (Brassicaceae), a perennial forb native to high elevation sties in the Rocky Mountains. We predicted that low elevation populations, which evolved under reduced water availability relative to their high elevation counterparts, would have elevated fitness in water limited treatments and high elevation populations would express the greatest fitness under well-watered conditions. Similarly, we hypothesized that high elevation populations that evolved in nutrient poor soils would have greater performance under nutrient limitation. We then tested the extent to which the environmental context influence life history tradeoffs. We hypothesized that under limited resource supply, allocation of resources results in trade-offs across fitness components. Additionally, we examine whether stressful environments enhance or restrict the adaptive potential of populations by comparing genetic variation under stressful and benign conditions. By exposing *B. stricta* individuals from a broad elevational gradient to variation in soil nutrient and soil water stress in the greenhouse, we directly test the extent to which global change influences correlations between fitness components.

Methods

Focal system

Boechera stricta (Brassicaceae) is a short-lived perennial forb that grows in montane habitats of the Rocky Mountains in North America, from Utah to Alaska (Rushworth et al., 2011). The Colorado Rocky Mountains are home to genetically diverse populations of *B. stricta* (Kiefer et al., 2009). Natural populations of *B. stricta* are adapted to local environments across latitudes, elevations, and soil water gradients (Anderson et al., 2013; Lee and Mitchell-Olds, 2013; Anderson et al., 2015). *Boechera stricta* is primarily self-pollinating (average FIS = 0.89, Song et al., 2006) and has gravity dispersed seeds. In this species, foliar N content increases with elevation (Anderson and Gezon, 2015). In addition, in this region, soil moisture increases and soil nitrate availability declines with elevation (Ranelli et al., 2015). Taken together, these data suggest that natural populations could differ in nutrient acquisition or allocation strategies.

The direct effects of climate change have implications for soil biogeochemical cycling of organic carbon and inorganic nutrients (Seneviratne et al., 2010; St.Clair and Lynch, 2010), including limiting phosphorous and nitrogen availability to plants (Agren et al., 2012). A simulation model found that increased mean annual temperatures reduced soil available phosphorous primarily by decreasing organic phosphorous (Hou et al., 2018). Elevated temperature and increased precipitation accelerate organic matter turnover (Conant et al., 2011; Fyfe et al., 2017), releasing plant available nitrogen (Kuzyakov, 2010). In northern latitudes and alpine regions, variability in interannual snowpack exposes soil to an increased number of freeze/thaw cycles, prematurely releasing nitrogen before plants emerge (Henry, 2008; Groffman et al., 2011; Gao et al., 2017; Templer et al., 2017). These abiotic stresses imposed by climate change could alter fitness trade-offs across environments and across life history.

Prior to initiating this experiment, we collected seeds from natural populations that span a broad elevational gradient (2499 – 3530m) around the Rocky Mountain Biological Laboratory (Gothic, Colorado; Appendix S1 see the Supplementary Data with this article) to capture genetic variation in resource acquisition and allocation strategies. We grew field-collected seeds for one generation in the greenhouse to standardize maternal effects and generate full-sibling maternal families to use in our experiments.

Experimental design

To evaluate the consequences of resource supply for phenotypic and genetic correlations, we experimentally manipulated water and nutrient resource availability in the greenhouse at the University of Georgia. We quantified fitness components to examine life history trade-offs such as growth vs. survival. In March 2017, we exposed plants from 35 maternal families (one family per source population) to factorial manipulations of three water levels and two nutrient availability levels. As very young seedlings would not have survived water stress, we first germinated seeds in benign conditions, misting with water daily to ensure survival to the juvenile stage. When individuals were 8 weeks old, we randomized plants into a factorial experiment, assigning six siblings per family to three water and two nutrient availability treatments (N = 6 siblings× 35 families × 3 water levels × 2 nutrient levels = 1,260 individuals). In 51 cases, seeds failed to germinate, or seedlings died prior to randomization, resulting in a total of 1,209 experimental plants in the analysis (Appendix S2). Water treatment levels included: control (watered 7 days/week), moderate drought (watered 2 days/week), and severe drought treatments (watered 1 day/week).

Hand watering delivered ~3 mm of water per treatment application, such that control, moderate drought, and severe drought received ~90, 24, and 12 mm of water per month, respectively. As a point of comparison, average annual summer (June-August) precipitation from 2000-2018 at the Rocky Mountain Biological Laboratory (elevation: 2895m) was 51.20 ± 18.30 mm (mean ± S.D.), with a range of 29.21 mm - 90.80 mm (b. barr, long-term weather data). We verified the efficacy of our water treatments by sampling soil moisture as percent volume water content (%VWC) before the weekly nutrient application with a Hydrosense II (Campbell Scientific, Logan, Utah) from 4 randomly selected individuals per block (Fig. S1.2). No visible size differences were detected at the time of collection of data on volumetric water content (personal obs., R. MacTavish). At a mid-elevation (2890m) field common garden at the Rocky Mountain Biological Laboratory, summer volumetric water content averaged 10% (standard deviation: 5%; range 0-39%) from 2014-2018. Thus, the moderate drought stress (mean ± S.E.: 10.51 %± 2.33%) we imposed reflects average soil water availability in the field, and the well-watered (17.52 %± 2.71%) and severe drought stress (4.65 % ± 1.71 %) fall within the range of variability in the field.

We applied two nutrient treatments once weekly as a mixture of water-soluble Peter's Professional General Purpose Fertilizer™ 20N-10P-20K at the concentrations of 110 ppm N (1x; 1 ppm = 1 mg/L), and 1100 ppm (10x). We selected fertilizer concentrations to be stressful vs. moderate (low vs. medium) based on previous studies of nutrient stress in *B. stricta* (Siemens et al., 2010; Siemens et al., 2012). We applied high nutrient levels early in life history to simulate nutrient pulses that occur at snowmelt when seedlings first emerge (Groffman et al., 2001; Groffman et al., 2011; Gavazov et al., 2017), and to enhance seedling establishment (Villar-Salvador et al., 2012; Gan et al., 2015). After vernalization, we reduced nutrient levels to a low (1x) and high (10x) 5 and 50 ppm N respectively through the remainder of the study to reflect high vs. low values documented across elevational gradients (Ranelli et al., 2015; He et al., 2016; Drollinger et al., 2017).

We rotated blocks around the greenhouse benches weekly to minimize non-experimental environmental variation. Juvenile plants experienced three weeks of watering treatments with nutrient application before an 8-week vernalization at 4°C in June 2017, during which water was supplied equally across treatments by bottom watering. Vernalization is necessary to induce flowering in this system (Anderson et al., 2011). After observing furled leaf development at the beginning of the second growing season, we applied a Magnesium and Calcium only micronutrient mixture to all plants once every 8 weeks at 110 ppm in water.

Fitness components We measured the following fitness components: survival to the end of the 20-week experiment, the probability of reproduction, fecundity among individuals that reproduced, and growth rate. We quantified fecundity as the number of mature siliques (fruits) per individual, and Relative Growth Rate (RGR) using initial and final leaf lengths: (ln(final leaf length) – ln(initial leaf length))/ elapsed time in months.

Statistical analyses To evaluate whether populations from different elevations have adapted locally to abiotic conditions, we evaluated fitness as a function of elevation of origin, drought treatment, nutrient treatment and all 2- and 3-way interactions. As our fecundity data were zero-inflated, we adopted a hurdle model approach, in which we first tested the probability of reproduction in a logistic regression framework (logit link) and then used a gamma distribution with a log link to test fecundity (number of fruits) among individuals that successfully reproduced. We analyzed data at both the individual- and the family-level in Proc Glimmix (SAS ver. 9.4), with random effects for source population (both analyses) and block (individual-level analysis). To analyze the probability of reproduction at the family-level, we summarized the number of individuals that successfully reproduced over the number of individuals planted for each source population. We generated family-level LSMEANs for fecundity (number of fruits) in generalized linear mixed models in Proc Glimmix with a random effect for block. Family- and individual-level analyses generated nearly identical results; we present the family-level analyses in the Results and the individual-level analyses in Appendix S3.

To assess whether life history trade-offs varied across environmental contexts, we analyzed trade-offs as a function of drought treatment, nutrient treatment, and drought by nutrient interactions. We modeled phenotypic correlations using family-level LSMEANs as well as individual-level data in a mixed linear model framework by regressing one trait against another in SAS 9.4 (Proc Glimmix), with fixed effects for drought and nutrient environments, and all two- and three-way interactions among drought treatment, nutrient treatment, and trait values. We incorporated maternal family as a random effect to account for nonindependence among families exposed to different treatments (both analyses) and block as a random effect in the individual-level models. We tested trade-offs between the probability of reproduction and RGR, as well as fecundity (# of fruits among individuals that reproduced) and RGR. We were unable to model trade-offs between survival and other fitness components because we have no fecundity or growth data on individuals that died. As we used the same data to evaluate two trade-offs, we adjusted our alpha ($\alpha = 0.05/2 = 0.025$) to correct for multiple comparisons (Chen et al., 2017).

We estimated broad-sense heritability and analyzed genetic correlations with a Bayesian approach known as the animal model, implemented in the MCMCglmm package of R 3.5.2 (Wilson et al., 2010). This approach uses a mixed model restricted maximum likelihood (REML) process to analyze individual-level data while considering genetic relationships between individuals. We extracted G-matrices (genetic variance and covariance matrices) to examine genetic trade-offs in different environments and determined statistical significance as 95% credible intervals not overlapping zero (Wilson et al., 2010). In *B. stricta* and other selfing species, selection operates on total genetic variance, not simply additive genetic variance (Roughgarden, 1979).

We estimated broad-sense heritability for fitness components by fitting univariate models with random effects for individual plant ("animal" in the coding for the MCMCglmm models) and block across the 6 resource supply environments (3 water x 2 nutrient). This analytical approach links phenotypic data to a pedigree file, which identifies the parents of each individual plant, thereby enabling estimation of heritability.

We then compared genetic covariance across the 6 resource supply environments (3 water x 2 nutrient) using MCMCglmm posterior mean and credible intervals to construct distributions and test for overlapping regions at $\alpha = 0.05$. After testing each trade-off × drought and nutrient combination, we found no significant differences due to treatment for any trade-offs. Therefore, we used results from our phenotypic correlations described above as a guide to examine whether drought influenced genetic covariance for Reproduction vs. RGR and Fecundity vs. RGR. To do so, we fitted multivariate models of fitness component trade-off × drought treatment to model the G-matrix across watering treatment. We could not analyze trade-offs between survival and other fitness components because we lacked fecundity and growth data on plants that died during the experiment.

Results

Fitness consequences of abiotic stress: The probability of reproduction varied significantly as a function of nutrient stress and nutrient by source elevation in both family-level (Table 1.2; Fig. 1.2) and individual-level (Appendix S4.2 and S5.2) analyses. In the high nutrient treatment, the probability of reproduction increased significantly with elevation of origin for both analyses, but we found no relationship between this fitness component and source elevation in the low nutrient treatment (source elevation × nutrient treatment: $F_{1,164}=6.23$, $p=0.013$, Fig. 1.2).

Family- and individual-level analyses of fecundity (# of fruits) among individuals that successfully reproduced generated nearly identical results (Tables 1.2 and Appendix S4.2; Fig. 2.2 and Appendix S6.2). A significant interaction between source elevation and nutrient treatment ($F_{1,115}$=10.09, p=0.0019) revealed a positive relationship between fecundity and elevation of origin in the high nutrient treatment and no relationship in the low nutrient treatment (Fig 2.2). This fecundity pattern in the high nutrient treatment is in the opposite direction from what we found for the probability of reproduction. The fecundity analysis also uncovered a significant interaction between source elevation and drought treatment ($F_{2,115}$=7.09, p=0.0013). Concordant with expectations, under severe drought stress, fecundity declined with source elevation in both family- and individual-level analyses (Fig. 3.2, Appendix S7.2), such that plants from arid low elevation population exhibited enhanced fitness in severe drought conditions relative to those from humid high elevation populations. We found no significant relationship between fecundity and source elevation under moderate drought and well-watered conditions (Fig. 3.2, Appendix S7.2).

Phenotypic correlations: Resource supply altered the magnitude of life history trade-offs. Nutrient availability influenced the relationship between reproductive success and RGR at both the family-level (nutrient treatment × RGR: $F_{1,163}$ = 5.91, p=0.016; Table 2.2, Fig. 4.2) and individual-level (nutrient treatment × RGR: $F_{1,998}$ = 9.22, p=0.0025; Appendix S8 and S9). Plants in both nutrient treatments showed a negative relationship between the probability of reproduction and RGR, yet higher nutrient levels intensified the magnitude of this negative pattern (Fig. 4.2).

Water availability influenced the relationship between fecundity and RGR, at the family level (drought treatment × RGR:$F_{2,114}$ = 5.48, p=0.0054; Table 2.2), such that a negative relationship emerged only under severe drought and no significant relationship appeared under moderate drought stress or well-watered conditions (Fig. 5.2). Individual-level analyses did not find this drought by treatment interactions, instead a negative relationship emerged between fecundity and RGR across all treatments (main effect of RGR: $F_{1,458} = 13.88$, $p=0.0002$; Appendix S8.2 and S10.2).

Heritability: Broad-sense heritability values ranged from 0.033 - 0.997 (Table 3.2). Consistent with expectations, the lowest heritability values were for fecundity (all treatments) and survival (all drought treatments under low nutrient levels). For all fitness components, we found high overlap in 95% credible intervals across treatment levels, except for survival, for which heritabilities were depressed in the low nutrient treatment relative to the high nutrient treatment. Thus, our dataset provides no evidence that abiotic stress either enhances or depletes genetic variation in fitness traits.

Genetic covariances: We found significant negative genetic covariances indicative of trade-offs between growth and the probability of reproduction under severe water stress in low nutrient conditions, and under all watering treatments (control, moderate drought, and severe water stress) at the moderate nutrient level (Table 4.2). Our analyses did not reveal any significant genetic covariances between growth and fecundity among individuals that successfully reproduced.

Discussion

In our study, drought and nutrient stresses contributed to local adaptation and the environment affected phenotypic correlations and genetic covariances between fitness components. Field studies have documented local adaptation to elevation in this system (Anderson et al., 2015; Wadgymar et al., 2017a). Concordant with expectations, in this greenhouse experiment, we found that low elevation ecotypes had elevated fecundity under drought stress compared with high elevation ecotypes. This tradeoff indicates that water stress contributes significantly to local adaptation in these populations. The results of our tests for local adaptation to nutrient stress were more equivocal. Contrary to predictions, the probability of reproduction increased with source elevation under higher nutrient levels. However, patterns were consistent with expectations when we examined fecundity among individuals that successfully reproduced: Fecundity declined with source elevation under higher nutrient levels, which could reflect local adaptation to high soil nutrient levels in low elevation locations. Both nutrient and drought treatments influenced the relationships between growth and reproductive success.

Our findings highlight that variation in resource allocation and fitness trade-offs depend on the environment (Riska, 1986; Van Noordwijk and de Jong, 1986; Houle, 1991; Roff, 2002). In the sand cricket, *Gryllus firmus*, negative genetic correlations between key traits only emerged under food restriction, indicating that low resource supply induced life history trade-offs (King et al., 2011). Few studies have empirically tested the effect of resource supply on phenotypic or genetic correlations of fitness components, although the environment clearly influences the direction and magnitude of other trait correlations (Sgro and Hoffmann, 2004). Service and Rose (1985) found that limiting food resources shifted the magnitude of genetic correlations between fecundity and starvation resistance for *Drosophilia melanogaster.* Despite significant heritability in fitness components under severe water restriction in this study, phenotypic trade-offs between growth and reproduction could constrain adaptation in stressful environments if selection favors both increased growth and fecundity. Climate change has already caused severe winter and summer droughts in many regions, including the native range of *B.*

stricta (Rangwala et al., 2012; IPCC, 2014; Fyfe et al., 2017). Our results suggest that families with high growth rates may have reduced fecundity during years of drought stress, which will intensify as climate change continues (Rangwala et al., 2012; IPCC, 2014; Fyfe et al., 2017; Schlaepfer et al., 2017). Furthermore, our results suggest that low elevation ecotypes, which are adapted to dry conditions, could have a fitness advantage over higher elevation ecotypes under increasing drought stress. Future studies will evaluate the extent to which fitness trade-offs restrict lifetime fecundity under drought.

Other studies have also observed that life history trade-offs can emerge under stressful conditions. For example, Villellas and Garcia (2018) examined the context-dependent nature of fitness trade-offs across natural populations of *Plantago major.* In their study, costs of reproduction manifested only in low soil fertility habitats (Villellas and García, 2018). Thus, stress could induce life history trade-offs that are not apparent when resource levels are abundant. Interestingly, Villellas and Garcia (2018) found greater evidence for costs of reproduction under increased precipitation, which could have been due to flooding stress in populations exposed to elevated precipitation. Populations of *Plantago coronopus* displayed negative genetic correlations between fitness components under drought stress in the greenhouse (Hansen et al., 2013). Additionally, Sherrard et al. (2009) found that water stress increased negative genetic correlations between physiological traits in *Avena barbata.* Stressful conditions likely reduce the amount of resources that individuals can acquire while simultaneously altering associations between fitness components and potentially reducing adaptive potential.

Genetic covariances were broadly congruent with phenotypic patterns for the trade-off between the probability of reproduction and growth rates. At the genetic and phenotypic levels, we found under higher nutrient levels, individuals with high growth rates had reduced probability of reproduction. In contrast, genetic and phenotypic patterns differed for the trade-off between fecundity and growth. At the phenotypic-level, allocation to growth reduced fecundity under severe drought; however, we found no evidence for significant genetic covariances between these fitness components under any treatment. We hypothesize that resource limitation drives the negative phenotypic correlations between growth and reproductive success in our study. In contrast, negative genetic covariances may result from intrinsic mechanisms including linkage and pleiotropy (Flatt and Hayland, 2011). Furthermore, negative genetic correlations can constrain evolution toward an optimal combination of trait values, potentially limiting a population's adaptive potential (Lande 1982, Etterson and Shaw 2001).

Fitness components experience strong selection, which leads to the expectation that genetic variation and heritability should be low for traits strongly associated with lifetime fitness (Mousseau and Roff, 1987b). Consistent with this prediction, heritability estimates were lower for fecundity than growth and the probability of reproduction, yet heritabilities in these three fitness components did not vary with environment. In contrast, heritabilities in survival were substantially lower under low nutrient levels than high nutrient levels across all drought treatments. Overall, this regional pool of plants appears to have sufficient genetic variation for adaptive responses to ongoing selection, but limited heritability could constrain adaptation to low nutrient levels. By exposing plants to stresses in the greenhouse that reflect conditions they experience in the field, we've increased the likelihood that our results reveal meaningful eco-evolutionary dynamics that occur in nature (Poorter et al., 2016). We focused this study on maternal families collected across a broad elevational and resource gradient to increase the genetic variation within our experiment. Future studies will test the extent to which genetic trade-offs and limited genetic variation could constrain within population adaptation to novel environments under field conditions.

Conclusions: By isolating the effects of nutrient and drought stresses on fitness in a diverse regional pool of accessions, our greenhouse experiment revealed local adaptation to severe drought, relatively high heritabilities across fitness components and environments, and fitness trade-offs at the phenotypic and genetic levels in distinct environments. In our study, resource supply altered the associations between fitness components. Under benign water supply and moderate drought stress, we found no relationship between fecundity and growth. In contrast, as drought increased in severity, we uncovered trade-offs, such that allocating resources to growth reduced investment in fecundity. Longitudinal studies have revealed that high interannual variability of water supply may obscure trade-offs (Agrawal et al., 2010; Zust and Agrawal, 2017). Our results show that water stress can shift genetic associations between fitness components, potentially constraining ongoing adaptation to stressful conditions despite significant heritability in fitness components (Hellman and Pineda-Krch 2007). As climate change continues to expose plants to stressful resource environments, genetic trade-offs that did not previously exist under benign conditions could restrict adaptive potential.

Tables and Figures

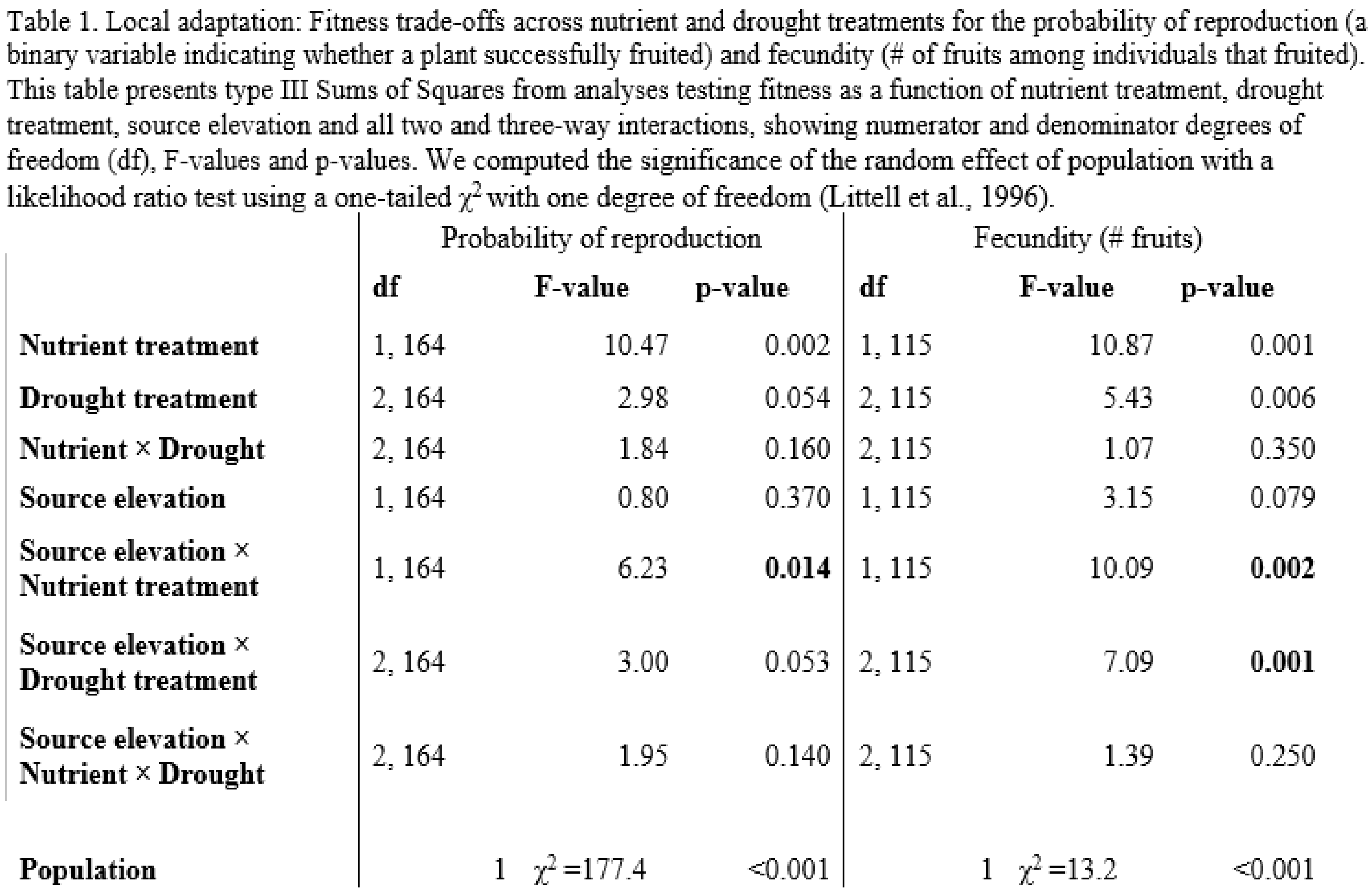

Table 1. Local adaptation: Fitness trade-offs across nutrient and drought treatments for the probability of reproduction (a binary variable indicating whether a plant successfully fruited) and fecundity (# of fruits among individuals that fruited). This table presents type III Sums of Squares from analyses testing fitness as a function of nutrient treatment, drought treatment, source elevation and all two and three-way interactions, showing numerator and denominator degrees of freedom (df), F-values and p-values. We computed the significance of the random effect of population with a likelihood ratio test using a one-tailed χ^2 with one degree of freedom (Littell et al., 1996).

	Probability of reproduction			Fecundity (# fruits)		
	df	**F-value**	**p-value**	**df**	**F-value**	**p-value**
Nutrient treatment	1, 164	10.47	0.002	1, 115	10.87	0.001
Drought treatment	2, 164	2.98	0.054	2, 115	5.43	0.006
Nutrient × Drought	2, 164	1.84	0.160	2, 115	1.07	0.350
Source elevation	1, 164	0.80	0.370	1, 115	3.15	0.079
Source elevation × Nutrient treatment	1, 164	6.23	**0.014**	1, 115	10.09	**0.002**
Source elevation × Drought treatment	2, 164	3.00	0.053	2, 115	7.09	**0.001**
Source elevation × Nutrient × Drought	2, 164	1.95	0.140	2, 115	1.39	0.250
Population	1	χ^2 =177.4	<0.001	1	χ^2 =13.2	<0.001

Table 2. Phenotypic associations of fitness components varied with treatment in family-level analyses. This table presents type III Sums of Squares from analyses evaluating the context dependent nature of phenotypic correlations, showing numerator and denominator degrees of freedom (df), F-values and p-values. We adjusted our alpha ($\alpha = 0.05/2 = 0.025$) to correct for multiple comparisons (analysis of 2 tradeoffs in one dataset). We highlight in bold significant p-values that fall below our adjusted α. We computed the significance of the random effect of population with a likelihood ratio test using a one-tailed χ^2 with one degree of freedom
(Littell et al., 1996).

	Probability of reproduction vs. RGR			Fecundity vs. RGR		
	df	*F*	*P*	df	*F*	*P*
RGR	1, 163	12.22	**0.001**	1, 114	8.96	**0.003**
Drought	2, 163	2.84	0.061	2, 114	4.46	**0.014**
Nutrient	1, 163	4.41	*0.0373*	1, 114	0.00	0.954
Drought × RGR	2, 163	3.17	0.045	2, 114	5.48	**0.005**
Nutrient × RGR	1, 163	5.91	**0.016**	1, 114	0.03	0.871
Drought × Nutrient	2, 163	0.52	0.598	2, 114	0.23	0.798
Drought × Nutrient × RGR	2, 163	0.57	0.566	2, 114	0.23	0.799
population	1	$\chi 2$ =168.9	<**0.001**	1	$\chi 2$ =5.39	**0.010**

Table 3. Broad-sense heritability (H^2) estimates across 6 resource combinations (3 water × 2 nutrient). Lower and Upper 95% credible intervals are represented as L95 and U95, respectively.

		RGR			Fecundity			Probability of reproduction			Survival		
Drought	Nutrient	H^2	L95	U95	H^2	L95	U95	H^2	L95	U95	H^2	L95	U95
Watered	Low	0.457	0.220	0.713	0.109	0.022	0.209	0.720	0.392	0.992	0.033	3.20e-5	0.170
Moderate	Low	0.282	0.109	0.479	0.098	0.022	0.193	0.849	0.571	0.995	0.048	1.07e-4	0.290
Severe	Low	0.198	0.053	0.352	0.117	0.022	0.222	0.875	0.505	0.996	0.031	3.90e-5	0.130
Watered	Moderate	0.360	0.137	0.612	0.137	0.024	0.257	0.843	0.620	0.966	0.839	0.571	0.990
Moderate	Moderate	0.275	0.096	0.465	0.138	0.033	0.258	0.810	0.565	0.962	0.823	0.457	0.980
Severe	Moderate	0.293	0.107	0.498	0.174	0.044	0.314	0.997	0.994	1.000	0.642	0.321	0.960

Table 4. Genetic covariances within each watering treatment for two fitness trade-offs: relative growth rate (RGR) vs. the probability of reproduction and RGR vs. fecundity (# of fruits produced among individuals that reproduced). We show genetic covariances between traits with lower and upper 95% credible interval values. Bold indicates significance.

		RGR vs. probability of reproduction			RGR vs. Fecundity		
Drought	Nutrient	*Covariance*	L95	U95	*Covariance*	L95	U95
Watered	Low	-6.75	-20.84	1.00e-01	-0.03	-0.07	0.001
Moderate	Low	-1.31	-5.27	5.50e-01	-0.02	-0.06	0.015
Severe	Low	**-1.19**	**-3.30**	**-2.16e-03**	-0.02	-0.05	0.002
Watered	Moderate	**-2.19**	**-5.44**	**-1.90e-01**	-0.01	-0.05	0.031
Moderate	Moderate	**-2.03**	**-6.14**	**-1.20e-01**	-0.02	-0.08	0.019
Severe	Moderate	**-1.70**	**-4.31**	**-8.00e-02**	-0.03	-0.08	0.018

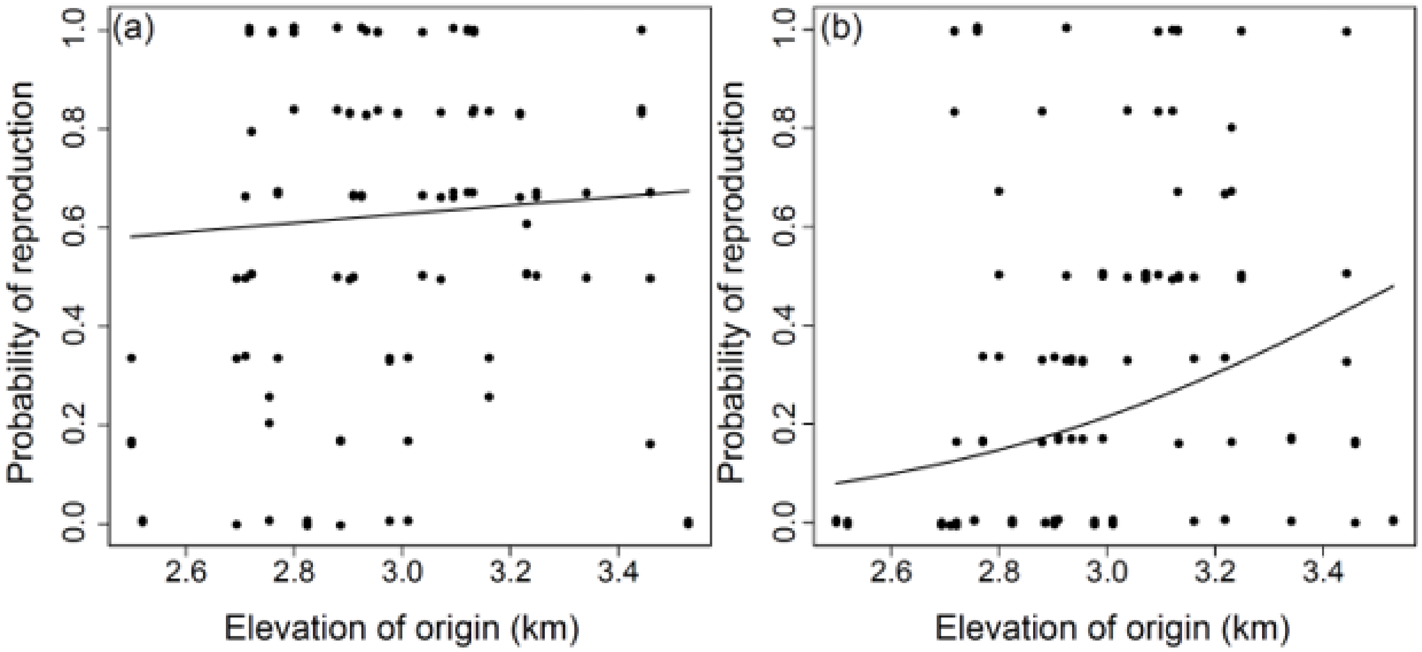

Fig. 1 Family-level analyses revealed a significant interaction between nutrient treatment and source elevation for the probability of reproduction. Under low nutrient levels (panel a), there was no evidence that the source elevation influenced reproductive success. In contrast, under higher nutrient levels, the probability of reproduction increased with source elevation. Data points represent family-means.

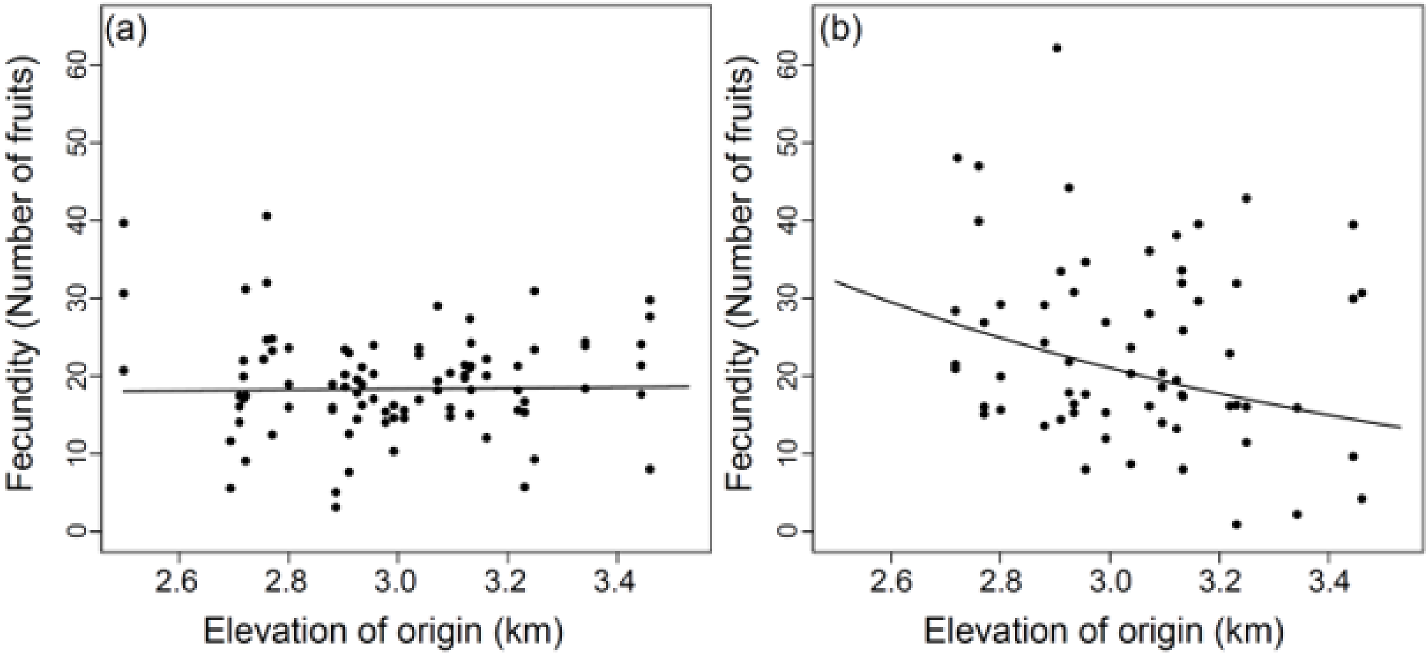

Fig. 2 Fitness trade-offs across nutrient levels also emerged for the fecundity component of fitness (# of fruits among individuals that reproduced successfully). We found no relationship between source elevation and fecundity in the low nutrient treatment (panel a). In contrast, in the higher nutrient treatment, fecundity declined with source elevation (panel b). Data points represent family-level LSMEANs for fecundity.

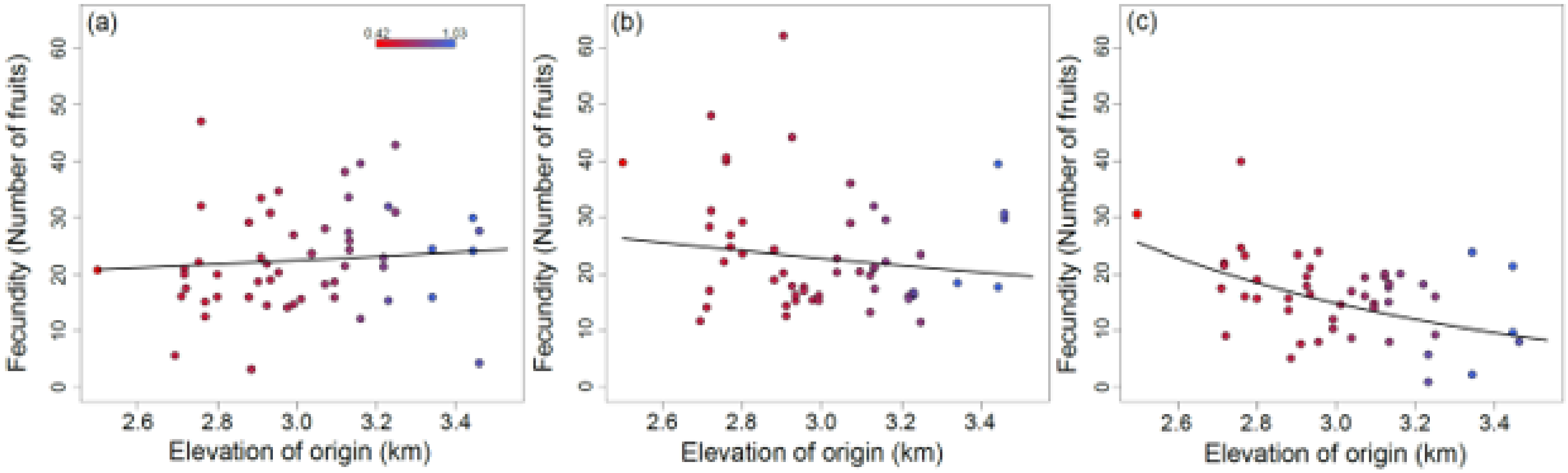

Fig. 3 Fecundity trade-offs across drought treatments. Under control well-watered conditions (panel a) and moderate drought stress (panel b), fecundity was not associated with source elevation. However, under severe drought (panel c), fecundity declined with increasing source elevation. We color coded the data points to indicate the Aridity Index (Mean Annual Precipitations/Mean Annual Potential Evapotranspiration) of each population based on climatic data extracted from WorldClim, with arid low elevation populations indicated with red and mesic high elevation populations indicated with blue. Data points represent family-level LSMEANs for fecundity.

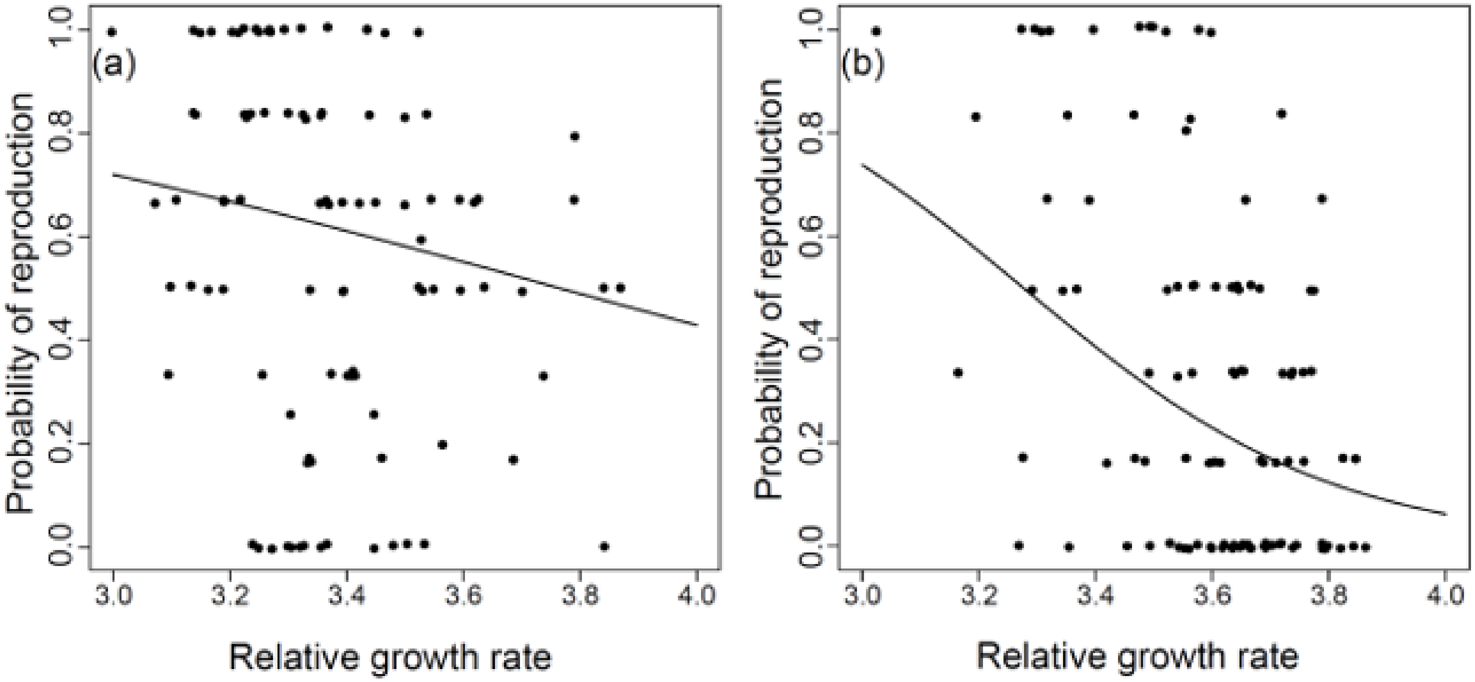

Fig. 4 Phenotypic correlations at the family-level between the probability of reproduction and relative growth rate revealed no significant relationship under low nutrient levels (panel a) and a negative association between these fitness components under higher nutrient levels (panel b). Data points represent family-level LSMEANs for relative growth rate and family-means for the probability of reproduction (# individuals that reproduced/ # planted for each family).

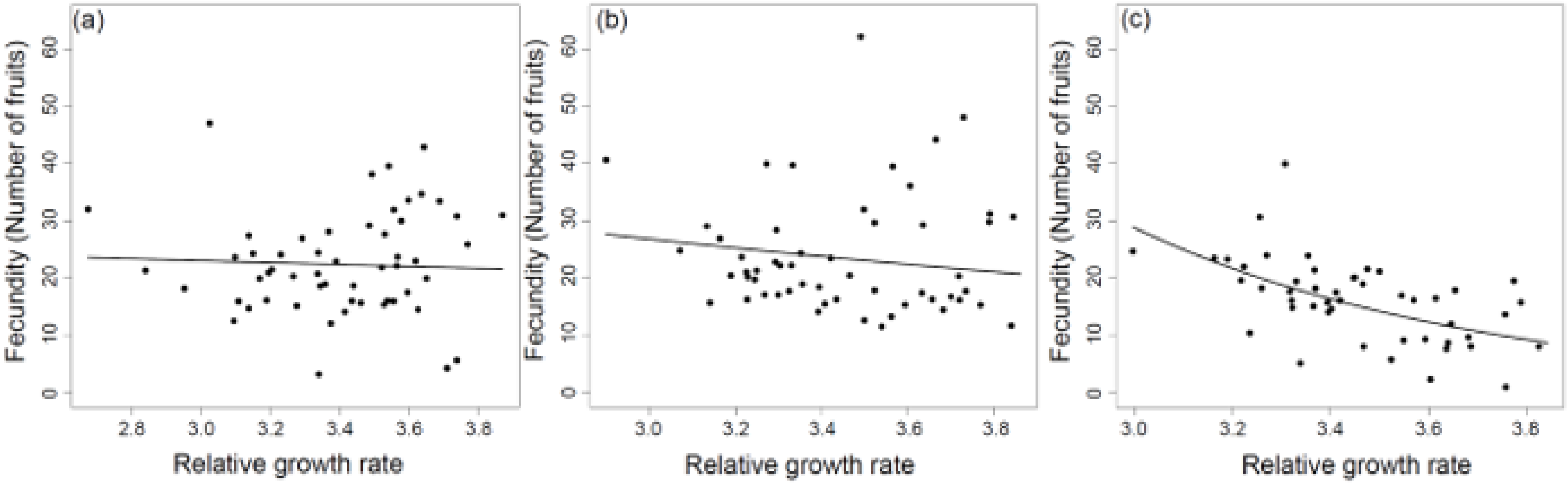

Fig. 5 Phenotypic correlations at the family-level between fecundity (# of fruits among individuals that reproduced successfully) and relative growth rate revealed context-dependent relationships. Under control conditions (panel a) and moderate drought (panel b), we found no significant relationship. However, under severe drought, a trade-off emerged across these two fitness components (panel c). Data points represent family-level LSMEANs for relative growth rate and fecundity.

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CHAPTER 3

WATER AND NUTRIENT AVAILABLITY EXERT SELECTION ON REPRODUCTIVE PHENOLOGY

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Abstract

Global change has altered resource availability to plants. In turn, these novel conditions can shift the adaptive landscape. We hypothesize that novel combinations of water and nutrient availability alter patterns of natural selection on reproductive phenology *Boechera stricta* (Brassicaceae) and influence the evolution of local adaptation in this system. We conducted a multifactorial greenhouse study using 35 accessions of *B. stricta* from an elevational gradient in the Rocky Mountains. We exposed full siblings to three soil water and two nutrient availability treatment levels reflecting contemporary and future conditions, and quantified fitness (seed count) and four genetically-correlated traits: the timing of first flowering, the duration of flowering, and height and leaf number at flowering. Selection favored early flowering and longer duration of flowering, and the genetic correlation between these traits accorded with the direction of selection. In most treatments, we found selection for increased height, but selection on leaf number depended on water availability, with selection favoring more leaves in well-watered conditions, and fewer leaves under severe drought. Fitness patterns generally accorded with local adaptation of low elevation genotypes to drought stress. Water and nutrient availability-imposed selection on all traits, and **G** matrix models revealed the potential for these traits to adapt to this selection. The extent of selection on size at flowering depended on the variable measured. Finally, selection can act strongly not only on the timing of first flowering but also on the duration over which an individual produces flowers.

Introduction

Selection acts on complex suites of genetically-correlated traits to shape adaptive evolution in natural populations (Roff, 2002; Sgro and Hoffmann, 2004; Latta, 2010). Anthropogenic climate change has imposed novel selection on plants (Karell et al., 2011; Hamann et al., 2018; Radchuk et al., 2019). Global temperatures have increased by 1.5°C since the industrial revolution (Allan et al., 2021). Additionally, projections suggest that arid regions will experience heightened and prolonged drought while mesic regions will experience intense storms with increased frequency (Hayhoe et al., 2008; Allan et al., 2021). Higher temperatures and altered precipitation expose plants to novel biotic communities and abiotic combinations (Fox et al., 1999; Adler et al., 2009; Lamarque et al., 2014). For example, increasing temperatures have shifted edaphic conditions, such that microbial processing of organic matter decomposition stimulates nutrient release from soils and uptake by faster growing roots (Rosenzweig and Hillel, 2000; Suseela et al., 2012; Classen et al., 2015). Additionally, increased evapotranspiration rates along with decreased precipitation reduce soil moisture content, amplifying the consequences of drought for plant functional traits and population persistence (Davies, 2006; Anderson, 2016). Thus, prolonged drought and altered nutrient availability may interact to alter patterns of natural selection on ecologically relevant traits. Investigating the capacity of natural populations to respond to novel environmental combinations imposed by climate change is critical to predicting evolutionary trajectories under future climate change scenarios (Jump and Peñuelas, 2005; Franks and Hoffmann, 2012; Hamann et al., 2018).

Multiple abiotic factors are shifting simultaneously under climate change (Allan et al., 2021). Water stress can favor earlier reproductive phenology as a mechanism of drought escape in plants (Franks, 2011). For example, *Brassica rapa* rapidly evolved drought escape strategies after multiple drought; however, drought escape became maladaptive under wetter periods caused by unpredictable storms associated with climate change (Hamann et al., 2018). In contrast, nutrient addition can delay flowering (Gutschick and BassiriRad, 2003; Vidal et al., 2014) and increase the size at reproduction (Müller et al., 2000; Burkle and Irwin, 2009). As plants encounter novel combinations of reduced soil water and increased nutrient availability, conflicting selection may negate adaptive responses. Experiments that manipulate single climate change factors at a time could have low power to predict biological responses to climate change. Given the importance of soil water and nutrient availability for plant fitness and trait expression (Wardle et al., 2004; Bardgett et al., 2013; Freschet et al., 2021), it is crucial to evaluate their joint effects on adaptive dynamics in the context of climate change.

Combined direct and indirect climate change forcings may act synergistically or antagonistically when considering long-term effects on soil nutrient availability in conjunction with increased nitrogen from anthropogenic land use changes (Seastedt et al., 2004). Soil microbial communities decompose organic matter, releasing inorganic nitrogen to available nitrogen through mineralization and sediment-bound phosphorus (Richardson and Simpson, 2011; Miransari, 2013). Elevated temperatures stimulate microbial activity while also contributing to soil drying, which can slow mineralization (Bradford et al., 2002; García-Palacios et al., 2013). A long-term warming experiment found available nitrate concentrations spike in the first 2 years but are reduced by 5 years, possibly due to organic matter depletion (Shaw and Harte, 2001). Rapid shifts in soil properties may outpace the adaptive response of some plant species, favoring others, leading to altered plant communities and potential for accelerated invasion of non-native species (Dukes and Mooney, 1999; Walther et al., 2002).

Boechera stricta (Brassicaceae) is a subalpine forb, which occurs across broad climatic gradients in the US and Canadian Rocky Mountains (Rushworth et al., 2011). High elevation ecosystems in the Rocky Mountains are characterized by short growing seasons, cool temperatures, and high soil moisture relative to arid low elevations, which experience longer, more intense summer drought (Dunne, 2003; Körner, 2007; Anderson and Wadgymar, 2020). Furthermore, soil nitrate available nitrogen (N) and K for plant use declines with increasing elevation (Ranelli et al., 2015; Vaidya et al., 2018). *Boechera stricta* expresses clear genetic clines in phenology in common gardens in the field, such that high elevation ecotypes flower early at short stature and low elevation genotypes flower late at greater heights (Wadgymar, Daws, et al., 2017). Flowering phenology in this system has likely evolved in response to variation in aridity, length of the growing season, and edaphic conditions across this gradient. In the field, the timing of snowmelt and soil water availability are tightly correlated (Anderson and Wadgymar, 2020), as water availability early in the growing season is primarily determined by winter snowpack (Fyfe et al., 2017). Indeed, *Boechera stricta* populations display strong local adaptation to elevation, latitude, and climate (Anderson et al., 2013, 2015; Lee and Mitchell-Olds, 2013; Anderson and Wadgymar, 2020). Here, we investigate soil moisture availability effects on the timing of phenological variables and examine the additive or synergistic effects of nutrient and water availability on local adaptation and selection on phenological traits.

Some plant species escape drought by flowering early in the growing season

(Franks, 2011; Hamann et al., 2018). Additionally, directional selection for earlier flowering occurs under a wide range of environmental conditions (Roff, 2002; Munguía-Rosas et al., 2011; Austen et al., 2017). This persistent pattern runs counter to the expectation that stabilizing selection should favor an intermediate optimal flowering phenology, as individuals that flower too early in the growing season risk reproduction during inclement conditions (such as late season frosts) or are too small and have insufficient resources to maximize seed production. In contrast, individuals that flower too late in the season may not have enough time to complete their life cycles prior to the onset of winter or other adverse conditions. In *B. stricta*, stabilizing selection via viability favored intermediate flowering phenology in a field experiment, but directional selection for earlier flowering emerged when considering viability and fecundity components of fitness cumulatively (Wadgymar, Daws, et al., 2017). It is possible that climate change has already shifted the selective landscape, favoring earlier flowering (Anderson et al., 2012). Alternatively, studies may miss critical selection operating on different life history stages by neglecting to consider the “invisible fraction” of individuals that die prior to trait expression, or by not capturing late flowering individuals (Hadfield, 2008; Mojica and Kelly, 2010; Austen et al., 2017; Wadgymar, Daws, et al., 2017). Additionally, apparent selection on well-studied traits, like flowering time, could mask the true targets of selection; this case could apply for reproductive phenology, as selection is often evaluated for flowering time but not for the duration of flowering (O’Neil, 1999; Munguía-Rosas et al., 2011; Austen et al., 2017). These two phenological traits are likely tightly genetically-correlated, as individuals that flower early in the growing season could also continue to produce flowers over a longer period of time. We sought to examine the extent of direct selection on reproductive phenology by quantifying four potentially correlated traits: timing and duration of flowering, and height and leaf number at first flowering.

We tested the extent to which soil water and nutrient availability impose selection on phenological traits. Second, we examined whether natural populations have evolved local adaptation in response to these abiotic factors. We hypothesize that these factors will interact synergistically to influence the direction and strength of selection on phenological traits so that selection under resource stress will favor early flowering. In contrast, in benign resource combinations we predict that selection will favor delayed flowering and increased size at flowering under elevated nutrient levels, such that individuals can acquire more resources prior to reproduction. Finally, we hypothesize that fitness will decline with source elevation under drought stress but increase with source elevation under well-watered, as low elevation populations evolved under hot arid conditions while high elevation populations experience cooler, moister conditions (Anderson and Gezon, 2015). Our multifactorial experiment explicitly evaluates the main and interactive effects of two key factors associated with global change.

Methods

Focal system

Boechera stricta (Brassicaceae) is a perennial herbaceous plant native to meadows and stream sides in the North American Rocky Mountains, from Utah to Alaska (Rushworth et al., 2011). Populations of *B. stricta* in the Colorado Rockies are genetically diverse (Kiefer et al., 2009). This species primarily self-pollinates (average

F_{IS} = 0.89) (Song et al., 2006) and has gravity- and wind-dispersed seeds. Natural *B. stricta* populations have adapted to historical local climates across latitudes and elevations (Anderson et al., 2013, 2015; Lee and Mitchell-Olds, 2013; Anderson and Wadgymar, 2020). For this experiment, we first collected seeds from *Boechera stricta* populations across a wide elevational gradient (2499 – 3450m) in sub-alpine meadows around the Rocky Mountain Biological Laboratory (Gothic, Colorado, USA; see appendix S1.2) and grew seeds for a generation in the greenhouse to homogenize maternal effects and produce full-sib maternal seed families.

Our experimental populations occur across gradients of soil moisture and nutrient availability. Anderson and Wadgymar (2020) installed volumetric water content and temperature sensors (ECH20 5TM, Meter Group) in each of five common gardens (2540 m to 3340 m in elevation), recording soil volumetric water content and temperature across multiple growing seasons. These sensors show that soil moisture increases with elevation and temperatures decrease (Dunne, 2003; Anderson and Wadgymar, 2020). Soil nitrogen (N), which is bioavailable for plants as nitrate, declines with elevation (Ranelli et al., 2015; He et al., 2016; Drollinger et al., 2017), as does soil K (Vaidya et al., 2018). In *Boechera stricta*, foliar N content increases with elevation (Anderson and Gezon, 2015), which could reflect variation in resource acquisition or allocation strategies across populations.

Experimental design

To examine divergent selection under variable resource availability, we experimentally manipulated water and nutrient resource supply in a greenhouse at the University of Georgia (Athens, GA). We exposed 35 maternal *Boechera stricta* families (one family per source population) to a factorial combination of three water and two nutrient availabilities to examine multiple concurrent effects of climate change on plant traits. In March 2017, we germinated seeds in Fafard #3B potting soil® (sungro.com) by planting 3 seeds per 2.5cm in diameter conetainer under benign moisture conditions, and misted seedlings daily to prevent desiccation. When plants reached 8 weeks old, we measured initial plant size and randomized individuals to blocks. Six siblings per family were assigned to each combination of three water and two nutrient availability treatments (N = 6 siblings x 35 families x 3 water levels = 1,260 individuals). Of those, 1,209 plants were retained for analysis after accounting for failure to germinate and death prior to treatment (MacTavish and Anderson, 2020).

In the experiment, we delivered ~3 mm of water per application such that control (watered 7 days/week), moderate drought (watered 2 days/week), and severe drought (watered 1 day/week) received ~90, 24, and 12 mm of water per month, respectively. We confirmed our soil moisture levels by measuring volumetric water content (%VWC) on a random subset of 4 similarly sized individuals per block on the driest day, before weekly watering (MacTavish and Anderson, 2020) using a Hydrosense II (Campbell Scientific, Logan, Utah). Differences in plant size were undetectable at that point (personal obs., R. MacTavish).

Natural population accessions were chosen from a soil moisture gradient ranging from xeric low elevation soils to mesic high elevation soils. Annual summer (June-August) precipitation at the Rocky Mountain Biological Laboratory (elevation: 2895m), which was 51.20 ± 18.30 mm (mean ± S.D.) ranging from 29.21 – 90.80 mm from 2000-2018 (b. barr , long-term weather data).Our treatment levels were like 2014-2018 average summer soil volumetric water content (VWC) at common garden field locations in the Rocky Mountain Biological Laboratory (Anderson and Wadgymar, 2020). The mid-elevation garden (2890m) averaged 10% VWC (standard deviation: 5%; range 0-39%). Our moderate drought stress (mean ± S.E.: 10.51 %± 2.33%) watering treatment reflects average soil water availability in the field, and the well-watered (17.52 %± 2.71%) and severe drought stress (4.65 % ± 1.71%) fall within the range of variability at high and low gardens, respectively (MacTavish and Anderson, 2020).

We applied nutrient treatments weekly as a mixture of water-soluble Peter's Professional General-Purpose Fertilizer™ 20N-10P-20K at concentrations of 110 ppm N (1 ppm = 1 mg/L), and 1100 ppm. Plant available nutrients are highly heterogeneous in space and time (Hodge, 2004; Giehl and von Wirén, 2014) thus, we selected fertilizer concentrations on a low and high range (0.2 and 1 g/L) corresponding to 5 and 50 ppm, previously shown to influence *B. stricta* trait expression (Siemens et al., 2010, 2012). As nutrient pulses occur immediately after snowmelt at the seed to seedling transition (Groffman et al., 2011; Gavazov et al., 2017), we applied high nutrient levels early in the life cycle. We applied drought and nutrient treatments for 3 weeks and then subjected them to 8 weeks of vernalization at 4^{o}C in June 2017 to induce flowering (Anderson et al., 2011). During vernalization, we supplied the same amount of water to all plants via bottom watering. After removing them from vernalization, we reduced nutrient levels to 5 and 50 ppm N to simulate high vs. low concentrations with increasing elevational gradients (Ranelli et al., 2015; He et al., 2016; Drollinger et al., 2017).

Weekly, we rotated blocks around greenhouse benches to reduce non-experimental environmental variation. We simulated three 16 week-long growing seasons, separated by two additional 8-week periods of vernalization. We observed signs of nutrient deficiency at the beginning of the second simulated growing season, at which point we applied a Magnesium and Calcium only micronutrient mixture to all plants once every 8 weeks at 110 ppm supplemental to weekly nutrient application.

Life history traits

During the first simulated growing season, we recorded phenological traits. We censused individuals every other day, recording survival and reproductive status. By recording the number of open flowers, height, and number of leaves on reproductive plants, we quantified the full flowering phenology schedule (day of first flowering, plant size as bolt height and leaf number at flowering, and duration of flowering). The day of first flowering represents the transition from vegetative to reproduction (Anderson et al., 2011). We calculated the timing of first flowering as the number of elapsed days between when plants were removed from vernalization and when they produced the first flower. We calculated peak flowering as the number of days after vernalization when the maximum number of flowers appeared on each plant. Days to first flower are highly correlated with days to peak flower (*Pearson's R*=0.91, p<0.0001); thus, we present analyses on days to first flower, which is a common metric that is highly responsive to climate change (Miller-Rushing and Primack, 2008). We calculated duration of flowering by subtracting the first day a flower appeared from the last day flowers were present. In the first season, 648 plants flowered, 944 plants flowered in season 2, and 194 plants flowered in season 3. We collected mature seeds from 546 plants in season 1,875 plants in season 2 and 164 plants in season 3.

Fitness components

To assess fecundity, we collected all fruits at maturity during all seasons, and quantified total seed set per individual by taking photographs of seeds on a white piece of paper and processing those images using ImageJ (Schneider et al., 2012) and Ilastik (Berg et al., 2019). Plants often allocate resources to reproduction and then abort seeds during development to enhance survival under resource stress (Leport et al., 2006; Hamann et al., 2021). We examined the viability of intact seeds in season 1 from 2 randomly selected reproductive individuals from each genotype (31) x nutrient (2) x water (3) combination for a total of n=250 plants (Appendix 1.3). Twenty seeds were haphazardly chosen per plant and tested for viability following Wadgymar et al. (2017) by first germinating seeds in petri dishes on filter paper for 14 days and counting the number of germinants. Ungerminated seeds could either be dormant or non-viable. Therefore, we exposed ungerminated seeds to a 1% solution of 2,3,5, triphenyltetrazolium-chloride, which stains living respiring tissue pink after 48 hours. Embryos were scored as non-viable (white or partially stained) or viable (fully-stained pink cotyledon and radicle).

Statistical analysis

Local adaptation

Lifetime seed production: To examine local adaptation, we analyzed individual-level lifetime fitness as a function of source elevation, drought treatment, and nutrient treatment and all 2- and 3-way interactions, with random effects for block and genotype. We calculated lifetime fitness by summing an individual’s seed production across all three simulated growing seasons. In this study, 211 individuals failed to set seeds, leading to zero-inflation in the distribution of total seed count. We, therefore, took a hurdle model approach in the R package glmmTMB 1.1.2.3 (Brooks et al., 2017) using a truncated negative binomial distribution. This approach simultaneously analyzes the probability of setting seeds in a mixed effects logistic regression, and then uses a negative binomial distribution to analyze seedset amongst individuals that produced $\geq$ 1 seed. We standardized source elevation to a mean of 0 and standard deviation of 1 to enable convergence, and incorporated quadratic effects of source elevation, as the residuals suggested non-linearity. For both components of hurdle model models, we assessed significance of random effects through likelihood tests contrasting models with and without each effect. We plotted these results using the R package ggplot2 ver. 3.3.5 (Wickham, 2016).

Seed viability: We calculated the total number of viable seeds as the sum of the germinants and fully-stained seeds. We then analyzed the probability of seed viability through logistic regression as a function of source elevation, drought treatment, nutrient treatment and all 2- and 3-way interactions, with random effects for block and genotype in a mixed effects logistic regression in the glmer function of the lme4 package ver. 1.1-27.1 (Bates et al., 2015) in R 4.0.3, visualized with the predictorEffect function of the R package effects (Fox and Weisberg, 2019)

Selection

Phenotypic selection analysis with individual-level data: To quantify selection gradients, we used a generalized linear mixed model analyzing individual-level fecundity (seed count) in the first simulated growing season as a function of source elevation, drought and nutrient treatments, and four plant traits measured in the first growing season: height and leaf number at flowering, flowering phenology (days to flower), and flowering duration using individual-level data. We incorporated random effects for block and genotype, and modeled all 2- and 3-way interactions between plant traits and drought and nutrient treatments to assess divergent selection across environments. We included source elevation as a covariate to account for unmeasured traits that also vary across elevation in this system (see also Wadgymar, Daws, et al., 2017).

Of the 616 individuals that flowered and had complete data for all four traits in the first growing season, 83 produced no seeds. Therefore, we again took a hurdle model approach in the glmmTMB R package (Brooks et al., 2017), first modeling the probability of setting seeds using a binomial distribution with logit link, and then analyzing fecundity with a negative binomial regression. When residuals suggested that selection could be non-linear, we included quadratic effects of specific traits. We standardized source elevation and all traits to a mean of 0 and standard deviation of 1 for model convergence, but we did not relativize fitness owing to the complex zero-inflated nature of this variable and the inability of negative binomial models to accommodate non-integer values. To visualize patterns of selection, we used the R package visreg vers. 2.6-0 (Breheny and Burchett, 2017), which plots partial residuals from multiple regressions while holding other predictors at their median values to evaluate the relationship between each trait and fitness. As visreg does not interface well with glmmTMB, we used the glmer function of the lme4 R package (Bates et al., 2015) to conduct mixed effects logistic regression and negative binomial models to generate visreg plots.

Genotypic selection analysis with family-level means: Phenotypic selection analyses restrict the dataset to individuals that successfully flowered, which could generate biased estimates of selection by neglecting to account for viability selection operating on the invisible fraction (Hadfield, 2008; Mojica and Kelly, 2010; Wadgymar, Daws, et al., 2017), i.e., individuals died prior to trait expression or did not flower and therefore failed to express flowering phenology traits. To account for these individuals, we conducted genotypic selection analyses (Rausher, 1992) by regressing family-level seed production in year 1 across all individuals on family level trait data. To generate family-level trait data, we regressed each trait separately on genotype, drought, and nutrient treatment, and all interactions in lmer models with random effects for block using the lmer function of the lme4 package. We extracted LSMEANs for each genotype by drought by nutrient combination using the emmeans package ver. 1.7.2 in R (Lenth, 2020). For fitness, we first calculated the proportion of individuals that reproduced for each genotype in each treatment combination (total number of individuals that reproduced /total number of individuals planted). We then modeled seed count amongst individuals that successfully set seeds using a gamma model in the glmer function of lme4, using the same predictors as we described for the calculation of LSMEANs for phenotypes, and extracting LSMEANs with the emmeans package.

We conducted genotypic selection analyses using these family-level LSMEANs. We calculated total fitness as the proportion of individuals that reproduced × seed count for each genotype by treatment combination. As in the phenotypic selection analysis above, we included drought and nutrient treatments, incorporated source elevation as a covariate, modeled all interactions between traits and treatments, and included a random effect for genotype using a negative binomial regression with the glmer.nb function of the lme4 package. Of the 162 rows (genotype by drought treatment by nutrient treatment combination) with complete records of phenological traits in the family-level dataset, only seven had total fitness values of 0; therefore, a hurdle model approach was unnecessary. We rounded total fitness to the nearest integer value as negative binomial models cannot include non-integer numbers; we standardized source elevation and all traits to a mean of 0 and standard deviation of 1. We used visreg to plot the selection gradients.

***G** matrix models*

We examined potential evolutionary responses to natural selection along with genetic correlations across phenological traits by modeling multivariate genetic variance-covariance (**G**) matrices using individual-level data in the R package MCMCglmm version 2.26, which uses Markov chain Monte Carlo methodology in Bayesian statistical framework (Hadfield, 2010). These **G** matrices allow us to estimate the Robertson-Price identity (Robertson, 1966; Price, 1970), which predicts the adaptive response to a single generation of selection by estimating the genetic covariance between relative fitness (w) and traits (**z**) from these multivariate models, where **z** is a vector of trait values. Although the Robertson-Price identity generally uses relative fitness (Morrissey et al., 2010), we modeled absolute fitness (seed count) because relativizing seed number resulted in a variable that included values of 0 and non-integer values. While Poisson and negative binomial distributions can accommodate zeros, they cannot accommodate non-integers.

Complementary models using a gaussian distribution for relative fitness produced very similar results to those presented in the main manuscript (Appendices 2.3 and 3.3).

We use the same subset of data as in the phenotypic selection analysis, which included all individuals with a full suite of phenotypic data and fitness (total seed count, including values of 0 for individuals that flowered but failed to set seed). We simultaneously modeled total seed count, source elevation, leaf number and height at first flowering, the timing of first flowering, and the duration of flowering. The genetic variance of source elevation is not biologically meaningful, but we retained source elevation in the model to evaluate potential genetic covariances with traits of interest. We specified the random effects for full-sib families (equivalent to total genetic variance, V_g) and block (V_b) the *us* covariance structures (Hadfield, 2010). As *Boechera stricta* has a selfing mating system, selection operates on total genetic variance (not simply additive genetic variance, Roughgarden, 1979), allowing to model genetic variance-covariance matrices based on full-sibling maternal families. We first evaluated each of the six treatment combinations separately to examine how selection and genetic correlations manifest in each environment. We then conducted a model combining all traits and modeling drought and nutrient treatments, and their interactions, as fixed effects. This final model permits inferences about selection and genetic correlations across all environments. For each model, we ran models for 2×10^6 MCMC iterations, discarded the first 30% of iterations (6×10^5) for burn-in, and retained 2,000 iterations for estimating genetic (co)variances and 95% credible intervals. We assessed the statistical significance of (co)variance estimates across environments through examination of whether 95% credible intervals overlapped with zero ($\alpha = 0.05$).

Results

Local adaptation

Lifetime seed production: We conducted a hurdle model to assess local adaptation in lifetime seed production. In the first logistic regression, we found that the probability of reproduction varied as a function of nutrient treatment by quadratic effects of source elevation (χ^2=4.66 d.f.=1, p=0.0307; Appendix 4.3). This interaction emerged because of significant negative quadratic effects of source elevation under high nutrient levels in all three drought treatments, indicating that mid-elevation genotypes had the greatest probability of reproducing in those treatments (Fig. 1.3). We found no effect of source elevation on the probability of reproduction under low nutrient conditions. In the negative binomial regression of fecundity amongst individuals that set seed across the three simulated growing seasons, we found a significant three-way interaction between drought treatment, nutrient treatment, and the quadratic effect of source elevation (χ^2=8.39, d.f.=2, p=0.015, Appendix 4.3). This interaction arose owing to the decline in fecundity with source elevation across all drought levels in the low nutrient treatment, negative quadratic curvature under well-watered conditions and moderate drought stress in the high nutrient treatment, and a slightly positive quadratic curve in the severe drought stress and high nutrient combination (Appendix 5.3).

Seed viability: We found a significant three-way interaction among source elevation, drought and nutrient treatment (Appendix 6.3). Consistent with local adaptation to drought stress, the probability of seed viability declined by 81% for every 1 km increase in source elevation under severe drought stress and low nutrient levels (odds ratio: 0.19; 95% confidence interval: 0.048, 0.74, p=0.017) and by 99.6% for every 1 km increase in source elevation under severe drought and high nutrients (OR: 0.0044; 95% confidence interval: 0.00062, 0.031, $p<0.0001$; Appendix 4.3, Fig. 2.3). We found no effects of other treatments on seed viability (Appendix 4.3, Fig. 2.3).

Selection

Phenotypic selection analysis with individual-level data: In the logistic regression model, directional selection favored early flowering across all treatments ($\chi^2=5.74$, d.f. =1, $p=0.017$, Appendix 7.3) and longer flowering duration under low nutrient levels (flowering duration X nutrient treatment: $\chi^2=4.35$, d.f. =1, $p=0.037$, Fig. 3.3, Appendix 8.3).

Amongst individuals that successfully set seeds, directional selection favored increased height at first flowering ($\chi^2=18.25$, d.f.=1, $p<0.0001$, Fig 4.3. and Appendix 6.3). Furthermore, the extent of selection on leaf number varied as a function of drought treatment ($\chi^2=9.74$, d.f.=1, $p=0.0077$, Appendix 9.3). Directional selection favored greater leaf number at flowering in the well-watered treatments, and reduced leaf number at flowering in the severe drought treatment. We found no evidence of selection on this trait in the moderate drought treatment. Although slight curvature suggestive of stabilizing selection seems possible in the well-watered, high nutrient treatment, we found only marginally significant ($p=0.06$) quadratic effects of leaf number in a complementary model; therefore, we only retained the linear term in the final model.

Genotypic selection analysis with family-level means: This model of total fitness using genotypic means revealed significant three-way interactions among drought and nutrient treatments with flowering duration ($\chi^2=9.7$, d.f.=1, $p=0.0078$, Fig. 5.3, Appendix

10.3) and with height at flowering (χ^2=8.9, d.f.=1, p=0.0115). Directional selection favored longer flowering duration in the severe drought, high nutrient treatment only (negative binomial β = 1.02 ± 0.26, z=3.9, p<0.0001, Fig. 5.3). We found selection for increased height at flowering under all treatment combinations, except in two cases. Under well-watered and high nutrient conditions, there was no significant selection on height. In contrast, under severe drought and high nutrient, selection favored reduced height at flowering (negative binomial β = -0.77 ± 0.35, z=-2.22, p=0.026).

***G** matrix (genetic variance-covariance) models*

We modeled seven multivariate **G** matrices: one averaged across all treatments, and one for each of the six treatment combinations. Each model showed significant genetic variance in fitness and all phenological traits (Appendix 2.3). We found several instances of significant genetic covariances between absolute fitness and phenological traits, indicating that this population could adapt to selection in that treatment. For example, in two environments, we found evidence of both genetic covariances between fitness and early flowering, along with fitness and prolonged duration of flowering. These two treatment combinations were well-watered, high nutrient conditions (genetic $\mathrm{cov}_{\mathrm{fitness,\ flowering\ time}}$=-4.9, 95% credible interval: -6.4, -2.7; genetic $\mathrm{cov}_{\mathrm{fitness,\ flowering\ duration}}$=1.79, 95% CI: 0.071, 3.4), and severe drought and high nutrient levels (genetic $\mathrm{cov}_{\mathrm{fitness,\ flowering\ time}}$=-5.3, 95% CI: -8.3, -2.8; genetic $\mathrm{cov}_{\mathrm{fitness,\ flowering\ duration}}$=2.4, 95% CI: 0.77, 4.2). In both cases, the models also revealed a significant negative genetic covariance between flowering time and duration (well-watered, high nutrient: $\mathrm{cov}_{\mathrm{flowering\ time,\ flowering\ duration}}$= -0.57, 95% CI: -1.0, -0.009; severe drought, high nutrient: $\mathrm{cov}_{\mathrm{flowering\ time,\ flowering\ duration}}$= -0.65, 95% CI: -1.2, -0.19). In the moderate drought stress, low nutrient environment, the

G matrix suggested a potential evolution of accelerated flowering and greater stature at flowering (genetic $\text{cov}_{\text{fitness, flowering time}}$=-0.318, 95% CI: -0.63, -0.016; genetic $\text{cov}_{\text{fitness, height}}$=0.49, 95% CI: 0.13, 0.84). In all three drought levels in the high nutrient treatment, we found a negative genetic covariance between height at flowering and source elevation (Appendices 2.3 and 3.3), consistent with genetic clines in this trait in field studies, in which high elevation genotypes flower at short stature (Wadgymar, Daws, et al., 2017). Interestingly, leaf number at flowering showed the opposite pattern (positive genetic covariance with elevation: 0.15; 95% CI: 0.024, 0.28) in the well-watered high nutrient environment, and there was a negative genetic covariance between height and leaf number at flowering in the overall model averaged across all treatments (-0.24, 95% CI: -0.440- 0.027).

Discussion

Our study uncovered strong selection on phenological traits, which often depended on soil water levels and nutrient availability. By examining selection on phenological traits in response to water and nutrient levels in the greenhouse, we were able to control for other abiotic and biotic factors that vary through time in the field and influence the environment that an individual experiences upon transitioning to flowering and the resources available for setting seeds (e.g., Austen and Weis, 2015). This approach allowed us to disentangle the fitness effects of four key phenological traits: timing of first flowering, duration of flowering, and two metrics of size at flowering. Across systems, numerous studies have documented selection favoring earlier flowering (Munguía-Rosas et al., 2011; Austen and Weis, 2015; Wadgymar, Daws, et al., 2017), which could arise if shorter winters and advancing springs under climate change have shifted the adaptive landscape (e.g., Anderson et al., 2012; Bock et al., 2014). In some cases, it is also possible that selection on genetically-correlated, yet under-studied traits like the duration of flowering could account for this pervasive selection for early reproduction (Austen et al., 2017). Indeed, we found selection favored longer flowering periods across multiple environments and fitness components in phenotypic and genotypic selection analyses. Additionally, our genetic covariance models indicate that selection and the genetic response to selection can act simultaneously on both the timing of first flowering and the duration of flowering. These traits are genetically-correlated, such that early flowering individuals flowered across a greater duration of the season. This genetic correlation is in the same direction as the joint vector of selection for early flowering and longer duration, suggesting both that this correlation could accelerate the genetic response to selection for both traits (Etterson and Shaw, 2001) and that it could be challenging to quantify direct selection on each trait without experimentally manipulating flowering schedules (e.g., Austen and Weis, 2015).

Investigations of selection on flowering phenology in environments that differ in resource availability could shed light on how climate change could alter flowering phenology. In our experiment, increased flowering duration augmented fitness in most treatments. Additionally, the timing of first flowering has advanced by >2 weeks since the mid-1970s in *B. stricta* populations in Colorado (Wadgymar et al., 2018) where selection strongly favors earlier reproduction (Wadgymar, Daws, et al., 2017). The genetic correlation between flowering time and duration in concert with climate-change mediated selection for accelerated reproduction (Anderson et al., 2012; Colautti et al.,

2017) could hasten the evolution of phenological strategies that are adaptive in novel climates.

Size at flowering

Plant size at flowering is often genetically-correlated with the timing of first flowering, such that individuals either invest resources in early flowering, in which case they are small, or they invest in growth, in which case they flower later at larger sizes (Roff, 2002). This trade-off can constrain the evolution of early flowering large plants, but the extent of the trade-off could depend on how plant size is measured. Here, we evaluated both plant stature and leaf number at flowering, which we found to be negatively genetically correlated in our overall **G** matrix model. A field study found that plant height at flowering declined with source elevation (Wadgymar et al., 2017), and our **G** matrix models revealed congruent genetic clines in height under high nutrients for both the well-watered and severe drought environments. In benign conditions (well-watered/high nutrient), the genetic cline in leaf number went in the opposite direction. These results indicate that high elevation genotypes may flower at shorter height but with greater numbers of leaves than low elevation genotypes, suggesting difference in resource allocation strategy. These patterns await testing in field common gardens.

Selection strongly favored greater height at flowering across most resource levels. However, selection on leaf number depended on drought treatment, with selection for increased numbers of leaves in well-watered conditions and decreased leaf number in severe drought. In the native Colorado environments of our *B. stricta* accessions, aridity declines with elevation (Anderson and Wadgymar, 2020). Divergent selection of this sort could favor higher leaf numbers in mesic high elevation locations and the opposite pattern could emerge in arid low elevation populations. This pattern of selection aligns with our aforementioned preliminary results of a genetic cline in leaf number. Height at flowering is frequency used as a rapid assessment of size at reproduction in eco-evolutionary studies (Munguía-Rosas et al., 2011; Garnier and Navas, 2012). Height can be positively correlated with pollination traits under selection as in *Lobelia cardinalis* (Johnston, 1991), *Myrecophila christinae* (Parra-Tabla and Vargas, 2004), and *Brassica rapa* (Zu and Schiestl, 2017). The observed pattern of selection for increased height at flowering in *B. stricta*, a highly selfing plant, is likely not mediated by pollinators. Instead, we propose that the genetic cline of declining height with elevation in our study and field studies (Wadgymar, Daws, et al., 2017) is driven by strong selection for early reproduction in high elevation locations where the growing season is very short, in conjunction with genetic trade-offs between height and timing of flowering (Bemmels and Anderson, 2019). We did not manipulate the length of the growing season nor vary conditions temporally across the season, and thus cannot directly evaluate how these agents of selection influence trait evolution. We encourage other researchers to consider alternative metrics of plant size at reproduction beyond height alone.

Local Adaptation

Remarkably few field or lab experiments have manipulated abiotic or biotic agents of selection to examine the factors that favor the evolution of local adaptation (Wadgymar, Lowry, et al., 2017; Briscoe Runquist et al., 2020; Hargreaves et al., 2020). *Boechera stricta* exhibits extensive local adaptation across elevation, latitude, and water gradients (e.g., Lee and Mitchell-Olds, 2011, 2013; Anderson et al., 2015; Anderson and Wadgymar, 2020), and yet it can be challenging to dissect the specific conditions that drive local adaptation because environmental conditions can covary. For example, early snowmelt is often associated with increased aridity (Anderson and Wadgymar, 2020). In this study, we sought to decouple these variables and isolate the effects of water and nutrient availability on fitness and selection. Studies of local adaptation typically do not examine whether seeds produced by experimental individuals are viable. In doing so, we found that seed viability declined with source elevation under severe water stress, concordant with local adaptation to water stress in *B. stricta* (Lee and Mitchell-Olds, 2011, 2013; Anderson and Wadgymar, 2020). Local adaptation to historical water availability could come at a fitness cost under contemporary climates as aridity increases (Anderson and Wadgymar, 2020).

The greater magnitude of the negative slope between viability and source elevation in the high nutrient treatment suggests that local adaptation to nutrient availability can manifest under severe drought. As nutrient levels decline with elevation in some portions of the native range of *B. stricta* (Ranelli et al., 2015) we would expect that low elevation genotypes would have higher fitness under high nutrient availability, but we found somewhat more nuanced patterns. For example, in the high nutrient treatment, mid-elevation genotypes had optimal probability of setting seed across drought treatment levels, and greater seedset in well-watered and moderate drought stress treatment. In no scenario did we find that fitness increased with source elevation under high nutrient levels. However, as soil nutrients tend to be heterogeneous across landscapes (Hodge, 2004; Gruber et al., 2013), additional information is needed about the soil nutrient levels of the source populations of our genotypes to examine the true extent of local adaptation to nutrient availability. Together, these results suggest that future work is needed on gradients in nutrient availability across elevation and that drought stress may be a more prominent driver of local adaptation across elevation.

Conclusions: Our multifactorial manipulations in the greenhouse revealed that soil water and nutrient availability impose selection on genetically correlated phenological traits, and that the magnitude and direction of selection can vary with environment. Additionally, we found local adaptation to soil water availability, suggesting that local adaptation in the field could evolve in response to variation in both the timing of (Anderson and Wadgymar, 2020) as well as the direct effects of water levels during the growing season.

Figures

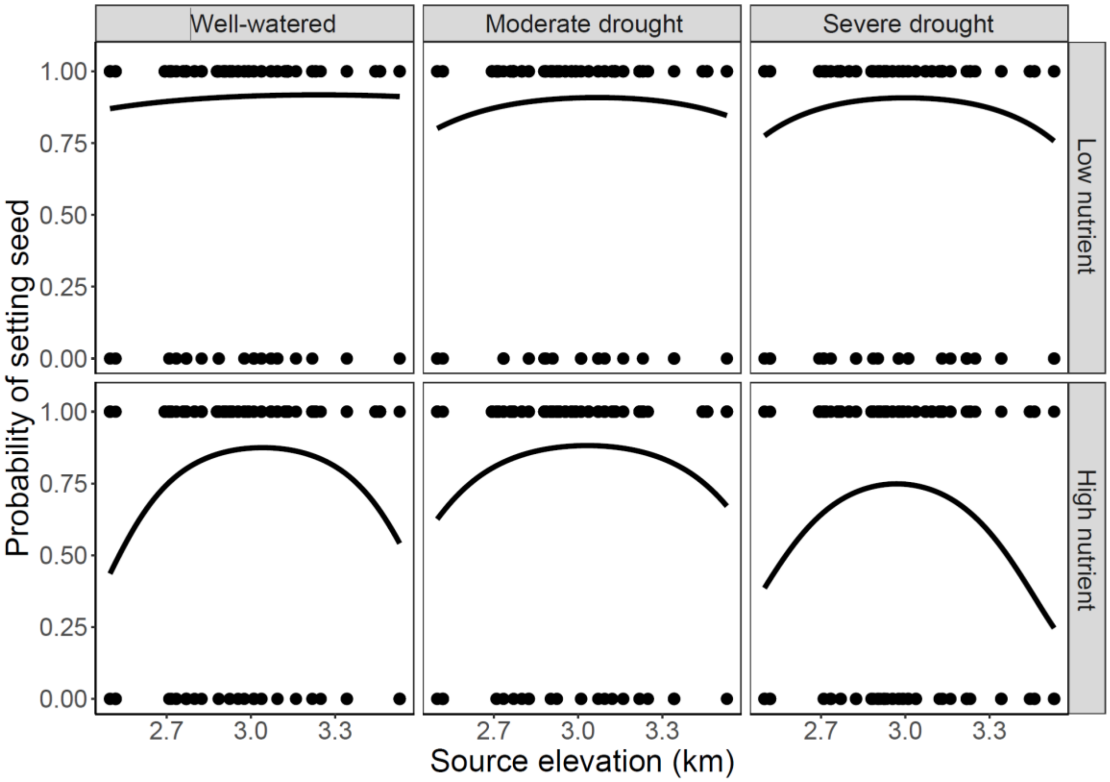

Figure 1.3. The probability of reproduction varied as a function of nutrient treatment by quadratic effects of source elevation. Under high nutrient levels in all three drought treatments, mid-elevation genotypes had the greatest probability of setting seeds. We found no relationship between the probability of seed set and source elevation under low nutrient levels.

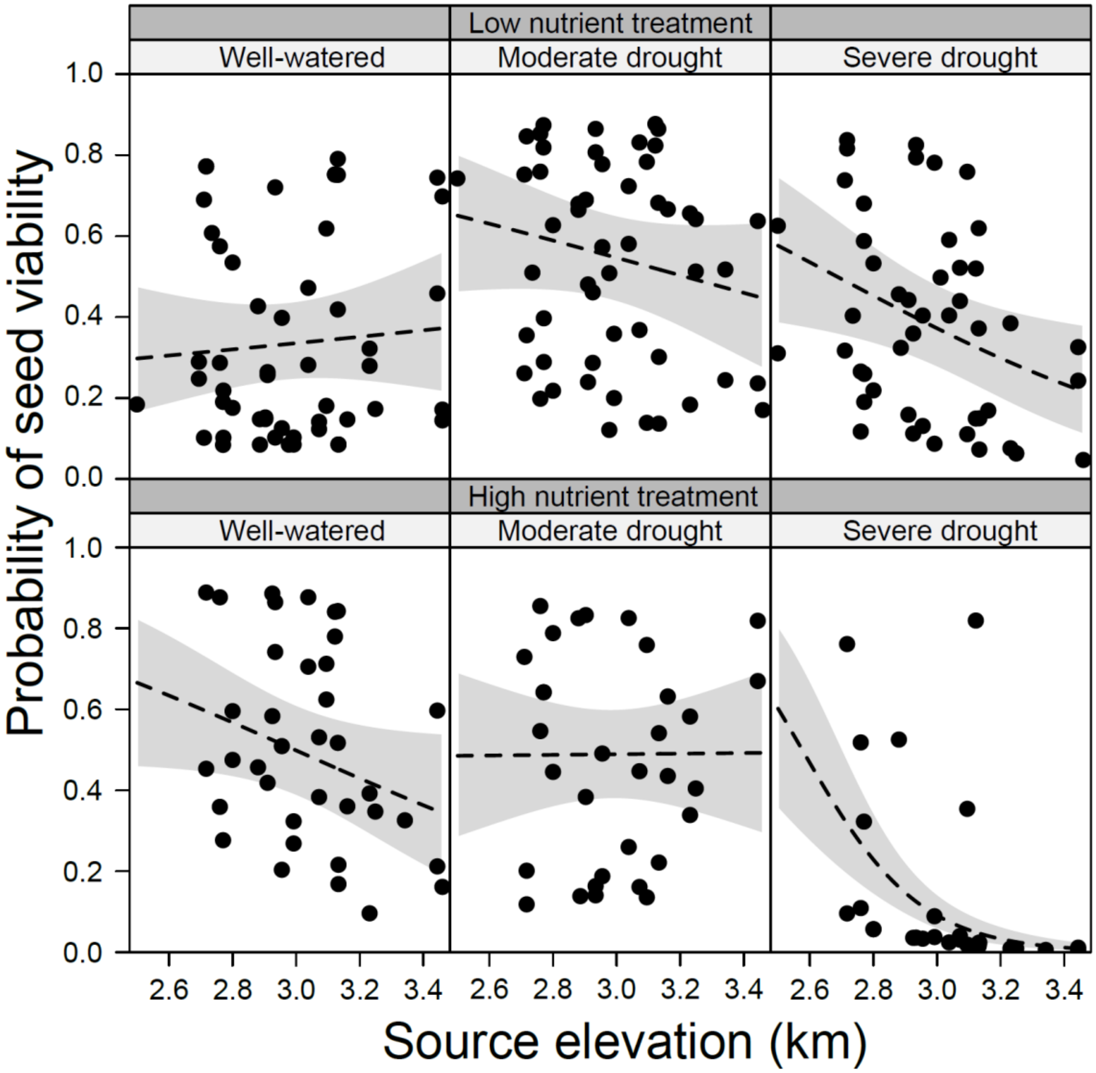

Figure 2.3. The probability of setting viable seed declines with elevation under severe drought conditions in a logistic regression model. The probability that a seed was viable decreased by 81% for every 1 km increase in source elevation in severe drought and low nutrient treatment, and by 99.6% for every 1 km increase in severe drought and high nutrient treatment. There was no effect of elevation on the probability of seed viability in other treatments.

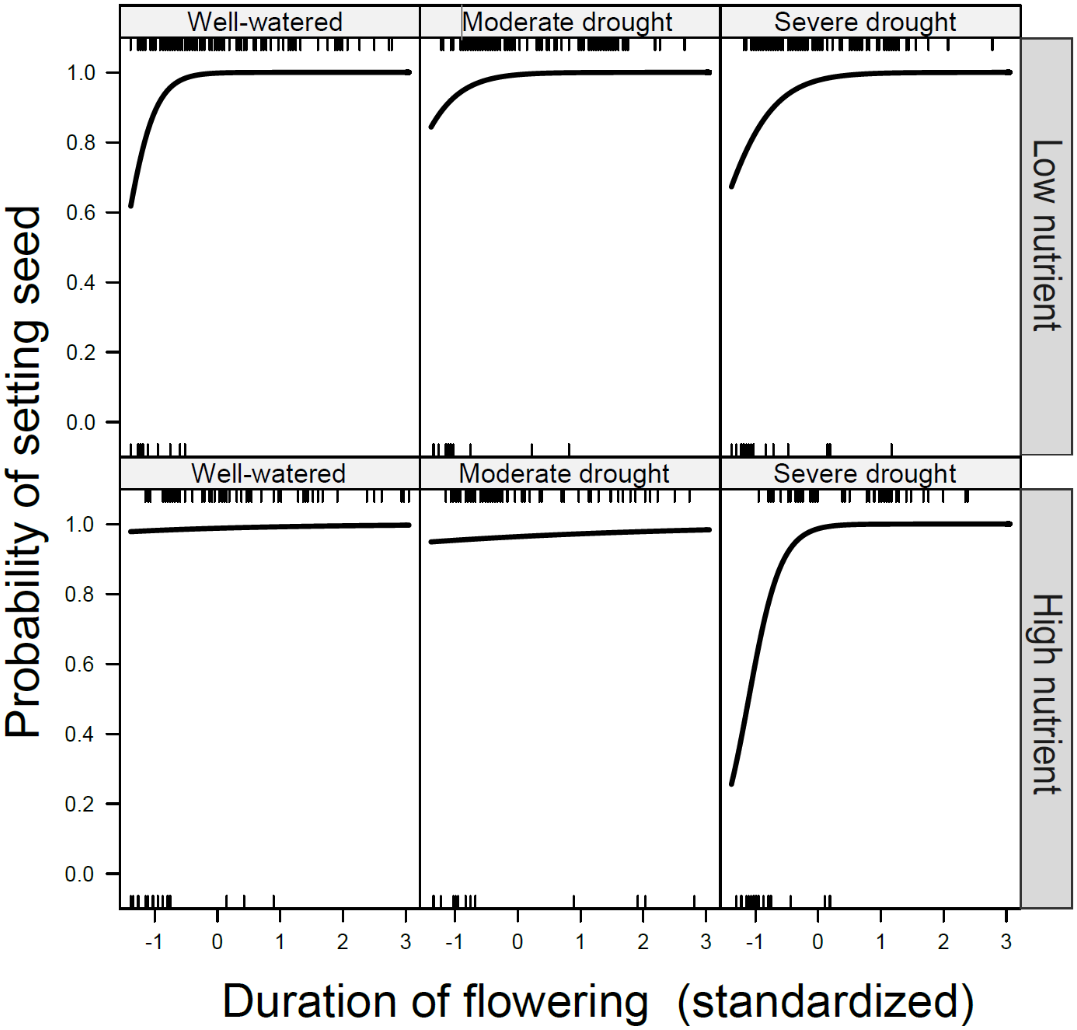

Figure 3.3. Directional selection favored longer flowering under low nutrient levels and in the severe drought and high nutrient treatment combination logistic regression of the probability of setting seed. The tick marks represent data points for individual plants. We standardized trait data to a mean of 0 and standard deviation of 1 to ensure model convergence.

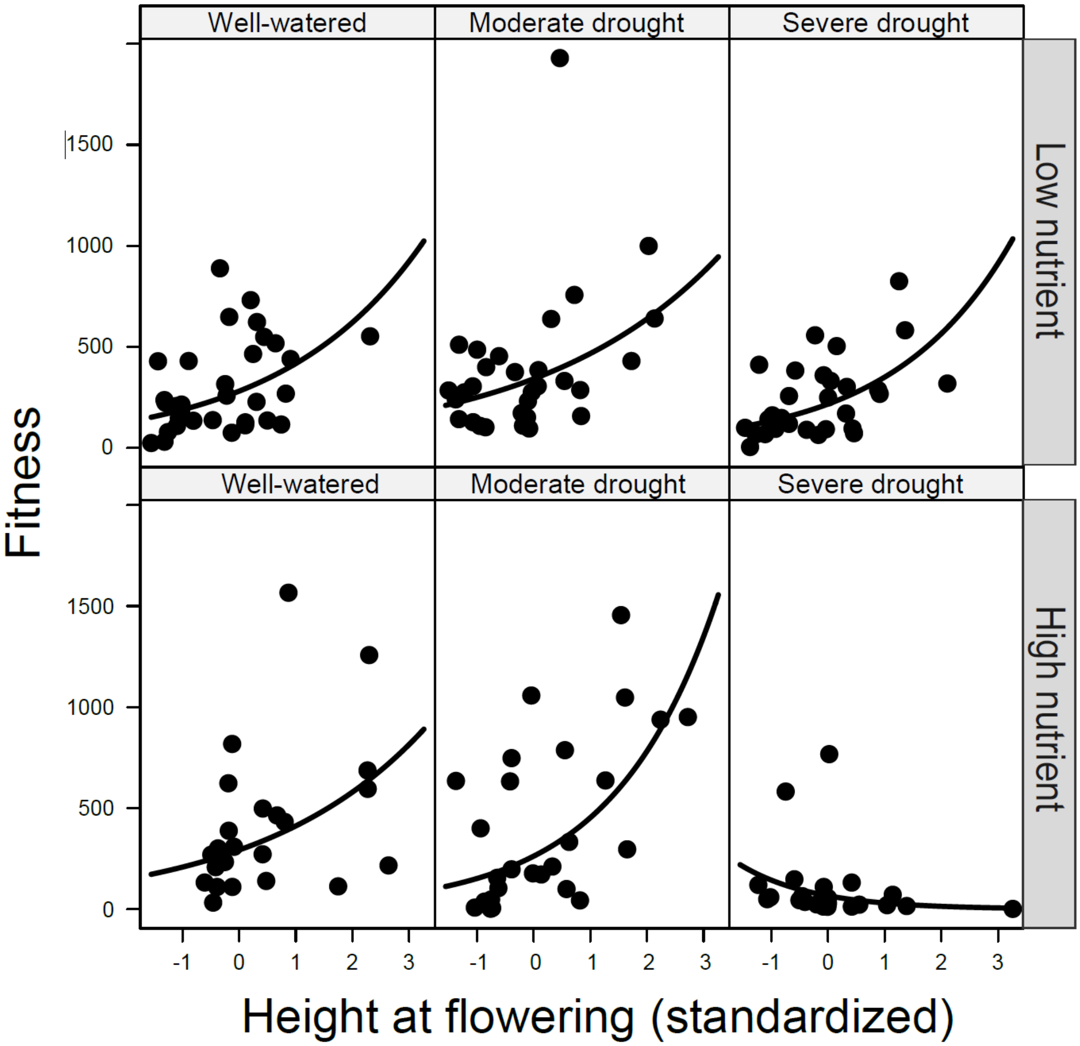

Figure 4.3. Selection favored increased height at first flower across water and nutrient availability combinations in genotypic selection analyses, except under severe drought and high nutrient where there was selection for reduced height, and under well-watered, high nutrient conditions where no selection was found. We standardized trait data to a mean of 0 and standard deviation of 1 to ensure model convergence.

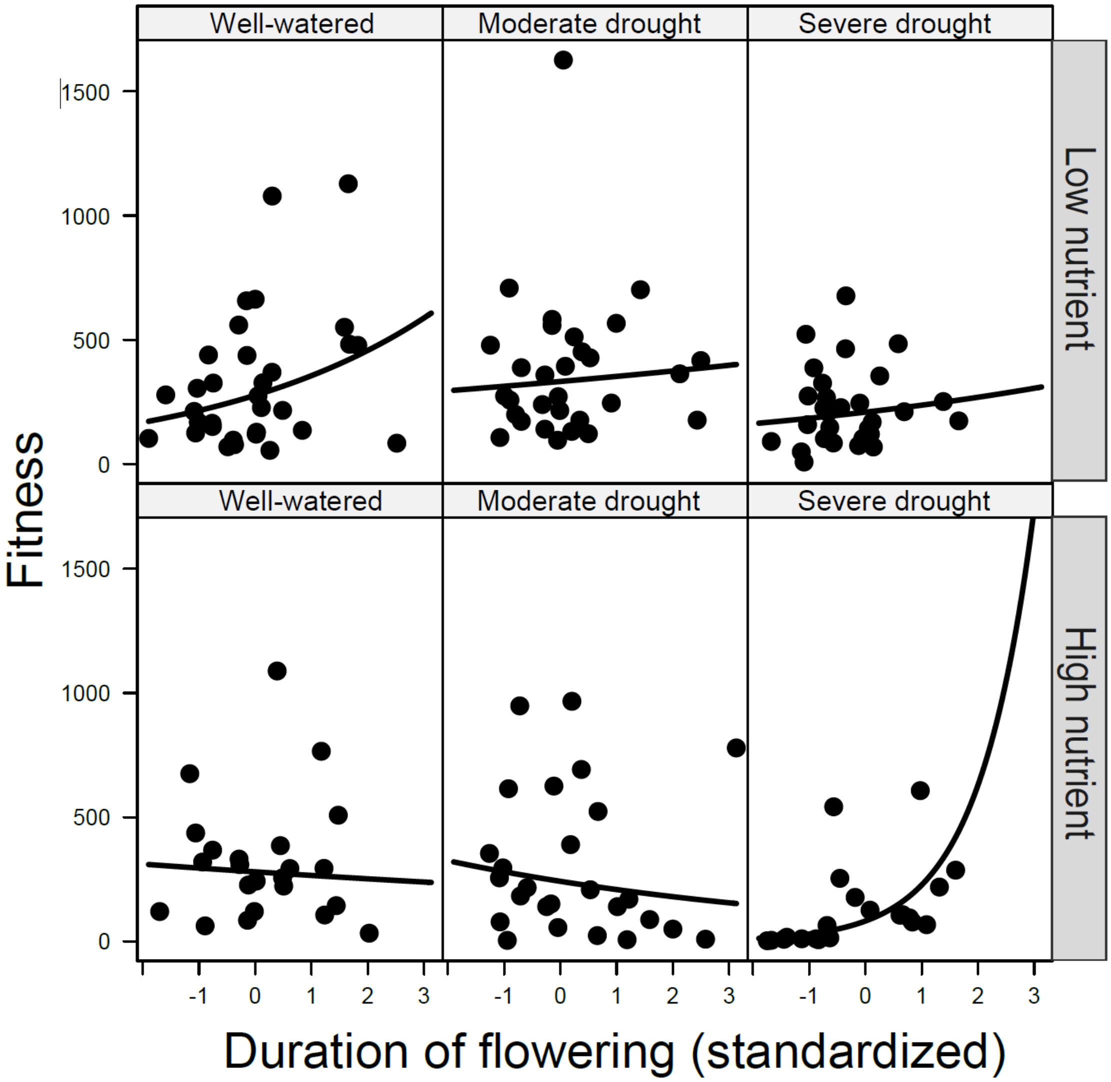

Figure 5.3. Directional selection favored longer flowering in severe drought and high nutrient treatment availability in genotypic selection analyses, but there was no evidence for selection in other treatments. We standardized trait data to a mean of 0 and standard deviation of 1 to ensure model convergence.

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CHAPTER 4

ADAPTIVE PLASTICTY OF LIFE HISTORY TRAITS IN RESPONSE TO WATER AND NUTRIENT AVAILABILITY

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[1] MacTavish, R.M., Anderson, J.T.

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[1] First author in co-authored manuscript

Abstract

Plasticity can facilitate population persistence under rapidly changing environments if populations have evolved plasticity in response to conditions that are shifting. We assessed the adaptive potential of plasticity in accessions of *Boechera stricta* (Brassicaceae) originating from populations distributed across a broad elevational gradient. We exposed these plants to variation in soil water and nutrient availability in a greenhouse setting and examined reproductive phenology. High nutrient levels delayed flowering, and plastic shifts in the timing of first flowering augmented fitness, demonstrating that plasticity in this trait was adaptive. Additionally, plasticity in flowering time plasticity increased with source elevation. In addition, water and nutrient treatments interacted to influence plant size at flowering, as measured through the number of leaves at first flower. Finally, stabilizing selection favored intermediate values of plasticity in flowering duration. Our study highlights the need to conduct multifactorial studies to examine the role of plasticity in biological responses of natural populations to climate change.

Introduction

Natural plant populations risk local extinction as climate change continues to expose them to novel environmental conditions (Anderson, 2016). Global temperatures have risen by 1.5°C since the industrial revolution (Allan et al., 2021). Additionally, climatic projections suggest that arid regions will experience prolonged periods of severe drought while mesic regions will be subject to more frequent and intense storms (Hayhoe et al., 2008; Allan et al., 2021). Temperature and precipitation patterns alter soil water and nutrient availability (Shaw and Harte, 2001; Sheffield and Wood, 2008). As climate change rapidly introduces novel environmental combinations, phenotypic plasticity can facilitate population persistence, potentially buying time for populations to adapt to new conditions (Chevin et al., 2010; Diamond and Martin, 2021). Moreover, plasticity can generate new phenotypic combinations that may be adaptive under novel conditions (Phenning, 2021).

Altered temperature and precipitation regimes will affect abiotic resources essential for plant growth and reproduction (Rosenzweig and Hillel, 2000; Suseela et al., 2012; Classen et al., 2015). In topographically-variable regions, soil moisture varies with rates of evapotranspiration, precipitation, and soil conditions including soil type (Tito et al., 2020). In response to variable soil conditions, many plant species shift their life history and functional traits via phenotypic plasticity (Sultan, 2000, 2003; Anderson and Gezon, 2015; Acasuso-Rivero et al., 2019). For example, soil moisture induces a plastic response in reproductive timing and leaf functional traits in two desert annuals (Angert et al., 2010) and adaptive plasticity enables *Polygonium* species to persist across soil moisture gradients (Sultan and Bazzaz, 1993). Furthermore, soil moisture drives nitrogen availability to plants (Delgado-Baquerizo et al., 2015) and allocation to root foraging (Jobin et al., 2020). These abiotic soil factors in concert with biotic factors like microbial communities result in spatio-temporal heterogeneity of soil nutrient pools (Hodge, 2004). Plant phenological and architectural traits exhibit extensive plasticity to soil nutrient availability (Pigliucci et al., 1997; Pigliucci and Schlichting, 1998; Sultan, 2004; Gupta and Narayan, 2012), which may induce stronger plastic responses than water (Pigliucci et al., 1995; Volis et al., 2002). Plasticity may facilitate population persistence as soil constituents are rapidly altered if populations have evolved plasticity in response to those conditions (Zettlemoyer and Peterson, 2021).

Plasticity can be adaptive when individuals experience multiple conditions across their lifetimes or when their progeny disperse into habitats that differ from those of their parents (Baythavong, 2011; De Kort et al., 2020). However, plasticity can also be a maladaptive stress response (Ghalambor et al., 2007). Trait plasticity itself can evolve when a genotypic by environmental variation is present, confers a fitness advantage, and the plant can sense and respond to changing conditions (Anderson and Mitchell-Olds, 2011; Draghi and Whitlock, 2012; Palacio-López et al., 2015; Draghi, 2019), yet the evolution of adaptive plasticity can be constrained by costs and limitations, especially in novel environments (van Kleunen and Fischer, 2005; Murren et al., 2015). For example, genetic trade-offs across traits can restrict the evolution of plasticity (Gianoli and Palacio-López, 2009). Nevertheless, adaptive plasticity could enhance survival to novel environmental conditions (Sultan, 2004). Thus, testing the fitness consequences of plastic trait variation is crucial for generating robust predictions about the evolution of plasticity, especially under novel conditions.

Heightened seasonal variation in could favor increased thermal plasticity (Dobzhansky, 1950; Janzen, 1967). This climatic variability hypothesis (Janzen, 1967) has primarily been tested in ecotherms across latitude gradients (Naya et al., 2008; Molina-Montenegro and Naya, 2012; Gutiérrez-Pesquera et al., 2016; Shah et al., 2017). Very few studies explore whether plasticity increases with latitude or elevation in plant systems (Ghalambor et al., 2006). In many regions globally, abiotic conditions change precipitously across elevation, with increasing snow depth, delayed spring snowmelt date, reduced temperatures at higher elevations (Tito et al., 2020). In addition, in many regions, soil moisture and nutrient available also shift with elevation (Wallace et al., 1996; Ranelli et al., 2015). These abiotic differences have pronounced consequences for the biotic community, as species composition and abundance can also differ dramatically with elevation (McCain and Grytnes, 2010; Ranelli et al., 2015; Tito et al., 2020). Clinal trait variation can evolve in response to variable conditions across the range (Kooyers et al., 2015; Wadgymar, Daws, et al., 2017). Detecting genetic clines in plasticity across climatic gradients could illuminate which agents of selection shaped the evolution of plasticity (Trussell, 2000; Wund, 2012; Wadgymar, Daws, et al., 2017; Ensing and Eckert, 2019). Nevertheless, few studies to-date have examined geographic variation in the extent of plasticity (Gratani, 2014; Wadgymar, Daws, et al., 2017).

We hypothesize that water stress and nutrient availability interact to induce plasticity in phenological traits. To test this hypothesis, we exposed accessions of *Boechera stricta* originating from a broad elevational gradient to a multifactorial manipulation of water and nutrient levels over multiple simulated growing seasons in the greenhouse. We predict that: 1) Soil nutrient and water availability will induce plasticity in phenology, 2) genetically-based clines in plasticity exist, such that plasticity increases with source elevation, and 3) plasticity in phenology is adaptive. Additionally, as climate change progresses altering snowpack and snowmelt date, soil water and nutrients in the Western Rocky Mountains will become increasing variable (Leonard et al., 2021), which could further favor the evolution of plasticity. We expect to see genetic clines in plasticity like those detected in flowering time phenology in field populations (Wadgymar, Daws, et al., 2017) and in plasticity in germination traits in the field (Wadgymar, Mactavish, et al., 2018). Furthermore, we predict that plasticity in flowering duration across will be adaptive. Height and leaf number at first flower are metrics of size at reproduction that are often subject to selection (Anderson and Mitchell-Olds, 2011; Munguía-Rosas et al., 2011) and may reflect soil moisture and nutrient resources acquired proceeding flowering time (chapter 2). In field studies of *B. stricta*, directional selection favored increased height and leaf number at flowering (Anderson, Panetta, et al., 2012). Additionally, size at flowering varies clinally, such that high elevation genotypes flower sooner at short stature in comparison with their low elevation counterparts in field common gardens (Anderson and Gezon, 2015; Wadgymar, Daws, et al., 2017). Selection also favored greater height at first flower across soil moisture and nutrient combinations in the greenhouse where stressed plants flowered early at small heights before mortality (chapter 2). Furthermore, divergent selection favored more leaves at flowering in the mesic water and low nutrient environments and fewer leaves numbers in the severe drought high nutrient environments. We expect to find adaptive plasticity and a cline in plasticity for both plant height and number of leaves at transition to reproduction.

Methods

Focal system

Boechera stricta (Brassicaceae), a perennial forb found in alpine meadows and streamside throughout the Rocky Mountains of North America (Rushworth et al., 2011). Gravity and wind dispersed seed coupled with a primarily self fertilizing reproductive stragey results in high genetic differentiation between populations (F_{ST} = 0.56, Song et al., 2006). Additionally, local adaptation in *B. stricta* is evident across latitudes and elevational gradients (Rushworth et al., 2011; Anderson et al., 2013; Lee and Mitchell-Olds, 2013; Anderson and Gezon, 2015; Anderson and Wadgymar, 2020). We collected materal genetic lines from natural populations approximately every 30 meters along an elevational gradient (2499-3450m) in sub-alpine meadows near Rocky Mountain Biological Laboratory (Gothic, Colorado, USA, Appendix S1.2), and grew them in greenhouses conditions for one generation to homogenize maternal effects and generate full sibling progeny.

In this system, aridity and soil nitrate (a bioavailable source of soil N for plants) decrease with elevation (Ranelli et al., 2015; Anderson and Wadgymar, 2020). In *B. stricta*, foliar N content increases with elevation (Anderson and Gezon, 2015), which could reflect variation in resource acquisition strategies across populations (Bloom, 1986; Xu et al., 2009). Soil water availability is driven in part by soil type and snowpack depth which currently increase with elevation in the Colorado Rocky Mountains (Leonard et al., 2021) and summer precipitation (Lambert et al., 2010). Soil water is essential for nutrient assimilation into plants (Baligar et al., 2001; Dubey et al., 2021) and root foraging (Jobin et al., 2020). Experimental manipulations indicate that warming and frost/thaw cycles increase variability in snowpack depth, soil frost depth, temperature, and moisture variability in the early spring (Templer et al., 2017), suggesting that these conditions will become more variable under climate change. Reduced snowpack increases the frequency of freeze/thaw cycles, and warming directly influence biogeochemical cycling of nitrogen, releasing bioavailable soil N and excess N_2O emissions, potentially mismatching plant nutrient needs with available nutrients (Gao et al., 2017; Gavazov et al., 2017). In this experiment, we simulated these changing conditions in the greenhouse through soil moisture and nutrient treatment levels that reflect projected climates at mid-elevation sites for mid-century.

Experimental design

We examined adaptive plasticity to resource availability using a greenhouse manipulation of water and nutrient resource supply at the University of Georgia (Athens, GA). In March 2017, we germinated 6 individuals from 35 maternal *Boechera stricta* lines selected along the 1000 m elevational gradient at ~30 m intervals, one family per source population. Germinants received homogenous daily mist watering and potting soil nutrients (Sungro® Fafard® 3b) to ensure seedling survival. After 8 weeks, we measured initial plant size and randomly assigned individuals to one of six novel soil water and nutrient availability combinations. Six siblings per family were exposed to a factorial combination of 3 water and 2 nutrient availabilities (N = 6 siblings x 35 families x 3 water x 2 nutrient treatment levels = 1,260 individuals). A small portion of individuals failed to germinate or died prior to treatment reducing the experimental sample size to 1,209 total individuals (Appendix S2.2). Weekly, treatment combintion blocks were roated to reduce confounding environmetnal variation introduced by the greenhouse.

Nutrient pulses are available after snowmelt and amplified as freeze/thaw cycles acclorate with climate change (Groffman et al., 2011; Gavazov et al., 2017). Thus we grew plants for 3 weeks after germinating and applied a weekly mixture of water-soluble Peter's Professional General-Purpose Fertilizer™ 20N-10P-20K at concentrations of 110 ppm N (1 ppm = 1 mg/L), and 1100 ppm shown to influence *B. stricta* trait expression (Siemens et al., 2010, 2012) for an additional 3 weeks. Plants were then vernalized at 4^{o}C in June 2017 for 8 weeks under equal bottom watered conditions without fertilizer application to induce flowering cues (Anderson et al., 2011). The treatment phase after vernilization reflected field conditions while retaining a 10 fold increase between the low and high nutrient treatment, 5 and 50 ppm respectively, coinside with increasing elevation reflecting pore water extractible nitrates (Ranelli et al., 2015; He et al., 2016; Drollinger et al., 2017).

Water and nutrient availablity combinations, applied for 16 weeks simulating field summer growing season length, reflected conditions found along natural populations elevational gradient. Annual summer rainfall (June-August) at the Rocky Mountain Bioloical Laboratory (2895 m) from 2000-2018 was 51.20 ± 18.30 mm (mean ± S.D.) ranging from 29.21 – 90.80 mm (b. barr, long-term weather data). Experimental hand watering applied ~3 mm of water per application in benign well-watered treatments received ~90 mm monthly, while moderate drought (twice weekly) and severe drought (one weekly), received ~24 and 12 mm of water monthly. We confirmed watering treatment efficacy using a Hydrosense II (Campbell Scientific, Logan, Utah) to measure soil moisture as volumetric water content (%VWC). On the most severe drought day, before weekly watering application, we randomly selected 4 individuals per block with no observable differences in plant size at time of collection (personal obs., R. MacTavish). Our volumetric water content findings aligned with 2014-2018 summer averages at common gardens field locations in the Rocky Mountain Biological Laboratory, where mid-elevation garden (2890m) averaged 10% (standard deviation: 5%; range 0-39%) (Anderson and Wadgymar, 2020). Greenhouse moderate drought stress (mean ± S.E.: 10.51 %± 2.33%) treatment reflects average soil water availability in the field. Severe drought stress (4.65 % ± 1.71%) and well-watered (17.52 %± 2.71%) treatments fall within the range of variability at high and low gardens, respectively (MacTavish and Anderson, 2020).

Traits

To quantify reproductive phenology, we censused plants every other day during the simulated summer season to record the day of first flowering, duration of flowering, along with height and number of first flowering. During each census we recorded the number of open flowers which allowed us to calculate day of first flowering and duration of flowering. We considered flowers present from the first opening, quantified as pinhole size opening at bud, to visible silique (fruit) emergence. We calculated flowering time, marking the transition from growth to reproduction as the number of days between removal from vernalization to the first appearance of a flower. We recorded the number of leaves present at first flowering and plant height, measuring from soil level to the base of the inflorescence. Flowering duration is the number of days elapsed between the first appearance of a flower and the last day a flower transitioned to silique. We collected fruits at maturity and quantified the total number of seeds produced by spreading them on white paper, imaging, and counting objects using ImageJ (https://imagej.nih.gov/ij/).

Statistical analysis

Plasticity across resource environments - To test for plasticity of phenological traits we analyzed individual-level trait data in response to drought, nutrient, and 2-way interaction term along with random effects to account for genotype and block variation using a generalized linear mixed model framework in R with the “lme4” package ver. 1.1.28 (Bates et al., 2015) in R 4.0.2. We fitted flowering time, flowering duration, and number of leaves at first flower with a negative binomial distribution, and the remaining traits with a gamma distribution with log link. All traits were analyzed individually after failing to meet normal distribution assumptions of multivariate regression, MANOVA (Shapiro-Wilk’s test, $W = 0.814$, $p < 0.0001$) in the “mvnormtest” ver. 0.1-9 (Jarek, 2015). We corrected for co-linearity between traits by adjusting the alpha level account the number of traits ($a = 0.05/4 = 0.0125$), a conservative multiple-comparison correction (Chen et al., 2017). We used ‘ggplot’ and ‘dyplr’ in the “tidyverse” package to visualize plasticity (Wickham, 2019).

Selection on Plasticity – We tested whether plasticity confers a fitness advantage using across-environment multivariate genotypic selection analysis (Van Kleunen and Fischer, 2001). To do so, we first calculated genotypic means, LSMEANs, for each novel soil water and nutrient availability combination were extracted with the “emmeans” package ver. 1.7.2 in R (Lenth, 2020) by regressing traits on genotype, drought, nutrient, and all possible interaction terms along with random effect of block in the “lme4” package ver. 1.1.28 (Bates et al., 2015). We then calculated a phenotypic plasticity index (PI_{LSM}) (Valladares et al., 2006; Anderson et al., 2021). Valladares et al (2006) propose an easily calculated plasticity index that accounts for the magnitude of plasticity ($PI_{LSM} =$

$LSMEAN_{maximum} – LSMEAN_{minimum}/ LSMEAN_{maximum}$). When testing for adaptive plasticity across environments, using the plasticity index ($PI_{LSM} = LSMEAN_{E, high} – LSMEAN_{E, low}/ LSMEAN_{E, high}$) where $LSMEAN_{E, high}$ is the highest genotype-mean trait value and $LSMEAN_{E, low}$ is the lowest genotype-mean trait value value of the trait (Valladares et al., 2006). Relative distance plasticity indices were modeled using LSMEANS in the "Plasticity" package in R (Ameztegui, 2017).

We examined selection on plasticity and genetic clines in plasticity using PI_{LSM}. We regressed fitness average across environments on plasticity indices and scaled trait means to examine selection on plasticity independent from selection on correlated traits in the "MASS" package in R (Venables and Ripley, 2002). Fitness LSMEANs were calculated from seed set of reproductive plants in each population by water and nutrient combination. LSMEANs were then multiplied by the proportion of plants that survived and reproduced, was rounded to the nearest integer for a negative binomial distribution.

Genetic clines in plasticity –We assessed the genetic clines in plasticity by regressing the trait plasticity index (PI_{LSM_mod}) on elevation, scaled to a mean of 0 and standard deviation of 1. For each of the four trait plasticity indices, presenting positively skewed proportional data, we fitted generalized linear models with quasibinomial family. We corrected for co-linearity between traits by adjusting the alpha level account the number of traits (a = 0.05/5 = 0.01), a conservative multiple-comparison correction (Chen et al., 2017).

Results

Plasticity across environments – We found significant plasticity to resource environment (Table 1.4, Fig. 1.4). A water and nutrient availability interaction influenced leaf number at first flower ($F_{3,620}$ = 14.66, *p* = 0.0007) (Table 1.4, Fig. 1.4a). Low nutrient availability reduced leaf number at flowering across all water treatments compared with the high nutrient availability treatment. Under high nutrients, drought stressed plants had lower numbers of leaves than individuals in the moderate or well-watered treatment. High nutrient availability delayed flowering ($F_{3,639}$ = 33.90, *p* <0.0001, Table 1.4, Fig. 1.4b).

Selection on plasticity – We found directional selection for increasing plasticity in flowering time (χ^2=8.07, d.f.=1, p=0.0045), linear regression (β = 1.33 ± 0.63, p=0.0338) (Table 2.4, Fig. 2.4a), and stabilizing selection (γ = -11.47 ± 3.16, p=0.0003) for intermediate plasticities in flowering duration b (χ^2=5.37, d.f.=1, p=0.0204) , favored mid values of flowering duration plasticity, linear selection (β= 13.23 ± 0.3.57, p=0.0002) was retained for flowering duration only (χ^2=5.11, d.f.=1, p=0.0238) (Table 2.4, Fig. 2.4a).

Genetic clines in plasticity – Plasticity in flowering time increased with elevation (χ^2=7.84, d.f.=1, p=0.0051) (Fig. 3.4), but we found no clines in plasticity in other phenological traits.

Discussion

Our greenhouse experiment revealed adaptive plasticity in flowering time across nutrient treatments. Plastic variation in phenotypes can facilitate population persistence in response to novel soil nutrient levels in the context of climate change (De Kort et al., 2020). Additionally, we found a genetic cline in plasticity, such that plasticity in flowering phenology increased with source population elevation. This clinal pattern is consistent with Janzen's climatic variability hypothesis, which postulates that increased variation in temperature at higher latitudes should favor greater plasticity in thermal tolerance (Janzen, 1967). In the field, early snowmelt accelerated the timing of first flowering in experimental manipulations (Anderson and Gezon, 2015; Wadgymar et al., 2018) and long-term dynamics in natural populations (Anderson et al., 2012; Wadgymar et al., 2018). Climate change is inducing early flowering across numerous systems in spring-flowering species (Parmesan and Yohe, 2003; Anderson et al., 2012; Lustenhouwer et al., 2018; Wadgymar et al., 2018; Iler et al., 2021). Under climate change, available soil nitrogen will spike with warmed soils then deplete over 3-4 growing seasons as microbial turnover outpaces litter decomposition rates (Shaw and Harte, 2001). Thus, soil nutrients, in the short term, could offset the effects of earlier snowmelt timing and potentially delay flowering.

Phenotypic plasticity allows plants to adjust to environmental variation rapidly whereas evolution by natural selection can be a much slower process (Phenning, 2021). Plants can respond to novel climates by shifting their geographic ranges, adapting to new selection, or altering their phenotypes plastically (Davis et al., 2005; Chevin et al., 2010; Nicotra et al., 2010; Anderson et al., 2012; Valladares et al., 2014; Fox et al., 2019).

Tracking conditions geographically requires spatially-extensive dispersal, and could be restricted by biotic interactions (Brooker et al., 2007; Ash et al., 2017). Plasticity may prevent decline of local populations of inbred, low dispersal species like *B. stricta* until adaptation to novel conditions can occur (Pigliucci, 1996). Thus, phenotypic plasticity could aid short term population persistence as climate change rapidly introduces novel combinations of soil and water availability (Alpert and Simms, 2002; Nicotra et al., 2010; Fox et al., 2019).

Plasticity itself is a trait subject to selection and can evolve in response to temporal variation in conditions (De Kort et al., 2020). Adaptive plasticity in flowering phenology in *B. stricta* (Fig. 2) could be favorable as climate change increases variability in the length of the growing season (De Kort et al., 2020). Shorter growth seasons at higher elevations could also favor increased plasticity (Ensing and Eckert, 2019). For example, Additionally, a multispecies common garden study 800 m apart in elevation, representing a 4 °C direct temperature difference, high elevation plants increased plasticity in flowering time (Schmid et al., 2017). Furthermore, environmental stability at low elevations selects for reduced plasticity (i.e., canalization) in functional traits and phenology (Angert et al., 2010; Chevin et al., 2010). The cline in plasticity in flowering time is consistent with the hypothesis that high elevation plants investing in rapid resource acquisition to complete their life cycles in a short growing season (Wadgymar, Daws, et al., 2017; MacTavish and Anderson, 2020; Hamann et al., 2021; López-Goldar and Agrawal, 2021).

Flowering time plasticity is prevalent across systems and can be either adaptive or maladaptive (van Kleunen and Fischer, 2005; Levin, 2009). Adaptive divergence in plasticity can decrease fitness of locally adapted ecotypes in foreign environments (Donohue et al., 2001). For example, genotypes adapted to low soil moisture were more canalized and were strongly selected against under increased soil moisture in natural populations of *Impatiens capensis* (Donohue et al., 2001). In the field, *Boechera stricta* low elevation populations outperform local genotypes at higher elevations, suggesting that low elevation genotypes may migrate upslope to track climate change (Anderson and Wadgymar, 2020). Reduced plasticity found in low elevations *B. stricta* genotypes, which evolved in arid regions, may slow dispersal upslope when encountering nutrient resource environments conflicting with historical context.

In contrast to our predictions, plasticity was not adaptive for either of our metrics of size at flowering (height or the number of leaves). In a greenhouse study of *Arabidopsis thaliana*, soil moisture and nutrient availability induced plasticity in both traits (Pigliucci and Schlichting, 1998). We found no evidence for plasticity in height at first flower to soil water or nutrient availability. Nevertheless, leaf number at first flower shifted in response to environment, with greater leaf numbers produced in benign water and high nutrient environments.

We identified adaptive plasticity in flowering time, which could aid population persistence to novel environments as climate change advances (Zettlemoyer and Peterson, 2021). Selection favors earlier flowering (chapter 2) and increased plasticity in flowering time (this chapter). In addition, both flowering time and plasticity in this trait show clinal variation, with high elevation ecotypes flowering earliest and having the greatest extent of flowering time plasticity. *Boechera stricta* maintains plasticity in numerous traits across multiple environments, including plasticity in defense chemicals in response to herbivory (Wagner and Mitchell-Olds, 2018), functional traits in response to snow removal (Anderson and Gezon, 2015), and flower color response to soil potassium and herbivory (Vaidya et al., 2018). Climate change is disrupting abiotic and biotic agents of selection along elevational gradients in complex and context dependent ways (López-Goldar and Agrawal, 2021).

Adaptation, range shifts, and phenotypic plasticity will likely occur simultaneously in plant populations as climate change advances (Anderson et al., 2012; Des Marais et al., 2013). These responses likely occur simultaneously. For example, phenotypic plasticity could enable migrants to establish in upslope locations and could also enhance population persistence in situ as conditions continue to change. Studies of the adaptive potential of plasticity to climate change should examine phenotypic responses to multiple agents of selection across different traits to generate robust predictions (Wadgymar et al., 2017b). Future research questions may focus on the following questions: 1) How many generations can plasticity in ecologically important phenotypic traits remain adaptive? 2) Will climate change impose unique suites of abiotic and biotic selection that favors optimum trait values outside the limits of phenotypic plasticity? (Valladares et al., 2007) 3) Will genetic correlations between plasticity and correlated traits hinder the response time to climate change? Our findings and future research on adaptive plasticity in phenological traits will reveal if co-gradient variation and plasticity will likely aid or hinder range expansion when models incorporate multiple agents of selection imposed by climate change.

Tables and Figures

Table 1.4. ANOVA table for regression of life history and aboveground biomass traits. Multiple comparison correction applied by adjusting alpha (a= 0.05/4 = 0.0125). Bolded values indicate significance.

Individual level data

	Drought:Nutrient		Drought (main)		Nutrient (main)	
Trait	F	*p*	F	*p*	F	*p*
Height	6.1939	0.0452	0.4705	0.7904	0.0079	0.9293
Leaf number	**14.6550**	**0.0007**	2.3597	0.3073	3.6251	0.0569
Flowering time	0.7418	0.6901	3.3554	0.1868	**33.9004**	**<0.0001**
Flowering duration	0.8881	0.6414	3.9215	0.1407	1.8424	0.1747

Table 2.4. Selection on plasticity, all traits across all environments across all water and nutrient treatment combinations. Significance at $\alpha = 0.05$ indicated in bold.

Plasticity index (PI_{lsm})	χ^2	*p*-value
Height	3.2213	0.0727
Leaf number	2.0785	0.1494
Flowering time	**8.0680**	**0.0045**
Flowering duration	**5.3702**	**0.0205**

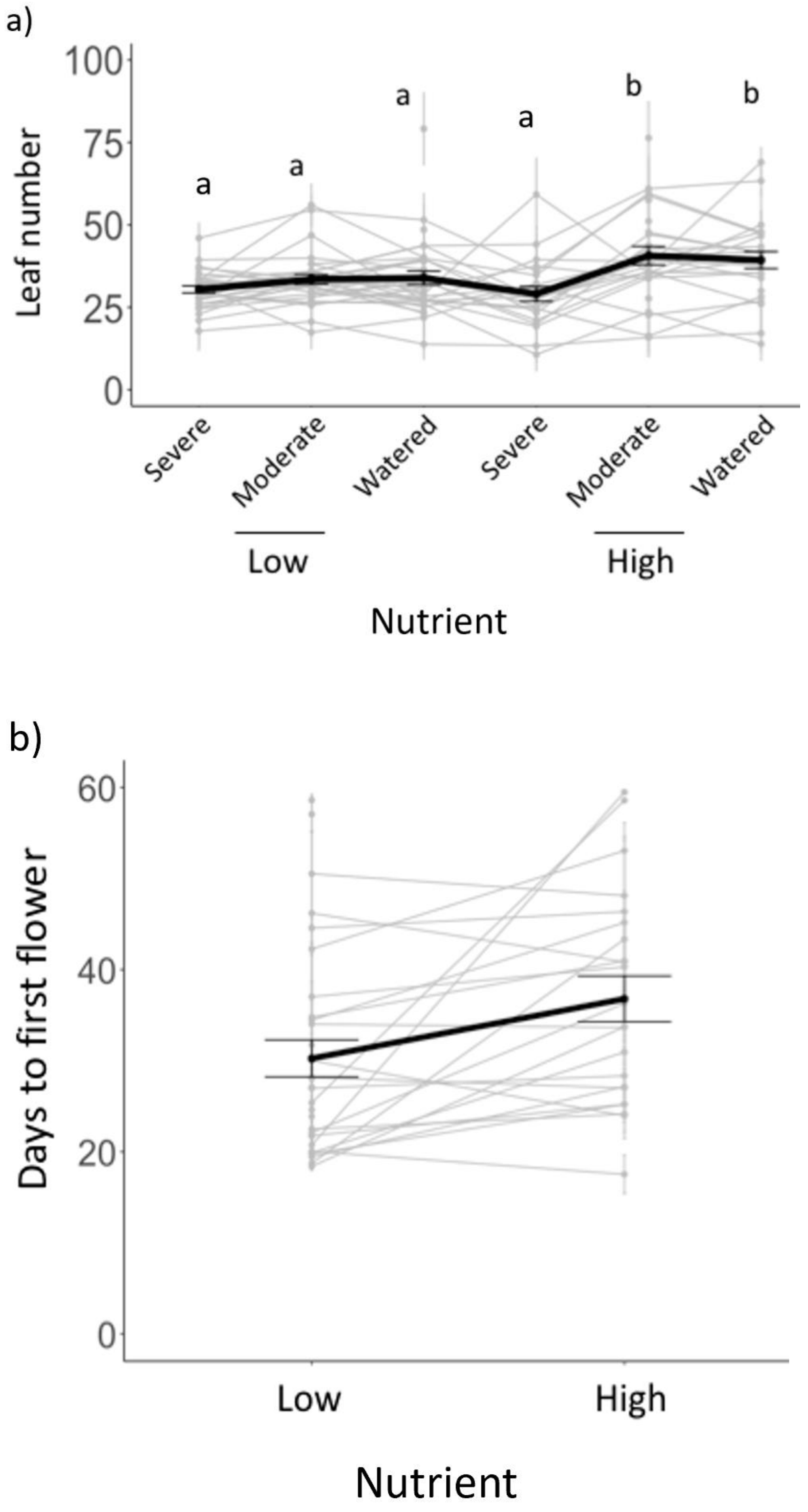
a)
Leaf number
100
75
50
25
0
a
a
a
a
b
b
Severe
Moderate
Watered
Severe
Moderate
Watered
Low
High
Nutrient
b)
Days to first flower
60
40
20
0
Low
High
Nutrient

Figure 1.4. Plasticity across soil resource availability treatments. A significant interaction between drought and nutrient availability was found in leaf number at first flowering (a). Additionally, flowering time plasticity was significant across soil nutrient level (b) letters indicate significantly different treatment means using Tukey’s post-hoc test. Overlain black line indicates individual-level overall mean response to treatment, grey lines indicate genotypic response.

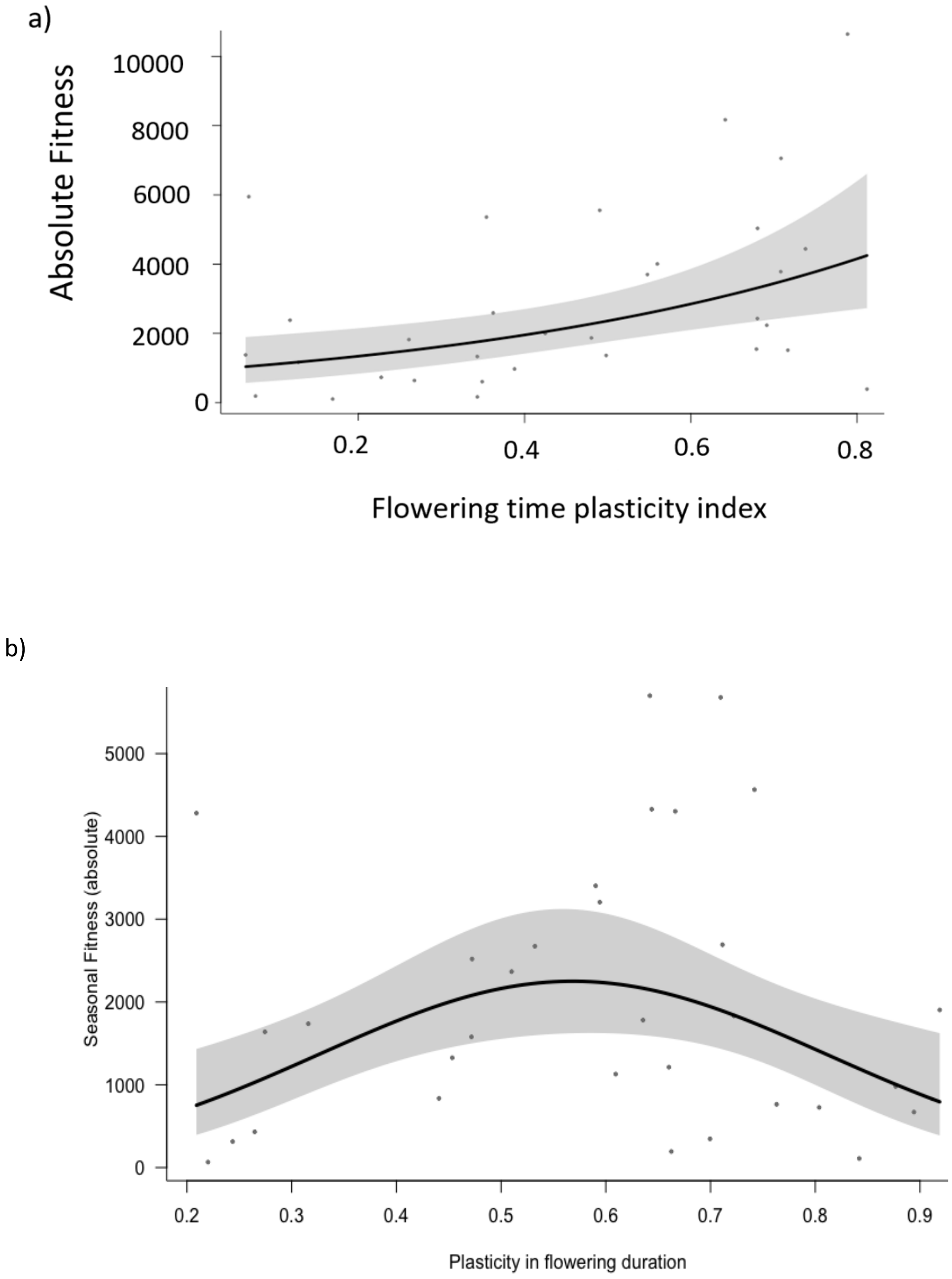

Figure 2.4. Selection on plasticity. Directional selection for increased plasticity in flowering time (a). Stabilizing selection favors mid values of flowering duration (b). Fitted regression line shown with 95% confidence intervals.

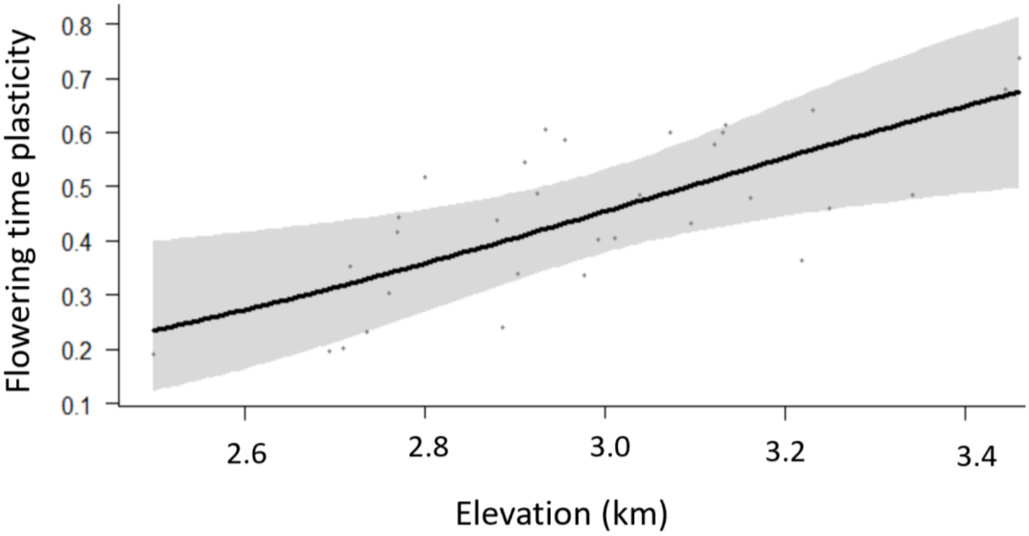

Figure 3.4. Genetic cline in flowering time plasticity. Flowering time plasticity (PI_{LSM}) significantly (p=0.0051) varied with Elevation (km). Fitted regression line shown with 95% confidence intervals.

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CHAPTER 5

CONCLUSIONS

Plants exhibit disparate life history strategies across and within species, ranging from fast growth and short lived to slow growth and long-life spans (Stearns, 1976). These life history strategies can evolve in response to local environmental conditions (Stearns, 1989; Roff, 2002). Climate change, industrial pollution, and agricultural runoff have altered soil resources (Jamieson et al., 2012), and likely impose novel selection on natural plant populations. Genetic correlations between traits can facilitate evolution if the joint vector of selection is in the same direction as the genetic correlation, or constrain evolution if selection opposes the direction of the genetic correlation (Etterson and Shaw, 2001). Additionally, adaptive phenotypic plasticity can aid population persistence in the short-term, and potentially increase the rate of adaptation to new conditions in the longer term (Zettlemoyer and Peterson, 2021).

I exposed accessions of *Boechera stricta* (Brassicaceae) from diverse natural populations to a large-scale multifactorial greenhouse manipulation of soil water and nutrient resource availability. My overarching objective was to evaluate the evolutionary consequences of altered water and nutrient resource availability for: 1) fitness trade-offs, 2) patterns of selection, and 3) phenotypic plasticity.

Resource supply altered the associations between fitness components (chapter 1). For example, trade-offs appeared under water stress, such that allocating resources to growth reduced investment in fecundity. Longitudinal studies have found that high interannual variability of water supply can obscure trade-offs (Agrawal et al., 2010; Zust and Agrawal, 2017). My results show that drought could constrain ongoing adaptation to stressful conditions despite significant heritability in fitness components (Hellmann and Pineda-Krch, 2007). As climate change continues to expose plants to stressful environments, genetic trade-offs that did not previously exist under benign conditions could restrict adaptive potential.

By isolating the effects of nutrient and drought stresses on fitness, my studies revealed local adaptation to severe drought, relatively high heritabilities across fitness components and environments, and fitness trade-offs at the phenotypic and genetic levels in distinct environments (chapter 2). Furthermore, fitness response generally accorded with local adaptation of low elevation genotypes to drought stress. I found local adaptation to soil water availability, suggesting that local adaptation in the field could evolve in response to variation in both the timing of snowmelt which drives early season soil water availability (Anderson and Wadgymar, 2020), as well as the direct effects of water levels during the growing season (chapter 3).

Magnitude and direction of selection was altered by soil resource availability and **G** matrix models revealed the potential for these traits to adapt to this selection (chapter 3). Soil water and nutrient availability exerted selection on all genetically-correlated phenological traits including flowering time, flowering duration, and size at flowering. Selection favored early flowering and longer duration of flowering, and the genetic correlation between these traits accorded with the direction of selection. In most treatments, we found selection for increased height, but selection on leaf number depended on water availability, with selection favoring more leaves in well-watered conditions, and fewer leaves under severe drought. The strength of selection on size at flowering depended on the variable measured. I found evidence that selection can act strongly not only on the timing of first flowering but also on the duration over which an individual produces flowers. Climate change imposes strong selection for earlier flowering across systems (Anderson, Panetta, et al., 2012), my results highlight unknown consequences of correlated traits associated with fitness like the length of the flowering period.

Adaptation, range shifts, and phenotypic plasticity will likely occur simultaneously in plant populations as climate change advances shifting soil resource availability (Anderson et al., 2012; Des Marais et al., 2013). I found strong adaptive plasticity in flowering time in response to nutrient treatment (chapter 4). Phenotypic plasticity in flowering time may allow populations to persist longer aiding adaptation by natural selection (Anderson and Gezon, 2015; Diamond and Martin, 2021). Phenotypic plasticity can also aid plant establishment as plants migrate to track favorable conditions (Anderson and Wadgymar, 2020; Zettlemoyer and Peterson, 2021).

My results enhance our ability to predict evolutionary responses to shifting resource availability. Soil water and nutrient availability influence fitness trade-offs in stressful environments and patterns of selection on critical life history traits. Additionally, plasticity may enhance short-term population persistence under climate change, especially in high elevation populations. However, genetic correlations, heritability, and selection estimates can vary between greenhouse and field (Campbell, 1996; Conner et al., 2003; Poorter et al., 2016) due to additional abiotic and biotic stresses in natural settings. I highlight the need to conduct multifactorial studies and investigate soil water and nutrient availability on life history evolution in the field to generate better models of evolutionary trajectories in response to rapid climate change.

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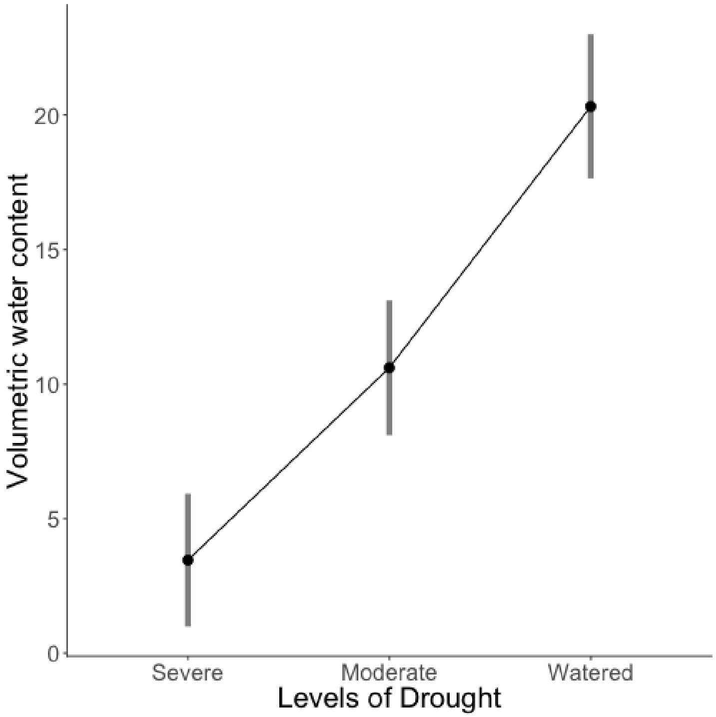
APPENDICES

Appendix S1.2. Spatial coordinates of selection populations spanning an elevational gradient in Gunnison Co. Colorado (USA).

No.	Elevation (m)	Population ID	Latitude	Longitude
1	2498.61	267	38 42.572	-106 48.092
2	2519.47	265	38 42.486	-106 48.171
3	2693.59	214	38 52.817	-106 59.867
4	2710	273	38 53.063	-106 59.501
5	2717.29	262	38 52.751	-106 59.910
6	2734.67	256	38 51.985	-106 54.766
7	2759.96	259	38 52.550	-106 54.552
8	2770	278	38 52.284	-106 53.878
9	2721.21	209	38 52.215	-106 57.118
10	2800	269	38 55.186	-107 02.088
11	2824.53	206	38 53.199	-106 57.688
12	2880	86	38 57.329	-106 59.308
13	2886	85	38 57.431	-106 59.300
14	2903	67	38 57.626	-106 59.548
15	2910	91	38 57.211	-106 59.545
16	2925	106	38 57.910	-106 59.605
17	2934	50	38 58.770	-107 00.030
18	2955	47	38 58.802	-106 59.978
19	2977	152	38 59.408	-107 00.695
20	2992	154	38 59.654	-107 00.881
21	3011	98	38 58.010	-106 59.380
22	3038	161	38 59.956	-107 01.265
23	3072	102	38 58.570	-106 59.370
24	3095	80	38 57.272	-106 58.222
25	3121.58	211	39 02.268	-107 03.758
26	3131	212	39 02.447	-107 03.922
27	3133	250	39 02.346	-107 03.818
28	3161	210	39 01.485	-107 03.038
29	3218.6	207	39 01.135	-107 02.970
30	3231	176	39 00.711	-107 02.499
31	3249	180	39 00.230	-107 00.433
32	3341.67	255	39 01.894	-107 04.708
33	3444	252	39 02.700	-107 06.699
34	3460	253	38 59.519	-107 03.956
35	3530	254	38 59.656	-107 03.931

Appendix S2.2. Genotypes with fewer than 6 siblings per genotype treatment combination. All remaining genotypes had N=6 siblings per treatment combination.

Drought_Nutrient Treatment	Genotype	Experimental Individuals
Moderate_Low		203
	180	3
	209	5
	257	4
Moderate_Moderate		203
	180	2
	210	4
	265	5
Severe_Low		203
	80	5
	180	3
	210	4
	265	5
Severe_Moderate		200
	98	5
	180	1
	210	3
Watered_Low		201
	176	5
	180	2
	210	3
	257	5
Watered_Moderate		199
	106	5
	176	5
	180	2
	210	3
	257	4
Total	1209	

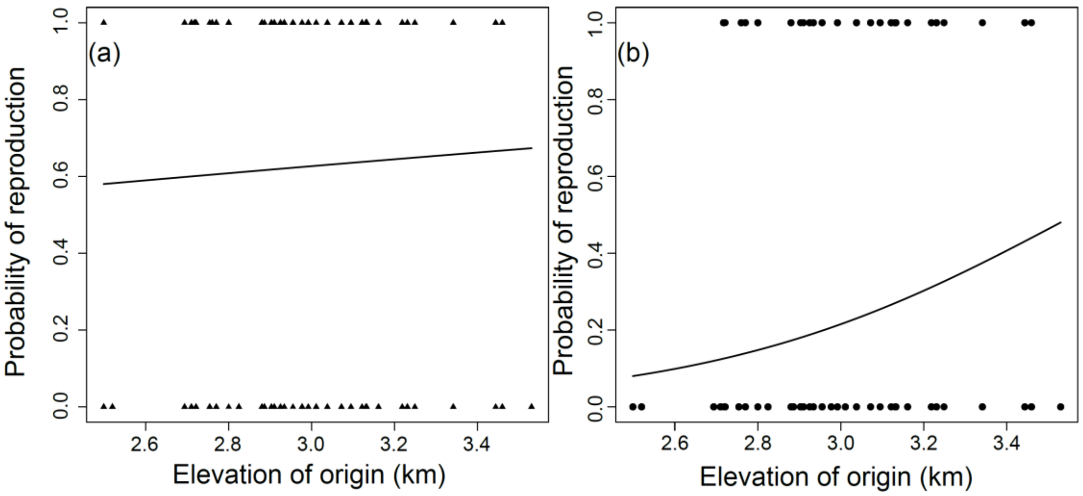


Appendix S3.2. Soil moisture as percent volumetric water content (%VWC). Drought treatment significantly (F=44.77, *p*=0.007) affected soil moisture.

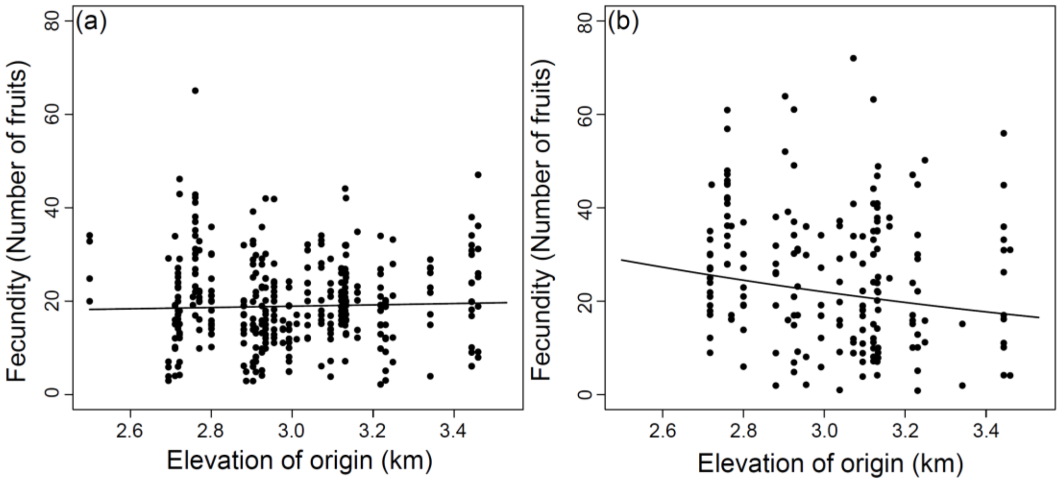
Appendix S4.2. Individual-level analyses of fitness trade-offs across nutrient and drought treatments for the probability of reproduction (a binary variable indicating whether a plant successfully fruited) and fecundity (# of fruits among individuals that fruited). This ANOVA table presents type III Sums of Squares from analyses testing fitness as a function of nutrient treatment, drought treatment, source elevation and all two and three-way interactions, showing numerator and denominator degrees of freedom (df), F-values and p-values. We computed the significance of the random effects of population and block with a likelihood ratio test using a one-tailed χ^2 with one degree of freedom (Littell et al., 1996). These individual-level analyses are highly concordant with the family-level analyses presented in the main text (Table 1.2). Continued page 132.

Appendix S4.2. continued.

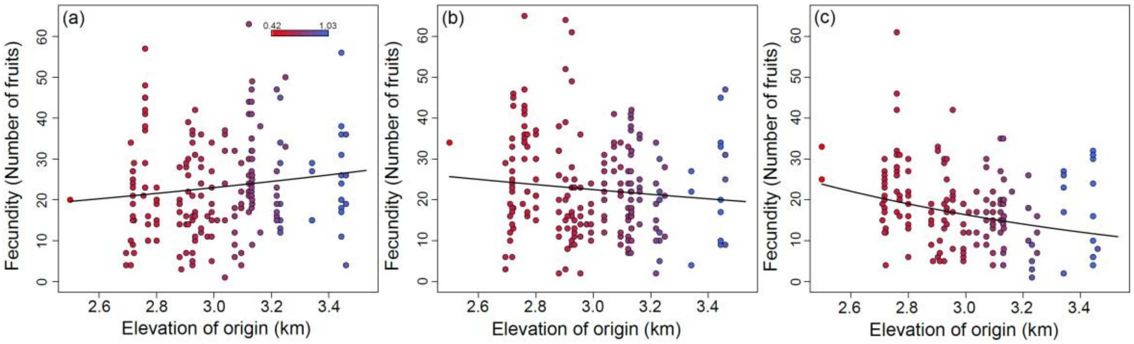
	Probability of reproduction			Fecundity (# fruits)		
	df	**F-value**	**p-value**	**df**	**F-value**	**p-value**
Nutrient treatment	1, 1134	10.42	0.001	1, 477	10.15	**0.002**
Drought treatment	2, 1134	2.98	0.051	2, 477	7.64	**0.001**
Nutrient × Drought	2, 1134	1.85	0.158	2, 477	1.15	0.318
Source elevation	1, 1134	0.81	0.369	1, 477	1.51	0.220
Source elevation × Nutrient treatment	1, 1134	6.20	**0.013**	1, 477	8.81	**0.003**
Source elevation × Drought treatment	2, 1134	3.00	0.050	2, 477	9.50	**<0.001**
Source elevation × Nutrient × Drought	2, 1134	1.96	0.141	2, 477	1.71	0.182
block	1	$\chi2$ =0.23	0.310	1	$\chi2$ =3.61	**0.029**
population	1	$\chi2$ =177.25	**<0.001**	1	$\chi2$ =41.77	**<0.001**



Appendix S5.2. Individual-level analyses of fitness trade-offs across nutrient levels were highly congruent with family-level analyses. No relationship between source elevation and the probability of reproduction emerged under low nutrient levels (panel a). However, in the higher nutrient treatment, the probability of reproduction increased with elevation of origin (panel b). Data points represent individual-level data.



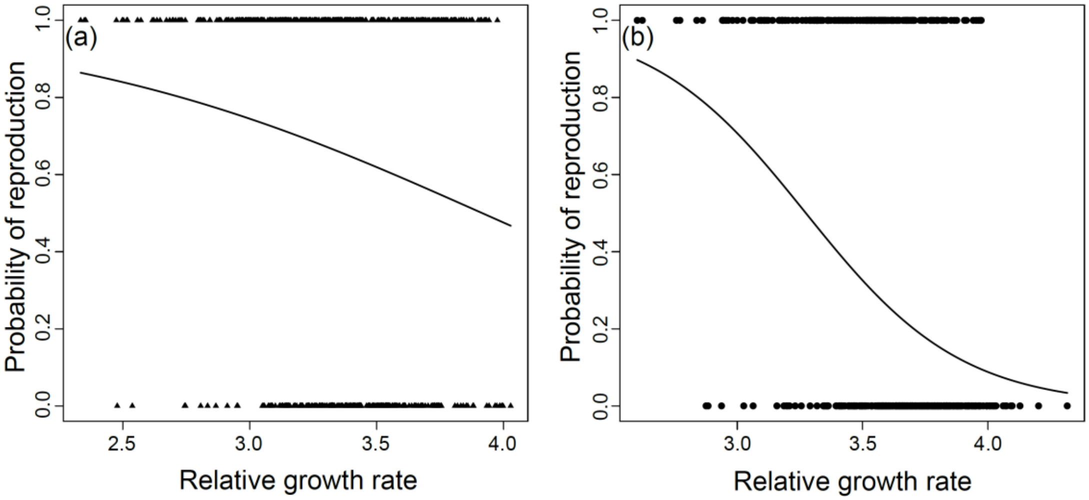
Appendix S6.2. As with the family-level analysis (Fig. 2), individual-level analyses of fecundity trade-offs across nutrient levels revealed no relationship between source elevation and fecundity (# of fruits among individuals that reproduced successfully) under low nutrient levels (panel a), and a decline in fecundity with source elevation (panel b). Data points represent individual-level data.



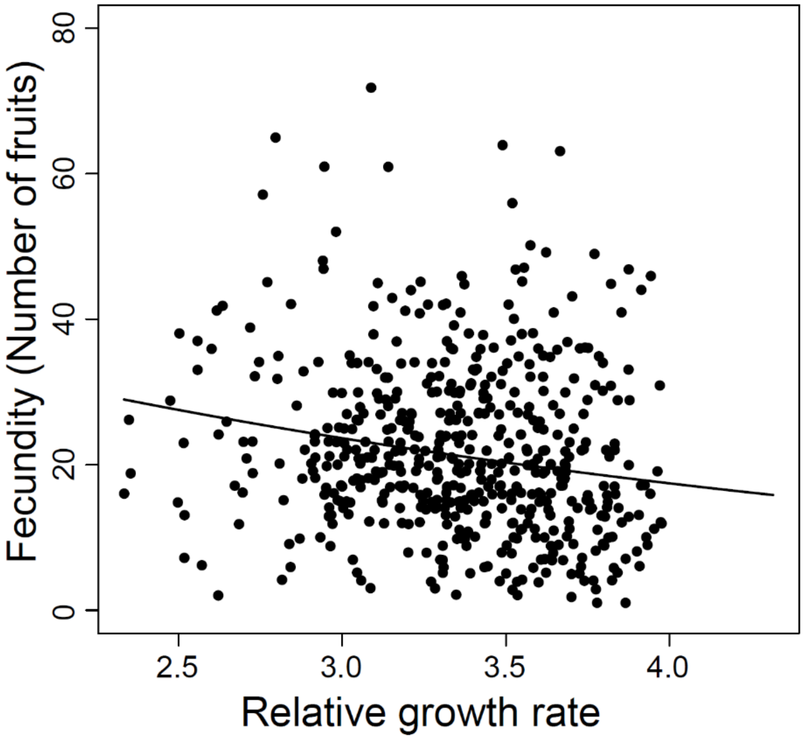
Appendix S7.2. Individual-level analyses of fecundity trade-offs across drought treatments. Under control well-watered conditions (panel a) and moderate drought stress (panel b), fecundity was not associated with source elevation. However, under severe drought (panel c), fecundity declined with increasing source elevation, as predicted. We color coded the data points to indicate the Aridity Index (Mean Annual Precipitations/Mean Annual Potential Evapotranspiration) of each population based on climatic data extracted from WorldClim, with arid low elevation populations indicated with red and mesic high elevation populations indicated with blue. Data points represent individual-level data.

Appendix S7. Phenotypic associations of fitness components varied with treatment in individual-level analyses. This table presents type III Sums of Squares from analyses evaluating the context dependent nature of phenotypic correlations, showing numerator and denominator degrees of freedom (df), F-values and p-values. We adjusted our alpha ($\alpha = 0.05/2 = 0.025$) to correct for multiple comparisons (analysis of 2 tradeoffs in one dataset). We highlight in bold significant p-values that fall below our adjusted α. We computed the significance of the random effect of population with a likelihood ratio test using a one-tailed χ^2 with one degree of freedom (Littell et al., 1996)

	Probability of reproduction vs. RGR			Fecundity vs. RGR		
	df	*F*	*P*	df	*F*	*P*
RGR	1, 998	36.12	**<0.001**	1, 458	13.88	**<0.001**
Drought	2, 998	0.01	0.988	2, 458	0.86	0.425
Nutrient	1, 998	6.19	**0.013**	1, 458	4.74	0.030
Drought × RGR	2, 998	0.04	0.960	2, 458	1.61	0.200
Nutrient × RGR	1, 998	9.22	**0.003**	1, 458	3.14	0.077
Drought × Nutrient	2, 998	0.46	0.630	2, 458	0.57	0.568
Drought × Nutrient × RGR	2, 998	0.48	0.620	2, 458	0.86	0.424
block	1	χ2 =2.07	0.075	1	χ2 =3.16	0.038
population	1	χ2 =153.5	**<0.001**	1	χ2 =34.02	**<0.001**



Appendix S9.2. Phenotypic correlations at the individual-level between the probability of reproduction and relative growth rate revealed a negative relationship between these two fitness components under low nutrient levels (panel a) and an even steeper negative association under higher nutrient levels (panel b). Data points represent individual-level data.



Appendix S10.2. Phenotypic correlations at the individual-level between fecundity (# of fruits among individuals that reproduced successfully) and relative growth rate revealed a trade-off between these two fitness components across all environments. Data points represent individual-level data.

Appendix 1.3. Number of plants used for each population x drought x nutrient combination to assess probability of viable seed. Unequal sample size reflects populations where less than 2 plants set seed. 250 total plants were used in the analysis.

	Severe Drought		Moderate Drought		Watered Daily	
Population	Low Nutrient	High Nutrient	Low Nutrient	High Nutrient	Low Nutrient	High Nutrient
47	2	1	2	2	2	2
50	2	1	2	2	2	2
67	NA	NA	2	2	2	NA
80	2	2	2	2	2	2
85	NA	NA	NA	NA	1	NA
86	2	1	2	2	2	1
91	2	NA	2	NA	2	1
98	1	NA	NA	NA	NA	NA
102	2	2	2	2	2	2
106	2	1	2	NA	NA	2
152	NA	NA	2	NA	1	NA
154	2	2	2	NA	2	2
161	2	1	2	2	2	2
176	2	1	2	2	2	2
180	2	1	2	1	1	1
206	NA	NA	NA	NA	NA	NA
207	NA	NA	NA	NA	NA	NA
209	2	NA	2	1	2	NA
210	1	NA	1	2	1	1
211	2	2	2	NA	2	2
212	2	2	2	NA	3	2
214	NA	NA	NA	NA	2	NA
250	2	1	2	2	2	2
252	2	2	2	2	2	2
253	1	NA	1	NA	3	1
254	NA	NA	NA	NA	NA	NA
255	NA	1	2	NA	NA	1
257	NA	NA	1	NA	1	NA
259	3	2	3	1	2	2
262	2	2	2	2	2	2
267	2	NA	1	NA	1	NA
265	NA	NA	NA	NA	NA	NA
269	2	2	2	3	2	2
273	2	NA	2	NA	2	NA
278	2	1	2	2	2	1
TOTAL	48	28	53	32	52	37

Appendix 2 a-f. Absolute fitness Genetic variance-covariance **(G)** matrix modeling phenological traits, and source elevation in the R package MCMCglmm.Values on the diagonal represent total genetic variances, and values on the off diagonals represent total genetic covariance between relevant traits. Estimates shown with 95% credible limit in parentheses. We highlight in bold the significant estimates, whose 95% credible intervals do not span 0. The genetic variance of source elevation is not biologically meaningful, but we retained source elevation in the model to evaluate potential genetic covariances with traits of interest.

a. Across all treatments

	Fitness	Flowering time	Flowering duration	Height at flowering	Leaf no. at flowering	Source elevation
Fitness	**2.589** **(0.538, 7.996)**					
Flowering time	-0.260 (-0.981, 0.357)	**0.331** **(0.102, 0.658)**				
Flowering duration	-0.048 (-0.819, 0.554)	-0.002 (-0.222, 0.226)	**0.364** **(0.093, 1.034)**			
Height at flowering	0.526 (-0.036, 1.060)	-0.021 (-0.200, 0.175)	-0.120 (-0.368, 0.103)	**0.789** **(0.572, 1.006)**		
Leaf no. at flowering	-0.294 (-0.929, 0.264)	0.112 (-0.060, 0.307)	0.039 (-0.191, 0.288)	**-0.238** **(-0.441, -0.027)**	**0.421** **(0.128, 0.868)**	
Source elevation	-0.036 (-0.086, 0.010)	-0.006 (-0.023, 0.011)	0.001 (-0.019, 0.016)	-0.015 (-0.038, 0.006)	0.015 (-0.002, 0.032)	0.063 (0.056, 0.071)

b. Watered daily · Low Nutrient

	Fitness	Flowering time	Flowering duration	Height at flowering	Leaf no. at flowering	Source elevation
Fitness	**3.678** **(0.742, 5.995)**					
Flowering time	-0.287 (-0.765, 0.157)	**0.184** **(0.037, 0.374)**				
Flowering duration	0.299 (-0.507, 1.095)	-0.052 (-0.287, 0.125)	**0.806** **(0.153, 1.407)**			
Height at flowering	-0.005 (-0.459, 0.409)	0.004 (-0.112, 0.128)	-0.174 (-0.405, 0.039)	**0.522** **(0.304, 0.731)**		
Leaf no. at flowering	0.247 (-0.280, 0.751)	0.001 (-0.135, 0.134)	-0.032 (-0.284, 0.219)	-0.087 (-0.247, 0.059)	**0.551** **(0.283, 0.788)**	
Source elevation	-0.075 (-0.253, 0.094)	-0.032 (-0.073, 0.013)	-0.014 (-0.096, 0.067)	-0.042 (-0.104, 0.026)	0.043 (-0.022, 0.108)	0.198 (0.149, 0.25)

c. Watered daily · High Nutrient

	Fitness	Flowering time	Flowering duration	Height at flowering	Leaf no. at flowering	Source elevation
Fitness	**17.217** **(10.03, 25.772)**					
Flowering time	**-4.494** **(-6.403, -2.653)**	**1.537** **(1.029, 2.142)**				
Flowering duration	**1.792** **(0.071, 3.404)**	**-0.565** **(-1.033, -0.009)**	**0.557** **(0, 1.423)**			
Height at flowering	-0.470 (-1.474, 0.550)	0.228 (-0.075, 0.541)	-0.032 (-0.352, 0.253)	**0.711** **(0.351, 1.056)**		
Leaf no. at flowering	1.154 (-0.338, 2.674)	-0.392 (-0.843, 0.064)	0.092 (-0.286, 0.523)	-0.300 (-0.630, 0.061)	**1.148** **(0.269, 2.066)**	
Source elevation	-0.102 (-0.524, 0.359)	-0.00029 (-0.135, 0.139)	0.014 (-0.071, 0.111)	**-0.095** **(-0.192, -0.005)**	**0.147** **(0.024, 0.279)**	0.194 (0.132, 0.259)

d. Moderate Drought · Low Nutrient

	fitness	Flowering time	Flowering duration	Height at flowering	Leaf no. at flowering	Source elevation
fitness	**3.935** **(2.244, 5.766)**					
Flowering time	**-0.318** **(-0.633, -0.016)**	**0.131** **(0.046, 0.218)**				
Flowering duration	0.346 (-0.27, 0.937)	-0.019 (-0.161, 0.105)	**0.740** **(0.213, 1.108)**			
Height at flowering	**0.488** **(0.133, 0.844)**	-0.065 (-0.154, 0.018)	-0.031 (-0.215, 0.131)	**0.641** **(0.448, 0.835)**		
Leaf no.at flowering	-0.294 (-0.719, 0.176)	0.076 (-0.016, 0.162)	-0.032 (-0.239, 0.17)	-0.098 (-0.243, 0.043)	**0.363** **(0.122, 0.655)**	
Source elevation	-0.123 (-0.285, 0.024)	-0.019 (-0.052, 0.013)	-0.012 (-0.08, 0.061)	-0.060 (-0.12, 0.001)	0.011 (-0.043, 0.063)	0.188 (0.142, 0.235)

e. Moderate Drought · High Nutrient

	Fitness	Flowering time	Flowering duration	Height at flowering	Leaf no. at flowering	Source elevation
Fitness	**10.013** **(6.015, 14.598)**					
Flowering time	-0.862 (-1.832, 0.127)	**0.612** **(0.056, 1.17)**				
Flowering duration	0.088 (-0.88, 1.214)	-0.034 (-0.497, 0.319)	**0.776** **(0.00001, 1.729)**			
Height at flowering	0.610 (-0.13, 1.433)	0.041 (-0.263, 0.344)	-0.185 (-0.585, 0.139)	**0.756** **(0.307, 1.226)**		
Leaf no. at flowering	-0.652 (-1.625, 0.293)	-0.03 (-0.39, 0.313)	0.167 (-0.246, 0.628)	**-0.408** **(-0.779, -0.06)**	**1.102** **(0.406, 1.817)**	
Source elevation	-0.112 (-0.447, 0.221)	-0.00016 (-0.09, 0.087)	0.010 (-0.099, 0.111)	**-0.111** **(-0.218, -0.017)**	0.066 (-0.054, 0.18)	0.191 (0.135, 0.252)

f. Severe drought · Low Nutrient

	Fitness	Flowering time	Flowering duration	Height at flowering	Leaf no. at flowering	Source elevation
Fitness	**5.765** **(0, 10.673)**					
Flowering time	-0.556 (-1.395, 0.376)	**0.462** **(0.165, 0.77)**				
Flowering duration	0.509 (-0.318, 1.396)	-0.074 (-0.300, 0.145)	**0.493** **(0.139, 0.817)**			
Height at flowering	0.297 (-0.334, 0.987)	-0.069 (-0.292, 0.113)	-0.065 (-0.269, 0.150)	**0.792** **(0.463, 1.135)**		
Leaf no. at flowering	-0.326 (-0.904, 0.119)	0.036 (-0.112, 0.189)	-0.028 (-0.197, 0.112)	-0.053 (-0.211, 0.111)	**0.171** **(0.011, 0.389)**	
Source elevation	-0.133 (-0.350, 0.078)	-0.025 (-0.085, 0.033)	0.020 (-0.041, 0.082)	-0.054 (-0.132, 0.02)	0.026 (-0.016, 0.068)	0.174 (0.130, 0.228)

g. Severe drought · High Nutrient

	Fitness	Flowering time	Flowering duration	Height at flowering	Leaf number at flowering	Source elevation
Fitness	**21.941** **(10.940, 34.84)**					
Flowering time	**-5.321** **(-8.249, -2.787)**	**1.946** **(1.130, 2.834)**				
Flowering duration	**2.430** **(0.767, 4.203)**	**-0.654** **(-1.221, -0.187)**	**0.683** **(0.111, 1.342)**			
Height at flowering	-0.479 (-1.775, 0.747)	0.208 (-0.219, 0.571)	-0.063 (-0.334, 0.245)	**0.566** **(0.063, 1.055)**		
Leaf number at flowering	0.054 (-1.449, 1.418)	-0.204 (-0.684, 0.297)	0.021 (-0.303, 0.322)	-0.129 (-0.418, 0.129)	**0.696** **(0.075, 1.194)**	
Source elevation	-0.153 (-0.806, 0.518)	0.029 (-0.158, 0.216)	-0.001 (-0.125, 0.114)	**-0.106** **(-0.221, -0.002)**	0.081 (-0.035, 0.202)	0.231 (0.148, 0.321)

Appendix 3. Relative fitness genetic variance-covariance (G) matrix modeling for relative fitness, phenological traits, and source elevation in the R package MCMCglmm. Values on the diagonal represent the total genetic variances, and values on the off diagonals represent the total genetic covariance between the relevant traits. We show the estimate with the 95% credible limit in parentheses. We highlight in bold the significant estimates, whose 95% credible intervals do not span 0. The genetic variance of source elevation is not biologically meaningful, but we retained source elevation in the model to evaluate potential genetic covariances with traits of interest.

a. Across all treatments

	Fitness	Flowering time	Flowering duration	Height at flowering	Leaf no. at flowering	Source elevation
Fitness	**0.416** **(0.113, 0.954)**					
Flowering time	-0.113 (-0.411, 0.099)	**0.355** **(0.098, 0.771)**				
Flowering duration	0.039 (-0.179, 0.364)	0.001 (-0.265, 0.266)	**0.377** **(0.072, 1.048)**			
Height at flowering	0.215 (-0.01, 0.426)	-0.017 (-0.227, 0.21)	-0.106 (-0.336, 0.136)	**0.7999** **(0.544, 1.014)**		
Leaf no. at flowering	-0.150 (-0.397, 0.047)	0.120 (-0.055, 0.355)	0.029 (-0.217, 0.291)	**-0.230** **(-0.435, -0.027)**	**0.431** **(0.129, 0.901)**	
Source elevation	**-0.019** **(-0.037, -0.002)**	-0.006 (-0.022, 0.011)	0 (-0.019, 0.017)	-0.015 (-0.035, 0.01)	0.014 (-0.004, 0.032)	0.062 (0.054, 0.07)

b. Watered daily · Low Nutrient

	Fitness	Flowering time	Flowering duration	Height at flowering	Leaf no. at flowering	Source elevation
Fitness	**0.533** **(0.171, 0.922)**					
Flowering time	-0.063 (-0.228, 0.092)	**0.171** **(0.023, 0.351)**				
Flowering duration	0.053 (-0.259, 0.342)	-0.045 (-0.265, 0.15)	**0.875** **(0.229, 1.471)**			
Height at flowering	0.147 (-0.028, 0.337)	-0.002 (-0.122, 0.123)	-0.173 (-0.416, 0.031)	**0.535** **(0.343, 0.751)**		
Leaf no. at flowering	0.019 (-0.183, 0.214)	0.019 (-0.113, 0.143)	-0.028 (-0.244, 0.207)	-0.085 (-0.233, 0.048)	**0.537** **(0.273, 0.759)**	
Source elevation	-0.041 (-0.108, 0.028)	-0.032 (-0.071, 0.014)	-0.013 (-0.095, 0.072)	-0.043 (-0.112, 0.019)	0.044 (-0.023, 0.108)	0.200 (0.148, 0.251)

c. Watered daily · High Nutrient

	Fitness	Flowering time	Flowering duration	Height at flowering	Leaf no. at flowering	Source elevation
Fitness	**0.743** **(0.212, 1.182)**					
Flowering time	-0.406 (-0.838, 0.029)	**1.317** **(0.687, 2.018)**				
Flowering duration	0.130 (-0.198, 0.632)	-0.327 (-0.893, 0.157)	**0.648** **(0, 1.708)**			
Height at flowering	0.173 (-0.088, 0.427)	0.138 (-0.196, 0.48)	0.012 (-0.348, 0.309)	**0.669** **(0.329, 1.044)**		
Leaf no. at flowering	0.133 (-0.284, 0.566)	-0.285 (-0.815, 0.271)	-0.025 (-0.532, 0.458)	-0.235 (-0.561, 0.112)	**1.224** **(0.256, 2.109)**	
Source elevation	-0.054 (-0.145, 0.046)	0.001 (-0.116, 0.133)	0.017 (-0.089, 0.118)	**-0.094** **(-0.193, -0.014)**	**0.145** **(0.005, 0.282)**	0.196 (0.13, 0.266)

d. Moderate Drought · Low Nutrient

	Fitness	Flowering time	Flowering duration	Height at flowering	Leaf no. at flowering	Source elevation
Fitness	**0.512** **(0.241, 0.741)**					
Flowering time	-0.086 (-0.182, 0.008)	**0.115** **(0.038, 0.203)**				
Flowering duration	0.063 (-0.150, 0.254)	0.0001 (-0.119, 0.115)	**0.739** **(0.317, 1.119)**			
Height at flowering	**0.213** **(0.066, 0.354)**	-0.056 (-0.144, 0.021)	-0.038 (-0.203, 0.139)	**0.641** **(0.459, 0.831)**		
Leaf no. at flowering	-0.054 (-0.224, 0.115)	0.065 (-0.028, 0.155)	-0.035 (-0.234, 0.199)	-0.093 (-0.25, 0.05)	**0.412** **(0.128, 0.717)**	
Source elevation	**-0.063** **(-0.121, -0.005)**	-0.018 (-0.048, 0.013)	-0.012 (-0.086, 0.053)	-0.059 (-0.122, 0.006)	0.009 (-0.040, 0.067)	**0.188** **(0.144, 0.238)**

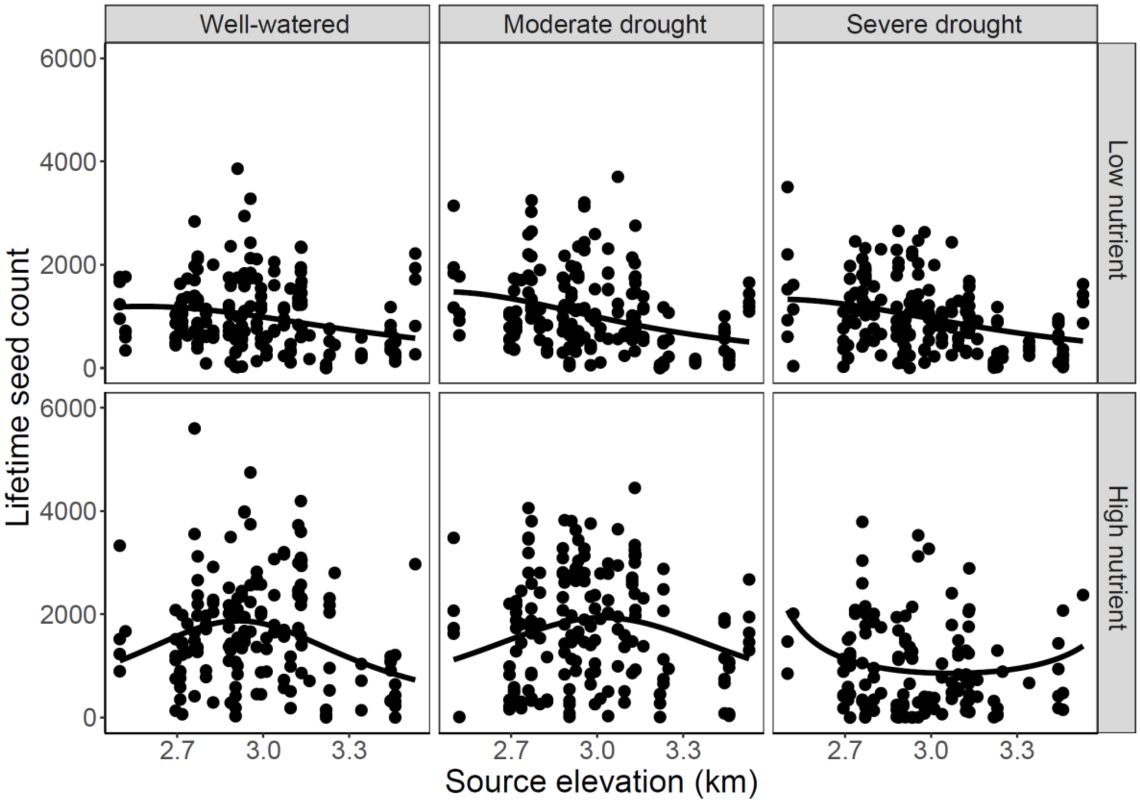
e. Moderate Drought · High Nutrient

	Fitness	Flowering time	Flowering duration	Height at flowering	Leaf no. at flowering	Source elevation
Fitness	**0.707** **(0.297, 1.080)**					
Flowering time	-0.117 (-0.381, 0.142)	**0.564** **(0, 1.111)**				
Flowering duration	-0.135 (-0.453, 0.208)	-0.013 (-0.433, 0.297)	**0.822** **(0, 1.713)**			
Height at flowering	**0.278** **(0.033, 0.562)**	0.056 (-0.241, 0.36)	-0.209 (-0.585, 0.104)	**0.825** **(0.402, 1.231)**		
Leaf no. at flowering	-0.204 (-0.496, 0.083)	-0.040 (-0.387, 0.285)	0.199 (-0.22, 0.645)	**-0.413** **(-0.795, -0.083)**	**1.171** **(0.454, 1.842)**	
Source elevation	-0.040 (-0.134, 0.046)	-0.002 (-0.088, 0.083)	0.010 (-0.089, 0.119)	**-0.111** **(-0.205, -0.011)**	0.067 (-0.044, 0.186)	0.192 (0.130, 0.254)

Appendix 4.3. Local adaptation using hurdle models, glmmTMB, to analyze lifetime fitness across three growing seasons as a function of linear and quadratic effects of source elevation, drought treatment, nutrient treatment, and all 2- and 3-way interactions. This approach first evaluates the probability of reproduction and then fecundity amongst individuals that successfully set seeds. We assessed the significance of random effects through likelihood ratio tests contrasting models with and without each effect. Highlighted in bold are p-values significant at $\alpha=0.05$.

Treatment	**Probability of reproduction**			**Fecundity**[*]		
	χ^2	df	p-value	χ^2	df	p-value
Source elevation (b)	0.18	1	0.6700	4.27	1	0.0390
Drought	10.46	2	0.0100	25.47	2	**<0.0001**
Nutrient	25.31	1	**<0.0001**	53.6	1	**<0.0001**
Source elevation (γ)	4.40	1	**0.0360**	0.12	1	0.7300
Source elevation (b) × Drought	1.78	2	0.4100	0.58	2	0.7500
Source elevation (b) × Nutrient	0.11	1	0.7400	12.09	1	**0.0005**
Drought × Nutrient	4.97	2	0.0800	20.37	2	**<0.0001**
Source elevation (γ) × Drought	0.92	2	0.6300	5.25	2	0.0700
Source elevation (γ) × Nutrient	4.67	1	**0.0307**	0.18	1	0.6700
Source elevation (b) × Drought × Nutrient	0.35	2	0.8400	2.86	2	0.2400
Source elevation (γ) × Drought × Nutrient	0.87	2	0.6500	8.39	2	**0.0151**
Random effects						
Block	2.66	1	0.1000	0.00	1	1.0000
Genotype	85.83	1	**<0.0001**	**250.99**	1	**<0.0001**

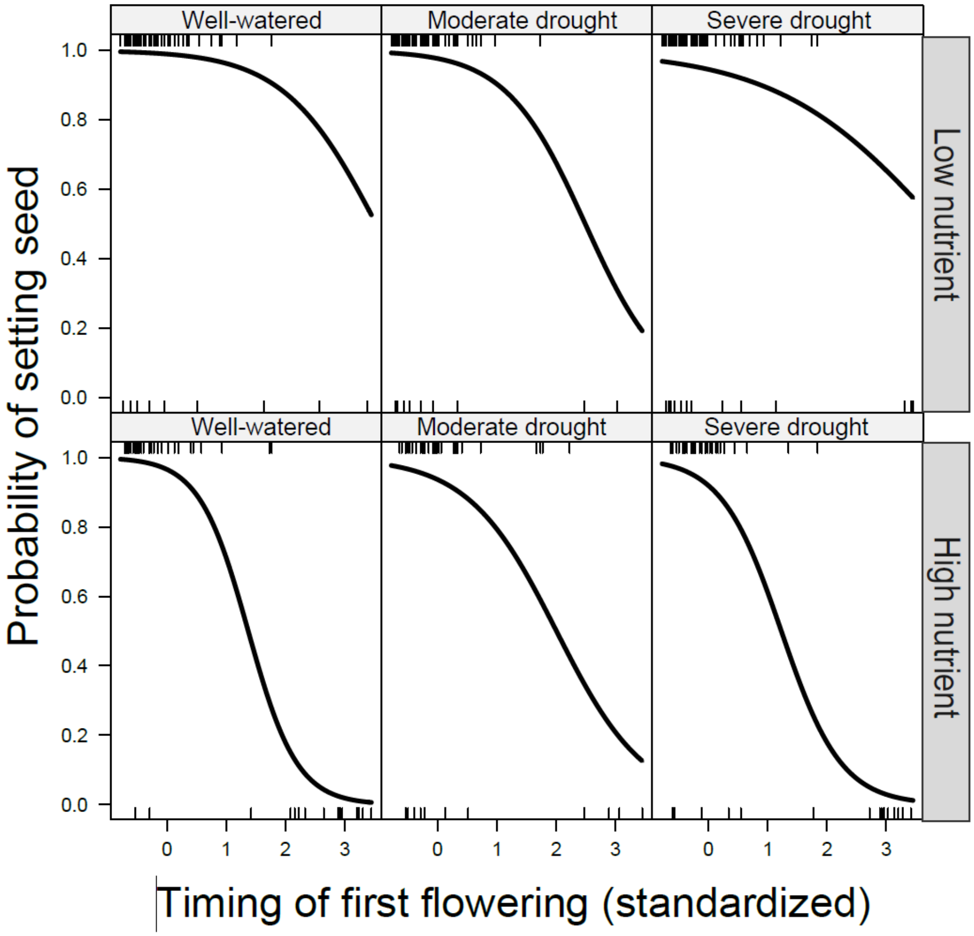
[*] Fecundity analyzed as seed set of individuals that successfully set seed.



Appendix 5.3. Lifetime seed count regressed as a function of source elevation. A decline in fecundity with source elevation detected across all drought levels in the low nutrient treatment. Negative quadratic curvature under well-watered conditions and moderate drought stress in the high nutrient treatment. Slight positive quadratic curve in the severe drought stress and high nutrient combination.

Appendix 6.3. Probability of seed viability as a function of source elevation, drought treatment, and nutrient treatment on a subset of seeds collected from the experiment. The data were analyzed using a mixed effects logistic regression. We assessed the significance of random effects through likelihood ratio tests contrasting models with and without each effect. Highlighted in bold are p-values significant at α=0.05.

Treatment	**Probability of viable seed**		
	χ^2	df	p-value
Source elevation	1.66	1	0.2
Drought	20.35	2	**<0.0001**
Nutrient	2.8	1	0.095
Source elevation × Drought	18.28	2	**0.0001072**
Source elevation × Nutrient	2.42	1	0.12
Drought × Nutrient	21.29	2	**<0.0001**
Source elevation × Drought × Nutrient	23.09	2	**<0.0001**
Random effects			
Block	60.13	1	**<0.0001**
Genotype	377.20	1	**<0.0001**

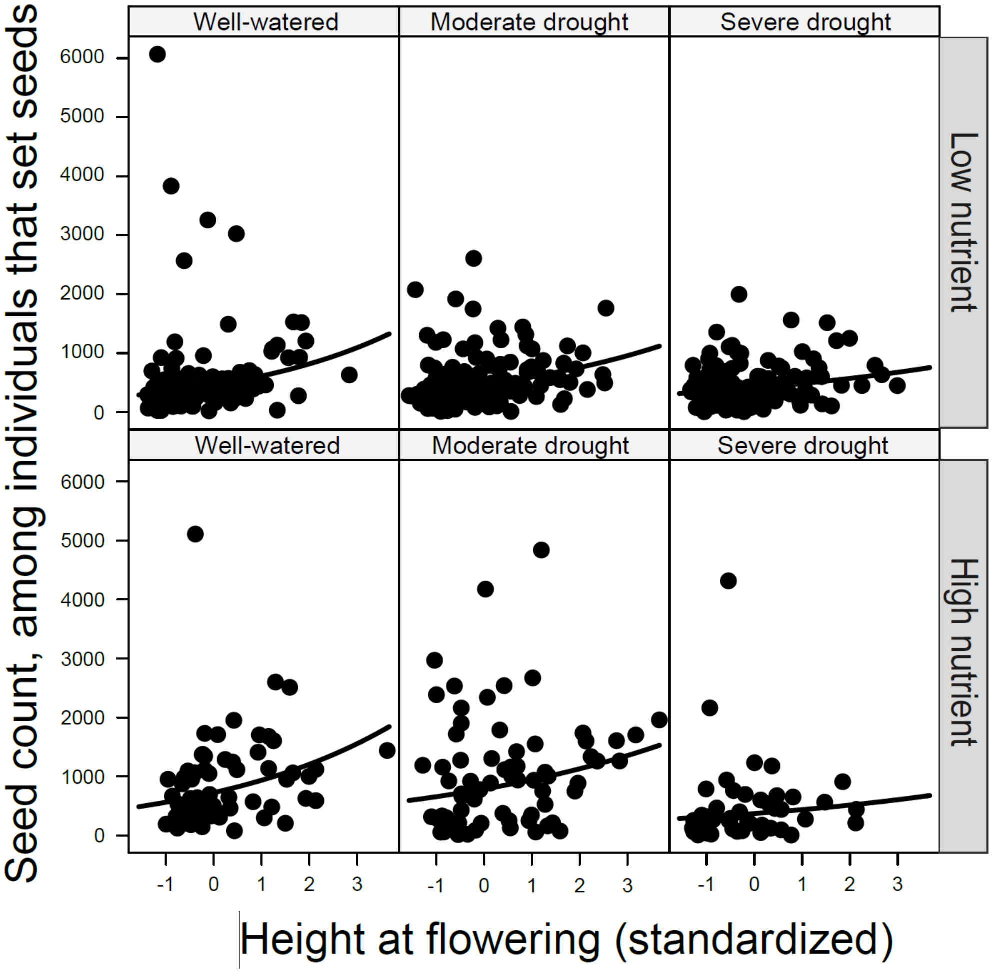


Appendix 7. Selection favored early flowering in all treatment combinations in a logistic regression modeling the probability of setting seeds among all individuals that flowered. Tick marks show individual-level data on the timing of flowering and success at setting seed.

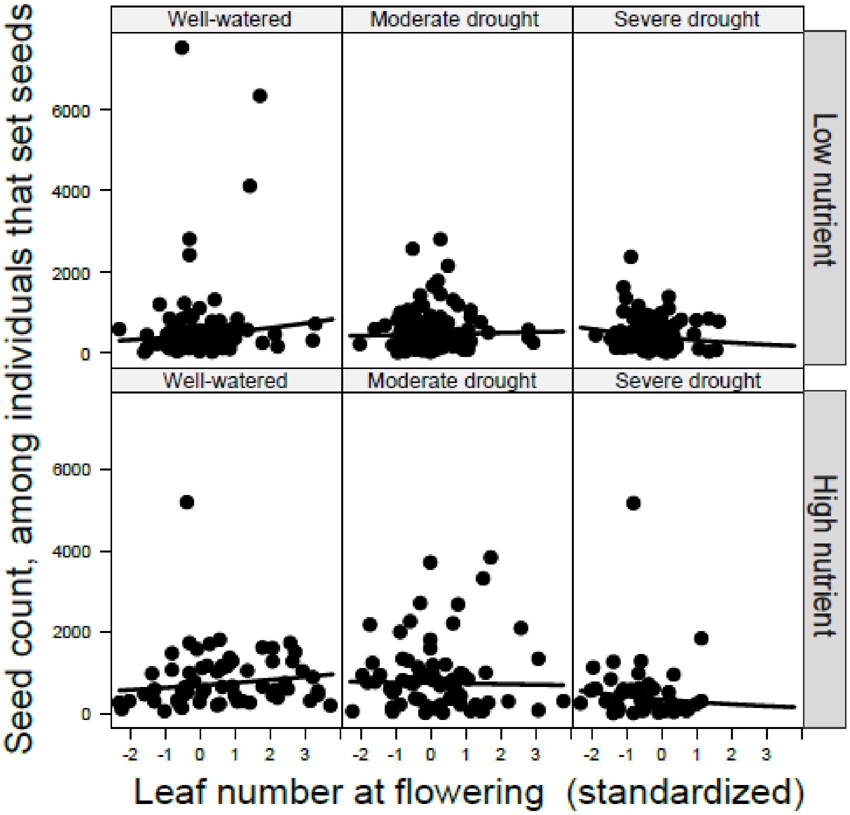
Appendix 8.3. Phenotypic selection evaluated with a hurdle model in glmmTMB measured during the first growing season of this study. This approach first evaluates the probability of reproduction and then fecundity amongst individuals that successfully set seeds. We assessed the significance of random effects through likelihood ratio tests contrasting models with and without each effect. Highlighted in bold are p-values significant at α=0.05.

[*] Fecundity analyzed as seed set of individuals that successfully set seed.

Treatment	**Probability of reproduction**			**Fecundity[*]**		
	χ^2	df	p-value	χ^2	df	p-value
Source elevation	3.24	1	0.072	6.04	1	0.0140
Drought	2.31	2	0.316	11.07	2	0.00395
Nutrient	2.24	1	0.135	10.66	1	0.00109
Flowering time	5.74	1	**0.017**	4.21	1	0.04012
Flowering duration	6.52	1	**0.011**	0.04	1	0.8300
Height	3.38	1	0.066	18.25	1	**<0.0001**
Leaf number	1.26	1	0.261	0.31	1	0.5800
Drought × Nutrient	0.95	2	0.622	8.74	2	**0.01264**
Drought × Flowering Time	1.44	2	0.486	0.44	2	0.8000
Nutrient × Flowering time	0.05	1	0.830	1.21	1	0.2700
Drought × Flowering duration	1.60	2	0.450	0.06	2	0.9700
Nutrient × Flowering duration	4.35	1	**0.037**	0	1	0.9500
Drought × Height	1.38	2	0.501	1.30	2	0.5200
Nutrient × Height	0.69	1	0.405	0.18	1	0.6700
Drought × Leaf number	5.06	2	0.080	9.74	2	**0.00766**
Nutrient × Leaf number	0.15	1	0.702	0.44	1	0.5100
Drought × Nutrient × Flowering time	2.10	2	0.351	1.18	2	0.5500
Drought × Nutrient × Flowering duration	4.94	2	0.084	2.91	2	0.2300
Drought × Nutrient × Height	1.83	2	0.401	0.07	2	0.9700
Drought × Nutrient × Leaf number	0.79	2	0.673	0.16	2	0.9200
Random effects						
Block	0.34	1	0.56	4.367	1	0.037
Genotype	142.24	1	**<0.0001**	1.36	1	0.24



Appendix 9: Fecundity selection favored increase height at flowering across all treatments.



Appendix 10: Fecundity selection favored more leaves at flowering in well-watered conditions and fewer leaves at flowering in severe drought conditions.

Appendix 11.3. Genotypic selection analysis of total fitness as a function of phenological traits, source elevation, drought treatment and nutrient treatment, and their interactions in a negative binomial regression. We assessed the significance of the random effect through likelihood ratio tests contrasting models with and without genotype. Highlighted in bold are p-values significant at α=0.05.

Treatment	**Total fitness[*]**		
	χ^2	df	p-value
Drought	4.504	2	0.105
Nutrient	1.909	1	0.167
Source elevation	0.750	1	0.386
Flowering time	0.193	1	0.661
Flowering duration	0.142	1	0.706
Height at flowering	2.861	1	0.091
Leaf number at flowering	0.104	1	0.747
Drought × Nutrient	4.976	2	0.083
Drought × Source elevation	0.337	2	0.845
Nutrient × Source elevation	0.540	1	0.462
Drought × Flowering Time	0.597	2	0.742
Nutrient × Flowering time	0.678	1	0.410
Drought × Flowering duration	0.847	2	0.655
Nutrient × Flowering duration	0.800	1	0.371
Drought × Height	0.546	2	0.761
Nutrient × Height	0.535	1	0.464
Drought × Leaf number	1.368	2	0.505
Nutrient × Leaf number	0.551	1	0.458
Drought × Nutrient × Source elevation	4.379	2	0.112
Drought × Nutrient × Flowering time	1.183	2	0.554
Drought × Nutrient × Flowering duration	9.713	2	**0.0078**
Drought × Nutrient × Height	8.927	2	**0.0115**
Drought × Nutrient × Leaf number	1.897	2	0.387
Random effect			
Genotype	38.90	1	**<0.0001**

[*] Total fitness assessed as probability of reproduction × seed production.