UNIVERSITY OF CALIFORNIA, MERCED

Phenotypic responses of a Sierra Nevada monkeyflower to climate variation and severe drought

A Thesis submitted in partial satisfaction of the requirements for the degree
Master of Science

in

Environmental Systems

by

Erin Elise Dickman

Committee in charge:
Professor Jessica L. Blois, Chair
Professor Steven J. Franks
Professor Jason P. Sexton

2016

The Thesis of Erin Elise Dickman is approved, and is acceptable in quality and form for publication on microfilm and electronically:

_____

Steven J. Franks, Ph.D

_____

Jason P. Sexton, Ph.D

_____

Jessica L. Blois, Ph.D
Committee Chair

University of California, Merced
2016

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEDMENTS

I would like to thank University of California, Davis undergraduate assistants Christopher Chen and Leticia Macias for many long hours of measuring and monitoring plants in the greenhouse. University of California, Merced undergraduate assistants Amanda Tse, Angelo Aragon, Alfredo Enriquez, William Higson, Yazmin Lomell, Sherman Yu, Jenna Heckel, and Tyler Rackelmann deserve recognition for their extensive help with processing samples in the lab. University of California, Davis greenhouse manager Ian Baker helped with greenhouse logistics. Graduate student Daniel Towes and lab manager Elizabeth Green assisted me in the lab and the field and provided constant encouragement. Dr. Molly Stevens and Dr. Emily Moran provided feedback on my experimental design and Molly additionally provided insightful edits on my drafts. Dr. Susan Mazer, Dr. Megan L. Peterson, Lorenzo Booth, and Joseph Crockett offered helpful suggestions in statistical data analysis. My committee members Dr. Jessica L. Blois and Dr. Steven J. Franks provided technical expertise and vital guidance. Special thanks go to Dr. Jason P. Sexton. He was an excellent adviser and enthusiastically coached me at every step in this process. His boundless positivity and creativity was crucial and I greatly appreciate his exemplary mentorship. Finally, completing this thesis would not have been possible without the unflagging support of my husband Garrett J. Dickman. He helped me extensively in the lab, field, and with feedback on my writing, and assured me that achieving this goal was possible.

This work would not have been possible without financial support of grants from the University of California Merced Natural Reserve System and the Valentine Eastern Sierra Reserve. Yosemite National Park, Sierra National Forest, and Rick Zappey provided access to these populations.

ABSTRACT

Title: Phenotypic responses of a Sierra Nevada monkeyflower to climate variation and severe drought
Student: Erin Elise Dickman
Degree: Master of Science, Environmental Systems
School: University of California, Merced 2016
Committee Chair: Professor Jessica L. Blois

As climatic conditions change, species will be forced to move or adapt to avoid extinction. Exacerbated by ongoing climate change, California recently experienced an exceptional drought from 2012-2014. To investigate whether an adaptive response occurred to this event, I conducted a "resurrection" study of the cutleaf monkeyflower (*Mimulus laciniatus*), an annual plant, by comparing trait responses of ancestral seed collections ("pre-drought") with contemporary descendant collections ("drought"). Plants were grown under common conditions to test whether this geographically-restricted species has capacity to respond to climate stress across its species range. My research examined if traits shifted in predicted ways in response to recent, severe drought and if the responses varied by climate region. I found days to emergence (i.e. seedling emergence from soil) in the drought generation were significantly fewer as compared to the pre-drought generation. Additionally, trait variation in days to emergence was reduced in the drought generation, which may suggest that a selective event occurred. Days to first flower differed significantly by region and increased with elevation, suggesting climate adaptation across the species range. The drought generation plants were larger and had greater reproduction as compared to the pre-drought generation, which may be attributed to earlier germination of these populations in the greenhouse. My results demonstrate that rapid shifts in trait means are possible within populations, including peripheral populations of a plant species with a relatively restricted range, consistent with climate adaptation. This study highlights the need for better understanding of rapid adaptation as a means for plant communities to withstand climate change.

INTRODUCTION

Global climate change presents a serious and immediate threat to ecosystem structure and function (Sala et al. 2000, Loarie et al. 2009). While evidence exists that humans have had an impact on natural ecosystems since the early Holocene (Lyons et al. 2016), the current rates of climate change are unprecedented (Diffenbaugh and Field 2013). Under changing climates, species will be forced to move or adapt to avoid extinction, with some studies already documenting climate-driven declines in biodiversity (Loarie et al. 2009, Harrison et al. 2015).

Plant responses to climatic change, such as range shifts (Walther et al. 2002, Parmesan and Yohe 2003, Root et al. 2003, Kopp and Cleland 2014, Wolf et al. 2016) and adaptation (Hairston et al. 1999, Parmesan 2006, Franks et al. 2007, Franks 2011, Sultan et al. 2013), can be rapid. However, little is known about how climate change affects populations across their range, especially at their low and high elevation range limits. In particular, the extremes of a species range (i.e. elevation, latitude) are important to understand as they are where range expansion or contraction may occur (Hampe and Petit 2005). The lowest elevation populations, referred to as the “rear edge,” may face the warmest and driest conditions. These populations may exhibit local extirpation (i.e., "trailing edge") and may be disproportionally affected by climate change, resulting in range contraction (Hampe and Petit 2005, Bridle and Vines 2007, Aitken et al. 2008, Sexton et al. 2011, Bertrand et al. 2011). Others have argued that high elevation populations, referred to as the “leading edge,” are highly vulnerable to rapid climate changes (Wookey et al. 2009) due to competitive pressure (Wolf et al. 2016), and amplified warming at high elevation regions (Wang et al. 2013). This warming effect may facilitate upslope range expansion (Hampe and Petit 2005, Aitken et al. 2008, Bertrand et al. 2011, Kopp and Cleland 2014) but is accompanied by a reduction in available habitat (i.e., surface area) (Pauli et al. 2003). Alternatively, some have expressed uncertainty that range position dictates plant community response to climate change (Bertrand et al. 2011, Rangwala and Miller 2012, Bjorkman et al. 2016) or stated that species responses to climate change will differ by individual species, and can include downslope range shifts (Rapacciuolo et al. 2014). Range-restricted or endemic species may be particularly vulnerable as we know that species with small ranges are at higher risk of extinction (Pimm and Raven 2000, Parmesan 2006, Dirnböck et al. 2011).

Vulnerability to climate shifts is related to the amount of genetic variation present in a population, upon which natural selection can act. Populations at species range limits may be smaller in size and lack sufficient genetic variation to respond to changing climates (Kirkpatrick and Barton 1997, Holt et al. 2003, Dawson et al. 2010). In contrast, populations at species range limits may have substantial genetic variation (Holt and Gomulkiewicz 1997, Sexton et al. 2011), and may already have some degree of local climate adaptation that could provide critical genetic variation to other populations within

the species' range (Holt and Gomulkiewicz 1997, Hampe and Petit 2005, Sexton et al. 2011). Other studies have shown that climate-driven population dynamics, rather than genetic differentiation between populations, is a leading factor shaping plant population structure and species range limits (Hampe and Petit 2005, Sexton et al. 2016).

A critical measure of species' responses to climate stress is timing their developmental stages to maximize limited resources and increase their chance of survival to reproduce (Cleland et al. 2007). Phenology studies are increasingly being recognized as a way to elucidate the effects of climate change on populations (Parmesan and Yohe 2003, Root et al. 2003, Menzel et al. 2006, Cleland et al. 2007, Hudson and Keatley 2009, Anderson et al. 2012). Selection for faster development and/or earlier flowering due to elevated CO_2 (Springer and Ward 2007), dry soil (Ivey and Carr 2012), and reduction in precipitation (Franks et al. 2007) has been documented in some systems and can facilitate drought escape in shortened growing seasons. Changes in phenology offer organisms a way to adapt to the stress of climate change, however these changes may be asynchronous with the surrounding ecosystem and maintenance of biodiversity (Hudson and Keatley 2009). Critical photoperiod is the primary control over phenology in temperate climates, with temperature as a secondary moderating effect (Körner and Basler 2010). Photoperiod is not affected by climate, and as snowpack declines and peak runoff dates shift earlier in the growing season there could be a mismatch between germination cues and resource availability, leading to reduced fitness (Anderson et al. 2012). Warmer climates have resulted in reductions in insulating snowpack and increased incidence of frost damage leading to reduced abundance of plants that flower, reduced flowers per individual, and increased incidence of flower bud abortion (Inouye et al. 2002, Saavedra et al. 2003). In this vein, reducing sensitivity to photoperiod can be adaptive with changes to the climate (Franks and Hoffmann 2012).

A relatively new technique, called the "resurrection" approach has emerged to document trait shifts (e.g. phenology), due to contemporary evolution (Hairston et al. 1999, Franks et al. 2007, 2008, Sultan et al. 2013). This approach takes ancestral and descendent propagules collected from a population and raises them in a common environment. Differences in phenotype between ancestors and descendants provide evidence of evolutionary change that has taken place in the interval between the two collections. An advantage of this approach is that it distinguishes between phenotypic plasticity and evolution (Etterson et al. 2016). Resurrection studies have helped to document the phenomena of rapid adaptation as a means to persist under climate change.

Exacerbated by the global trend of hotter and drier climates, California recently experienced an exceptional drought beginning in 2012 and containing the driest 12-month period on record between 2013-2014 (National Climate Data Center, Swain et al. 2014). Not only did this drought exceeded the historic record, but the drought in 2014 has an estimated return interval of 700-900 years, and the cumulative drought of 2012-2014 has an estimated return interval of over 1200 years (Robeson 2015).

The California Sierra Nevada is home to a great diversity of endemic species living along its steep elevational gradients. To investigate the effect of the recent, severe drought on the adaptive response of plants across their species range, I conducted a resurrection study of the Sierra endemic cut-leaf monkeyflower, *Mimulus laciniatus*.

My research examined two questions:
1) Have traits shifted in predicted ways in response to the recent, severe drought?; and
2) If there are trait shifts, do responses vary by climate region?

I compared phenological and morphological responses of ancestor and descendant seed collections, collected at two separate years at the same populations. I grew seeds from nine populations across the species range, including its elevational extremes representing the leading and rear edge regions, from ancestors (hereafter referred to as "pre-drought generation"), collected in years with typical precipitation in 2008 or earlier, and descendants (hereafter referred to as "drought generation"), collected in an exceptional drought year, 2014. These plants were grown in a greenhouse under common conditions.

A discovery that these populations are able to rapidly shift their traits to a phenotype that may be favored by hotter and drier conditions would be an important contribution to our understanding of climate adaptation. Conversely, finding that traits do not differ within this time period would lend credibility to concerns that plants will not be able to adequately track climate change (Loarie et al. 2009), or perhaps that the recent drought did not exert a strong selective pressure on *M. laciniatus*. We know that climate change is a driver of evolution (Etterson et al. 2016), and determining whether populations at low and high elevation range limits are equally able as intermediate populations to evolve rapidly would be a novel and important contribution to the science of species range limits. With modern climate change, understanding adaptive responses, across a species range, is crucial to the goal of conserving biodiversity and ecosystem function.

STUDY SYSTEM AND METHODS

Study system

Mimulus laciniatus (A. Gray) is an annual herbaceous plant endemic to the western slope of the central Sierra Nevada, California, and limited in its distribution due to its habitat requirements (Sexton and Dickman 2016). It primarily inhabits snowmelt seeps and moss patches on granite outcrops between ca. 900 – 3,270 m, many of which progressively dry during the growing season (Figure 1). *M. laciniatus* spans several biotic zones in the Sierra Nevada, including the foothill woodland, the montane mixed-conifer, and the subalpine and alpine communities (Gray 2013). It is a winter annual that germinates during the late fall and winter rains characteristic of its Mediterranean climate.

It develops a small basal rosette of leaves through the winter, flowers during the spring or early summer and senesces in the dry late spring or summer depending on elevation (Cowling et al. 1996). It gets its name from its highly lobed and dissected leaves presumed to be adapted to hot and exposed environments (Ferris et al. 2014, 2015). It is primarily self-pollinating (95%; Ferris et al. 2014), though it can be visited by bees and other insects (Sexton et al. 2011). *Since M. laciniatus* is largely self-pollinating, maternal and epigenetic effects may be important components of its adaptive response (e.g., coping with environmental stress) (Germain et al. 2013).

We collected seeds from nine *M. laciniatus* populations at two points in time, which I refer to as the pre-drought generation and drought generation, respectively (Table 1). The pre-drought generation seeds were collected in 2006 for all populations with the exception of Hwy 168 (L2) and Hetchy Sign (I2), which were collected in 2005, and Jackass Meadow (I3), which was collected in 2008. The drought generation seeds were collected in 2014 for all populations. The nine localities, within three climate regions (three, low elevation-edge; three intermediate; and three, high elevation-edge), span elevations from the lowest at 947 m to the highest at 3095 m and represent the entirety of the species elevational range. Additionally, two populations are located at the low-elevation species range edge (L1, L2) and two are located at the high-elevation species range edge (H2, H3), which represent the rear edge and leading edge portions of the species range, respectively. These populations are located within Yosemite National Park, Sierra National Forest and private property (Figure 2).

Greenhouse Experiment

To assess seed viability I conducted cut tests of seeds from 30 randomly drawn maternal families from pre-drought and drought generations (Ooi et al. 2004). All generations' seeds were examined under a dissecting microscope and appeared to have a normal endosperm and a live embryo, which indicates viability (Bonner and Russell 1974, Baskin and Baskin 2014).

I planted field-collected seeds from 30 maternal families per site, from nine populations (three, low elevation-edge; three intermediate; and three, high elevation-edge) with replicates for pre-drought and drought generations. As the drought generation experienced an extreme climate and had low seed yield, there were two populations, May Lake (H1) and Mammoth Edge (H2), from which I could not derive 30 maternal families. I planted 12 maternal families for H1 and 14 maternal families for H2 for a total of 510 maternal families for the experiment. For one site, H2, no field-collected seed were available in the pre-drought generation so I planted seed that had been self-pollinated for one generation after field collection.

Seeds were randomly sown into Sunshine Mix potting soil (Greenhouse Megastore, Danville, Illinois, USA) in eight trays with 72-cell, black, plastic planters using a randomized block design. Ten seeds from each maternal family were chosen at random and were sown into a cell, except where seed collections did not contain ten

seeds and fewer were planted. If multiple seedlings emerged, seedlings were thinned until only one maternal family was represented per cell.

After sowing, I added 1cm of sand mulch to the top of each cell, filled the tray bottom with water, covered the tray with a black plastic lid, and placed trays in a 4 °C vernalization cabinet for 11 days (Friedman and Willis 2013) at University of California, Davis (UCD) Controlled Environment Facility.

After vernalization, I moved trays to the UCD greenhouse on February 17, 2015. Plants received natural light and moderate ambient temperatures between 18.5-30.1 °C. Trays were filled with reverse osmosis water as needed to maintain saturated soil. Once per week they received a nutrient mix water that contained a 1.3% concentration of fertilizer (Grow More Inc., Gardena, California, USA), magnesium sulfate and calcium nitrate.

Plants were surveyed weekly from March 9-September 25, 2015, for phenology, morphology, and floral traits. Once a healthy seedling was growing in a cell, the individual closest to the center was selected and the other seedlings were documented and thinned. Phenology was recorded at the most advanced stage on the plant: 1) seedling (emerged from soil, vegetative), 2) budding (flower buds present), 3) flowering (at least one open flower was present), 4) fruiting (at least one fruit was present), or 5) dead (dry, senesced) (Jonas and Geber 1999, Franks et al. 2007, Schneider and Mazer 2016). Using these data I calculated days to emergence, defined as the first day when a plant was observed in a cell; days to flower, defined as the first day a flower is observed on a plant; and days to first flower, defined as the number of days between emergence and flowering (Jonas and Geber 1999, Franks et al. 2007, Schneider and Mazer 2016). There were some instances when a plant recorded as “bud” one week had a mixture of fruits and flowers the next. In such instances the stage was entered as "flower."

Height can be a measure of reproductive investment (Ostertag et al. 2015). Thus I recorded maximum height (cm) achieved by each plant. For plants that infringed on neighboring cells I stabilized them with a wooden stake and ties. Before measuring plant height stakes were removed. One basal leaf was also collected from the most basal node when a plant was fruiting and photographed for analysis of specific leaf area (SLA), the ratio of leaf area to dry mass (mm^2/mg). SLA can be a measure of resource allocation (Mooney and Dunn 1970, Ostertag et al. 2015). Generally there is a negative relationship between leaf size and resource availability, and smaller leaves are advantageous in hot and dry climates (Mooney and Dunn 1970, Dolph and Dilcher 1980, Peñuelas and Matamala 1990). Reduced SLA (i.e. thicker and/or smaller leaves), has been found to increase drought tolerance, nutrient retention, and water-use efficiency (Mooney and Dunn 1970, Ackerly et al. 2002).

By June 1, 2015, 36.86% of the original, untreated cells germinated, and were largely senescing. This left 63.14% of maternal families planted that had not germinated. In order to test seed viability and confirm dormancy of those that did not germinate, I

exposed these cells to two experimental treatments. I moved all living plants from their cells and transplanted them to new, identical trays. The original, untreated trays then contained only cells that had not germinated. Half of trays (178 cells) received a gibberellic acid solution (5 ml per cell of 200 ppm concentration) applied to the soil surface and 24 hours later were rinsed with running water for three minutes. Gibberellic acid is a growth hormone that can be used to overcome dormancy and promote germination. The other half of trays (172 cells) were returned to the 4 °C vernalization chamber for six days with black lids, and then seven more days without lids, and were subsequently returned to the greenhouse. All trays were vernalized before their initial placement in the greenhouse to break dormancy. I hypothesized that perhaps photoperiod or temperatures were not ideal in the greenhouse in the early spring to promote germination for all populations and that a second round of vernalization may help to break dormancy. The plants that grew initially, prior to the gibberellic acid or second vernalization, will hereafter be referred to as "untreated" group; those receiving the gibberellic acid will be referred to as "GA" group; and those receiving the second vernalization will be referred to as "vernalized" group. Plants were allowed to grow until September 25, 2015 when the majority had senesced and the experiment ended.

Reproductive data

Plants were harvested when all fruits reached maturity and the plant senesced. Plants were clipped at the soil surface, excluding roots. Total number of fruits were counted, removed, collected, and weighed. I weighed the fruits collected from each individual using an aluminum weigh boat and weigh paper on an electronic microbalance scale (Mettler Toledo Classic Plus AB265-S/FACT). Any loose seed, broken pieces of fruit or dried flower parts were also included. Sexton et al. (2011) found total seed mass was highly correlated with fruit mass ($r = 0.929$; $df = 139$; $P < 0.0001$). Fruit mass has been used as a proxy for fitness (Sandring and Ågren 2009, Sexton et al. 2011, Vergeer and Kunin 2013), and I also used it as the lifetime fitness proxy in my study. Non-reproductive aboveground biomass was placed into a drying oven at 60°C for 48 hours, then weighed. The weight of the single leaf harvested from each plant for the SLA analysis was added to the total. To assess SLA, leaf photos were processed using Image J software to obtain the area (Schneider et al. 2012).

Accounting for maternal effects

Maternal effects, defined as the effect of environmentally induced trait expression in one generation on trait expression in a subsequent generation (Roach and Wulff 1987, Heger et al. 2014), can affect experimental results. However, maternal effects (also referred to as "transgenerational effects"), can be an important adaptive mechanism in the wild (Galloway and Etterson 2007, Germain et al. 2013). They can also be an important and inseparable component of phenotypic genetic variance, especially for a highly selfing species such as *M. laciniatus* (Conner and Hartl 2004). In plants, a common maternal effect might result in resource partitioning with effects on seed mass (e.g., seeds of pre-drought populations weigh more and have greater resources than those of drought

populations). Nevertheless, in the absence of a refresher generation in this experiment I addressed maternal effects in several ways described below.

Growing field collected seed in the greenhouse can provide a realistic measure of trait expression in natural populations (Rice et al. 2013). To further account for potential maternal effects, I estimated mean individual seed mass for a subset of maternal families that had ample seeds to measure (406 maternal families out of the 510), which captures ca. 79.6% of maternal families planted. *Mimulus laciniatus* seeds are tiny (generally < 1 mm) and so I calculated mean seed mass by weighing 10-30 field collected seeds per family (to 0.01 mg), for both pre-drought seeds and drought seeds, on an electronic microbalance scale (Mettler Toledo Classic Plus AB265-S/FACT) and divided mass by number of seeds weighed. Mean seed mass is included as a covariate in my data analysis models to account for potential maternal effects (Jonas and Geber 1999, Schneider and Mazer 2016). Additionally, my study design includes independent maternal families as seed replicates within populations, with three replicate populations sampled from each region: low, high, and intermediate elevations. Thus, replication should reduce (although not completely remove) potential bias from maternal effects on regional and generational responses during analysis.

Climate data

To estimate climate values, I obtained data for each population, extrapolated from the United States Geologic Survey Basin Characterization Model (270 m resolution) (Flint and Flint 2014). I obtained water year data for the year of seed collection at each population from the pre-drought and drought collection years. I used the United States Geologic Survey definition of water year, defined as the period from October 1 of the previous year to September 30 of the current year (United States Geological Survey 2016). This period is when the Sierra Nevada receives the majority of its precipitation and represents the conditions under which seeds germinate, grow, and reproduce. I obtained total water year precipitation (mm), mean maximum annual temperature and mean minimum annual temperature (°C), and climatic water deficit (CWD; mm). Mean maximum temperature is calculated by taking the maximum monthly temperature averaged annually. Mean minimum temperature is calculated by taking the minimum monthly temperature averaged annually. CWD is defined as the evaporative demand exceeding available soil moisture, calculated by subtracting actual evapotranspiration from potential evapotranspiration (Flint and Flint 2014). I also obtained 30-year annual averages (1981-2010) for precipitation and temperature maximum and minimum for each population. I imported these data into R Version 0.99.903 (R Core Team 2016) and calculated precipitation and temperature anomaly by subtracting the 30-year annual water year average from values of the water year of seed collection to obtain a departure from climate normals. Since plants tend to be locally adapted, largely driven by climate (Clausen et al. 1941, Leimu and Fischer 2008, Hereford et al. 2009), understanding climate departures from normal could help to identify a selective pressure on survival and fitness.

Statistical analysis

To detect differences in phenological traits (i.e. days to emergence and first flower), between regions and generations, I conducted survival analyses using Cox Proportional Hazards models (Fox 2001). These analyses can accept censored values, which in this experiment were individuals that never emerged when testing time to emergence and individuals that emerged but never flowered when testing differences in days to first flower. Germination treatments (untreated, GA, vernalization) were analyzed separately since their treatments could have influenced their growth and because they included different time periods. Population, nested within region, was initially included in the analyses but did not improve model fit under model selection (see below) so population effects were subsequently excluded. I fit models for response variables days to emergence, days to flower and days to first flower; included region, generation (pre-drought or drought), and region by generation interaction as explanatory variables; and mean seed mass and tray as random effects. Results did not differ qualitatively among models so I selected the models without random effects and conducted post-hoc, multiple comparison adjustment tests. Significance of explanatory variables was tested using likelihood ratio tests. The survival analyses were conducted in R Version 0.99.903 (R Core Team 2016) using the survival package (Therneau 2015). I calculated multiple comparisons (Tukey-adjusted) using the Multcomp package (Hothorn et al. 2008). Significant differences were determined using $\alpha = 0.05$.

For analyses of morphological traits, all variables were transformed using average ranks (Conover and Iman 1981) because standard transformations did not sufficiently meet the assumptions of parametric analyses. I created a Pearson correlation matrix in R using the Hmisc package (Harrell Jr and Dupont 2016) to examine if any traits are highly correlated.

I used a REML model (Shaw 1987) with body-size or fitness-related traits (total plant mass, fruit mass, vegetative biomass, number of fruits, plant height, specific leaf area) as response variables; region or population, generation (pre-drought or drought), region or population by generation interaction, and germination treatment as explanatory variables; and mean seed mass and tray as random variables. Initially, I included population nested within region but the models would not run due to insufficient degrees of freedom. Due to this limitation, I ran population and regional effects separately. Inclusion of mean seed mass did not allow these models to run so to further examine the effect of mean seed mass on morphology-related traits I conducted an ANCOVA of each trait with mean seed mass as an explanatory variable and tray as a random variable. Mean seed mass was not significant for any trait ($0.648 \leq P \leq 0.928$) and was removed from final models. For all models I obtained Akaike information criteria (AIC) scores to aid in model selection. Tukey HSD post-hoc tests were performed to determine which groups were driving significant differences. All analyses were conducted in JMP® Pro (Version 12.0.1. SAS Institute Inc., Cary, NC, 1989-2007) and were restricted to plants that emerged.

Finally, I conducted Levene's tests of homogeneity of variance in R (R Core Team 2016), using the car package (Fox and Weisberg 2011), to determine if trait variance differed by generation within a population or region, or by geographic position within the species range. For a highly selfing plant like *M. laciniatus*, variance among full-sibling families (e.g., used to estimate broad-sense heritability, not estimated here due to lack of replication within maternal families), which includes maternal effects, is the most relevant measure of genetic variance (Conner and Hartl 2004). Thus, I used differences in within-population trait variance as a proxy for differences in trait genetic variance. I also calculated the coefficient of variation (CV) for each trait using the raster package (Hijmans and van Etten 2012) to estimate trait variance among maternal families. These data were used to determine whether or not peripheral populations have equivalent genetic variation to intermediate populations upon which natural selection can act, and whether variance was reduced during the drought of 2012-2014.

RESULTS

Climatic variation among generations

The Sierra Nevada has a Mediterranean climate, characterized by cool wet winters and warm dry summers. It also features irregular El Niño-Southern Oscillation cycles that results in wet or dry years, and few that are average (Barbour et al. 2007). The climate leading up to the year of collection for the pre-drought generation was largely average, within the typical range of variability (Table 2). In contrast, the climate leading up to the year of collection for the drought generation was exceptionally hot and dry, across all three regions, which represent the elevational extremes of the species range for *M. laciniatus*. To focus on climate for seeds produced in the field, I report differences between the generations for the water year of seed collection (Table 2).

Total water year precipitation was lower for drought generation populations than pre-drought generation populations. All pre-drought generation populations had a positive precipitation anomaly, that is, deviation (i.e., increased precipitation) from the 30-year mean at that locality, which ranged from 275.1 mm for population L1 to 592.2 mm for population H1, with the exception of population I3, which was -367.3 mm, however those seeds were collected in 2008 (Figure A1). Drought generation populations all had negative precipitation anomaly (i.e. decreased precipitation) ranging from a minimum of -288.8 mm for population H2 to a maximum of -776.8 mm for population H1, which represent 36.6% to 55.8% reductions in precipitation, respectively (Table 2). The mean precipitation in the low region pre-drought was 1156.1 mm and in the drought generation was 419.3 mm; a dramatic reduction in precipitation. The mean precipitation in the high region pre-drought was 1474.6 mm and in the drought generation was 553.4 mm, which is a large reduction in precipitation. Additionally, the normal variation in precipitation between the extremes of the species range (i.e. low and high elevation populations) was not seen during the severe drought. The precipitation received at low

and high elevation populations only differed by 134.1 mm in 2014 as opposed to the pre-drought generation where low and high elevation populations differed by 318.6 mm (Table 2).

Moisture stress was higher for all drought generation populations. Drought generation populations had a greater CWD than pre-drought generation populations, from a minimum of 2.2 mm for population I3 to a maximum of 599.4 mm for population L2 (Figure A2; Table 2).

Maximum temperature (Tmax) was higher for all drought generation populations than pre-drought generation populations. Pre-drought generation Tmax anomalies ranged from -0.7 °C for population L2 to 0.4 °C for population I3. Drought generation population Tmax anomaly all demonstrated increases from the 30-year means, from a minimum of 1.4 °C for population H1 to a maximum of 2.8 °C for population L3, which represent 13.2% and 14.5 % increases in Tmax, respectively (Figure A3; Table 2). Populations H2 and H3 experienced the greatest percentage increase in Tmax relative to their 30-year mean: 23.0% and 20.9%, respectively. Nevertheless, the absolute increase in temperature in these populations was less than that of population L3.

Minimum temperature (Tmin) was more variable for all drought generation populations as compared to pre-drought populations. The pre-drought generation Tmin anomaly ranged from -0.4 °C for population L2 to 1.5 °C for population H2. Drought generation Tmax anomaly was more variable and had a minimum of -0.8 °C for population I3 to 3.1 °C for population L3, which represent a -27.0% decrease and a 58.1% increase in Tmin relative to their 30-year mean, respectively (Figure A4; Table 2). Although the absolute increase in degrees C in Tmin was less than population L3, population I2 experienced the greatest percentage increase, 66.0%, in Tmin relative to its 30-year mean. Note that in the drought generation all three high region populations experienced substantial increases in Tmin relative to their 30-year means: 65.0% for population H1, 47.1% for population H2, and 22.5% for population H3.

The largest reduction in overall precipitation in 2014 was at a high elevation population, which suffered a reduction of 55.7% from the 30-year mean. However two populations in the low region and two in the intermediate region suffered reductions over 50% as well (Table 2). Thus, the drought affected all populations across the species range. Tmax was higher for all populations in the drought generation, with the greatest increases (ca. 20-23%) in the high elevation populations. Tmin was more variable among regions and generations, yet the high region populations all showed a substantial increase in Tmin for the pre-drought and drought generations, relative to the 30-year means, which means that winter was not as cold as in the past. Taken together, all drought generation populations were moisture-limited due to very hot and dry conditions. The high elevation populations experienced the greatest change in climate due to the drought as compared to the low and intermediate populations.

Growth by generation and climate regions

Germination varied greatly among treatments and regions. In the untreated group, 36.9% of maternal families germinated but among the regions the high elevation populations had lowest germination (14.9%) (Table 3, Table 4). Although trays were placed in the greenhouse early in the growing season for natural populations of *M. laciniatus* (mid-February), high elevation populations in the field are often under snow until May or even later when photoperiod is substantially longer. In contrast, low and intermediate elevation populations had very similar germination (42.0% and 43.1%, respectively), which indicates that the photoperiod in the greenhouse most closely mimics day length of germination under field conditions. The difference in germination between regions implies that high elevation plants have stricter germination cues. Despite regional differences, almost all untreated plants that germinated, flowered (94.7%). The GA group had the highest germination (94.9% of individuals treated) but only a little over half flowered (54.4%). The vernalized group had the lowest germination (25.6% of individuals treated) and the lowest flowering rate (38.6%). The GA and vernalized plants were placed in the greenhouse in early June, so there may have been an inadequate photoperiod to cue flowering in these individuals in middle-to-late summer (i.e., generally decreasing photoperiods).

The GA results rule out seed viability as the cause for low germination in other groups. Furthermore, these results verify there are biologically-driven dormancy traits that the GA treatment was able to overcome, but the second vernalization treatment could not completely overcome. After accounting for mortality and data errors I recorded data for 401 individuals (78.6% overall germination), each representing a unique maternal family.

For phenological traits, results for days to flower and days to first flower were qualitatively similar (i.e., the same effects were significant) for all analyses and thus days to flower data are not presented here. For morphological traits, the Pearson correlation matrix revealed that two pairs of traits are highly correlated (non-reproductive biomass and total mass; number of fruits and fruit mass; Table C1). These traits had a correlation coefficient > 0.9 (P=0.0001, P=0.012, respectively), and so I do not present model results for non-reproductive biomass and number of fruits.

Phenotypic response between generations

Drought generation plants emerged significantly earlier than pre-drought generation plants in the untreated group, which is consistent with drought avoidance for *M. laciniatus* (Sexton et al. 2011). Days to emergence differed significantly by generation (DF=1, X^2=9.003, P=0.003; Figure 4) in the untreated group but differences were not significant in the GA group or the vernalized group (Figure 3; Table 5). In the untreated group, mean day of emergence was 4.93 days earlier for the drought generation than pre-drought generation; for the GA group 0.99 days earlier, and for the vernalized group 1.08 days earlier. The high elevation region had low germination in the untreated group and

due to small sample size, and so generational effects for those results will not be explored further. Levene's tests for days to emergence provided evidence that genetic variation was reduced in the drought generation. The untreated group differed significantly by generation (DF=1, F=5.143, P=0.024; Table B1), with a lower CV for the drought generation (CV=38.319) than the pre-drought generation (CV=77.318) (Table B2). For the Levene's tests of days to emergence within the untreated group, the generation effect within each region was significant for the intermediate region (DF=1, F=4.542, P=0.036; Table B1), with the drought generation (CV=4.718) demonstrating a lower CV than the pre-drought generation (CV=90.636) (Table B2). Other Levene's tests were not significant (Table B1).

Days to first flower did not significantly differ by generation in the untreated group, GA group, or vernalized group (Figure 5; Table 5). Mean days to first flower in the drought generation was 2.92 days later in the untreated group, 1.39 days earlier in the GA group, and 21.10 days earlier, in the vernalized group, relative to the pre-drought generation. The great difference in days to flower in the vernalized group may be an artifact of small sample size in the group (see above). Levene's tests were also not significant (Table B1).

There were significant differences in morphological traits of mean fruit mass, mean total plant mass and mean maximum height between generations. Drought generation plants were generally larger and had greater reproduction than pre-drought generation plants.

Mean fruit mass differed significantly by generation at the population level (P=0.0001, F ratio=15.383, DF=1, DFDen=265.6; Figure 6; Table 6) and at the regional level (P=<.0001, F ratio=19.566, DF=1, DFDen=277.9; Figure 7; Table 6). The drought generation plants had greater mean fruit mass than the pre-drought generation in each region. The intermediate region plants produced the greatest fruit mass, followed by the low region and then high region. Levene's test for homogeneity of variance in mean fruit mass by generation within each region was significant for the low elevation region (DF=1, F=4.162, P=0.044; Table C2). The drought generation (CV=121.533) had a lower CV than the pre-drought generation (CV=166.644) (Table C3). Other Levene's tests were not significant (Table C2).

Mean total plant mass differed significantly between generations by population (P=0.002, F ratio=9.388, DF=1, DFDen=262.9; Table 6) and region (P=0.001, F ratio=10.707, DF=1; Figure 8; Table 6). The drought generation had significantly greater mean total plant mass than the pre-drought generation across the species range. Levene's tests were not significant (Table C2).

Corresponding to the results from fruit mass and total plant mass, mean maximum height also differed significantly between generations at the population level (P=0.034, F ratio=4.307, DF=1, DFDen=364.2; Table 6) and regional level (P=0.020, F ratio=5.497, DF=1, DFDen=376.3; Figure 9; Table 6). Maximum height was greater in drought

generations than the pre-drought generations at both population and regional levels. The pre-drought generation had similar maximum height across the species range but drought generation plants in the intermediate region were larger than low and high regions, respectively. Levene's tests were not significant (Table C2).

Mean SLA did not differ between generations at population or regional levels (Table 6). Levene's test for homogeneity of variance of specific leaf area was not significant between generations (Table C2).

Phenotypic response by climate region

The interaction between population and generation or between region and generation were not significant for any trait that I examined (Table 5, Table 6). Thus, response to drought did not depend on the location within species range (i.e. certain regions did not respond more strongly than others).

The phenological traits I studied did not respond in the same way to climate region. Days to emergence did not significantly differ by region in the untreated group, GA group or vernalized group (Table 5). However, days to first flower differed by region in the untreated group (DF=2, X^2=12.494, P=0.002; Figure 5; Table 5), which suggests days to emergence is related to climate adaptation. Mean days to first flower was 21.80 days for the low region, 34.53 days for the intermediate region, and 43.68 days for the high region. Days to first flower did not differ significantly in the GA or vernalized groups (Table 5). Levene's test of days to first flower showed significant differences between regions and corroborates model results. Region differed significantly in the untreated group (DF=2, F=7.387, P=0.001; Table B1). The CV was highest for intermediate region (CV=63.395), followed by low region (CV=43.417), and high region (CV=36.676; Table B2). This result suggests that intermediate regions have greater genetic variation in this trait than low and high elevation limits. Other Levene's tests were not significant (Table B1).

There were significant differences in the morphological traits mean fruit mass, mean total plant mass and mean maximum height between generations at population and regional levels. Generally, drought generation plants were larger and had greater reproduction than pre-drought generation plants in each region.

Drought generation plants had greater mean fruit mass than pre-drought generation plants in each region. The intermediate region plants produced the greatest fruit mass, followed by low and high regions. Fruit mass differed significantly at the population level (P<0.0001, F ratio=8.974, DF=8, DFDen=266.2) and by germination treatment (P<0.0001, F ratio=65.719, DF=2, DFDen=223.4; Table 6). Fruit mass also differed significantly at the regional level (P=0.0002, F ratio=8.913, DF=2, DFDen=278.5), and by germination treatment (P<0.0001, F ratio=53.656, DF=2, DFDen=221; Table 6). Levene's test for mean fruit mass by generation within each

region was significant for the low elevation region (DF=1, F=4.162, P=0.044; Table C2). The drought generation (CV=121.533) had a lower CV than the pre-drought generation (CV=166.644) (Table C3). Other Levene's tests were not significant (Table C2).

Mean total plant mass differed significantly by germination treatment and at population and region levels. Total plant mass differed significantly at the population level (P<0.0001, F ratio=5.613, DF=8, DFDen=264.1) and by germination treatment (P<0.0001, F ratio=15.705, DF=2, DFDen=194.2; Table 6). Total plant mass also differed significantly at the regional level (P=0.016, F ratio=4.170, DF=2, DFDen=276.4; Table 6) and by germination treatment (P<0.0001, F ratio=12.329, DF=2, DFDen=193.7). At the regional level, the intermediate region had the largest total plant mass followed by high and low regions. Levene's test for total plant mass was significant only between populations (DF=8, F=2.425, P=0.015; Table C2). Population I3 had the lowest CV (CV=77.715) and population H1 had the highest CV (CV=143.124; Table C3).

Mean maximum height differed significantly by population and region, and germination treatment. Mean maximum height differed significantly at the population level (P=0.0009, F ratio=3.405, DF=8, DFDen=364.2) and by germination treatment (P<0.0001, F ratio=46.223, DF=2, DFDen=262.4; Table 6). Maximum height also differed significantly at the regional level (P=0.003, F ratio=5.926, DF=2, DFDen=376) and by germination treatment (P<0.0001, F ratio=46.329, DF=2, DFDen=268.4). Levene's test was significant only between populations for mean maximum height, which suggests there are equal variances among geographic regions. Levene's test for mean maximum height by population was significant (DF=8, F=2.327, P=0.019; Table C2). Population I1 had the lowest CV (CV=45.028; and population H3 had the highest CV (CV=82.595; Table C3).

Mean SLA did not differ by region, although there were significant differences by germination treatment. SLA differed significantly by germination treatment at the population level (P<0.0001, F ratio=31.123, DF=2, DFDen=106.2; Table 6) and at the regional level (P<0.0001, F ratio=29.877, DF=2, DFDen=95.39; Table 6). Levene's test for homogeneity of variance of SLA was not significant between germination treatments or any other response variables (Table C2).

DISCUSSION

I tested the adaptive response of a native species to a severe drought at its high and low elevation range limits, compared to the intermediate portion of the range. These results demonstrate that peripheral and intermediate populations are capable of responding to severe drought. The interactions of generation with population or region were not statistically significant, indicating no difference among populations across the entirety of

the species elevational range to respond to severe drought. These results indicate that the adaptive response capabilities of peripheral populations may have been underestimated as a means to respond to climate change. Previous studies have demonstrated that plants can adapt rapidly (Parmesan 2006, Franks et al. 2007, Franks 2011, Sultan et al. 2013), although several tested only a portion of the species range and/or used a non-native species as their study system. Alternatively, some research has found that peripheral populations are unable to respond quickly or effectively to a strong selective pressure due to lack of genetic variation (Holt et al. 2003, Pujol and Pannell 2008, Dawson et al. 2010).

I expect that observed phenotypic differences were largely due to genetic (evolutionary) shifts by using the resurrection study approach. Generally, days to emergence were significantly fewer in the drought generation than the pre-drought generation, consistent with an adaptive response to drought. We know that rapid increases in temperature are associated with a reduction in genetic variation for traits affected by climate (Jump and Peñuelas 2005). The reduced trait variance in days to emergence observed in the drought generation suggests that a natural selection event has occurred. Earlier emergence is an adaptive trait in dry climates because it provides opportunity for plants to maximize earlier spring runoff and complete their life cycle prior to desiccation.

Considering days to emergence response by climate region, the largest reduction in trait variation in the drought generation was observed in the intermediate region, perhaps where drought selection response was the strongest. Intermediate elevations tend to receive the most precipitation due to orographic lift and higher frequency of freezing temperatures at high elevations (Stephenson 1998, Urban et al. 2000). Thus, low and high elevation populations normally experience climate extremes and may already be better adapted to drought episodes. The GA and vernalized groups also had reduced mean days to emergence in the drought generation, although these differences were not statistically significant. The vernalized group had a small sample size of plants emerged, which may have caused a lack of statistical power to detect significant differences.

The untreated group most closely matched the photoperiod plants would experience in the field, especially at lower elevations, that encounter a shorter, early spring photoperiod (i.e. February-May) and temperate, wet conditions. The majority of the untreated plants flowered. For plants in the *M. guttatus* complex, of which *M. laciniatus* is a member, flowering time is strongly influenced by photoperiod and is genetically-based (Friedman and Willis 2013). GA and vernalized groups experienced decreasing photoperiods over time and hotter, wet conditions (i.e. June-September), which may have decreased flowering rates in this set.

Days to first flower differed significantly by region, which showed a clear signal suggestive of elevation-based local adaptation by means of flowering time variation. The low region had the fewest days to first flower, followed by the intermediate region, and high region, which corroborates other studies that have linked phenology to climate

adaptation in *M. laciniatus* (Sexton et al. 2011, Friedman and Willis 2013). Additionally, trait variation in days to first flower significantly differed between regions in the untreated group. The intermediate region demonstrated the highest trait variation, followed by low and high regions, respectively. This supports the 'abundant center hypothesis', where higher genetic variation and ideal climate may result in plant populations that exhibit greater growth and reproduction (Wang et al. 2013). Furthermore, this supports the hypothesis that peripheral populations have less genetic variation and therefore may have reduced capacity to respond to selective pressures (Hampe and Petit 2005, Angert et al. 2008; but see Sagarin and Gaines 2002, Sagarin et al. 2006). In this trait I found significant differences by region, but not between generations. This may mean that days to first flower were less crucial to drought adaptation than days to emergence and that this trait was under less selective pressure. Days to emergence differed by generation but not by region, while days to first flower differed by region but not generation.

Selection for a smaller body size, and potentially less reproduction, is a typical response to drought (Sheridan and Bickford 2011). However, my results showed the opposite (i.e. drought generation plants grew larger and reproduced more). Since the drought generation emerged earlier in greenhouse conditions, they had more time to grow, which resulted in larger body size and greater reproduction, across all three regions. Earlier emergence leading to greater biomass and fitness has been documented in other studies (Sexton et al. 2011, Germain et al. 2013). Drought might have selected genotypes that emerge earlier and grow faster, where in greenhouse conditions they were able to maximize growth and produce more fruit. I did not conduct a drought experiment, but if I had limited water availability, a common occurrence in the field where *M. laciniatus* grows (Sexton et al. 2011, Peterson et al. 2013, Ferris 2014), I would expect the fast-emerging drought generation to exhibit greater fitness than the pre-drought generation.

Fruit mass, total plant mass and maximum height also differed significantly by population and region. The intermediate region populations grew larger and produced more fruits than low and high regions in the drought generation. In the pre-drought generation the intermediate region had the greatest mean fruit mass and maximum height. All regions had similar total plant mass. This may be because central elevations of the range represent the most favorable environment for *M. laciniatus*. That is to say, these environments have reduced temperature and moisture extremes and as a result have a longer potential growing season and have evolved genotypes that take advantage of opportunities to grow longer. The high elevation region had the smallest mean fruit mass in both pre-drought and drought generations. Previous work has found plant size tends to be smaller at high elevations due to challenging environmental conditions and shortened growing season (Clausen et al. 1941, Conover and Schultz 1995). Thus, drought plants at the "leading edge" are not yet demonstrating the response of evolving a larger body size to take advantage of the lengthened growing season. Nevertheless, low sample sizes at high elevations due to low emergence makes it difficult to draw inter-generation conclusions for these populations.

Levene's tests for homogeneity of variance of morphological traits were mostly not significant. However, total plant mass was significant between populations, which suggests there is variability in traits determined by location. Also, Levene's test of fruit mass by generation within each region was significant only for the low elevation region. The drought generation had a lower CV than the pre-drought generation, which suggests a selective event may have occurred. This is contrary to the expectation that peripheral populations would be less responsive to selection, although there is limited evidence of peripheral populations being more responsive to climate selection (Sheth and Angert 2015). SLA did not respond as other morphological traits did. This suggests that specific leaf area was not under strong selection due to drought or other factors. This may be because *M. laciniatus* is an annual plant and was able to shift its phenology to escape drought rather than change leaf morphology (i.e. reduce SLA) to tolerate drought, a strategy documented in perennials (Ackerly et al. 2002).

Climate is second to photoperiod as a determinant of phenology and has a strong selective pressure on plants (Clausen et al. 1941, Linhart and Grant 1996, Jump and Peñuelas 2005, Körner and Basler 2010). Advancement of spring has been attributed to climate change in other studies: Menzel et al. (2006) documented 2.5 days per decade, Parmesan and Yohe (2003) documented 2.3 days per decade, and Root et al. (2003) 5.1 days per decade. In my study I observed a mean of 2.33 days earlier emergence (averaged over three germination treatments) over an eight-year span between two generations. Given the severity of drought from 2012-2014 it is likely that climate played a role in the shift in days to emergence between pre-drought and drought generations. A reduction in days to emergence in the drought generation is expected given several years of exceptional drought. This pattern has been documented (Franks et al. 2007), though not over the entirety of a native species range. These results suggest emergence response cues can be variable and are potentially under selection for drought adaptation. It is possible natural selection has reduced sensitivity to photoperiod in time to emergence, which would be adaptive in hotter, drier conditions (Franks and Hoffmann 2012). Timing of flowering may also be under selection during drought, as it is for climate adaptation, but timing does not appear to be as responsive to drought as the timing of emergence.

Climate change is causing the growing season to lengthen, which some plants can take advantage of to expand their populations or increase fecundity, whereas others suffer declines (Guisan and Theurillat 2000, Kopp and Cleland 2014). In my study, all drought generation populations experienced extreme climate conditions across the species range. It appears the largest changes to climate, relative to the 30-year means, were experienced by high elevation populations, with a combination of less precipitation and much warmer temperatures, which has been documented in other systems (Wang et al. 2013). At the high elevation region, both pre-drought and drought generations demonstrated substantial increases in Tmin relative to 30-year mean, which suggests winters are not getting as cold as in the past. This suggests extreme climate events may induce stress on all *M. laciniatus* populations, yet it may have increased magnitude at high elevations. High elevation populations may be disproportionally affected by climate change because they are sensitive to increases in temperature and new competitors, and are geographically

separated by discontinuous terrain. Furthermore, shifts upslope in climate envelopes will be accompanied by a decrease in habitat area (Guisan and Theurillat 2000, Inouye et al. 2002), and environmental factors beyond temperature may inhibit range shifts (Bjorkman et al. 2016).

There are several caveats that should be considered when interpreting my results. I documented a significant reduction in days to emergence that would be adaptive in hotter and drier climates, accompanied by a reduction in trait variance in the drought generation. This may indicate that a selective event occurred, however, an alternative explanation for this reduction in variance could be due to genetic drift (Conner and Hartl 2004). If observed changes were due to genetic drift, I would predict a random reduction in genetic diversity across all or most traits, which is not necessarily adaptive (e.g., emergence time would be expected to lengthen instead of shorten in some populations of the drought generation). Therefore, while genetic drift possibly contributed to my results, it seems unlikely given that most Levene's tests were not significant.

Another caveat is that only one generation was grown, which increases the possibility that transgenerational effects influenced these results. Maternal effects can last several generations (Roach and Wulff 1987), which can be adaptive, and some studies have found that dry or otherwise stressful maternal growth conditions can have benefits for offspring through earlier emergence and increased fitness (Germain et al. 2013). Enhanced offspring quality through increased seed mass is one common maternal effect in plants (Roach and Wulff 1987). I accounted for such maternal effects by using mean maternal seed mass as a covariate in my models and found that it was never significant. Additionally, by growing seed from three replicate populations per region, and observing significant variance among maternal families between populations (i.e. variance differences between populations), it is unlikely that maternal effects are solely driving trait differences observed between generations. Furthermore, faster seedling emergence has been shown to be under strong natural selection in a fast-drying low-elevation environment (Sexton et al. 2011), which supports the hypothesis that the observed trait shifts would be adaptive under drought.

There is the possibility that seed quality in the pre-drought generation may have caused low germination. Seed quality and longevity is known to decline with seed age. Quality declines initially with a loss in plant vigor after germination and decline continues with time until ultimately the seed loses the ability to germinate (Harrington 1972). Seed storage conditions also affect seed longevity (Harrington 1972, Baskin and Baskin 2014). My seeds were stored in cool, dry conditions under very low humidity, which are ideal for maintenance of seed longevity. Seed quality could also affect results if a non-random portion of seeds did not germinate and thus their correlated traits are not represented; i.e. the "invisible fraction" effect (Grafen 1988). However, the cut tests coupled with results from the GA group, in which nearly every cell that was treated germinated within a few days of each other, suggest that seed quality or invisible fraction effects did not influence these results. Moreover, although not statistically significant, days to first flower were fewer for the pre-drought generation, which is inconsistent with

the idea of reduced vigor in these older seeds. To my knowledge no one had previously attempted to apply GA to soil containing sown seeds (it is typically applied to seed prior to sowing). This knowledge could be a useful tool for future studies diagnosing the above germination issues.

As a final caveat, the possibility exists that *M. laciniatus* may have a multi-year seed bank, which could mean that some proportion of field collected seed was not a result of that water-year's reproduction, but rather, a preceding year (Franks et al. 2007). However, longevity of the seed bank in *M. laciniatus* populations is not expected to be long-lived (Sexton and Dickman 2016), although this requires further study.

CONCLUSION

The discovery that there can be a rapid adaptive response is an important finding that contributes to our understanding of plant species distribution and persistence under climate change. Few studies have attempted to resurrect older genotypes and compare them to contemporary populations, though this approach is gaining recognition as an important way to empirically test the ramifications of climate change (Franks et al. 2008). I encourage subsequent studies to contribute to Project Baseline (Etterson et al. 2016) or other seed bank programs to facilitate further research.

Future investigation into selection for adaptive genotypes will be necessary to improve our understanding of climate adaptation in dynamic systems. Future work could expand to test fitness of *M. laciniatus* under various levels of simulated drought and to determine effects of habitat specificity (i.e. soil type, soil moisture, aspect, etc.). Additional research could investigate the number of consecutive years of climate anomaly needed to affect selection and if severe reductions in precipitation across all elevations will drive traits to become more similar (i.e. phenotypes that are successful at low elevations become advantageous at high elevations).

Many climate models predict an increasingly hot and dry future in California, with temperature increases of 1.5-1.8 °C by 2100 and substantial reductions in precipitation (Cayan et al. 2008, Ackerly et al. 2015). Forecasts also predict a greater proportion of precipitation falling as rain rather than snow (Cayan et al. 2008), which will compress timing of water availability. Thus, there will likely continue to be strong directional selection for traits and phenotypes that correspond with drought tolerance or escape (Jump and Peñuelas 2005, Franks et al. 2007, Schneider and Mazer 2016, Etterson and Mazer 2016). However, if climate continues to become hotter and drier, and intense directional selection continues, subsequent reductions in genetic variation may make adaptation increasingly difficult or impossible (Jump and Peñuelas 2005, Anderson et al. 2012).

Scientists, land managers and conservation organizations are actively seeking tools and knowledge to aid their efforts to stem biodiversity loss and support species persistence in the face of climate change. While my results are encouraging--that species may be able to rapidly evolve consistent with severely changing climates--the limit of their adaptive capabilities are not known and species range limits often represent niche limits (Hargreaves et al. 2014, Lee-Yaw et al. 2016, Sexton and Dickman 2016). Rapid shifts in phenology may be adaptive under climate change but can have negative repercussions for human health, agricultural systems and the economy if changes are asynchronous with the ecosystem (Hudson and Keatley 2009, Dawson et al. 2011, R.K. Pachauri and L.A. Meyer (eds.) 2014). Species that have special habitat requirements, small populations, poor reproduction, limited gene flow among populations, and/or are enduring new pathogens or competitors may be unable to adapt to multiple stressors and may be candidates for assisted migration or prescriptive gene flow (Loarie et al. 2008, Aitken et al. 2008, Sexton et al. 2011). It is crucial that we conduct vulnerability assessments for plant species to estimate risk of decline or extirpation and to consider possible management actions (Dawson et al. 2011, Pacifici et al. 2015). The potential for rapid adaptation provides hope for species persistence, however it has limitations and given the velocity of climate change may not be enough to ensure long-term survival of the species.

FIGURES

Figure 1. From Sexton et al. 2016. Upper left- *Mimulus laciniatus* flower; Upper right- *M. laciniatus* plant growing on moss patch; Lower- Granite outcrop seep habitat.

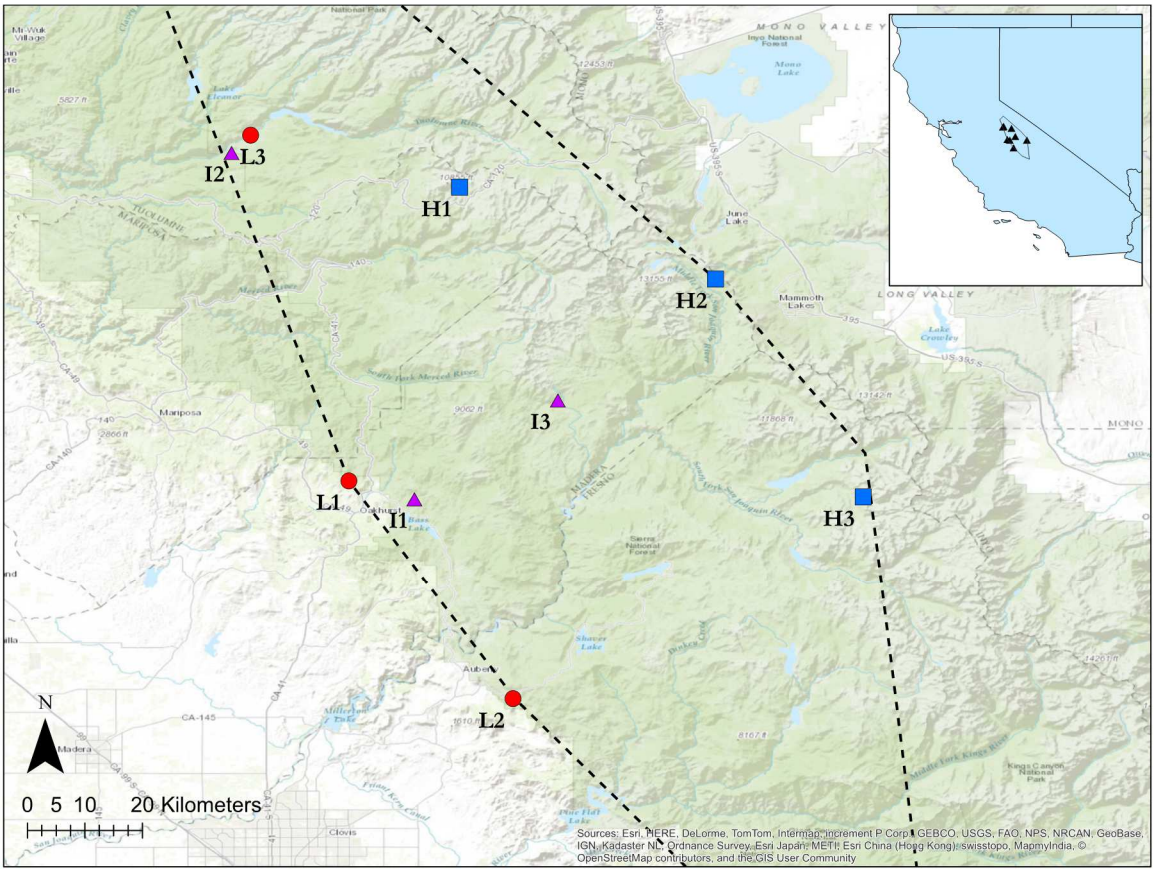

Figure 2. Map of study locations. Black dotted line indicates extent of *M. laciniatus* species range. The red circles denote the three low-elevation populations located at the low edge of the species range, labeled L1-3. The purple triangles denote the three intermediate populations to the species range, labeled I1-3. The blue squares denote the three high-elevation populations at the high edge of the species range, labeled H1-3. Inset map shows location of study populations within the central portion of the Sierra Nevada Mountains, California.

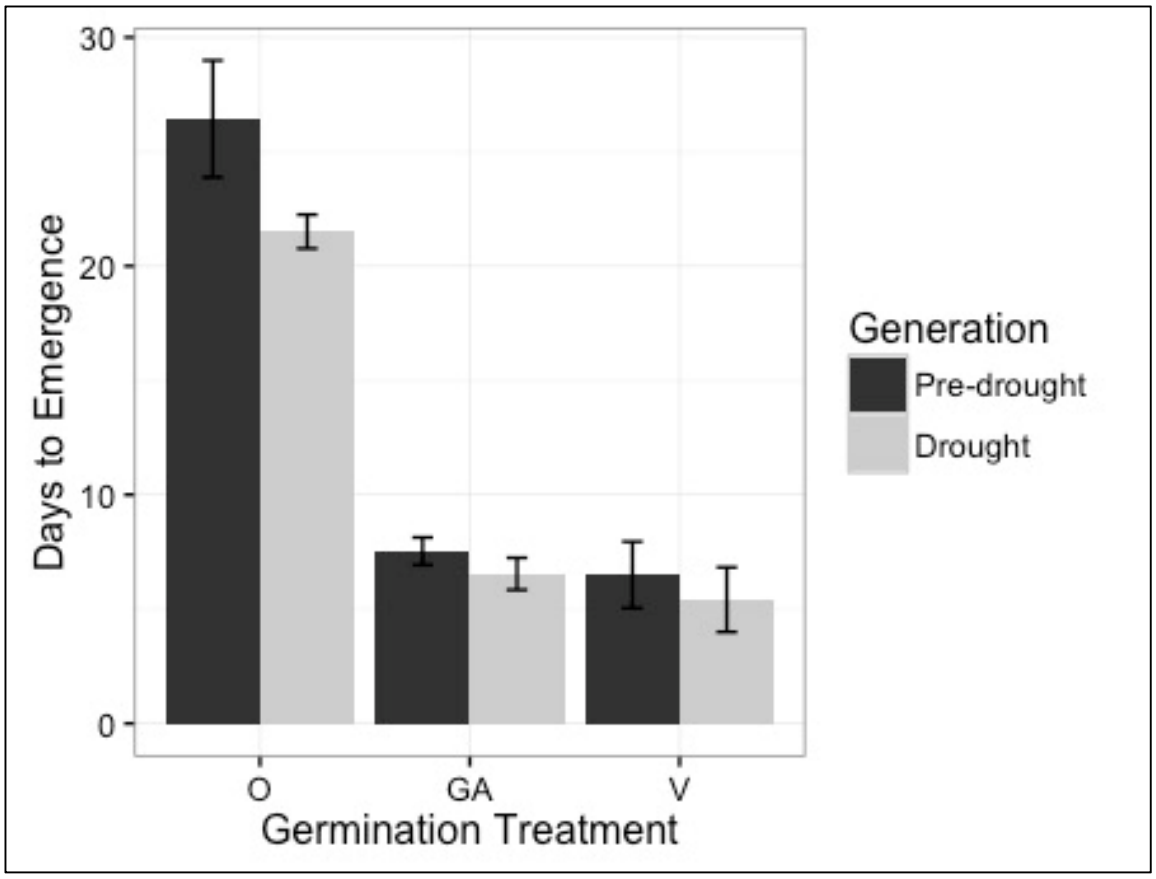

Figure 3. Days to emergence by germination treatment and generation (O=untreated group, GA= Gibberellic Acid group, V=vernalized group).

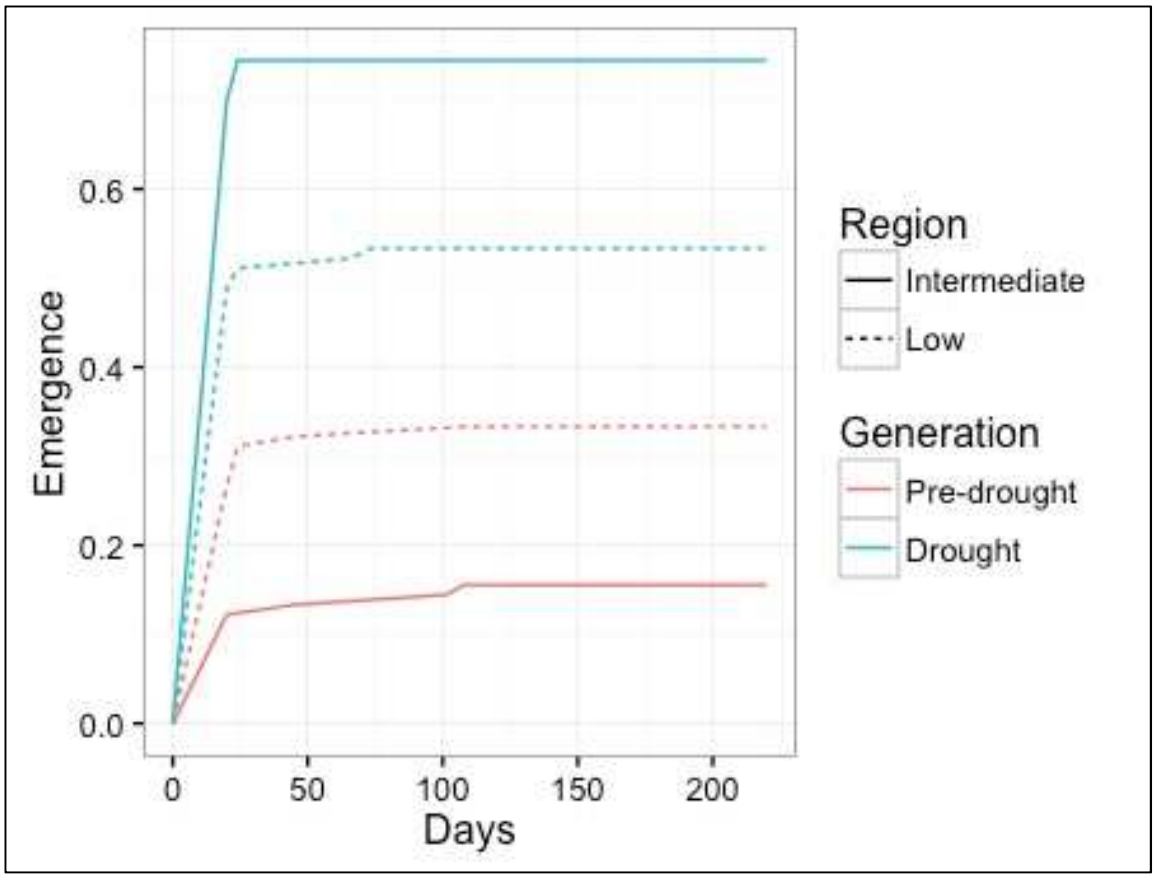

Figure 4. Time to emergence by region and generation in the untreated group. The y-axis represents the proportion emerged and the x-axis represents days since start of experiment. High region is omitted due to low sample size.

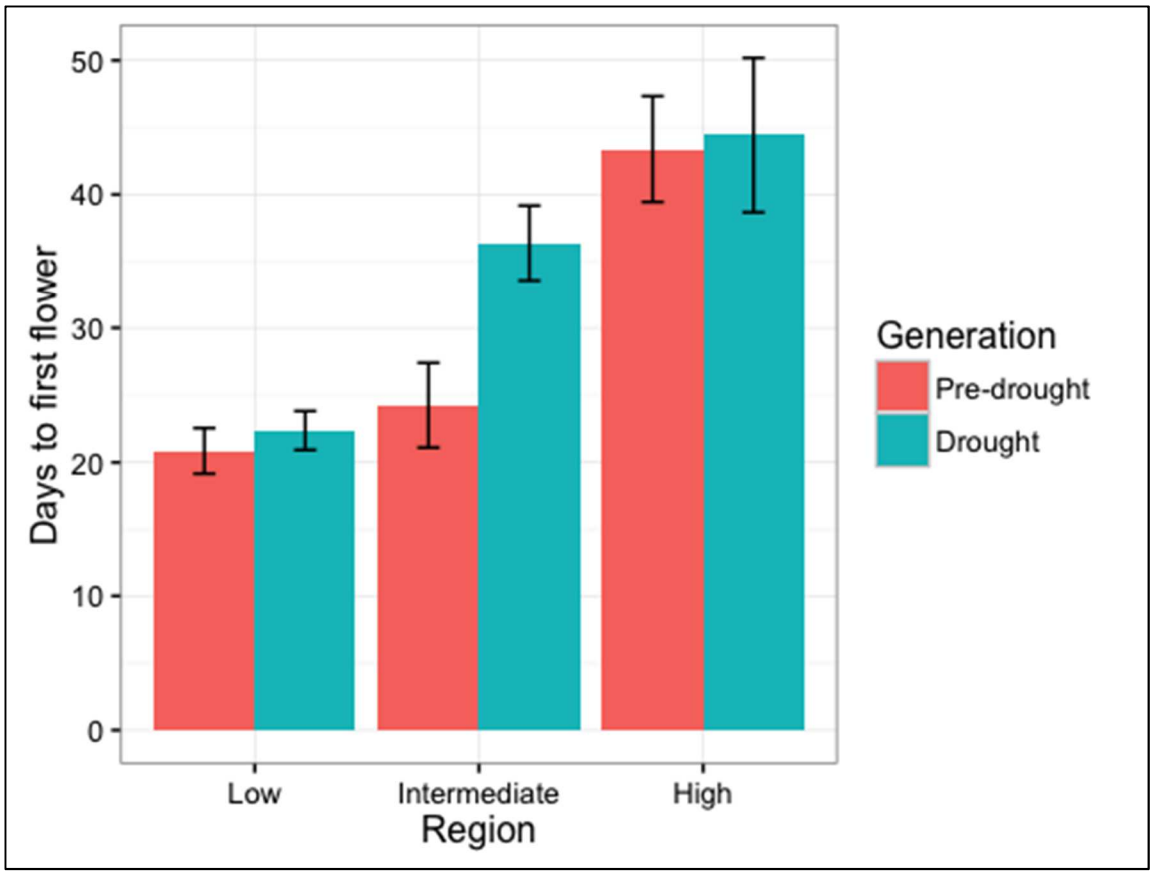

Figure 5. Days to first flower by region and generation in the untreated group.

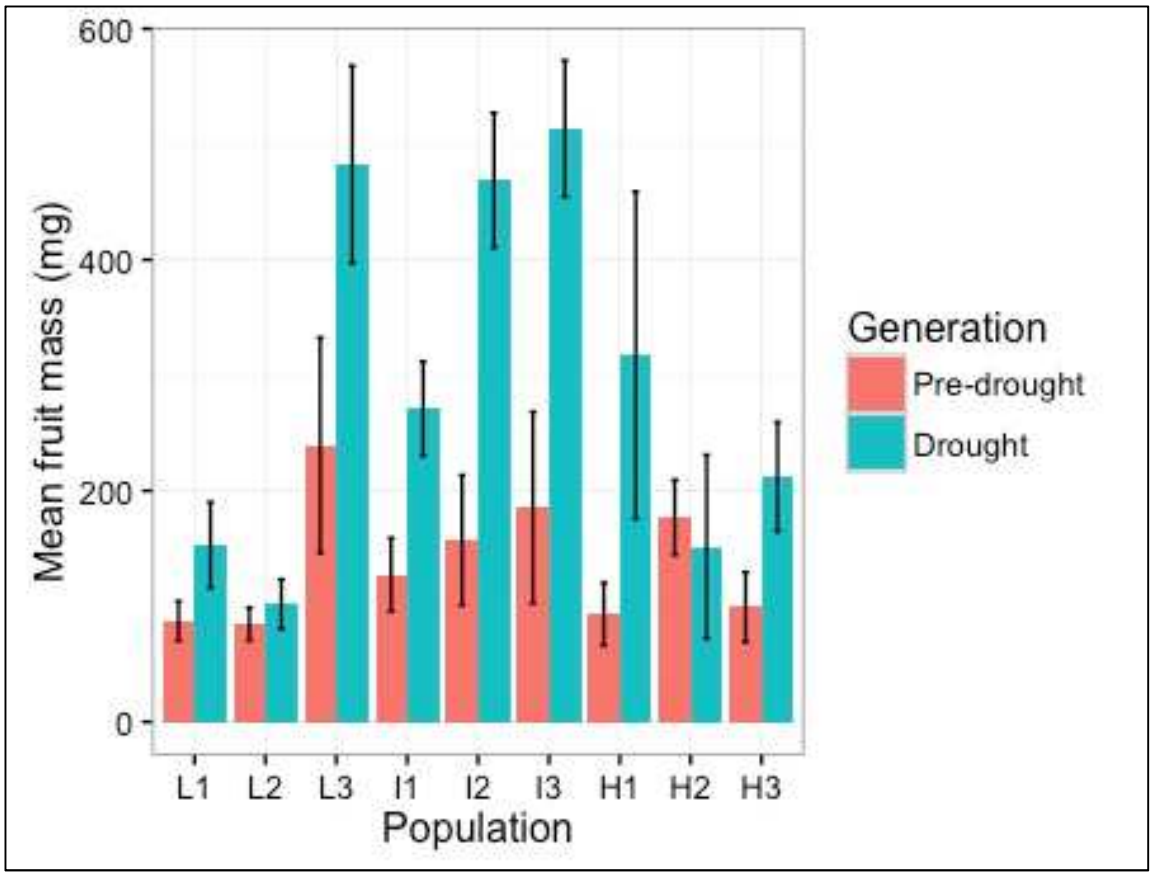

Figure 6. Mean fruit mass by population and generation.

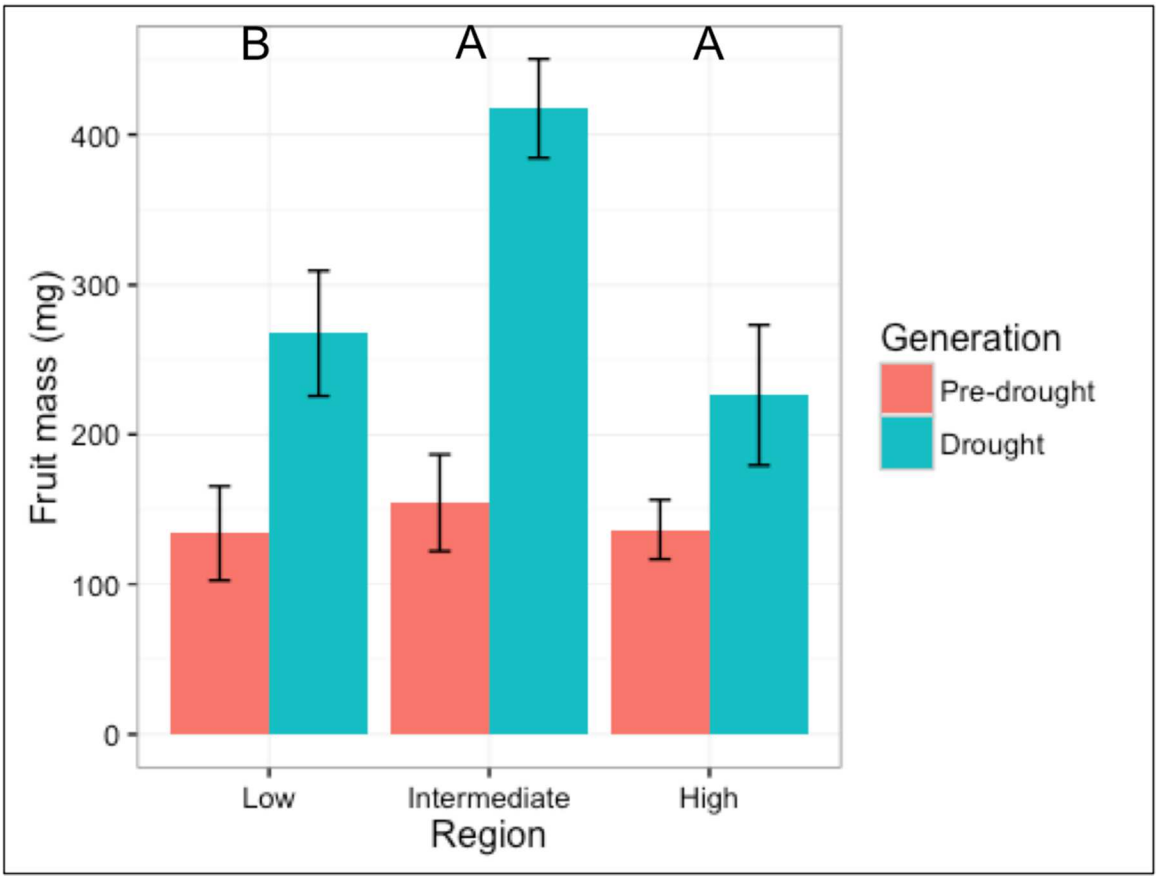

Figure 7. Mean fruit mass by region and generation. Letters represent Tukey's HSD significant differences at $P < 0.05$.

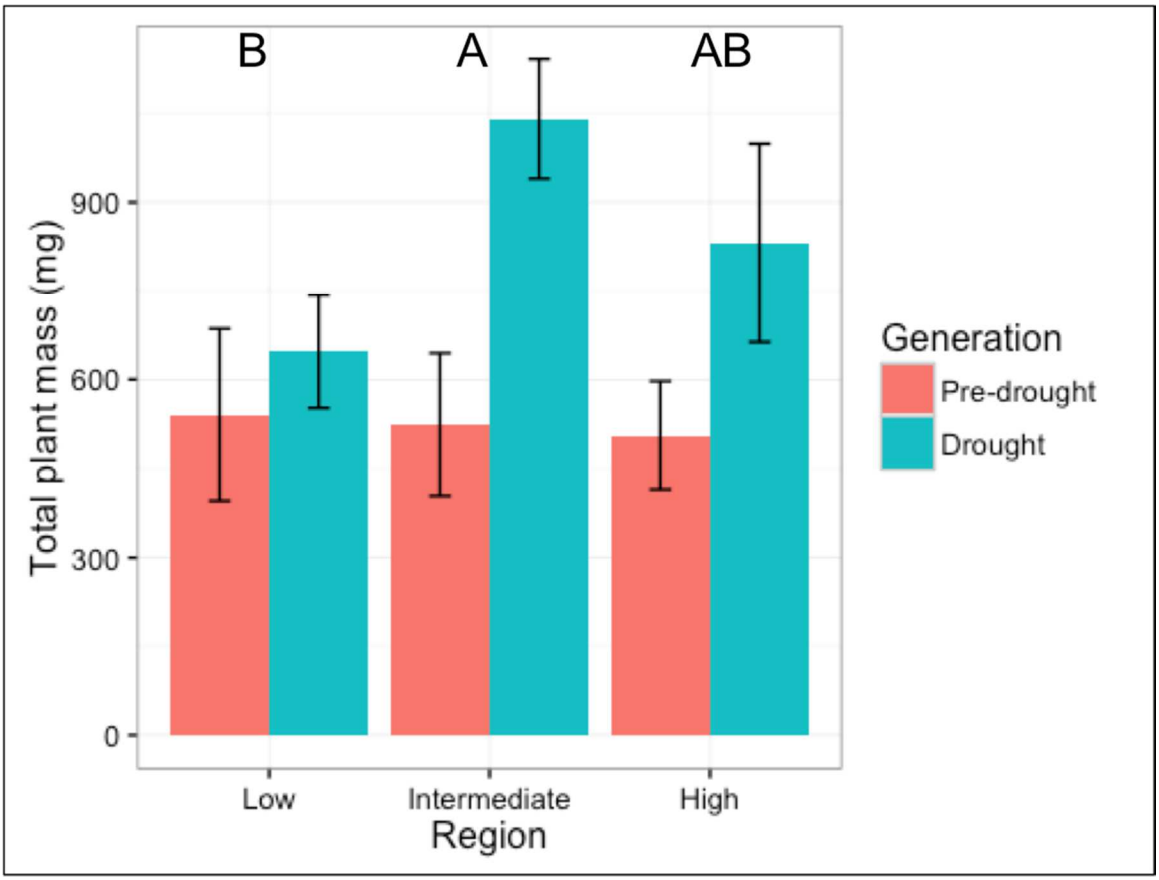

Figure 8. Mean total plant mass by region and generation. Letters represent Tukey's HSD significant differences at $P < 0.05$.

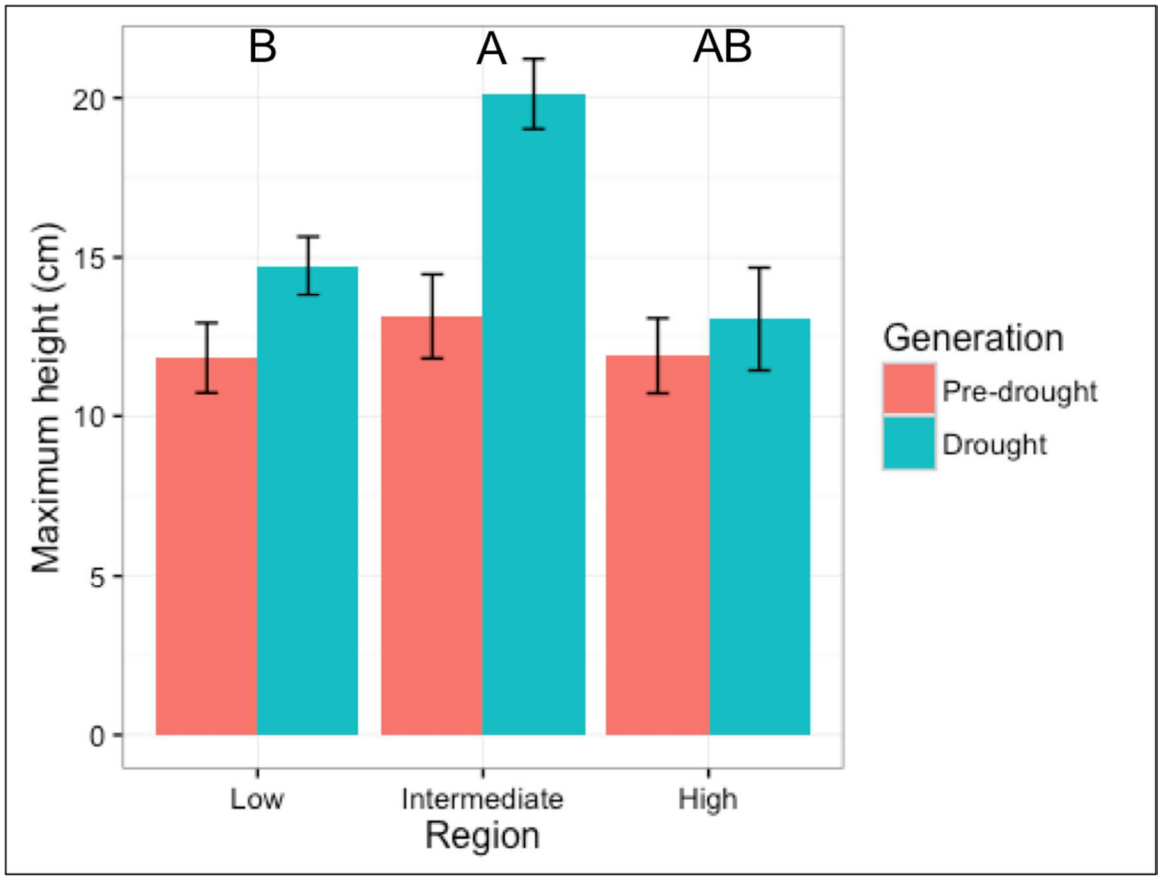

Figure 9. Mean maximum height by region and generation. Letters represent Tukey's HSD significant differences at $P < 0.05$.

TABLES

Table 1. Study population names, locations and elevations. The three lowest elevation sites have been assigned population codes of L1-3. The three intermediate sites have been assigned population codes of I1-3. The three highest elevation sites have been assigned population codes H1-3.

Population Code	Region	Locality	Latitude (DD)	Longitude (DD)	Elevation (m)
L1	Low	Zappey Property, Madera County	37.38136	-119.66533	947
L2	Low	Highway 168, Fresno County	37.03977	-119.40857	1000
L3	Low	Poopenaut Valley, Tuolumne County	37.92159	-119.81907	1020
I1	Intermediate	McLeod Flat, Madera County	37.35265	-119.56288	1280
I2	Intermediate	Hetchy Sign, Tuolumne County	37.89389	-119.84903	1400
I3	Intermediate	Jackass Meadow, Madera County	37.50694	-119.33867	2200
H1	High	May Lake, Mariposa County	37.8402	-119.49213	2774
H2	High	Mammoth Edge, Madera County	37.69661	-119.0919	3049
H3	High	Mt. Hilgard, Fresno County	37.35627	-118.86088	3095

Table 2. BCM model climate values for the water year for all study populations in pre-drought and drought seed collection years, (Ppt is precipitation; Tmax is maximum temperature; Tmin is minimum temperature).

Population	Climatic water deficit (mm)	Total water year precip. (mm)	Percent precip deviation from 30-yr mean	Mean annual Tmax (°C)	Percent Tmax deviation from 30-yr mean	Mean annual Tmin (°C)	Percent Tmin deviation from 30-yr mean	30 yr mean annual ppt (mm)	30 yr mean Tmax (°C)	30 yr mean Tmin (°C)
L1										
Ancestors	890.15	1109.58	0.33	22.34	0.00	8.39	0.12	834.47	22.26	7.50
Descendants	1037.98	394.79	-0.53	23.87	0.07	8.75	0.17			
L2										
Ancestors	508.84	1054.68	0.41	20.44	-0.03	8.81	-0.04	749.66	21.17	9.19
Descendants	1108.27	351.50	-0.53	22.69	0.07	8.75	-0.05			
L3										
Ancestors	671.34	1303.89	0.38	19.33	0.02	5.52	0.03	947.43	19.01	5.34
Descendants	757.93	511.51	-0.46	21.77	0.15	8.44	0.58			
I1										
Ancestors	886.05	1370.47	0.36	19.82	0.00	8.01	0.15	1009.46	19.74	6.99
Descendants	954.82	458.27	-0.55	21.86	0.11	7.23	0.03			
I2										
Ancestors	525.47	1353.10	0.43	17.58	-0.04	3.87	-0.07	946.39	18.30	4.16
Descendants	750.78	544.24	-0.42	19.67	0.07	6.90	0.66			
I3										
Ancestors	775.54	781.88	-0.32	14.58	0.02	3.69	0.20	1149.20	14.23	3.08
Descendants	777.78	521.05	-0.55	16.41	0.15	2.25	-0.27			
H1										
Ancestors	166.83	1985.16	0.43	11.05	0.02	-1.20	-0.53	1392.92	10.86	-2.57
Descendants	424.82	616.10	-0.56	12.29	0.13	-0.90	-0.65			
H2										
Ancestors	294.09	1066.41	0.35	9.20	-0.01	-1.01	-0.59	790.03	9.26	-2.49
Descendants	386.82	501.27	-0.37	11.39	0.23	-1.32	-0.47			
H3										
Ancestors	272.93	1372.34	0.37	8.43	-0.02	-2.52	-0.33	1003.11	8.60	-3.74
Descendants	351.34	542.81	-0.46	10.40	0.21	-2.90	-0.23			

Table 3. *M. laciniatus* plant germination by population and germination treatment (U=untreated group; GA=Gibberellic acid group; V=vernalized group).

Population	Seed collection year	Number of maternal families planted	Number of plants that grew-U	Proportion that grew-U	Number of plants that grew-GA	Proportion that grew-GA	Number of plants that grew-V	Proportion that grew-V	Total number that grew	Proportion total plants that grew
L1	2006	30	14	0.467	9	0.300	2	0.012	25	0.833
L2	2005	30	10	0.333	10	0.333	3	0.017	23	0.767
L3	2006	30	7	0.233	12	0.400	5	0.029	24	0.800
I1	2006	30	11	0.367	6	0.200	3	0.017	20	0.667
I2	2005	30	2	0.067	14	0.467	1	0.006	17	0.567
I3	2008	30	1	0.033	15	0.500	7	0.041	23	0.767
H1	2006	30	2	0.067	16	0.533	4	0.023	22	0.733
H2	2006	30	15	0.500	7	0.233	4	0.023	26	0.867
H3	2006	30	2	0.067	14	0.467	3	0.017	19	0.633
L1	2014	30	20	0.667	6	0.200	2	0.012	28	0.933
L2	2014	30	9	0.300	10	0.333	1	0.006	20	0.667
L3	2014	30	19	0.633	4	0.133	1	0.006	24	0.800
I1	2014	30	23	0.767	3	0.100	0	0.000	26	0.867
I2	2014	30	25	0.833	4	0.133	1	0.006	30	1.000
I3	2014	30	19	0.633	5	0.167	2	0.012	26	0.867
H1	2014	12	2	0.167	10	0.833	0	0.000	12	1.000
H2	2014	18	2	0.111	12	0.667	1	0.006	15	0.833
H3	2014	30	5	0.167	12	0.400	4	0.023	21	0.700
Totals		510	188	0.369	169	0.949	44	0.256	401	0.786

Table 4. *M. laciniatus* plant germination by region and germination treatment.

Region	Untreated	GA	Vernalized
Low	79	51	14
Intermediate	81	47	14
High	28	71	16
Total	**188**	**169**	**44**

Table 5. Cox proportional hazards model results for phenological data. Values in bold were significant at α= 0.05.

		Days to emergence			Days to First Flower		
Explanatory variables	Germination Treatment	DF	X^2	P value	DF	X^2	P value
Region	Untreated	2	3.814	0.149	2	12.494	**0.002**
Generation	Untreated	1	9.003	**0.003**	1	0.127	0.722
Region*Generation	Untreated	2	2.488	0.288	2	2.989	0.224
Region	GA	2	1.202	0.548	2	2.303	0.316
Generation	GA	1	0.284	0.594	1	0.028	0.868
Region*Generation	GA	2	0.938	0.626	2	1.012	0.603
Region	Vernalized	2	2.078	0.354	2	3.495	0.174
Generation	Vernalized	1	0.777	0.378	1	0.626	0.429
Region*Generation	Vernalized	2	0.630	0.730	2	3.426	0.180

Table 6. REML model results for morphological traits. Population-level results on the left and region-level results are on the right. Values in bold were significant at α= 0.05.

Population level					**Region level**				
Response variable	DF	DF Den	F Ratio	P value	Response variable	DF	DF Den	F Ratio	P value
Fruit mass					**Fruit mass**				
population	8	266.2	8.974	**<.0001**	region	2	278.5	8.913	**<.0001**
generation	1	265.6	15.383	**0.000**	generation	1	277.9	19.566	**<.0001**
generation x population	8	266.0	0.464	0.881	generation x region	2	278.1	0.789	0.455
germination treatment	2	223.4	65.719	**<.0001**	germination treatment	2	221.0	53.656	**<.0001**
Total plant mass					**Total plant mass**				
population	8	264.1	5.613	**<.0001**	region	2	276.4	4.170	**0.016**
generation	1	262.9	9.388	**0.002**	generation	1	275.4	10.707	**0.001**
generation x population	8	263.8	0.616	0.764	generation x region	2	275.9	0.565	0.569
germination treatment	2	194.2	15.705	**<.0001**	germination treatment	2	193.7	12.329	**<.0001**
Maximum height					**Maximum height**				
population	8	364.2	3.405	**0.001**	region	2	376.0	5.926	**0.003**
generation	1	262.4	4.307	**0.039**	generation	1	376.3	5.497	**0.020**
generation x population	8	364.3	0.384	0.929	generation x year	2	376.5	0.348	0.706
germination treatment	2	262.4	46.223	**<.0001**	germination treatment	2	268.4	46.329	**<.0001**
Specific leaf area					**Specific leaf area**				
population	8	160.6	1.302	0.246	region	2	173.0	2.324	0.101
generation	1	161.6	0.103	0.749	generation	1	174.0	0.201	0.654
generation x population	8	160.6	1.637	0.118	generation x region	2	172.8	1.259	0.287
germination treatment	2	106.2	31.123	**<.0001**	germination treatment	2	95.4	29.877	**<.0001**

LIST OF REFERENCES

Ackerly, D. D., W. K. Cornwell, S. B. Weiss, L. E. Flint, and A. L. Flint. 2015. A Geographic Mosaic of Climate Change Impacts on Terrestrial Vegetation: Which Areas Are Most at Risk? PloS one 10:e0130629.

Ackerly, D., C. Knight, S. Weiss, K. Barton, and K. Starmer. 2002. Leaf size, specific leaf area and microhabitat distribution of chaparral woody plants: contrasting patterns in species level and community level analyses. Oecologia 130:449–457.

Aitken, S. N., S. Yeaman, J. A. Holliday, T. Wang, and S. Curtis-McLane. 2008. Adaptation, migration or extirpation: climate change outcomes for tree populations. Evolutionary Applications 1:95–111.

Anderson, J. T., D. W. Inouye, A. M. McKinney, R. I. Colautti, and T. Mitchell-Olds. 2012. Phenotypic plasticity and adaptive evolution contribute to advancing flowering phenology in response to climate change. Proceedings of the Royal Society of London B: Biological Sciences 279:3843–3852.

Angert, A. L., H. D. Bradshaw Jr, and D. W. Schemske. 2008. Using Experimental Evolution to Investigate Geographic Range Limits in Monkeyflowers. Evolution 62:2660–2675.

Barbour, M., T. Keeler-Wolf, and A. A. Schoenherr. 2007. Terrestrial Vegetation of California, 3rd Edition. University of California Press.

Baskin, C. C., and J. M. Baskin. 2014. Seeds: ecology, biogeography, and evolution of dormancy and germination., 2nd edn.(Elsevier/Academic Press: San Diego, CA).

Bertrand, R., J. Lenoir, C. Piedallu, G. Riofrío-Dillon, P. de Ruffray, C. Vidal, J.-C. Pierrat, and J.-C. Gégout. 2011. Changes in plant community composition lag behind climate warming in lowland forests. Nature 479:517–520.

Bjorkman, A. D., M. Vellend, E. R. Frei, and G. H. Henry. 2016. Climate adaptation is not enough: warming does not facilitate success of southern tundra plant populations in the high Arctic. Global Change Biology.

Bonner, F. T., and T. E. Russell. 1974. Liriodendron tulipifera L. Seeds of woody plants of the United States. USDA For Serv, Washington, DC:508–511.

Bridle, J. R., and T. H. Vines. 2007. Limits to evolution at range margins: when and why does adaptation fail? Trends in Ecology & Evolution 22:140–147.

Cayan, D. R., E. P. Maurer, M. D. Dettinger, M. Tyree, and K. Hayhoe. 2008. Climate change scenarios for the California region. Climatic Change 87:21–42.

Clausen, J., D. D. Keck, and W. M. Hiesey. 1941. Regional Differentiation in Plant Species. The American Naturalist 75:231–250.

Cleland, E. E., I. Chuine, A. Menzel, H. A. Mooney, and M. D. Schwartz. 2007. Shifting plant phenology in response to global change. Trends in Ecology & Evolution 22:357–365.

Conner, J. K., and D. L. Hartl. 2004. A primer of ecological genetics.:xv + 304 pp.

Conover, D. O., and E. T. Schultz. 1995. Phenotypic similarity and the evolutionary significance of countergradient variation. Trends in Ecology & Evolution 10:248–252.

Conover, W. J., and R. L. Iman. 1981. Rank transformations as a bridge between parametric and nonparametric statistics. The American Statistician 35:124–129.
Cowling, R. M., P. W. Rundel, B. B. Lamont, M. Kalin Arroyo, and M. Arianoutsou. 1996. Plant diversity in mediterranean-climate regions. Trends in Ecology & Evolution 11:362–366.
Dawson, M. N., R. K. Grosberg, Y. E. Stuart, and E. Sanford. 2010. Population genetic analysis of a recent range expansion: mechanisms regulating the poleward range limit in the volcano barnacle Tetraclita rubescens. Molecular Ecology 19:1585–1605.
Dawson, T. P., S. T. Jackson, J. I. House, I. C. Prentice, and G. M. Mace. 2011. Beyond Predictions: Biodiversity Conservation in a Changing Climate. Science 332:53.
Diffenbaugh, N. S., and C. B. Field. 2013. Changes in Ecologically Critical Terrestrial Climate Conditions. Science 341:486–492.
Dirnböck, T., F. Essl, and W. Rabitsch. 2011. Disproportional risk for habitat loss of high-altitude endemic species under climate change. Global Change Biology 17:990–996.
Dolph, G. E., and D. L. Dilcher. 1980. Variation in Leaf Size with Respect to Climate in the Tropics of the Western Hemisphere. Bulletin of the Torrey Botanical Club 107:154–162.
Etterson, J. R., S. J. Franks, S. J. Mazer, R. G. Shaw, N. L. S. Gorden, H. E. Schneider, J. J. Weber, K. J. Winkler, and A. E. Weis. 2016. Project Baseline: An unprecedented resource to study plant evolution across space and time. American Journal of Botany 103:164–173.
Etterson, J. R., and S. J. Mazer. 2016. How climate change affects plants' sex lives. Science 353:32–33.
Ferris, K. G. 2014. The Genetics of Adaptation to a Harsh Granite Outcrop Environment in Mimulus.
Flint, L. E., and A. L. Flint. 2014. California Basin characterization model: a dataset of historical and future hydrologic response to climate change. US Geological Survey Dataset Release. doi 10:F76T0JPB.
Fox, G. A. 2001. Failure-time analysis. Design and analysis of ecological experiments:235–266.
Fox, J., and S. Weisberg. 2011. An {R} Companion to Applied Regression, Second Edition. Thousand Oaks CA: Sage.
Franks, S. J. 2011. Plasticity and evolution in drought avoidance and escape in the annual plant Brassica rapa. New Phytologist 190:249–257.
Franks, S. J., J. C. Avise, W. E. Bradshaw, J. K. Conner, J. R. Etterson, S. J. Mazer, R. G. Shaw, and A. E. Weis. 2008. The Resurrection Initiative: Storing Ancestral Genotypes to Capture Evolution in Action. BioScience 58:870–873.
Franks, S. J., and A. A. Hoffmann. 2012. Genetics of Climate Change Adaptation. Annual Review of Genetics 46:185–208.
Franks, S. J., S. Sim, and A. E. Weis. 2007. Rapid evolution of flowering time by an annual plant in response to a climate fluctuation. Proceedings of the National Academy of Sciences 104:1278–1282.
Friedman, J., and J. H. Willis. 2013. Major QTLs for critical photoperiod and

vernalization underlie extensive variation in flowering in the Mimulus guttatus species complex. New Phytologist 199:571–583.
Galloway, L. F., and J. R. Etterson. 2007. Transgenerational plasticity is adaptive in the wild. Science 318:1134–1136.
Germain, R. M., C. M. Caruso, and H. Maherali. 2013. Mechanisms and consequences of water stress–induced parental effects in an invasive annual grass. International Journal of Plant Sciences 174:886–895.
Grafen, A. 1988. On the uses of data on lifetime reproductive success. Reproductive success:454–471.
Guisan, A., and J.-P. Theurillat. 2000. Assessing alpine plant vulnerability to climate change: a modeling perspective. Integrated Assessment 1:307–320.
Hairston, N. G., W. Lampert, C. E. Cáceres, C. L. Holtmeier, L. J. Weider, U. Gaedke, J. M. Fischer, J. A. Fox, and D. M. Post. 1999. Lake ecosystems: Rapid evolution revealed by dormant eggs. Nature 401:446–446.
Hampe, A., and R. J. Petit. 2005. Conserving biodiversity under climate change: the rear edge matters. Ecology Letters 8:461–467.
Hargreaves, A. L., K. E. Samis, and C. G. Eckert. 2014. Are species' range limits simply niche limits writ large? A review of transplant experiments beyond the range. The American Naturalist 183:157–173.
Harrell Jr, F. E., and C. Dupont. 2016. Hmisc: Harrell Miscellaneous. R package version 3.17-4.
Harrington, J. F. 1972. Seed storage and longevity. Seed biology 3:145–245.
Harrison, S. P., E. S. Gornish, and S. Copeland. 2015. Climate-driven diversity loss in a grassland community. Proceedings of the National Academy of Sciences 112:8672–8677.
Heger, T., B. S. Jacobs, A. M. Latimer, J. Kollmann, and K. J. Rice. 2014. Does experience with competition matter? Effects of source competitive environment on mean and plastic trait expression in Erodium cicutarium. Perspectives in Plant Ecology, Evolution and Systematics 16:236–246.
Hereford, J., A. E. E. Elle, and E. M. A. Geber. 2009. A Quantitative Survey of Local Adaptation and Fitness Trade-Offs. The American Naturalist 173:579–588.
Hijmans, R. J., and J. van Etten. 2012. raster: Geographic analysis and modeling with raster data. R package version 2.0-12.
Holt, R. D., and R. Gomulkiewicz. 1997. How Does Immigration Influence Local Adaptation? A Reexamination of a Familiar Paradigm. The American Naturalist 149:563–572.
Holt, R. D., R. Gomulkiewicz, and M. Barfield. 2003. The phenomenology of niche evolution via quantitative traits in a "black-hole" sink. Proceedings of the Royal Society of London B: Biological Sciences 270:215–224.
Hothorn, T., F. Bretz, and P. Westfall. 2008. Simultaneous Inference in General Parametric Models. Biometrical Journal 50(3):346--363.
Hudson, I. L., and M. R. Keatley. 2009. Phenological Research: Methods for Environmental and Climate Change Analysis. Springer Science & Business Media.
Inouye, D. W., M. A. Morales, and G. J. Dodge. 2002. Variation in timing and abundance

of flowering by Delphinium barbeyi Huth (Ranunculaceae): the roles of snowpack, frost, and La Nina, in the context of climate change. Oecologia 130:543–550.

Ivey, C. T., and D. E. Carr. 2012. Tests for the joint evolution of mating system and drought escape in Mimulus. Annals of Botany 109:583–598.

Jonas, C. S., and M. A. Geber. 1999. Variation among populations of Clarkia Unguiculata (Onagraceae) along altitudinal and latitudinal gradients. American Journal of Botany 86:333–343.

Jump, A. S., and J. Peñuelas. 2005. Running to stand still: adaptation and the response of plants to rapid climate change. Ecology Letters 8:1010–1020.

Kirkpatrick, M., and N. H. Barton. 1997. Evolution of a Species' Range. The American Naturalist 150:1–23.

Kopp, C. W., and E. E. Cleland. 2014. Shifts in plant species elevational range limits and abundances observed over nearly five decades in a western North America mountain range. Journal of Vegetation Science 25:135–146.

Körner, C., and D. Basler. 2010. Phenology Under Global Warming. Science 327:1461–1462.

Lee-Yaw, J. A., H. M. Kharouba, M. Bontrager, C. Mahony, A. M. Csergő, A. M. Noreen, Q. Li, R. Schuster, and A. L. Angert. 2016. A synthesis of transplant experiments and ecological niche models suggests that range limits are often niche limits. Ecology letters 19:710–722.

Leimu, R., and M. Fischer. 2008. A Meta-Analysis of Local Adaptation in Plants. PLOS ONE 3:e4010.

Linhart, Y. B., and M. C. Grant. 1996. Evolutionary significance of local genetic differentiation in plants. Annual Review of Ecology and Systematics:237–277.

Loarie, S. R., B. E. Carter, K. Hayhoe, S. McMahon, R. Moe, C. A. Knight, and D. D. Ackerly. 2008. Climate change and the future of California's endemic flora. PloS one 3:e2502.

Loarie, S. R., P. B. Duffy, H. Hamilton, G. P. Asner, C. B. Field, and D. D. Ackerly. 2009. The velocity of climate change. Nature 462:1052–1055.

Lyons, K. S., K. L. Amatangelo, A. K. Behrensmeyer, A. Bercovici, J. L. Blois, M. Davis, W. A. DiMichele, A. Du, J. T. Eronen, J. Tyler Faith, G. R. Graves, N. Jud, C. Labandeira, C. V. Looy, B. McGill, J. H. Miller, D. Patterson, S. Pineda-Munoz, R. Potts, B. Riddle, R. Terry, A. Tóth, W. Ulrich, A. Villaseñor, S. Wing, H. Anderson, J. Anderson, D. Waller, and N. J. Gotelli. 2016. Holocene shifts in the assembly of plant and animal communities implicate human impacts. Nature 529:80–83.

Menzel, A., T. H. Sparks, N. Estrella, E. Koch, A. Aasa, R. Ahas, K. Alm-Kübler, P. Bissolli, O. 'ga Braslavská, A. Briede, F. M. Chmielewski, Z. Crepinsek, Y. Curnel, Å. Dahl, C. Defila, A. Donnelly, Y. Filella, K. Jatczak, F. Måge, A. Mestre, Ø. Nordli, J. Peñuelas, P. Pirinen, V. Remišová, H. Scheifinger, M. Striz, A. Susnik, A. J. H. Van Vliet, F.-E. Wielgolaski, S. Zach, and A. Zust. 2006. European phenological response to climate change matches the warming pattern. Global Change Biology 12:1969–1976.

Mooney, H. A., and E. L. Dunn. 1970. Convergent Evolution of Mediterranean-Climate

Evergreen Sclerophyll Shrubs. Evolution 24:292–303.
Ooi, M., T. Auld, and R. Whelan. 2004. Comparison of the cut and tetrazolium tests for assessing seed viability: a study using Australian native Leucopogon species. Ecological Management & Restoration 5:141–143.
Ostertag, R., L. Warman, S. Cordell, and P. M. Vitousek. 2015. Using plant functional traits to restore Hawaiian rainforest. Journal of Applied Ecology 52:805–809.
Pacifici, M., W. B. Foden, P. Visconti, J. E. M. Watson, S. H. M. Butchart, K. M. Kovacs, B. R. Scheffers, D. G. Hole, T. G. Martin, H. R. Akçakaya, R. T. Corlett, B. Huntley, D. Bickford, J. A. Carr, A. A. Hoffmann, G. F. Midgley, P. Pearce-Kelly, R. G. Pearson, S. E. Williams, S. G. Willis, B. Young, and C. Rondinini. 2015. Assessing species vulnerability to climate change. Nature Climate Change 5:215–224.
Parmesan, C. 2006. Ecological and Evolutionary Responses to Recent Climate Change. Annual Review of Ecology, Evolution, and Systematics 37:637–669.
Parmesan, C., and G. Yohe. 2003. A globally coherent fingerprint of climate change impacts across natural systems. Nature 421:37–42.
Pauli, H., M. Gottfried, T. Dirnböck, S. Dullinger, and G. Grabherr. 2003. Assessing the Long-Term Dynamics of Endemic Plants at Summit Habitats. Pages 195–207 *in* L. Nagy, G. Grabherr, C. Körner, and D. B. A. Thompson, editors. Alpine Biodiversity in Europe. Springer Berlin Heidelberg, Berlin, Heidelberg.
Peñuelas, J., and R. Matamala. 1990. Changes in N and S Leaf Content, Stomatal Density and Specific Leaf Area of 14 Plant Species during the Last Three Centuries of CO2 Increase. Journal of Experimental Botany 41:1119–1124.
Peterson, M. L., K. J. Rice, and J. P. Sexton. 2013. Niche partitioning between close relatives suggests trade-offs between adaptation to local environments and competition. Ecology and evolution 3:512–522.
Pimm, S. L., and P. Raven. 2000. Biodiversity: extinction by numbers. Nature 403:843–845.
Pujol, B., and J. R. Pannell. 2008. Reduced Responses to Selection After Species Range Expansion. Science 321:96–96.
R Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.
Rangwala, I., and J. R. Miller. 2012. Climate change in mountains: a review of elevation-dependent warming and its possible causes. Climatic Change 114:527–547.
Rapacciuolo, G., S. P. Maher, A. C. Schneider, T. T. Hammond, M. D. Jabis, R. E. Walsh, K. J. Iknayan, G. K. Walden, M. F. Oldfather, D. D. Ackerly, and S. R. Beissinger. 2014. Beyond a warming fingerprint: individualistic biogeographic responses to heterogeneous climate change in California. Global Change Biology 20:2841–2855.
Rice, K. J., J. D. G. Jr, A. R. Dyer, and J. K. McKay. 2013. Evolutionary ecology along invasion fronts of the annual grass Aegilops triuncialis. Biological Invasions 15:2531–2545.
R.K. Pachauri and L.A. Meyer (eds.)]. 2014. IPCC, 2014: Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth

Assessment Report of the Intergovernmental Panel on Climate Change. Page 151 pp. IPCC, Geneva, Switzerland.

Roach, D. A., and R. D. Wulff. 1987. Maternal Effects in Plants. Annual Review of Ecology and Systematics 18:209–235.

Robeson, S. M. 2015. Revisiting the recent California drought as an extreme value. Geophysical Research Letters 42:2015GL064593.

Root, T. L., J. T. Price, K. R. Hall, S. H. Schneider, C. Rosenzweig, and J. A. Pounds. 2003. Fingerprints of global warming on wild animals and plants. Nature 421:57–60.

Saavedra, F., D. W. Inouye, M. V. Price, and J. Harte. 2003. Changes in flowering and abundance of Delphinium nuttallianum (Ranunculaceae) in response to a subalpine climate warming experiment. Global Change Biology 9:885–894.

Sagarin, R. D., and S. D. Gaines. 2002. The "abundant centre"distribution: to what extent is it a biogeographical rule? Ecology letters 5:137–147.

Sagarin, R. D., S. D. Gaines, and B. Gaylord. 2006. Moving beyond assumptions to understand abundance distributions across the ranges of species. Trends in ecology & evolution 21:524–530.

Sala, O. E., F. S. Chapin, Iii, J. J. Armesto, E. Berlow, J. Bloomfield, R. Dirzo, E. Huber-Sanwald, L. F. Huenneke, R. B. Jackson, A. Kinzig, R. Leemans, D. M. Lodge, H. A. Mooney, M. Oesterheld, N. L. Poff, M. T. Sykes, B. H. Walker, M. Walker, and D. H. Wall. 2000. Global Biodiversity Scenarios for the Year 2100. Science 287:1770–1774.

Sandring, S., and J. Ågren. 2009. Pollinator-Mediated Selection on Floral Display and Flowering Time in the Perennial Herb Arabidopsis Lyrata. Evolution 63:1292–1300.

Schneider, C. A., W. S. Rasband, and K. W. Eliceiri. 2012. NIH Image to ImageJ: 25 years of image analysis. Nature Methods 9:671–675.

Schneider, H. E., and S. J. Mazer. 2016. Geographic variation in climate as a proxy for climate change: Forecasting evolutionary trajectories from species differentiation and genetic correlations. American Journal of Botany 103:140–152.

Sexton, J. P., and E. E. Dickman. 2016. What can local and geographic population limits tell us about distributions? American Journal of Botany.

Sexton, J. P., M. B. Hufford, A. Bateman, D. B. Lowry, H. Meimberg, S. Y. Strauss, and K. J. Rice. 2016. Climate structures genetic variation across a species' elevation range: a test of range limits hypotheses. Molecular ecology.

Sexton, J. P., S. Y. Strauss, and K. J. Rice. 2011. Gene flow increases fitness at the warm edge of a species' range. Proceedings of the National Academy of Sciences 108:11704–11709.

Shaw, R. G. 1987. Maximum-Likelihood Approaches Applied to Quantitative Genetics of Natural Populations. Evolution 41:812–826.

Sheridan, J. A., and D. Bickford. 2011. Shrinking body size as an ecological response to climate change. Nature Climate Change 1:401–406.

Sheth, S. N., and A. L. Angert. 2015. Artificial Selection Reveals High Genetic Variation in Phenology at the Trailing Edge of a Species Range. The American Naturalist 187:182–193.

Springer, C. J., and J. K. Ward. 2007. Flowering time and elevated atmospheric CO2. New Phytologist 176:243–255.

Stephenson, N. 1998. Actual evapotranspiration and deficit: biologically meaningful correlates of vegetation distribution across spatial scales. Journal of Biogeography 25:855–870.

Sultan, S. E., T. Horgan-Kobelski, L. M. Nichols, C. E. Riggs, and R. K. Waples. 2013. A resurrection study reveals rapid adaptive evolution within populations of an invasive plant. Evolutionary Applications 6:266–278.

Swain, D. L., M. Tsiang, M. Haugen, D. Singh, A. Charland, B. Rajaratnam, and N. S. Diffenbaugh. 2014. The extraordinary California drought of 2013/2014: Character, context, and the role of climate change. Bulletin of the American Meteorological Society 95:S3.

Therneau, T. 2015. A Package for Survival Analysis in S_. Version 2.38.

United States Geological Survey. 2016. Explanations for the National Water Conditions. http://water.usgs.gov/nwc/explain_data.html.

Urban, D. L., C. Miller, P. N. Halpin, and N. L. Stephenson. 2000. Forest gradient response in Sierran landscapes: the physical template. Landscape Ecology 15:603–620.

Vergeer, P., and W. E. Kunin. 2013. Adaptation at range margins: common garden trials and the performance of Arabidopsis lyrata across its northwestern European range. New Phytologist 197:989–1001.

Walther, G.-R., E. Post, P. Convey, A. Menzel, C. Parmesan, T. J. C. Beebee, J.-M. Fromentin, O. Hoegh-Guldberg, and F. Bairlein. 2002. Ecological responses to recent climate change. Nature 416:389–395.

Wang, Q., X. Fan, and M. Wang. 2013. Recent warming amplification over high elevation regions across the globe. Climate Dynamics 43:87–101.

Wolf, A., N. B. Zimmerman, W. R. L. Anderegg, P. E. Busby, and J. Christensen. 2016. Altitudinal shifts of the native and introduced flora of California in the context of 20th-century warming. Global Ecology and Biogeography 25:418–429.

Wookey, P. A., R. Aerts, R. D. Bardgett, F. Baptist, K. A. Bråthen, J. H. C. Cornelissen, L. Gough, I. P. Hartley, D. W. Hopkins, S. Lavorel, and G. R. Shaver. 2009. Ecosystem feedbacks and cascade processes: understanding their role in the responses of Arctic and alpine ecosystems to environmental change. Global Change Biology 15:1153–1172.

APPENDICIES

Appendix A- Climatic variation among generations

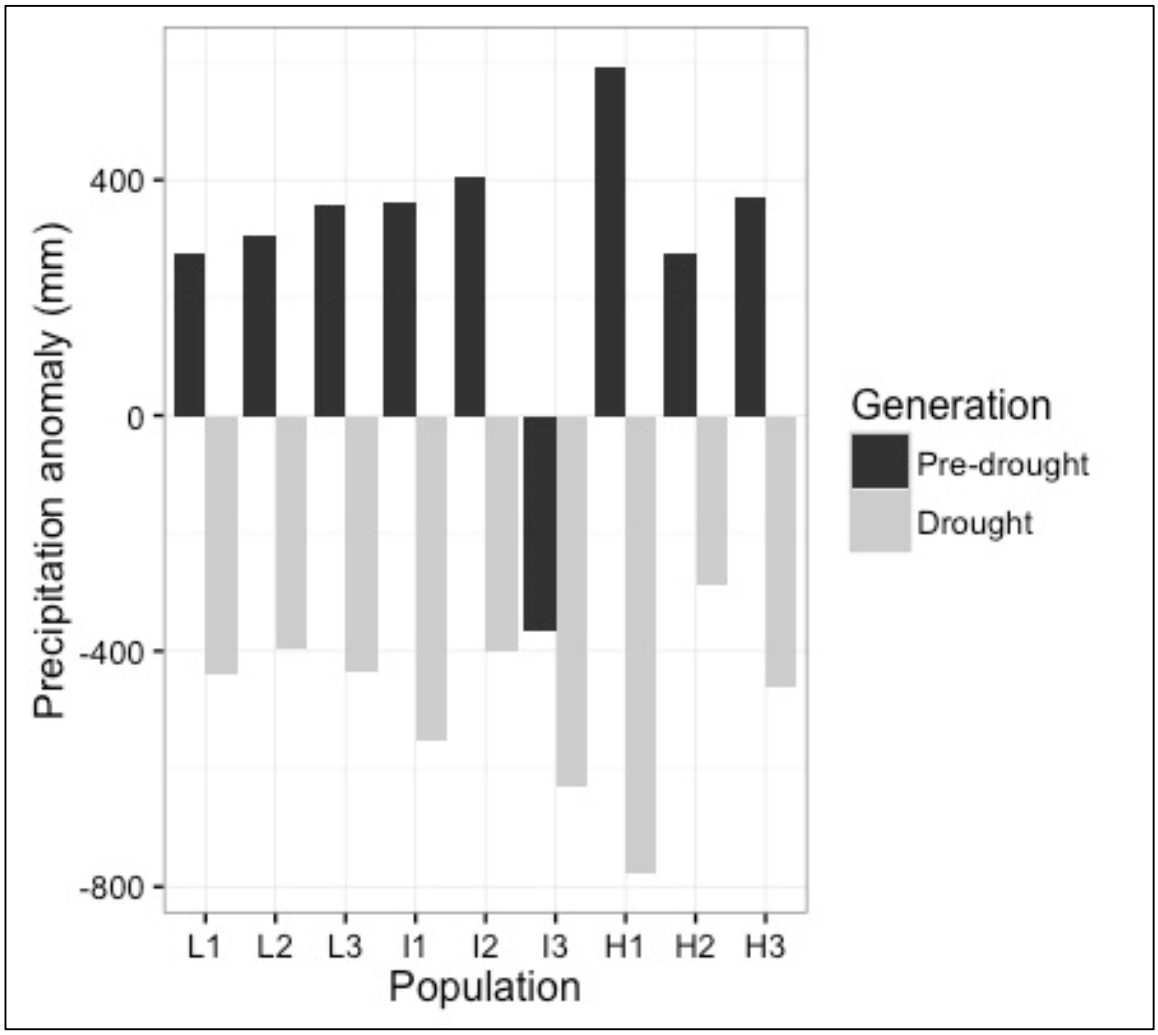

Figure A1. Precipitation anomaly for populations by generation, relative to the 30-year mean for each population.

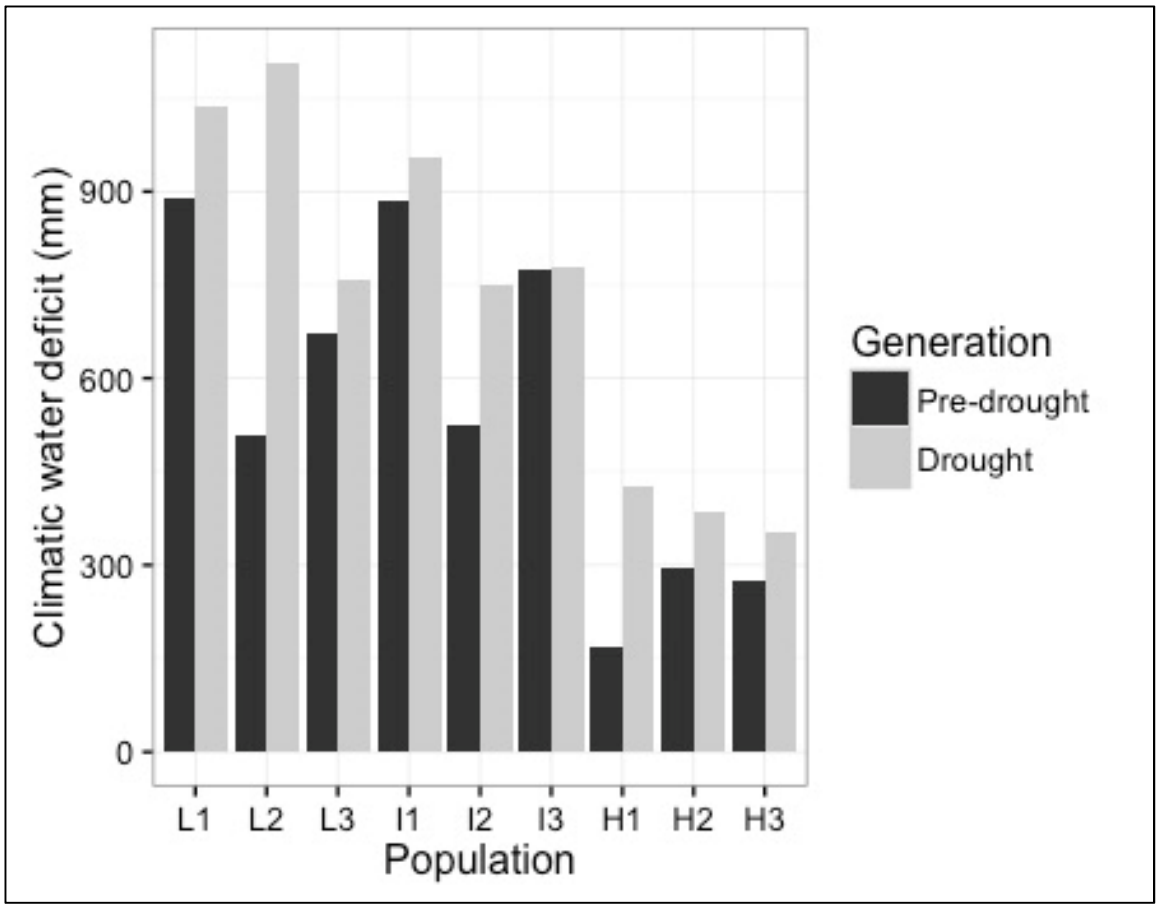

Figure A2. Climatic water deficit (CWD; mm) for populations by generation.

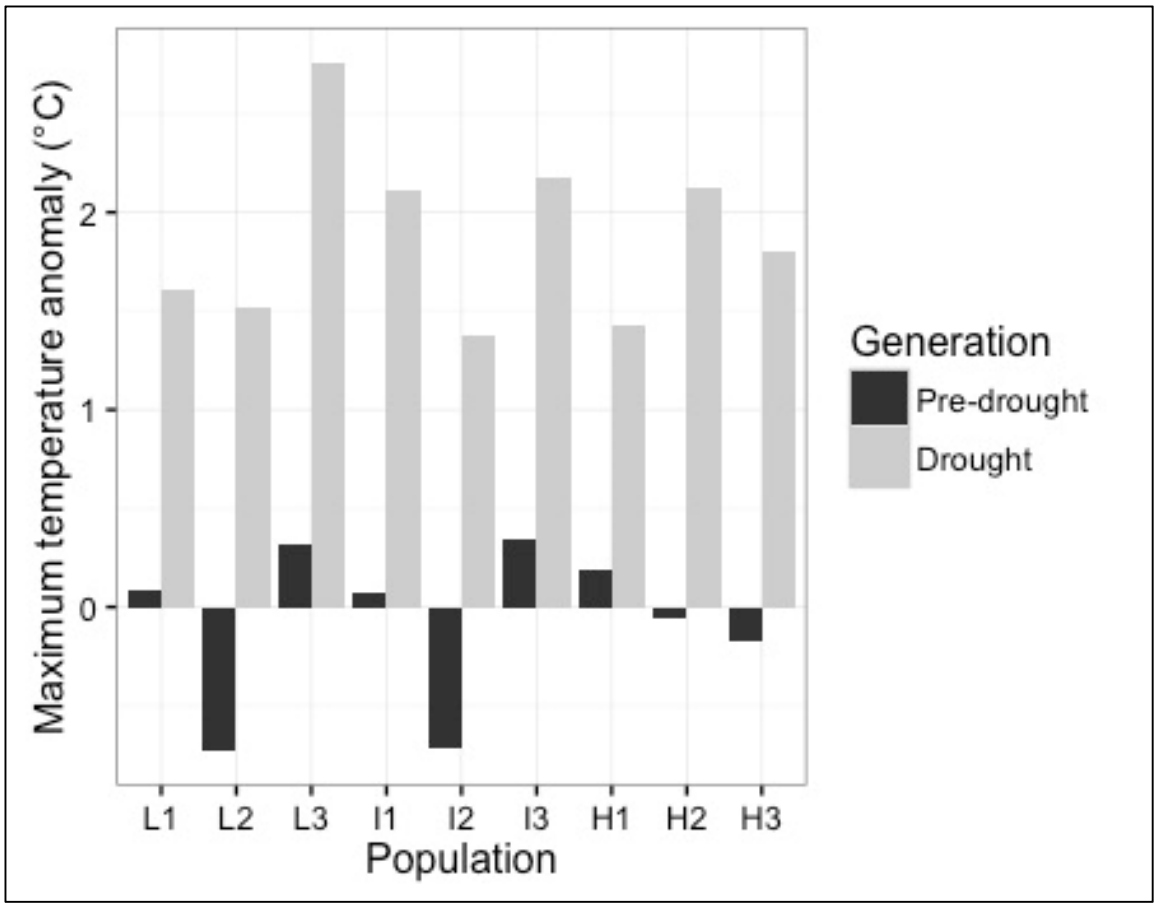

Figure A3. Maximum temerature anomaly for populations by generation, relative to the 30-year mean for each population.

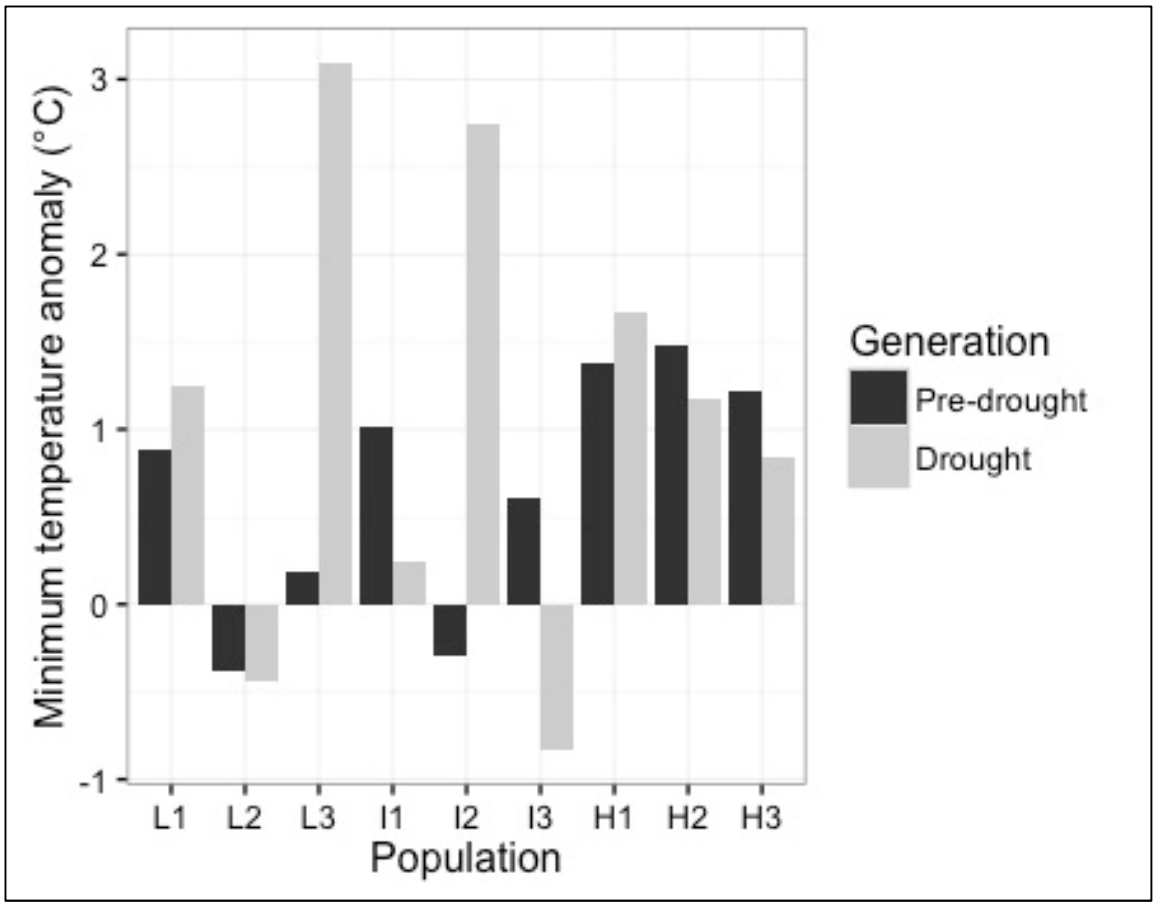

Figure A4. Minimum temperature anomaly for populations by generation, relative to the 30-year mean for each population (represented by 0 on y-axis).

Appendix B- Phenological traits statistics and results

Table B1. Levene's test results for phenology data. Values in bold were significant at α= 0.05.

			Days to Emergence			Days to First Flower		
Dataset	Explanatory Variable	Germination treatment	DF	F ratio	P value	DF	F ratio	P value
All	Region	Untreated	2	0.629	0.534	2	7.387	**0.001**
All	Generation	Untreated	1	5.143	**0.025**	1	0.327	0.568
Low region	Generation	Untreated	1	3.230	0.076	1	0.008	0.931
Intermediate region	Generation	Untreated	1	4.542	**0.036**	1	0.818	0.369
High region	Generation	Untreated	1	0.690	0.411	1	0.022	0.884
All	Region	GA	2	0.515	0.598	2	0.053	0.949
All	Generation	GA	1	1.145	0.286	1	0.147	0.703
Low region	Generation	GA	1	0.121	0.730	1	0.304	0.586
Intermediate region	Generation	GA	1	0.022	0.883	1	1.708	0.206
High region	Generation	GA	1	4.019	**0.049**	1	1.008	0.322
All	Region	Vernalized	2	0.309	0.736	2	0.066	0.936
All	Generation	Vernalized	1	0.025	0.876	1	2.720	0.119
Low region	Generation	Vernalized	1	0.381	0.549	1	1.473	0.312
Intermediate region	Generation	Vernalized	1	0.571	0.465	1	0.938	0.377
High region	Generation	Vernalized	1	2.406	0.143	1	0.542	0.515

Table B2. Phenological traits CV test results.

Dataset	Germination treatment	Days to Emergence	Days to First Flower
		CV	CV
All	Untreated	59.595	60.975
Pre-drought	Untreated	77.318	55.051
Drought	Untreated	38.319	63.068
Low region	Untreated	62.876	43.417
Intermediate region	Untreated	59.486	63.395
High region	Untreated	50.104	36.676
All	GA	82.012	45.400
Pre-drought	GA	81.118	44.279
Drought	GA	83.128	47.774
Low region	GA	83.303	49.704
Intermediate region	GA	82.342	40.229
High region	GA	81.249	44.881
All	Vernalized	119.735	50.405
Pre-drought	Vernalized	126.564	44.741
Drought	Vernalized	90.600	21.027
Low region	Vernalized	143.910	42.216
Intermediate region	Vernalized	117.137	41.603
High region	Vernalized	83.951	69.673

Appendix C- Morphological traits statistics and results

Table C1. Pearson correlation matrix for morphological traits. Values in bold were significant at α= 0.05.

	Total plant mass	Fruit mass	Number of fruits	Non-reproductive biomass	Maximum height	Specific leaf area
Total plant mass	1	**0.84**	0.59	**0.99**	0.71	**-0.96**
Fruit mass	**0.84**	1	**0.91**	0.75	0.75	-0.78
Number of fruits	0.59	**0.91**	1	0.47	0.47	-0.47
Non-reproductive biomass	**0.99**	0.75	0.47	1	0.67	**-0.96**
Maximum height	0.71	0.75	0.47	0.67	1	**-0.84**
Specific leaf area	**-0.96**	-0.78	-0.47	**-0.96**	**-0.84**	1

Table C2. Levene's test results for morphology data. Values in bold were significant at α= 0.05**.**

		Fruit mass (mg)			Total plant mass (mg)			Maximum height (cm)			Specific Leaf Area		
Dataset	Explanatory Variable	DF	F ratio	P value	DF	F ratio	P value	DF	F ratio	P value	DF	F ratio	P value
All	Population	8	1.6720	0.1050	8	2.4254	**0.0151**	8	2.3269	**0.0190**	8	0.9292	0.4938
All	Region	2	0.4561	0.6342	2	0.2510	0.7782	2	1.1116	0.3301	2	0.5766	0.5628
All	Generation	1	2.3120	0.1295	1	0.0445	0.8331	1	0.1016	0.7501	1	0.0678	0.7949
Low region	Generation	1	4.1620	**0.0438**	1	<.0001	0.9918	1	0.5563	0.4570	1	0.7424	0.3916
Intermediate region	Generation	1	2.8195	0.0960	1	0.4097	0.5235	1	3.3166	0.0708	1	0.0400	0.8421
High region	Generation	1	1.4352	0.2351	1	1.2712	0.2636	1	1.2033	0.2751	1	2.6276	0.1140

Table C3. Morphological traits CV statistics.

	Fruit mass (mg)	Total plant mass (mg)	Maximum height (cm)	SLA
Dataset	CV	CV	CV	CV
All morphology data	111.9498	118.6080	68.9049	194.0588
Pre-drought	133.6519	156.2648	77.3412	206.5125
Drought	91.9971	96.6076	60.2538	185.8650
Low region	140.4152	147.8135	64.8086	211.6658
Intermediate region	86.2970	97.3062	61.1271	63.6856
High region	108.0433	114.2578	81.3996	155.8741
L1	116.4031	101.8528	52.0764	42.0976
L2	73.7699	102.2845	67.4955	80.1551
L3	106.7993	119.6724	69.1438	227.7043
I1	85.5708	93.4461	45.0279	35.7055
I2	82.6010	83.9614	57.0504	55.9627
I3	77.6642	77.7150	74.7115	105.4403
H1	143.4157	143.1236	82.1052	172.1403
H2	91.1232	101.6042	80.1141	43.0823
H3	94.6654	99.2327	82.5949	83.9510