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Divergent phenological, morphological, and fitness responses to climate and herbivory across elevations in a native California wildflower

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Divergent phenological, morphological, and fitness responses to climate and herbivory across elevations in a native California wildflower

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ABSTRACT

Plants vary in their phenological and morphological responses to abiotic and biotic conditions along environmental gradients like elevation and latitude, and this variation often reflects local adaptation. However, rapid climate change poses new challenges for many biological systems, threatening population viability, species persistence, and diversity. In this dissertation, I investigated phenological, morphological, and fitness responses to climate and herbivory across elevations in the native California wildflower, *Streptanthus tortuosus*. In Chapter 1, I explored relationships between snowmelt timing, reproductive phenology, and fitness along a steep elevation gradient at Lassen Volcanic National Park. My findings show that flowering phenology generally tracks snowmelt timing but suggest that populations may be approaching their limits in plastic phenological responses to snowmelt timing. Furthermore, fitness is generally maximized at earlier snowmelt times unless warm, wet conditions extend the growing season. In Chapter 2, I explored responses to herbivory and drought in 8 populations that vary in elevation, climate, and herbivory pressure at their home environment. I conducted a common garden experiment manipulating simulated herbivory and drought and measured phenological and fitness responses. I found that individuals from lower elevations display higher herbivory tolerance, especially when water availability is high. These patterns are consistent with local adaptation along an elevation gradient and are best explained by herbivory pressure and growing season length experienced by populations in the field. In Chapter 3, I studied variation in morphological traits along elevation and latitudinal gradients across the species range. Using phenotypic and genomic data collected and sequenced from 20 populations across climatic and elevation gradients grown in a common garden, I found that morphological traits vary in tandem along multiple parallel elevation gradients. Specifically, individuals at low elevations tend to be taller with thinner leaves and fewer branches, while plants at high elevations are shorter with thicker leaves and more branches. These results are concordant with a large body of literature describing a fast-slow continuum in morphological traits across elevations. Overall, my dissertation research demonstrates that *S. tortuosus* populations display variation in phenological, morphological, and fitness responses to precipitation and herbivory regimes across elevations and reveals patterns consistent with local adaptation. Under continued climate change, low elevation *S. tortuosus* populations may be better equipped to cope with herbivory and drought than high elevations, though high elevation populations may be robust to expected changes in the short term. The presence of extensive genetically based intraspecific trait variation may aid *S. tortuosus* in adaptive evolutionary response to climate change; alternatively, a lack of gene flow from warm-adapted low elevation populations may hinder evolutionary rescue as populations at high elevations experience warmer temperatures and increased drought stress. Accurate and robust predictions of responses to ongoing changes in environmental conditions will require further research investigating intraspecific phenological, morphological, and fitness responses in variable environments.

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CHAPTER 1

Earlier snowmelt advances flowering with varying consequences for fitness mediated by growing season conditions in a native California wildflower

INTRODUCTION

Climate change has affected the timing of seasonal life history events, or phenology, across taxa and ecosystems (Parmesan & Yohe, 2003). Climate-induced shifts in snowmelt timing have profoundly influenced growing season conditions, flowering phenology, and fitness for plants in alpine regions (Høye et al., 2007; Inouye, 2020), environments that are especially sensitive and vulnerable to climate change impacts (Høye et al., 2007; Nogués-Bravo et al., 2007). Although shifts to earlier flowering in response to climate change have been well documented (Parmesan & Yohe, 2003; Sherry et al., 2007; Bertin, 2008), phenological responses to climate change vary considerably among species (Parmesan, 2007; Crimmins et al., 2010; CaraDonna et al., 2014) depending on factors like sensitivity to climate and extent of warming (e.g., Love & Mazer, 2021). Species that fail to shift their reproductive phenology in response to changing climatic conditions are at greater risk of decline (Willis et al., 2008; Thomson, 2010). By assessing how variation in climate affects phenological shifts across environmental gradients, we can gain insight into the potential for climate change to affect population persistence while also identifying populations most at risk to these factors.

High elevation environments are a useful study system for evaluating climate change responses. Abiotic conditions on mountains are highly heterogeneous and can vary greatly over short spatial and temporal scales, and within and among seasons (Stanton et al., 1994). Alpine regions have experienced declines in snow cover extent, mass, and duration as well as earlier seasonal snowmelt (Mote et al., 2005; Huning & AghaKouchak, 2018; Lute et al., 2022). Further, snowpack levels are projected to continue to decrease by >50% in the western US by the end of the century (Lute et al., 2022). Snowpack and snowmelt timing are key abiotic factors influencing the growing season conditions for plants in alpine ecosystems, and these environmental characteristics interact to affect local patterns of phenology (Galen & Stanton, 1993; Stanton et al., 1997; Anderson, Inouye, et al., 2012). Timing of snowmelt also influences abiotic conditions such as the temperature, photoperiod, and water availability individuals experience during growth and reproduction (Inouye et al., 2002; Wadgymar et al., 2018). Despite heterogeneity in abiotic conditions on mountains, overall patterns in snowmelt timing and temperature are generally correlated with elevation in predictable ways, with warmer low elevation localities accumulating less snow and experiencing earlier snowmelt (Hunsaker et al., 2012; Vorkauf et al., 2021). Because snowmelt timing typically covaries with temperature and other abiotic variables across elevation gradients, disentangling the effects of proximate factors through which climate change affects phenology and fitness can be difficult (Price & Waser, 1998; Keller & Körner, 2003; Wadgymar et al., 2018). Given evidence for diverging plant responses to temperature and snowmelt (Iler et al., 2013), evaluating plant responses in a system in which snowmelt is partially decoupled from temperature can allow for a better understanding of plant responses to particular seasonal cues.

Flowering phenology often tracks snowmelt timing (Høye et al., 2007; Inouye, 2008; Hülber et al., 2010; Iler et al., 2013; Anderson & Gezon, 2015), and as snowpack declines, earlier flowering in response to earlier snowmelt has been widely observed (Lambert et al., 2010; Kudo, 2020). However, the extent of phenological responses to climate change varies across species depending on the magnitude of change in cues that plants use to time reproduction and sensitivity to those cues (Wadgymar et al., 2018). Studies have also demonstrated nonlinear phenological responses to climate change (Inouye, 2008; Steltzer et al., 2009; Iler et al., 2013, 2017), particularly a pattern in which phenological responses become less sensitive with increasing change. These studies attribute such nonlinear responses to the rate of snowmelt timing exceeding the ability for phenology to track change, indicating that populations may be reaching a threshold in their ability to respond. Evaluating the fitness consequences of such nonlinear responses, which are expected to occur more frequently with ongoing climate change, will have important implications for population persistence as environmental conditions continue to shift (Steltzer et al., 2009; Iler et al., 2017). Snow can also indirectly affect flowering timing responses through effects on and interactions with other proximal cues for flowering like temperature and photoperiod (Keller & Körner, 2003). Snowpack levels may indirectly influence phenology by dictating water availability and length of the snow-free period during the following growing season (Sloat et al., 2015; Huning & AghaKouchak, 2018). For example, low snowpack and earlier snowmelt may be associated with increased drought stress, which can in turn affect selection on flowering timing.

Fitness responses to changing snowpack and phenological shifts may vary considerably (Abeli et al., 2012; Campbell, 2019; Anderson & Wadgymar, 2020). For example, individuals flowering earlier can be more vulnerable to damaging frosts (Inouye, 2008) or may have less time to allocate growth to reproduction and flower at a smaller size, resulting in lower fecundity (Colautti et al., 2017). Alternatively, advancing flowering timing could increase performance by allowing plants to complete reproduction before they are exposed to herbivory, drought, or late-season frost (Pilson, 2000; Franke et al., 2006; Giménez-Benavides et al., 2007). Indeed, studies have demonstrated declines in vital rates associated with flowering either too early or too late (Inouye, 2000; Colautti et al., 2017). Growing season conditions also strongly mediate fitness consequences of phenological responses. For example, theory regarding optimal flowering timing posits that short seasons select for early flowering and long seasons select for later flowering, due to a tradeoff between flowering early at a small size when conditions are favorable or flowering later at a larger size at risk of encountering unfavorable late season conditions (Weis et al. 2014). Alternatively, one might expect to observe an intermediate fitness optimum as expected by life history theory, which predicts stabilizing selection on age at reproduction, particularly when variation in conditions produces fluctuating selection over time (McGlothlin, 2010). For plant species that require outcrossing for reproduction, fitness may be optimized at intermediate flowering times when one would expect flowering synchrony and therefore pollinator visitation and availability to be at its highest (Augspurger, 1981; Wolowski et al., 2017). Elucidating the links between snow regime, plant phenology, and fitness is critical for understanding biological response to climate change in snow-dominated regions.

In this study, we explore the roles of snow and temperature in cueing reproductive phenological response and the subsequent consequences for fitness in the native California forb *Streptanthus tortuosus* (Brassicaceae). This species is ideal for studying life history responses to climate change because it exhibits remarkable variation in life history strategy both within and across populations and a large elevational range. This variation is strongly linked to temperature and precipitation cues (Gremer et al., 2020a; b), both of which are predicted to shift in California with climate change (Wright et al., 2016; Pathak et al., 2018; Swain et al., 2018; Luković et al., 2021). We examine how snowmelt and growing season conditions are correlated with phenology and fitness across four populations along an elevational and snowmelt gradient at Lassen Volcanic National Park over three years. The high interannual variation in seasonal conditions as well as partial decoupling of snowmelt and temperature across elevations due to unusually warm temperatures at high elevations in this study system offers an opportunity to explore plant responses to climate across populations and time. Specifically, we address the following questions: (1) How does flowering timing vary in relation to snowmelt timing?, and

(2) What are the consequences of snowmelt timing and phenology for fitness? We also seek to understand how these relationships vary among years. We predicted that phenology would track snowmelt timing, with individuals experiencing early snowmelt flowering earlier than those in late-melt patches. We expected that individuals in late-snowmelt patches that flower later in the season would have lower fitness due to a restricted growing season and exposure to unfavorable late-season conditions like drought and frost. Consistent with life history theory, we expected an intermediate fitness optimum for flowering phenology across time, especially if there is high interannual variation in growing season length (McGlothlin, 2010) and because this is a primarily outcrossing species (Preston, 1990; 1991).

METHODS

Study species and populations

Streptanthus tortuosus Kellogg (Brassicaceae) is a native forb that inhabits dry, rocky slopes and outcrops from southern California to southern Oregon (Preston, 1991; Calflora, 2014). Across its range, *S. tortuosus* populations tend to be discontinuously distributed and are located across a broad elevational range from 200 m to 4100 m (Calflora, 2014). All populations experience a Mediterranean climate characterized by winter precipitation and hot, dry summers, and are exposed to strong intra- and inter-annual variability in temperature and precipitation across the species range. (Baldwin, 2014; Rundel et al., 2016).

We conducted an observational field study from 2018-2020 investigating the relationship between variation in snowpack and temperature with phenology and fitness at Lassen Volcanic National Park (Lassen hereafter; Shasta County, CA; 40.46660, -121.50811) located in northern California at the southern end of the Cascade Range. For this study, we focus on four high elevation *S. tortuosus* populations (populations numbered 1-4 from low to high elevation, Figure

1.1, Suppl. 1.1). In Lassen populations, most individuals display a biennial life history in which individuals germinate after snowmelt, grow throughout the summer, and overwinter as rosettes that flower in the spring or summer of their second year; rarely, some individuals will continue to survive and flower in future seasons as iteroparous perennials (E. Suglia, *unpubl. data*). Lassen is an active volcanic region with precipitation occurring primarily as snowfall, with snow cover typically lasting from the onset of precipitation in the fall or winter through spring to summer (November - June), including some permanent patches of snow at higher elevations (Dove et al., 2012).

Populations experience interannual variation in snowpack (Suppl. 1.1) and maximum temperature (Suppl. 1.1), resulting in variation in climatic water deficit (cwd; a cumulative metric for drought stress) (Suppl. 1.1) across an elevation gradient, making this study system useful for exploring plant responses to climate change. To characterize variation in snowmelt and seasonal temperatures across populations along the elevational gradient, we deployed temperature sensors (Thermochron iButton DS1921G-F5#, Embedded Data Systems, Lawrenceberg, KY, USA) buried ~3 cm at each site (n=2-16) to measure soil temperature every 3 hours. Since soil temperatures stabilize at 0°C under snowpack, we quantified the snowmelt date as the first day on which temperatures began to vary from 0°C (Lute et al., 2022). There were occasional brief periods of snow cover after snowmelt (<2 days total, indicated by temperatures re-stabilizing around 0°C), likely due to wind re-depositing snow, but otherwise plots were melted out for the rest of the season. In 3 of 36 plot-year combinations, soil temperature data were lacking, in which case, we estimated snowmelt date using data from geographically and topographically similar plots (Suppl. 1.1). We defined snow cover date as the first 24-hour period when temperatures stabilized to 0°C (when the standard deviation of 8 consecutive temperature readings was <1). We calculated snow cover date for fall 2018 and 2019 and compared our metrics with those calculated from snowpack data from a Lassen weather station (Lower Lassen Peak Station; 40.46660, -121.50811; 2541 m a.s.l.; CDEC 2022), in which snow cover day of year was defined as the first day of the first period of 5 consecutive days with snow depth greater than 2 inches (Lute et al., 2022). The metrics corresponded well across datasets, with 0-4 days of discrepancy between metrics calculated from our temperature data versus the weather station data each year.

At Lassen, snowmelt timing does not correlate linearly with elevation: during the years of our study snowpack melted last at a mid-elevation population (3) and earlier at low and high elevation extremes (Figure 1.1, see *Results*), possibly owing to volcanic thermal warming of the latter. When we analyzed the relationship between snowmelt and elevation using a model including elevation and year as main effects, a quadratic effect of elevation on snowmelt timing and the interaction between the quadratic effect and year, with year coded as a factor were significant.

To further characterize local abiotic conditions in relation to snowmelt timing, we quantified growing season conditions experienced by individuals using temperature sensor data and downscaled climate data for 2018 - 2020 (California Basin Characterization Model, CA BCM, Flint et al., 2021; 2023). We calculated growing season length as days from snowmelt to snow cover for each population in each year. We also calculated average maximum and minimum temperatures, Growing Degree Days (GDD), and accumulated photoperiod experienced during the time from snowmelt to mean peak flowering for each population every year (Suppl. 1.2). Average maximum and minimum temperatures were calculated from daily site level averages. In 2019, temperature data was missing from DOY 207-216 at population 1 (the lowest elevation site) due to a lost sensor, so we estimated temperatures during those days using average differences between temperatures at that site versus those at the nearest location with a temperature sensor (at population 2; the second-lowest elevation site). GDD is defined as the amount of heat units accumulated over a threshold base temperature needed for plant growth by the average peak flowering time per population per year. We calculated GDD by extrapolating from daily maxima and minima temperature for each population using the R package pollen (*gdd* function; set base temperature = 5°C and maximum base temperature = 45°C; Nowosad 2019). We defined accumulated photoperiod as summed day length values from the day of snowmelt to mean peak flowering for each population (calculated using the *daylength* function in package chillR; Luedeling 2022). We quantified drought stress using Climatic Water Deficit (cwd) across the snow-free period each year. cwd was calculated as the evaporative demand that exceeds available water (potential evapotranspiration - actual evapotranspiration; in units of mm H_2O) (Flint et al. 2021) and was estimated using downscaled climate data (Basin Characterization Model, BCM, Flint et al., 2021) for each population summed across months of the snow-free growing season (May - November in 2018 & 2020; June - November in 2019).

We analyzed variation in these growing season conditions across populations and years using Principal Components Analysis (PCA, *prcomp* function in base R), and tested for differences using PERMANOVA on Bray-Curtis dissimilarities in growing season conditions with 999 permutations (*adonis2* function in R vegan package; Oksanen et al. 2017) (Table 1.1, Figure 1.1b, Suppl. 1.2). We conducted all analyses in R statistical software, version 4.2.1 (R Core Team, 2023).

Flowering phenology and fitness

To measure plant phenology and fitness, we tracked 30-100 flowering plants per population per year that we individually marked with bird bands or aluminum tags at each of four populations by establishing 2-6 plots containing 1-2 subplots each. Due to the patchiness of plant distribution across years, we assigned subplot locations in a non-random, haphazard fashion each year to sample flowering individuals in the area, randomly sampling up to 100 individuals per subplot. Plot locations remained constant from year to year except when there were insufficient numbers of flowering individuals within a plot, at which time plots were expanded to encompass additional subplots (within 20m of original plot). Plot sizes ranged from 5-10m x 10-20m and were marked with flagging tape tied to nails at each corner.

To quantify phenology, we counted floral buds, open flowers, and successful fruits (those visibly containing seeds) every 7-10 days throughout the growing season (Suppl. 1.4). We defined peak flowering time as the day on which individuals had the greatest number of open flowers (Schemske, 1977; Hof et al., 1999), and recorded first and last flowering as the day of first and last observed open flower, respectively. We measured fecundity by counting the number of fruits throughout the growing season each year and used maximum fruit set per individual as an estimate of seed production. Individuals had dynamic patterns of fruit development and dehiscence throughout the season, so using this maximum flower or fruit counts metric as a proxy for fecundity is a useful approximation of maximum potential for successful offspring despite fluctuation in numbers due to fruit loss and gain over time.

To analyze relationships between snowmelt timing and peak flowering time and whether responses varied across years, we used mixed models including scaled snowmelt day of year and year as main effects, as well as their interaction. To account for structure in the data based on location, we included random effects for plot nested within population. We tested several different flowering time responses (first, peak, and last flowering), but all results were qualitatively similar, so we chose peak flowering (date on which the population had the maximum number of viable flowers) as our primary metric for flowering time because timing of peak flowering offers insights about flowering synchrony. We also explored nonlinear effects of snowmelt date (Iler et al., 2013) by testing for a quadratic effect of scaled snowmelt date on peak flowering responses and the interaction between a quadratic effect and year. We omitted population 1 in 2019 and 2020 from this analysis because of low replication of flowering individuals (n=0-3, Suppl. 9). We used likelihood ratio tests on nested models to evaluate the significance of main effects and interactions.

To determine how snowmelt and phenology related to fitness, we used hurdle models to analyze relationships between the probability of reproducing and fruit production in relation to snowmelt. We first analyzed the probability of reproducing (making one or more fruits) using a mixed model (function *glmer* in R package lme4, binomial distribution with a logit link, Bates et al. 2015) including scaled snowmelt date, year, and their interaction as main effects. For plants that did successfully reproduce, we then evaluated the effect of snowmelt date on fruit production using a negative binomial mixed model (function *glmer.nb* in R) with scaled snowmelt date, year, and their interaction as main effects. Both models included plot nested with population as a random effect. We used Akaike's Information Criterion (AIC) to compare fit between Gaussian, negative binomial, and Poisson distributions for fruit production, and chose negative binomial because it had the lowest AIC score (Johnson & Omland, 2004). We supplemented the hurdle model with an analysis exploring reproductive fitness, defined as the plot-level mean probability of reproducing multiplied by fruit production, in relation to scaled snowmelt timing and scaled growing season length. Models were built separately for snowmelt timing and growing season length, which included interactions with year, as well as the random effect for plot nested within population.

We used a hurdle model to test the relationship between peak flowering time and fitness. For the first part of the model relating the probability of reproducing to peak flowering time, we used a mixed model (function *glmer* in R package lme4, binomial distribution with a logit link, Bates et al. 2015) with peak flowering date, year, and their interaction as main effects. To test whether there was an intermediate fitness optimum for peak flowering timing (stabilizing selection) across years for plants that successfully reproduced, we used a mixed model (function *glmer.nb* in R) that included fecundity as a response variable, a quadratic term for peak flowering date as a main effect, and year as a covariate. All models included random effects of plot nested within population.

RESULTS

Snowmelt timing across elevations

Populations varied in snowmelt timing, with low and high elevation populations melting out earlier than intermediate populations (quadratic effect: F = 6.7665, P = 0.035, Figure 1.2). Snowmelt timing varied among years (F = 9.2973, P = 0.0107, Figure 1.2), with earliest snowmelt in 2018 (DOY 123-171) and latest snowmelt in 2019 (DOY ranging from 164-223). However, the rank order of snowmelt timing remained similar among populations across the study period (elevation x year: F = 0.9661, P = 0.441; Figure 1.2). Populations varied in subsequent seasonal conditions after snowmelt, especially in cwd, which also varied by year (Figure 1.1, Suppl. 1.2). Growing season length varied from 114-196 days throughout the study but did not vary significantly by year (Suppl. 1.2).

Flowering phenology in relation to snowmelt

Peak flowering for individuals in plots with earlier snowmelt was significantly earlier than those with later snowmelt in all years (Figure 1.2, Table 1.1). A significant quadratic term for snowmelt date reveals a nonlinear relationship between snowmelt and flowering time such that flowering advances with early snowmelt, but the rate of change in flowering response is greatest when snowmelt is late and responses are reduced at very early snowmelt times (Figure 1.2, Table 1.1). Despite differences in snowmelt timing, phenology responses to that timing were similar across years (Figure 1.2, marginally significant main effect of year).

Fitness in relation to snowmelt timing and phenology

Individuals in plots that experienced earlier snowmelt were more likely to reproduce (Figure 1.3a, Table 1.2) and had higher fecundity in 2018 and 2019 but lower fecundity in 2020 (Figure 1.3b, Table 1.2), which was the warmest, wettest year. The effect of snowmelt date on reproductive fitness varied by year: individuals in plots that experienced earlier snowmelt had higher fitness in 2018 and 2019 and lower fitness in 2020 (interaction between snowmelt DOY and year: $\chi^2 = 5.0322$, P = 0.025; Fig 4a, Table 1.2). Individuals in plots that experienced longer growing seasons had higher overall mean reproductive fitness ($\chi^2 = 8.28$, P = 0.004; Fig 4b, Table 1.2) and the strength of this relationship increased over the years of the study (year: $\chi^2 = 6.45$, P = 0.011; Fig 4b, Table 1.2).

Individuals with earlier peak flowering times were more likely to reproduce in 2018-2019 but less likely to reproduce in 2020 (significant interaction between snowmelt timing and year; Figure 2.5a, Table 1.2). Fruit production was highest at intermediate flowering times and fecundity responses to flowering time varied across years ($\chi^2 = 9.3784$, P = 0.002; Figure 2.5b, Table 1.2).

DISCUSSION

Our study provides key insights into plant phenology and fitness responses to climate in a system with brief growing seasons and remarkable interannual variation in abiotic conditions. In this system, snowmelt dictates the start of the growing season and early snowmelt advances flowering, a consistent finding in alpine systems (Inouye, 2008; Anderson & Gezon, 2015). We also found nonlinear phenological responses to snowmelt, suggesting that *S. tortuosus* may be approaching a threshold in climate sensitivity which may limit its ability to respond to even earlier snowmelt projected with climate change (Iler et al., 2013, 2017). However, we found evidence that early snowmelt can confer fitness benefits in a system with a relatively brief snow-free period unless the growing season is extended by wet conditions. These findings are in contrast to recent studies on the effects of climate change on alpine systems in North America showing that declines in snowpack negatively affects individual fitness and population viability (Campbell, 2019; Iler et al., 2019; Anderson & Wadgymar, 2020). Taken together, these results suggest that in these types of variable environments with high snowpack, local abiotic factors and seasonal conditions such as moisture and temperature regimes after snowmelt mediate fitness responses to climate change and imply that future changes in precipitation regime will have differing consequences depending on local and seasonal variation in conditions.

While *S. tortuosus* generally advanced flowering with earlier snowmelt, responses were attenuated with very early snowmelt times, as evidenced by the reduced slope at earlier snowmelt dates (Figure 1.2). Some studies attribute this type of nonlinear response to insufficient accumulation of heat for flowering (Inouye, 2008; Steltzer et al., 2009). However, this is unlikely to be a limiting factor at Lassen, where high snowpack leads to a relatively late onset of the growing season when temperatures are warm. Indeed, we observe nonlinear relationships across all years of the study despite high interannual variation in temperature and snowmelt. Rather, a more likely explanation is one posited by Iler and colleagues that such nonlinearity indicates that populations are reaching a limit to plastic phenological response at very early snowmelt dates (Iler et al., 2013, 2017). For example, one long-term study found that snowmelt advanced at a faster rate than phenology, suggesting that phenological sensitivity to snowmelt was not enough to keep pace with the rate of environmental change (Wadgymar et al., 2018). Such nonlinear relationships are likely to become more common as climate change progresses (Iler et al., 2013; Steltzer et al., 2009), and may threaten population persistence (Willis et al., 2008). Therefore, further studies investigating nonlinear trends will be important for understanding phenological responses and the consequences for population persistence in the coming decades.

Despite consistent nonlinear phenological responses to snowmelt, the effect of snowmelt timing on fitness depended on year, suggesting that seasonal conditions mediate fitness responses. Due to high snowpack levels at Lassen, plants experienced a relatively brief snow-free period, which we define as the growing season length in this study. Growing season length plays a key role in selection for optimal flowering time (Weis et al., 2014), which posits that short seasons select for early flowering (Weis et al. 2014). Indeed, our finding that the probability of reproducing was higher with earlier snowmelt and earlier flowering in 2018 and 2019 aligns with that prediction. However, defining the growing season as the snow-free period may not fully capture the conditions driving growth and development in the season. For example, the timing and extent of water availability during the snow-free period will also define growing conditions. In snowmelt-driven systems, reduced snowpack and early snowmelt can lead to increased drought stress or an abbreviated growing season due to late-season drought, which may also select for earlier flowering (Franks et al., 2007; Hamann et al., 2018). This may explain why we found the opposite pattern in 2020, in which plants had a higher probability of reproducing with later peak flowering and higher fecundity in response to later snowmelt time. In 2020, all populations experienced lower drought stress (cwd) due to late spring rains and summer thunderstorms, allowing plants that did reproduce to opportunistically take advantage of wetter conditions to extend their time for growth (effectively lengthening the growing season) and maximize reproductive effort. This is consistent with our finding that mean reproductive fitness was higher in plots that experienced longer growing seasons.

While probability of reproducing varied in response to snowmelt and flowering timing across years (Figure 1.3), fruit production was highest for plants that reached peak flowering at intermediate times across all years of the study (Figure 2.5). Though the intermediate fitness peak is not steep, results are consistent with signatures of weak stabilizing selection for flowering phenology. Stabilizing selection is expected if selection fluctuates over life stages or generations (McGlothlin, 2010), which may be the case for these populations due to high interannual variation in growing season length, herbivory exposure, and pollinator visitation. Higher fecundity at intermediate flowering time may also indicate that flowering synchrony is important for pollinator visitation to maximize reproductive output in *S. tortuosus*, a primarily outcrossing species (Preston, 1990; 1991). The differences we observed in probability of reproducing versus fruit production in response to peak flowering time may indicate different constraints for these processes. Indeed, probability of producing any successful fruits was consistently higher with early snowmelt, though again patterns reveal attenuated responses at earlier snowmelt times. However, maximizing reproductive success may depend more on successfully timing peak flowering to coincide with flowering times for others in the population, pollinator visitation, or likely both. Such synchrony is likely mediated by growing season temperature and water availability, and possibly other cues such as photoperiod, driving both flowering phenology as well as pollinator activity. On the other hand, the shallow slopes leading to fitness peaks may indicate that fitness is somewhat robust to the exact timing of peak flowering, which may belie decreased sensitivity to pollinator phenology in a species that is largely visited by generalists (Preston, 1994). These findings provide insights into the effects of variable abiotic and biotic conditions on fitness under climate change and highlight the importance of investigating the effects of climate change on multiple components of reproductive fitness.

The persistence of these *S. tortuosus* populations in the face of climate change will depend partially on their sensitivity to changing temperature and snowmelt and their ability to respond as conditions change. Studies have shown that plants respond to climate change by accelerating phenology through both phenotypic plasticity and adaptive evolution (Anderson, Inouye, et al., 2012). Recent studies have shown that earlier snowmelt reduces survival and population viability (Iler et al., 2019), disrupts local adaptation (Anderson & Wadgymar, 2020), and causes population declines (Campbell, 2019). Here we find that components of reproductive fitness can increase with early snowmelt, but those patterns vary across years with very different subsequent growing conditions. Differences in local climate in our study system may explain some of these discrepancies. Populations at Lassen experience high annual snowpack (Dove et al., 2012), such that despite declines in snowpack over recent decades, thresholds or tipping points related to reduced snowpack and early snowmelt may still be a ways off. Though our study was conducted over only three years, long-term studies on systems like ours could provide insights into whether such a threshold exists and if so, document population dynamics as a system approaches a tipping point in real time. Additionally, the ability for individuals to respond to warmer and wetter conditions in 2020 and take advantage of the longer growing season to maximize fecundity may suggest that high climate responsiveness could benefit fitness in this species (Willis et al., 2008). Interestingly, the plastic phenological responses we observed are contrary to evidence from herbarium specimens that *S. tortuosus* populations from cooler environments are less phenologically sensitive to climate change than those from warmer regions (Love & Mazer, 2021). This may be due to differences in spatial and temporal extent of these studies, since our field study focused on high elevation populations in a subset of the broader distribution of the species. Alternatively, it could be explained by the unusually warm temperatures individuals at Lassen experience throughout the growing season despite their high elevation. However, as the climate continues to change and snowpack decreases dramatically, the snow-free period is expected to start earlier as well as become longer, warmer, and drier, and populations may reach a limit in either their capacity to resist drought or to shift flowering timing earlier to escape drought. Additionally, if snowmelt continues to shift earlier, then at some point early-season frost exposure may eventually impact reproductive success (Inouye, 2008; Pardee et al., 2018). Therefore, although we may see short-term fitness gains of earlier snowmelt, the negative consequences of declining snowpack observed in other studies may be a harbinger of what is to come with continued climate change in systems such as Lassen, which is projected to experience drastic declines in snowpack (>60% Snow Water Equivalent) over the coming decades (Lute et al., 2022). Conducting experiments manipulating snowpack would be an excellent next step to test for causality in the correlative relationships we found in our observational study. Overall, our results are consistent with theory predicting that plasticity will facilitate short-term climate change responses (Jump & Penuelas, 2005; Ghalambor et al., 2007; Chevin et al., 2010; Hendry, 2016), although it is unknown whether they would be sufficient for long-term success. On the other hand, populations that experience high interannual variability such as Lassen may be able to cope with some of these changes (Huang et al., 2015; Nadeau et al., 2017).

This study highlights the value of assessing phenological and fitness responses to snowmelt in the context of environmental conditions, especially in environments with high interannual variation in snowpack and partially decoupled temperature and snowmelt across elevations. Our results reveal that in systems like Lassen, very high snowpack and phenological responses may buffer plants from some of the negative effects of climate change. Nonetheless, as snowmelt continues to decrease, the fitness declines already being documented in response to climate change in systems with lower snowpack may foreshadow what is next for these populations at Lassen. Synthesizing global studies of phenological response to climate change in alpine regions, similar to the GLobal Observation Research Initiative in Alpine environments (GLORIA) network (https://www.gloria.ac.at/network/general) would greatly improve our understanding of the mechanisms driving divergent responses across systems/regions. An outstanding question at the forefront of evolutionary ecology is whether populations will be able to respond to continued change, and the roles of phenological plasticity and adaptive evolution in that change (Anderson, Panetta, et al., 2012; Fox et al., 2019). Accurate and robust predictions of responses to ongoing changes in environmental conditions will require more studies disentangling the effects of phenological responses to climate on fitness in variable environments.

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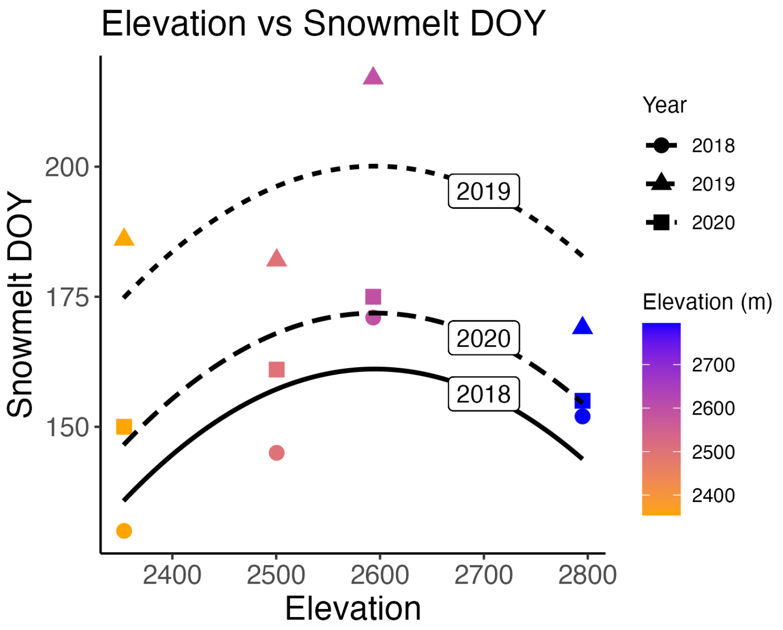
FIGURES

Figure 1.1. Snowmelt timing across an elevation gradient at Lassen

Variation in (a) snowmelt timing and (b) Principal Components Analysis describing variation in growing season conditions across the elevational cline at Lassen from 2018-2020. (a) Points represent snowmelt day of year for each population averaged across plots within a site each year. The relationship between snowmelt and elevation did not vary across years (interaction: P = 0.44). Colors indicate the elevation at each population. Circles represent 2018 means; triangles represent 2019 means; squares represent 2020 means. (b) Principal Components Analysis describing variation in growing season conditions across populations and years of the study. First two principal components (PCs) illustrated on the x and y axes. Growing season variables include growing season length (season_length), photoperiod, Growing Degree Days (gdd), minimum and maximum temperature (tmin and tmax, respectively), and Climatic Water Deficit (cwd). PC1 (51.12%) describes metrics related to growing season length such as the length of the snow-free period and photoperiod accumulated by peak flowering. PC2 (30.53%) largely describes variation related to drought metrics like climatic water deficit and maximum temperature. Shapes indicate populations (circles for 1; triangles for 2; squares for 3; plus signs for 4). Colors indicate years (red for 2018; green for 2019; blue for 2020). Data source was Flint et al. (2021).

A)


Elevation vs Snowmelt DOY
Snowmelt DOY
200
175
150
2400
2500
2600
2700
2800
Elevation
2019
2020
2018
Year
2018
2019
2020
Elevation (m)
2700
2600
2500
2400

B)

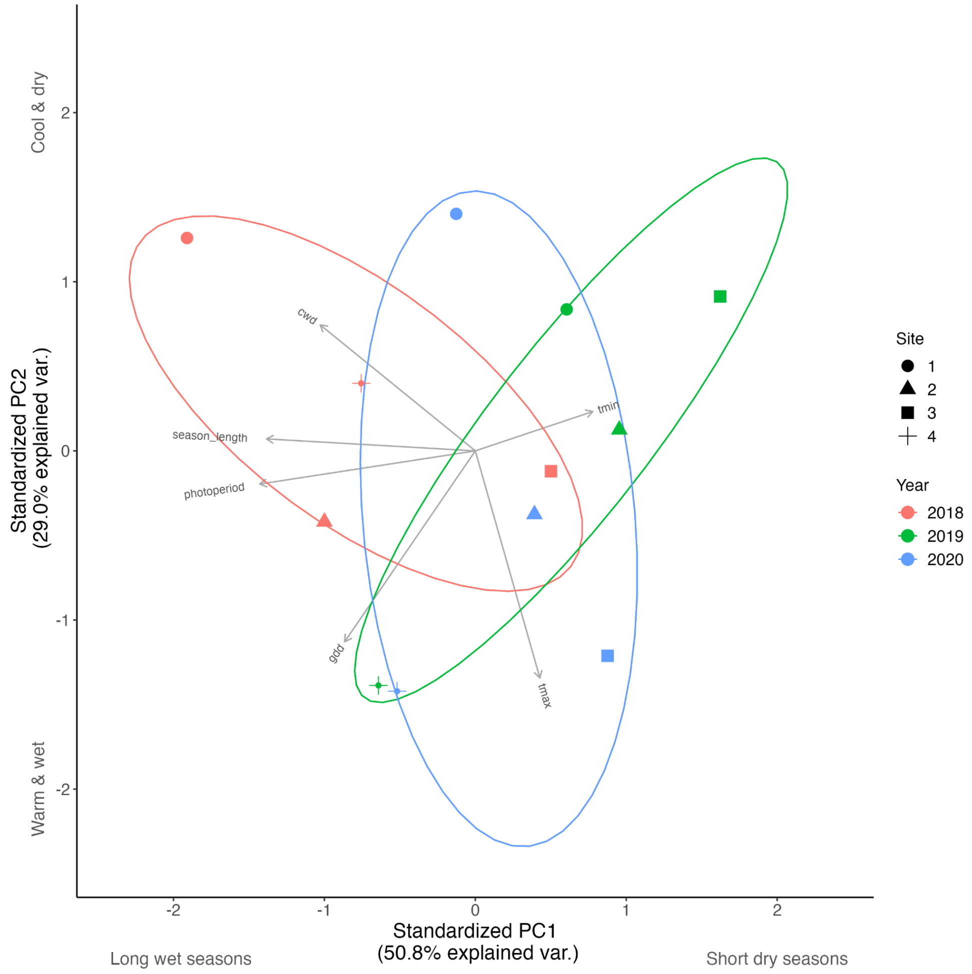

Cool & dry
Warm & wet
Standardized PC2
(29.0% explained var.)
2
1
0
-1
-2
cwd
season_length
photoperiod
gdd
tmax
tmin
-2
-1
0
1
2
Standardized PC1
(50.8% explained var.)
Long wet seasons
Short dry seasons
Site
1
2
3
4
Year
2018
2019
2020

Figure 1.2. Snowmelt day of year vs peak flowering

Snowmelt day of year vs peak flowering day of year. Peak flowering is the day of year on which the maximum number of open flowers were counted per individual. Points indicate individuals. Colors indicate elevation at each population. Shapes indicate years of the study (circles for 2018; triangles for 2019; squares for 2020). Relationships are significantly curvilinear for all years (refer to results).

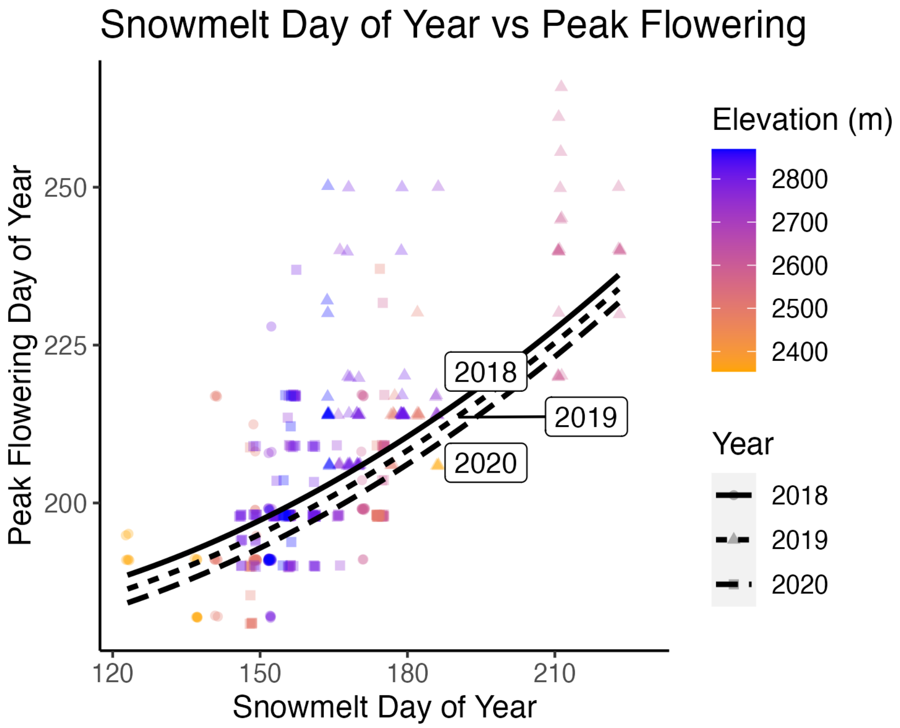


Table 1.1. Snowmelt vs phenology summary model output

Results from mixed model for peak flowering time; statistics are results from likelihood ratio tests.

Predictor	χ^2	**Pr (> χ^2)**
Snowmelt date	74.27	**<0.001**
Snowmelt date^2	22.17	**<0.001**
Year	3.06	*0.08*
Snowmelt date x Year	0.05	0.83
Snowmelt date^2 x Year	1.54	0.21

P < 0.05 (in bold)
P < 0.1 (in italics)

Figure 1.3. Fitness in relation to snowmelt timing

Fitness in relation to snowmelt timing for *Streptanthus tortuosus*. (a) Probability of reproducing (making successful fruits) and (b) fecundity, measured as maximum number of fruits. Points indicate individual plant fitness. Lines indicate trends predicted from linear models for each year. Colors indicate elevation at each population. Shapes indicate years of the study (circles for 2018; triangles for 2019; squares for 2020).

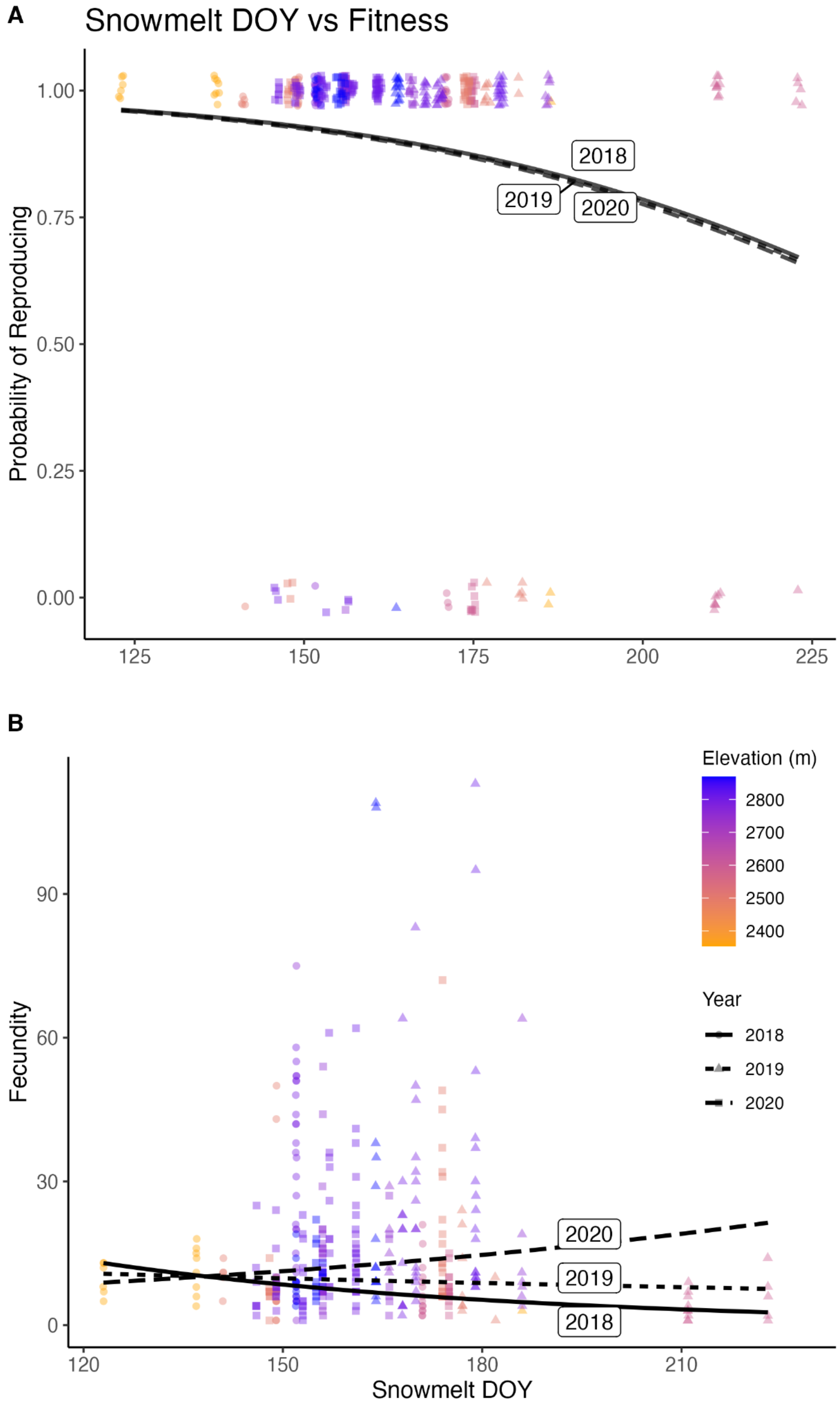


Figure 1.4. Snowmelt and growing season length vs reproductive fitness

Snowmelt DOY (a) and (b) growing season length in relation to overall reproductive fitness (plot-level mean probability of reproducing multiplied by fruit production) in *Streptanthus tortuosus*. Points indicate individual plant fitness. Lines indicate trends predicted from linear models for each year. Colors indicate elevation at each population. Shapes indicate years of the study (circles for 2018; triangles for 2019; squares for 2020).

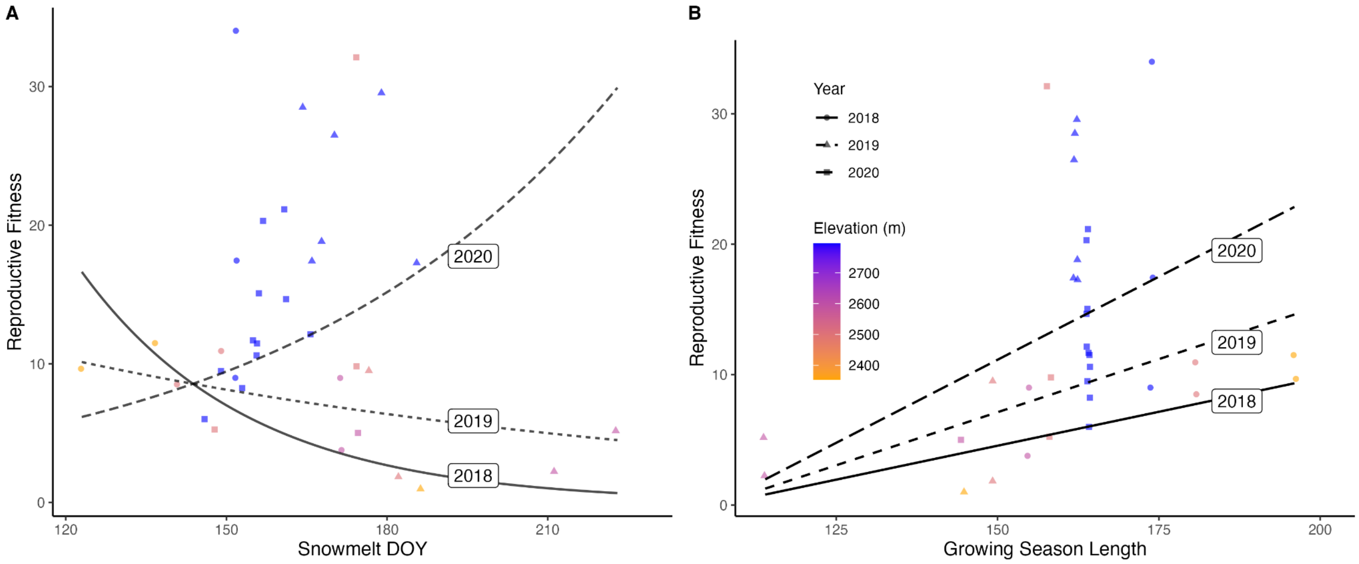


Figure 1.5. Probability of reproducing and fecundity in relation to flowering time

Probability of reproducing (a) and (b) fecundity in relation to peak flowering day of year in *Streptanthus tortuosus*. Points indicate individual plant fitness. Lines indicate trends predicted from linear models for each year. Colors indicate elevation at each population. Shapes indicate years of the study (circles for 2018; triangles for 2019; squares for 2020).

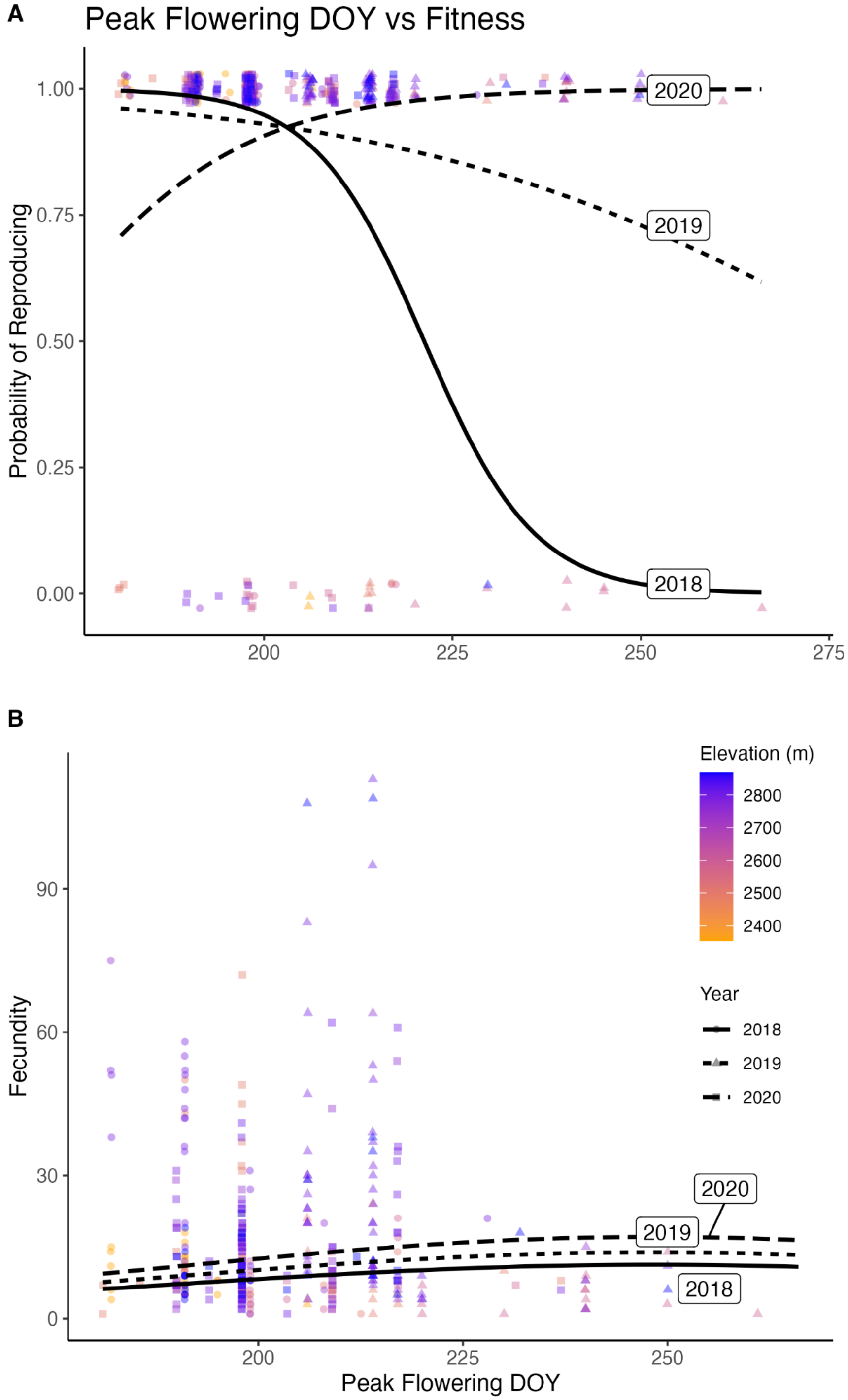


*Table 1.2. **Fitness responses summary model output***

Results from mixed linear models for fitness; statistics are results from likelihood ratio tests.

Response	**Predictor**	**χ^2**	**Pr (> χ^2)**
Probability of reproduction	Snowmelt date	5.44	**0.02**
Probability of reproduction	Snowmelt date x Year	1.92	0.17
Probability of reproduction	Year	0.01	0.92
Fecundity	Snowmelt date x Year	4.29	**0.04**
Probability of reproduction	Peak flowering x Year	13.78	**<.001**
Fecundity	Peak flowering	10.09	**0.006**
Fecundity	(Peak flowering)	13.78	**<.001**
Fecundity	Year	9.38	**0.002**
Reproductive fitness	Growing season length	8.28	**0.004**
Reproductive fitness	Year	6.45	**0.011**
Reproductive fitness	Snowmelt date x Year	5.0322	**0.025**

$P < 0.05$ (in bold)

SUPPLEMENTAL MATERIALS

Supplement 1.1. Study species and populations

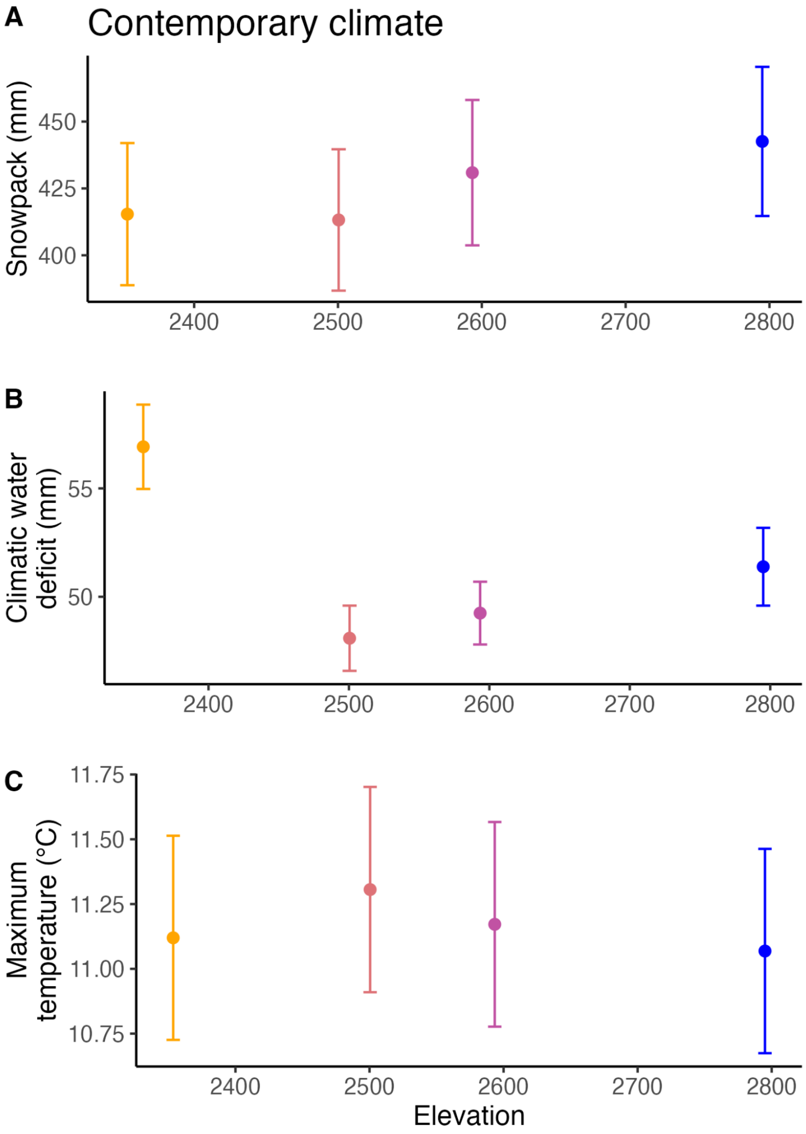
Lassen population localities and elevation

Lassen population locality elevation (meters a.s.l.) and GPS coordinates (latitude and longitude).

Population	**Elevation**	**Latitude**	**Longitude**
4	2795	40.48005	-121.50366
3	2593.41664	40.47471	-121.50486
2	2500.45718	40.46563	-121.51487
1	2353.55075	40.4664	-121.52311

Lassen long-term contemporary climate

Lassen population long-term contemporary a) Snowpack, b) Climatic water deficit, and c) Maximum Temperature. Points represent 30-year averages (1990-2020) and lines represent means +/- 1 sd for each population. Downscaled data (270m resolution) was extracted from output from the California Flint Basin Characterization Model (Flint et al., 2021; 2023). (b-d) Colors indicate the elevation at each population.



Field census dates

Field survey dates at Lassen populations each year of the study.

Year	Survey 1	Survey 2	Survey 3	Survey 4	Survey 4	Survey 5	Survey 6	Survey 7
2018	6/30-7/1	7/9-7/10	7/18	7/28-7/29	8/5-8/6/1	8/16	8/27	
2019	7/25-7/26	8/2	8/8	8/18	8/28	9/7	9/18	9/28
2020	6/29	7/8	7/16	7/27	8/4	8/13	8/24	

Manually assigning estimated snowmelt dates

We were missing data on 3 out of 36 total plot-year combinations due to loss of temperature sensors in the field: population 4 plot 2.5 in 2018, population 4 plot 1.5 subplot “left” in 2020 and population 2 plot 1 in 2020. We assigned population 4 plot 2.5 in 2018 to a snowmelt date of 6/1/18, because the two other plots we marked at that site in that year (population 4 plot 1 and population 4 plot 2) also melted out on that day. We assigned population 4 plot 1.5 subplot “left” to 6/4/20 because it was the median of all plots in that population (population 4) in that year, as well as very close to the median of the two nearest plots, population 4 plot 1.75 subplot “right” and population 4 plot 1.75 subplot “left”, which melted out on 6/1 and 6/9, respectively (median for those two plots was 6/5). We assigned population 2 plot 1 in 2020 to 6/22/20, because the nearest plot (population 2 plot 1.5) melted out on that date in 2020, and these two plots are very similar geographically and topographically (~ 5 meters apart and 3 meter elevation difference, E. Suglia*, pers. obsv.*). In 2019, both plots melted out on the same day.

Replication of flowering individuals by year and site

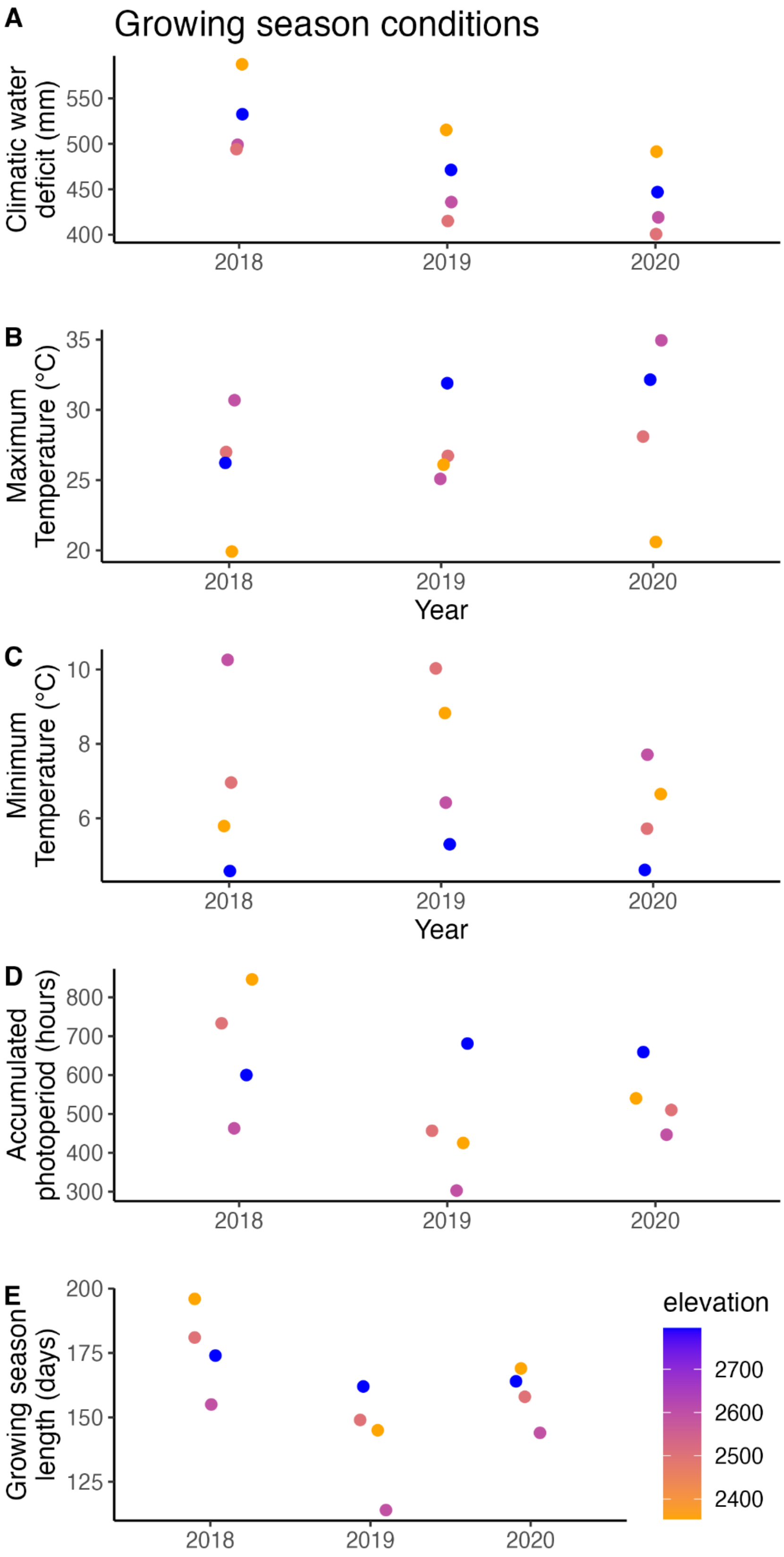
Number of flowering individuals at each site every year of the study.

Site	**Year**	**n**
3	2018	17
3	2019	18
3	2020	25
2	2018	27
2	2019	32
2	2020	10
1	2018	13
1	2019	3
1	2020	0
4	2018	120
4	2019	39
4	2020	70

Supplement 1.2. Growing season conditions during years of the study

Populations experienced the wettest and warmest conditions (lowest cwd and highest maximum temperatures) in 2020. Growing seasons were generally shorter in 2019 (due to higher snowpack and later snowmelt) and longer in 2018. Populations experienced the widest range of conditions in 2019 (they occupied the largest breadth of climate space), and the narrowest range of conditions in 2020. Populations occupied similar climate space relative to each other across the study period. Population 3 (the second-highest-elevation population; 2593m) consistently melted out later and had the shortest growing season across years. Population 1 (the lowest elevation population; 2353m) experienced the greatest level of drought stress (highest climatic water deficit) across years. In 2019 and 2020, population 4 (the highest elevation population; 2795m) experienced the longest growing seasons and accumulated the most GDD compared with other populations.

Figure caption: a) Climatic Water Deficit (cwd) during the snow-free period, b) average maximum temperature from snowmelt to peak flowering, c) average minimum temperature from snowmelt to peak flowering, d) accumulated photoperiod from snowmelt to peak flowering, e) and growing season length (snowmelt to snow cover) across populations and years of the study. (a-e) Colors indicate elevation at each population. Numerical values for climate variables shown in the accompanying table.


A
Growing season conditions
Climatic water deficit (mm)
400
450
500
550
2018
2019
2020
B
Maximum Temperature (°C)
20
25
30
35
2018
2019
2020
Year
C
Minimum Temperature (°C)
6
8
10
2018
2019
2020
Year
D
Accumulated photoperiod (hours)
300
400
500
600
700
800
2018
2019
2020
E
Growing season length (days)
125
150
175
200
2018
2019
2020
elevation
2700
2600
2500
2400

Site	**Year**	**cwd**	**GDD**	**Maximum Temperature**	**Minimum Temperature**	**Photoperiod**	**Growing season length**
3	2018	498.95	482.75	30.69	10.26	462.95	155
3	2019	435.93	244.00	25.09	6.42	302.84	114
3	2020	419.13	494.21	34.95	7.71	446.50	144
2	2018	494.12	607.00	27.00	6.96	733.26	181
2	2019	415.14	416.38	26.73	10.03	456.71	149
2	2020	400.73	428.88	28.10	5.72	510.18	158
1	2018	587.49	484.88	19.92	5.79	846.19	196
1	2019	515.33	374.61	26.10	8.83	425.28	145
1	2020	491.44	326.25	20.60	6.65	540.15	169
4	2018	532.55	447.50	26.23	4.58	600.12	174
4	2019	471.28	660.50	31.90	5.30	681.09	162
4	2020	446.92	621.13	32.15	4.61	659.11	164

Notes on defining and calculating growing season conditions

Population 1 in 2019 and 2020 had low replication of flowering individuals (n=0-3, Suppl. 1.1), so we estimated peak flowering date for population 1 in those years by calculating the mean peak flowering time in each year in days from snowmelt to peak flowering across populations, then assigning peak flowering date as snowmelt date plus mean days from snowmelt to peak flowering for population 1.

Because these analyses focused on population level differences and due to the resolution of the downscaled climate data, we used population means of snowmelt dates and temperatures.

Growing season condition linear models

We used linear models to evaluate how each growing season metric (growing season length, maximum and minimum temperatures, GDD, photoperiod, and cwd) varied across site and year by including site and year as main effects, as well as their interaction.

Table shows results from analyses exploring covariation in growing season conditions, population, and year. Metrics for abiotic growing season conditions were growing season length, climatic water deficit, minimum and maximum temperature, growing degree days, and accumulated photoperiod. Statistics are P values for each predictor regressed against site and year.

Predictor	**Site**	**Year**
Climatic Water Deficit (cwd)	**0.001**	**<0.001**
Growing Degree Days (GDD)	0.34	0.72
Growing season length (snow-free period)	0.25	0.19
Accumulated photoperiod	0.58	0.29
Minimum Temperature (°C)	0.26	0.66
Maximum Temperature (°C)	*0.05*	0.14

$P < 0.05$ (in bold)
$P < 0.1$ (in italics)

Populations and years varied in cwd ($P = 0.016$; $P = 0.004$) and populations varied by maximum temperature ($P = 0.047$) but not by year ($P = 0.139$). However, we did not find variation in growing degree days, growing season length, photoperiod accumulation, or minimum temperature across populations and years.

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CHAPTER 2

Herbivory and drought interact to affect fitness and life history in a native California plant

INTRODUCTION

Anthropogenic climate change is rapidly altering natural systems and exposing organisms to increasingly extreme, unpredictable, and novel combinations of abiotic and biotic conditions (Williams et al., 2007; Williams & Jackson, 2007; IPCC, 2023). The shifts in temperature and precipitation predicted with climate change, especially increasing drought (Wright et al., 2016; Pathak et al., 2018; Swain et al., 2018; Luković et al., 2021), pose significant challenges for plants. For example, altered timing and conditions of the growing season can affect phenology and fitness, with implications for evolution of life history traits and population persistence (Inouye, 2008; Franks, 2011; Anderson & Wadgymar, 2020; Blumenthal et al., 2020; DeMarche et al., 2020; Inouye, 2020). Climate change will also interact with biotic stressors like herbivory to affect these patterns, which could lead to no-analog community assemblages (Parmesan, 2006) and expose plants to increased herbivory pressure (Meineke et al., 2019; Hamann et al., 2021a). Moreover, plants are often exposed to multiple stressors simultaneously and evolve adaptations to localized abiotic and biotic conditions along environmental gradients (Anderson et al., 2015; Colautti et al., 2017; Defossez et al., 2018; Anderson & Wadgymar, 2020).

Plants may vary in their responses to selective pressures like herbivory and drought as a result of trade offs in how they allocate resources to maximize lifetime fitness (Hamann et al., 2021b). Such trade-offs in response to abiotic and biotic shifts and resulting fitness consequences will depend on localized environmental conditions as well as on how the past environment has shaped population responses (Rasmann et al., 2014b; Moreira et al., 2018). For example, whether or not plants reproduce after herbivory may depend on the time remaining in the growing season: individuals experiencing long growing seasons might have sufficient time to reproduce before unfavorable end-season conditions while those in short growing seasons may delay reproduction to subsequent growing seasons (Brys et al., 2011; Hamann et al., 2018; Rauschkolb et al., 2022). Patterns and trade-offs in reproductive responses to herbivory across environments also depend on life span; for example, annual plants may face particularly intense selective pressure to time reproduction appropriately because their life cycles are limited to one growing season. Understanding the constraints to climate change responses across environmental clines is essential for predicting population persistence in variable environments.

Flowering timing is a critical life history trait shaped by past environmental selective pressures that can strongly impact reproductive fitness and influence responses to shifting conditions (Augspurger, 1981; Rathcke & Lacey, 1985; Pilson, 2000; Inouye, 2008; Hamann et al., 2021a). Growing season length often varies along environmental clines such as elevation and latitude, and accordingly, trade-offs between flowering timing and size have been shown to adaptively vary within species across such clines (Colautti et al., 2017; Sacristán-Bajo et al., 2023). For example, during brief growing seasons at higher elevations, plants may flower as early as possible due to strong selection to complete the life cycle rapidly (Freeman et al., 2003; Anderson & Gezon, 2015) while those with longer growing seasons at lower elevations may delay flowering to accumulate biomass and maximize reproductive output by flowering later at a larger size (Cohen, 1976; Kozłowski, 1992; Weis et al., 2014; Colautti et al., 2017). In variable environments, timing and intensity of stressors during reproduction may interact with growing season length to influence responses. Herbivory and drought are two important stressors that influence flowering timing and fitness and can shape local adaptation across a species range. Some plants tolerate or compensate for herbivory by recouping losses in performance sustained by herbivores through rapid regrowth of reproductive axillary meristems (McNaughton, 1983; Wallace et al., 1985; Bullock et al., 1994; Ramula et al., 2019), while others experience fitness losses through reduced survival and fecundity when eaten, especially when large mammalian herbivores remove entire stems, flowers, and fruits (Whigham, 1990; Bergelson & Crawley, 1992; Rooney & Waller, 2003). The direction and magnitude of compensation in response to herbivory is often mediated by ecological context such as the timing of damage relative to flowering time and the end of the growing season, resource availability, and the degree and frequency of tissue damage (Hawkes & Sullivan, 2001; Wise & Abrahamson, 2007, 2008; Ramula et al., 2019). Different models predict herbivory tolerance to be maximized under higher resources like water and light availability (compensatory continuum hypothesis; Maschinski & Whitham, 1989), lower resources (growth rate model; Hilbert et al. 1981), or either, depending on whether the resource in question limits plant (re)growth (limiting resource model; Wise & Abrahamson, 2005). For example, in Mediterranean environments where plants flower during hot, dry summers that end with terminal drought, late herbivory may be more likely to interact with drought to produce negative fitness effects. On the other hand, perennial plants may respond to herbivory by reverting to vegetative growth and attempting reproduction in a future season (Knight, 2003), which puts them at risk of exposure to unfavorable conditions.

Water availability is an especially critical resource affecting plant responses to herbivory, and drought can hinder compensation, especially in seasonal environments (Lennartsson et al., 1998; Levine & Paige, 2004; Ramula et al., 2019). However, whether and how drought affects plant responses to herbivory depends on how past environmental stressors shaped selection. If herbivory and drought historically exerted convergent selection pressure (Coughenour, 1985; Milchunas et al., 1988; Cingolani et al., 2005), plants may evolve certain functional traits in response to both stressors, reducing fitness losses when plants face them both simultaneously. For instance, life history traits like flowering early or displaying annual behavior may facilitate escape from both herbivory and drought (Díaz et al., 2007; Krimmel &

Pearse, 2016). Alternatively, evolving under drought-prone conditions may confer fitness benefits in response to drought but trade off with reduced tolerance to herbivory (Pearse et al., 2017). Variation in historical selective forces across environments can therefore influence patterns in local adaptation to herbivory and drought, with implications for current and future phenology and fitness responses under climate change.

Finally, timing of herbivory damage in relation to flowering time and drought also affects herbivory responses (Maschinski & Whitham, 1989; Strauss & Agrawal, 1999; Knight, 2003, 2007; Akiyama & Ågren, 2012; Pearse et al., 2017). If herbivory removes reproductive structures, delays flowering, or occurs late in the season, plants may run out of time to reproduce. Short growing seasons, steep fitness costs to later flowering, or annual life history schedules may intensify these costs.

In this study, we investigate phenological and fitness responses to experimental herbivory, drought, and their interaction across 8 populations of the native California forb *Streptanthus tortuosus* (Brassicaceae). These populations differ in climate and herbivory pressure across a large elevational range (Figure 2.1) and previous work has shown that *S. tortuosus* exhibits life history variation both within and across populations in response to differences in temperature and precipitation cues (Gremer et al. 2020a, b). We experimentally manipulate water availability and herbivory in a common garden to determine whether and how home environment and varying resource levels affect life history and fitness responses. Specifically, we ask (1) How does climate of origin affect first flowering time?, (2) How do climate of origin, water availability, and herbivory affect peak flowering time?, and (3) How do climate of origin and herbivory pressure of origin affect reproductive fitness in response to water availability and herbivory? Based on prior studies, we hypothesized that herbivory should delay flowering (Freeman et al., 2003; Jordan et al., 2015) and drought should accelerate flowering (Sherrard &

Maherali, 2006; Franks, 2011). We expected drought and herbivory to have negative effects on fitness and that herbivory would intensify the negative effects of drought on fitness, and vice versa. We also expected that when exposed to abiotic and biotic stressors, plants from regions that experience similar stressors in their environment of origin would outperform those that do not. More specifically, we predicted that under low water availability, plants from dry environments would outperform plants from more mesic environments, and that when clipped, plants from environments that experience higher herbivory pressure would outperform plants from regions less exposed to herbivory.

METHODS

Study system

Streptanthus tortuosus Kellogg (Brassicaceae) is a widespread forb native to California, where it inhabits elevations from 200 m to 4100 m and latitudes from southern California to southern Oregon (Preston 1991; Calflora 2014). Individuals occupy exposed rocky outcrops and slopes and populations tend to be discontinuously distributed (Preston 1991; Calflora 2014). Across the species range, populations experience a breadth of abiotic and biotic conditions including variation in drought and herbivory. All populations experience a Mediterranean climate characterized by winter precipitation and hot, dry summers, and therefore are exposed to strong interannual variation in temperature and precipitation across the species range (Baldwin, 2014; Rundel et al., 2016). Like others in Brassicaceae, *S. tortuosus* interacts with a variety of generalist and specialist herbivores (Shapiro, 1981) and sustains high levels of herbivory (Figure 2.1b; Suppl. 1; Karban & Courtney, 1987). Species across the *Streptanthus* clade often experience apical browsing by deer and rabbits as well as flower, fruit, and foliar damage by aphids, pierid caterpillars and beetles (Shapiro, 1981; Karban & Courtney, 1987; Strauss & Cacho, 2013). Herbivory intensity and type varies in the field but often negatively impacts fitness in *S. tortuosus*, including completely defoliating smaller plants (Karban & Courtney, 1987) and causing complete seed loss in reproductive individuals (Shapiro, 1981).

In the field, individuals experience a range of herbivory regimes, including insect chewing and mammalian browsing at various life stages targeting leaves, buds, flowers, fruits, and stems (Suppl. 2.1). Populations experience decreasing herbivory as elevation increases (Figure 2.1b, Suppl. 2.1), as has been found in other study systems (Galmán et al., 2018; Moreira et al., 2018). Populations also vary in life history, phenological, and morphological traits in the field, making this study system valuable for exploring plant responses to climate (Gremer et al. 2020a, b).

S. tortuosus also displays remarkable variation in life history traits both within and among populations. High elevation populations are mostly composed of biennials but also some iteroparous perennials, and low elevation populations are largely annuals but can also feature biennials and iteroparous perennials. This variation is maintained in a common garden, suggesting a genetic basis for some of this variation (E. Suglia*, unpubl. data*).

We sampled from populations across a latitudinal and elevational gradient in California that experience varying abiotic and biotic conditions (Figure 2.1; Suppl. 2.1). All populations except for Table Mountain (TM2) are biennial or iteroparous; TM2 is primarily annual. Seeds for the study were collected as maternal seed families at each population from 2014-2020. Prior to the start of the experiment, seeds were stored dry at room temperature (~21°C).

Home environmental conditions

To understand plant responses to experimental drought and herbivory treatments in the context of population origin, we characterized variation in local climate, focusing on growing season conditions experienced by populations using downscaled climate data for 1990-2020 (Flint et al. 2021). We retrieved monthly climate variables related to temperature and precipitation for each population from the Flint Basin Characterization Model, which downscales PRISM (PRISM Climate Group, Oregon State University, http://www.prism.oregonstate.edu/) data to a 270m resolution for the California hydrologic region (Flint et al. 2021). The metrics we used were climatic water deficit (cwd), precipitation (ppt in mm H_2O), maximum and minimum air temperature, (tmax and tmin in °C, respectively), soil-water storage (str; amount of water stored in the soil), and snowpack (pck in mm H_2O; snow water equivalent) (Suppl. 2.1). Climatic water deficit (cwd) is a metric for drought stress calculated as the evaporative demand that exceeds available water (potential evapotranspiration - actual evapotranspiration; in units of mm H_2O). Soil-water storage is the amount of water stored in the soil (precipitation + snowmelt - actual evapotranspiration - recharge - runoff; in units of mm H_2O). We calculated a summary metric for all variables by summing the monthly values during the water year (September - August) and averaging over years. To characterize how long plants have to complete their life cycles across an elevation gradient, we quantified growing season length as the number of months from snowmelt to snow cover (first month with pck = 0 to first month with pck > 0) at high elevations (sites: CP2, LV3, SQ3, WL2, YO1) and months with rainfall (first month with ppt > 25 mm to last month with ppt > 0) at low elevations (sites: BH, TM2, KC2) and averaged over 1990-2020. Then, we analyzed variation in climate across populations using Principal Components Analysis (PCA, *prcomp* function in base R) including the climate metrics as well as growing season length and elevation. PC1 (70.72%) described variation along an elevation gradient, from warm, low elevation sites with long growing seasons to cool high elevation sites with short seasons. The strongest loadings for PC1 were elevation (0.40), growing season length (-0.40) and temperature

(-0.41 for both tmn and tmx). PC2 (22.36%) described variation related to drought metrics along a dry to wet gradient, and soil water storage (0.72) and climatic water deficit (-0.48) loaded most strongly (Figure 2.1c, Suppl. 2.1).

To relate population phenology and fitness responses to herbivory pressure experienced by plants at their location of origin, we surveyed for presence of herbivory damage on approximately 60 (n=26-67; Suppl. 2.1) plants per population for all source populations in the field near peak reproduction (when the majority of plants were post-flowering) and quantified herbivory pressure as the proportion of individuals with herbivory damage present in each population.

Experimental design

We conducted an outdoor pot experiment on 8 populations of *S. tortuosus* investigating the effects of herbivory and drought on phenology and fitness (Figure 2.1). To understand how herbivory and drought interact to affect phenology and fitness, we experimentally exposed plants to varying watering levels and simulated herbivory at the University of California, Davis growing facilities in May-August 2021 and measured phenological and fitness traits. We conducted this experiment on outdoor benches (Suppl. 2.2), exposing plants to ambient temperatures and day length while controlling the water regime. Plants were exposed to 2 herbivory and 5 drought treatments in a fully factorial design.

To simulate mammalian herbivory, we randomly assigned individuals to either a control (no treatment) or tissue removal treatment. We implemented the herbivory treatment during the phenophase at which individuals had approximately 50% buds and 50% other reproductive structures (flowers and fruits), because mammalian herbivory in the field was common at this phenophase (E. Suglia, *unpubl. data*). Due to variation in individual flowering rates, we clipped plants at the same phenophase but not on the same date. In the field, herbivores typically browse the topmost parts of the plant, likely because the tender new growth at branch tips is most palatable and accessible (E. Suglia*, pers. obsv.,* Suppl. 2.1). To simulate mammalian herbivory, we carefully clipped at the internode directly below the lowest reproductive structure on each branch using scissors, ensuring no buds, flowers, or fruits remained. For plants that did not reproduce, we clipped half of the leaf tissues from the top half of the plant.

We created field-informed watering treatments by calculating mean monthly soil water storage per population (October-May) averaged over 1990-2020 using the Flint database (Flint et al., 2021). We implemented five treatments spanning from 42% lower than the driest site (SQ3) to 62.5% higher than the wettest site (KC2), with levels at: (41.1 mm/mo or 1.37 mm/day, 76.25 mm/mo or 2.54 mm/day, 111.25 mm/mo or 3.70 mm/day, 146.25 mm/mo or 4.7875 mm/day and 333.615 mm/mo or 11.12 mm/day) (Figure 2.2, Suppl. 2.1). The high water treatment was greater than that experienced by most populations even in high precipitation years, except LV3 (Figure 2.2, Suppl. 2.1). We skewed water treatments drier to better match drought conditions projected under climate change.

We included 7-8 maternal families from each of the 8 populations in the experiment (Suppl. 2.2). we staggered plantings with high elevation populations (LV3, SQ3, CP2, WL2, YO1, KC2) planted 10/6-10/7/20 and low elevation populations (BH, TM2) planted 11/16/20 so that we could initiate the experiment on all plants simultaneously owing to different vernalization requirements (Gremer et al. 2020b; Gremer, *unpublished data*). (Suppl. 2.2). We planted 3 seeds per maternal family in cone-tainers (164 ml cone-tainer pots, Stuewe and Sons, Corvallis, Oregon, USA) filled with 2 parts UC Davis potting soil (1:1:1 parts sand, compost, and peat moss with dolomite) and 1 part coarse 16 grit sand, as this species is found in well-drained soil, then covered soil with approximately 1 cm of sand. After planting, pots were watered in and immediately stratified at 4°C in a chamber at Storer Hall, UC Davis (covered with aluminum foil to block out light). we stratified plants from all populations for 6 weeks, watering as needed to maintain soil moisture, and moved plants to the UC Davis Controlled Environment Facility (12/28/20 for low elevation; 11/19/20 for high elevation) to germinate in 16/8 hr light/dark cycles and temperatures cycling between 24°C daytime maximum and 16°C nighttime minimum. We thinned to one germinant per pot by keeping the plant closest to the center of the cone-tainer. After transplanting, we fertilized plants once per week for three weeks with a dilute fertilizer mixture (equivalent to ~25% strength Hoagland's solution). Once plants had reached 1 cm tall, we placed plants into a chamber set at 4°C for vernalization (high elevation: 3/5/21; low elevation: 4/12/21; Suppl. 2.2).

On 5/27/2021, we assigned plants randomly to treatments and bench locations, then moved plants to an outdoor lathhouse at ambient temperatures at the Orchard Park facility to acclimate to outdoor temperatures for 5 days before moving them to outdoor benches and assigning trays into four blocks by bench quadrant (Suppl. 2.2). We fertilized plants once and hand watered to maintain moisture, then watered twice daily through emitters inserted into each cone-tainer for one week (Suppl. 2.2). After this initial acclimation to the common garden environment, watering treatments were initiated in a stepwise fashion to acclimate plants to watering treatments and avoid drought shock (Suppl. 2.2). During periods of extreme heat (maximum temperatures exceeding 40°C), we gave plants supplemental water to offset drought stress. Because plants were grown outdoors under increasing summer heat, water availability decreased throughout the course of the experiment, similar to drying experienced by plants in the field.

All plants experienced catastrophic drought during a hot spell from 7/29 - 8/3 (Suppl. 2.2) owing to automatic watering software failure. On 8/3, all plants were hand-watered thoroughly to revive as many as possible, and we monitored plants carefully daily for signs of drought damage, then conducted a mortality survey on 8/11 to quantify which plants died from the drought event. Approximately 40% of plants died because of this accidental dry-down (n=204). We collected fitness data every 4-9 days, the last of which was the day the accidental dry-down began (7/29/21).

Flowering phenology and fitness responses

We tracked individual reproductive phenology by recording onset of flowering every 1-4 days between 6/14 - 7/30/21 and counting the number of open flowers every 4-9 days from 6/28 - 7/29/21. We scored first flowering as the date the first flower opened. Plants generally initiated flowering before drought treatments were fully in place and before we clipped any plants in the herbivory treatment (Suppl. 2.2), so we did not expect to see any treatment effects in onset of flowering responses; thus, first flowering should primarily reflect differences owing to population origin and any block effects.

We also quantified individual peak flowering as the day of year (DOY) on which the individual accumulated 50% of the total flowers it produced in the experiment (Iler et al., 2013; Castillioni et al., 2022). We chose peak flowering because it is a less-biased measure of flowering phenology than first flowering, both for estimating dates themselves (Miller-Rushing & Primack, 2008) and for shifts in phenology in response to climate (CaraDonna et al., 2014). We first quantified flowering time distributions by fitting curves to observations of number of flowers per individual using a locally weighted scatterplot smoother with the loess function from the stats package (R Core Team, 2023; Rivest et al., 2021). We set the smoothing factor and curve degree at 0.75 and 2, respectively, based on visual inspection of curve fits. When the predicted number of flowers was <0, we adjusted values to zero. We used the fits from these locally smoothed curves to calculate the number of open flowers in 0.1-day intervals. Next, we calculated cumulative sums of flower counts for each individual from the fitted loess curves using the cumsum function (R Core Team, 2023) and calculated peak flowering as the day on which 50% of flowers were first observed.

We measured fitness by counting successful fruits (those visibly containing seeds) every 4-9 days from 6/28 - 7/29/21. We quantified individual fecundity as the number of fruits produced by an individual before the catastrophic drought event (Suppl. 2.2).

Statistical analyses

Flowering in relation to home environment and treatments

To evaluate whether population climate affected the probability of flowering, we used mixed models including home climatic water deficit (cwd) as a main effect and block as a random effect (function *glmer* in R package lme4, binomial distribution with a logit link, Bates et al. 2015). As expected, there were no effects of treatments on first flowering since treatments were imposed afterwards (Suppl. 2.2). We used likelihood ratio tests on nested models to evaluate the significance of main effects.

We also used mixed models with drought treatment and population as main effects, as well as their interaction (function *glmer* in R package lme4, Gaussian distribution, Bates et al. 2015) to assess more general effects of population origin, which include not only historic and recent climatic regime but also other factors like historic herbivory pressure, genetic differences and much more. We also included a random effect for block (Suppl. 2.2).

First flowering time was right-skewed, so we log-transformed the data to meet the assumption of normality for linear regression. We omitted populations BH and KC2 from this analysis due to low replication of flowering individuals (n=1-4, Suppl. 2.3). We also removed 3 individuals from the first flowering analysis that bloomed after receiving treatments (n=2) or after the accidental drought event (n=1). We used likelihood ratio tests (function *anova* in the base R stats package) coding population as a fixed effect, as populations were chosen based on elevation. If we found an effect of population, we replaced population coded as a factor with its loading on each PC axis as continuous variables to explore whether climate explained population variation in responses. We used Akaike's Information Criterion (AIC) to compare fit between models that included PC score loadings, and chose the model with the lowest AIC score (Johnson & Omland, 2004).

To explore how population climate, herbivory pressure, and experimental treatments affected peak flowering phenology, we used mixed models including main effects for treatments and population and a random effect for block on peak flowering time. We also tested for interactions between treatment and population and substituted population for PC axis loadings of climate variables when population was significant in the model. We then used AIC scores and R2 values to determine whether population or climate best explained variation in flowering timing. The only primarily annual population TM2 was a strong outlier in phenology responses; therefore, we omitted TM2 from peak flowering analyses (see Suppl. 2.5 for analysis including this pop).

Reproduction in response to home environment and treatments

To determine reproduction in response to treatments and home environment, we used hurdle models to analyze relationships between the probability of reproducing and fruit production in relation to drought treatment, herbivory treatment, and population. We first analyzed the probability of reproducing (making one or more fruits) using a mixed model (function *glmer* in R package lme4, binomial distribution with a logit link, Bates et al. 2015) including drought treatment, herbivory treatment, population, and their interaction as main effects. For plants that did successfully reproduce, we then evaluated the effect of treatments on fruit production using a negative binomial mixed model (function *glmer.nb* in R) with snowmelt date, year, and their interaction as main effects. To avoid confounding effects of the accidental drought event, we used fruit number on DOY = 210, the day before we measured mortality from the accidental drought (Suppl. 2.2) as our metric for fecundity in this analysis. We used Akaike's Information Criterion (AIC) to compare fit between Gaussian, negative binomial, and Poisson distributions for fruit production, and chose negative binomial because it had the lowest AIC score (Johnson & Omland, 2004). For each model, if we found an effect of population, we substituted population for continuous metrics including PC loadings, specific climate variables, and herbivory pressure at home. Both models included block as a random effect.

We supplemented the hurdle model with an analysis exploring reproductive fitness, defined as the probability of reproducing multiplied by fruit production, in relation to treatments and home environment, at the level of block (bench quadrant, Suppl. 2.2). We calculated this metric at the block level because many individuals made zero fruits, so calculating it at the individual level would result in many zeroes, making the metric more statistically difficult to use. We used a fixed effects model (function *lm* in R) and included main effects for drought treatment, herbivory treatment, and population coded as a factor, and tested for interactions between main effects. We chose not to use a mixed model with block as a random effect to avoid redundancy because we calculated reproductive fitness at the block level. We chose to use a negative binomial distribution for fitness because it had the lowest AIC score when compared with Gaussian and Poisson distributions (Johnson & Omland, 2004). For each response variable, we competed the models using continuous metrics against each other and selected the model with the lowest AIC score.

RESULTS

Flowering in relation to home environment

Populations from drier environments (higher cwd) were less likely to flower in our common garden (Suppl. 2.4, Table 2.1; Suppl. 2.3). First flowering time varied along PC1: cooler, high elevation populations with shorter growing seasons flowered faster/earlier than warmer, low elevation populations with longer growing seasons ($\chi^2 = 23.39$, $P < 0.001$, Suppl. 2.4, Table 2.1. Populations from even lower values of PC1 (KC2 & BH) had few flowering individuals, likely because the vernalization pretreatment failed to satisfy flowering requirements (Suppl. 2.3). The mid-high elevation populations flowered at an intermediate time and LV3, the highest elevation and latitude population, flowered earliest (Suppl. 2.4). Peak flowering time varied marginally among populations but not among treatments (Table 2.1).

When the outlier population TM2 was included in the peak flowering analyses, populations varied significantly in peak flowering time and this variation was explained by climatic gradients related to elevation and moisture. Peak flowering varied along PC1 in the same way as first flowering: cooler high elevation populations with shorter growing seasons had earlier peak flowering than those from warmer, low elevation populations with longer growing seasons. Additionally, drier populations (those with higher cwd) flowered marginally later than wetter populations (Suppl. 2.5).

Reproduction in response to home environment and treatments

Probability of reproducing

Clipped plants were more likely to reproduce if they were from a low elevation environment with high herbivory pressure/longer growing seasons except for plants in the driest watering treatment, and greater experimental water availability generally increased the probability of reproduction (Figure 2.3, Table 2.2). There was also an interaction between mean herbivory rates at the field site and experimental water availability; for populations that had lower herbivory pressure at home (LV3 & CP2), experimentally increasing water availability reduced the probability of reproducing (Figure 2.3, Table 2.2).

Fecundity

There was a marginal three-way interaction between scaled drought treatment, herbivory treatment, and population ($\chi^2 = 9.59$, $P = 0.09$, Figure 2.4, Table 2.2). Drought always decreased fecundity for plants in the control treatment, and clipped plants always had lower fecundity than undamaged plants (Figure 2.4, Table 2.2); however, the degree to which fruit production declined in response to herbivory depended on both population and water availability (Figure 2.4, Table 2.2). Surprisingly, clipped LV3, SQ3, and YO1 had lower fecundity with higher experimental watering (Figure 2.4, Table 2.2). When continuous loadings from PC1 of climate variables were substituted for the factor population, the 3-way interaction disappeared, suggesting that factors that vary by population besides climate may contribute to the observed interaction.

Reproductive fitness

Herbivory reduced overall reproductive fitness (probability of reproduction x fruit production at the block level), drought marginally reduced reproductive fitness, and populations from wetter locations (higher soil water storage-str) had marginally lower reproductive fitness (Figure 2.5, Table 2.2). Interestingly, there was an interaction between herbivory treatment and population, but not between herbivory treatment and soil water storage, suggesting that populations varied in response to herbivory due to factors unrelated to the climate metrics we tested.

DISCUSSION

Theory posits that predominantly abiotic drivers govern phenological responses in high elevations and latitudes with short, variable growing seasons while biotic cues drive responses at lower elevations, which experience relatively more climatically stable seasonal conditions (Wolkovich et al., 2014). Our results provide some evidence consistent with these expectations: we found that abiotic factors (elevation, growing season length, and temperature) explain variation in first flowering along an elevation gradient, but that both abiotic (growing season length) and biotic (herbivory pressure) factors in the home environment explain response to herbivory and drought in a common garden environment. Understanding the relative importance of abiotic and biotic factors driving selection on phenology and fitness along environmental gradients will be critical for predicting population persistence in response to climate change, and is an area of study being actively explored (Wolkovich et al., 2014).

We find that the home environment profoundly impacts phenology and fitness responses to herbivory and drought. Flowering phenology under common garden conditions was earlier for populations from higher elevations, and populations from low elevations with high herbivory pressure and long growing seasons best tolerated herbivory, especially under high water availability. These results are consistent with local adaptation along an elevation gradient related to herbivory pressure and growing season length. Overall, this study provides evidence for population variation in flowering timing and responses to multiple stressors in *S. tortuosus* along an elevation gradient, which can have important implications for future evolutionary responses. Our results indicate that under continued climate change, abiotic and biotic cues will interact with historical environmental conditions to shape phenology and fitness responses in this species, with consequences for population persistence.

Plants from cooler, high elevation environments with shorter growing seasons flowered more rapidly than those from warmer, low elevation populations with longer growing seasons. Such phenotypic variation maintained in a common garden is consistent with local adaptation of timing of flowering onset along an elevation gradient (Hall & Willis, 2006; Anderson et al., 2013; Leinonen et al., 2013). These patterns also align with optimal flowering theory, which predicts that in seasonal environments, selection will favor earlier flowering in short seasons (Cohen, 1976; Kozłowski, 1992; Weis et al., 2014). For example, one study found that accessions of *Arabidopsis thaliana* from cooler climates flower more quickly under warm growing conditions than lines from warmer areas (Hoffmann et al., 2005). The authors attribute this result to selection to complete growth in a shorter growing season. Indeed, our population with the shortest time to onset of flowering was LV3, which experiences abbreviated growing seasons in the field due to high snowpack in the spring and the onset of winter conditions in the fall (Dove et al., 2012) and flowers immediately upon snowmelt (*unpublished data - Lassen MS*). On the other hand, the lowest elevation population, TM2, started flowering last, which may indicate that individuals from this population are selected to take advantage of longer growing seasons, possibly by accruing more biomass before transitioning to reproduction. It is also the only primarily annual population in this dataset.

If high elevation *S. tortuosus* populations initiate flowering earlier because they are locally adapted to brief growing seasons, one might expect that they would respond to herbivory by rapidly regrowing reproductive structures to complete their life cycles within the growing season. However, we instead found that high elevation populations with shorter growing seasons were generally less likely to attempt reproduction following clipping. This surprising result may be explained by the iteroparous perennial life history strategy displayed by some *S. tortuosus* individuals at higher elevations. If clipped individuals have too little time to regrow reproductive structures and complete reproduction before the growing season ends, but have the capacity to perennate, they may instead re-allocate growth to vegetative structures and attempt to reproduce in a following season (Brys et al., 2011). Selection should favor perennating over flowering late if late flowering incurs higher fitness costs than reproducing in a future growing season. In seasonal environments, flowering too late can expose plants to late season drought, increased herbivory, or early winter conditions like frost and snow (Pilson, 2000; Franke et al., 2006; Franks et al., 2007; Giménez-Benavides et al., 2007; Hamann et al., 2018; Rauschkolb et al., 2022). Similarly, optimal flowering theory also predicts an asymmetrical fitness function such that flowering too late incurs higher fitness costs than flowering too early, and entails a risk of total reproductive failure (Weis et al., 2014). Other studies have found that plants at higher elevations displayed lower tolerance because browsing reduced the time available for regrowth in the remainder of the growing season (Freeman et al., 2003). Grazing on perennial plants can reduce transition probability of surviving and flowering the next year (Brys et al., 2011), and those that do survive and flower may experience diminished reproductive output (Knight, 2003) or even population decline (Knight, 2007; Knight et al., 2009). Indeed, the lowest elevation population, TM2, is the only population that displays a majority annual life history strategy, which may explain why it was more likely to reproduce after clipping. Further, TM2 also has the longest growing seasons, providing more time to re-grow and complete reproduction following clipping. Therefore, the increased tolerance to herbivory at lower elevations we observed is consistent with local adaptation to growing season length.

Plant tolerance to herbivory may also decline with source elevation due to variation in factors covarying with elevation besides growing season length, such as herbivory pressure or resource gradients. For example, individuals from low elevation populations which evolved under an environment with high exposure to herbivory were more likely to successfully reproduce. These results are consistent with local adaptation to herbivory pressure along an elevation gradient, a pattern which has also been detected in other studies investigating intraspecific differences in herbivory responses (Rasmann et al., 2014b; Anderson et al., 2015).

Variation in resource availability across an elevation gradient may also drive differences in responses among populations and/or across watering treatments. If plants from low elevation populations evolved in higher-resource environments (Callaway et al., 2002; Rasmann et al., 2014a; Moreira et al., 2018), our results align with models predicting selection for increased investment in fast growth traits in response to herbivory when the cost of replacing tissue is low (Herms & Mattson, 1992; Stamp, 2003; Cipollini et al., 2017; Züst & Agrawal, 2017; Hahn et al., 2021). If life history decisions like flowering and reproducing in response to herbivory covary with growing season length, and individuals use water availability as a cue to determine time remaining in the growing season, then we would have expected high elevation plants to display greater herbivory tolerance at higher water levels. The fact that we observe the opposite in our study may indicate that *S. tortuosus* plants use cues other than or in addition to water to determine remaining time left in the growing season, such as photoperiod, heat, or photothermal accumulation (Aono & Kazui, 2008; Wilczek et al., 2010; Cober et al., 2014; Wadgymar et al., 2018).

A caveat to our results is that the extreme accidental drought may have influenced plant responses. In effect, the accidental drought artificially truncated the growing season at a time when photoperiod and thermal cues were not indicative of end of season, possibly creating maladaptive plant responses. Additionally, the well-watered treatment exposes plants to higher water availability than mean conditions populations experience in the field, which may minimize the biological relevance of responses to our experimentally imposed well-watered treatment, though the highest watering treatment is similar to water availability levels in peak water years for all but one population (LV3; Figure 2.2).

Plants in California are expected to experience increased drought stress, lower rainfall, and more erratic and unpredictable abiotic conditions with future climate change (Wright et al., 2016; Pathak et al., 2018; Swain et al., 2018; Luković et al., 2021). Plant-herbivore interactions under climate change are much more difficult to predict, but have already undergone climate-driven alterations with diverse phenological and fitness consequences for both the plants and herbivores (Post & Forchhammer, 2008; Robinson et al., 2017; Hamann et al., 2021a; Rauschkolb et al., 2022). Consistent with paleontological records showing higher rates of herbivory during warmer periods, a recent meta-analysis found that drought stress directly and indirectly induces greater food consumption by herbivores and further suggests that heightened temperatures and drought may increase florivory (Hamann et al., 2021a). Ultimately, the degree to which specific environmental characteristics mediate variation in phenological and fitness responses and the presence of adaptive plastic and genetic variation in these traits will determine whether these populations will persist in the face of climate change.Overall, our study indicates that the changes in drought and herbivory pressure expected with climate change are likely to pose significant challenges to *S. tortuosus* at high elevations, resulting in fitness losses that may threaten population persistence.

This study highlights the value of assessing intraspecific phenological and fitness responses to herbivory and drought in the context of home environment. Our results reveal that the shifting abiotic and biotic conditions predicted with climate change may differentially affect populations across a species range, depending on factors like home herbivory pressure, growing season length, and life history strategy. Possible local adaptation to such factors is likely to play a key role in enabling or hindering adaptive evolution and therefore persistence under climate change. Further research investigating variation in the effects of interactions between altered abiotic and biotic conditions across environmental gradients can facilitate identification of vulnerable populations in the face of climate change.

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FIGURES

Figure 2.1 Population map and home herbivory pressure and climate

Population (a) map, (b) herbivory pressure at home across an elevation gradient and (c) Principal Components Analysis describing contemporary climate across populations. (a) Points represent populations and colors indicate the elevation at each population. (b) Herbivory pressure, defined as the proportion of individuals with herbivory damage present in each population when surveyed near peak reproduction. Points represent populations and colors indicate the elevation at each population. (c) Principal Components Analysis describing variation in population abiotic conditions across populations from 1990-2020. First two principal components (PCs) illustrated on the x and y axes. Growing season variables include climatic water deficit (cwd), precipitation (ppt in mm H_2O), maximum and minimum temperature (tmax and tmin, respectively), soil water storage (str), and snowpack (pck). PC1 (72%) describes variation along an elevation gradient and related metrics such as growing season length and temperature. PC2 (19.09%) describes variation related to drought metrics like soil water storage and climatic water deficit. Points represent populations and colors indicate the elevation at each population. Data source was Flint et al. (2021; 2023).

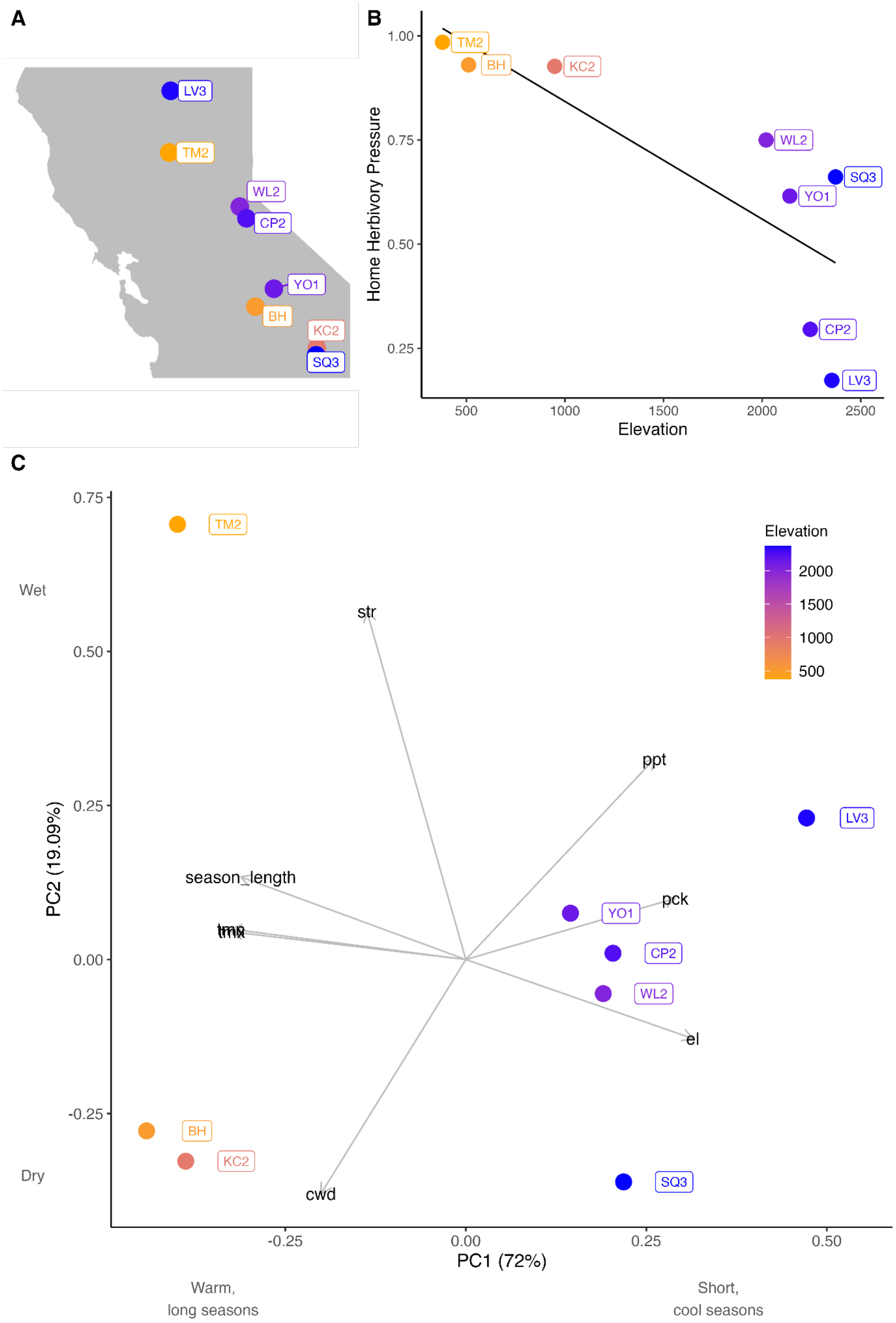

A
LV3
TM2
WL2
CP2
YO1
BH
KC2
SQ3
B
Home Herbivory Pressure
1.00
0.75
0.50
0.25
TM2
BH
KC2
WL2
SQ3
YO1
CP2
LV3
500
1000
1500
2000
2500
Elevation
C
0.75
0.50
0.25
0.00
-0.25
Wet
Dry
PC2 (19.09%)
TM2
str
ppt
LV3
season_length
tmp
tmx
pck
YO1
CP2
WL2
el
BH
KC2
cwd
SQ3
Elevation
2000
1500
1000
500
-0.25
0.00
0.25
0.50
PC1 (72%)
Warm,
long seasons
Short,
cool seasons

Figure 2.2. Experimental watering levels compared to home precipitation

Treatment watering amounts compared with total precipitation at home population (ppt in mm H_2O) during the peak water year (October-May). Points represent populations, shapes represent precipitation metric (circles = mean precipitation from 1990-2020; X's = maximum precipitation from 1896-2020), and lines represent watering amounts dispensed in treatments. Points colored by elevation. Lines colored by amount of water for each treatment. Calculations for dispensing water to plants in the experiment were rate-based, so the amount dispensed daily during the experiment is equal to the mean amount a plant would receive daily in the field based on the total precipitation during the peak water year. Therefore, the total volume of water plants received in the experiment, if summed daily for 8 months, would equal the amount of water for each treatment represented by the lines on the graph. Data source was Flint et al. (2021; 2023).

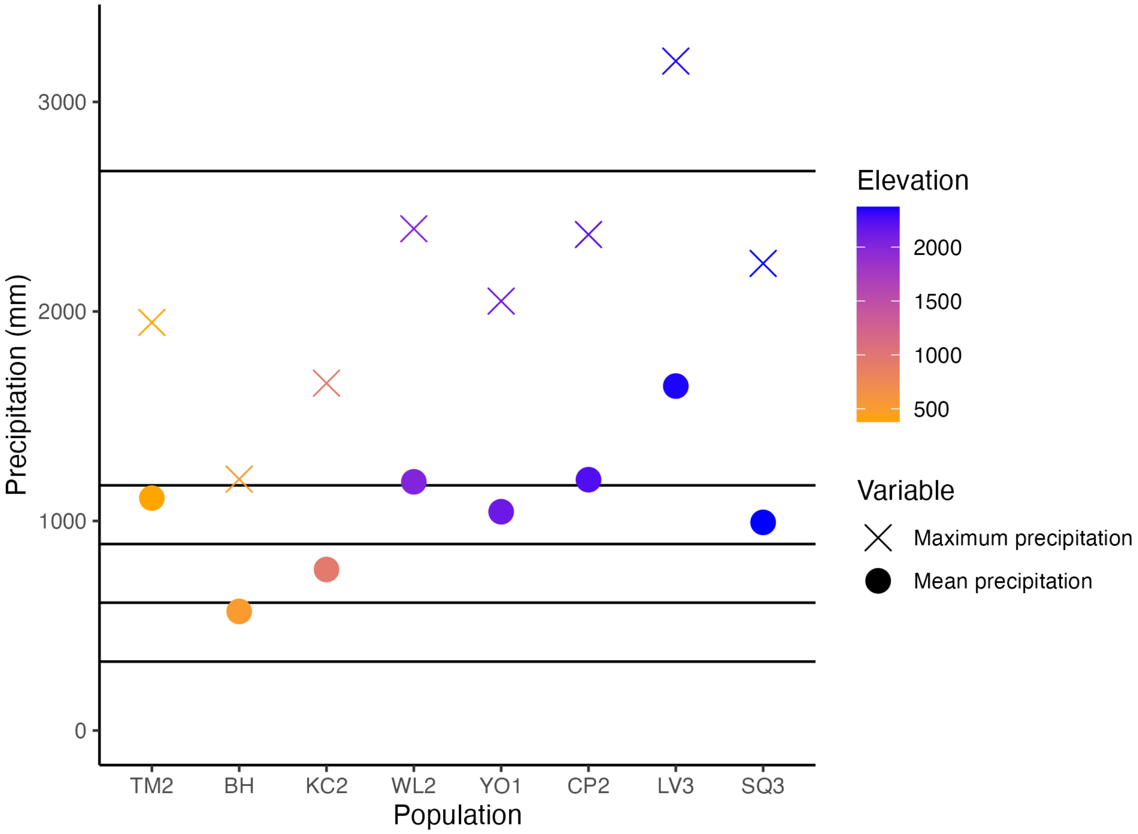


Table 2.1. Flowering responses summary model output

Results from mixed linear models for flowering time; χ^2 and Pr (> χ^2) statistics are results from likelihood ratio tests. Black vertical lines separate different models that include different main effects for population (Population coded as factor versus climate PC1 versus cwd).

	Herbivory Treatment x Drought Treatment x Population		Herbivory Treatment x Population		Drought Treatment x Herbivory Treatment		Drought Treatment x Population		Drought Treatment		Population		PC1		cwd	
Response	χ^2	**Pr(>χ^2)**	χ^2	**Pr(>χ^2)**	χ^2	**Pr(>χ^2)**	χ^2	**Pr(>χ^2)**	χ^2	**Pr(>χ^2)**	χ^2	**Pr(>χ^2)**	χ^2	**Pr(>χ)**	χ^2	**Pr(>χ)**
Probability of flowering	1.74	0.94	4.04	0.98	2.56	0.92	3.10	0.99	0.37	0.54	170.11	**<.001**	--	--	95.74	**<.001**
Log first flowering	--	--	--	--	--	--	--	--	--	--	21.44	**<.001**	4.524	**0.03**	--	--
Peak flowering	3.35	0.50	3.92	0.42	1.20	0.27	0.46	0.98	0.05	0.83	3.63	*0.07*	--	--	--	--

$P < 0.05$ (in bold)
$P < 0.1$ (in italics)

Table 2.2. Reproductive fitness responses summary model output

Results from fixed models for reproductive fitness defined as probability of reproducing x fecundity. Statistics are results from likelihood ratio tests.

	Herbivory Treatment x Drought Treatment x Population		Herbivory Treatment x Drought Treatment x Growing season length		Herbivory Treatment x Drought Treatment x Herbivory pressure	
Response	**χ^2**	**Pr(>χ^2)**	**χ^2**	**Pr(>χ^2)**	**χ^2**	**Pr(>χ^2)**
Probability of reproduction	5.44	0.36	9.78	**0.002**	5.58	**0.02**
Fecundity	9.59	*0.09*	--	--	--	--

	Herbivory Treatment x Drought Treatment x Population		Herbivory Treatment x Population		Drought Treatment x Herbivory Treatment		Drought Treatment x Population		Herbivory Treatment x Soil Water Storage		Drought Treatment		Herbivory Treatment		Soil Water Storage	
Response	**χ^2**	**Pr(>χ^2)**	**χ^2**	**Pr(>χ^2)**	**χ^2**	**Pr(>χ^2)**	**χ^2**	**Pr(>χ^2)**	**χ^2**	**Pr(>χ^2)**	**χ^2**	**Pr(>χ^2)**	**χ^2**	**Pr(>χ^2)**	**χ^2**	**Pr(>χ)**
Reproductive fitness	8.26	0.14	21.69	**0.02**	8.27	0.22	12.73	0.24	1.46	0.23	3.34	*0.06*	50.84	**<.001**	3.45	*0.06*

$P < 0.05$ (in bold)
$P < 0.1$ (in italics)

Figure 2.3. Probability of reproducing

Probability of reproducing in relation to home herbivory pressure across herbivory and watering treatments for *Streptanthus tortuosus*. Top panel shows control herbivory treatment and bottom panel shows herbivory treatment. Colors indicate experimental watering level (in mm H_2O) for each drought treatment, and lines indicate trends predicted from linear models for each drought treatment. Points indicate means for probability of reproducing for each treatment combination per population.

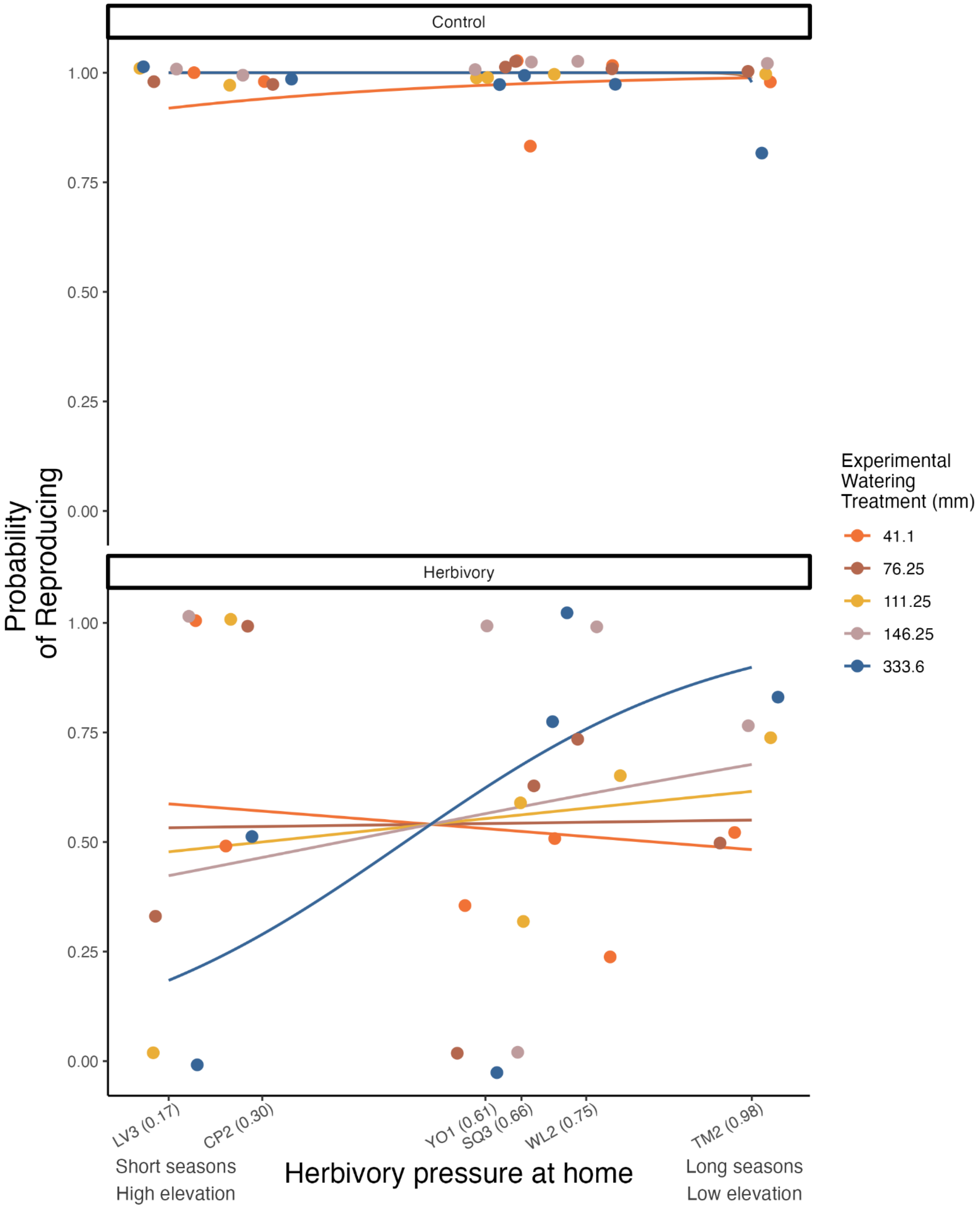


Figure 2.4. Fecundity

Fecundity in relation to experimental watering treatment across populations and herbivory treatments for *Streptanthus tortuosus*. Panels show responses by population (in order of increasing elevation). Colors indicate herbivory treatment (green = control, pink = herbivory), and lines indicate trends predicted from linear models for each herbivory treatment. Points indicate fecundity for each individual.

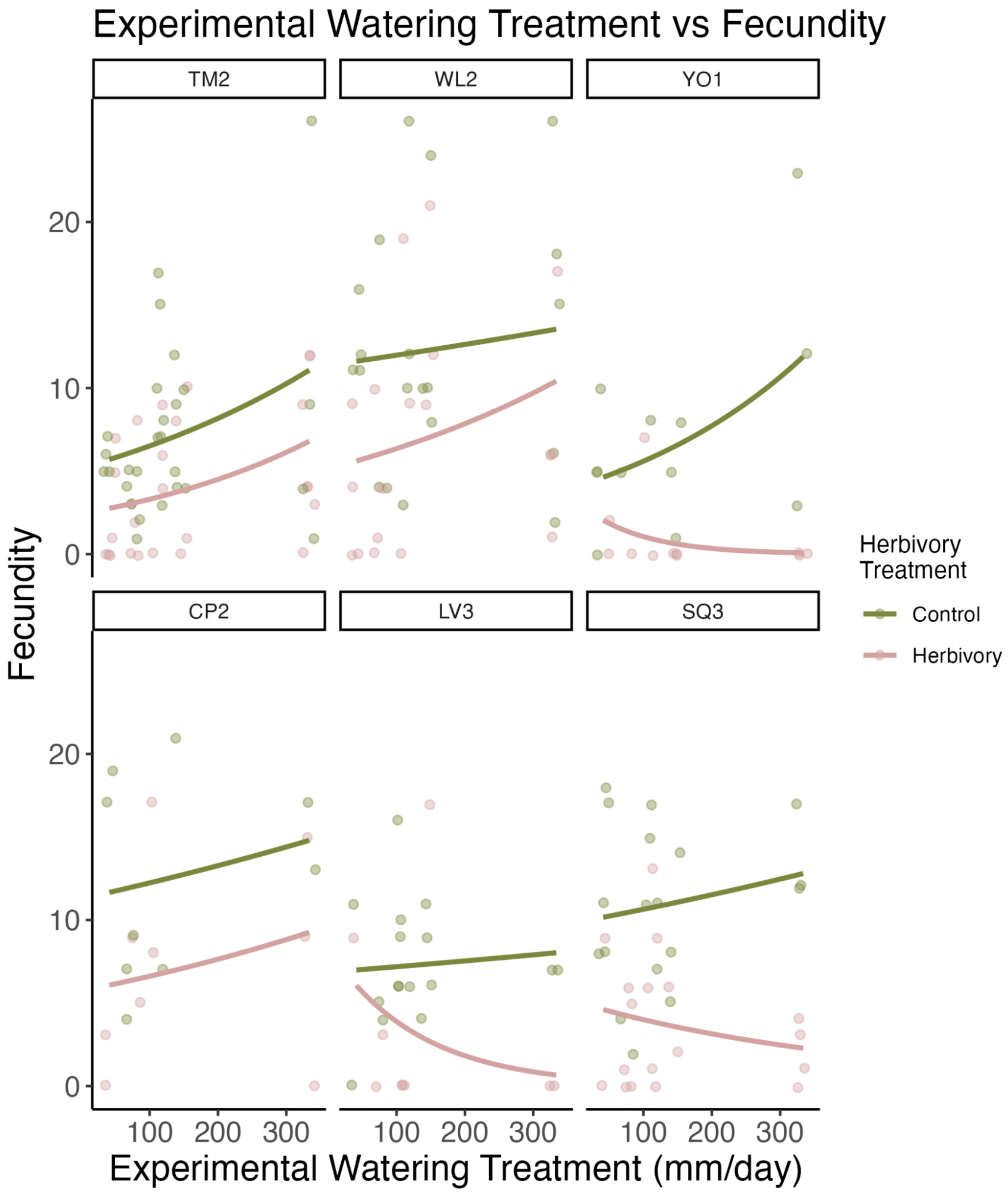
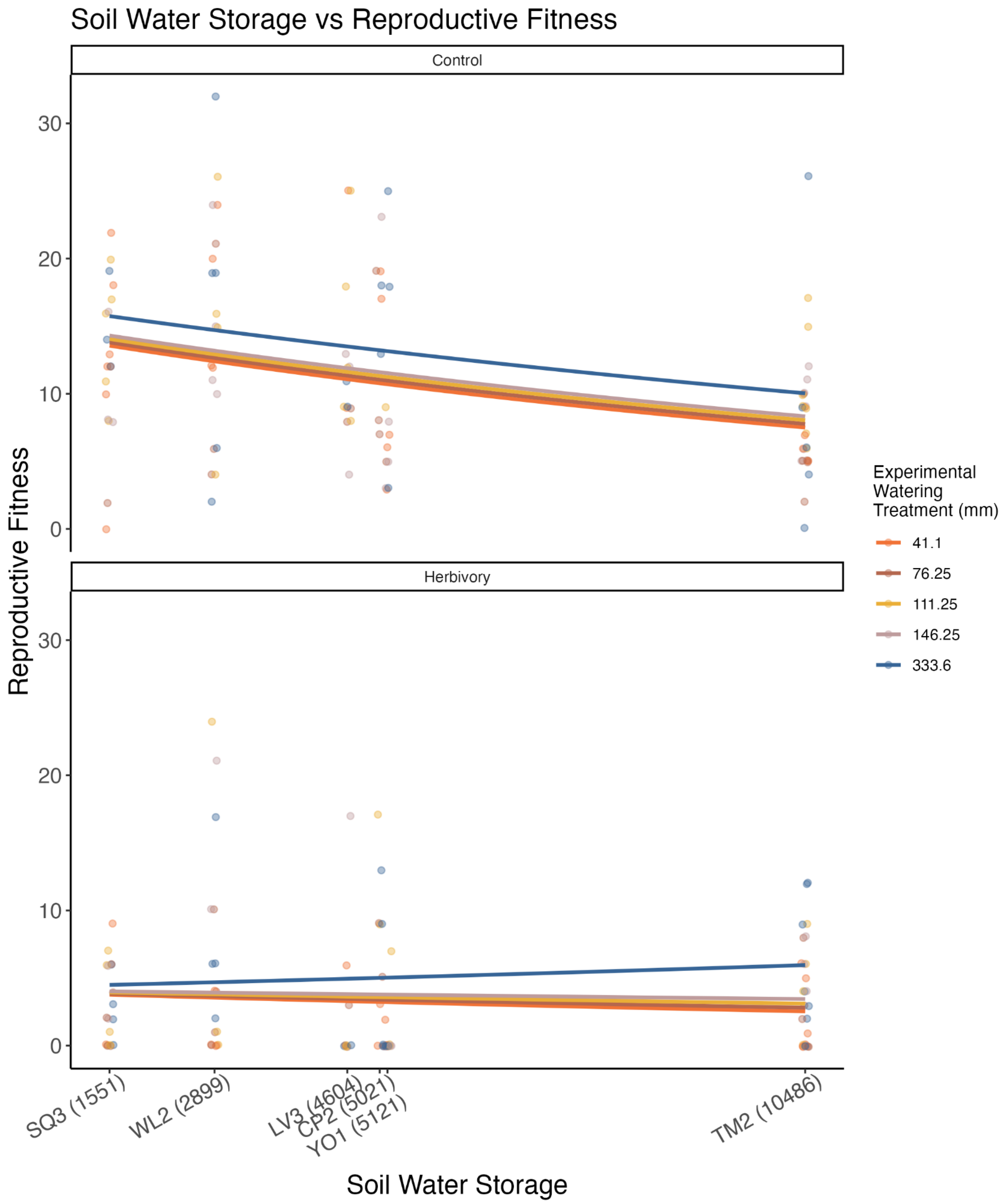


Figure 2.5. Reproductive fitness

Reproductive fitness (probability of reproducing x fecundity) in relation to population field soil water storage (str) across experimental watering treatments for *Streptanthus tortuosus*. Top panel shows the control treatment and bottom panel shows the herbivory treatment. Colors indicate experimental watering level (in mm H_2O) for each drought treatment, and lines indicate trends predicted from linear models for each drought treatment (linear regression models were conducted at the block level). Points indicate observed reproductive fitness calculated for each individual.



SUPPLEMENTAL MATERIALS

Supplement 2.1. Population localities, climate, and herbivory pressure

Population locality and environment of origin

Population locality, elevation, GPS coordinates, season length, herbivory pressure, and climate metrics. Climate variables are Climatic Water Deficit (cwd), precipitation (ppt in mm H_2O), snowpack (pck in in mm H_2O), soil-water storage (str), and minimum and maximum temperature (tmn, and tmx, respectively). Shown here are sums of monthly climate metric values during the water year (September to August), averaged across years. Data source for climate metrics was Flint et al. (2021; 2023).

Population	**Elevation**	**Lat**	**Long**	**Season length**	**cwd**	**ppt**	**pck**	**str**	**tmn**	**tmx**	**Herbivory pressure**
BH	511.43	37.41	-119.96	8.11	869.88	0.00	555.43	4077.78	100.43	271.25	0.93
CP2	2244.13	38.66	-120.13	5.35	719.80	2302.09	1203.84	5021.37	12.02	154.58	0.30
KC2	947.93	36.82	-118.84	8.64	958.13	4.66	757.76	3983.49	99.04	241.05	0.92
LV3	2353.55	40.47	-121.52	4.74	656.35	4789.92	1676.09	4604.96	-18.32	128.22	0.17
SQ3	2372.45	36.72	-118.85	4.42	694.35	1415.88	991.45	1551.74	12.98	155.56	0.66
TM2	379.153	39.59	-121.55	9.12	631.03	0.00	1090.92	10486.12	110.76	262.64	0.98
WL2	2020.12	38.83	-120.25	4.97	616.72	1599.65	1188.62	2899.47	28.30	167.73	0.75
YO1	2140.51	37.66	-119.62	5.13	598.69	1323.11	1035.35	5121.73	25.63	171.02	0.62

Climate PCA loadings

Variable	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**
cwd	-0.2551939	-0.4832851	0.67414809	-0.378076	-0.0125791	-0.2647976	-0.1681481	0.0731068
pck	0.36888715	0.12627689	0.54879515	0.33748407	-0.4768188	0.41840705	-0.1461307	-0.095551
ppt	0.33228936	0.41420322	0.37345147	0.16204096	0.63552284	-0.2758232	-0.0611969	0.26188439
str	-0.1743423	0.71835024	0.04742393	-0.5439755	-0.3355283	-0.1268226	-0.1635347	-0.0035424
tmn	-0.4135843	0.06275604	-0.0371141	0.11875835	0.34399472	0.40577367	-0.7060505	-0.1674416
tmx	-0.4123818	0.05711304	-0.0142691	0.35201572	-0.2385522	-0.0091454	-0.0054249	0.80345531
el	0.39984877	-0.1630938	-0.1324644	-0.524798	0.12171681	0.5111995	-0.0921244	0.48573122
season_length	-0.3962285	0.17058266	0.28897286	-0.0869767	0.25683184	0.48640982	0.64254156	-0.0861248

Herbivory pressure field surveys

We combined data from several different studies to estimate herbivory pressure experienced by populations in the field. All sampling methods involved recording herbivory presence/absence on individuals at the same phenophase within previously established transects or plots.

For the populations BH, KC2, SQ3, TM2, WL2, and YO1, we designed and implemented a survey specifically for the purposes of this study that we conducted once at each site in 2021 near peak reproduction (when most plants were post-flowering). The sampling protocol originally included more detailed data on the level and type of damage present on different organ types, but was then simplified to a presence/absence metric for this study.

For the population LV3, we used the same protocol as described above but collected data during weekly surveys during the growing season in 2018 and filtered to include only data collected on the day on which the majority of individuals were post-flowering so plant phenophase was consistent across sampling locations.

For the population CP2, herbivory presence/absence data was collected on 88 individuals near peak reproduction in 2021.

Population	**Total # individuals**	**# Individuals with herbivory present**	**Survey date**
BH	43	40	6/23/21
CP2	88	26	7/28/21
KC2	41	38	8/15/21
LV3	52	9	8/27/18
SQ3	62	41	8/14/21
TM2	65	64	5/23/21
WL2	60	45	7/27/22
YO1	26	16	8/30/21

Herbivory at different phenological stages in the field

Top row, left-right: Vegetative rosette, Flowering plant, Plant in fruit
Bottom row, left-right: Herbivorized plants at same phenological stages as top row

Supplement 2.2. Experimental setup and methods details

Experiment replication

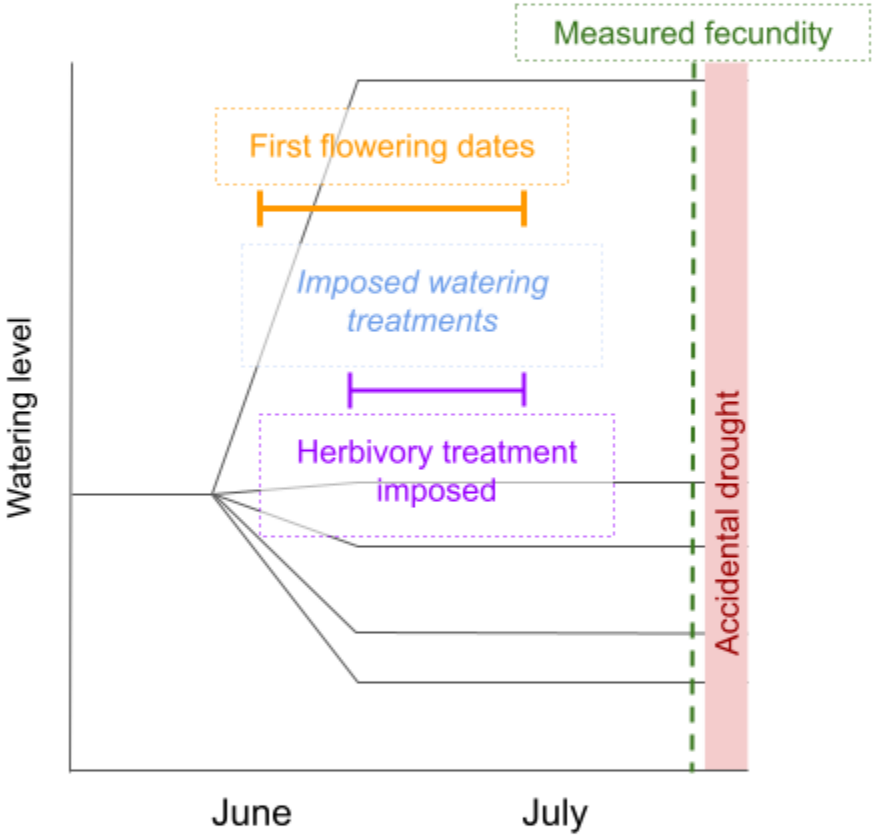
Plants that survived to be included in the experiment (alive after 6/11/21 or DOY 162; n = 503 total) by population and maternal family.

Population	**Seed year**	**# Maternal families**	**Total # individuals**
BH	2018	15	77
CP2	2020	18	64
KC2	2017	7	19
LV3	2016	11	81
SQ3	2017	12	74
TM2	2020	15	65
WL2	2020	15	66
YO1	2019	20	57

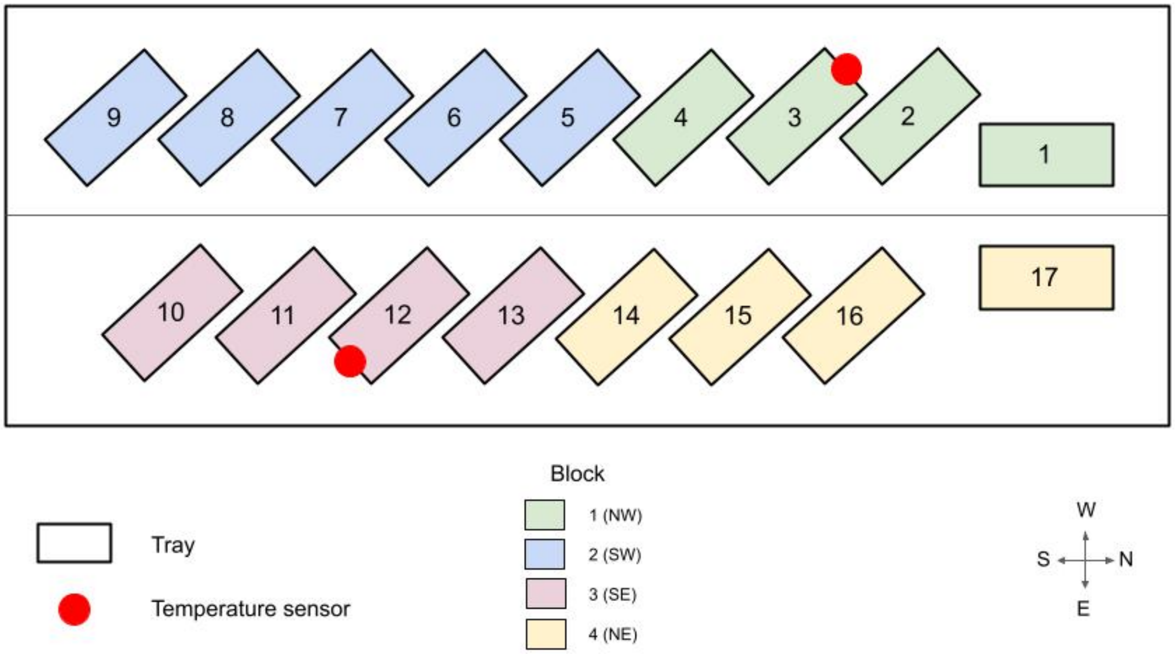
Planting timeline

	October			November			December			January			February			March			April			May		
High elevation planting (LV3, SQ3, CP2, WL2, YO1, KC2)																								
Stratification	🟩	🟩	🟩	🟩																				
Inductive conditions					🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩								
Vernalization																	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
Low elevation planting (BH, TM2)																								
Stratification					🟩	🟩	🟩	🟩	🟩															
Inductive conditions										🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩				
Vernalization																					🟩	🟩	🟩	🟩

Experiment timeline



Experiment setup on outdoor bench



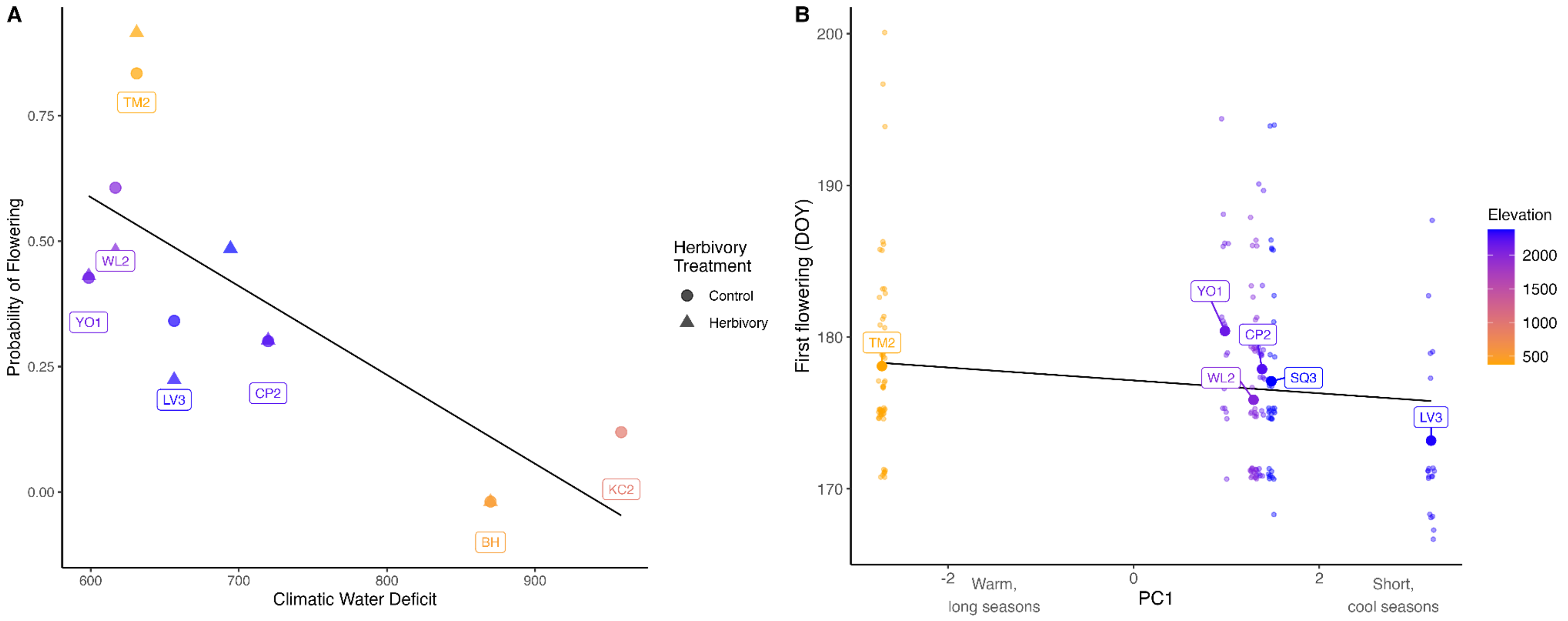
Supplement 2.3. Probability of flowering across populations

Table with number of flowering individuals, total number of individuals, and % flowering per population.

Population	**# Flowering individuals**	**Total # individuals**	**Proportion flowering**
BH	0	77	0
KC2	2	19	0.11
CP2	19	64	0.30
LV3	23	81	0.29
YO1	25	57	0.44
WL2	37	66	0.56
SQ3	37	74	0.5
TM2	57	65	0.88

Supplement 2.4. Flowering in relation to home climate

Climate versus (a) probability of flowering and (b) first flowering. (a) Climatic water deficit versus probability of flowering. Shapes indicate herbivory treatment (circles = control, triangles = herbivory). Points indicate probability of flowering for each population in each herbivory treatment. Line indicates linear model trend prediction for probability of flowering. (b) Population score on PC1 axis versus first flowering DOY. Small points indicate individuals and larger points indicate population means. Line indicates linear model trend prediction for first flowering. Colors indicate elevation at each population.



Supplement 2.5. Peak flowering responses including TM2 - summary model output

Results from mixed linear models for peak flowering time; χ^2 and Pr (> χ^2) statistics are results from likelihood ratio tests.

	Herbivory Treatment x Drought Treatment x Population		Herbivory Treatment x Population		Drought Treatment x Herbivory Treatment		Drought Treatment x Population		Drought Treatment		Population		PC1		cwd	
Response	χ^2	**Pr(>χ^2)**	χ^2	**Pr(>χ^2)**	χ^2	**Pr(>χ^2)**	χ^2	**Pr(>χ^2)**	χ^2	**Pr(>χ^2)**	χ^2	**Pr(>χ^2)**	χ^2	**Pr(>χ)**	χ^2	**Pr(>χ)**
Peak flowering	3.72	0.59	5.98	0.31	0.41	0.52	1.39	0.93	0.13	0.71	13.82	**0.02**	5.01	**0.03**	2.92	*0.09*

$P < 0.05$ (in bold)
$P < 0.1$ (in italics)

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CHAPTER 3

Morphological traits in *S. tortuosus* diverged in tandem across multiple elevational clines

INTRODUCTION

Understanding the drivers of population differentiation in traits of ecological importance is a central goal in evolutionary biology (Karhunen et al., 2014). Species and populations often vary along environmental gradients in ways that match local conditions. For example, plant architecture and leaf morphology can vary along elevation and latitudinal gradients (Körner 2003; Read et al., 2014; Halbritter et al., 2018), and this phenotypic variation frequently reflects adaptive divergence (Luo, Dong, et al., 2015; Ye et al., 2020). Although examples of adaptive evolutionary responses to environmental variation are ubiquitous (Kawecki & Ebert, 2004; Leinonen et al., 2008; Hereford, 2009; Savolainen et al., 2013), the relative roles of neutral versus adaptive processes in shaping differentiation continue to be debated (O'Hara, 2005; Karhunen et al., 2014; Wadgymar et al., 2022; Chung et al., 2023). Similarly, ascertaining how often such divergence occurs in the presence of continued gene flow versus via convergent evolution (Monty & Mahy, 2009; Bolnick et al., 2018; Marin et al., 2020) is an active area of study and will be crucial for understanding the potential for future evolutionary response in the face of climate change.

Environmental conditions vary steeply with elevation, and plants in alpine regions have evolved many adaptations to harsh abiotic conditions like low temperatures, high levels of ultraviolet radiation, and brief growing seasons (Körner 2003; Halbritter et al., 2018). For example, species and populations at higher elevations frequently develop shorter stature to protect from high winds and smaller, thicker leaves to increase resistance to frost and cold (Körner et al., 1989; Luo et al., 2015a; Halbritter et al., 2018). Small, thick leaves can help plants endure lower temperatures, higher solar irradiation, and wind exposure at high elevations (Tian et al., 2016; Liu et al., 2020). To cope with low temperatures, plants often decrease leaf size and increase leaf thickness to protect internal tissues from exposure and minimize heat loss at higher elevations (Körner 2003). Also, thicker leaves can protect from water loss, increase water use efficiency, and reduce transpiration, which may provide adaptive benefits under lower water availability at higher elevations (Guo et al., 2017). Shorter stature may enable plants to reduce damage from strong winds and take advantage of warmer temperatures near the soil surface (Turesson, 1922; Körner, 2003). Several studies have therefore posited that "alpine dwarfism" is adaptive (Byars et al., 2007; Gonzalo-Turpin and Hazard, 2009); in support of this, one field transplant study found that *Arabidopsis* individuals with a locally evolved dwarfism mutation achieved higher fitness at high elevations than those without it (Luo et al., 2015b).

Morphological traits can trade off along clines to create syndromes in plant architecture and leaf traits that maximize fitness (Wright et al., 2004; Díaz et al., 2016; Dammhahn et al., 2018; Sartori et al., 2019). At the whole organism level, the fast-slow continuum describes trade-offs in phenotypes resulting from variation from resource-acquisitive to resource-conservative traits along a low-high resource gradient that has been observed across taxa worldwide (Dammhahn et al., 2018). Such trade-offs can result in a relatively small number of evolutionarily viable trait combinations at specific locations along environmental gradients. For example, one study found that three-quarters of plant trait variation can be captured in a two-dimensional multivariate trait space: the first PC axis reflects the size of whole plants and their parts, and the second represents the leaf economics spectrum (Díaz et al., 2016). Overall, coordinated trait frameworks generally predict that morphological traits will vary from fast to slow with increasing elevation (Díaz et al., 2016; Dammhahn et al., 2018; Halbritter et al., 2018).

Though plant traits along elevation gradients often vary in similar ways, this variation may arise through different evolutionary mechanisms. When distinct ancestral populations repeatedly colonize higher elevations, they may evolve similar trait patterns either via continued gene flow or through convergent evolution while reproductively isolated (Thompson et al., 1997; Conte et al., 2015). Studies in natural and lab settings have shown that when replicate populations colonize similar habitats, evolutionary trajectories often yield inconsistent outcomes (Von Wettberg et al., 2008; Bolnick et al., 2018). Therefore, whether phenotypic evolution is repeatable and to what extent it depends on starting genetic variation is a central question in evolutionary biology and an area of active study (Agrawal, 2017; Bolnick et al., 2018; Fang et al., 2020; Heckley et al., 2022). Populations are already migrating to new habitats and facing novel ecological challenges due to climate change, such as plants adapting to novel environments at higher latitudes and elevations, but less is known about exactly how evolution of traits will proceed and the consequences for population persistence. Understanding whether evolution of key traits follows parallel trajectories over similar environmental gradients will thus be crucial for predicting biological responses to climate change.

While it is well understood that evolution by natural selection plays a key role in driving population divergence and local adaptation (Kawecki & Ebert, 2004; Leinonen et al., 2008), neutral processes like mutation, gene flow, and genetic drift can also contribute to phenotypic differentiation (Lande, 1992). For example, the stochastic effects of genetic drift in small populations can produce random variation in phenotypes across the landscape that do not necessarily match phenotypic optima for those environments (Jones et al., 1968; Spitze, 1993). In lieu of time-intensive and logistically challenging reciprocal transplant field studies (Kawecki & Ebert, 2004; Blanquart et al., 2013), methods to test for adaptive divergence by comparing quantitative trait variation (Q_{ST}) to neutral genetic divergence (F_{ST}) such as Q_{ST}-F_{ST} have proliferated in the past several decades (O'Hara & Merilä, 2005; Whitlock, 2008; Ovaskainen et al., 2011; Leinonen et al., 2013). One new approach, Q_{PC} (Josephs et al., 2019), offers substantial flexibility because it tests for excess phenotypic variance using the principal components of the genotypes and can therefore be performed on individuals grown in a common garden without knowledge of parentage (e.g. from wild-collected seed).

Here, we explore patterns in landscape population structure, investigate whether morphological traits have adaptively diverged, and elucidate relationships between traits, climate, and geography in the native California forb *Streptanthus tortuosus*. Populations of this species occur across a wide elevational gradient, making it an interesting study system for investigating questions related to adaptive phenotypic evolution and evolutionary repeatability. Additionally, *S. tortuosus* populations vary substantially in traits related to environmental conditions such as relative growth rate, leaf morphology, and life history strategy (Gremer et al., Bontrager et al., *unpublished*). Specifically, we ask: Have morphological traits adaptively diverged, and if so, does this divergence relate to climate? We predict that plants from higher elevation, colder environments will display more compact, stress tolerant morphologies consistent with a "slow" resource allocation strategy such as shorter stature, thicker leaves, and more branches and leaves, and that these traits have adaptively diverged. By investigating the evolutionary history of this species in relation to its realized climatic niche, this research can offer insights into traits and divergence for past and current climates, which may have implications for responses under future climate change.

METHODS

Study System

Streptanthus tortuosus Kellogg (Brassicaceae) is a widespread native wildflower that occupies bare habitats and exposed rocky outcrops across a broad elevational (200 m to 4100 m) and latitudinal range within the California Floristic Province (CFP) (Preston, 1991; Calflora 2014). Populations span from southern California to southern Oregon and tend to be discontinuously distributed (Preston 1991; Calflora 2014). The Streptanthoid complex is thought to have originated from southern warm-adapted taxa and subsequently expanded northward, possibly facilitated by the onset of a Mediterranean climate 2-5 Ma in the CFP (Raven & Axelrod, 1978; Cacho & Strauss, 2014). All populations experience the winter precipitation that characterizes Mediterranean environments (Baldwin, 2014; Rundel et al., 2016), though low elevation populations experience a winter growing season and hot, dry summers whereas high elevation populations have spring and summer growing seasons after snowmelt (Gremer, Chiono, et al., 2020). Variation in traits like flowering time and morphology among populations has been observed in the field and is maintained in a common garden (E. Suglia, M. Bontrager, *unpubl. data*), suggesting population differentiation. Gene flow in this species may be hampered by its patchy distribution across the landscape but could also be enhanced by its reliance on generalist pollinators (Preston, 1994). We studied 20 populations across the species range that experience conditions spanning the species' climatic niche (Figure 3.1; Suppl. 3.1). We collected seeds from maternal families at each population from 2014-2020 which were stored dry at room temperature before the experiment was initiated (~21°C).

Population climate

To understand how variation in morphological traits relate to home climate, we quantified variation in local conditions experienced by populations using downscaled climate data for 1990-2020 (Flint et al., 2021; 2023). We retrieved monthly climate variables for each population from the Flint Basin Characterization Model, which downscales PRISM (PRISM Climate Group, Oregon State University, http://www.prism.oregonstate.edu/) data to a 270m resolution for the California hydrologic region (Flint et al., 2021; 2023). The metrics we used were climatic water deficit (cwd), precipitation (ppt in mm H_2O), maximum and minimum air temperature, (tmax and tmin in °C, respectively), soil-water storage (str; amount of water stored in the soil), and snowpack (pck in mm H_2O; snow water equivalent) (Suppl. 3.1). Climatic water deficit (cwd) is a metric for drought stress calculated as the evaporative demand that exceeds available water (potential evapotranspiration - actual evapotranspiration; in units of mm H_2O). Soil-water storage is the amount of water stored in the soil (precipitation + snowmelt - actual evapotranspiration - recharge - runoff; in units of mm H_2O). We calculated a summary metric by summing the monthly values for precipitation metrics and averaging values for temperature metrics over all years (Suppl. 3.1). To characterize how long plants have to complete their life cycles across an elevation gradient, we quantified growing season length as the number of months from snowmelt to snow cover (first month with $pck = 0$ to first month with $pck > 0$) at sites with >10 mm snowfall per year on average and months with rainfall (first month with $ppt > 25$ mm to last month with $ppt > 0$) at sites with <10 mm snowfall per year and averaged over 1990-2020. Season length was highly correlated with elevation (-0.77; Suppl. 3.1).

Common Garden Planting

To understand how phenotypic and genetic traits vary among *S. tortuosus* populations, we grew plants in a common garden at the University of California, Davis, and measured morphological traits related to climatic adaptation, particularly elevation. We conducted this experiment in a lathhouse, exposing plants to ambient temperatures and day length while controlling the water regime.

We included 13-15 maternal families from each of the 20 populations (except SHA, for which only 6 families were available; Suppl. 3.1) in the common garden growout. On 10/17/20, we planted 3 seeds per maternal family in cone-tainers (164 ml cone-tainer pots, Stuewe and Sons, Corvallis, Oregon, USA) filled with 2 parts UC Davis potting soil (1:1:1 parts sand, compost, and peat moss with dolomite) and 1 part coarse 16 grit sand, then covered with approximately 1 cm of sand. After planting, pots were watered in and immediately stratified at 4°C in a chamber at Storer Hall, UC Davis under dark conditions to satisfy chilling requirements (stratification) for germination (Gremer, Chiono, et al., 2020). We stratified seeds for 6 weeks, watering as needed to maintain soil moisture, and on 11/30/20 moved plants to the UC Davis Controlled Environment Facility to germinate in 16-hour days at 24°C during the day and 16°C at night. Depending on population-wide germination levels, if multiple individuals germinated per pot, we either transplanted extra individuals into new cone-tainers to supplement sample sizes or thinned to one germinant per pot, keeping the plant closest to the center of the cone-tainer. After transplanting, we fertilized plants weekly for three weeks with a dilute fertilizer mixture (equivalent to ~25% strength Hoagland's solution). Once plants had reached 1 cm tall (on 5/4/21), we placed plants back into the 4°C chamber for vernalization for 11 weeks. Due to low germination levels for SHA and YO11, we supplemented plants for these populations from a different experiment (5 YO11 and 13 SHA individuals) which were planted and reared under identical conditions except were in inductive conditions for 16 fewer days and vernalized for 48 additional days. On 7/22/21, we assigned plants to randomized locations, moved them to a lathhouse, and watered and fertilized by hand as needed while installing drip irrigation emitters into each cone-tainer to water the plants for the remainder of the growout.

Trait measurement

To characterize how morphological traits vary along climatic gradients, we took several size measurements on all plants between 7/26-8/3/21, after vernalization but before flowering initiated. To evaluate variation in leaf size across and elevation gradient, we measured leaf thickness (Cornelissen et al., 2003) by using calipers midway between the margin and the midrib at the widest part of the most recently fully expanded and hardened leaf (as in Witkowski & Lamont, 1991) and measured the length of the longest leaf on the plant, not including the petiole. To explore variation in apical dominance and meristem allocation, we counted the total number of branches and measured height as the distance from the soil surface to the highest point. Finally, to assess plant size, we counted the total number of leaves and measured stem diameter, traits which are often correlated with biomass (Picard et al., 2012; Paul et al., 2016; J. Sun et al., 2019). We measured stem diameter with calipers to the nearest hundredth of a millimeter at the soil surface.

Sequencing and assembly

To assess population structure and neutral genetic divergence, we collected leaf tissue to perform DNA extraction, sequencing, and assembly. First, we collected two 50-100 mg samples of tissue into 1.5ml microcentrifuge tubes from 9/28-10/22/2021 by clipping the petiole carefully without damaging apical or axillary meristems. Ideally, we chose 1-2 young, perfect, less than fully expanded leaves on side branches, but if this was not possible, we collected the highest quality tissue available. We placed samples directly into a dewar of liquid nitrogen while transported to a -80 °C freezer in Davis, CA, where tissues were stored until we extracted DNA for sequencing.

We subsampled 192 randomly chosen individuals from 6-10 maternal families per population for sequencing. We ground frozen tissues by hand in liquid nitrogen and extracted

DNA using the DNEasy Plant Mini Kit (Qiagen, Valencia, California, USA). We sent samples to the UC Davis Genome Center to prepare genotyping by sequencing (GBS) libraries and sequence samples on an Illumina NovaSeq platform.

We used the Stacks v2.62 (Catchen et al., 2013; Rochette & Catchen, 2017; Rochette et al., 2019) pipeline designed for paired-end GBS for cleaning, demultiplexing, alignment, and variant detection. We aligned reads using the sister species *S. diversifolius* as a reference genome. The reference *S. diversifolius* genome that we used is a draft genome created with the assembly program Allpaths-LG and short read sequencing data. The draft assembly was then scaffolded using Hi-C sequencing data and the Hi-C scaffolding program YaHS. We also used the Stacks "populations" program to generate and export SNP data in vcf and TreeMix format for further analyses. We filtered out rare variants by setting a minimum allele frequency of 0.01 (--min-maf 0.01).

Patterns of population differentiation and spatial genetic structure

To assess sequencing and alignment quality, we used samtools to quantify the average number of paired reads per individual (function *flagstat*) as well as average reads mapped to the reference genome and average coverage across reference genome scaffolds (function *coverage*) (Danecek et al., 2021). To estimate population genetic structure, we calculated pairwise F_{ST} (Wright, 1949; Holsinger & Weir, 2009) for each pair of populations using the "populations" module in Stacks. We also visualized population structure by conducting a Principal Components Analysis (PCA) on the SNP data. To do so, we used the function *glPca* from the R package adegenet (Jombart, 2008). We identified six outliers mislabeled by population and used the PCA to reassign them to their putative populations. Results from analyses with these outliers reassigned versus left as their original population assignment were concordant. We also estimated ancestry coefficients using sparse non-negative matrix factorization with the *snmf* function from the package LEA (Frichot & François, 2015). To determine the most probable number of K ancestral populations, we used the function *LEAce* in the wrapper package sambaR (De Jong et al., 2021) to calculate cross-entropy loss for K values of 2-14 with 50 iterations each. Finally, we evaluated isolation by distance (IBD) using a Mantel test to assess the correlation between pairwise F_{ST} and Euclidean geographic distance.

To evaluate evolutionary relationships between populations, we constructed a phylogeny using TreeMix v1.13 (Pickrell & Pritchard, 2012) and rooted the tree using *S. diversifolius* as an outgroup. To account for linkage disequilibrium, we conducted the analysis on different partitions of the dataset by separating into blocks of 1000 SNPs using the -k parameter in TreeMix (-k = n SNPs window size). To judge confidence in the tree topology, we generated a bootstrap replicate by resampling blocks of 500 SNPs using the -bootstrap parameter in TreeMix.

Morphological trait variation in relation to climate and phylogeny

To explore whether populations evolved coordinated trait strategies across environmental gradients, we examined how morphology relates to climate in multivariate space using Canonical Correlation Analysis (CCA) (Hotelling 1936; Anderson 1984). The morphological traits we used were leaf thickness, stem diameter, height, length of longest leaf, and number of leaves; the climate variables we used were elevation, cwd, growing season length, str, tmx, and ppt.

Adaptive divergence in morphological traits

To determine whether morphological traits are adaptively differentiated among populations in relation to climate, we used an extension of Q_{ST}-F_{ST} called Q_{PC}, which applies principal component analysis to the relatedness matrix in order to test for adaptive divergence in polygenic traits (Josephs et al., 2019). Like traditional Q_{ST}-F_{ST} methods, Q_{PC} tests for phenotypic divergence in quantitative traits that exceeds expected levels of neutral genetic divergence. However, Q_{PC} detects adaptive differentiation by identifying correlations between the phenotypic trait of interest and any relatedness PCs generated from the kinship matrix that are significantly different from that expected under neutral evolution. The utility of Q_{PC} for our study is that it tests for adaptive differentiation on individuals without prior knowledge of relatedness between individuals, lending itself well to studies on plants grown from field-collected seed with unknown parentage. We tested for signatures of adaptive divergence in leaf thickness, stem diameter, height, length of longest leaf, number of leaves, and number of branches. Because our phylogeny showed geographic structure aligning with southern, central, and northern populations, we performed Q_{PC} both on the entire dataset and on two subsets of populations grouped by latitude: 1) the northern clade and 2) the central and southern clades combined. To conduct the Q_{PC} analyses, we first constructed the K matrix using the function *myK* in the quaint package in R (Josephs et al., 2019). We then ran Q_{PC} using the function calcQpc, for which we defined PCs 1:10 as those used to test for selection (parameter myM) and defined PCs 11:n (where n = number of individuals) as those used to estimate V_A (parameter myL).

RESULTS

Climatic variation across population source environments

To characterize variation in climate across populations, we used Principal Components Analysis (PCA, *prcomp* function in base R) including all previously described climate metrics as well as growing season length and elevation. PC1 (59.82%) described variation along an elevation gradient, from warm, low elevation sites with long growing seasons to cool high elevation sites with short seasons. The strongest loadings for PC1 were temperature (-0.44 for both tmn and tmx), elevation (0.43), snowpack (0.41), and growing season length (-0.38). PC2 (24.2%) described variation related to drought metrics along a wet to dry gradient, and climatic water deficit (0.62), soil water storage (-0.58), and precipitation (-0.45) loaded most strongly (Figure 3.1b, Suppl. 3.1).

Patterns of population differentiation and spatial genetic structure

After sequencing and alignment, there were an average of 11,693,050 paired reads per individual and an average of 64% of the reads mapped to the reference genomes. Average coverage across reference genome scaffolds was 7.43X. After filtering for missingness, 74,934 biallelic SNPs were retained on the dataset of 192 samples.

Patterns of relatedness in *S. tortuosus* populations over time suggest that lineages at different latitudes migrated into higher elevation environments. Individuals cluster tightly by population (high population structure; Figure 3.2, Suppl. 3.2) and populations that are closer together geographically are more closely related (IBD; Mantel test: $P < 0.001$; Figure 3.2b). The maximum likelihood TreeMix reconstruction shows populations group into three distinct clades by latitude: northern (sites: IH, DPR, TM2, WV, SHA, & LV), central (sites: WL & CP), and southern (sites: SQ, KC, YO, & BH) (Figure 3.2a). Structure models also identified strong clustering by geography (Figure 3.3; Suppl. 3.3). The latitudinal division in lineages is most evident at $K = 2$, and a third cluster defining additional variation by longitude is resolved at $K = 3$. At $K = 3$ and $K = 4$, admixture within the northern and central clades is uncovered. The cross-entropy test revealed that populations have complex structure, with $K = 14$ most strongly supported (Suppl. 3.3).

The PCA performed on the SNP data revealed that PC1 explains 19.95% of the variation, PC2 explains 7.42%, and PC3 explains 6.18%, and all three genetic PC axes relate to environmental gradients (Figure 3.4a-b). PC1 describes variation along a geographic gradient largely from south to north and correlates most strongly with latitude (0.82), precipitation (0.78), and longitude (0.70). PC2 describes variation along a gradient from dry to wet and is most correlated with cwd (-0.71) and soil water storage (0.51). PC3 describes variation along an elevation gradient from high, cool locations to low, warm locations, correlating with elevation (-0.71), and maximum and minimum temperature (0.68 and 0.64, respectively).

Adaptive divergence in morphological traits

The Q_{PC} analyses provided support for adaptive divergence within the southern clade in height along PC4 (correlated with cwd (-0.36), season length (0.35), and latitude (0.26); $P = 0.03$, Figure 3.5a) and number of leaves along PC8 (correlated with maximum temperature (0.21), elevation (0.18), and minimum temperature (-0.18); $P < .0001$, Figure 3.5b; Table 3.1). We did not detect signatures of adaptive differentiation in leaf thickness, stem diameter, length of longest leaf, or number of branches in either the southern clade or the combined northern and central clades. Despite correlations between these traits and climatic gradients like elevation and latitude, variation in these morphological traits did not seem to be sufficient to overcome substantial population structure.

Morphological trait variation in relation to climate

Morphology varied significantly with climate in *S. tortuosus* ($P < 0.0001$): plants from higher elevation locations with colder temperatures and shorter growing seasons were branchier and shorter with more, thicker, smaller leaves and thicker stems (Figure 3.4c). The first canonical variate explains 60.5% of the variation in the data and primarily describes differences in leaf thickness along elevation (correlation between first morphological and climate canonical variates = 0.65; Figure 3.4c).

DISCUSSION

As *S. tortuosus* populations diverged, they expanded into higher elevations multiple times, evolving thicker leaves and stems, shorter stature, and more branches and leaves that are consistent with adaptation to harsher abiotic conditions in alpine environments (Figure 3.2; Figures 3.4; 3.5; 3.6). Populations cluster into three distinct lineages by latitude, and our data suggest there were multiple instances of evolution into high elevations (Figure 3.2; Figure 3.3). Despite high population structure, evolutionary patterns in morphological trait divergence along elevational clines display similar patterns among populations within both the northern and southern clades. Though we detected signatures of adaptive divergence in height and number of leaves, whether variation in morphological traits reflects local adaptation to elevation across the *S. tortuosus* clade is unclear, possibly due to high neutral population structure overwhelming signatures of phenotypic variation. Overall, our study indicates that multiple lineages of *S. tortuosus* populations adaptively evolved morphological differences along elevation clines, with implications for population persistence and spatial distribution as they face new ecological challenges with climate change across the species range.

Despite strong genetic divergence, we observe parallel patterns in morphological trait evolution across multiple elevation clines, which has implications for trait adaptation as well as repeatability of evolution in plant morphology as populations migrate upwards in elevation. If ancestral *S. tortuosus* populations experienced reproductive isolation early on as clades at different latitudes migrated to higher elevations, and that divergence was maintained throughout their evolutionary history, parallel evolution in morphological traits along multiple elevation clines may indicate independent instances of convergent evolution (Stern, 2013). Other studies have also found convergent evolution along multiple elevation gradients both among and within species (Read et al., 2014; Y.-B. Sun et al., 2018). However, similar evolutionary trajectories in plant traits along elevation gradients may also reflect gene flow among populations (Wei et al., 2013; Wang & Bradburd, 2014; Sexton et al., 2016; Wu et al., 2020)). Thus, the patterns we observed may be due to convergent evolution, long distance migration (i.e. introgression of adaptive alleles), or both. For example, low elevation populations may have been adapted to cooler environments during glacial periods, and low elevation morphologies may have evolved at the trailing edge of the species range as it moved upslope during the last interglacial period. Further research investigating gene flow and long-distance migration events would be needed to quantify the extent to which morphological trait patterns along elevation clines evolved independently among clades. Additionally, molecular genetics studies could uncover whether similar genetic mechanisms underlie similar phenotypic responses to elevation across clades. Investigating parallel evolution in continuous phenotypic variation across multiple elevation gradients can offer insights into the predictability of evolution and facilitate a deeper understanding of future evolutionary responses under climate change.

Our study reveals the profound influence of climate on the morphological and genetic differentiation of *S. tortuosus* populations; however, whether the variation we observed in plant traits reflects local adaptation to elevation is unclear. On one hand, we detected signatures of adaptive divergence for number of leaves and height in southern populations, despite high levels of genetic population structure, which can overwhelm signatures of phenotypic variation. Additionally, environmental variation that contributes to trait variation will reduce the power of Q_{PC} to detect adaptive divergence because it will inflate variance at later PCs (Josephs et al., 2019). Therefore, the fact that we detected significant phenotypic variation in height and number of leaves in the southern clade consistent with adaptive divergence may provide particularly strong evidence that genetic differentiation in these traits is adaptive, as has been found in numerous previous studies (Kawecki & Ebert, 2004; Blanquart et al., 2013; Halbritter et al., 2018). On the other hand, Q_{PC} did not uncover differentiation in number of branches, stem diameter, or leaf length that exceeded expectations for neutral genetic variation, and we found no significant patterns in any trait when analyzing all populations together. Therefore, though the patterns we detected are consistent with local adaptation to elevation in morphological traits, other explanations are also possible.

The morphological trait variation we observed across an elevation cline is largely consistent with theoretical expectations and empirical findings in the literature. Studies typically attribute such trait variation to local selective pressures to adapt to more stressful abiotic conditions like cold temperatures, frost, and high winds (Körner 2003; Halbritter et al., 2018). For example, thicker leaves can reduce heat and water loss (Tian et al., 2016; Guo et al., 2017) and shorter stature keeps plant organs closer to warmer temperatures at the soil surface (Körner 2003). Overall, the combination of traits we observed – more branches and leaves, short stature, and thicker leaves and stems at higher elevations – produce a plant architecture that forms more compact plants closer to the soil surface. These lower, dense canopies create better microhabitats, like trapping heat and nutrients, to enable plants to better cope with more stressful conditions at higher elevations (Körner et al., 1989). Together, the evolutionary patterns in *S. tortuosus* morphology across elevations are consistent with both the fast-slow continuum (Dammhahn et al., 2018) and plant-specific leaf economics spectrum (LES), which generally predict species will evolve slower, resource-conservative traits like thicker, longer-lived leaves in harsh environments, where tissue replacement is more costly (Wright et al., 2004). Furthermore, *S. tortuosus* also exhibits variation in life history schedules within and among populations. Low elevation populations are largely composed of annuals, high elevation populations typically exhibit biennial life histories, and intermediate populations contain individuals displaying both strategies. These life history strategies vary plastically in response to germination timing and exposure to cold (Gremer et al., 2020b; Bontrager, *unpublished data*). Therefore, our results also align with studies linking physiological and functional traits along the fast-slow continuum with variation in life history strategy (Adler et al., 2014).

Our results also reveal a limited extent of evolution in morphological traits along a latitudinal gradient: fewer leaves and branches and marginally thicker stems at higher latitudes. Surprisingly, the patterns in branch and leaf number contradict those across elevation, even though high elevation and high latitude environments share many environmental characteristics like cooler temperatures, fewer resources, and harsher conditions. Incongruent patterns across elevation and latitude do occur (e.g., Kooyers et al., 2015). These contradictory results suggest that it is the environmental factors that differ between high elevations and latitudes that drive selection on branch and leaf number rather than those factors that vary similarly. Across the *S. tortuosus* range, elevation relates strongly to temperature while latitude is more correlated with variables related to moisture, which may explain the differences we observed in morphological patterns. Furthermore, differences in morphological patterns across latitude compared with elevation may be driven by variation in gene flow patterns among lineages: isolation is much greater for a given distance in the Northern clade than in the Southern clade. This may be due to the more discontinuous population distributions at low elevations in the Northern clade, while the more continuously distributed high elevation populations may experience higher gene flow. Disentangling the mechanisms driving differences in variation along environmental clines can facilitate a deeper understanding of the ways in which organisms will respond to ecological challenges under climate change either in situ or as they migrate into new environments to track their optimal niches.

Climate change is expected to cause increased temperatures, drought, and extreme weather events in California (Wright et al., 2016; Pathak et al., 2018; Swain et al., 2018; Luković et al., 2021), posing significant challenges to adaptation and population persistence. Phenotypic plasticity plays an important role in plant responses to climate change, but adaptive evolutionary response is likely to become increasingly necessary as abiotic conditions continue to change rapidly (Jump & Peñuelas, 2005). Therefore, understanding how adaptive divergence arises in populations is essential for predicting future biological responses to climate change, with implications for species distributions. This may offer insights into future responses as more organisms migrate to higher latitudes and elevations to keep pace with changing climate as their optimal niches shift. Obtaining a window into evolution across elevational and latitudinal clines will not only be useful for understanding future evolutionary responses but also for generating hypotheses regarding genetic mechanisms driving evolutionary processes. In *S. tortuosus*, the presence of extensive genetically based intraspecific phenotypic variation in morphological traits may aid in adaptive evolutionary response to climate change. Alternatively, a lack of gene flow from warm-adapted low elevation populations may hinder evolutionary rescue as populations at high elevations experience warmer temperatures and increased drought stress. A meta-analysis of climate change adaptation indicated that distributional changes along elevational gradients is lagging when compared to that across latitude (Chen et al., 2011).

This study highlights the value of investigating intraspecific patterns in morphological traits across elevation gradients and linking those patterns with evolutionary history. Our results suggest that *S. tortuosus* populations repeatedly evolved shorter, branchier, more compact plant architectures with thicker leaves as they colonized high elevation environments, and that several of these morphological traits adaptively diverged within the southern clade in association with climatic variation along an elevation gradient. Further research investigating the extent to which *S. tortuosus* populations are locally adapted to their environments, standing genetic variation in adaptive traits, and patterns of gene flow will be essential for understanding population persistence in the face of climate change. Studies such as ours elucidating the adaptive value of continuous variation in coordinated trait strategies along parallel environmental gradients can provide insights into the repeatability of evolutionary patterns in continuous traits across steep environmental gradients as well as shed light on adaptation to harsh environments in general.

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FIGURES

Figure 3.1. Population map and climate PCA

Population (a) map and (b) Principal Components Analysis describing contemporary climate across populations. (a) Points represent populations and colors indicate the elevation at each population. (b) Principal Components Analysis describing variation in population abiotic conditions across populations from 1990-2020. First two principal components (PCs) illustrated on the x and y axes. Growing season variables include climatic water deficit (cwd), precipitation (ppt in mm H_2O), maximum and minimum temperature (tmax and tmin, respectively), soil water storage (str), and snowpack (pck). PC1 (59.82%) describes variation along an elevation gradient and related metrics such as growing season length and temperature. PC2 (24.2%) describes variation related to drought metrics like soil water storage and climatic water deficit. Points represent populations and colors indicate the elevation at each population. Data source was Flint et al. (2021; 2023).

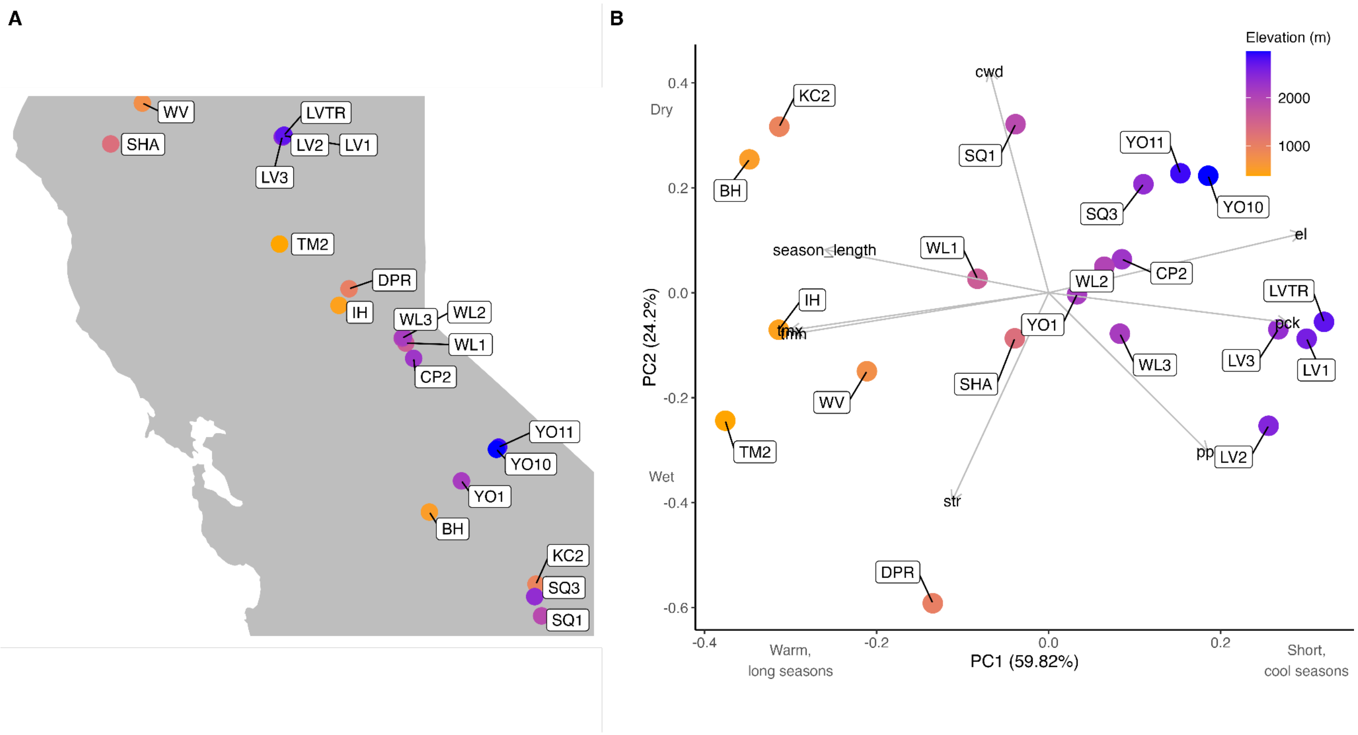
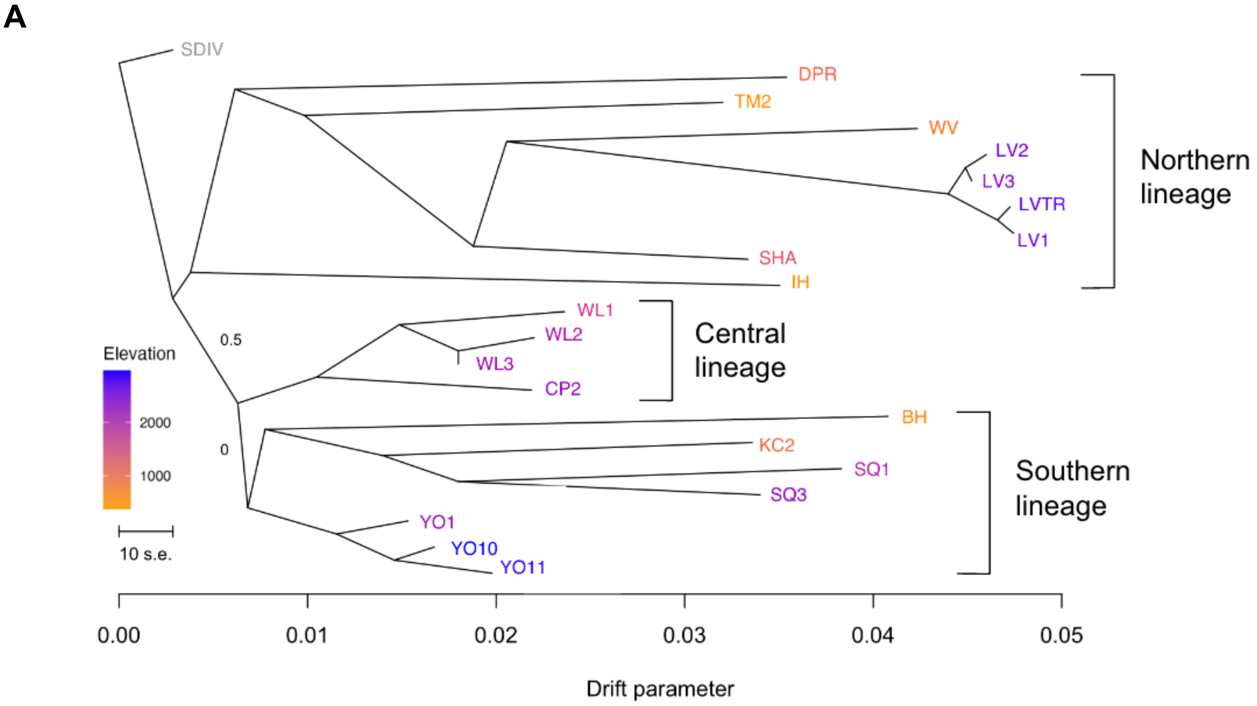


Figure 3.2. Population differentiation and spatial genetic structure
(a) Maximum likelihood phylogenetic tree inferred with TreeMix with no migration events allowed. The tree reconstruction delineated three lineages across a latitudinal cline, labeled as “northern,” “central,” and “southern” clades. The sister species *S. diversifolius* was used as an outgroup. (b) Isolation by distance depicted by geographic distance versus F_{ST} across pairs of populations. Each point is a pair of populations. Points are colored by elevation of the first population (outline) and second population (fill) being compared. Lines depict trends by clade (solid = central, short dash = northern, long dash = southern).



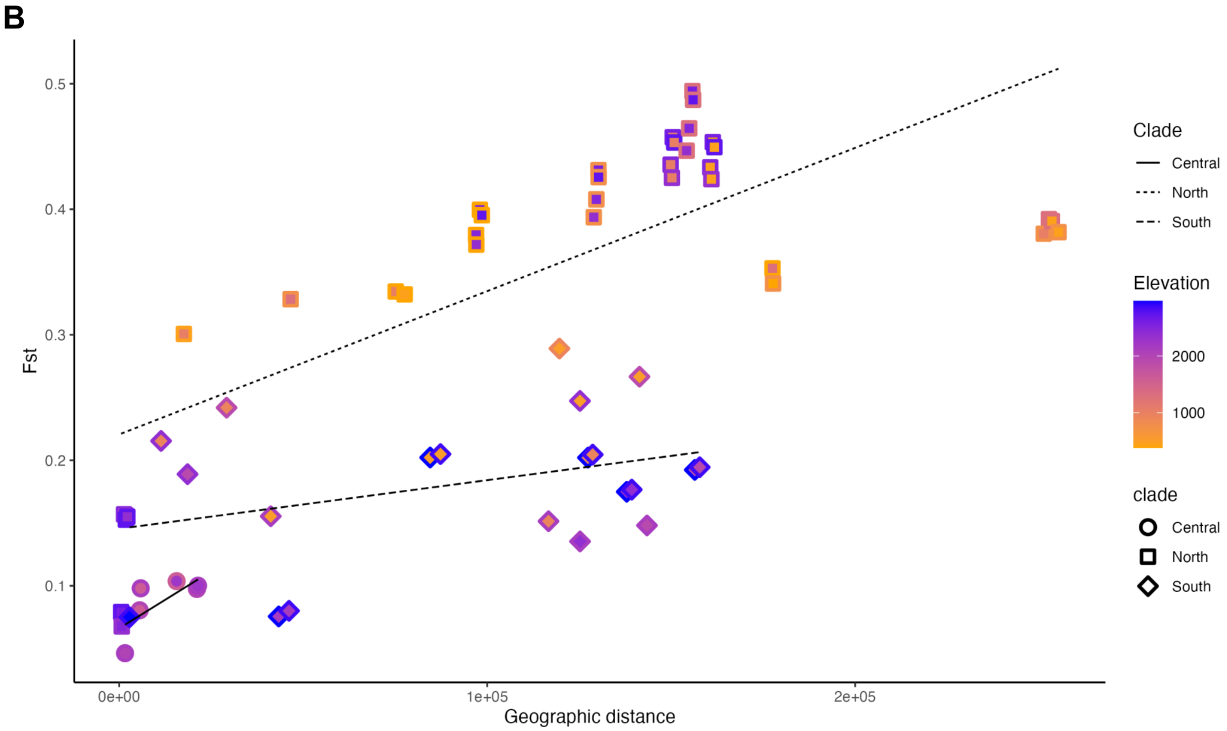

B
Fst
0.5
0.4
0.3
0.2
0.1
0e+00
1e+05
2e+05
Geographic distance
Clade
Central
North
South
Elevation
2000
1000
clade
Central
North
South

Figure 3.3. ***Admixture analyses***

Clustering of genotypes from structure analysis on neutral genetic variation (K=2-4). Structure plots of individuals grouped by population (ordered by increasing latitude) comparing grouping across K values.

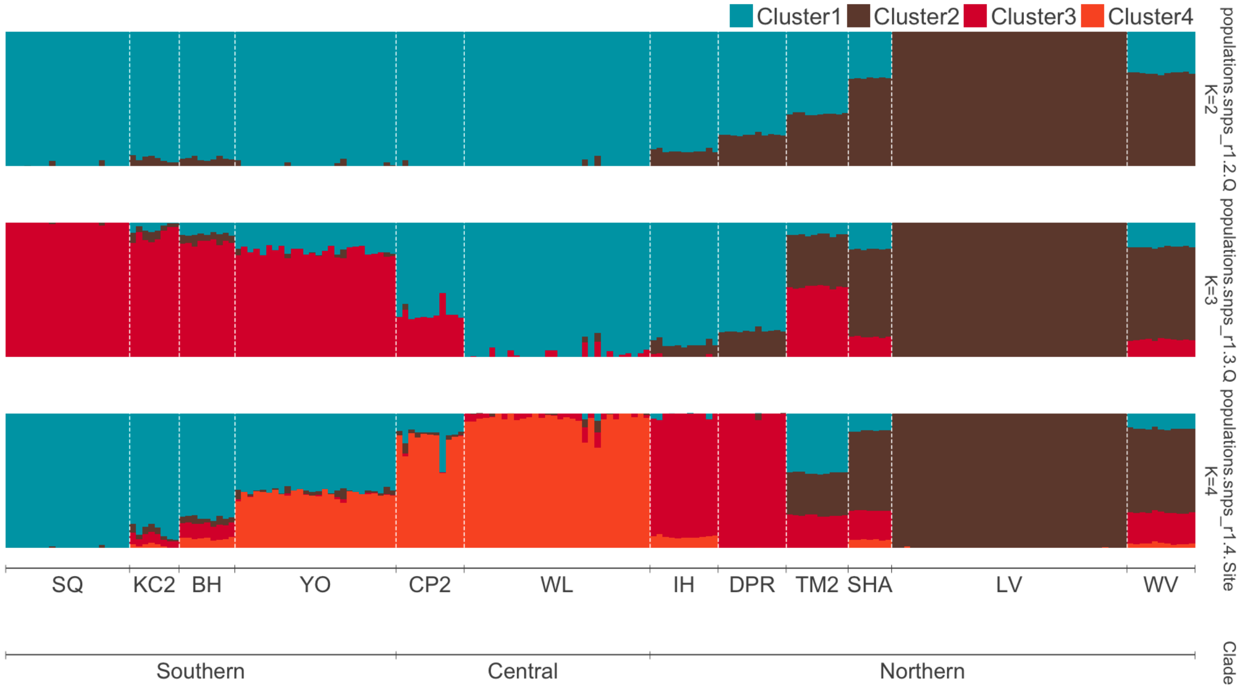
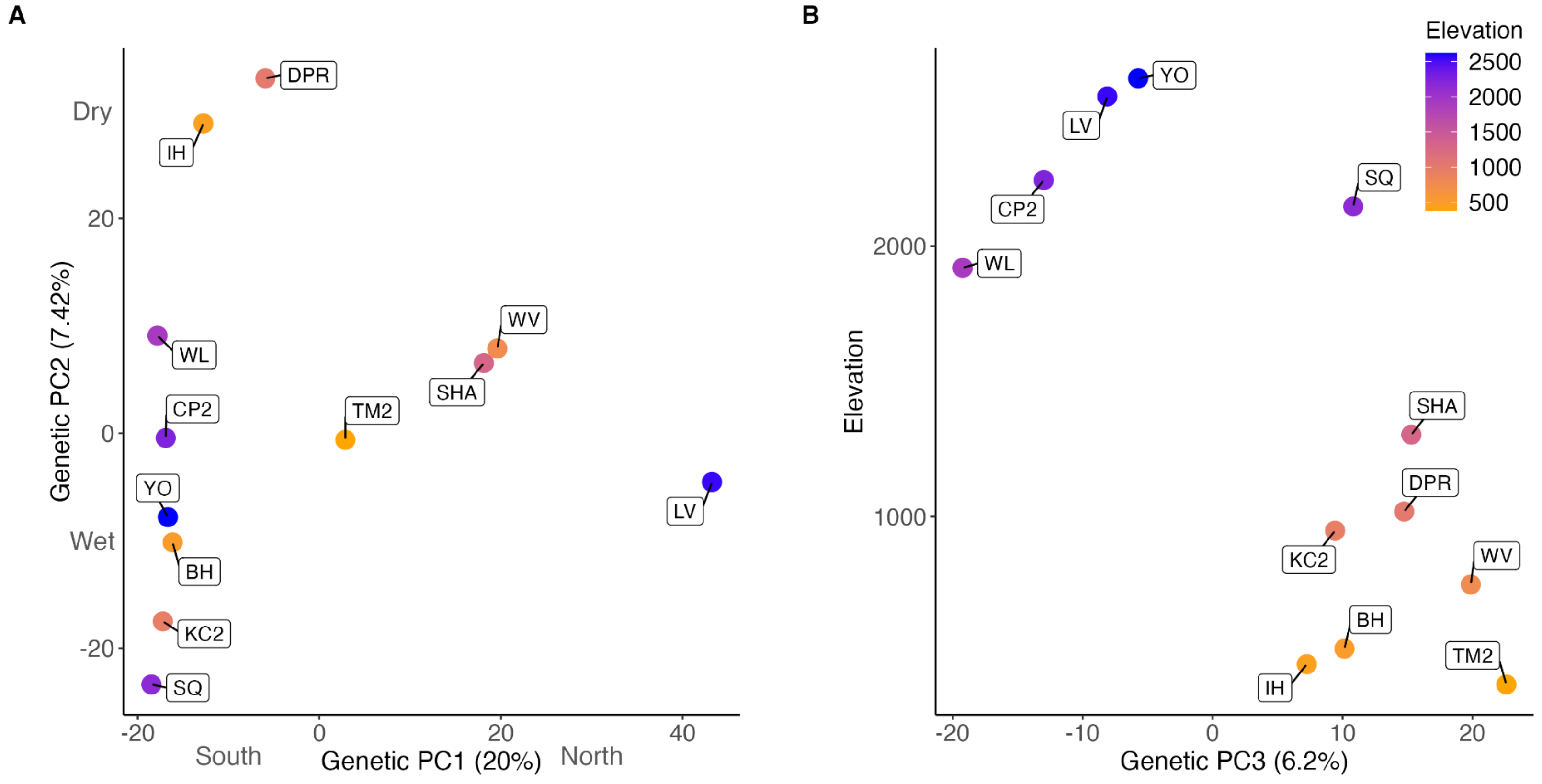


Table 3.1. Summary of results from morphological trait analyses

Trait	**Climate-trait relationships; CCA**	**Qpc - all populations**	**Qpc - split populations by clade along latitude**
Leaf thickness	Increases with elevation	No	No
Stem diameter	Increases with elevation and latitude	No	No
Height	Decreases with elevation	No	Yes - central + southern clade populations, PC4 P = 0.02
Length of longest leaf	Decreases with elevation	No	No
Number of leaves	Increases with elevation; decreases with latitude	No	Yes - central & southern clade populations, PC8 P < .0001
Number of branches	Increases with elevation; decreases with latitude	No	No

Figure 3.4. Genetic-trait-climate multivariate space

(a) Genetic data PC1 vs PC2, (b) PC3 vs elevation, and (c) multivariate morphological trait space with climate loadings overlaid from canonical correlation analysis. (a) genetic PC1 (20%; correlates with a latitudinal cline from south to north) vs PC2 (7.52%; correlates with moisture from dry to wet) from PCA conducted on SNP variant data. (b) genetic PC3 (6.2%) versus elevation. Points indicate populations colored by elevation. (c) morphological-climate canonical covariates 1 (60.5%; primarily describes variation between elevation and leaf thickness;) and 2 (18.8%). Arrow direction indicates loading and length indicates strength. Morphological trait loadings are colored green and climatic metric loadings are colored blue.



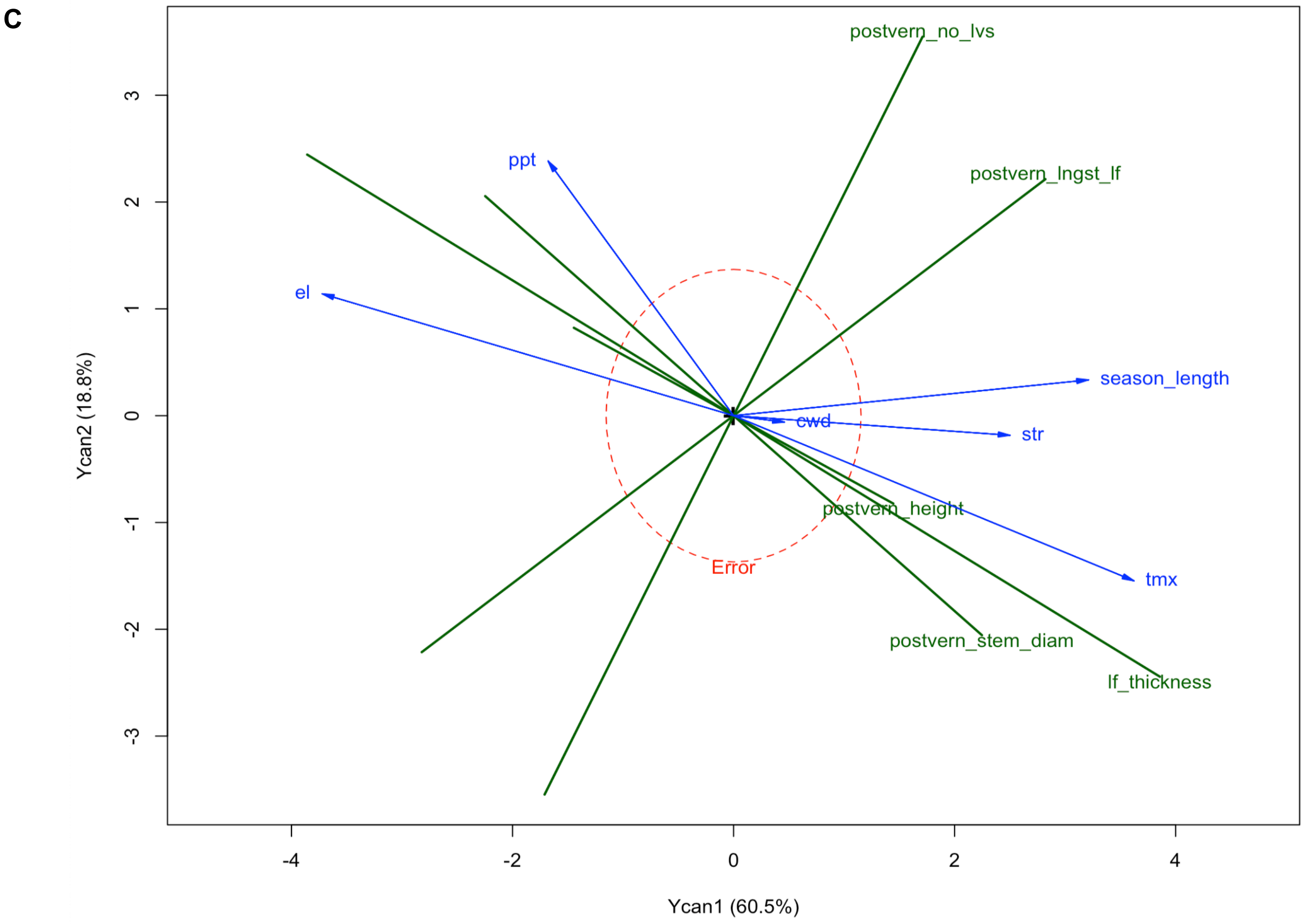

C
postvern_no_lvs
postvern_lngst_lf
ppt
el
season_length
cwd
str
postvern_height
tmx
Error
postvern_stem_diam
lf_thickness
Ycan2 (18.8%)
Ycan1 (60.5%)
3
2
1
0
-1
-2
-3
-4
-2
0
2
4

Figure 3.5. Adaptive divergence in morphological traits

Adaptive divergence detected in morphological traits in the southern clade. Variation in (a) height and (b) number of leaves that exceeds neutral expectations along PC4 and PC8 of the kinship matrix, respectively. Grey dashed lines represent neutral expectation/CIs. Black isolines depict observed variation of (a) height and (b) number of leaves along the kinship matrix PC axis of interest (PC4 and PC8, respectively).

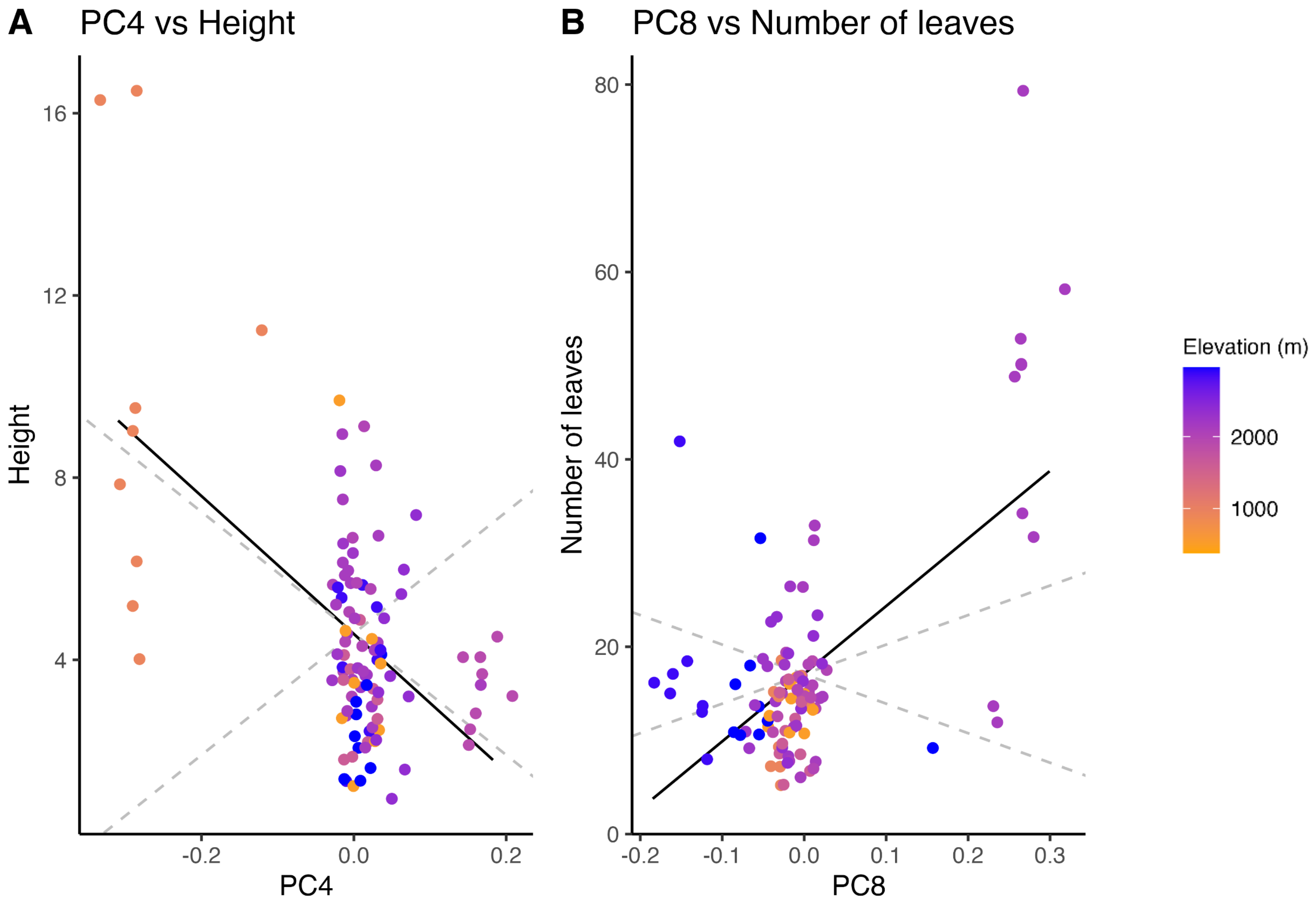
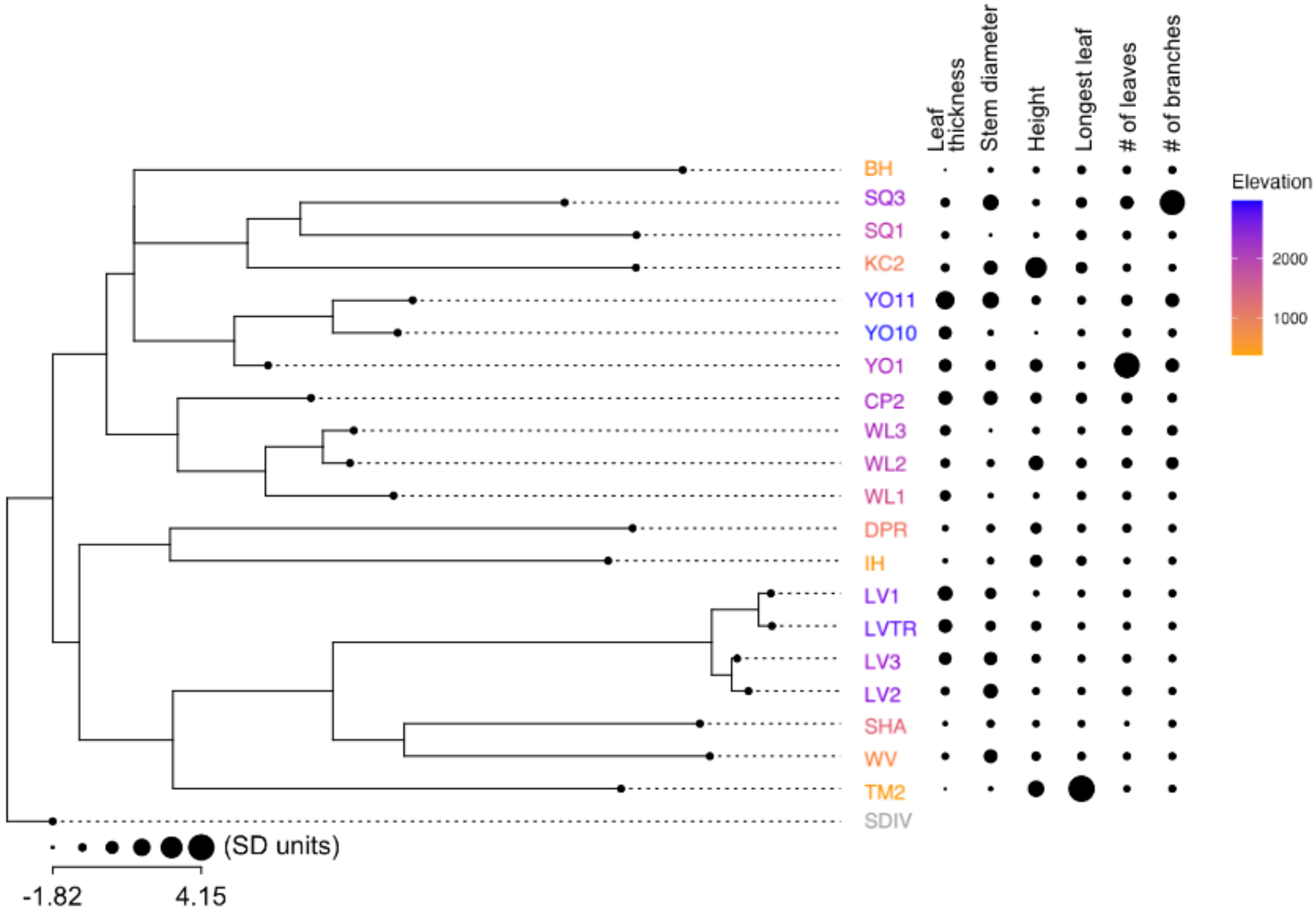


Figure 3.6. Morphological variation across the phylogeny

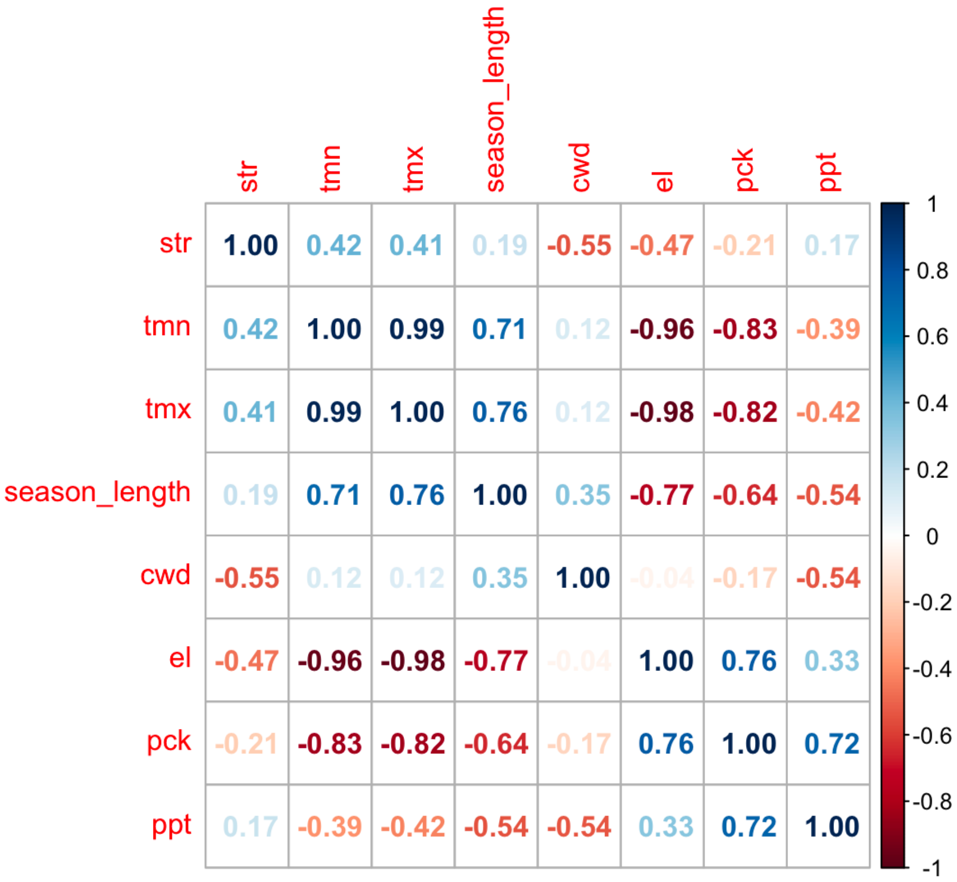
Morphological trait variation across the *S. tortuosus* phylogeny. Dot size indicates variation in standardized mean leaf thickness, stem diameter, height, length of longest leaf, number of leaves, and number of branches measured in plants grown in a common garden for each population across the phylogeny. Branch tip colors indicate elevation at each source population.



SUPPLEMENT

Supplement 3.1. Population locations and climate metrics

Correlation of climate metrics



	str	tmn	tmx	season_length	cwd	el	pck	ppt
str	1.00	0.42	0.41	0.19	-0.55	-0.47	-0.21	0.17
tmn	0.42	1.00	0.99	0.71	0.12	-0.96	-0.83	-0.39
tmx	0.41	0.99	1.00	0.76	0.12	-0.98	-0.82	-0.42
season_length	0.19	0.71	0.76	1.00	0.35	-0.77	-0.64	-0.54
cwd	-0.55	0.12	0.12	0.35	1.00	-0.04	-0.17	-0.54
el	-0.47	-0.96	-0.98	-0.77	-0.04	1.00	0.76	0.33
pck	-0.21	-0.83	-0.82	-0.64	-0.17	0.76	1.00	0.72
ppt	0.17	-0.39	-0.42	-0.54	-0.54	0.33	0.72	1.00

Population locality and environment of origin

Population locality, elevation, GPS coordinates, season length, and climate metrics. Climate variables are Climatic Water Deficit (cwd), precipitation (ppt in mm H_2O), snowpack (pck in in mm H_2O), soil-water storage (str), and minimum and maximum temperature (tmn, and tmx, respectively). Shown here are summary monthly climate metric values from 1990-2020. Data source for climate metrics was Flint et al. (2021; 2023).

Site	**Pop**	**Elev**	**Lat**	**Long**	**Season length**	**cwd**	**ppt**	**pck**	**str**	**tmn**	**tmx**
Table Mountain 2	TM2	379.15	39.59	-121.55	7.80	20193.06	34909.41	0.00	335555.81	9.61	22.78
Iowa Hill	IH	454.13	39.09	-120.92	7.64	17951.61	32607.01	69.73	154618.34	8.57	22.22
Ben Hur	BH	511.43	37.41	-119.96	6.72	27836.01	17773.91	0.00	130488.98	8.71	23.52
Weaverville	WV	748.86	40.74	-123.00	6.94	16064.96	31120.89	9663.07	214346.98	5.01	19.39
Kings Canyon 2	KC2	947.93	36.82	-118.84	7.45	30660.23	24248.25	148.97	127471.81	8.59	20.90
Drum Powerhouse Rd	DPR	1018.59	39.23	-120.82	4.49	10010.41	44452.27	3364.68	367741.78	7.70	20.21
Shasta	SHA	1302.96	40.41	-123.34	5.65	18546.33	49187.89	31163.78	101994.53	4.61	17.90
Wrights Lake 1	WL1	1613.84	38.79	-120.21	6.24	21017.26	37866.35	21361.59	139004.07	4.28	17.22
Sequoia 1	SQ1	1921.04	36.56	-118.78	5.49	27051.23	28594.00	19164.16	55465.86	3.53	15.69
Wrights Lake 2	WL2	2020.12	38.83	-120.25	4.97	19734.91	38035.83	51188.95	92783.04	2.45	14.55
Wrights Lake 3	WL3	2137.76	38.84	-120.24	4.81	17889.44	37769.51	59302.90	174783.15	1.90	13.82
Yosemite 1	YO1	2140.51	37.66	-119.62	5.13	19158.03	33131.06	42339.37	163895.23	2.22	14.83

Carson Pass 2	CP2	2244.13	38.66	-120.13	5.35	23033.61	38523.01	73666.98	160683.83	1.04	13.41
Lassen Volcanic 3	LV3	2353.55	40.47	-121.52	4.74	21003.13	53634.74	153277.36	147358.67	-1.59	11.12
Sequoia 3	SQ3	2372.45	36.72	-118.85	4.42	22219.23	31726.28	45308.00	49655.71	1.13	13.49
Lassen Volcanic 2	LV2	2500.46	40.47	-121.51	4.74	17743.83	53065.56	152474.58	252178.97	-1.44	11.31
Lassen Volcanic 1	LV1	2593.42	40.47	-121.50	4.71	18171.60	54076.71	159001.60	108776.61	-1.61	11.17
Lassen Peak Trail	LVTR	2741.39	40.48	-121.50	4.71	18961.10	55907.74	163310.01	89835.18	-1.78	11.07
Yosemite 11	YO11	2872.30	37.94	-119.23	5.65	20834.26	24246.60	65309.20	100793.53	-2.84	10.54
Yosemite 10	YO10	2968.30	37.92	-119.25	5.48	21125.63	26579.35	74422.98	99066.43	-3.28	10.06

Climate PCA loadings

Variable	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**
cwd	-0.10	0.62	0.42	-0.16	0.58	-0.18	0.16	-0.05
ppt	0.27	-0.45	0.62	0.28	-0.04	-0.49	0.11	-0.12
pck	0.41	-0.08	0.48	-0.28	0.02	0.66	-0.26	0.11
str	-0.17	-0.58	-0.15	-0.60	0.49	-0.11	0.06	-0.04
tmn	-0.44	-0.12	0.15	0.29	0.22	-0.06	-0.62	0.50
tmx	-0.44	-0.11	0.15	0.21	0.06	0.31	-0.17	-0.77
Elevation	0.43	0.17	-0.23	-0.15	0.07	-0.36	-0.68	-0.35
Season length	-0.38	0.12	0.31	-0.55	-0.61	-0.24	-0.13	-0.01

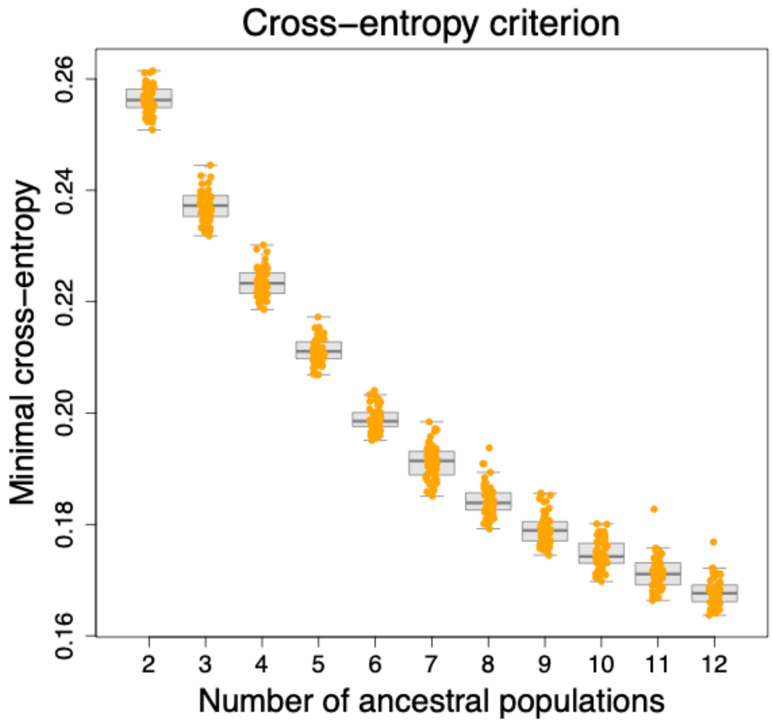
Supplement 3.2. Population differentiation and spatial genetic structure

Number of variant private alleles per population

Population	# Private alleles	# Individuals
YO11	569	9
KC2	2181	9
CP2	818	10
YO1	889	10
WL2	250	10
IH	963	10
WV	2182	11
LV1	18	9
SHA	1472	8
YO10	491	9
WL3	140	10
SQ3	2317	10
DPR	896	10
LV3	43	9
LVTR	38	10
WL1	296	10
SQ1	1089	9
BH	2953	9
TM2	2376	11
LV2	5	9

Supplement 3.3. Cross-entropy analysis

Cross-entropy results indicating the most probable number of ancestral populations for K = 2-14 with 50 repetitions each. Created using the sambaR package in R.



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