# Lagging Adaptation to Climate Supersedes Local Adaptation to Herbivory in an Annual Monkeyflower*

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ABSTRACT: While native populations are often adapted to historical biotic and abiotic conditions at their home site, populations from other locations in the range may be better adapted to current conditions due to changing climates or extreme conditions in a single year. We examine whether local populations of a widespread species maintain a relative advantage over distant populations that have evolved at sites better matching the current climate. Specifically, we grew lines derived from low- and high-elevation annual populations in California and Oregon of the common monkeyflower (*Erythranthe guttata*) and conducted phenotypic selection analyses in low- and high-elevation common gardens in Oregon to examine relative fitness and the traits mediating relative fitness. Californian low-elevation populations have the highest relative fitness at the low-elevation site, and Californian high-elevation populations have the highest relative fitness at the high-elevation site. Relative fitness differences are mediated by selection for properly timed transitions to flowering, with selection favoring more rapid growth rates at the low-elevation site and greater vegetative biomass prior to flowering at the high-elevation site. Fitness advantages for Californian plants occur despite incurring higher herbivory at both sites than the native Oregonian plants. Our findings suggest that a lag in adaptation causes maladaptation in extreme years that may be more prevalent in future climates, but local populations still have high growth rates and thus are not yet threatened.

## Introduction

The prevalence of local adaptation, the greater fitness of native populations relative to nonnative populations in their local habitat, is hotly contested between different subdisciplines of evolutionary biology (Hendry and Gonzalez 2008). Meta-analyses of reciprocal transplant experiments demonstrate that ~29% of such comparisons find evidence of maladaptation (Hereford 2009), and this number is likely an underestimate due to biases in experimental design and reporting. Maladaptation may result from many factors, including small population sizes, immigration of less fit alleles, constraining genetic architectures, and temporal variation in the environment (reviewed in Crespi 2000). The prevalence of maladaptation is likely rising as populations are increasingly confronting novel or unusual conditions as climatic change alters both the average values and the year-to-year variances of many environmental parameters (Lane et al. 2012; Mills et al. 2013; Pachauri et al. 2014). In extreme cases of such maladaptation, population growth rates may drop below replacement levels, and extirpation could occur (Jump and Penuelas 2005; Reich and Oleksyn 2008; Anderson 2016).

A key signature for documenting climate-mediated patterns of maladaptation is an adaptation lag. When the climate changes as fast or faster than the rate at which adaptive evolution proceeds within a population, a pattern emerges where local populations have lower average fitness in their native habitats than populations from sites at which historical conditions better match the contemporary environment (Aitken et al. 2008; Hoffmann and Sgrò 2011; Wilczek et al. 2014). For instance, pioneering work in *Arabidopsis thaliana* using a continental-scale array of common gardens has dem-

onstrated that selection in contemporary environments favors accessions derived from populations in historically warmer climates over accessions derived from the geographically close populations (Wilczek et al. 2014). Adaptation lags should be avoidable if populations can rapidly adapt to novel conditions (i.e., evolutionary rescue) or acclimate in response to environmental disturbance (e.g., Franks et al. 2007; Anderson et al. 2012; CaraDonna et al. 2014; Fitzpatrick et al. 2016), particularly if migration often introduces adaptive alleles from sites more similar to current conditions (Fitzpatrick et al. 2016). However, few studies have explicitly tested for adaptation lags due to changing climates or tested whether fitness differences between local and distant populations match expectations set by the divergence between historical and contemporary climates.

Understanding how phenotypic variation gives rise to population-level differences in fitness that contribute to adaptation lag may be as important as determining the prevalence of climate maladaptation (Etterson and Shaw 2001; Etterson 2004a, 2004b; Frank et al. 2017). When we observe adaptation lag in common gardens, we expect that phenotypes that have diverged between local and distant populations will be the primary causes of fitness differences and that directional selection should favor phenotypic values more similar to those of the distant population. Depending on the distance of the distant population mean from the contemporary local optimum, the distant population may be experiencing directional or stabilizing selection for the same trait(s). However, if no heritable variation exists in the local population or if strong genetic correlations exist between important phenotypes in the local population, there may be little potential for evolutionary rescue (Etterson and Shaw 2001; Peterson et al. 2017).

Although identifying the biotic and abiotic differences between sites that lead to patterns of local adaptation or maladaptation is typically a challenging process due the multivariate nature of environments (Siepielski et al. 2017), in the case of climate change there are clear expectations about which factors drive maladaptation. Climate models predict with confidence that the future will bring longer, warmer growing seasons such that the average population will soon experience a climate that resembles historical conditions of more southern or lower-elevation populations (Pachauri et al. 2014). In contrast, predicting how biotic interactions with local competitors, herbivores, or mutualists will respond to the same trends is far more difficult. Such interactions may remain stable, may change but more slowly than the climate, or may cease to exist, and all of these potential disconnects between shifts in the abiotic and biotic agents of selection may slow the pace of local adaptation further (Parmesan 2006; Hothorn et al. 2008; CaraDonna et al. 2014). For instance, patterns of relative fitness in reciprocal transplant experiments may be mismatched such that distant populations are better adapted to current abiotic conditions than native populations but still suffer greater levels of herbivory or lower pollination success.

Theory predicts that natural selection should be most efficient in widespread species with short generation times, large population sizes, and extensive levels of standing genetic variation. Thus, we least expect to observe climate maladaptation in systems that fulfill these criteria unless environmental conditions are changing at a pace even swifter than adaptation could proceed even in these most malleable species. Here, we test this prediction using annual populations of the common monkeyflower (*Erythranthe guttata*, or *Mimulus guttatus* prior to recent taxonomic revision), a species that exhibits each of these qualities. This wildflower species is found from central Mexico to central Alaska at elevations from 0 to 3,500 m asl as both an annual and a perennial (Vickery 1978; Wu et al. 2008). Annual populations typically occur below 2,000 m asl in habitats like seeps, meadows, and moist rock walls that have seasonal water supplies (Lowry et al. 2008; Friedman et al. 2015; Kooyers et al. 2015), and they can harbor exceptional levels of sequence diversity (Puzey et al. 2017) and phenotypic diversity (Hall et al. 2006; Kooyers et al. 2015). During the last 30 years, climatic conditions across the range of *E. guttata* have changed dramatically, including a historically severe drought in California from 2012 to 2016. Consequently, climatic conditions during the *E. guttata* growing season in central Oregon are becoming more similar to historic conditions in the Sierra Nevada, with earlier growing seasons due to lower snowpack and higher temperatures (Kunkel 2004; Gergel et al. 2017).

Extensive previous research has highlighted the traits and environmental patterns that are likely to be important for adaptation to climate change in *E. guttata*. Many traits exhibit clinal patterns of variation among annual *E. guttata* populations along elevation and latitudinal gradients, suggesting that local or regional adaptation is strong (Kooyers et al. 2015), and several key abiotic and biotic selective factors likely drive these patterns. For instance, annual populations are highly dependent on water availability, with growing seasons beginning following spring rains or snowmelt and ending with terminal drought. Because the timing of water availability changes dramatically across the range of *E. guttata*, growing season timing and duration also vary dramatically and are associated with differences in growth rate, flowering time, and water use efficiency (Mojica et al. 2012; Kooyers et al. 2015). *Erythranthe guttata* also interacts with a wide variety of generalist and specialist herbivores (Rotter and Holeski 2017) and sustains high levels of herbivory, especially in regions with longer growing seasons (Ivey et al. 2004; Kooyers et al. 2017). Traits that aid in herbivore defense, including trichomes (Colicchio 2017) and chemical defenses (Holeski et al. 2013; Keefover-Ring et al. 2014), are a significant resource allocation cost (Kooyers et al. 2017).

Here, we examine patterns of fitness and trait variation among populations of annual *E. guttata* when grown in two common gardens that differ in climate, growing season timing, and herbivore communities. We ask whether there is evidence consistent with an adaptation lag such that populations sampled from sites with historical climates more similar to the contemporary garden conditions are better adapted than populations sampled from sites nearby the common garden. In addition, using two sets of advanced-generation interpopulation crosses for phenotypic selection analysis, we determine which traits affect fitness under current conditions and whether directional selection is favoring evolution toward new phenotypic optima. Finally, we assess whether biotic interactions with herbivores favor native populations or distant populations and how correlations between life-history and defense traits may impact future adaptation.

## Methods

### Study Populations and Experimental Germplasm

We developed a suite of intra- and interpopulation outbred families derived from low- and high-elevation populations in the central Sierra Nevada and the central Oregon Cascades. These populations differ dramatically in environmental conditions during the growing season as well as in morphological, physiological, and reproductive traits (Kooyers et al. 2015; table S1; tables S1–S6 are available online). Below, these populations are referred to as Low CA, High CA, Low OR, and High OR.

Maternal lines within populations were selfed for one or more generations in the greenhouse to remove maternal effects prior to inclusion in intra- and interpopulation crosses. To avoid exposing inbreeding depression, as is common in *Erythranthe guttata*, we constructed outbred intrapopulation families by crossing individuals descended from unique maternal lineages within each population (Hall and Willis 2006). We created three intrapopulation crosses per population and also planted one to three inbred lines from each population in the field (table S2). To examine phenotypic selection in panels with broader ranges and more independent assortment of trait variation, we also constructed two sets of $F_4$ interpopulation outbred families derived by initially crossing (1) Low CA and High CA parent plants and (2) Low OR and High OR parent plants (fig. A1; figs. A1–A7 are available online). In brief, each panel started from a cross between a single individual from each parent population; a single $F_1$ individual was selfed to create an $F_2$ population; random pairings of $F_2$ plants were crossed to each other to create $F_3$ families; and, finally, an individual from each $F_3$ family was randomly paired and crossed with an individual from another $F_3$ family to create outbred $F_4$ families (fig. A1; Rock-

man and Kruglyak 2008). This design resulted in 49 $F_4$ families for the California cross and 56 $F_4$ families for the Oregon cross.

### Common-Garden Experiments

To examine patterns of local adaptation, adaptation lag, and phenotypic selection, we planted intra- and interpopulation outbred families in common gardens at two seepy meadows in the central Oregon Cascades proximal to the Oregon populations (fig. 1A). The low-elevation site was near Lookout Point Dam (lat. 43.91667, long. 122.75603; 277 m asl; fig. 1B). The high-elevation site was near the Browder Ridge Trailhead (lat. 44.37348, long. 122.13055; 1,246 m asl; fig. 1C). Seeds from three intrapopulation crosses, three maternal lines from each population, and each of the interpopulation outbred families were planted in Fafard 3B potting soil in two waves, one per common-garden site. After stratification in a 4°C room at the University of Oregon for 7 days, plants were germinated over 2 weeks in the University of Oregon greenhouse, where they experienced the natural day length and diurnal temperature cycles with the same phase but lower amplitude than the outdoor conditions. Flats were misted daily and maintained under humidity domes for the first 7 days to improve germination. Germination was recorded daily. Germination of seeds sown for use at the high-elevation site was unexpectedly low for a number of lines that had sufficient germination when prepared for the low-elevation site, limiting the number of lines eventually transplanted at the high-elevation site (table S2). Seedlings (low-elevation site: $n = 1,216$; high-elevation site: $n = 1,200$) were transplanted into the soil context of the low-elevation site on April 6–8, 2014, and of the high-elevation site on May 21, 2014. A block design was used to minimize effects of microhabitat variation. At transplant, most plants had expanded the first set of true leaves, similar to the developmental stage of the native plants. Each block (high-elevation site: $n = 10$ blocks; low-elevation site: $n = 11$ blocks) included one individual per outbred interpopulation family and multiple individuals from each outbred intrapopulation family. In total, 1,125 plants successfully established at the low-elevation site, and 621 plants successfully established at the high-elevation site (table S2).

We measured a suite of phenological, morphological, and physiological traits for each plant. Plants were surveyed every 2 days for survival and number of new flowers for the whole growing season. Flowering time was measured as the time from germination to first flowering. At first flower, we scored plant height, stem diameter, corolla length, corolla width, corolla height, throat width, tube length, peduncle length, branch number, leaf number, leaf herbivory (across all leaves, estimated to the nearest 5%), and presence/absence of trichomes on the stem and leaves. In addition, to measure leaf relative water content (i.e., [saturated mass −
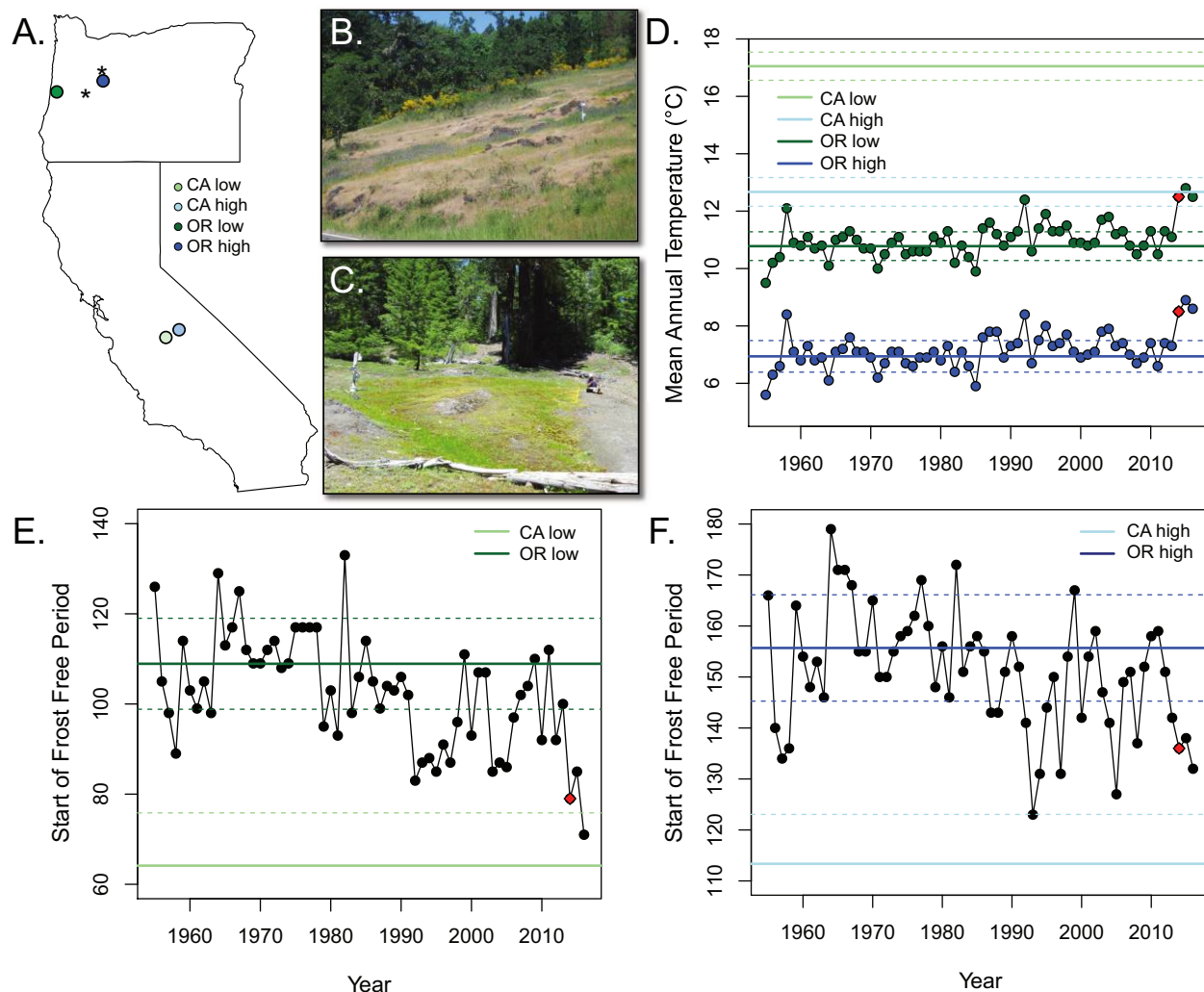
**Figure 1:** *A*, Geographic locations of parent populations (colored points) and locations of field sites (black asterisks) in Oregon. *B*, *C*, Pictures of low-elevation Oregon field site at Lookout Point Dam (*B*) and Browder Ridge Trailhead (*C*) taken in June of 2014. *D*, Mean annual temperature data from 1955 to 2016 at low- and high-elevation field sites. Dark green points represent the low-elevation site, and blue points represent the high-elevation site. Red points represent the 2014 field season. Solid horizontal lines signify the average mean annual temperature for each parent site from 1955 to 1990, and dashed lines represent the standard deviation around this mean. *E*, *F*, Beginning of the frost-free period averages for the low-elevation (*E*) and high-elevation (*F*) Oregon sites. Horizontal lines represent historical averages from 1955 to 1990 (solid lines) and standard deviations around these averages (dashed lines) for each parental population site.

dry mass]/saturated mass), we removed the larger second true leaf and placed it in a petri dish with deionized water overnight. Then, after being patted dry with paper towels, we measured saturated leaf mass. Leaves were then dried in glassine envelopes at 65°C for ≥3 days and weighed. We also recorded total number of flowers as a fitness measure. We note that our experimental design had a minor but uniform impact on reproductive fitness values, as we removed one bud per flowering plant for DNA extraction to facilitate future studies of the genetics of adaptation. All phenotype data have

been deposited in the Dryad Digital Repository (https://dx.doi.org/10.5061/dryad.039v4j5; Kooyers et al. 2019).

## Statistical Analyses

*Environmental Data.* To examine how climatic conditions during the experiment related to both recent years and historical averages at each parental collection site, we downloaded climate data including historic normal (1960–1990) as well as annual (1955–2016) summaries for each parent

population and each field site from ClimateWNA (http://www.climatewna.com/; Wang et al. 2016). ClimateWNA includes many annual, seasonal, and monthly variables (>200), including beginning of frost-free period, which is a relevant measure of growing season start date for *E. guttata* populations and closely related to our previously published metrics of growing season start date (Kooyers et al. 2015). To ground truth these data, we also downloaded monthly temperature and precipitation normals from 1980 to 2014 from National Oceanic and Atmospheric Administration (NOAA) weather stations located proximally to each parental population (NOAA National Climate Data Center; station details are provided in table S1). Data prior to 1980 were not available for all populations. In addition, hourly measurements of temperature, precipitation, and soil moisture were recorded within each field site during the experimental period with a HOBO U30 NCR data logger and associated sensors (Onset, MA).

*Fitness Comparisons.* To test for patterns consistent with local adaptation within regions and/or adaptation lag across regions, we calculated differences in survival to flowering, number of flowers produced, relative fitness, and absolute fitness between outbred intrapopulation cross types within both sites. Absolute fitness was calculated as the total number of flowers produced; plants that did not produce flowers were included with a value of zero. Relative fitness was calculated for each individual by dividing its absolute fitness by the average absolute fitness across the intrapopulation crosses in either the low- or the high-elevation site. We also counted number of seeds for each individual; however, the low-elevation site had intense fruit and/or seed predation, preventing accurate seed counts for this site. Seed counts at the high-elevation site were moderately correlated with number of flowers produced ($r^2 = 0.42$, $P < .0001$; fig. A2). We assessed how parental line and site differences impacted variation in fitness with generalized mixed linear models (GLMMs) implemented via restricted maximum likelihood in the lme4 package (ver. 1.1-10; Bates et al. 2014). Parental population, field site, and their interaction were modeled as fixed effects, while block within field site and family within parental population were modeled as random effects.

To determine the best-fitting distribution and link for our data, we screened three model types: a Gaussian distribution with an identity link, a Gaussian distribution with a log link, and a Poisson distribution with a log link. We then chose the best-fitting model as assessed by quantile-quantile plots, histograms of residuals, and Akaike information criterion values. Statistical significance of parent population, field site, and population × site interaction was assessed by ANOVA with type III sum of squares using the Wald $\chi^2$ test in the car R package (ver. 2.2-1; Fox et al. 2013). To better characterize patterns of adaptation within each experimental habitat, we also ran the same GLMM analyses for each

field site independently without the field site or population × site fixed effects. We tested for significant differences between parental populations using Tukey's tests implemented via the glht() function in the multcomp R package (ver. 1.4-6; Hothorn et al. 2008). If native populations are better adapted to each respective field site, we expect statistically significant parent × site interaction term in the GLMMs with the native Oregon population having the highest fitness. However, if adaptation lag is occurring across regions, we expect the population from the most historically similar climate to have the highest fitness (i.e., elevation-matched California populations).

*Trait Variation.* To explore which traits may explain the observed patterns of adaptation, we examined variation in morphological, phenological, and physiological traits within and between parental lines grown at each site. Given the observed covariance between many of the traits measured, we conducted a principal component analysis (PCA) using the pcaMethods package (ver. 1.60.0; Stacklies et al. 2007) for all quantitative traits and including all individuals from both the intra- and the interpopulation crosses with morphological data. Missing values were imputed using the svdImpute function. PC1 for this PCA (38.3% of variance; termed morphology PC1 hereafter) is effectively a measure of overall plant size, with higher values associated with tall plants with large leaves and flowers (table S3). PC2 for this PCA (17.9% of variance; termed morphology PC2 hereafter) is associated with a trade-off between phenological timing and morphology, with greater values indicating plants that take longer to flower and flower at later nodes but are generally larger at flowering.

We generated GLMMs by including the same fixed and random effects, choosing the best-fitting distributions and links, and testing for significance as described above for the fitness comparisons. Although we ran similar GLMMs on multiple traits, we do not report results after imposing a multiple testing correction, as this procedure can increase false negatives when traits are correlated (García 2004). Instead, we first ran GLMMs with morphology PC1 and PC2, and then we used these results to guide which traits were most important to include in subsequent GLMMs. Nonetheless, we note that even when we impose strict multiple testing criteria, the vast majority of our significant effects do remain statistically significant. Best-fitting distributions and links are reported in table S4.

*Phenotypic Selection Analysis.* Due to past divergence, multiple traits covary between populations, making it challenging to disentangle specific targets of selection. Therefore, we used the interpopulation outbred $F_4$ families generated from crosses between low- and high-elevation California and Oregon parents to conduct phenotypic selection analysis via

partial regression of traits on relative fitness (Lande and Arnold 1983) and to assess phenotypic correlations among traits that may influence responses to selection. Separate phenotypic selection analyses were run for each field site and for each set of hybrid lines. Relative fitness was calculated from total flower number for each plant (including plants that produced zero flowers). Unlike above, relative fitness was calculated separately for each $F_4$ population at each site. All traits used in this analysis except relative fitness were standardized to a mean of 0 and a variance of 1 within each field site × interpopulation cross combination. Selection gradient coefficients were obtained by least squares regression using the lm() call in R with relative fitness as the response variable and flowering time, plant height, leaf damage, floral PC1, relative water content, and leaf number at flowering as predictor variables. To detect stabilizing or disruptive selection, we also included quadratic terms for each trait and report $\gamma$ as the respective model coefficients multiplied by 2 (Lande and Arnold 1983; Stinchcombe et al. 2008). This specific subset of traits was chosen given the fitness relationships found for the intrapopulation crosses and to avoid introducing trait pairs with high genetic correlations into this analysis. Statistical significance of all directional and quadratic regression coefficients was determined via ANOVA as with the above GLMMs. We expect patterns of directional selection toward optimal parental phenotypes for each field site in both $F_4$ crosses. If an optimal phenotype already exists in variation associated with an $F_4$ cross, there should be stabilizing selection around this phenotype. As with most phenotypic selection experiments, we note that our analyses may be biased by unmeasured fine-scale environmental variation that leads to indirect correlations between phenotype and fitness (i.e., Rausher 1992; Stinchcombe et al. 2002). To examine how phenotypic correlations may impact patterns of selection, we calculated Pearson correlations between family means for each mapping population at each site for all traits combinations using the Hmisc package (ver. 3.17-01; Harrell 2015).

*Biotic versus Abiotic Selection Pressures.* To examine whether plant-herbivore interactions parallel patterns of local adaptation in our field sites, we estimated herbivore damage (to the nearest 5%) at flowering as a performance metric. A single observer made all observations at the low-elevation field site, and three observers collected these data at the high-elevation site. To ensure that all observers were unbiased, we measured the amount of leaf area missing on a limited sample of photographed leaves with imageJ (National Institutes of Health; https://imagej.nih.gov/ij/). The correlation between field-assessed and computer-measured leaf damage was relatively low but consistent for all observers ($r = 0.34$). This low correlation was expected due to the fact that the computerized measure measured herbivory only on a

single (and least damaged) second true leaf that had been sampled for estimating leaf relative water content, while the field measure examined all leaves. A GLMM with a Poisson distribution and log link was used to model differences between the parental populations using intrapopulation families. Fixed and random effects were specified as for the trait GLMMs, and significance of fixed effects was determined via Wald $\chi^2$ tests as described above. A separate GLMM was used to determine differences between the two interpopulation outbred $F_4$ populations. If herbivory is an important selection pressure within a given site, plants with lower herbivory should have higher fitness. Deviations from this association may occur because other selection pressures are more important and/or because no multivariate phenotype exists to best match the current environment.

## Results

### *Above-Average Temperatures Lead to Early Growing Season Start Dates*

In Oregon, 2014 was the second warmest year on record with an average temperature 1.7°C above historical averages for 1900–2000 (NOAA National Climate Data Center). Data from both ClimateWNA (figs. 1*D*, A3; table S1) and our onsite sensors (fig. 2*A*, 2*B*) reflected this difference in minimum, average, and maximum daily temperatures during the growing season. While there was above-average precipitation early in the season, precipitation during the growing season was normal (low-elevation site) to slightly below average (high-elevation site) compared with the last 30 years (fig. A4). These data suggest that although 2014 was an abnormally warm year for Oregon, this season is still closer to historical Oregon temperatures and precipitation averages than historical California averages (fig. 1*D*). However, the abnormal combinations of high temperature with precipitation in Oregon in 2014 did lead to earlier declines in snowpack and soil moisture than typically observed at these field sites (fig. 2*C*, 2*D*). Thus, the growing season start date in 2014 at the low-elevation Oregon site was closer to the historical growing season start date at the low-elevation California site (fig. 1*E*, table S1), and the high-elevation Oregon growing season start date in 2014 started as close to the historical high-elevation California growing season start date as the historical high-elevation Oregon growing season start date (fig. 1*F*; table S1).

### *Nonnative but Elevation-Matched Populations Are Most Fit*

To examine the relationship between source location and adaptation to current environments, we compared fitness at high- and low-elevation common gardens in central Oregon for outbred intrapopulation families and inbred maternal lines derived from four annual *Erythranthe guttata* populations
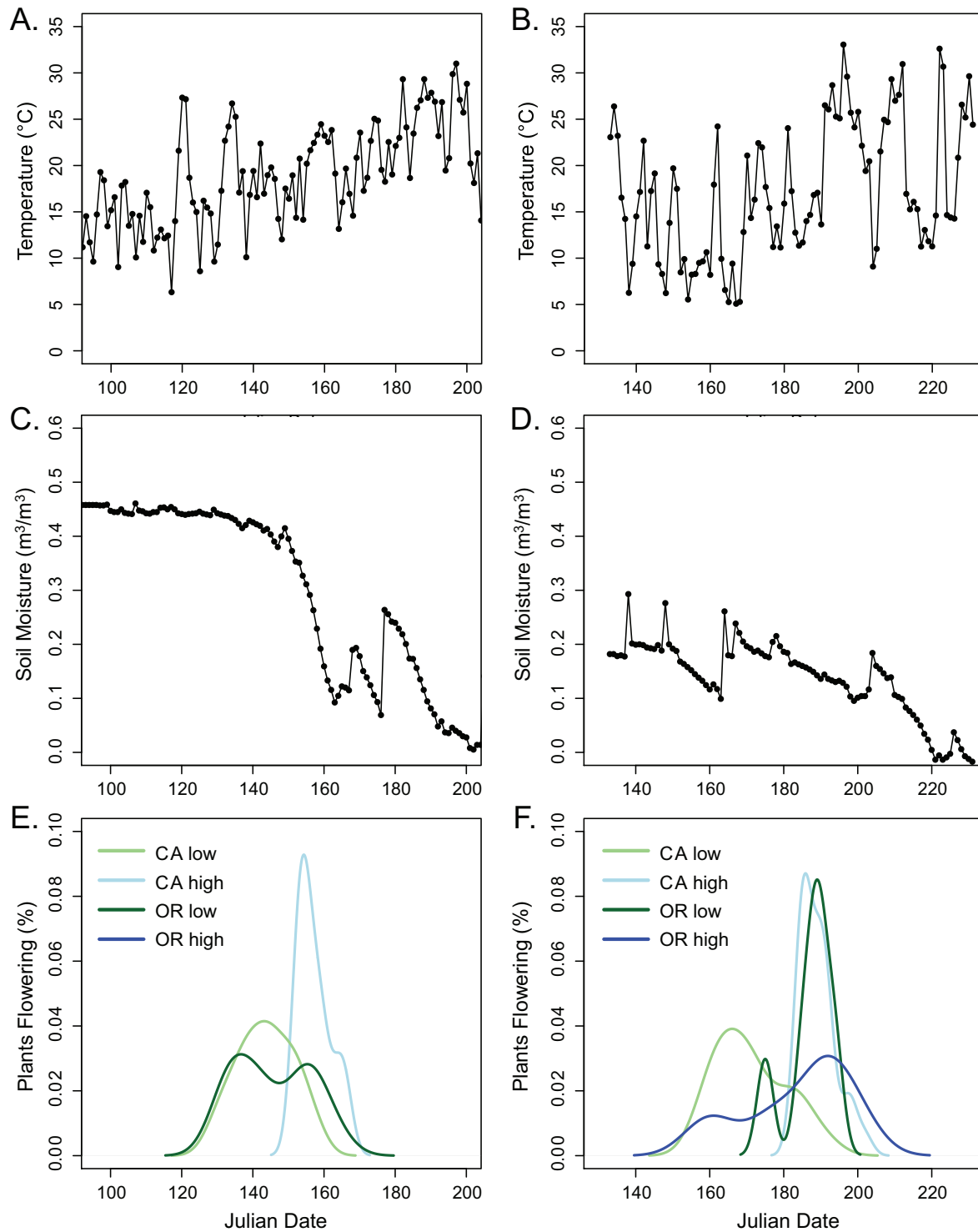
**Figure 2:** Environmental conditions and plant phenology at low- and high-elevation sites during the 2014 growing season. *A, B,* Temperature at noon each day during the growing season at the low-elevation (*A*) and high-elevation (*B*) sites. *C, D,* Soil water content at noon each day during the growing season at the low-elevation (*C*) and high-elevation (*D*) sites. *E, F,* Density plots depicting the timing of first flowering for individuals from each parent population at the low-elevation (*E*) and high-elevation (*F*) sites. Only two individuals from the high-elevation Oregon population survived to flowering at the low-elevation site, and they have been excluded from this graph.

sampled from low and high elevation in both Oregon and California. We found significant population × field site interaction effects for survival to flowering ($\chi^2 = 8.9$, $P = .03$; fig. 3A; table S4), number of flowers ($\chi^2 = 21.9$, $P < .001$; fig. 3B), absolute fitness ($\chi^2 = 30.5$, $P < .0001$; fig. A5), and relative fitness ($\chi^2 = 27.5$, $P < .0001$; fig. 3C), indicating that populations differed in their relative fitness ranking between sites. Notably, for all of these fitness measures the Oregon population most proximal to the field site was never the most fit of the four focal populations. Instead, with the exception of survival to flowering at high elevation, where the Low CA population performed best, the elevation-matched population from California was most fit (fig. 3B). When considering the low-elevation common garden in isolation, parental population strongly affected survival ($\chi^2 = 18.1$, $P < .001$) and relative fitness ($\chi^2 = 8.5$, $P = .034$). Low CA plants more frequently survived to flowering (Low CA [76%] > Low OR [51%] > High CA [28%] > High OR [7%]; table S4) and also produced more flowers (Low CA [mean ± SE, 2.5 ± 0.27] > Low OR [2.1 ± 0.44] > High CA [1.7 ± 0.92]). Only two High OR plants flowered at this site, but they produced an average of four flowers. Thus, when jointly considering survival and number of flowers to estimate lifetime relative fitness, Low CA plants produced 0.51 more flowers on average than Low OR plants, 1.44 more flowers than High CA plants, and 1.33 more flowers than High OR plants (fig. 3).

Considering the high-elevation field site in isolation, parental populations also significantly differed in number of flowers produced ($\chi^2 = 13.1$, $P = .004$) and relative fitness ($\chi^2 = 8.53$, $P = .036$) but not survival to flowering ($\chi^2 = 4.1$, $P = .25$). Like at the low-elevation site, the elevation-matched California population rather than the most proximal Oregon population was the most fit. Although most plants survived to flowering, High CA plants produced more flowers than any other population (High CA [5.6 ± 0.99] > High OR [4.2] > Low OR [2.9 ± 0.47] > Low CA [2.0 ± 0.53]) and also were more fit relative to any other population (High CA [1.26 ± 0.31] > High OR [0.79 ± 1.24] > Low OR [0.69 ± 1.73] > Low CA [0.47 ± 0.13]). These results indicate that low- and high-elevation Oregon populations are locally adapted to each site relative to each other but that each local Oregon population is maladapted compared with elevation-matched California populations.

### Different Life-History Strategies Facilitate Adaptation at Low- and High-Elevation Sites

To assess which traits may explain the differences in relative fitness among the focal populations, we examined patterns of trait variation at the high- and low-elevation field sites. Since many traits were highly covarying, we first examined morphological principal components. Although High CA and High OR plants were marginally larger at flower-
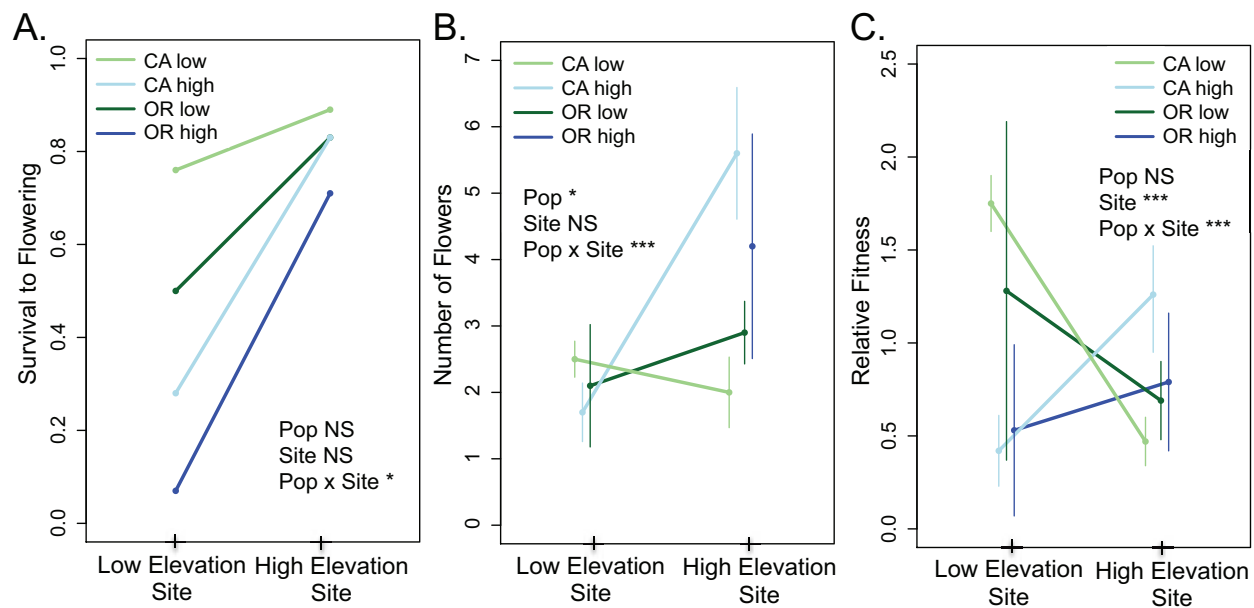


**Figure 3:** Mean values for low- and high-elevation parent populations from Oregon and California for survival to flowering (*A*), number of flowers produced (*B*), and relative fitness within low- and high-elevation field sites (*C*). Total number of flowers excludes individuals that did not flower. Relative fitness was calculated as the total number of flowers including zeros for plants that did not survive to flowering standardized by mean values within each plot. The statistics depicted were obtained from the generalized mixed linear models described in the text. *$^{*}P < .05$; $^{***}P < .001$; NS = not significant.

ing than low-elevation plants (morphology PC1, population: $\chi^2 = 6.9$, $P = .08$; fig. 4*A*), we found much stronger effects of population, field site, and their interaction on morphology PC2 (population: $\chi^2 = 58.2$, $P < .001$; site: $\chi^2 = 41.0$, $P < .001$; population × site: $\chi^2 = 11.0$, $P = .012$; fig. 4*B*), likely corresponding to differences in the trade-off between flowering time and vegetative growth. Low CA plants had the lowest morphology PC2 values at each site, indicating that they reached first flower more quickly and at an earlier node while producing fewer leaves and branches. In contrast, High CA plants had the highest morphology PC2 values at each site, indicating that they were slower to flower and achieved more vegetative growth prior to flowering.

To further parse the traits potentially contributing to these differences, we examined patterns of variation in several morphology PC–associated traits within and across field sites (table S4). As expected from differences in morphology PC2, we found strong population × site interactions for flower-

ing time ($\chi^2 = 10.8$, $P = .013$; fig. 4*C*), leaf number at flowering ($\chi^2 = 7.8$, $P = .049$; fig. 4*D*), and flowering node ($\chi^2 = 12.4$, $P = .006$; fig. 4*E*). Low CA plants flowered earliest in both plots, and all plants flowered more quickly at the high-elevation site than at the low-elevation site. Differences in flowering node and number of leaves were more complex but reflected slower transitions (i.e., more nodes and leaves prior to flowering) for High CA plants relative to all Oregon plants and for Oregon plants relative to Low CA plants. However, this slower transition did not necessarily extend to total allocation to vegetative growth before flowering, as plant height, branching, and leaf biomass did not differ between parents.

These results highlighted key phenotypic differences between parental populations that could be responsible for fitness differences. To test these hypotheses more rigorously, we conducted phenotypic selection analysis at each field site on two sets of outbred interpopulation F$_4$ families. These
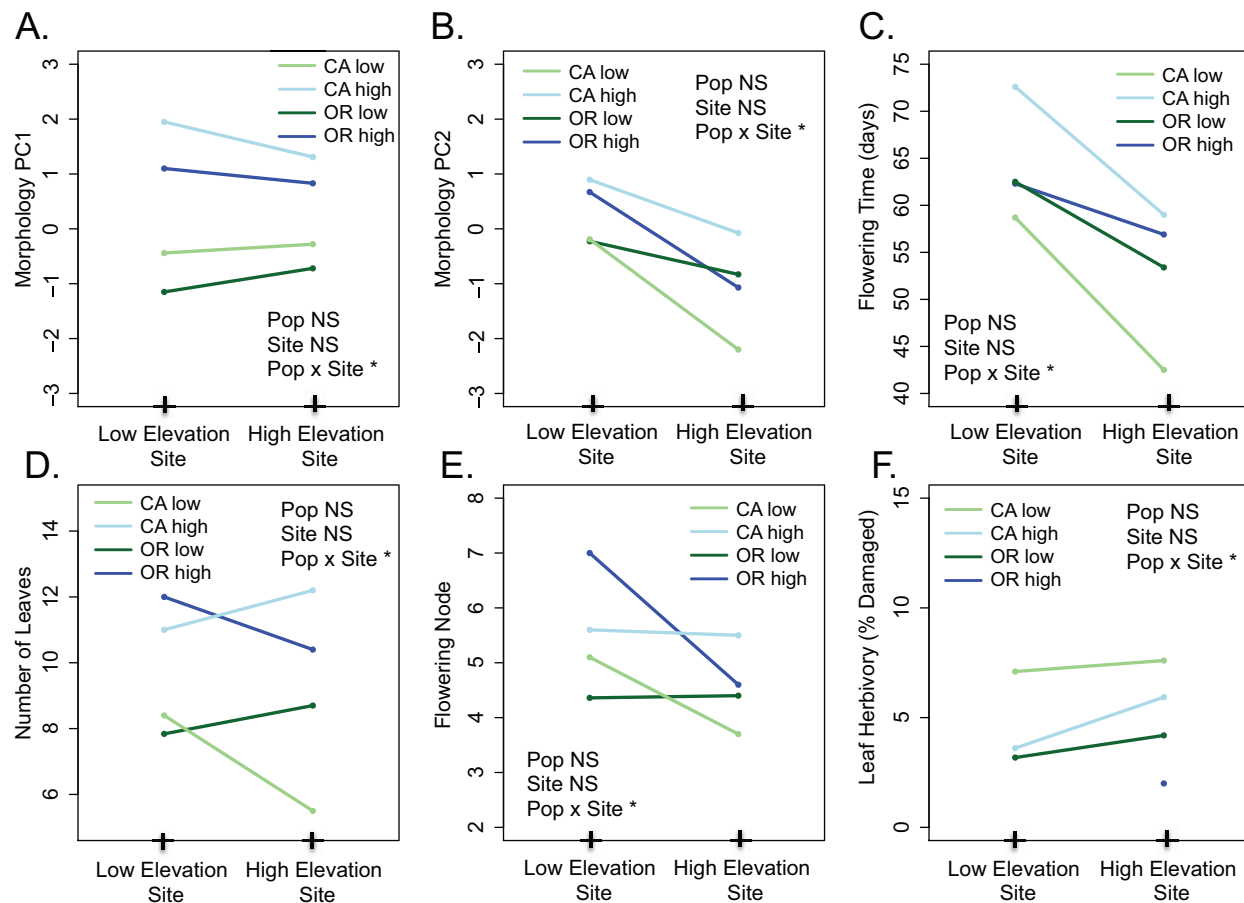


**Figure 4:** Mean values for low- and high-elevation parent populations from Oregon and California for morphology principal component (PC) 1 (*A*), morphology PC2 (*B*), flowering time (*C*), number of leaves at flowering (*D*), flowering node (*E*), and leaf damage (*F*). Population means are the average of all parental line means from each population. The statistics depicted were obtained from the generalized mixed linear models described in the text. *$P < .05$; NS = not significant.

families were derived from two crosses between low- and high-elevation parents, one with parents sampled from Low OR and High OR and the other with parents sampled from Low CA and High CA.

In the low-elevation common garden, we observed similar patterns of directional selection in both sets of outbred $F_4$'s (table 1), and these were consistent with our expectations given the phenotypic and fitness differences we observed among the four sets of intrapopulation families at this field site. We found strong directional selection for earlier flowering (OR $F_4$'s: $\beta = -0.2$, $P < .001$; CA $F_4$'s: $\beta = -0.22$, $P < .001$; fig. 5) and for taller plants at flowering (OR $F_4$'s: $\beta = 0.21$, $P = .03$; CA $F_4$'s: $\beta = 0.18$, $P = .001$). There were also significant patterns of directional and stabilizing selection for leaf number at flowering for the CA $F_4$'s (table 1). Low CA parental plants flowered the earliest, had high values of morphology PC1, and had the highest fitness in the low-elevation plot (figs. 3, 4), and we observed directional selection toward earlier flowering and fast growth rates in both $F_4$ populations (table 1).

Selection gradients differed more substantially between the CA and OR $F_4$ populations in the high-elevation common garden. Directional selection favored more leaves at flowering in both $F_4$ populations (OR $F_4$'s: $\beta = 0.2$, $P = .02$; CA $F_4$'s: $\beta = 0.26$, $P < .001$; table 1). However, OR but not CA $F_4$'s also revealed a quadratic selection gradient on leaf number, where producing too many leaves prior to flowering reduced fitness ($\gamma = 0.2$, $P = .03$) and weak directional selection for earlier flowering ($\beta = -0.17$, $P = .04$). These selection gradients suggest that OR $F_4$'s may have been limited more by the season-ending drought than CA $F_4$'s. CA $F_4$'s flowered an average of 4.9 days earlier than OR $F_4$'s, and they were also under directional and quadratic selection for plant height, with moderately tall plants generally favored ($\beta = 0.16$, $P < .001$; $\gamma = 0.16$, $P < .001$; fig. 5). In sum, these selection gradients suggest that timing reproduction late enough in the season to maximize vegetative biomass but early enough to avoid terminal droughts results in the highest fitness (figs. 2, 5). As at the low-elevation site, these patterns of selection are consistent with the phenotypic divergence and differences in relative fitness we observed between the four parent populations. Parent plants that flowered later (High CA) with a large number of nodes and leaves had higher fitness at the high-elevation site. Likewise, in both $F_4$ crosses we observe directional selection toward producing more vegetative biomass before flowering and observe directional selection for earlier flowering only in the later-flowering OR $F_4$'s.

To examine potential constraints on responses to selection due to correlations between traits, we calculated Pearson correlations between family means for each interpopulation $F_4$ cross at each site. There were moderate correlations between traits ($r < 0.8$ for all combinations; table S6) along

the same axes as observed in parental lines (e.g., taller plants have larger flowers, wider stems, and more leaves). Interestingly, there were no correlations between flowering time and most morphological phenotypes (table S6), indicating that flowering time may be unconstrained by correlations with other traits and thus able to evolve independently of growth rate.

### Elevation-Matched California Populations Achieve Highest Fitness in Oregon despite Sustaining Greater Herbivore Damage

To explore whether plant-herbivore interactions may contribute to the differences in relative fitness between the four focal populations, we also quantified leaf damage at flowering. There were moderate levels of leaf damage at both sites (low-elevation site: $\mu = 5.65\%$; high-elevation site: $\mu = 5.66\%$), and leaf damage was strongly affected by population and by a population × site interaction (population: $\chi^2 = 9.1$, $P = .029$; population × site: $\chi^2 = 21.4$, $P < .001$; figs. 4F, A6). Low OR plants incurred the least leaf damage ($3.2\% \pm 0.7\%$) in the low-elevation common garden, and High OR plants incurred the least leaf damage ($2\% \pm 1.2\%$) in the high-elevation common garden. Low CA plants were the most damaged at both sites (low-elevation site: $7\% \pm 1.3\%$; high-elevation site $7.6\% \pm 1.3\%$).

Thus, at both sites we observed a pattern contrary to expectation; plants more damaged by herbivory actually had higher fitness on average (fig. A7). Moreover, we did not detect significant directional or quadratic selection for leaf damage in either outbred $F_4$ population, indicating that differential leaf herbivory had little impact on relative fitness compared with other selective pressures (table 1). These unexpected observations may reflect genetic correlations between damage and other traits at each site. Consistent with this possibility, in the low-elevation common garden leaf herbivory has a positive phenotypic correlation with most growth phenotypes in both outbred $F_4$ panels; that is, taller plants were more fit but also had greater levels of herbivory (fig. 6A, table S6). In the high-elevation common garden, leaf herbivory was negatively correlated with flowering time (fig. 6B, table S6) for both sets of hybrid lines. These correlations of leaf herbivory with different traits at each site indicate that there may be trade-offs between allocation of resources to growth or reproduction versus defense that manifest in different ways in different environments.

### Discussion

*Patterns Consistent with Maladaptation and Adaptation Lag during an Extreme Climatic Event*

A primary signature of adaptation lag is that the local population has lower fitness than populations from other sites with historical environmental conditions that more closely

Table 1: Summary of phenotypic selection analyses at the low- and high-elevation field sites

| Site, trait | OR F$_4$'s | | | | | | | | CA F$_4$'s | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $\beta$ | SE | $\chi^2$ | P | $\gamma$ | SE | $\chi^2$ | P | $\beta$ | SE | $\chi^2$ | P | $\gamma$ | SE | $\chi^2$ | P |
| Low-elevation site: | | | | | | | | | | | | | | | | |
| Flowering time | −.20 | .05 | 18.5 | >.001 | −.01 | .07 | ... | ... | −.22 | .04 | 30.8 | >.001 | .09 | .07 | ... | ... |
| Plant height | .21 | .10 | 4.5 | .033 | −.08 | .04 | 3.0 | .08 | .18 | .06 | 10.5 | .001 | .06 | .05 | ... | ... |
| Leaf damage | .06 | .07 | ... | ... | −.08 | .06 | ... | ... | −.02 | .05 | ... | ... | −.02 | .06 | ... | ... |
| Floral PC1 | .10 | .06 | ... | ... | .08 | .08 | ... | ... | .04 | .04 | ... | ... | .03 | .05 | ... | ... |
| RWC | .07 | .06 | ... | ... | .03 | .03 | ... | ... | .08 | .06 | ... | ... | .01 | .01 | ... | ... |
| Leaf number | .14 | .08 | ... | ... | .03 | .06 | ... | ... | .21 | .08 | 7.0 | .008 | −.12 | .05 | 4.7 | .029 |
| High-elevation site: | | | | | | | | | | | | | | | | |
| Flowering time | −.17 | .09 | 4.0 | .044 | −.11 | .10 | ... | ... | −.08 | .04 | 3.6 | .059 | −.03 | .06 | ... | ... |
| Plant height | .07 | .09 | ... | ... | −.11 | .12 | ... | ... | .16 | .05 | 12.3 | >.001 | .16 | .05 | 11.8 | .001 |
| Leaf damage | .08 | .06 | ... | ... | −.12 | .09 | ... | ... | .03 | .04 | ... | ... | −.06 | .04 | ... | ... |
| Floral PC1 | .23 | .07 | 9.8 | .002 | .15 | .09 | ... | ... | .04 | .04 | ... | ... | .00 | .05 | ... | ... |
| RWC | −.01 | .12 | ... | ... | −.05 | .08 | ... | ... | .00 | .05 | ... | ... | .00 | .03 | ... | ... |
| Leaf number | .20 | .08 | 5.4 | .020 | .22 | .10 | 4.9 | .026 | .26 | .06 | 20.3 | >.001 | .06 | .06 | ... | ... |

Note: Both directional and quadratic selection gradients are reported as unstandardized values. All models had 1 degree of freedom. Bold text signifies statistical significance at $P < .05$, while italic text signifies statistically marginal associations. $\chi^2$ values and $P$ values are not reported for models with $P > .01$. PC = principal component; RWC = relative water content.

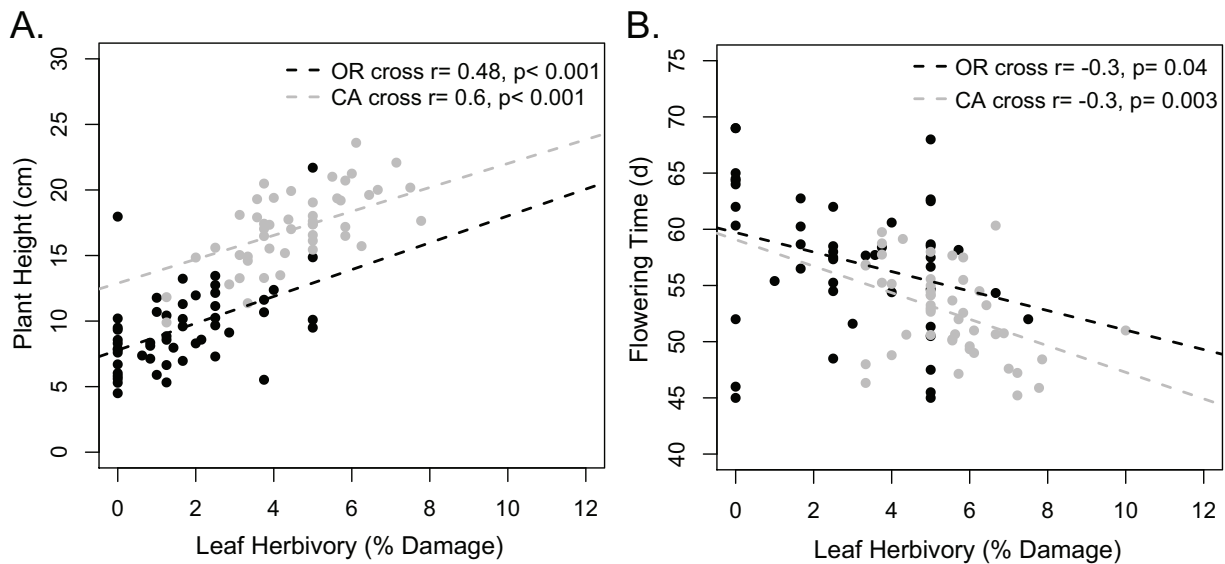## Low Elevation Site



## High Elevation Site
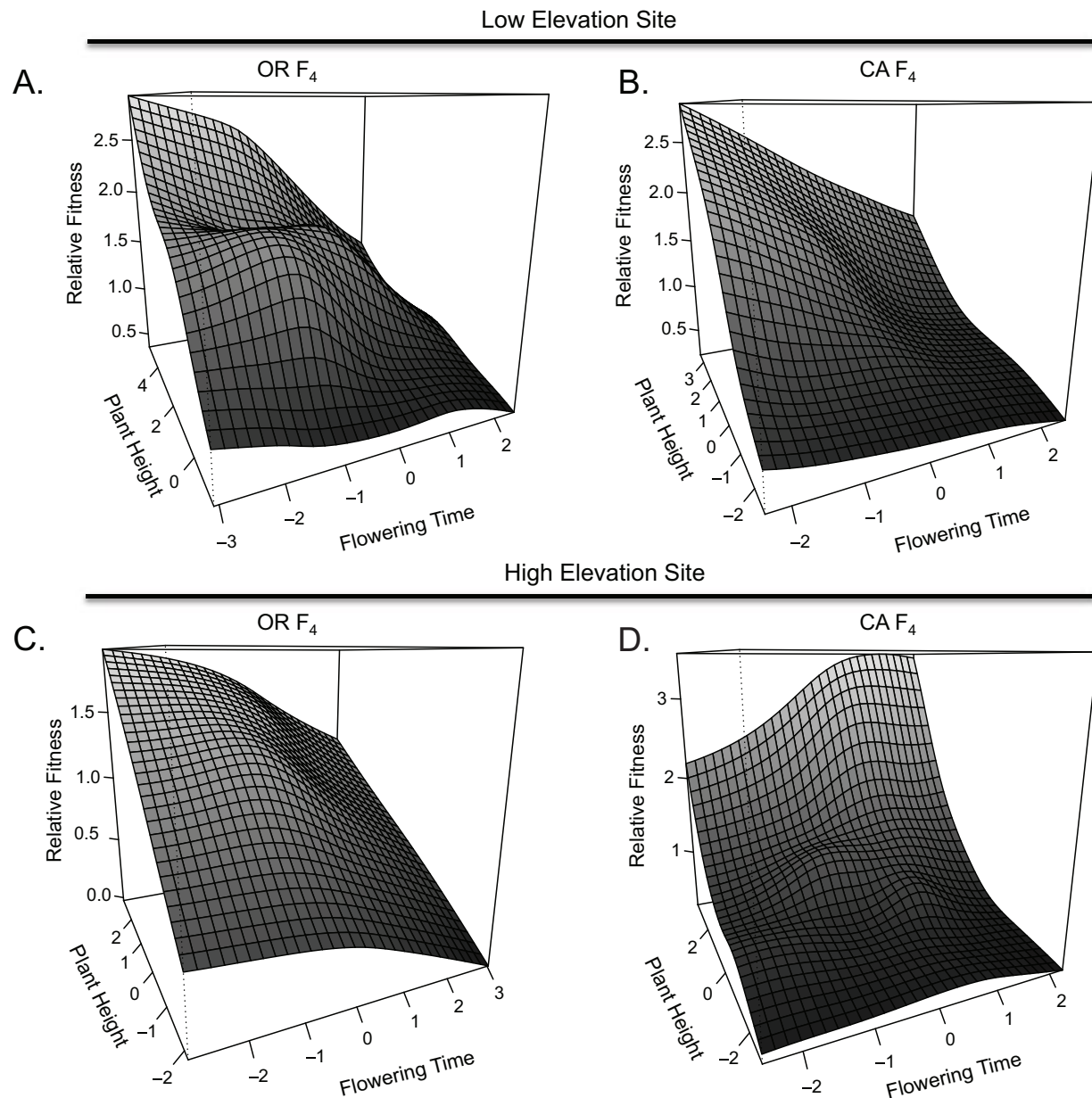


**Figure 5:** Cubic splines depicting the fitness landscape as a function of flowering time and plant height for Oregon and California interpopulation outbred $F_4$ crosses at the low-elevation site (*A, B*) and the high-elevation site (*C, D*). Shading indicates the value of relative fitness, with darker shading indicating lower fitness.

match current conditions (Wilczek et al. 2014). Here, we observe this pattern for annual populations of the common monkeyflower *Erythranthe guttata* during a historically atypical early growing season. One central result of our study is that populations geographically proximal to our common-garden sites in Oregon have lower fitness than populations sampled from similar elevations in California but with earlier growing seasons (figs. 1, 3). The pattern is consistent at both

the low- and the high-elevation field site. Low CA plants had the highest relative fitness at the low-elevation Oregon field site (Low OR plants had the second highest fitness), while High CA plants had the highest fitness at the high-elevation Oregon field site (again, High OR plants had the second highest fitness). This elevation-matched pattern of relative fitness suggests that no one parent population has a universally higher fitness, and it indicates that maladaptation is ob-

**Figure 6:** Relationships between leaf herbivory and flowering time at the high-elevation site (*A*) and leaf herbivory and plant height at the low-elevation site (*B*) for Oregon (gray) and California (black) interpopulation outbred $F_4$ crosses. Points represent family means, and trend lines are from linear regression of each variable on leaf herbivory.

servable even in a system with short generation times and ample genetic variation.

Several caveats to this conclusion merit discussion. First, it is important to consider whether the signal associated with adaptation lag could be a result of maladaptation for reasons other than abnormal climatic conditions, as observing patterns of maladaptation is not uncommon (Leimu and Fischer 2008; Hereford 2009). Local adaptation should be very likely in an annual outcrossing plant with large effective population sizes occurring across steep elevation gradients. Indeed, considering the High and Low OR populations alone, we do observe local adaptation across this short elevation gradient, and past work has also shown local adaptation within *E. guttata*, albeit between ecotypes (Hall and Willis 2006; Peterson et al. 2016). This suggests that the signature of maladaptation is unlikely to be due to high levels of gene flow relative to the strength of selection but is more likely to be due to the atypical selective environment experienced during the 2014 field season. Second, we also want to highlight the caveat that our sampling design may not have adequately reflected the pool of standing variation at each parental site (Puzey et al. 2017). A limited number of parents were used to generate our various crosses; only a single High OR family survived the initial transplant into the low-elevation site, and only two High OR families survived transplant into the high-elevation site. However, even if the High OR population is excluded, it is still clear that geographically close populations have significantly lower fitness than more distant populations in our study. Additionally, the interpopulation outbred $F_4$ families derived from distant California popula-

tions had higher average fitness than the interpopulation outbred $F_4$ families derived from more local Oregon populations at both low- and high-elevation sites (table S5). Thus, after reflecting on these caveats, we still consider our interpretation of our findings as consistent with adaptation lag to be well supported.

A major criterion for documenting adaptation lag is that the current climatic conditions at field sites must be more similar to the historic conditions at the more distant sites than to local historic conditions. While the temperatures at the Oregon field sites were well above normal temperature during the *E. guttata* growing season (figs. 1*D*; A3), matching the average temperatures in western Oregon expected for 2070–2099 (St. Clair and Howe 2007), our analyses of climate data suggest that 2014 temperature and precipitation at either field site were not closer to the historical elevation-matched climatic conditions of our California source populations than the historical conditions of our Oregon source populations (fig. 1*D*). However, these above-average temperatures did translate into marked differences in growing season timing and duration. For instance, snow melted out at our high-elevation site nearly a month earlier than during average years (early May), forcing us to start our planting at the high-elevation site far earlier than has been done for other *E. guttata* experiments at this site (Hall and Willis 2006; Hall et al. 2010; Mojica et al. 2012). Above-average temperature combined with low precipitation also caused earlier reductions in soil moisture than normal, altering the growing season duration (fig. 2). These shifting growing seasons largely parallel the earlier timing of the growing seasons in California

populations with growing season start dates at Oregon field sites closer to California population norms than norms at the same sites (fig. 1*E*, 1*F*). Interestingly, low-elevation Oregon populations typically have an earlier growing season than high-elevation Oregon populations but did not have higher relative fitness at the high-elevation Oregon site. We suggest that both the low-elevation Oregon population and the low-elevation California population had too early of a growing season start date at the high-elevation Oregon site, as flowering immediately came at the cost of putting on valuable vegetative biomass prior to flowering (as the high-elevation California population did) that could have allowed plants to complete reproduction in low soil moisture conditions.

### Life-History Traits Are the Primary Targets under Selection in Changing Environments

Determining which traits are under selection and responsible for differences in fitness provides insight into causes of maladaptation and potential for future adaptation. Several phenotypes differ between parental populations in a manner consistent with the selection gradients we estimated on interpopulation outbred $F_4$ crosses, suggesting that these traits are the most likely to contribute to patterns of (mal)adaptation. For instance, at both sites Low CA plants were the fastest to flower, were the most likely to flower, and had the second-highest plant height at flowering (High CA was taller), and earlier flowering and larger size were also favored by directional selection at the low-elevation site for both sets of $F_4$ crosses (fig. 5). The native Low OR plants were the second fastest to flower but had a much lower growth rate and were shorter at flowering. These results are not necessarily surprising, as the cool, moist, and relatively homogeneous conditions typical of Oregon springs are usually ideal for a resource-acquisitive growth strategy, but flowering earlier in the year should become more favorable as winters become shorter and milder. This pattern parallels the phenology shifts observed in many other plant species (Willis et al. 2008; CaraDonna et al. 2014). Other studies have observed significant variation in critical photoperiod in annual *E. guttata* (Friedman and Willis 2013; Kooyers et al. 2015), and to the extent that these differences contribute to differences in seasonal phenology seen here, this may be valuable variation for adapting to future climates.

At the high-elevation site, the rapid growth and early reproduction strategy of Low CA plants was much less successful. Low CA plants flowered prior to and during an early season drop in soil moisture (~160 Julian days; fig. 2) caused by early cessation of snowmelt, which was later relieved with multiple days of rain (at our high-elevation site) and snow (at even higher elevations). Instead, High CA plants, which were the last to flower and largest at flowering of the parental outbred families, had the highest fitness. Phenotypic selection analyses do not show evidence for selection for later flowering; rather, both sets of outbred $F_4$'s have directional selection for producing more leaves prior to flowering. This larger size at flowering allowed plants to thrive once snowmelt subsided and terminal drought conditions began. High OR plants had the second-highest number of leaves at flowering, had the second-highest plant height at flowering, and generally flowered earlier than High CA plants. However, there was quite a bit of variation in life-history traits for the High OR population (even with only a single outbred line), with plants from this population being among the first and last to produce a first flower. This is consistent with previous work showing that large year-to-year fluctuations in climate maintain life-history variation in this population (Mojica et al. 2012), and local adaptation may have been more apparent had we been able to conduct common-garden transplant experiments across multiple seasons, allowing us to examine geometric mean fitness.

The result that High CA plants with a relatively slow transition to flowering had the highest fitness at the high-elevation site was surprising. Previously studies had found that rapid flowering is a key component of fitness success in the short growing seasons associated with the high-elevation Cascades (Hall and Willis 2006; Hall et al. 2010). In fact, High OR populations typically had the most rapid time to flower in flowering-permissive greenhouse conditions in a common-garden study of populations surveyed throughout the range of *E. guttata* (Kooyers et al. 2015). However, the growing season in 2014 in the high-elevation site began far earlier than average—native *E. guttata* plants in the high-elevation population were producing true leaves before the snow would have typically melted at this site in late May. This growing season is more reminiscent of high-elevation California populations, where snow at similar elevation populations typically melts by late April or early May. This work suggests that understanding the genetic variation associated with life-history trade-offs as well as shifts in the commencement and duration of growing seasons will be important for predicting evolutionary rescue in annual plants.

### Herbivore Pressure May Be Less Important than Abiotic Conditions in Extreme Climatic Years

While changing abiotic conditions at Oregon sites may favor plants with phenotypes more similar to California populations, substantial differences in native herbivore and plant communities may still favor native plants. We find strong population differentiation for leaf herbivory, where the proximal Oregon populations have the lowest levels of leaf damage at each site (fig. 4*F*). Previous comparisons of Oregon and California *E. guttata* populations have documented that these regional groups differ in level and composition of their primary chemical defense compounds, phen-

ylpropanoid glycosides (PPGs), in leaves (Kooyers et al. 2017). The rank order of average leaf damage at the low-elevation site corresponds directly to the average amount of constitutive PPGs (but not constitutive trichome density; Kooyers et al. 2015) found in each population. This association is consistent with the inferences that PPGs do deter herbivores, and past selection has likely favored the evolution of greater herbivore defense in the Oregon populations than in the California populations.

However, in the 2014 common gardens, levels of leaf damage did not correspond well with the relative fitnesses of the parental populations. In fact, we find that $F_4$ families with greater levels of herbivory had higher fitness (fig. A7). This unexpected positive relationship may be primarily explained by underlying correlations between herbivory and life-history traits such that plants that invest less in defense may have higher herbivory but are also able to grow faster or flower sooner (fig. 6). This mismatch between herbivory and relative fitness may suggest that herbivory is not currently an important selection pressure or is less important compared with other selection pressures. Although herbivore damage is common in annual *E. guttata* populations, it has rarely been considered a major selective factor compared with abiotic factors (but see Ivey et al. 2004; Colicchio 2017; Kooyers et al. 2017). These results are highly relevant for understanding maladaptation within these populations because if they reflect a strong genetic correlation between defense and flowering, then future evolutionary responses could be constrained such that genotypes capable of rapid development and resisting herbivores would be slow and difficult to achieve (Etterson and Shaw 2001).

### Assessing Maladaptation in a Changing Climate

Our interpretation of climate-mediated maladaptation in annual *E. guttata* populations provides an interesting case for classifying the precise meaning of maladaptation (sensu Hendry and Gonzalez 2008). Lower absolute and relative fitness of both native populations relative to more distant populations at each site suggests that the Oregon populations do indeed show evidence of maladaptation. However, maladaptation may not necessarily be the correct term here, as every population has average absolute fitness that would far exceed replacement level. On average, an *E. guttata* plant at the high-elevation site that produced a single flower would produce 32.4 seeds (fig. A2), leading to $\lambda \gg 1$. Even if seedling establishment rates are low, this value is well above replacement level, suggesting that local populations remain well adapted to their sites.

Nonetheless, we still find a pattern of divergence among populations consistent with the existence of maladaptation even in this annual outcrossing species with high effective population sizes and high levels of standing phenotypic and genetic variation that should exhibit outstanding potential for evolutionary rescue. Although our study was conducted at two sites for just one extreme season, we would emphasize that these results may have enhanced explanatory value, as extreme years can have disproportionate effects on long-term population demography (e.g., Grant and Grant 1993), and we anticipate that such conditions will become more frequent in the future. An open topic raised by these findings is determining what factors best explain this maladaptive pattern. One possible explanation is that genetic constraints due to correlations among traits decrease the efficiency of selection to shifting climates (Etterson and Shaw 2001). Additionally, fluctuating selection across multiple seasons whereby alleles that are favorable in some seasons are disfavored in other seasons may be another source of constraint (Mojica et al. 2012; Troth et al. 2018). Alternatively, it may simply be that there has been limited opportunity in the local populations for adaptation to extreme conditions like those experienced during this field season since they have been too infrequent historically. In other words, past selection has been too weak or too soft to allow evolution of specialist genotypes that perform well in extreme conditions or generalists genotypes that can respond plastically to extreme climate conditions. These possibilities—constraint versus lack of opportunity—have very different ecological implications for the capacity of populations to adapt to future climates. Thus, we would advocate for more studies like the one we report here, that is, studies that ask whether natural populations of widespread and genetically tractable systems exhibit patterns consistent with adaptation lag.

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*Special Feature Editor: Joseph Travis*



Dr. Nic Kooyers is an evolutionary biologist whose research focuses on determining the prevalence of and mechanisms underlying local adaptation in natural plant populations. Much of his work investigates potential responses of plant populations to climate change in the western United States. Photo credit: Nic Kooyers.