American Journal of Botany

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RESEARCH ARTICL

INVITED SPECIAL ARTICLE

For the Special Issue: Life Without Water

Population responses to a historic drought across the range of the common monkeyflower (*Mimulus guttatus*)

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Manuscript received 15 April 2020; revision accepted 26 August 2020.

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Citation: Kooyers. N. J., K. A. Morioka, J. M. Colicchio, K. S. Clark, A. Donofrio, S. K. Estill, C. R. Pascualy, I. C. Anderson, et al. 2021. Population responses to a historic drought across the range of the common monkeyflower (Mimulus guttatus). *American Journal of Botany*. 108(2): 1–13.

doi:10.1002/ajb2.1589

PREMISE: Due to climate change, more frequent and intense periodic droughts are predicted to increasingly pose major challenges to the persistence of plant populations. When a severe drought occurs over a broad geographical region, independent responses by individual populations provide replicated natural experiments for examining the evolution of drought resistance and the potential for evolutionary rescue.

METHODS: We used a resurrection approach to examine trait evolution in populations of the common monkeyflower, *Mimulus guttatus*, exposed to a record drought in California from 2011 to 2017. Specifically, we compared variation in traits related to drought escape and avoidance from seeds collected from 37 populations pre- and post-drought in a common garden. In a parallel experiment, we evaluated fitness in two populations, one which thrived and one which was nearly extirpated during the drought, under well-watered and dry-down conditions.

RESULTS: We observed substantial variation among populations in trait evolution. In the subset of populations where phenotypes changed significantly, divergence proceeded along trait correlations with some populations flowering rapidly with less vegetative tissue accumulation and others delaying flowering with greater vegetative tissue accumulation. The degree of trait evolution was only weakly correlated with drought intensity but strongly correlated with initial levels of standing variation. Fitness was higher in the post-drought than pre-drought accessions in both treatments for the thriving population, but lower in both treatments for the nearly extirpated population.

CONCLUSIONS: Together, our results indicate that evolutionary responses to drought are context dependent and reflect the standing genetic variation and genetic correlations present within populations.

KEY WORDS adaptation; common garden; drought avoidance; drought escape; *Erythranthe guttata*; evolutionary rescue; phenology; phenotypic evolution; *Phrymaceae*; resurrection experiment.

Periodic shortages of water are a ubiquitous challenge and significant selective agent for nearly all plant species (Engelbrecht et al., 2007; Juenger, 2013; Siepielski et al., 2017). Such droughts are regularly predicted to become more severe and frequent in many regions as human-mediated climate change alters the timing and amount of precipitation around the globe (Dai, 2013; IPCC, 2014).

Indeed, these predictions have been proving true as multiple droughts with recurrence intervals previously estimated at >1000 years have occurred in the last few decades (Gallant and Gergis, 2011; Robeson, 2015). Determining the ability and speed of plant populations to adapt to changing precipitation patterns and extreme droughts is a shared goal of botanists, climate scientists, and ecologists. Resurrection studies are one important tool for assessing the potential for phenotypic evolution and evolutionary rescue in response to severe selection pressures (Davison and Reiling, 1995; Sultan et al., 2013; Franks et al., 2018). In these studies, seeds from many plants are collected before and after populations endured a selection pressure and then grown in a common environment. Shifts in phenotypic means and variances are posited to reflect phenotypic evolution associated with the selective event. Past resurrection studies examining phenotypic evolution during severe droughts have demonstrated drought-related phenotypic evolution (Franks et al., 2007; Dickman et al., 2019; Lambrecht et al., 2020), indicated that evolution of plasticity to drought may play a limited role in this adaptation (Franks, 2011), and documented drought-associated genomic differentiation (Franks et al., 2016).

Many resurrection studies conducted to date have followed an experimental design that leverages a large number of maternal lines from a small number of populations to examine phenotypic evolution (reviewed by Franks et al., 2018). This approach maximizes the power to detect low-magnitude signatures of selection within one or few populations by providing precise estimates of trait distributions both before and after the selective pressure. In contrast, resurrection studies with an experimental design that incorporates a more limited number of maternal lines from a large number of populations have several distinct advantages. By comparing phenotypic responses across many populations, these studies can document how often phenotypic evolution occurs in the same traits in different populations and how consistent the direction of phenotypic evolution is across populations. In doing so, this sampling design facilitates tests that may provide insight into which extrinsic and intrinsic mechanisms underlie local variation in evolutionary responses. For instance, the intensity of a common selection pressure can vary between populations and thus influence the direction or magnitude of the phenotypic response (i.e., Nevo et al., 2012). Additionally, because initial conditions vary among populations that have historically adapted to spatial heterogeneity (e.g., in aridity or drought frequency), populations may vary in how well they tolerate the selection pressures, how far phenotypic means are offset from new adaptive optima, and the amount of standing genetic variation segregating for relevant traits (Bossdorf et al., 2008; Knapp et al., 2008; Agashe et al., 2011).

Plants have evolved a variety of adaptive strategies for coping with limited water availability, and trait values that confer increased drought resistance are expected to rise in frequency during a severe drought. Physiological adaptations that increase drought resistance are often classified into three different syndromes that each incorporate a specific set of traits (Ludlow, 1989; Kooyers, 2015; Volaire, 2018). First, drought or dehydration escape involves rapid growth and reproduction to complete a life cycle prior to a terminal drought. Second, drought- or dehydration-avoidant plants have traits that enhance water-use efficiency, allowing maintenance of homeostasis during drought. Finally, drought tolerance involves physiological adaptations that permit survival through a drought with the intent to reproduce following the drought and in extreme cases can involve complete cellular dehydration. While these strategies are not mutually exclusive (i.e., Bouzid et al., 2019), physiological and genetic trade-offs are often thought to preclude the evolution of multiple strategies (McKay et al., 2003; Des Marais et al., 2014). Resurrection studies that have examined how herbaceous plant species have evolved in response to contemporary droughts have most commonly documented evolution of more pronounced drought escape through earlier flowering (Franks et al., 2007; Lambrecht et al., 2020) or more rapid emergence from seeds (Dickman et al., 2019). However, drought avoidance or tolerance strategies may be more beneficial when drought onset occurs too early in the growing season for any reproduction to take place or when drought stress is short in duration or mild in intensity (Kooyers, 2015).

Resurrection studies can be even more powerful when pre- and post-stressor generations of seed are grown in multiple environments (e.g., well-watered and water-limited conditions), as doing so can reveal cryptic adaptation to the stressor and facilitate estimation of phenotypic selection in relevant conditions (Kawecki and Ebert, 2004; Blanquart et al., 2013). Although reproducing a drought event predicted to occur only every 500 years in field conditions is challenging, manipulative greenhouse experiments that attempt to recreate drought conditions can be helpful for testing key hypotheses about how directly phenotypic evolution relates to drought adaptation (Knight et al., 2006; Mojica et al., 2016). For instance, if the most ecologically important phenotypes were not measured, increased survival or reproduction may be observed post-drought in the absence of phenotypic evolution (Franks et al., 2018). Higher fitness achieved through phenotypic adaptation during drought may also lead to higher fitness in more typical water conditions (i.e., universal adaptation). Alternatively, severe drought could result in lower fitness of post-drought populations as there may be entire years without reproductive success or drastic reductions in population size during drought could lead to increased inbreeding and/or genetic drift.

Annual populations, such as those found in the common yellow monkeyflower (*Mimulus guttatus*), are at increased risk of extirpation due to severe events. *Mimulus guttatus* is a model species for ecological genomics that occupies moist areas in the western United States from coastal bluffs along the Pacific Ocean to seepy meadows and rock walls at high elevation in the Sierra Nevada and Cascade Mountain ranges (Wu et al., 2008; Yuan, 2019). Most inland populations found below 2000 m are facultatively annual populations that have ephemeral water supplies from spring rain or snowmelt that dry up during hot Mediterranean-like summers. These annual populations exhibit a drought escape strategy to complete a life cycle before summer drought begins (Hall and Willis, 2006; Kooyers et al., 2015; Troth et al., 2018). Populations from the most arid portion of the range, the Central Valley of California, also exhibit some evidence of a drought avoidance response with more succulent leaves and greater water-use efficiency than elsewhere in the range (Kooyers et al., 2015). Future trait evolution in annual *M. guttatus* populations is predicted to occur rapidly because these populations harbor some of the highest levels of genetic diversity observed in plant species (Friedman et al., 2015; Twyford and Friedman, 2015; Puzey et al., 2017).

From 2012 through 2017, *M. guttatus* populations in California experienced one of the worst droughts on record, and temperatures during this period were well above historical averages, exacerbating the drought (Griffin and Anchukaitis, 2014; Robeson, 2015). The most intense portion of this drought occurred in water years 2013–2015 with snowpack reaching a 500-year low in 2014 (Belmecheri et al., 2016). The drought extended north into Oregon and Washington in 2014 and 2015, and the spatial heterogeneity of this drought imposed on existing variation in seasonal water availability throughout the range of *M. guttatus* provides a rich context for examining patterns of phenotypic evolution during sustained drought. Greater levels of phenotypic evolution may occur in populations that experienced more intense droughts. Alternatively, drought intensity might matter less than the historical precipitation regime of a site or the amount of heritable trait variation within each population.

In this study, we investigated patterns of phenotypic evolution and adaptation in *M. guttatus* during this historic drought using a resurrection approach that combines a large common garden experiment with a more focused manipulative study. Leveraging seed collections made before and after the drought from the same 37 populations, we addressed the following questions: (1) How common across populations is phenotypic evolution due to the drought? (2) Is phenotypic evolution limited by correlations between traits or do traits evolve independently from one another in different populations? (3) What factors best predict which populations evolved similar phenotypic responses? (4) How does phenotypic evolution correspond to differences in fitness in normal and drought environments? Our results suggest that evolutionary responses to severe drought are not universal and are somewhat predictable based on properties of the population and the drought.

MATERIALS AND METHODS

Population collections, environmental data collection, and resurrection experiments

A historic drought event afflicted western North America from 2012 to 2017. We collected seeds from 37 populations pre-drought in 2011 and post-drought in 2016 or 2017 for a total of 539 maternal lines (mean: 7.3 lines/population/year; Fig. 1). When ripe seeds were not available at the time a site was visited, entire plants were taken back to the greenhouse facilities at Duke University or University of California, Berkeley, and seed was collected from field-fertilized fruits once they matured or from fruits produced by hand-pollination and selfing. Post-drought collections consisted of multiple years because the drought did not end in 2016 for southern California populations, and consequently, many of these populations did not establish in 2016. We used 2016 collections whenever possible (Appendix S1). We obtained latitude, longitude, and elevation values for each population, and this information was subsequently used to extract environmental metadata from ClimateWNA (Wang et al., 2016) including historical averages for both temperature and precipitation variables (1980–2010) and contemporary temperature and precipitation data from each year between 2011–2017. To classify the intensity and duration of the drought at each site, monthly precipitation data was summated for each water year (October–September) during the drought and compared to historical average annual precipitation (1980–2010). Since the drought ended earlier in some areas of the range relative to others (i.e., Oregon vs. California), we summarized the intensity of the entire drought by adding up the relative precipitation deficits from the most extreme years of the drought (water years between 2013–2015). We refer to this measure below as relative drought intensity, and it qualitatively matches the United States Drought Monitor's metrics of drought severity (Appendix S2).

To determine whether phenotypic evolution occurred during the drought, we took a resurrection experimental approach. Seeds from each maternal line collected above were planted in 2.5″

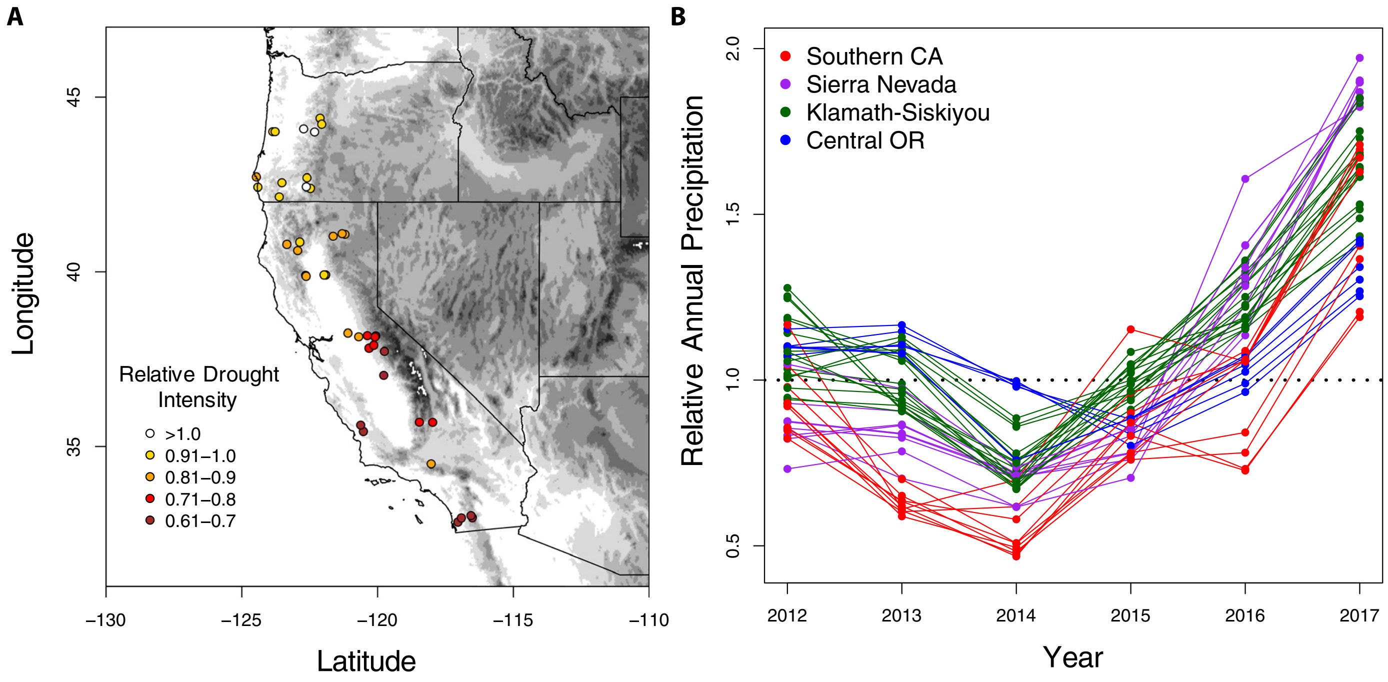

FIGURE 1. Spatial variation in the intensity and duration of the 2011–2017 drought for annual *Mimulus guttatus* populations. (A) Locations of each population sampled in the resurrection experiment. Marker color corresponds to the relative amount of precipitation in water years 2013–2015 compared to historical averages. White-black raster corresponds to the elevation of the site (low to high, respectively). (B) Relative precipitation compared to historical average at each *M. guttatus* population in each water year during the drought. Yearly and historical climate data (1980–2010) were downloaded from ClimateWNA.

pots with Sunshine Mix #1 (Sun Gro Horticulture, Agawam, MA, USA) and cold-stratified in total darkness under humidity domes for 9 d at 4°C in a walk-in growth room (Environmental Growth Chambers, Chagrin Falls, OH, USA). Subsequently, the chambers were adjusted to 16 h, 21°C day/8 h, 19°C night cycles with light intensity increasing or decreasing over the 30 min after dawn or before dusk, respectively, and germination was surveyed each day. Time to germination was slightly lower on average for plants collected in 2011 compared to 2016 plants, but populations collected in 2011 and 2017 had similar germination times (Appendix S3). This finding suggests that there is limited nonrandom mortality among collection years during storage that could influence our conclusions on phenotypic evolution, i.e., the invisible fraction problem (Weis, 2018). Following 16 d with daily misting, seedlings were moved to the greenhouse and grown under supplemental lighting to maintain 16 h day/8 h night conditions (Oxford Tract Facility, University of California, Berkeley, CA, USA). Greenhouse temperatures were set to 21°C day/12°C night; however, cooling was often not sufficient to counteract ambient heating, and maximum observed daytime temperatures could reach up to 27°C. Seedlings were thinned to two plants per maternal line, at most, with the second seedling transplanted into its own pot. Pots were randomized into flats containing 32 pots, and flats were randomized across greenhouse benches. Throughout the experiment, each flat was bottom-watered to maintain constant water availability, and flats were rotated every 2 d across greenhouse benches to limit microenvironmental effects. We examined four phenotypes related to drought escape and avoidance. We surveyed germination and flowering daily and report flowering time as the time from germination to the time of first flower opening. At time of first flower, we recorded the node of the first flower, plant height at flowering, and the leaf length and width of the second true leaf.

Phenotypic evolution during drought

To examine patterns of phenotypic evolution in traits following drought across populations, we used a general linear model framework implemented in R v3.5.3 (R Foundation for Statistical Computing, Vienna, Austria). We used univariate models to examine each individual trait as a separate response variable with population, collection year, and the population × year interaction as factors. We also include seed area, which is highly correlated with seed mass ($N = 27$, $r^2 = 0.85$; Appendix S4) as a covariate in this analysis to partly account for maternal effects since we did not conduct a refresher generation in the common garden to eliminate these effects prior to initiating the resurrection experiment. Seed area was calculated for each line using an image of ~20 seeds/line. Images were taken using an Apple iPhone XR or a Google Pixel 3 attached to an Olympus SZ30 stereomicroscope using a universal cell phone adaptor. Images of a ruler were taken to normalize the ratio of pixels to millimeters and calculate seed size. Variation between ruler images from different batches of seed photos was <0.5%. Images were batch processed in ImageJ (Fiji distribution; Schneider et al., 2012) using a custom script. For any maternal line where two individuals germinated, we averaged phenotypes within the maternal line. Statistical significance was assessed via two-way ANOVA utilizing type III sum of squares implemented in the car package v3.0-2 (Fox et al., 2013). We did not employ any correction for multiple comparisons as our phenotypes are highly correlated. If phenotypic evolution occurred in the same direction for all populations, we expect a significant effect of collection year on a phenotype. If populations differ in phenotypes but drought imparted no evolutionary change in phenotype, then we expect a significant effect of population in our analysis. If phenotypic evolution occurred in different directions or to different magnitudes in different areas of the range, we expect a significant interaction effect.

To determine whether drought resistance evolved via the independent evolution of multiple phenotypes or via distinct drought resistance trait syndromes, we examined correlations between traits across years and populations. Pearson correlation coefficients were assessed using the Hmisc package v4.0-2 to examine changes in correlations between traits between pre- and post-drought populations (Harrell, 2015). As some phenotypes were highly correlated, we conducted a PCA with imputation of missing phenotypes via the pcaMethods package version 1.74 (Stacklies et al., 2007). All traits described above were included. Traits were z-score-transformed before the PCA. Strong correlations among traits that exhibit phenotypic evolution would suggest that trait evolution may be constrained into distinct syndromes.

We next investigated potential drivers of spatial variation in phenotypic evolution during drought by modeling how the absolute magnitudes of phenotypic differences between pre- and post-drought samples relate to potential causal factors. First, to examine whether geographic variation in the severity of drought impacts the extent of phenotypic evolution, we assessed the association between relative drought intensity and absolute change in trait PC1 between pre- and post-drought populations via linear regression. Then, to test whether populations that inhabit more historically arid areas are preadapted to drought and thus possibly needed to evolve less to adapt to the drought, we assessed whether the annual heat-moisture index of a population is associated with absolute change in trait PC1 between pre- and post-drought populations via linear regression. Annual heat-moisture index is calculated as (mean annual temperature + 10)/(mean annual precipitation/1000) (Wang et al., 2016). We dropped any population from this data set that had less than three maternal lines in either the pre- or post-drought collection (26 populations included). Finally, to test whether the magnitude of broad-sense heritable phenotypic variation segregating within a population impacts the magnitude of phenotypic evolution, we extracted phenotypic variation (squared standard deviation) in flowering time and in vegetative traits (first principal component of variation in nine traits including stem diameter, leaf measurements, etc.) for each population from a previous common garden experiment. This prior common garden experiment was also conducted with seed derived from the 2011 collections from most of the same populations (23 populations overlapped, Kooyers et al., 2015), and incorporated an additional generation in a common environment to reduce maternal effects. We examined the association between phenotypic variation within a population to the change in trait PC1 between pre- and post-drought populations via linear regression. We hypothesize that if any of these factors impact the extent of phenotypic evolution during the drought, there should be a significant association in each linear regression.

Evolution of fitness differences during drought

Since not all phenotypic differences result in differences in fitness and we did not survey many phenotypes that could impact drought adaptation in our larger common garden study, we conducted a second manipulative experiment to examine adaptation to drought conditions. For this experiment, we selected two populations from the Central Valley of California that experienced some of the worst drought conditions, BEL (37.039833, −119.77382; 196 m a.s.l.) and MEDX (37.816633, −120.313667; 344 m a.s.l.). The BEL population maintained its size throughout the drought, while MEDX had a decreasing population size that consisted of less than 24 individuals producing seeds during drought years (N. Kooyers, personal observation). For each population, we selected 8–10 maternal lines pre- and post-drought. We grew these lines in a common garden on growth shelving (14 h day/10 h night at 23°C) for one generation to reduce maternal effects. Germination for 2011 lines was lower than 2016 lines, resulting in loss of several maternal lines. We selfed each line to maintain pre- and post-drought lines to use in the next generation. Because *M. guttatus* is known to have severe inbreeding depression, we also produced outbred lines by randomly crossing post-drought lines within each population and also randomly crossing pre- and post-drought lines within each population.

Both selfed and outcrossed lines were used in a manipulative experiment conducted on growth shelving within a single walk-in growth chamber (14 h day/10 h night at 19°C). Seed stratification was conducted as in the above experiment, and germination was induced in flats covered with humidity domes with daily misting of all plants. Three replicates of each maternal line were randomized across flats, and flats were rotated every 3 d. There were two treatments, a well-watered control treatment with bottom watering as needed and a dry-down treatment with flats given 1 L of water 14 d after germination and not watered again. This treatment resulted in a consistent dry down across flats such that relative water content was reduced to less than 10% at ~10 d after the start of the dry down (Appendix S5). Our final data set consisted of 284 plants with an average of 6.3 maternal lines per population per treatment (Appendix S6).

Numerous phenotypes were measured for each plant in the manipulative experiment to compare with the resurrection experiment and to compare to fitness in each treatment. Flowering time was scored as above. At flowering, we measured plant height, node of the flower, number of leaves, number of branches, length and width of a second true leaf, and corolla width, length and height. A single second true leaf was also taken at flowering and weighed immediately (wet mass). Each leaf was placed in DI water for >12 h and then weighed again (turgid mass). Leaves were then dried at 65°C for 4 d and weighed to calculate dry mass. Relative water content was measured as 100 × (wet mass − dry mass)/(turgid mass − dry mass). We scored four phenotypes as various fitness proxies: end-of-experiment branch number, end-of-experiment plant height, flower number when all plants in the dry down treatment had senesced (40 d after drought treatment initiated), and aboveground biomass (dry mass after drying at 65°C for >7 d).

To examine whether pre- and post-drought populations differed in fitness, we conducted a series of general linear mixed models (glmm) using the lme4 v1.1-21 package (Bates et al., 2014). First, to determine whether cross type (outcrossed and selfed) needed to be taken into account, we modeled each fitness trait within univariate glmm models with population, cross type, and treatment as fixed effects and maternal line and flat as random variables. Model fit for all models was assessed by examining histograms of residual values, and aboveground biomass was then log-transformed to improve model fit. Statistical significance of fixed effects on fitness was assessed via ANOVA with type III sum of squares calculated via the Kenward-Roger approximation implemented via lmerTest package v3.1-0 (Kuznetsova et al., 2017). Because cross type (outcrossed or selfed) was highly significant for every fitness trait measured (see results below), we analyzed data sets for selfed and outcrossed lines separately. To assess whether there was phenotypic evolution for fitness traits (i.e., adaptation) in either treatment for selfed lines, we conducted univariate glmm models for each fitness measure with fixed effects for population, treatment, and collection year with maternal line and flat as random variables. For outcrossed lines, collection year represented outcrossed 2011–2016 lines or 2016–2016 crosses. Statistical significance was assessed by ANOVA and lmerTest as above. If adaptation occurred during the drought, there should be higher fitness in post-drought populations. However, this difference may only appear in treatments with limited water availability; in our design, this difference would lead to a significant drought treatment by collection year interaction.

To determine whether any of the assessed phenotypes could drive differences in fitness, we first conducted a trait PCA to reduce the dimensionality of our data (termed trait2PCA below). Methods for this PCA mirrored the PCA described above, and this PCA included all traits measured in the manipulative experiment. To examine whether trait2PCA axes impacted fitness, we explored two different models. First, we replaced fitness in the above univariate linear mixed models with either trait2PC1 or trait2PC2. If a given trait2PC impacts fitness, we expect to see similar effects of population, collection year, and drought treatment on variation in the trait. Second, we assess associations between both trait2PCs with fitness using a linear mixed model implemented in lme4. We model fitness as a univariate response variable and a trait2PC1, trait2PC2, and their interaction as fixed factors and line and block as random factors. This approach has the benefit of testing phenotype–fitness associations independently of our treatments, years collected, or populations. Significance for both approaches was tested via ANOVA and lmerTest as above.

RESULTS

Drought intensity and duration

The historic drought disproportionately impacted some populations over others across the range of *M. guttatus*. The drought lasted the longest and was most intense for southern California populations, stretching from water year 2012 to water year 2017 and reaching <50% relative precipitation in 2014 (Fig. 1). While the drought was historically long and intense in the Sierra Nevada, it ended earlier there than in the southern California populations (i.e., in 2016 rather than 2017). Further north in Oregon, there is evidence for drought conditions only in 2015, and drought conditions were not nearly as severe (i.e., were closer to historical averages; Appendix S2).

Magnitude and direction of phenotypic evolution—Both the magnitude and direction of phenotypic evolution in response to the drought varied dramatically between populations. Models demonstrated that each of the five phenotypes measured varied significantly across the range regardless of the drought (Appendix S7). There were interaction effects between collection year and population for both flowering time ($F_{1,35} = 3.18$, $P < 0.0001$) and flowering node

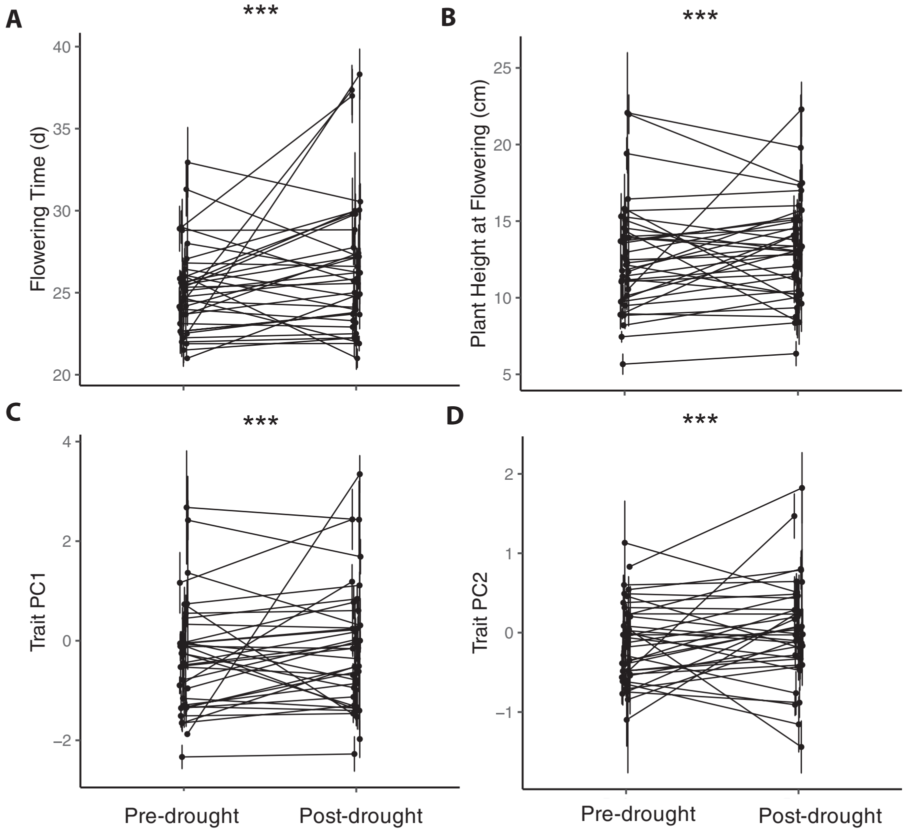

FIGURE 2. Phenotypic differences for each population in pre- vs post-drought collections for flowering time (A), plant height at flowering (B), trait PC1 (C), and trait PC2 (D). Each point and line represent a single population. Error bars represent standard error. Asterisks indicate level of statistical significance for a population × collection year interaction effect: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

($F_{1, 35} = 2.01$, $P = 0.0008$), indicating the different populations had different evolutionary responses to the drought (Fig. 2; Appendix S1). There was also a significant effect of year on flowering time after accounting for the interaction effect ($F_{1, 1} = 6.94$, $P = 0.008$), suggesting that selection for later flowering was more common than earlier flowering across populations. Seed area was not a significant covariate in any model, which suggests a limited role for maternal provisioning in determining trait variation (Appendix S7).

Phenotypes were generally moderately correlated with one another with the strongest correlation between flowering time and flowering node ($r^2 = 0.51$; Appendix S8). Range-wide correlations between all traits before and after the drought were very similar (Appendix S8). To understand how phenotypic correlations may influence phenotypic evolution due to drought, we conducted a PCA that included all five phenotypes and all individuals both pre- and post-drought. The PC1 axis (trait PC1; 55.6% of variance) corresponds to allocation into vegetative biomass vs. early reproduction with higher values corresponding to larger plants and later flowering times (Appendix S9). The PC2 axis (trait PC2; 23.2% of the variation) corresponds to an association between flowering time and leaf size with higher values corresponding to later flowering plants with smaller leaves (Appendix S9). As with the individual phenotypes above, there was a significant interaction of collection year and population on both trait PC1 ($F_{1, 35} = 1.97$, $P = 0.001$; Fig. 2C) and trait PC2 ($F_{1, 35} = 1.72$, $P = 0.008$; Fig. 2C, D). These results suggest that individual populations may have had different phenotypic responses to drought, but these responses involved multiple phenotypes changing in parallel directions. Indeed, in each population with a significant change in flowering time during the drought, there was also an opposing difference in plant height at flowering. That is, populations evolving earlier flowering were shorter at flowering (Fig. 3).

Predictive factors for phenotypic evolution

We evaluated whether the intensity of drought, the annual heat moisture index, or the amount of heritable phenotypic variation for flowering or plant size impacted the extent to which populations exhibited phenotypic evolution in response to drought. First, examining each factor individually, we found that while drought intensity and annual heat moisture index were not correlated with the magnitude of response to drought on trait PC1 or trait PC2 (Fig. 4), the amount of initial phenotypic variation for flowering time in each population was correlated to differences in both trait PC1 ($F_{1, 21} = 8.1$, $P = 0.009$; Fig. 4C) and trait PC2 ($F_{1, 21} = 14.3$, $P = 0.001$; Fig. 4F). This correlation also held for initial phenotypic variation for plant size (Vegetative PC1 from Kooyers et al. 2015; Appendix S10). We then used multiple regression to assess the relative influence of relative intensity of drought, annual heat moisture index, or heritable phenotypic variation on the magnitude of phenotypic responses to drought observed across populations. The best model via AIC included all three factors and all interactions aside from the three-way interaction (Full model AIC = 20.3). This model explained 62% of the variation in trait PC1 response and included significant interactions between heritable phenotypic variation in flowering time and both AHM and relative drought intensity (Appendix S11). A model that includes only the effect of amount of heritable phenotypic variation in flowering time was the fourth-best model (AIC = 25.0). However, looking at BIC instead flips the rank order of the top four models where the best model only includes the effect of initial amount of phenotypic variation in flowering time, although BIC values are similar for the top three models (BIC = 28.4–29.4). In combination, these data suggest that all three factors are important for determining how strongly populations evolve in response to drought stress, but the amount of heritable phenotypic variation is likely the most important.

Adaptation and plasticity in a manipulative experiment

Phenotypic evolution does not necessarily lead to increased fitness under either drought or well-watered conditions. Thus, to examine how the drought impacted the evolution of fitness, we

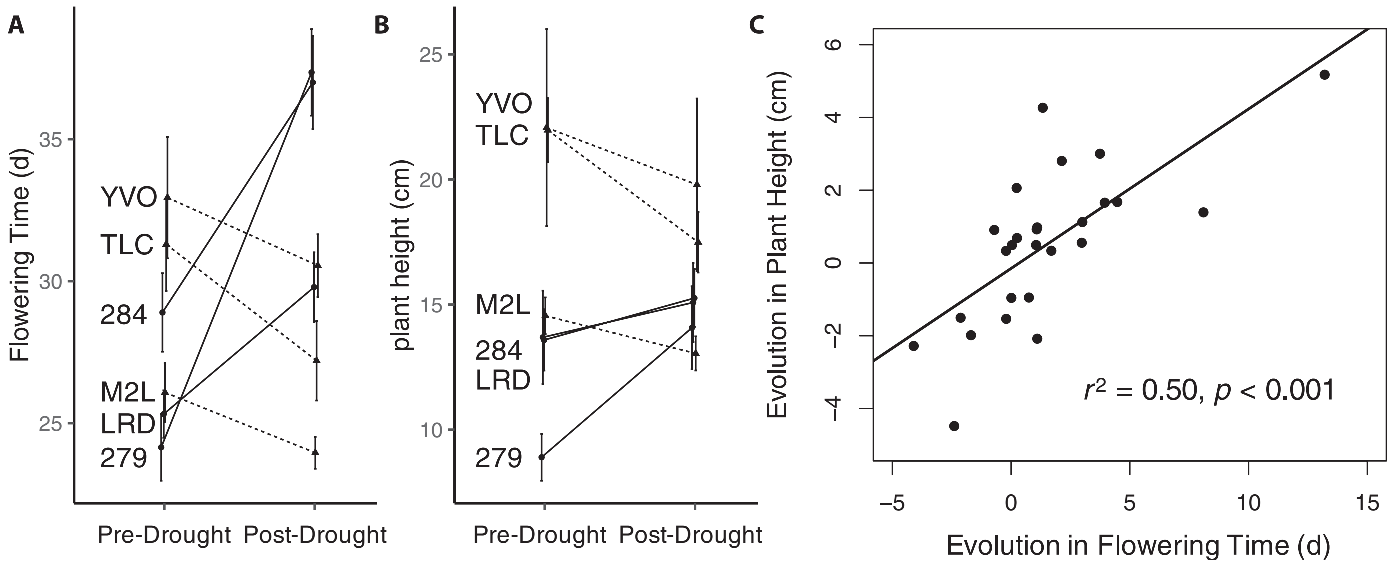

FIGURE 3. Phenotypic differences for six populations that statistically differed pre- to post-drought in either flowering time (A) or plant height at flowering (B). These populations exhibited the most extreme responses in the data set. Note that all populations that involved earlier flowering also were shorter at flowering. Error bars represent standard error. (C) Correlation between pre- to post-drought changes in flowering time and plant height for each population. Populations with a sample size of fewer than three maternal lines in the pre- or post-drought collections were excluded.

conducted an experiment with dry-down and well-watered treatments and measured the fitness of pre- and post-drought populations for two populations that experienced severe drought in the lower foothills of the Sierra Nevada in central California (BEL and MEDX). The dry-down treatment severely impacted fitness. Plants produced ~22 fewer flowers on average in the dry-down treatment relative to the control treatment. There was a significant interaction of population and collection year on number of flowers (Pop:Year $F_{1,\,23.9} = 4.99$, $P = 0.035$) where the plants descended from post-drought collections from the BEL population had higher fitness than plants descended from pre-drought collections, while the opposite was true for the MEDX population (Fig. 5; Appendix S12). There were also marginal or significant population by collection year interactions on each of the other three fitness phenotypes measured (Appendix S13). Because *M. guttatus* often has significant inbreeding depression, we also created outbred lines and exposed them to the same treatments. Type of cross had a large effect on fitness ($F_{1,\,6.8} = 17.6$, $P < 0.001$) with outbred lines always having higher fitness than inbred lines. While there was an interaction between type of cross and treatment ($F_{1,\,226.9} = 11.9$, $P > 0.001$), outbred lines from each population had similar qualitative fitness patterns as the inbred lines. That is, BEL lines had higher fitness in post-drought than pre-drought conditions and vice versa for MEDX lines. However, there was not a statistically significant interaction between population and cross type (2011 × 2016 or 2016 × 2016), possibly due to the lower sample size for the outbred lines relative to the inbred lines (Appendix S6).

In this manipulative experiment, we also measured several traits to examine how phenotypic evolution was associated with fitness differences. In the larger resurrection study above, both BEL and MEDX exhibited no significant phenotypic evolution during the drought. All phenotypes that were measured in both experiments (i.e., flowering time, plant height, flowering node) were quite similar between experiments (Appendix S14). In the manipulative experiment, we assessed more drought-related phenotypes than in the larger resurrection experiment above. As in the resurrection experiment, a PCA suggests that most phenotypes were highly correlated. The PC1 axis (trait2 PC1; 45.4% of variation) corresponded to a vegetative growth at flowering axis with higher values indicating plants had larger leaves, larger flowers, were taller, and had more branches. The PC2 axis (trait2 PC2; 16.8% of variation) loaded heavily on flowering time and leaf size with higher values corresponding to later flowering and smaller leaves. Neither of the trait2 PC axes exhibited similar population × year interactions to those observed within the fitness models above (Appendix S15). This result suggests that none of these traits underlie the differences in fitness observed between pre- and post-drought collected lines (Appendix 16). However, both trait2 PC axes were strongly associated with number of flowers (trait PC1 $F_{1,\,245.1} = 38.6$, $P < 0.001$; trait2 PC2 $F_{1,\,255} = 8.5$, $P = 0.003$; trait2 PC1: trait2 PC2 $F_{1,\,261.3} = 10.6$, $P = 0.001$). Together, these results indicate that the phenotypes we measured are important for fitness, but that they did not contribute to the fitness differences observed in either condition between pre- and post-drought collected lines. Thus, these results suggest that we have yet to identify the phenotype(s) responsible for the evolution of fitness in response to drought by the BEL and MEDX populations.

DISCUSSION

Determining the propensity, magnitude, and direction of phenotypic evolution following severe drought is essential for understanding how populations will respond to and cope with similar environmental challenges. Here, we have combined resurrection and manipulative experimental approaches to leverage seed collections made before and after the recent historic drought in the western United States to examine whether and how plant populations can adapt to an extreme contemporary climate event that is expected to occur with increased frequency going forward (Dai,

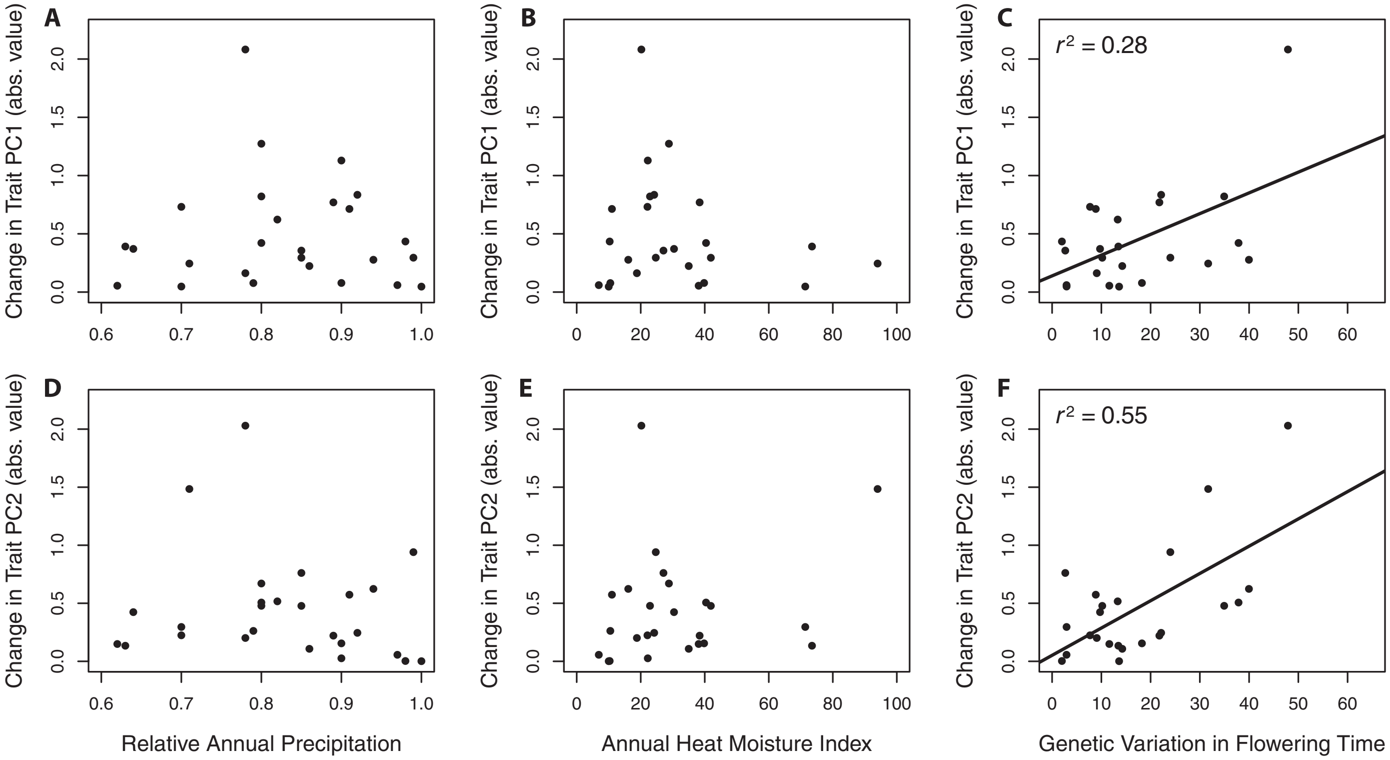

FIGURE 4. Scatterplots visualizing the relationship between absolute change in trait PC1 (A–C) or trait PC2 (D–F) and drought intensity (A, D), heat moisture annual index (B, E) or phenotypic variation in flowering time in a previous experiment (C, F). Each point is a population mean. Regression lines are shown only for statistically significant relationships.

2013). Our results indicate that some, but not all, annual *M. guttatus* populations from this range-wide sample evolved differences in reproductive timing and patterns of vegetative growth during the drought (Fig. 2). Trait evolution was highly correlated across populations. Populations that evolved earlier flowering also flowered at an earlier node, were shorter, and had smaller leaves (Fig. 3). These patterns of multivariate evolution are consistent with drought escape and avoidance strategies. However, the direction of phenotypic evolution was not consistent across the range as some populations evolved toward a stronger drought escape strategy and others toward a drought avoidance strategy. Although the direction of phenotypic evolution was not predictable, a number of factors—including the amount of heritable phenotypic variation present within a population prior to drought, the intensity of the drought, and historical aridity of the site—were associated with the absolute magnitude of evolution during the drought. The amount of heritable variation had the highest association with phenotypic evolution, where populations with greater variation had larger evolutionary responses to the drought (Fig. 4). In addition to these findings, our manipulative drought experiments suggest that evolutionary responses of additional unmeasured traits likely contribute to changes in fitness that evolved over the drought period. Below we discuss these results in the context of findings from other resurrection experiments, knowledge of our study species, and theoretical predictions on the evolution of functional strategies.

Both drought escape and avoidance evolved during the CA drought

Perhaps the most striking result from the resurrection experiment was the diversity of evolutionary responses we observed. Several populations evolved toward earlier flowering, a more drought escape-like strategy (Figs. 2, 3). Faster time to flowering was highly correlated with being shorter at flowering and producing smaller leaves (Fig. 3). This correlation was not unexpected as there were significant correlations between these traits pre-drought, but these traits are not developmentally constrained because there are populations that exhibit early flowering and are relatively large at flowering (i.e., BEL and SAA; Kooyers et al., 2015). Notably, populations that exhibited this evolutionary response were all located in mountain ranges of central and northern California rather than in the southern California regions, which had the most severe and longest drought. Evolution of an enhanced drought escape strategy was not unexpected given previous studies documenting drought escape as an important strategy to cope with seasonal drought by annual *M. guttatus* populations (Hall and Willis, 2006; Mojica et al., 2012; Kooyers et al., 2015; Troth et al., 2018). Across the range, flowering is associated with growing season duration where populations with shorter growing seasons typically require fewer days to flower in inductive day lengths (Kooyers et al., 2015). In areas with short growing seasons, year to year variation in precipitation causes fluctuating selection on flowering time, with early flowering plants having the highest fitness in low precipitation years (Nelson et al., 2018; Troth et al., 2018). Evolutionary enhancement of drought escape responses has also been extensively documented in other herbaceous plant species, most notably in two intensively studied southern California populations of *Brassica rapa* (Franks et al., 2007, 2016). Notably, the drought escape response in *B. rapa* also involved substantial correlated evolution of phenotypes as varied as stem width (Franks and Weis, 2008) and fungal susceptibility (O'Hara et al., 2016).

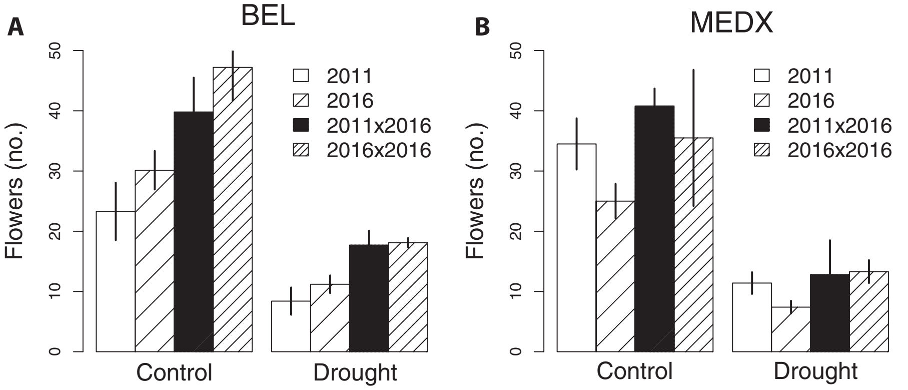

FIGURE 5. Differences in fitness in BEL (A) and MEDX (B) in well-watered and dry down conditions. Bars display averages, and error bars represent standard error for a factor combination. The 2011 lines are pre-drought collected lines, and the 2016 lines are post-drought collected lines. Each of these lines was selfed within a refresher generation prior to this manipulative experiment. Outbred lines (2011 × 2016 and 2016 × 2016) were crossed within populations during the refresher generation to reduce inbreeding effects.

Although evolution of a multivariate drought escape strategy following severe drought is a common response by herbaceous annual plant species, our findings suggest we should not expect this to be a ubiquitous response. Indeed, several *M. guttatus* populations surprisingly evolved later flowering and greater vegetative growth before flowering during the 2010s drought period (Fig. 3). These populations may be evolving toward a more drought avoidance-like strategy in which accumulating more vegetative biomass prior to flowering either enables the plant to stay alive for long enough to complete reproduction during a terminal drought and/or confers greater water use efficiency. Populations that had this evolutionary response to the recent drought occur in parts of the range that suffered the highest drought intensities (Southern California and the Sierra Nevada). Interestingly, two of the populations that exhibited this response most drastically, 279 and LRD, were also populations that did not establish during at least 1 year during the drought. Specifically, 279 did not establish in 2014–2016 (visited each year by N. Kooyers), and LRD did not establish in 2016 (visited in 2016 and 2017 by N. Kooyers). Thus, drought may favor greater seed dormancy and consequently prevention of germination as an alternate means to escape periodic drought. A strong genetic association between greater seed dormancy and later flowering time has been established in *Arabidopsis* (Auge et al., 2018). However, at least one other population that evolved this more drought avoidant syndrome did establish each year during the drought, suggesting that more than one of these postulated adaptive explanations may be at play.

Why did some populations evolve while others did not?

We did not detect statistically significant signatures of phenotypic evolution for the majority of populations that we surveyed (>80%) even though these populations experienced an extreme drought event. Lack of an observed response is atypical in the resurrection ecology literature. Most published studies report some pattern of phenotypic evolution (Franks et al., 2018), although publication bias may come into play and also few studies have taken an approach similar to ours of examining a modest number of individuals from many populations (but see Vigouroux et al., 2011; Van Dijk and Hautekèete, 2014; Kuester et al., 2016). Our relatively small sample sizes for each population may reduce our experimental power and only allow detection of moderate to large shifts in trait means over time within populations. Still, the limited amount of phenotypic evolution observed during the drought was unexpected given that *M. guttatus* has substantial standing genetic and phenotypic variations that should facilitate rapid adaptation to changing conditions (Friedman et al., 2015; Kooyers et al., 2015; Puzey et al., 2017 but see Kooyers et al., 2019).

Some other aspects of our experimental design could bias our findings toward overestimating phenotypic evolution. Modeling predicts that maternal effects via transgenerational plasticity to drought are likely to evolve in a portion of the range (the Sierra Nevada) because seasonal temperature and precipitation parameters are temporally autocorrelated (Colicchio and Herman, 2020). We attempted to control maternal provisioning by including seed size as a covariate in our models, but did not conduct a refresher generation to more fully control for maternal environmental effects. We note that the years when we collected the seed (i.e., the maternal environment) were relatively similar in terms of historical norms. Precipitation conditions were normal in 2011 for the entire range and normal to above normal in 2016 and 2017 in the populations that we collected in each of those years (Appendix S2). Even if conditions were exactly the same between years, grandparental effects could drive phenotypic differences between generations in our experiment as grandparents of the post-drought generation experienced drought-like conditions, while the pre-drought generation experienced more climatically normal conditions (Appendix S2). Not surprisingly, seed areas were different between pre- and post-drought populations in that most populations had smaller seeds post-drought (Appendix S17). However, the populations that had large pre- to post-drought changes in seed area were not the same populations where phenotypic evolution occurred for other traits during the historic drought, as demonstrated by the relatively minute effects of seed area in our models (Appendix S7). These results suggest that the observed patterns of phenotypic evolution are likely not due to maternal effects but instead that adaptive plasticity via maternal provisioning may play a buffering role in some of the populations where we observed little phenotypic evolution.

In addition to these methodological explanations, several potential biological explanations may have influenced our finding that few populations exhibited patterns of phenotypic evolution. First, these results could be an accurate depiction of stochastic population responses to severe but spatially variable selection. We intentionally surveyed populations across a large environmental gradient including central Oregon localities where the drought only lasted a single year (Fig. 1). None of the central Oregon populations exhibited results consistent with phenotypic evolution. However, our spatial sampling design cannot be the only explanation, as many of the populations that experienced extreme drought also revealed no evidence of phenotypic evolution. Second, in some of these areas, the drought may have been so severe that populations either could not establish or that there were uniformly drastic fitness consequences of the drought across phenotypes, i.e., reproduction was equally low and random with respect to the traits we measured. While this may be more likely in some southern California populations, we visited many of the Sierra Nevada populations every year during the drought and found that most of them did establish every year (N. Kooyers, personal observations). The seed bank persistence of *M. guttatus* is unknown, but seeds are viable for at least 5 years at room temperature on a laboratory shelf.

Third, we may be missing phenotypic evolution because we did not measure all relevant phenotypes. Since the resurrection experiment included a massive number of maternal lines and populations (final data set $N = 936$ individuals), we triaged phenotyping to score the few traits like flowering time and plant height at flowering that we anticipated to be most critical for drought escape and avoidance-related strategies based on previous work (Franks and Weis, 2008; Wu et al., 2010; Kooyers et al., 2015). In our manipulative experiment, we measured several additional phenotypes including relative water content, flower size differences, and leaf biomass. However, even with this extra effort, differences in fitness between the pre- and post-drought collections for the two populations included were largely unexplained by the phenotypic differences observed (Appendix S16). Future experimental work on these populations will focus on other phenotypes associated with drought resistance in other systems, including seed dormancy, stomatal density, water-use efficiency, or tolerance to wilting (Yu et al., 2008; Des Marais et al., 2014; Bouzid et al., 2019; Dickman et al., 2019). Finally, we may have observed only limited phenotypic evolution due to phenotypic plasticity. Some populations may express the traits measured with sufficient plasticity to cope with severe natural drought conditions and thus obviate any evolutionary response (Heschel et al., 2004). We consider plasticity to be an unlikely explanation on its own as few traits in the manipulative experiment had significant treatment effects and because the natural drought was severe enough to cause noticeable declines in population size. However, variation in plasticity in different populations should be explored using a design that follows a larger set of populations than our manipulative experiment, as plasticity may be an important buffer mechanism in changing climates. Most likely, a combination of these explanations explain our limited observations of phenotypic evolution following this intense natural drought.

Multiple intrinsic and extrinsic factors influence magnitude of phenotypic evolution

Despite a relatively limited number of populations exhibiting large magnitude phenotypic changes, patterns of variation among populations in the absolute magnitude of evolutionary response to drought may provide further clues about the factors influencing evolutionary responses. Our results indicate that the single most explanatory factor for the magnitude of evolutionary response is the amount of heritable phenotypic variation that a population possesses before the onset of selection. In this study, populations with greater phenotypic variance in flowering time before the drought had larger absolute responses in both trait PC1 and trait PC2 (Fig. 4). This result is predicted by many theoretical models and empirical data examining responses to extreme selection events (i.e., Hairston et al., 2005; Barrett and Schluter, 2008; Grant et al., 2017). However, the amount of heritable phenotypic variation had little to do with the direction of the phenotypic shift as about equal number of populations evolved lower or higher values of trait PC1 and trait PC2.

While we do not observe direct correlations between relative drought intensity or site aridity and the magnitude of responses, multivariate regressions do indicate that interactions among these factors may play a role in determining the magnitude of phenotypic evolution. A direct correlation between relative drought intensity and the absolute magnitude of phenotypic evolution is likely weakened by inclusion of populations that did not establish during the drought. Removing the 2017 populations from the drought does not make this correlation significant, but there is a positive trend between drought intensity and magnitude of evolution. The impact of the population's history and/or preadaptation to drought is less clear. Although one might expect that annual populations that already flower very rapidly to escape terminal droughts would not be able to evolve greater drought escape responses, our data do not support this hypothesis. This hypothesis is likely oversimplified as it presupposes that a population mean is at a static optimum rather than segregating for standing variation that is maintained as the population hovers around a temporally fluctuating optimum. Additional phenotypic selection experiments (e.g., Troth et al., 2018) are needed to quantify the evolutionary limits of drought escape, and future manipulative experiments will be helpful for parsing the relative roles and interactions of each of these different factors in facilitating or constraining phenotypic evolution.

Adaptation and evolutionary rescue during extreme climatic events

The two populations that were selected for the manipulative experiment were chosen independent of the larger resurrection study because they had opposite population dynamics during the natural drought. Even though both populations inhabit sites in the foothills of the Sierra Nevada Mountains and encountered similar intensity drought conditions, BEL is a gigantic population (100s of individuals) that had a very similar appearance pre- and post-drought whereas MEDX was a relatively small population pre-drought (<150 individuals) that was greatly reduced following the drought (<20 reproductive individuals in 2016 and 2017). Both populations were smaller during the drought. Data from the manipulative experiment support the different demographic trajectories of these populations. BEL lines post-drought had higher fitness both in control and dry down conditions than pre-drought lines while pre-drought MEDX lines had higher fitness in both conditions than post-drought lines (Fig. 5). This pattern was qualitatively similar in both inbred and outbred lines and suggests that both population demography and trait variation are important factors for adaptation to extreme events.

While the findings in the manipulative experiment are derived from observations made on only two populations, they reinforce our conclusions from the initial resurrection experiment, and both experiments have important implications in the context of evolutionary rescue—the ability of populations to recover from an environmental pressure via evolution. First, our results support that evolutionary rescue may be likely to occur in populations with more individuals and greater standing genetic variation (Carlson et al., 2014). Greater than 60% of the variation in the magnitude of phenotypic evolution was predictable by knowing the amount of heritable phenotypic variation, contemporary drought intensity, and historical environmental conditions. This suggests that surveys of population-level heritable phenotypic variation can aid in understanding responses to extreme events, at least in an annual monkeyflower. Second, although population structure analyses indicate that historical gene flow between populations at this distance has been substantial (Kooyers et al., 2015), it likely does not operate at a temporal scale that could be beneficial for rescue via gene flow for severe but punctuated events (i.e., genetic rescue). Third, we were unable to associate fitness differences with phenotypic differences in the manipulative experiment (Appendix S16). This result suggests that there could be many more ecologically important phenotypes than we could hope to measure, and there may be multiple trajectories that could lead to evolutionary rescue. This result also indicates that field experiments and manipulative experiments are essential for developing our understanding of the extent to which adaptation can help plant populations respond to extreme events.

While studies with our experiment design are rare, there have been a substantial number of similar resurrection experiments examining how changing climates have altered either phenotypes or genomes over longer time periods (Nevo et al., 2012; Thomann et al., 2015; Gómez et al., 2018). These experiments represent different kinds of selection pressures—either an extreme pulse in the case of a severe selection event or a prolonged shift fluctuating toward a new optimum. This difference in duration and intensity of the selective agent could have a drastic impact on the degree of phenotypic evolution and potential for evolutionary rescue. Notably, a population subject to an intense pulsed selection pressure may be more subject to stochastic demographic events or complete loss of fitness than a population subject to a prolonged selection pressure. Thus, we need to promote a proactive approach to empirically evaluating how populations will respond to both kinds of selection pressures. Doing so is particularly important in the case of understanding population-level responses to changing precipitation regimes, as there may be both long term shifts in mean precipitation as well as increases in the frequency and intensity of drought that plant populations must endure.

ACKNOWLEDGMENTS

We thank Cameron Yuki, Thanassis Tetradis, Nina Guttierez, Alex Ripperton, Marcos Ortigoza, and Aarushi Ratna for research assistance and the UC Berkeley greenhouse staff for help with plant growth. This article was greatly improved through review by the Kooyers and Blackman lab groups and two anonymous reviewers. Support for this project was provided by the University of California, Berkeley, the University of Virginia, the University of South Florida, and the University of Louisiana, Lafayette and National Science Foundation grant (IOS-1558035) to N.J.K. and B.K.B., a National Science Foundation grant to N.J.K. (OIA-1920858), a National Science Foundation Postdoctoral Fellowship in Biology to B.K.B. (DBI-0905958), and an RET fellowship to M.H. from the Teachers Researching Evolution program of the NSF BEACON Center for the Study of Evolution in Action.

AUTHOR CONTRIBUTIONS

N.J.K. and B.K.B. designed the experiment. N.J.K. and B.K.B. performed all fieldwork. J.M.C., K.A.M., S.K.E., C.R.P., M.H., and C.C. managed and collected all data for the resurrection experiment, while K.S.C., A.D., and N.J.K. collected data for the manipulative experiment. J.M.C. and I.C.T. wrote the script for seed area analysis. N.J.K. performed the statistical analyses. N.J.K. and B.K.B wrote the paper together, and all authors edited and approved of the manuscript before submission.

DATA AVAILABILITY

Complete data sets containing all of the phenotypes measured on each individual plant for the resurrection experiment and the manipulative experiments is available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.98sf7m0gv) (Kooyers et al., 2020). All code and a tutorial for assessing seed size from images is available on the Blackman Lab github (https://github.com/BlackmanLabUCB).

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

APPENDIX S1. Locality, historical climate, and phenotypic information from the resurrection experiment for each population.

APPENDIX S2. Intensity of California drought via United States Drought Monitor.

APPENDIX S3. Time to germination for each collection year.

APPENDIX S4. Relationship between seed mass and seed area.

APPENDIX S5. Soil water loss during the dry down manipulation.

APPENDIX S6. Fitness and phenotype summary statistics for each treatment, population, and collection year for the manipulative experiment.

APPENDIX S7. Univariate general linear models from resurrection experiment with seed area covariate.

APPENDIX S8. Correlations between traits in the resurrection experiment.

APPENDIX S9. PCA loadings for both the resurrection experiment and the manipulative experiment.

APPENDIX S10. Intrinsic and extrinsic factors underlying phenotypic evolution in response to the CA drought.

APPENDIX S11. AIC and BIC from multiple regression models examining contributions of initial genetic variation, relative drought intensity, and annual heat moisture index to phenotype evolution.

APPENDIX S12. ANOVA summaries for fitness-related phenotypes in the manipulative experiment.

APPENDIX S13. The effect of population, collection year, and treatment on fitness-related phenotypes in the manipulative experiment.

APPENDIX S14. Comparison between phenotypic means for BEL and MEDX between experiments.

APPENDIX S15. ANOVA table examining effects of population, collection year, and treatment on trait2PC1 and trait2PC2.

APPENDIX S16. Patterns of phenotypic variation in the manipulative experiment.

APPENDIX S17. Means for seed area in pre and post-drought populations.

LITERATURE CITED

Agashe, D., J. J. Falk, and D. I. Bolnick. 2011. Effects of founding genetic variation on adaptation to a novel resource: Genetic diversity facilitates adaptive niche shift. *Evolution* 65: 2481–2491.

Auge, G. A., L. K. Blair, A. Karediya, and K. Donohue. 2018. The autonomous flowering-time pathway pleiotropically regulates seed germination in *Arabidopsis thaliana*. *Annals of Botany* 121: 183–191.

Barrett, R., and D. Schluter. 2008. Adaptation from standing genetic variation. *Trends in Ecology & Evolution* 23: 38–44.

Bates, D., M. Machler, B. M. Bolker, and S. Walker. 2014. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* 67: 1–48.

Belmecheri, S., F. Babst, E. R. Wahl, D. W. Stahle, and V. Trouet. 2016. Multi-century evaluation of Sierra Nevada snowpack. *Nature Climate Change* 6: 2–3.

Blanquart, F., O. Kaltz, S. L. Nuismer, and S. Gandon. 2013. A practical guide to measuring local adaptation. *Ecology Letters* 16: 1195–1205.

Bossdorf, O., A. Lipowsky, and D. Prati. 2008. Selection of preadapted populations allowed *Senecio inaequidens* to invade Central Europe. *Diversity and Distributions* 14: 676–685.

Bouzid, M., F. He, G. Schmitz, R. E. Häusler, A. P. M. Weber, T. Mettler-Altmann, and J. de Meaux. 2019. *Arabidopsis* species deploy distinct strategies to cope with drought stress. *Annals of Botany* 124: 27–40.

Carlson, S. M., C. J. Cunningham, and P. A. H. Westley. 2014. Evolutionary rescue in a changing world. *Trends in Ecology & Evolution* 29: 521–530.

Colicchio, J. M., and J. Herman. 2020. Empirical patterns of environmental variation favor adaptive transgenerational plasticity. *Ecology and Evolution* 10: 1648–1665.

Dai, A. 2013. Increasing drought under global warming in observations and models. *Nature Climate Change* 3: 52–58.

Davison, A. W., and K. Reiling. 1995. A rapid change in ozone resistance of Plantago major after summers with high ozone concentrations. *New Phytologist* 131: 337–344.

Des Marais, D. L., L. C. Auchincloss, E. Sukamtoh, J. K. McKay, T. Logan, J. H. Richards, and T. E. Juenger. 2014. Variation in *MPK12* affects water use efficiency in Arabidopsis and reveals a pleiotropic link between guard cell size and ABA response. *Proceedings of the National Academy of Sciences, USA* 111: 2836–2841.

Dickman, E. E., L. K. Pennington, S. J. Franks, and J. P. Sexton. 2019. Evidence for adaptive responses to historic drought across a native plant species range. *Evolutionary Applications* 12: 1569–1582.

Engelbrecht, B. M. J., L. S. Comita, R. Condit, T. A. Kursar, M. T. Tyree, B. L. Turner, and S. P. Hubbell. 2007. Drought sensitivity shapes species distribution patterns in tropical forests. *Nature* 447: 80–82.

Fox, J., M. Friendly, and S. Weisberg. 2013. Hypothesis tests for multivariate linear models using the car package. *R Journal* 5: 39–52.

Franks, S. J. 2011. Plasticity and evolution in drought avoidance and escape in the annual plant *Brassica rapa*. *New Phytologist* 190: 249–257.

Franks, S. J., E. Hamann, and A. E. Weis. 2018. Using the resurrection approach to understand contemporary evolution in changing environments. *Evolutionary Applications* 11: 17–28.

Franks, S. J., N. C. Kane, N. B. O'Hara, S. Tittes, and J. S. Rest. 2016. Rapid genome-wide evolution in *Brassica rapa* populations following drought revealed by sequencing of ancestral and descendant gene pools. *Molecular Ecology* 25: 3622–3631.

Franks, S. J., S. Sim, and A. E. Weis. 2007. Rapid evolution of flowering time by an annual plant in response to a climate fluctuation. *Proceedings of the National Academy of Sciences, USA* 104: 1278–1282.

Franks, S. J., and A. E. Weis. 2008. A change in climate causes rapid evolution of multiple life-history traits and their interactions in an annual plant. *Journal of Evolutionary Biology* 21: 1321–1334.

Friedman, J., A. D. Twyford, J. H. Willis, and B. K. Blackman. 2015. The extent and genetic basis of phenotypic divergence in life history traits in *Mimulus guttatus*. *Molecular Ecology* 24: 111–122.

Gallant, A. J. E., and J. Gergis. 2011. An experimental streamflow reconstruction for the River Murray, Australia, 1783–1988. *Water Resources Research* 47: W00G04.

Gómez, R., B. Méndez-Vigo, A. Marcer, C. Alonso-Blanco, and F. X. Picó. 2018. Quantifying temporal change in plant population attributes: insights from a resurrection approach. *AoB PLANTS* 10: ply063.

Grant, P. R., B. R. Grant, R. B. Huey, M. T. J. Johnson, A. H. Knoll, and J. Schmitt. 2017. Evolution caused by extreme events. *Philosophical Transactions of the Royal Society, B, Biological Sciences* 372: 20160146.

Griffin, D., and K. J. Anchukaitis. 2014. How unusual is the 2012–2014 California drought? *Geophysical Research Letters* 41: 9017–9023.

Hairston, N. G., S. P. Ellner, M. A. Geber, T. Yoshida, and J. A. Fox. 2005. Rapid evolution and the convergence of ecological and evolutionary time. *Ecology Letters* 8: 1114–1127.

Hall, M. C., and J. H. Willis. 2006. Divergent selection on flowering time contributes to local adaptation in *Mimulus guttatus*. *Evolution* 60: 2466–2477.

Harrell, F. E. 2015. Hmisc: Harrell miscellaneous. R package version 3.17-1. Website: https://cran.r-project.org/web/packages/Hmisc/index.html.

Heschel, M. S., S. E. Sultan, S. Glover, and D. Sloan. 2004. Population differentiation and plastic responses to drought stress in the generalist annual *Polygonum persicaria*. *International Journal of Plant Sciences* 165: 817–824.

IPCC. 2014. Climate Change 2014: Synthesis Report. In Core Writing Team, R. K. & Meyer, L. A. [eds.], Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, 151pp. IPCC, Geneva, Switzerland.

Juenger, T. E. 2013. Natural variation and genetic constraints on drought tolerance. *Current Opinion in Plant Biology* 16: 274–281.

Kawecki, T. J., and D. Ebert. 2004. Conceptual issues in local adaptation. *Ecology Letters* 7: 1225–1241.

Knapp, A. K., C. Beier, D. D. Briske, A. T. Classen, Y. Luo, M. Reichstein, M. D. Smith, et al. 2008. Consequences of more extreme precipitation regimes for terrestrial ecosystems. *BioScience* 58: 811–821.

Knight, C. A., H. Vogel, J. Kroymann, A. Shumate, H. Witsenboer, and T. Mitchell-Olds. 2006. Expression profiling and local adaptation of *Boechera holboellii* populations for water use efficiency across a naturally occurring water stress gradient. *Molecular Ecology* 15: 1229–1237.

Kooyers, N. J. 2015. The evolution of drought escape and avoidance in natural herbaceous populations. *Plant Science* 234: 155–162.

Kooyers, N. J., J. M. Colicchio, A. B. Greenlee, E. Patterson, N. T. Handloser, and B. K. Blackman. 2019. Lagging adaptation to climate supersedes local adaptation to herbivory in an annual monkeyflower. *American Naturalist* 194: 541–557.

Kooyers, N. J., A. B. Greenlee, J. M. Colicchio, M. Oh, and B. K. Blackman. 2015. Replicate altitudinal clines reveal that evolutionary flexibility underlies adaptation to drought stress in annual *Mimulus guttatus*. *New Phytologist* 206: 152–165.

Kooyers, N. J., K. A. Morioka, J. M. Colicchio, K. S. Clark, A. Donofrio, S. K. Estill, C. R. Pascualy, et al. 2020. Data from: Population responses to a historic drought across the range of the common monkeyflower (*Mimulus guttatus*). *Dryad Digital Repository* https://doi.org/10.5061/dryad.98sf7m0gv.

Kuester, A., A. Wilson, S.-M. Chang, and R. S. Baucom. 2016. A resurrection experiment finds evidence of both reduced genetic diversity and potential adaptive evolution in the agricultural weed *Ipomoea purpurea*. *Molecular Ecology* 25: 4508–4520.

Kuznetsova, A., P. B. Brockhoff, and R. H. B. Christensen. 2017. lmerTest package: tests in linear mixed effects models. *Journal of Statistical Software* 82: 1–26.

Lambrecht, S. C., A. K. Gujral, L. J. Renshaw, and L. T. Rosengreen. 2020. Evolutionary and plastic changes in a native annual plant after a historic drought. *Ecology and Evolution* 10: 4570–4582.

Ludlow, M. M. 1989. Strategies of response to water stress. Structural and functional responses to environmental stresses, 269–281. SPB Academic Publishing, The Hague, Netherlands.

McKay, J. K., J. H. Richards, and T. Mitchell-Olds. 2003. Genetics of drought adaptation in *Arabidopsis thaliana* I. Pleiotropy contributes to genetic correlations among ecological traits. *Molecular Ecology* 12: 1137–1151.

Mojica, J. P., Y. W. Lee, J. H. Willis, and J. K. Kelly. 2012. Spatially and temporally varying selection on intrapopulation quantitative trait loci for a life history trade-off in *Mimulus guttatus*. *Molecular Ecology* 21: 3718–3728.

Mojica, J. P., J. Mullen, J. T. Lovell, J. G. Monroe, J. R. Paul, C. G. Oakley, and J. K. McKay. 2016. Genetics of water use physiology in locally adapted *Arabidopsis thaliana*. *Plant Science* 251: 12–22.

Nelson, T. C., P. J. Monnahan, M. K. McIntosh, K. Anderson, E. MacArthur-Waltz, F. R. Finseth, J. K. Kelly, and L. Fishman. 2018. Extreme copy number variation at a tRNA ligase gene affecting phenology and fitness in yellow monkeyflowers. *Molecular Ecology* 28: 1460–1475.

Nevo, E., Y.-B. Fu, T. Pavlicek, S. Khalifa, M. Tavasi, and A. Beiles. 2012. Evolution of wild cereals during 28 years of global warming in Israel. *Proceedings of the National Academy of Sciences, USA* 109: 3412–3415.

O'Hara, N. B., J. S. Rest, and S. J. Franks. 2016. Increased susceptibility to fungal disease accompanies adaptation to drought in *Brassica rapa*. *Evolution* 70: 241–248.

Puzey, J. R., J. H. Willis, and J. K. Kelly. 2017. Population structure and local selection yield high genomic variation in *Mimulus guttatus*. *Molecular Ecology* 26: 519–535.

Robeson, S. M. 2015. Revisiting the recent California drought as an extreme value. *Geophysical Research Letters* 42: 6771–6779.

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

Siepielski, A. M., M. B. Morrissey, M. Buoro, S. M. Carlson, C. M. Caruso, S. M. Clegg, T. Coulson, et al. 2017. Precipitation drives global variation in natural selection. *Science* 355: 959–962.

Stacklies, W., H. Redestig, M. Scholz, D. Walther, and J. Selbig. 2007. pcaMethods a bioconductor package providing PCA methods for incomplete data. *Bioinformatics* 23: 1164–1167.

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

Thomann, M., E. Imbert, R. C. Engstrand, and P.-O. Cheptou. 2015. Contemporary evolution of plant reproductive strategies under global change is revealed by stored seeds. *Journal of Evolutionary Biology* 28: 766–778.

Troth, A., J. R. Puzey, R. S. Kim, J. H. Willis, and J. K. Kelly. 2018. Selective trade-offs maintain alleles underpinning complex trait variation in plants. *Science* 361: 475–478.

Twyford, A. D., and J. Friedman. 2015. Adaptive divergence in the monkey flower *Mimulus guttatus* is maintained by a chromosomal inversion. *Evolution* 69: 1476–1486.

Van Dijk, H., and N.-C. Hautekèete. 2014. Evidence of genetic change in the flowering phenology of sea beets along a latitudinal cline within two decades. *Journal of Evolutionary Biology* 27: 1572–1581.

Vigouroux, Y., C. Mariac, S. De Mita, J.-L. Pham, B. Gérard, I. Kapran, F. Sagnard, et al. 2011. Selection for earlier flowering crop associated with climatic variations in the Sahel. *PLoS One* 6: e19563.

Volaire, F. 2018. A unified framework of plant adaptive strategies to drought: crossing scales and disciplines. *Global Change Biology* 24: 2929–2938.

Wang, T., A. Hamann, D. Spittlehouse, and C. Carroll. 2016. Locally downscaled and spatially customizable climate data for historical and future periods for North America. *PLoS One* 11: e0156720.

Weis, A. E. 2018. Detecting the "invisible fraction" bias in resurrection experiments. *Evolutionary Applications* 11: 88–95.

Wu, C. A., D. B. Lowry, A. M. Cooley, K. M. Wright, Y. W. Lee, and J. H. Willis. 2008. *Mimulus* is an emerging model system for the integration of ecological and genomic studies. *Heredity* 100: 220–230.

Wu, C. A., D. B. Lowry, L. I. Nutter, and J. H. Willis. 2010. Natural variation for drought-response traits in the *Mimulus guttatus* species complex. *Oecologia* 162: 23–33.

Yu, H., X. Chen, Y.-Y. Hong, Y. Wang, P. Xu, S.-D. Ke, H.-Y. Liu, et al. 2008. Activated expression of an *Arabidopsis* HD-START protein confers drought tolerance with improved root system and reduced stomatal density. *Plant Cell* 20: 1134–1151.

Yuan, Y. 2019. Monkeyflowers (*Mimulus*): new model for plant developmental genetics and evo-devo. *New Phytologist* 222: 694–700.