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RESEARCH ARTICLE

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# Unique drought resistance strategies occur among monkeyflower populations spanning an aridity gradient

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## Abstract

**Premise:** Annual plants often exhibit drought-escape and avoidance strategies to cope with limited water availability. Determining the extent of variation and factors underlying the evolution of divergent strategies is necessary for determining population responses to more frequent and severe droughts.

**Methods:** We leveraged five *Mimulus guttatus* populations collected across an aridity gradient within manipulative drought and quantitative genetics experiments to examine constitutive and terminal-drought induced responses in drought resistance traits.

**Results:** Populations varied considerably in drought-escape- and drought-avoidance-associated traits. The most mesic population demonstrated a unique resource conservative strategy. Xeric populations exhibited extreme plasticity when exposed to terminal drought that included flowering earlier at shorter heights, increasing water-use efficiency, and shifting C:N ratios. However, plasticity responses also differed between populations, with two populations slowing growth rates and flowering at earlier nodes and another population increasing growth rate. While nearly all traits were heritable, phenotypic correlations differed substantially between treatments and often, populations.

**Conclusions:** Our results suggest drought resistance strategies of populations may be finely adapted to local patterns of water availability. Substantial plastic responses suggest that xeric populations can already acclimate to drought through plasticity, but populations not frequently exposed to drought may be more vulnerable.

KEYWORDS

common garden, common yellow monkeyflower, *Erythranthe guttata*, flowering time, functional trait syndromes, genetic correlations, heritability, *Mimulus guttatus*, plant ecophysiology, plasticity, quantitative genetics, water-use efficiency

Climate change is predicted to increase variation in precipitation around the world, regionally causing more frequent and extreme droughts (Dai, 2013; Pachauri et al., 2014; Diffenbaugh et al., 2015, 2017). Drought and low water availability are central selection pressures with most plant species experiencing low water availability at some point in their life (Siepielski et al., 2017). However, in a changing environment where species may experience conditions beyond their historic extremes, plant populations may have to move, acclimate, or adapt to persist or thrive (Aitken et al., 2008; Panetta et al., 2018). A central endeavor for ecologists and evolutionary biologists is determining how populations and/or species will respond to such extreme conditions and requires examining the potential for acclimation and adaptation by exploring the diversity of mechanisms that species use to counter low water availability as well as how such variation is geographically distributed.

Drought resistance in plants has been classified into three non-mutually exclusive strategies—drought escape, drought avoidance, and drought tolerance (Ludlow, 1989; Kooyers, 2015; Volaire, 2018). Annual plant species primarily exhibit drought escape or avoidance strategies. Species exhibiting drought escape grow rapidly and reproduce quickly to complete their life cycles prior to drought conditions. Species with a drought avoidance strategy resist dehydration during drought by increasing water-use efficiency through more efficient gas exchange (Des Marais et al., 2014), using stored nutrients (Rodrigues et al., 1995), and investing in traits, like trichomes, that limit evapotranspiration (Ehleringer and Mooney, 1978). These strategies are assumed to reflect the more general leaf economics spectrum, with drought escape having a resource-acquisitive growth strategy and drought avoidance employing a resource-conservative growth strategy (Reich, 2014). Importantly, either drought escape or avoidance can occur constitutively when a plant does not experience water stress or can occur as a plastic response to limited water availability (Des Marais et al., 2013). While these strategies are not mutually exclusive, they may also be governed by physiological trade-offs or genetic trade-offs between traits. For instance, drought-escaping plants must open stomata to perform the gas exchange necessary for photosynthesis and growth, which theoretically also limits the ability for plants to prevent transpiration through leaves (Geber and Dawson, 1990; McKay et al., 2003). However, these trade-offs do not seem to be universal and may depend on the environment where a plant grows and/or the genetic architecture of traits (Kooyers et al., 2015).

While variation in drought resistance strategies and associated phenotypes has been widely documented both intra- and interspecifically, there is limited understanding of how herbaceous plants have adapted to variation in water availability and propensity for drought across natural populations (Juenger, 2013; Kooyers, 2015). A basic prediction is that populations that have historically experienced more water stress because they occur in more arid environment or have experienced more frequent or severe droughts should have greater drought resistance (Kooyers et al., 2015, 2021; Mojica et al., 2016; Monroe et al., 2019). However, this prediction elicits more questions about the mechanisms of drought resistance: Does greater drought resistance come through more extreme versions of a particular drought resistance strategy, or are there specific strategies that are favored under different drought regimes? Where and when does plasticity evolve? And, can the physiological or genetic trade-offs that occur between strategies be "broken" to produce greater drought resistance? These questions need to be addressed to determine evolutionary responses to drought. One appropriate way of exploring these questions is examining heritable differences and plasticity in traits associated with drought resistance within species that exist across substantial gradients in water availability (Brouillette et al., 2014; Des Marais et al., 2017; Solé-Medina et al., 2022).

The common yellow monkeyflower (*Mimulus guttatus* syn. *Erythranthe guttata*; Phrymaceae) is a model species for ecological genetics that exists from the Sonoran Desert in northern Mexico to the temperate rain forests of the Pacific Northwest and Alaska (Wu et al., 2008). Annual populations of *M. guttatus* occur inland in areas with ephemeral water sources such as thin-soiled meadows, rock walls, and drainages. Water is the primary determinant of growing season phenology, as growing seasons begin with winter/spring rain or snowmelt and end when sites dry out during summer droughts. Annual populations exhibit a drought escape strategy characterized by rapid growth and flowering prior to terminal drought (Hall and Willis, 2006; Wu et al., 2010; Kooyers et al., 2015; Lowry et al., 2019). Populations with shorter growing seasons tend to have quicker times to flowering from germination (Kooyers et al., 2015), and there is clear temporal heterogeneity in selection on flowering time corresponding to length of the growing season (Troth et al., 2018).

However, population-level studies have also identified variation in a number of other traits associated with drought avoidance that covary with gradients in water availability or growing season dynamics including specific leaf area (SLA), succulence, trichome density, and water-use efficiency (Kooyers et al., 2015). Interestingly, the trade-offs between drought escape and avoidance traits only appear in certain populations, although this conclusion is derived from experiments that do not explicitly examine trade-offs under water-limited conditions and have limited sampling (Kooyers, 2015; Kooyers et al., 2015). These patterns of variation in drought resistance phenotypes were predictive of phenotypic evolution during a historic California drought. Specifically, populations with more standing genetic variation in flowering time had greater phenotypic evolution following the drought (Kooyers et al., 2021). Yet, relatively few populations evolved during this historic drought, and the direction in which plants evolved (i.e., toward greater drought escape or avoidance) was not associated with the constitutive phenotypic variation present in the populations before the drought. These conclusions suggest there are potentially important drought resistance phenotypes that have not been surveyed, that there is variation in heritability or genetic correlations that may impact phenotypic evolution in natural populations, or that plasticity could play a more prominent role than expected.

Here we examined patterns of plasticity and genetic variation found in five *M. guttatus* populations distributed across a spatial gradient in aridity and annual precipitation. We used a manipulative experiment and a midparent–offspring quantitative genetic experimental design to address four questions. First, is there plasticity in drought escape and avoidance traits and does plasticity differ in magnitude among populations? Second, is there constitutive variation among populations in drought escape or avoidance traits? We predicted that populations that experience greater drought stress will exhibit more extreme drought resistance phenotypes. Third, are drought escape and avoidance traits heritable and does heritability differ among populations? Fourth, is there a genetic correlation between drought escape and avoidance traits and, if so, does this correlation differ among populations? Our results suggest that there is a more diverse set of phenotypes associated with drought resistance than previously assumed with different populations varying in strategies and predicted responses to early terminal drought.

# MATERIALS AND METHODS

We conducted a manipulative experiment and a quantitative genetics experiment leveraging field-collected seed from 30–50 maternal lines per population from five *M. guttatus* populations distributed across a precipitation gradient extending from southern California to central Oregon (Figure 1; Appendix S1). These populations were chosen to reflect the diversity of growing season starting times and durations as well as differences in aridity during the growing season (Figure 1B, C). Lines were chosen from seed collections made in 2013, 2016, and 2017, and historical climate data was extracted from ClimateWNA (1960–1990; Wang et al., 2016).

## Experimental design

Our experimental design consisted of three generations: a refresher generation followed by an experiment manipulating water availability to examine variation in drought resistance traits between populations, followed by an $F_1$ generation to examine patterns of heritability and genetic correlations between traits. In the refresher generation, seedlings from each maternal line (30 lines/population) were grown under well-watered conditions in a common garden to limit maternal effects in downstream generations. Seeds were planted in 2.5-inch pots filled with water-saturated Farfard 3B potting mix (Sun Gro Horticulture, Agawam, MA, USA). Pots were randomized in flats (32 pots/flat) with humidity domes before being cold-stratified at 4°C for 7 days. Flats were placed on grow shelving under a Hydro Crunch T5 growth light system with a program of 16-h day:8-h nights at room temperature (23–25°C). Humidity domes were removed after 7 days, and extra germinants were transplanted to new pots. Flats were rotated every 2–3 days to minimize microenvironment effects. Each plant was self-fertilized by hand pollination, and the resulting seeds were used in the manipulative experiment below.

Our manipulative experiment consisted of a well-watered and a dry-down treatment that mimics an early terminal drought. We planted two replicate pots from 30 maternal lines from each of the five populations using the same germination conditions as in the refresher generation (150 lines/treatment; 300 total pots). Some of these lines did

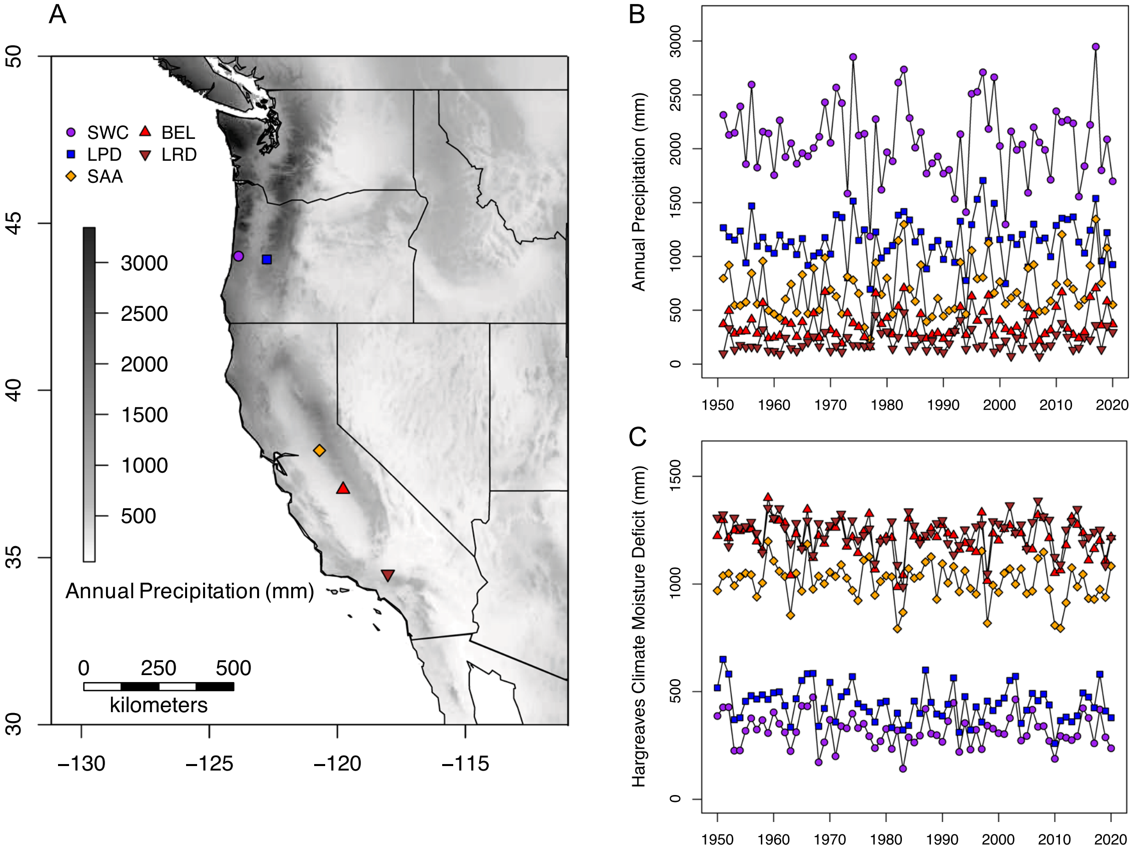

**FIGURE 1** Locations and metadata associated with the five sampled *Mimulus guttatus* populations across the western United States (A). Shapes and colors represent the locations of each population. The black to white background raster corresponds to annual precipitation in millimeters at each site (1960–1990 historical averages). Yearly annual precipitation data (B) and Hargreaves climate moisture deficit (C) for each population. Hargreaves climate moisture deficit is an aridity-related metric that examines the difference between potential evapotranspiration and dependable precipitation with higher values indicating greater moisture deficit.

not germinate and were replaced by extra germinants from other lines to maintain the same number of pots in each flat (ave. 22 lines per population, range 18–27). Following stratification, flats were placed in AR-66L2 growth chambers (Percival Scientific; Perry, IA, USA) with light intensity of 360 μmol $m^{-2}$ $s^{-1}$ set at 22°C with a 16-h day length. Flats were randomly thinned to a single plant per pot, and pots were randomized within flats corresponding to either the well-watered or the dry-down treatment. Flats were rotated within the growth chambers every 2 days. The dry-down treatment began 18 days after planting. Well-watered flats continued to be bottom-watered every 2 days with soil consistently saturated. This treatment reflects the moist conditions of *M. guttatus* during the middle of a growing season. Flats in the dry-down treatment had any remaining water removed and 2 L of water added via bottom watering. These flats were not watered for the rest of the experiment. We assessed volumetric water content daily for three random positions in each treatment using a Delta-T SM150T Soil Moisture Meter (Dynamax, Houston, TX, USA). Care was taken to avoid touching the plant and walls of the pots with the probe. Voltage was converted to volumetric water content using standards for a peat-based potting mix.

## Trait measurements

Germination and flowering were assessed daily throughout the experiment. Flowering time was calculated as the days from germination to opening of the first flower. At time of first flower, we measured plant height, branch number, leaf number, flowering node, and flower morphology. Plant height was the distance from the apical meristem to the lowest part of the stem touching the soil. Flowering node referred to the node on the main stem where the first flower appeared. If the first flower was on a branch, the node on the branch was added to the node on the main stem where the branch originated. Corolla length, width, and height were measured following Fishman et al. (2002) and Kooyers et al. (2015). We destructively sampled a single second true leaf on each plant between day 30–32 of the experiment for leaf-level morphological and physiological measures. Cut leaves were placed petiole down in deionized water. After 24 h, the wet leaf mass of the leaf was measured (termed wet leaf mass) and a picture of the leaf next to a 1 × 1 cm red square was taken to assess leaf area using ImageJ (Easlon and Bloom, 2014). Leaves were dried at 65°C for 5 days and then reweighed (dry leaf mass). To calculate specific leaf area (SLA), we divided the leaf area by dry leaf mass. Relative water content (RWC) was calculated by subtracting dry leaf mass from wet leaf mass and then dividing by wet leaf mass. Finally, dried leaf tissue was ground at 1200 rpm for 30 s using a Fast Prep 96 homogenizer (MP Biomedicals, Santa Ana, CA, USA), packaged into tin containers and shipped to Stable Core Isotope Laboratory at Washington State University for $\delta^{13}C$ and $\delta^{15}N$ analysis. The data for $\delta^{13}C$ are expressed relative to the standard Vienna Pee Dee belemnite ($R_{PDB}$), where $\delta^{13}C$ (‰) = ¼ ($R_S/R_{PDB}$ − 1) × 1000. Since many of our traits were correlated, we conducted a principal component analysis including all traits with imputation of missing values using the pca() function in the pcaMethods v package (Stacklies et al., 2007). All traits were *z*-score-transformed before the PCA.

To examine heritability and genetic correlations of the above traits, we used a midparent–offspring experimental design. We crossed maternal lines within each population from the well-watered treatment in the manipulative experiment above. The number of $F_1$ crosses varied between populations (ave. 26, range 21–31), and we made an effort to include as many lines as possible. We planted two individuals per $F_1$ cross. The $F_1$ generation was grown as described above for the well-watered treatment, and we measured the same traits.

## Statistical analyses

To examine patterns of trait variation and plasticity within and among populations, we evaluated the manipulative experiment using linear models and two-factor ANOVA. For each trait, we created a linear model using the lm() function with the trait as the response variable and population, treatment, and population × treatment interaction as factors. Significance of each factor was determined via ANOVA using a type III sum of squares using the Anova() function in the car package (Fox et al., 2013). Significance of the population term indicates that there is variation among populations for a particular trait in both the dry-down and well-watered treatment. Significance of the treatment term indicates that there is a trait difference between the well-watered and dry-down treatments across populations (i.e., the trait is plastic and responds to water stress). Significance of the interaction term indicates that there were differential responses to the dry-down treatment for different populations. We did not adjust *P*-values for multiple comparisons post hoc because nearly all of the traits we measured were moderately to highly correlated. Further, significant *P*-values were not used as absolute indications of a trait's importance for drought resistance, but rather as an indication to further dissect relationships among populations and treatments.

While differentiation among populations for a particular trait indicates that a trait is broadly heritable, estimating narrow-sense heritability ($h^2$) requires a quantitative genetics experimental design. We examined patterns of heritability for each trait using midparent–offspring regressions. Midparent values were calculated as the average value of the two parents from the well-watered experiment. We first pooled data from all populations and then subsequently examined heritability in each population to explore patterns of variation. Narrow-sense heritability of a trait is equal to the slope of the regression line that describes the midparent and offspring association (Falconer and Mackay, 1996). We conducted linear regressions using the lm() function and extracted slopes and standard errors from the resulting summaries. We described a trait as heritable only if the slope of the regression line was positive and significantly different from zero. We note that our experimental design has the caveat that the parent generation was inbred, while the offspring generation was largely outbred. This difference may lower the covariance in phenotypes between generations, potentially causing lower narrow-sense heritability.

We explored relationships among drought-escape- and drought-avoidance-associated traits through assessing phenotypic correlations within the manipulative experiment followed by determining genetic correlations among traits within the framework of the midparent–offspring experimental design. We considered flowering time and $\delta^{13}$C to be the traits most closely aligned to drought escape and avoidance, respectively, but we also examined correlations across traits to help determine which other traits may facilitate or constrain drought escape or avoidance. To determine phenotypic correlations, we conducted pairwise linear mixed models between traits measured in the well-watered treatment in the manipulative experiment. In each model, one trait was the response variable, the second was the independent variable, and population was included as a random factor. We assessed the level of variation among populations by examining the variation associated with population relative to the total variation. Linear mixed models were conducted using lmer() in the lme4 package (Bates et al., 2014). For traits with high levels of among population variation, we examined Pearson correlations within each population using the rcorr() function within the Hmisc package (Harrell, 2015). Finally, we assessed genetic correlations between traits using the midparent–offspring experimental design. The genetic correlation coefficient between traits was assessed via the following equation (Falconer and Mackay, 1996):

$$r_A = \frac{\mathrm{cov_{xy}}}{\sqrt{(\mathrm{cov_{xx}cov_{yy}})}}.$$

In this equation, the $\mathrm{cov_{xy}}$ refers to the average covariance between two traits, x and y, calculated in both the parent (i.e., the experimental manipulation) and offspring ($F_1$) generation. The terms $\mathrm{cov_{xx}}$ and $\mathrm{cov_{yy}}$ refer to the covariance between a particular trait in the parent and offspring generations. Midparent values were used for traits values in the parent generation.

## RESULTS

The dry-down treatment had a dramatic effect on both soil volumetric water content and plants compared to the well-watered treatment (Appendix S2). There were clear treatment effects that in some cases differed among populations (Table 1). Fewer plants flowered in every population in the dry-down treatment compared to the well-watered treatment, although the difference in survival varied among populations with SAA and LRD having the lowest and highest survival, respectively (Figure 2A). In the dry-down treatment, plants were shorter at flowering (Treatment: $F_{1,158} = 4.23$, $P = 0.04$), had higher water-use efficiency (Treatment: $F_{1,194} = 6.8$, $P = 0.01$), and had higher C:N ratios (Treatment: $F_{1,190} = 12.8$, $P < 0.001$) than in the well-watered treatment. Finally, although not statistically significant, plants from all populations also flowered earlier on average in the dry-down treatment (Figure 2B, Table 1), this plasticity was particularly pronounced in the populations from the driest sites, LRD and BEL. The lack of statistical significance for flowering time was likely due to high variation in flowering time in the well-watered treatment.

Patterns of plasticity also differed between populations. The extent of plasticity in plant height at flowering also varied among populations (Population × Treatment: $F_{4,158} = 2.57$, $P = 0.04$) and was likely driven by differences in growth rate and flowering time. The dry-down treatment decreased average growth rates in the two Sierra Nevada populations, BEL and SAA, while increasing growth rates in the most arid population, LRD, and in LPD. The most mesic population, SWC, had nearly no difference in growth rate between treatments. Multiple measures of flower morphology (corolla length and height) had statistically significant contrasting patterns of plasticity between treatments driven by a single population, SAA (Appendix S3). This population had smaller flowers in dry-down treatment than in the well-watered treatment. Together, these results demonstrate that plasticity plays a clear role in drought resistance in nearly every population, but the specific traits and magnitude of plasticity differs among populations.

### Patterns of variation in constitutive drought escape and avoidance traits

There were also clear constitutive differences in drought resistance traits among populations (Table 1). Rather than continuous differences across an aridity gradient, these differences were driven by a single population from the most mesic site, SWC, which has a more resource conservative strategy than the other populations. SWC has the slowest growth rate of any population, produces smaller, denser leaves with lower relative water content and lower C:N ratio, and also produces the smallest flowers on average (Figure 2; Appendices S3, S4). Consequently, SWC plants flower at an earlier node and are shorter at flowering than the other populations. In all other populations, variation between treatments exceeded variation among populations.

### Synthesizing variation in plastic and constitutive trait responses

Because many of the traits observed are at least moderately correlated (Appendix S5), we next conducted PCA to assess

**TABLE 1** ANOVA summary table from experimental manipulation experiment.

| | Population | | | Treatment | | | Population × Treatment | | |
|---|---|---|---|---|---|---|---|---|---|
| **Trait** | ***F*** | **df** | ***P*** | ***F*** | **df** | ***P*** | ***F*** | **df** | ***P*** |
| Flowering time | 0.4 | 4158 | 0.782 | 1.3 | 1158 | 0.254 | 1.5 | 4158 | 0.217 |
| Plant height | 1.8 | 4158 | 0.123 | **4.2** | **1158** | **0.041** | **2.6** | **4158** | **0.040** |
| Growth rate | **2.5** | **4158** | **0.043** | 2.2 | 1158 | 0.136 | 1.4 | 4158 | 0.248 |
| Flowering node | **3.6** | **4158** | **0.008** | 0.1 | 1158 | 0.737 | 1.0 | 4158 | 0.422 |
| Number of branches | 0.9 | 4158 | 0.451 | 0.0 | 1158 | 0.844 | 0.6 | 4158 | 0.695 |
| Number of leaves | 1.5 | 4158 | 0.196 | 1.7 | 1158 | 0.195 | 1.1 | 4158 | 0.376 |
| Corolla length | 1.5 | 4158 | 0.194 | 0.7 | 1158 | 0.398 | **2.4** | **4158** | **0.049** |
| Corolla width | 2.3 | 4158 | 0.063 | 0.0 | 1158 | 0.890 | 1.9 | 4158 | 0.109 |
| Corolla height | 1.9 | 4158 | 0.120 | 1.6 | 1158 | 0.210 | **3.6** | **4158** | **0.008** |
| Dried leaf mass | **5.9** | **4194** | **<0.001** | 3.5 | 1194 | 0.062 | 1.2 | 4194 | 0.331 |
| Leaf area | **7.8** | **4194** | **<0.001** | 1.6 | 1194 | 0.209 | 1.2 | 4194 | 0.302 |
| RWC | **3.3** | **4194** | **0.012** | 0.0 | 1194 | 0.839 | 0.3 | 4194 | 0.883 |
| SLA | **5.8** | **4158** | **<0.001** | 1.0 | 1158 | 0.325 | 1.4 | 4158 | 0.248 |
| C:N ratio | **3.2** | **4190** | **0.014** | **12.8** | **1190** | **<0.001** | 2.3 | 4190 | 0.062 |
| δ13C | 0.0 | 4194 | 1.000 | **6.8** | **1194** | **0.010** | 0.7 | 4194 | 0.617 |
| δ15N | **4.6** | **4190** | **0.001** | 1.6 | 1190 | 0.210 | 2.0 | 4190 | 0.104 |
| PC1 | **3.8** | **4158** | **0.005** | 0.4 | 1158 | 0.525 | **3.0** | **4158** | **0.022** |
| PC2 | 0.4 | 4158 | 0.799 | **10.7** | **1158** | **0.001** | 0.4 | 4158 | 0.840 |

*Notes*: df: numerator, denominator degrees of freedom. Bold values indicate significant significance at $\alpha = 0.05$. Number of leaves and leaf area were log-transformed before the analysis to better meet model assumptions. RWC = relative water content; SLA = specific leaf area.

the extent to which drought resistance traits may evolve as a syndrome vs. independently. The PC1 axis (31.8% of variation, Appendix S6) reflects a trade-off between flowering time and plant size, where higher values indicate later flowering but taller plants with larger leaves, more leaves, more branches, and larger flowers (i.e., a life history axis). The PC2 axis (13.9% of the variation; Appendix S6) reflects a spectrum of drought resistance. Higher values of PC2 reflect plants that flower earlier with higher water-use efficiency, high C:N ratios, lower SLA, and lower relative water content than plants with lower values. PC1 varied between populations (Population: $F_{4,158} = 3.8$, $P = 0.005$), driven by the difference between SWC and other populations (Figure 3A, B). There was also a population by treatment effect on PC1 (Population × Treatment: $F_{4,158} = 3.0$, $P = 0.02$; Figure 3A, B) reflecting that LRD and SAA had higher values of PC1 in the wet treatment, while the opposite was true for the other populations (Figure 3C). There was a significant treatment effect for PC2 (Treatment: $F_{1,158} = 10.7$, $P = 0.001$; Figure 3A, B), where all populations had higher average PC2 values in the dry-down treatment. However, the most mesic site, SWC, has little difference in PC2 among treatments. This axis reflects the plasticity in drought resistance noted in the first paragraph of the results. Together, these results indicate that variation in life history traits is not highly correlated with variation in drought resistance traits and that phenotypic syndromes related to these organismal functions are potentially capable of evolving independently of one another.

## Heritability of drought escape and avoidance traits

Although variation within and between populations in drought resistance phenotypes reflects the outcomes of past selection, determining how phenotypes may respond to natural selection requires understanding patterns of heritability and genetic correlations between traits. Midparent–offspring regressions suggest that 15 of 17 traits measured had some degree of heritability (only $\delta^{15}$N and RWC did not; Figure 4; Appendices S7, S8). Heritability estimates range from 0.156 ± 0.08 (number of branches) to 0.845 ± 0.06 (plant height at flowering) with morphological traits (plant height, corolla measurements, leaf area and mass, etc.) generally being more heritable than physiological traits (SLA, $\delta^{13}$C, C:N ratio, etc.). We note that we measured heritability only in a well-watered environment and that heritability may decrease in the dry-down treatment, as we observed less phenotypic variation in that treatment

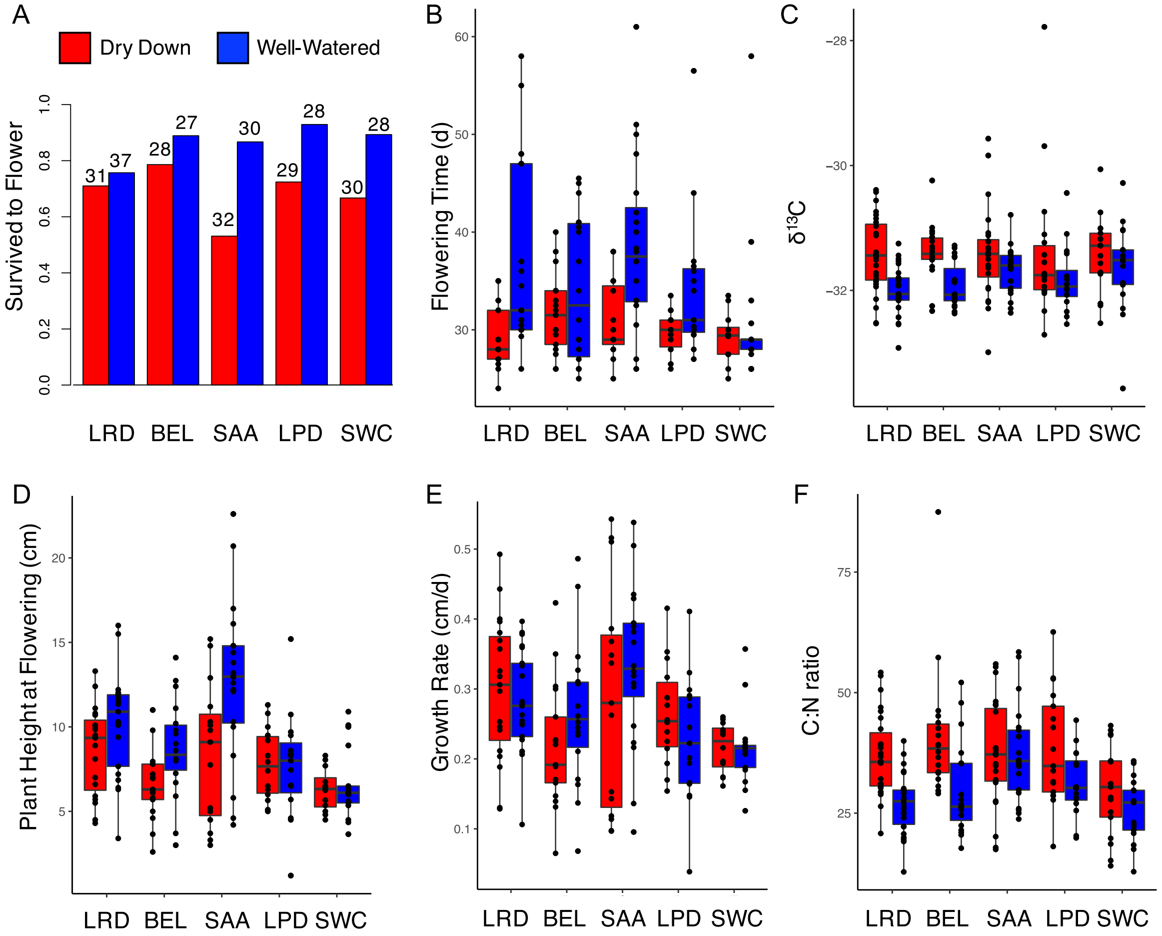

**FIGURE 2** Variation across population in treatments in key phenotypes including survival to flowering (A), flowering time (B), $\delta^{13}C$ (C), plant height at flowering (D), growth rate (E), and C:N ratio (F). Numbers above bars represent total number of plants in each category. Each point represents a line mean. Populations are presented in order of most xeric to most mesic (left to right). Box edges in boxplots represent the interquartile range, center line represents the median, and upper and lower whiskers are the largest value either greater or less than, respectively, 1.5 times the interquartile range. Each point represents a line mean. Details on statistical significance can be found in Table 1.

(Figures 2 and 3; Appendix S1). Although subsampling by population markedly decreases our sample size and thus our power to detect statistical differences from zero, heritabilities were generally consistent by trait between populations (Figure 4; Appendix S7) with a few noteworthy exceptions. Most notably, heritability in leaf traits (wet and dry leaf mass, SLA, and RWC) were variable among populations with higher heritability in the driest population (LRD) and often the most mesic populations (LPD, SWC). Additionally, $\delta^{13}C$ was only heritable when examining all populations together, suggesting that most variation is contained among populations rather than within populations, at least in the well-watered treatment.

## Phenotypic and genetic correlations

Phenotypic and genetic correlations ranged from weak to strong and were not necessarily consistent between well-watered and dry-down treatments. In both treatments, there were moderately strong positive phenotypic correlations between morphological measures (plant height, number of branches, leaf size, leaf number, corolla measures, etc.; Appendices S9, 10) where taller plants had larger leaves, larger flowers, and more branches. The only physiological measure phenotypically correlated with any of these morphological phenotypes was C:N ratio, where larger plants had higher C:N ratios. However, phenotypic correlations between many morphological phenotypes and flowering time differed in direction between well-watered and dry-down treatments (Figure 5). In the well-watered treatment, earlier flowering was strongly correlated with shorter plants with smaller flowers. However, in the dry-down treatment, earlier flowering was correlated with taller plants with larger flowers. Genetic correlations between traits were only calculated using the well-watered treatment and were generally in the same direction, but of stronger magnitude than the phenotypic correlations between traits in the manipulative experiment (Appendix S11).

Our data set was less powerful for examining differences in phenotypic correlations between populations; however, there were clear differences among populations in some

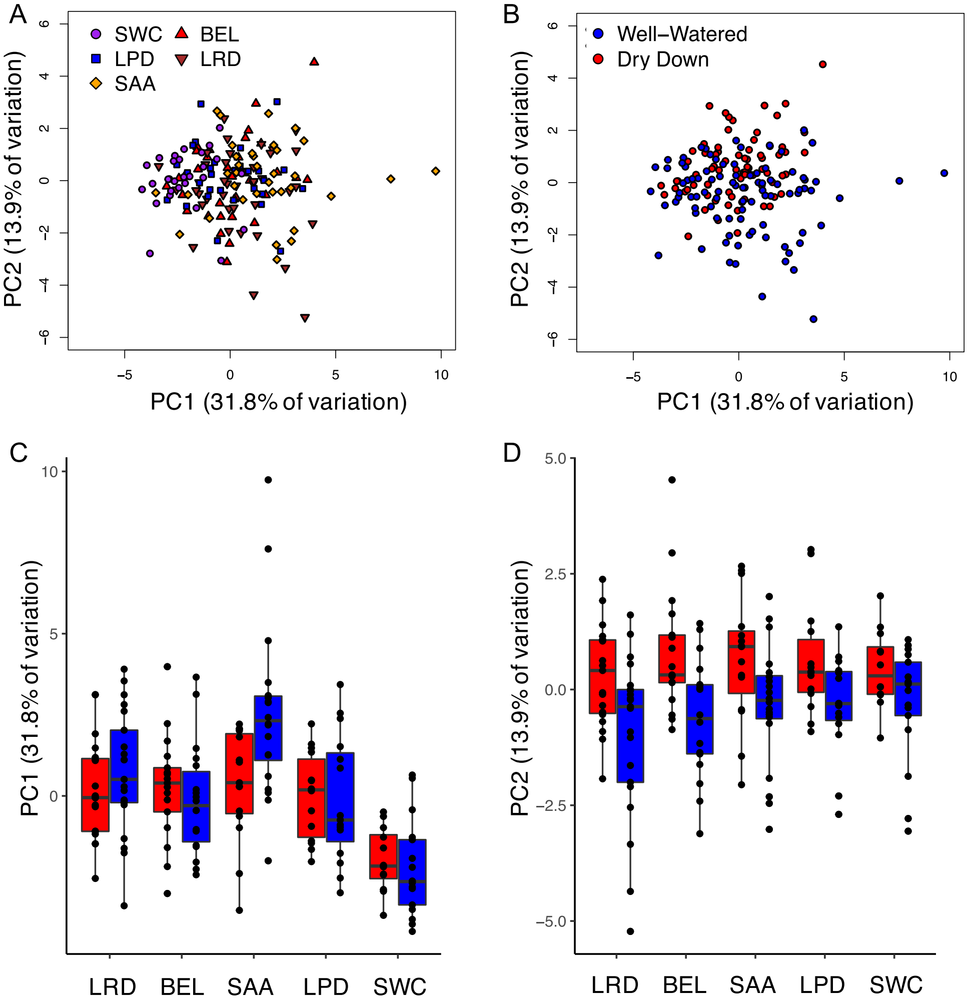

**FIGURE 3** Principal component analysis of trait variation across populations and treatments. Scatterplots depict variation in PC1 and PC2 separated by population (A) or treatment (B). Greater values of PC1 strongly associated with later-flowering plants that are larger at flowering. Greater values of PC2 are associated with earlier flowering with higher water-use efficiency, higher C:N ratios, lower SLA, and lower relative water content. Each point represents a line mean. Boxplots show the interactions between populations and treatment for PC1 (C) and PC2 (D). Box edges in boxplots represent the interquartile range, center line represents the median, and upper and lower whiskers are the largest value either greater or less than, respectively, 1.5 times the interquartile range.

correlations (Figure 6; Appendix S12). While there was no correlation between flowering time and $\delta^{13}$C in either the well-watered or dry-down treatments, this lack of a pattern appears to be an artifact of combining multiple populations with correlations in different directions (Figure 6). In the populations from the most mesic areas (SWC, LPD), there was a positive correlation between flowering time and $\delta^{13}$C where earlier flowering plants had lower water-use efficiency (Appendix S13). In Sierra populations (SAA, BEL), there were nonsignificant trends in the opposite direction where earlier-flowering plants had higher water-use efficiency. In the single Southern California site (LRD), there was no pattern. Correlations between flowering time and $\delta^{13}$C were higher for each population in the dry-down treatment than in the well-watered treatment. Together, these results suggest the correlation between drought escape and avoidance strategies exists only in some populations and is partially dependent on environmental conditions.

# DISCUSSION

While hypotheses regarding the existence of drought resistance strategies were first proposed more than 30 years ago, these strategies have rarely been examined in herbaceous plants across wide environmental gradients, incorporated plastic responses or examined heritability—key factors for predicting future responses to selection. Our results indicate a diversity of drought resistance strategies exist across the range of annual monkeyflowers and that these strategies do not fit neatly into classically described, and often considered mutually exclusive, drought escape or avoidance strategies. The most prominent pattern across populations was plasticity in response to our simulated terminal drought. All populations exhibited some similar physiological responses including increasing water-use efficiency, creating leaves with relatively less nitrogen to carbon, and reproducing at shorter stature. However,

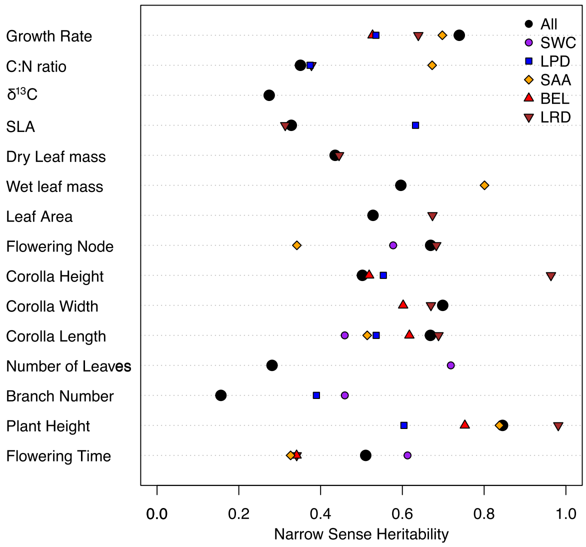

**FIGURE 4** Estimates of narrow-sense heritability for each trait calculated via midparent–offspring regressions. Black points represent data pooled across populations; colored dots represent estimates of heritability in each population. Only populations with statistically significant midparent–offspring regressions are shown.

patterns of plasticity in growth rate, timing of flowering, and investments into reproductive tissue differed among populations. We observed rangewide variation in each of the drought resistance traits measured, with differences between populations often driven by differences in the most mesic population, which employed a more resource conservative strategy than all other populations. Nearly every trait measured was moderately to highly heritable, and many traits were highly correlated with each other. Together, these results suggest that annual *M. guttatus* populations have adapted to heterogeneity in timing and severity of terminal droughts and have substantial variation to respond to natural selection for moderately earlier terminal droughts. Below, we discuss patterns of variation and plasticity of trait variation in the context of trait syndromes and future responses to selection in a changing climate.

## Heterogeneity among populations in drought resistance

Our populations demonstrate strong differences in both their constitutive drought resistance and plastic responses to terminal drought. The clearest pattern of variation among populations was the difference from the most mesic population, SWC, compared to all other populations (Figure 2; Appendix S3). SWC grows slowly, produces small flowers, and produces smaller but more costly leaves that contain more nitrogen and less water. Moreover, SWC was less plastic for almost all traits, including flowering time and water-use efficiency, than the other populations. These results indicate that SWC has a more resource conservative strategy than other populations (Reich, 2014). Some of these patterns of trait variation also suggest that the strategy of SWC is more drought avoidant than in the other populations despite SWC being in the most mesic environment. We also note that SWC is the only rockwall population and this could contribute to a habitat more xeric than expected. However, we hypothesize that these traits may have evolved in response to a different stressor—herbivory. Previous work on these same populations found that SWC has the highest herbivory pressure of any population surveyed across a latitudinal gradient and the highest concentration of the dominant chemical defense class, phenylpropanoid glycosides (PPGs), of any population across the range (Kooyers et al., 2017). In contrast, the populations in the Central Valley of California (BEL and SAA) constitutively produce almost 10-fold lower levels of PPGs, have higher C:N ratios, produce more leaves, grow more rapidly, and are taller at flowering than SWC plants. While results from this study cannot determine whether a more resource conservative or acquisitive strategy preforms better during drought or during early dry-down, previous field studies using these populations suggest that SWC plants perform more poorly than more resource acquisitive populations (BEL) when dry-down occurs early (Kooyers et al., 2019).

Aside from SWC, all other populations could be classified as having resource acquisitive strategies with significant plasticity in response to dry-down conditions. Each population flowered earlier at a shorter height, increased water-use efficiency, and produced leaves with higher C:N ratios in dry-down conditions relative to well-watered conditions. While faster reproduction and increased water-use efficiency are common responses to water-limited conditions, C:N ratios often decrease in response to limited water as carbon becomes limiting (Pinheiro and Chaves, 2011). While the same phenotypes responded to dry-down conditions across populations, there were differences in the magnitude and direction of the response. While flowering was highly variable in the well-watered treatment, all populations flowered rapidly in the dry-down treatment. Some populations flowered earlier by speeding up growth rate and flowering at the same node (LRD, LPD). Others slowed the growth rate and flowered at an earlier node (BEL, SAA). Interestingly, these differences in strategies were not necessarily geographically structured—while BEL and SAA are in the foothills of the Sierra Nevada, LRD and LPD are more than 600 km apart. This geographical disjunct could reflect that the strategy shared by BEL and SAA could have evolved locally and has not yet been introduced to other regions. Alternatively, the patterns we found could reflect local adaptation to the specific drought regimes in the foothills of the Sierra Nevada.

While the plasticity associated with growth allocation and physiology influenced each population to a certain degree, plasticity in flower size was only observed in a single population. A Sierra population, SAA, had smaller flowers

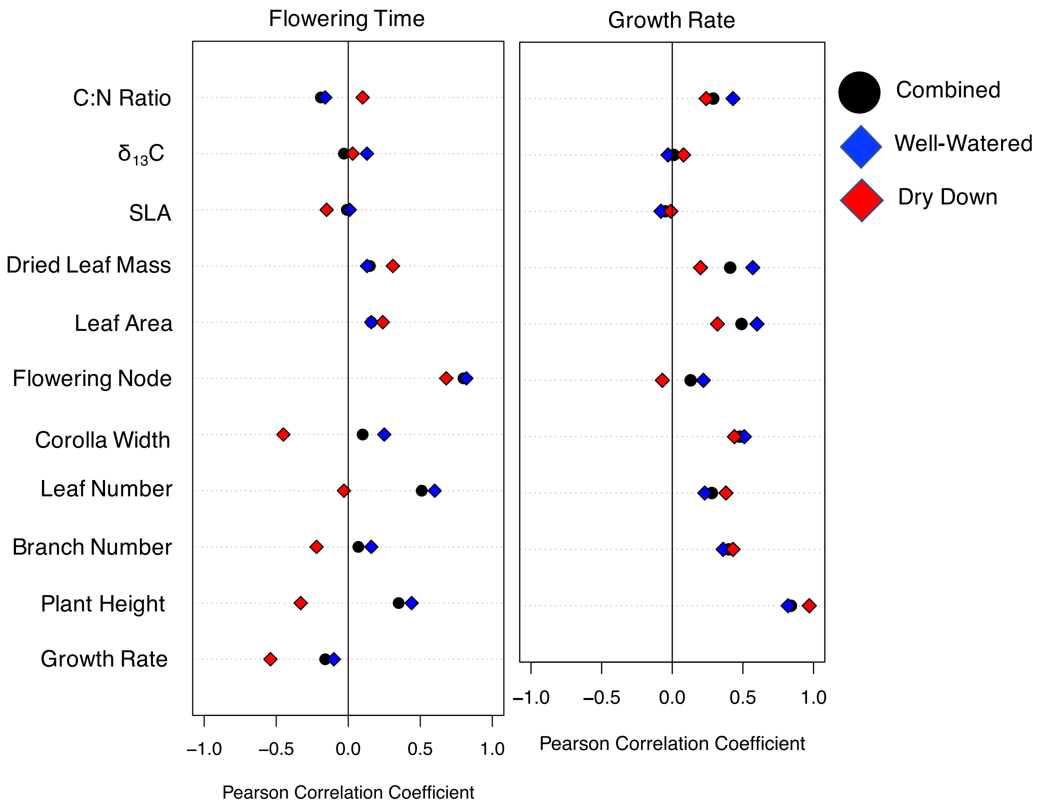

**FIGURE 5** Phenotypic correlations between all heritable traits and either flowering time (left) or growth rate (right) in the well-watered and dry-down treatments. Black circles represent Pearson correlation coefficients for a pooled data set that includes both treatments and all populations. Blue and red diamonds represent Pearson correlation coefficients between traits within the well-watered and dry-down treatments, respectively. All Pearson correlation coefficients are included regardless of whether correlations differed significantly from 0.

in the dry-down treatment than in the well-watered treatment. Other systems also produce smaller flowers in response to stressful conditions including drought (Lambrecht et al., 2017, 2020). This plasticity could be a response to more limited nutrients because flowers represent a costly investment for a plant. However, smaller flowers could also result in mating system shifts toward greater selfing to ensure reproduction. Regardless of mechanism, our results demonstrate that there are multiple types of trait plasticity employed in response to restricted water availability and that more xeric populations had greater plasticity. Notably, this pattern is the opposite observed in *Quercus faginea*; many of its drought resistance traits are less plastic in more xeric habitats (Solé-Medina et al., 2022). Future studies should analyze these results in relation to patterns of fitness in different natural environments to better understand the fitness benefits and trade-offs associated within each of these strategies.

The degree of plasticity was surprising for an annual species known for a “live fast, die young” strategy in ephemeral environments associated with yearly terminal droughts (Ernande and Dieckmann, 2004). However, the nature of a terminal drought as a relatively gradual environmental shift within the lifespan of the organism with clear cues is ideal for the evolution of plasticity (Fusco and Minelli, 2010). Notably, such gradual environmental transitions can simultaneously act as a selection pressure for greater plasticity in some traits and as a directional selection pressure for other traits (Garland and Kelly, 2006). We suggest that it should not be surprising for a species with extensive genetic and phenotypic variation (Kooyers et al., 2015; Puzey et al., 2017) to also have high levels of variation in plasticity and thus the ability to evolve strong responses to appropriate cues. We hope our results provide the impetus for future optimization modeling detailing when plasticity should evolve given gradual vs. immediate cues.

## Escaping traditional drought syndromes

The assortment of phenotypes into trait syndromes that represent non-mutually exclusive functional strategies for drought resistance has been predicted for decades (Ludlow, 1989; Kooyers, 2015; Volaire, 2018). Annual *M. guttatus* has largely been classified as a drought-escaping species that times its life history events to evade a yearly terminal drought (Hall and Willis, 2006; Wu et al., 2010; Kooyers et al., 2015). Our results support annual *M. guttatus* as a drought-escaping species; however, the degree to which drought escape occurred as a response to dry-down conditions rather than a constitutive pattern was

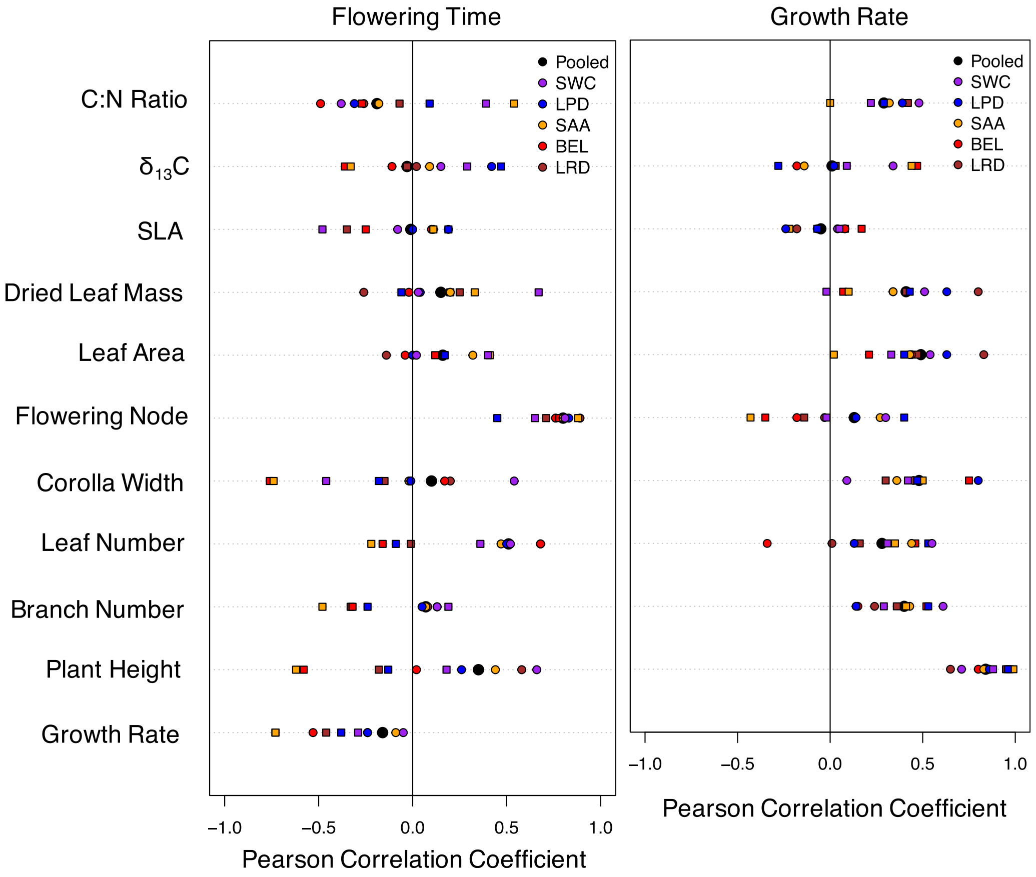

**FIGURE 6** Phenotypic correlations between all heritable traits and either flowering time (left) or growth rate (right) across populations. Black points represent Pearson correlation coefficients for a pooled data set that includes both treatments and all populations. Colored points refer to each population with circles signifying the correlations in the well-watered treatment for a population and squares signifying the correlation in the dry-down treatment for a population. All Pearson correlation coefficients are included regardless of whether correlations differed significantly from 0.

surprising. Previous evidence strongly supported the existence of a constitutive drought escape strategy, as observed in other herbaceous plants (e.g., Brouillette et al., 2014). For instance, flowering time in well-watered conditions varies clinally across the range of annual *M. guttatus* with populations that have shorter growing seasons flowering more quickly (Kooyers et al., 2015).

Instead of a constitutive response, we observed strong drought escape *and* avoidance responses to the dry-down treatment. Previous evidence for a drought escape response was equivocal with several studies finding evidence for faster flowering in dry-down or drought treatments (Galloway, 1995; Murren et al., 2006; Mantel and Sweigart, 2019) and others finding no pattern (Wu et al., 2010; Ivey and Carr, 2012). Our results provide an explanation for this inconsistency—there is geographic variation not only in the magnitude of the responses, but also in the physiological mechanism. That is, drought escape responses for some xeric populations including faster growth rates before flowering, while others include slowing growth rates and flowering at an earlier node (SAA). Presumably, slowing growth rates could also allow for improved water-use efficiency through better-regulated transpiration, potentially explaining the correlated drought escape and avoidance response. However, this would not explain how populations that respond to dry down with faster growth also have increased water-use efficiency.

Our results deviate from those in the drought literature in variation that we see in correlations between traits. A physiological trade-off between rapid growth to escape drought while maintaining high water-use efficiency is often hypothesized because stomata must be open to allow for flux of $CO_2$ for photosynthesis, but this same process also facilitates transpiration from the leaf (Geber and Dawson, 1990). Empirical evidence for this physiological trade-off comes from studies of *Arabidopsis thaliana*, where antagonistic pleiotropy of the *FRI* locus maintains a trade-off between flowering time and water-use efficiency (McKay et al., 2003, 2008; Lovell et al., 2015) and studies of other annual species (Geber and Dawson, 1990). However, several studies, including others in *Arabidopsis* and *M. guttatus*, have found limited evidence or even correlations in the opposite direction (Ivey and Carr, 2012; Wolfe and Tonsor, 2014; Kooyers, 2015; Kooyers et al., 2015). Our results suggest that this correlation may indeed exist in some populations (LPD, potentially SWC), particularly under dry-down conditions (Figure 6; Appendix S5) with the caveat that we were only able to estimate phenotypic correlations rather than genetic correlations in the dry-down treatment. We note that this result is relatively robust, as we find significant correlations despite having relatively limited sample sizes compared to typical quantitative genetics designs. Notably the populations exhibiting positive correlations between flowering time and water-use efficiency are the most mesic populations in our study and likely do not experience growing seasons severely shortened by terminal droughts very often. These populations are also the most similar to *Arabidopsis* in habitat and multivariate phenotypes (smaller and denser leaves, smaller height, low C:N ratio, etc.). We hope these observations spur future experiments delving into the physiological mechanisms underlying correlations between drought escape and avoidance.

## Responses to selection in a changing climate

Future climates for all populations in this study are likely to be prone to more frequent and severe droughts (Diffenbaugh et al., 2015). While we do not connect phenotypes to fitness to model how populations are likely to change in future environments, our results suggest that these populations should have both acclimation and adaptive responses to future droughts or shorter growing seasons. Our results demonstrate that plasticity in response to drought conditions is already common across populations and occurs through multiple different mechanisms. However, evolution of greater plasticity (higher magnitude responses) may be limited as independent mechanisms occurring in different populations may not be compatible with one another. For instance, a cross between a population that exhibits a drought escape response via faster growth rates and a population that flowers earlier by slowing development and flowering at an earlier node may not produce offspring with an enhanced drought escape response. This difference has noteworthy implications for assisted gene flow because sourced populations may not provide useful genetic variation into the threatened population (Aitken and Whitlock, 2013). Populations also possess the potential for adaptation through natural selection because substantial variation exists in nearly every trait and all traits except for RWC and $\delta^{15}$N are at least moderately heritable. Our data is less useful for understanding how genetic correlations may impact future evolution because we only were able to measure genetic correlations in the well-watered treatment. Future work is needed to determine whether observed shifts in phenotypic correlations between treatments hold for genetic correlations (Figure 5) or whether, like our phenotypic correlations and other quantitative genetic studies (Wood and Brodie, 2015), genetic correlations differ between populations (Figure 6).

Our results can also be interpreted in the context of a resurrection experiment in *M. guttatus* after the 2013–2017 historic drought in California (Kooyers et al., 2021). The majority of the 37 populations surveyed in this resurrection study had no evidence for phenotypic evolution following the drought. These 37 populations included two of the three California populations surveyed here (BEL and SAA), while LRD evolved to flower earlier and at a taller height following the drought. However, BEL had greater fitness post-drought than pre-drought in both well-watered and dry-down environments. Our results suggest that a major reason that more evolution was not observed was that California populations have significant plasticity responses that can ameliorate water stress. Future field studies that incorporate fitness data with water manipulations will be necessary to determine whether, where, and when the plastic responses observed in this experiment have costs and benefits as well as establish whether there is an intensity of water stress beyond which plasticity no longer affords plants the capacity to survive and reproduce.

## AUTHOR CONTRIBUTIONS

J.A.F. and N.J.K. conceived the ideas and designed the methodology. J.A.F., B.I.D., and C.M.P. collected the data. J.A.F., S.D.H., and N.J.K. analyzed the data. J.A.F. and N.J.K. wrote the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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## DATA AVAILABILITY STATEMENT

All data sets from the manipulative experiment and midparent–offspring experiment have been deposited ~~on~~ the Dryad Digital Repository (https://doi.org/10.5061/dryad.qrfj6q5mq).

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**Appendix S1**. Population summary statistics for phenotypic and climatic data.

**Appendix S2**. Volumetric water content within the well-watered and the dry-down treatments.

**Appendix S3**. Variation among treatments and populations in flower morphology.

**Appendix S4**. Variation among treatments and populations in leaf-level physiology.

**Appendix S5**. Phenotypic correlations pooled across treatment and populations in the manipulative experiment.

**Appendix S6**. Variance proportions and loadings scores in the principal component analysis.

**Appendix S7**. Narrow-sense heritability of all traits across pooled and population-subsetted data sets.

**Appendix S8**. Midparent–offspring regressions for all traits.

**Appendix S9**. Phenotypic correlations in well-watered treatment.

**Appendix S10**. Phenotypic correlations in the dry-down treatment.

**Appendix S11**. Genetic correlations between traits from midparent–offspring experiment.

**Appendix S12**. Phenotypic correlations by population.

**Appendix S13**. Phenotypic correlations between flowering time and $\delta_{13}$C in each population by treatment.

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