Patterns of Natural Variation in Drought Escape and Drought Avoidance in the Common Monkeyflower

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Abstract

Climate change has caused increasing temperatures and drier climates over the past decade. This has especially affected the western coast of North America, bringing even drier and hotter climates. This makes it difficult for plant life to prosper in these areas. One way that plants fight these drought conditions is through drought resistance traits. These drought resistance traits include flowering early and reproducing early before drought conditions onset or increasing the efficiency of water use by tissues and the stomata of the plant. While these traits are both beneficial in fighting drought conditions, they often negatively impact each other. This negative tradeoff is generally found due to genetics that control the physiology of the stomata. These genes can be affected by environmental conditions or genetic correlations. In this thesis, we use *Mimulus guttatus* to determine how heritability, genetic correlations, and environmental conditions affect the expression drought escape and drought avoidance traits. We discovered that there is variation in the expression of these traits between Oregon and California populations. We also find significant variation in the heritability, genetic correlation, and plasticity of morphological traits. These results together suggest that local adaptation is occurring at specific environmental sites. These results can help us predict how *Mimulus guttatus*, and possibly other plant species, will adapt to the drier and hotter temperatures brought about by climate change.

Chapter 1: Introduction/Background

In order to avoid the detrimental effects of changing climates, organisms can respond via a number of different mechanisms. Some of these responses include dispersing to other areas, plasticity of traits, adaptation to new environmental conditions, and/or maladaptation of the conditions (Etterson & Shaw, 2001; Kooyers et al., 2019). However, anthropogenic climate change is changing environments at a faster rate than species can adapt, causing increasing variation in temperature and precipitation levels worldwide. In the western US, the environment is changing with hotter temperatures, lower precipitation, and longer, more extreme droughts. (Davis, Shaw, & Etterson, 2005; Etterson & Shaw, 2001). Although plants of the western US have adapted to the face seasonal droughts and the hot temperatures of this area, climate change may bring more extreme changes that these plants may struggle to adapt and survive through (Thorne, Boynton, Flint, & Flint, 2015).

One potential effect of climate change is that clines in ecological traits related to adaptation to temperature or precipitation may shift towards northern latitudes and/or higher elevations (Davis et al., 2005; Etterson, 2004). This shift in clines has been predicted in *C. fasciculata* where it is expected that states like Minnesota are likely to exhibit the current climate of Kansas in the future while the future Kansas environment will become warmer (Etterson, 2004). This change can be especially devasting on southern populations, as the warmer climate can also bring drier conditions leading to higher levels of water stress. Southern populations may be more likely to struggle and go extinct in these warmer conditions (Davis et al., 2005). While southern populations will struggle due to warmer conditions, northern populations are more likely to struggle due to competition with native or invasive species better suited to the contemporary

environment or due to interactions with novel species (Etterson, 2004; Etterson & Shaw, 2001; Kooyers et al., 2019). Southern populations that are well adapted to their environment may move northwards as the northern climates become more habitable to their adaptations. Ultimately, we may observe declining adaptation in specific populations, but we may also see limited shifts in species ranges as well as extirpation of some populations (Etterson, 2004; Etterson & Shaw, 2001; Kooyers et al., 2019).

Due to the changing climate, many environments are seeing drastic shifts in moisture and aridity. One of these shifts include the onset and severity of droughts (Cook, Mankin, & Anchukaitis, 2018). Many areas, especially the west coast of the United States are experiencing more droughts with increasing severity (Cook et al., 2018; Davis et al., 2005; Wang, Hamann, Spittlehouse, & Carroll, 2016). In order for plants to survive these conditions, they may implement a variety of drought resistance strategies. Drought resistance strategies are often grouped into three non-mutually exclusive syndromes including drought escape, drought avoidance, and drought tolerance (Ludlow 1988). Drought escape strategies involve a rapid development to reproductive maturity in order to reproduce before the onset of a drought. A phenotypic trait example of this strategy would be a faster time to flowering, hereafter termed flowering time (Kooyers, 2015). If plants are exhibiting a drought avoidance strategy, they will reduce transpiration by closing stomata to prevent excessive water loss in an effort to increase water-use-efficiency (WUE). WUE is often approximated by $\delta^{13}C$ which is a less common isotope of Carbon. In short, less water to be transpired while the stomata is open, the more $\delta^{13}C$ is that is measured. This allows it to be a measure of how much water a plant which is a way to measure water use efficiency (Franks, 2011; Kooyers, 2015; Lewis et al., 2010)

Some plants also employ drought tolerant strategies by develop tissue that can withstand dehydration and grow following drought stress. These plants acquire this tolerance through osmotic adjustment and produce molecules that help stabilize cells and keep them alive as they experience water stress (Kooyers, 2015). For the purpose of this thesis, we focus primarily on drought escape and drought avoidance as these are the two primary resistance strategies employed by annual herbaceous plants.

While these drought resistance strategies each work well in combating drought, they may negatively impact the expression of other traits - which may include traits of an opposing drought resistance strategy. Drought escape and drought avoidance are an example of a well-known physiological tradeoff. Their physiological tradeoff occurs between flowering time, a drought escape trait, and WUE, a drought avoidance trait. Flowering time is the measurement of how long it takes a plant to produce its first flower from time of germination while WUE is a measurement of how well plants can hold onto water in dehydrating conditions (D. L. Des Marais et al., 2014; Kooyers, Greenlee, Colicchio, Oh, & Blackman, 2015). This physiological tradeoff is a consequence of the photosynthetic process in plants. Plants require the uptake of both CO_2 and water for photosynthesis to occur. Most water is absorbed through the roots of the plant while CO_2 diffuses into the leaves of a plant through little pores on the leaves' surface called a stomata (D. L. Des Marais et al., 2014). However, while the stomata is open, transpiration also occurs which results in water loss. To prevent water loss, plants can increase WUE by closing the stomata on the leaves for longer which reduces transpiration. As a consequence, this reduces the amount of CO2 uptake the longer the stomata are closed (D. L. Des Marais et al., 2014; Kooyers, Donofrio, Blackman, &

Holeski, 2020; Kooyers et al., 2015). This physiological mechanism is why we generally see a tradeoff or phenotypic correlation between the drought escape and drought avoidance (Franks, 2011; Kooyers et al., 2015).

Such phenotypic tradeoffs (also termed phenotypic correlations) can be caused by environmental constraints, genetic correlations, developmental correlations, or a combination of the three. Genetic correlations are a measure of the proportion of variance shared by two traits (Gardner & Latta, 2007). Genetic correlations are a result of either pleiotropy, where the same genetic variant controls variation in multiple traits, or linkage, where genetic variants that occur physically close to one another in the genome segregate together more often than expected by chance. In the case of drought escape vs avoidance, antagonistic pleiotropy could occur where a single allele at a locus increases drought escape at the cost of drought avoidance or vice versa(David L. Des Marais, Hernandez, & Juenger, 2013). Genetic correlations can also be dependent on the environment in that the environment can cause selection that increases or decreases a genetic correlation (Sgrò and Hoffman 2004). Genetic correlations are important to consider when considering responses to selection including selection that could occur from more frequent or severe droughts. Both positive and negative correlations can be a cause of tradeoffs between traits which may constrain evolution and/or limit the plasticity of traits, notably including drought escape and drought avoidance. If the tradeoff between drought escape and drought avoidance is largely caused by a negative genetic correlation between the two, this could severely restrict the ability of populations to adapt to the warming climates. For instance, populations with a strong negative genetic correlation between flowering

time and WUE could only evolve earlier flowering at the expense of lower WUE, or greater WUE at the expense of later flowering.

Other factors that have a crucial role in an individual's ability to adapt to warming climates include heritability and plasticity. Heritability refers to how much of the genetic variation between generations in a specific environment is controlled by additive genetic variation (Falconer & Mackay, 2009). Plasticity refers to how much traits vary when grown in separate environments and can have a separate genetic basis than heritability (David L. Des Marais et al., 2013; Falconer & Mackay, 2009; Franks, 2011; Gutteling, Riksen, Bakker, & Kammenga, 2007). Heritability and plasticity are important to consider when predicting how different populations will respond to the changing climates. In *C. fasciculata,* researchers discovered that the presence of heritable traits and genetic correlations impeded adaptive evolution to a warmer and drier climate (Davis et al., 2005; Etterson, 2004). Based on this data, one prediction on how response to climate change will be is that populations with higher plasticity and less negative correlations between drought resistant traits and fitness traits are more likely to adapt sufficiently to the fast rate of climate change (Davis et al., 2005; David L. Des Marais et al., 2013; Etterson, 2004; Etterson & Shaw, 2001).

The model species we used to analyze drought escape and drought avoidance was the common yellow monkeyflower (*Mimulus guttatus*). *Mimulus guttatus* is a species of monkeyflower that is unique due to its widespread distribution and its ecology. It is found in a variety of environments ranging from xeric to mesic areas (Wu et al., 2008). It can be found worldwide but is most notably found along the west coast of North American ranging from Mexico to Canada (Wu et al., 2008). *Mimulus guttatus* has become a

widespread model species for evolutionary genetic studies, ecological studies, inbreeding depression, and adaptation. It has become a highly used model species due to its short growing time, high genetic diversity, large geographic distribution, and small genome (J. Puzey & Vallejo-Marín, 2014; Wu et al., 2008). Due to this large range and variation in environments, *Mimulus guttatus* has extreme variation in morphology, phenology, and physiology. For example, *Mimulus guttatus* contains both perennial and annual populations, can range from several inches to several feet in height, and employ different drought resistance strategies (Hall & Willis, 2006). These large variations within and between populations contribute to its success as an invasive species in widespread locations such as Scotland and New Zealand (J. Puzey & Vallejo-Marín, 2014). *M. guttatus* also has some of the highest levels of genetic variation observed with in a population of any angiosperm (J. R. Puzey, Willis, & Kelly, 2017) potentially due to substantial temporal and spatial heterogeneity in environments(Troth, Puzey, Kim, Willis, & Kelly, 2018).

Annual *M. guttatus* is found in inland regions in the western US. Growing seasons start with spring rains or snowmelt and end with terminal droughts. There is substantial variation in growing season timing and duration across the range (Kooyers, 2015; Kooyers et al., 2015). These growing season dynamics create substantial water deficits that individual plants must respond to and an important selection pressure across the range is water limitation at the end of the growing season. These selection pressures have led to variation within drought resistance strategies across the range (Kooyers et al. 2015). This large variation provides a framework that allows us to investigate how specific environmental differences, latitudinal differences, or genetic differences create

local adaptation and/or genetic differentiation in both physiological and morphological traits.

In this thesis we examine, through manipulative experimentation and quantitative genetics, patterns of trait plasticity, variation, heritability, and genetic correlations between traits for drought resistance traits in *Mimulus guttatus*. We predict that different populations will have evolved different mechanisms to combat drought stress including through constitutive and plastic drought escape and drought avoidance phenotypes. Specifically, we examine how patterns of trait variation, trait plasticity and genetic correlations vary across populations spanning a latitudinal gradient from southern California to central Oregon. Clinal variation in any of these three variables across this gradient would suggest environmental factors differing across this gradient potentially cause adaptive differentiation. However, lack of a patterns in variation, plasticity, or genetic correlations do not necessarily suggest that selection for drought resistance has not occurred, but rather that populations could either finely adapt to local environments or may adapt in many different ways to the same stressors. Together our experimental results present a picture of how *M. guttatus* has historically adapted to variation in water availability and how it could evolve in response to future climatic changes.

Chapter 2: Manipulative Drought Experiment

This chapter explores the variation and plasticity of both morphological and physiological traits when exposed to environments with different of water deficit. Previous studies have often observed a negative correlation between drought escape and drought avoidance traits, presumably as the result of a well-known physiological tradeoff for C3 plants. That is, plants that grow quickly to escape the worst effects of a drought often are much worse at altering water use efficiency to avoid desiccation. This tradeoff likely reflects the physiology of the stomata. When the stomata is open, more uptake of carbon dioxide occurs which ultimately facilitates growth. The carbon dioxide then enters the Calvin Cycle and undergoes reductive conversion into monosaccharides. These monosaccharides can then be used as building blocks for larger structures within the plant or can be used for energy (Fridlyand and Scheibe 1999). Generally, the higher the carbon dioxide uptake, the more structural molecules available for growth and development. Therefore, a higher stomatal conductance will lead to increased CO_2 uptake which then leads to more monosaccharide synthesis. Higher levels of monosaccharides increase the rate of growth and development resulting in a shorter time to develop reproductive capabilities. This commonly results in faster time to first flowering (Fridyland and Scheibe 1999, Shavrukov et al. 2017)

However, there is a cost to keeping stomata open as transpiration also occurs through the opening of stomata. This loss of water results in less water available for photosynthesis and other major cellular processes. It also negatively impacts water use efficiency (WUE) which is a key component of drought avoidance strategies in plants (Kooyers, 2015). While a negative correlation between drought escape and drought avoidance is the general trend as demonstrated by populations of *Arabidopsis thaliana,*

Brassica rapa, and *Panicum hallii* (Kooyers, 2015), this is not always the case. *Mimulus guttatus* is one such species that does not exhibit this negative correlation across its populations. Some populations from *Mimulus guttatus* show the opposite of this general trend by exhibiting a positive correlation between drought escape and drought avoidance under well-watered conditions (Kooyers et al., 2015). One possible explanation for this is that a plant defense trait, total PPGs, correlates with flowering time and this could create a three-way tradeoff (Kooyers et al., 2020). With some additional component to the tradeoff between drought escape and drought avoidance, it would be impossible for populations of *Mimulus guttatus* to express both drought escape and drought avoidance. However, there can be other reasons that may explain the absence of this tradeoff. One reason could be due to the plants being grown in well-watered conditions where there is little need to close stomata in response to limited water. Furthermore, populations across the range may have similar negative correlations between these traits, but pooling these populations together produces a positive correlation as an artifact of sampling (Kimball, Gremer, Huxman, Lawrence Venable, & Angert, 2013).

In this chapter, we look at the evolution and plasticity of drought escape and drought avoidance traits in *Mimulus guttatus* by performing a manipulative experiment with a dried-down treatment and a well-watered/control treatment. This experiment allows us to examine variation in phenotypes associated with drought escape and avoidance across populations and water availability conditions. We address four main questions within this experiment. First, is there variation in traits associated with drought escape and drought avoidance between treatments, and/or across populations? Second, is there a phenotypic correlation between traits associated with drought escape and drought

avoidance? Third, does the availability of water impact the existence or magnitude of this correlation between drought escape and drought avoidance? Finally, do different populations have different correlations between drought escape and drought avoidance traits? We hypothesize that the populations from more drought stressed environments will not exhibit a phenotypic correlation between drought escape and drought avoidance traits, but the populations with less historical frequency of drought stress and lower aridity will exhibit a stronger correlations. We predict that we will see a general trend that flowering time, a proxy for measuring drought escape, will be significantly earlier in all populations when subjected to water stress. We also predict to see a general trend that $\delta^{13}C$ will be significantly higher when lines are subjected to water stress as plants begin to close their stomata to regulate water loss.

Methods

Population Sampling

We used field collected seeds from three populations (BEL, LRD, SAA) originating from an arid environment in central California and two populations (LPD, SWC) originating from a more temperate environment in the coastal mountains and Willamette Valley of Oregon. The California populations have a higher mean annual temperature and were more arid than the Oregon populations (Table 1). The growing seasons also differ between the populations. The low elevation populations in California (LRD, BEL, and SAA) have growing seasons that last about three months between the months of February and May. The low elevation populations of Oregon (LPD, SWC) have a longer growing season between the months of late March to July. The Oregon

populations germinate during winter or spring rains and terminate due to a seasonal drought. The seeds used for this experiment was collected from 25-50 maternal lines from each population in 2013, 2016, or 2017.

Table 1: Location and climate data of the different populations.

Population	Latitude	Longitude	MAT	AHM (m)	CMD (mm)
BEL	37.039833°N	119.77382°W	17.6°C	71.4	1155
LRD	34.506467°N	118.0276°W	15.8°C	119.4	1188
SAA	38.143317°N	120.6973°W	15.9°C	27.1	786
LPD	43.916665°N	122.75603°W	11.3°C	18	279
SWC	44.01415°N	123.85997°W	11.4°C	11	396

Data is based on the period between from 1981 – 2010. Data is taken from Wang et al. 2016. MAT stands for mean annual temperature. AHM stands for annual heat-moisture index. CMD stands for Climate moisture deficit.

Refresher Generation

We grew a "refresher" generation first instead of directly proceeding to the manipulative experiment to reduce any maternal effects during the experiment (Fishman et al. 2002). For the refresher generation, seeds from each maternal line were grown under well-watered conditions on growth shelving under Philips growing lamps (Philips: 479626). Plants experienced 16hr day: 8hr night conditions with light intensity set at set at 6500K lumens for nine lamps. These lines were selfed using hand pollination techniques to give rise to the parental generation. This included using tweezers to extract anthers out of one of the flowers followed by crushing the anthers to release the pollen.

The tweezers were then rubbed against the pollen to pick up the pollen. Next, the tweezers were rubbed against the stigma of another flower on the same line. The corolla of the fertilized flower was then removed, and the peduncle of the flower was labeled. Tweezers were sterilized with 70% ethanol between each use. Seeds were collected four weeks after selfing date and placed in coin envelopes for use in the below manipulative experiment

Manipulative Experiment

The overarching goal of the manipulative experiment was to examine genetic variation within and between populations in traits related to drought escape and drought avoidance. The two conditions in this experiment were the control and dry down treatments. The control was well-watered throughout the experiment while the dry down treatment stopped receiving water 18 days after the start of the experiment to simulate the terminal drought conditions found in the Mediterranean environment where *Mimulus guttatus* is found.

For the manipulative experiment, we used the seed that was obtained by selfing the refresher generation. For each of our five populations, we planted two replicates of thirty maternal lines with one plant in the control treatment and the second plant in the dry down treatment. This equated to 150 total lines per treatment and 300 total plants and maxed out the space in our growth chambers. We planted 4-5 seeds per pot. We placed multiple seeds per pot to provide a better chance that each line would have a germinate (Fishman et al. 2002). We used 2.5” pots with holes on the bottom to allow for water absorption through bottom-watering. Pots were filled with Fafard 3B soil (Sun Gro; Pine

Bluff, Arizona) and placed in 1020 flats (Hummert International, St. Louis, Missouri). Pots were randomized within treatment by assigning a random number that correlated with what flat and position they would be placed in. The flats were saturated with water, covered with humidity domes, and subjected to cold stratification at 4° Celsius. After one week, the plants were taken out and placed in a Percival AR-66L2 growth chambers with light intensity of 360 μmol/m²/s set at 22° Celsius with a 16-hour day length. Day one of the experiment was the date when pots were placed in the growth chamber. For the first week, pots were misted daily using tap water. After the first 7 days, we removed the humidity domes (Kooyers et al. 2020). On day 14 of the experiment, we removed any extra germinants in each pot, thinning to one plant per pot. Extra germinants for a given pot were transplanted to new pots to replace pots where no plants germinated. Additional transplantation was done to ensure that all flats had the same number of germinants to reduce microvariation between the flats.

We had two treatments that manipulated water availability in this experiment, a well-watered control treatment and a dry down treatment. For the first 18 days of the experiment, both the control and experimental condition experienced the same water treatment. On day 18, the treatment group underwent a dry-down treatment to simulate the natural dry down process. To perform the dried down treatment, we first emptied water from all five flats within the treatment group and then added 2L of water to each flat. This would be the final time that the treatment group would receive water during the duration of the experiment. The control treatment continued to be bottom watered throughout the entire experiment and did not experience water deficit. Flats in both the

control and experimental groups were rotated daily to eliminate spatial microvariation within the growth chamber.

Measurement of traits

A variety of morphological and phenotypic traits were measured on every plant throughout the experiment. Several traits were measured on the day of flowering, and we assessed flowering daily. Flowering time was measured as the time it took for the first flower to appear from germination date. Flowering was considered to occur when the bottom corolla lips opened to reveal the stigma and anthers. Plant height was measured as the distance from the apical meristem to the lowest part of the stem touching the soil. Branch number was measured as the number of branches present at first flowering. Leaf number was measured as the number of leaves present at first flowering. Corolla length, width, and height were measured on following the procedures in Fishman et al. (2002) at time of flowering. Flowering node referred to the node on the main stem that 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 occurred.

Some traits were not measured at the day of flowering. These traits involved leaf physiological measurements that required destructive sampling of the second true leaves of a plant. Second true leaves were collected between days 30-32 of experiment and placed in tackleboxes filled with deionized water. Second true leaves were cut off the main stem and placed with the peduncle face down in the water. After 24 hours, the wet leaf mass of the leaves was measured (termed wet leaf mass) and a picture of the leaf next to a 1 x 1 cm red square was taken. The leaf was then placed in a coin envelope and

dried in a 65°C oven. We measured dry leaf mass of each leaf after five days in the oven or until no additional weight loss was measurable (Kooyers et al. 2015). Succulence was calculated by dividing the wet leaf mass by the dry leaf mass. Leaf area was calculated by analyzing the areas of the 1 x 1 cm square and leaf in the picture using the program ImageJ (Easlon & Bloom, 2014). To calculate SLA, we divided the leaf area by dried leaf mass. For $\delta^{13}C$ and $\delta^{15}N$ analysis, we first grinded leaves up using beads, grinding tubes, and a fast prep 96 tissuelyzer (MP Biomedicals, Santa Ana, California) at 1200 rpm for 30 seconds. If leaves were too small to be grinded for risk of losing sample, we instead packaged the entire leaf into the tin containers. We aimed to package between 0.3 to 1.1 grams of leaf tissue in each tin. We then sent the samples off to the Stable Core Isotope Laboratory at Washington State University for standard procedures of $\delta^{13}C$ and $\delta^{15}N$ analysis (Lewis et al 2010).

Statistics

We assessed summary statistics for each trait for the entire dataset as well as broken down into each treatment and each population using R. To determine whether a particular trait statistically differed between treatments or populations, we built univariate general linear models using the lm() function with the specific trait as the response variation and population, treatment, and a 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. Significance of the population term indicates that populations statistically differ for a particular trait. Significance of the treatment term indicates that a trait difference between the well-watered and dry down treatments across populations (i.e. the trait is plastic and responses to water stress). Significance of the

interaction term indicates that there were differential responses to the dry down treatment for different populations. We examined pairwise correlations between traits across the dataset as well as broken down into different populations and treatments using similar general linear models as above with one trait treated as the response variable and the other as the independent variable. This method is equivalent to assessing Pearson correlations between traits.

Validation of Dry Down Treatment

To quantify our experimental treatments, we measured soil moisture within the control and experimental flats. Starting at day 18 of the experiment, the day we began the dried down treatment, we measured soil moisture daily. We randomly selected three positions every day and measured the soil moisture of those same positions for every flat. A Delta T SM150T Soil moisture kit (Dynamax, Houston TX) was used to measure soil moisture. To measure soil moisture, the probe was stuck into the soil, near the plant, away from the walls of the pot about an inch to two inches deep. This recorded the soil moisture in milliVolts (mV). We then converted the data from mV to V and then converted this data into soil moisture percentage using the following equations:

Equation 1: $\sqrt{\varepsilon} = =1+14.4396V-31.2578V^2+49.0575V^3-36.5575V^4+10.7117V^5$

Equation 2: $\theta = (\sqrt{\varepsilon} - \alpha_0)/\alpha_1$

Equation 3: % volumetric = $100 * \theta$

We used the peat mix value of 1.16 and 7.09 as the α_0 and α_1 values in the equation. After soil moisture percentages were obtained, averages for each treatment were calculated for each day's results.

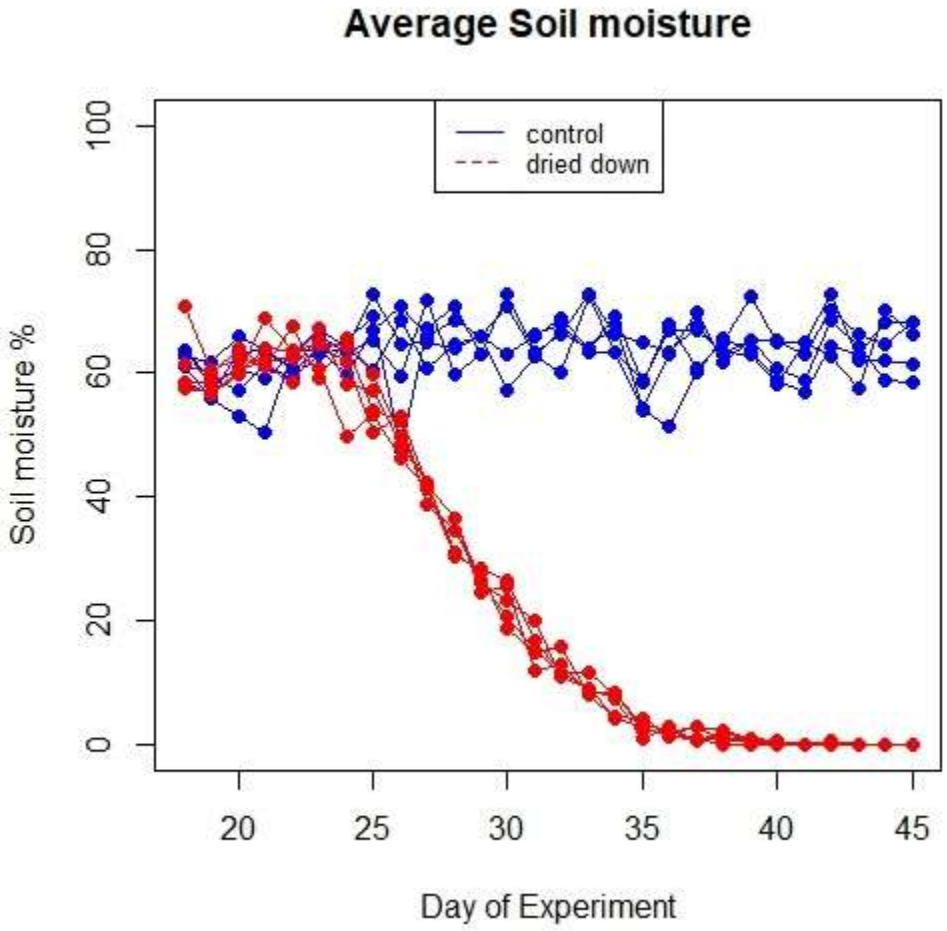

Figure 1. Average soil moisture within flats for the dry down and the well-watered treatment the five blue lines represent the average soil moisture for each of the five control flats measured daily. The five red lines represent the average soil moisture within its pots for each of the five dry-down flats measured daily.

Results

Variation in drought escape and avoidance across populations and treatments

In our experimental conditions, plants exhibited significant variation in drought escape, as measured by flowering time, and in drought avoidance, as measured by $\delta^{13}C$. Both flowering time and $\delta^{13}C$ were impacted by the dry down treatment (Figure 4). Plant in the well-watered treatment flowered after an average of 35 days (sd: 8.61, min: 25, max: 61) while plants in the dry-down treatment flowered after an average of 30 days (sd: 3.4, min: 24 max: 40). There was less variation in $\delta^{13}C$, but still a significant treatment effect . Plants in well-watered treatment had an average $\delta^{13}C$ of -31.83 (sd: 0.49, min: -33.57, max: -30.28) while plants in dry down had an average $\delta^{13}C$ of -31.41 (sd: 0.72, min: -32.99, max: -27.78).

There was also substantial variation among populations in drought escape. Flowering time varied significantly between populations with SWC flowering the earliest on average with 31 days (sd: 7.64, min: 26, max: 58) and SAA flowering the latest on average with 38 days (sd: 9.05, min: 26, max:61) in well-watered conditions. BEL, LPD, and LRD also had averages of 34 days (sd: 7.5, min:25, max:46), 34 days (sd: 7.58, min:27, max: 57), and 37 days (sd: 9.2, min:26, max:58) in well-watered conditions. In dried down conditions, we found that the Oregon populations did not flower significantly earlier with SWC having an average flower time of 29 days (sd: 2.59, min: 25, max: 34) and LPD having an average flower time of 30 (sd: 2.13, min: 26, max: 34). The California populations did have a significant difference in flower time between

treatments with BEL having an average of 32 days (sd: 4.08, min: 26, max: 40), LRD having an average of 29 days (sd: 3.04, min:24, max: 35), and SAA having an average of 31 days (sd: 4.01, min: 25, max: 38) (Figure 4).

Alternatively, drought avoidance, measured by $\delta^{13}C$, did not vary among populations and the treatment effect was similar across all five populations. In the well-watered conditions, the $\delta^{13}C$ values for the populations included BEL with an average of -31.91 (sd: 0.36, min: -32.37, max: -31.28), LPD with an average of -31.83 (sd: 0.53, min: -32.54, max: -30.44), LRD with an average of -32.01 (sd: 0.38, min:-32.92, max: -31.25), SAA with an average of -31.68 (sd: 0.38, min: -32.36, max: -30.79), and SWC with an average of -31.64 (sd: 0.71, min: -33.57, max: -30.28). There was a significant treatment effect with $\delta^{13}C$ being significantly higher in all populations in dried down conditions. The $\delta^{13}C$ in these populations included BEL with an average of -31.39 (sd: 0.46, min: -32.33, max: -30.24), LPD with an average of -31.41 (sd: 1.12, min: -32.71, max: -27.78), LRD with an average of -31.43 (sd: 0.6, min: -32.53, max: -30.39), SAA with an average of -31.41 (sd: 0.74, min: -32.99, max: -29.57), and SWC with -31.41 (sd: 0.59, min: -32.53, max: -30.06). Together, these summary statistics suggest that there is substantial variation in drought escape both between treatments and between the Oregon and California populations. However, there was no substantial variation in drought avoidance strategies across the populations, but there was variation based on treatment conditions within this experiment.

Phenotypic correlation between drought escape and drought avoidance

We examined the correlations between drought escape and avoidance at multiple scales across our experimental design. We first examine global patterns across treatments and populations. There was no association between flowering time and $\delta^{13}C$ ($r^2 < 0.01$; Fig. 2) suggesting there is no correlation between drought escape and drought avoidance. We then subset the data by treatment conditions to see whether the well-watered treatment could be masking a potentially significant correlation. In both the control and treatment condition, there was no correlation between flowering time and $\delta^{13}C$ ($r^2 < 0.01$, Fig. 3).

To address whether spatial variation can affect the presence or type of phenotypic correlation, we examined phenotypic correlations across all combinations of treatments and populations. Only a single population (SWC) had a correlation between flowering time and $\delta^{13}C$, and this correlation only occurred in the dry down treatment (Figure 5). As predicted, this was a negative correlation where plants that flowered earlier had lower water use efficiency.

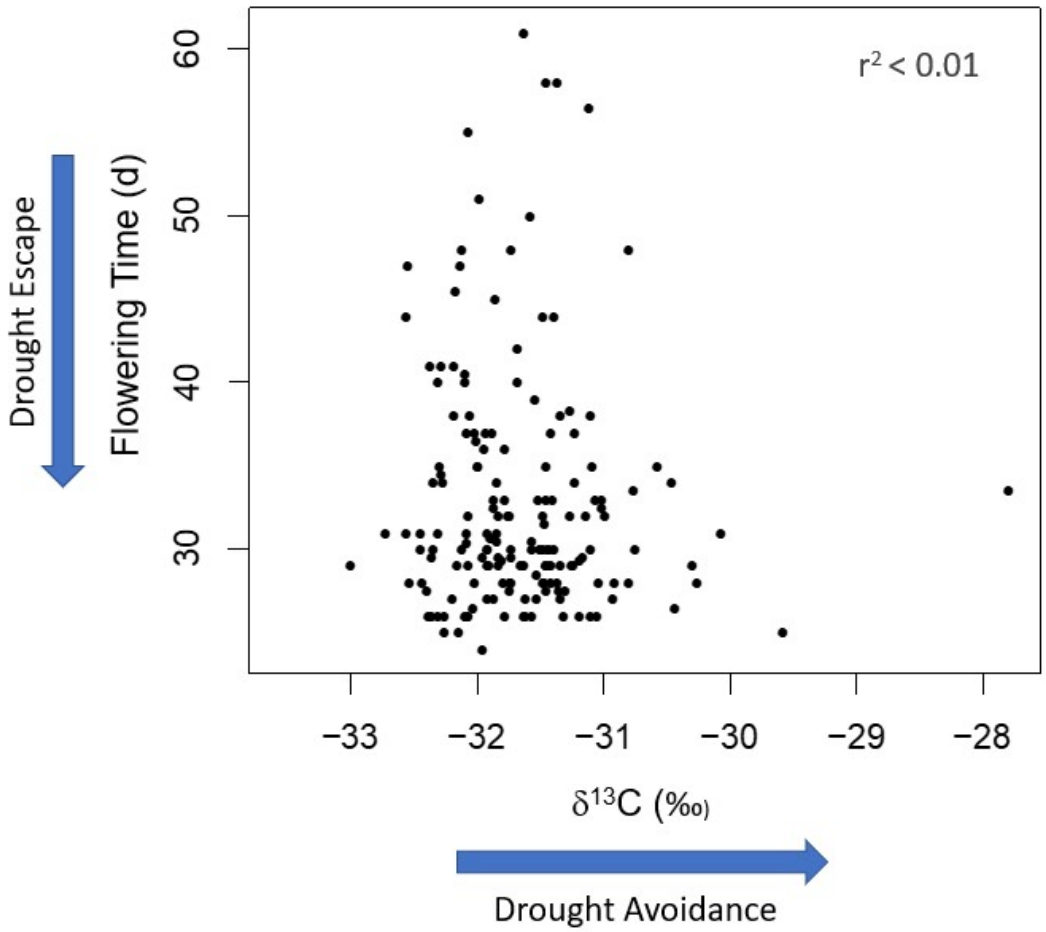

Figure 2: Correlation between drought avoidance trait, δ13C, and drought escape trait, flowering time. Data is combined from all populations from both treatments.

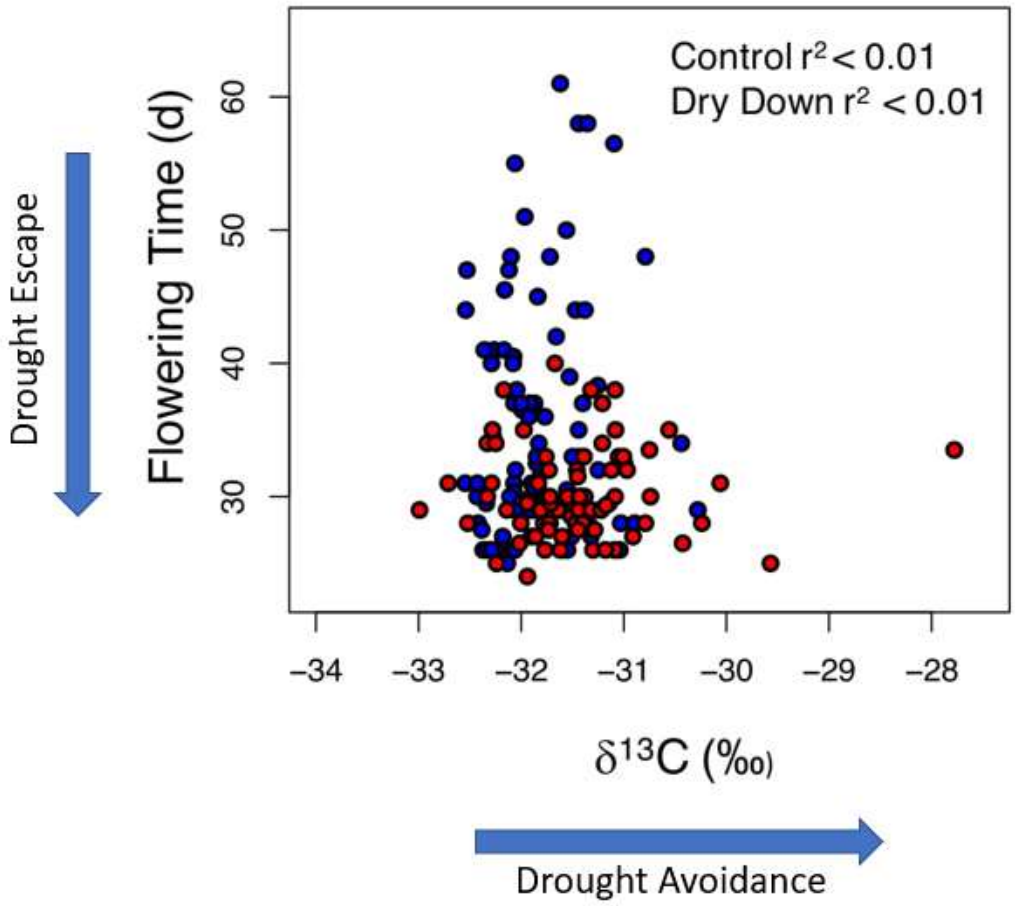

Figure 3: Correlation between drought escape trait (flowering time) and drought avoidance trait (δ13C) within each treatment group. Red dots refer to samples from dry down treatment. Blue dots refer to samples from control treatment.

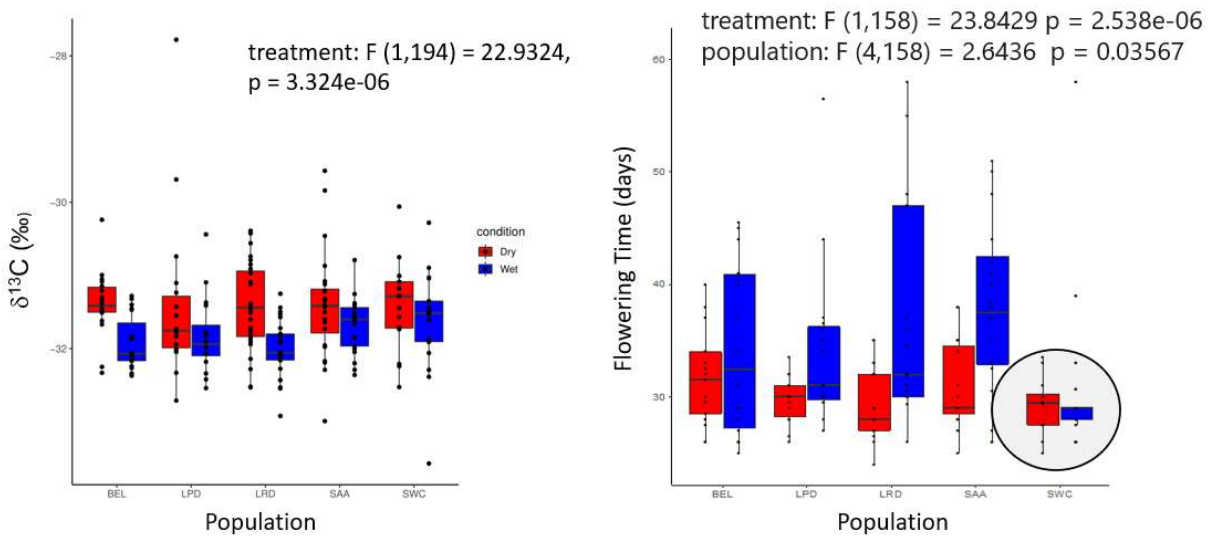

Figure 4: A) Plasticity and variation of δ13C between populations and between different treatments. B) Plasticity and variation of flowering time between populations and between different treatments. LPD and SWC are not statistically significant in graph B. The circled population in graph B is the population represented in Figure 5.

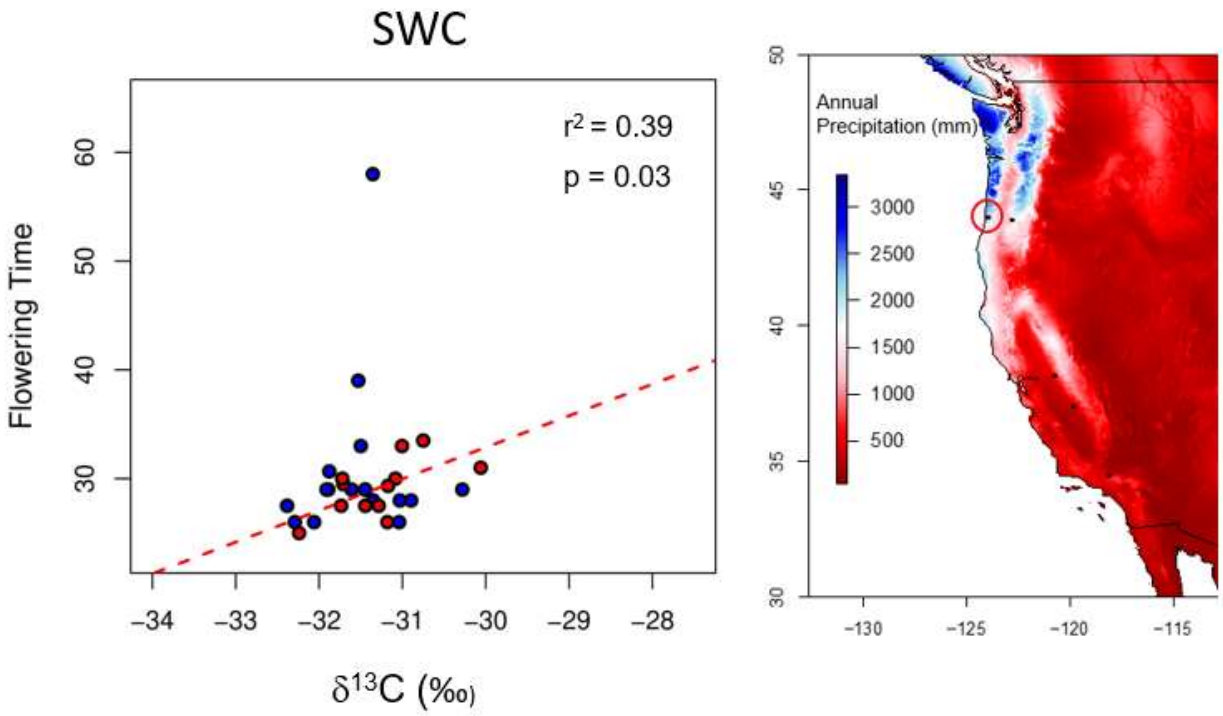

Figure 5: Correlations between drought escape and drought avoidance were population and treatment dependent. Correlation between drought escape and drought avoidance within a temperate, Oregon population within both control and dried-down treatment (A). Regression line represents the correlation within the dried down treatment. r^2 value and p-value represent the correlation within the dried down treatment. Red dots refer to lines from the dry down treatment. Blue dots refer to lines from the control treatment. B) Map of five populations used within experiment and annual precipitation levels as background raster. Black dots represent each population. SWC population is circled in red.

Discussion

This manipulative experiment reveals that substantial levels of variation exists in flowering time across the species range and a modest amount of within population variation exists in $\delta^{13}C$. Below we examine these patterns of variation in the context of evolution of drought resistance.

Substantial difference between wet and dry treatments for flowering time and $\delta^{13}C$ suggest that both of these traits are plastic and respond to water deficits. $\delta^{13}C$ was significantly higher in dry down conditions for all populations, indicating that plants have greater water use efficiency in the dry down conditions. However, we saw the most plasticity within the California populations for δ13C. This suggests that California populations may be able to adjust phenotypes based on the environmental conditions in a particular year to best utilize resources. This may be expected because the California populations live in harsher water-stress conditions and deal with more severe droughts than the Oregon populations. Having greater plasticity in drought resistant traits would better allow the population to survive long enough to reproduce when the more severe droughts occur.

However, plasticity in flowering time differed across populations with only the California populations having significant differences due to treatment. The CA populations exhibited earlier flowering time when subjected to water-stress condition. Also, there was higher variability of flowering time between the California populations. These results suggests that the California populations have a greater drought escape response to water-stress. The Oregon populations differed from the California

populations in response to water-stress. While these populations showed significant plasticity in response to the dry down treatment for $\delta^{13}C$, they did not show any significant plasticity or variation in relation to flowering time. Surprisingly, one of the populations (SWC) actually had earlier flowering in the control treatment. This is also the same and only population that exhibited a negative correlation between flowering time and δ13C. These results are similar to plasticity in flowering time and water use efficiency in *Arabidopsis thaliana*. In *A. thaliana*, there is significant plasticity in both flowering time and water use efficiency. However, these traits are negatively correlated with flowering time being the favored trait for combating drought. This means that it is common for earlier flowering to occur at the expense of water use efficiency (Kenney, McKay, Richards, & Juenger, 2014).

Our manipulative experiment revealed that the expected tradeoff between drought escape and drought avoidance was absent when pooling populations and treatments as well as when pooling within treatments. These results suggests that these traits can respond independently to future selection, such as the more frequent and severe droughts expected under climate change. However, when the data is broken down by population, one of the Oregon populations (SWC) exhibited the negative correlation, but only within the dry down treatment. These results could be explained due to the tradeoff with defense, independent evolution of traits, or for the control group having no water stress. Interestingly, the SWC population is also the wettest population in our experiment and occurs in the temperate rainforest of the Oregon coast. This may indicate that this population experiences the least water stress and may suggests that SWC may have experienced less historical selection on drought resistance strategies. Combining the fact

that the wettest population exhibits a negative correlation between flowering time and δ13C while the California populations do not, suggests that the genetic correlation between flowering time and δ13C may evolve to be lower in areas of severe and/or frequent water stress.

The negative phenotypic correlation measured may be heavily influenced by environmental conditions, specifically water-stress. This stressor may be vital to the presence of a phenotypic correlation. Without this stressor present, the phenotypic correlation between drought escape and drought avoidance may not occur which would explain why only the dried down treatment for the SWC population exhibited a phenotypic correlation.

Further research should include investigating defense traits and their relationship to drought escape and drought avoidance traits. While flowering time and $\delta^{13}C$ may have evolved independently of each other with these populations, defense may still be linked to one or both traits. If defense is as linked to both drought escape and drought avoidance, we would expect the California populations that had a positive correlation between δ13C and flowering time to have a tradeoff with plant defense. While it is possible for the tradeoff with defense to be between both drought escape and drought avoidance, we would expect to see a more significant tradeoff between defense traits such as PPGs and WUE since these are both physiological traits. Future studies should investigate the presence of phenotypic correlations between these three traits while also investigating whether these phenotypic correlations have a genetic basis. Discovering if the linkage between defense, drought escape, and/or drought avoidance varies between populations

would provide more information on how *Mimulus guttatus* evolves and responds to different environments.

Chapter 3: Heritability and Genetic Correlations Analysis

In this chapter, I explore the heritability, covariance, and genetic correlations of the different morphological and phenotypic traits of *Mimulus guttatus*. Heritability is an estimate that is used to determine how much of a trait is controlled by the expression of an organism's genes and thus the extent that a trait can be inherited. It is denoted as a value between zero and one with a value being closer to zero suggesting more of an environmental emphasis on the trait while a value closer to one suggests a higher emphasis from genetic variation on the trait. If heritability estimate is calculated to be a negative value, it suggests that the trait is not heritable. Heritability can be calculated two different ways. Narrow sense heritability refers to the proportion of phenotypic variance that is determined by the additive effect of genes from the parents (Falconer & Mackay, 2009). Narrow sense heritability is calculated by dividing the additive genetic variation in a trait by phenotypic variance in a trait. This is denoted by the equation $h^2 = V_a/V_p$. Broad sense heritability is another method of calculating heritability that focuses on how much of the phenotypic variance can be attributed to genotypic variation rather than allelic variation, i.e. this measure includes additive variation, dominance variation and epistatic variance (Falconer & Mackay, 2009). This is calculated by dividing the genotypic variance by the phenotypic variance. This is denoted by the equation $h^2= V_g/V_p$.

We chose to use narrow sense heritability as our method of calculation due to its distinct advantages. Narrow-sense heritability is the only measure that can be accurately used to predict responses to selection or to predict trait values between generations because both dominance variation and epistatic variance are not inherited through a single parent. That is, both dominance and epistasis require knowing both parent's genotypes and the interactions among alleles. If a trait is not heritable in a narrow-sense,

the trait cannot respond to selection pressures and produce offspring that can be better suited for those environmental selection pressures. We also can calculate narrow-sense heritability through an unmeasured genotype approach by examining phenotypic variation between related individuals. Specifically, the slope of a regression between midparent and offspring for a trait is equal to the narrow sense heritability of the particular trait.

While the heritability measures we discuss above typically refer to a single trait, some traits are negatively or positively correlated with each other, and this can also impact responses to selection. Phenotypic correlations refer to association between traits within a particular group or population. For instance, when individuals in a group have phenotype with high values, another phenotype also has high values or low values. A positive correlation would be when there is a positive association between traits while a negative correlation would be when there is a negative association between traits. Phenotypic correlations can be a result of environmental influence, genetic influence, or a combination of the two (Waitt & Levin, 1998). For instance, traits may occur in certain combinations because past selection within a population selects for those combinations. Alternatively, correlations among traits may occur because the two traits share a genetic basis or physical linkage within the genome. This type of correlation is referred to as a genetic correlation.

Two different genetic mechanisms can cause genetic correlations: 1) genetic correlations can occur when genes for each trait are located close to one other in the genome. Their close proximity to one another means they are more likely to be inherited in subsequent generations by avoiding being split up due to crossing over that occurs

during meiosis (Falconer & Mackay, 2009). Second, genetic correlations can be caused by pleiotropy where the same allele at a particular locus that increases one trait, decreases that other trait. While we explore the phenotypic and genetic correlations between traits within this chapter, we cannot untangle the mechanism underlying genetic correlations with our experimental design. Genetic correlations are important because they can also impact the response to selection of a population. They can do this by limiting adaptation through generations of heritable traits. Basically, traits can be limited in their response because of their genetic linkage to another trait (David L. Des Marais et al., 2013).

In this chapter, we explore patterns of heritability and trait correlations within *Mimulus guttatus*. There are currently no quantitative genetics studies that have documented whether drought escape and drought avoidance phenotypes are narrowly heritable or genetically correlated in *M. guttatus* despite the importance of these phenotypes in understanding the ecology of an important model system. Other plant evolutionary models that have examined the inheritance and genetic basis of drought escape and drought avoidance include the model system *Arabidopsis thaliana* (Kooyers, 2015; Mckay, Richards, & Mitchell-Olds, 2003). Within *Arabidopsis*, studies have identified a negative genetic correlation between drought escape and drought avoidance that manifests at the genetic level due to antagonistic pleiotropy (Kooyers, 2015).

We address four main questions using a basic quantitative genetics design. First, we examine the heritability of ecologically important traits, including those associated with drought escape and avoidance. Second, we address whether phenotypic correlations exist between traits and determine which of these phenotypic correlations were genetically based. Third, we investigate whether heritability and genetic correlations

between traits were similar or differed between populations that inhabit different environments. Finally, if there are differences across populations, we examine which factors predict this variation in heritability and genetic correlations.

Methods/Statistical Analysis

To measure heredity of traits, we used midparent-offspring regressions to determine the heritability of individual traits and to assess genetic correlations among traits. The parent generation was the well-watered treatment within the manipulative experiment from Chapter 2. We compared the mid-parent value (the average between the two parents for a specific trait) from the parental generation to the value of the same trait in the F_1 generation. This is one of the most accurate methods of conducting parent-offspring regressions in the absence of maternal effects. To construct the F_1 generation, we crossed lines within the control treatment of the dry down experiment. We crossed lines within populations and chose specific lines to maximize the variation in flowering time and $\delta^{13}C$. Specifically, we focused on crossing lines that held opposing extremes in flowering time and $\delta^{13}C$ because we wanted to incorporate the maximum amount of variance from our parent generation that could be included in our offspring population to avoid spurious correlations. Such spurious correlation could exist if we only chose lines that were specific to one end of the spectrum for a trait as this design conflate similarities due to environmental similarity with similarity due to relatedness.

We calculated heritability for all phenotypes measured in the parental generation in Chapter two. The list of phenotypes can be found in Chapter 2. R v4.1.1 was used to calculate heritability and genetic correlations between traits and create display plots.

Narrow sense heritability of a trait is equal to slope of regression line that describes the midparent and offspring association (Falconer & Mackay, 2009). We conducted linear regressions using the lm() function and extracted slopes and standard errors from the resulting summary of the linear regression. To be described as heritable, slopes must be positive and statistically different from zero.

We also calculated both phenotypic and genetic correlations between traits. We calculated phenotypic correlations by examining the Pearson correlation between phenotypes of individuals grown just in the wet treatment of the parent generation reported in Chapter two. Specifically, we used the the rcorr() function within the *Hmisc* package in R to compute Pearson correlations and assess whether correlations were statistically different from zero. To calculate genetic correlations between traits, the following equation was used:

$$r_A = \frac{cov_{xy}}{\sqrt{(cov_{xx}\, cov_{yy}}}$$

In this equation, cov_{xy} is the covariance between the two traits, cov_{xx} is the offspring-midparent covariance in trait one and cov_{yy} is the offspring-midparent covariance in trait two. A positive genetic correlation indicates that the two traits are often inherited together and individuals with higher values of one trait will have higher values of the second trait. A negative genetic correlation indicates that individuals with high values of one trait will have low values of the other trait. If no genetic correlations between traits are present, that means the traits could respond to selection independently of one another.

Results

Heritability

In analyses pooling all populations, we found that most of our traits had moderate to high heritability (Table 1, Figure 1). Flowering time had a moderate heritability with 0.51 (se: 0.08) suggesting that drought escape can respond to selection. $\delta^{13}C$ had a low-moderate heritability with 0.27 (se: 0.1) indicating the drought avoidance also can be selected between generations. The majority of the morphological traits had to moderate-high levels of heritability. Plant height had the highest heritability of all traits with 0.85 (se: 0.06). Flowering node also had a high heritability with 0.67. Leaf area, wet leaf mass, and dry leaf mass both had moderate levels of heritability with 0.53, 0.52, and 0.44, respectively. Leaf number had a low heritability with 0.28 All three corolla measurements exhibited moderate to high heritability. The only traits that did not exhibit any heritability were succulence and branch number. Most of the traits measured exhibited some level of heritability which indicates that they could potentially respond to selection pressures.

Phenotypic and genetic correlations

We pooled phenotype data from individuals across populations and examined the phenotypic correlations between traits during the parent generation. We found no significant phenotypic correlation between flowering time and $\delta^{13}C$ suggesting no presence of a correlation between drought escape and drought avoidance (Table 2).

Flowering time had moderate phenotypic correlations with plant height (r = 0.44) and leaf number (r = 0.6). Flowering time and flowering node were highly correlated (r = 0.82). Flowering time also had a low phenotypic correlation with corolla measurements. The main drought avoidance trait, $\delta^{13}C$, exhibited no significant phenotypic correlations with any other trait. Outside of drought resistant traits we found many significant phenotypic correlations between morphological traits. Generally, larger plants had larger corollas, flowered at higher nodes, had larger leaves, and more leaves at flowering. Plant height had moderate phenotypic correlations with branch number, leaf number, flowering node, leaf area, wet and dry leaf mass, and corolla measurements as would be expected for traits all involved in plant architecture. Many of these morphological traits also had some phenotypic correlations with the other traits listed above.

We found that many phenotypic correlations were genetically based and that there were also genetic correlations present when no phenotypic correlation was present (Table 2 and Table 3). With regards to drought escape and drought avoidance, we saw a slight negative genetic correlation with a value of -0.1. This suggests that $\delta^{13}C$ and flowering time have little to shared genetic basis and may be independently influenced by genetic and environmental factors. Drought escape traits were genetically correlated with many morphological traits. That is, flowering time had significantly higher genetic correlations with plant height, leaf number, node, and corolla measurements compared to the phenotypic correlations (Table 2 and Table 3). While $\delta^{13}C$ did not exhibit any phenotypic correlations with other traits, it did exhibit genetic correlations with some of these traits. $\delta^{13}C$ shared moderate genetic correlations with plant height (-0.42), leaf number (-0.48), and flowering node (-0.3). $\delta^{13}C$ also had high genetic correlations with

all three corolla measurements. All genetic correlations between $\delta^{13}C$ and morphological traits were negative and statistically significant. Morphological traits also exhibited substantial genetic correlations with each other. Interestingly, most genetic correlation was significantly higher than the phenotypic correlation (Table 2 and Table 3)

Variability with heritability and genetic correlations among populations

We found that several traits varied significantly among populations in heritability (Figures 2, 3, and 4). Many of these significant differences occurred between the Oregon populations (LPD, SWC) and the California populations (BEL, SAA, LRD). However, the populations that live in similar environments such as the Sierra populations (BEL and SAA) had similar heritabilities and genetic correlations for drought resistance and morphology. With regards to drought escape, we saw that the California populations all had similar heritabilities for flowering time. BEL had a heritability of 0.34 se: 0.16, LRD had 0.34 se: 0.13, and SAA had 0.3 se: 0.22 (Figure 2). The Oregon populations on the other hand, had different heritabilities for flowering time with LPD having a heritability of 0.06 se: 0.3 and SWC having a heritability of 0.61 se: 0.24. With regards to drought avoidance, we found that all three California populations had negative heritabilities for $\delta^{13}C$, which suggests no heritability of this trait within those populations (Figure 3). The Oregon populations also had low heritabilities of this trait with error estimate overlapping with zero (LPD: 0.13 se: 0.19; SWC: 0.20 se: 0.20). These highly variable estimate suggests there is limited heritability in drought avoidance in any population and suggests we may need higher sample sizes to detect differences.

Not only did we find variation in heritability of drought resistant traits, but we also found that heritability of some morphological traits differed between populations. The California populations had higher heritabilities of plant height than the Oregon populations with BEL, LRD, and SAA having heritabilities of 0.75 se: 0.18, 0.98 se: 0.27, and 0.84 se: 0.15 (Figure 4). Heritabilities for plant height within the Oregon populations, LPD and SWC, were 0.60 se: 0.16 and 0.33 se: 0.22, respectively. The opposite was true in relation to leaf number with the Oregon populations having higher heritability such as LPD having 0.55 +/ 0.33 and SWC having 0.72 se: 0.16. The California populations in comparison had BEL with 0.19 se: 0.2, LRD with 0.13 se: 0.13, and SAA with a negative heritability suggesting no heritability within that population.

We also found significant variation with the presence of genetic correlations between flowering time and $\delta^{13}C$ between populations. The Oregon populations had similar genetic correlation values that did not differ from zero in SWC (-0.02) and LPD (-0.1). The Sierra populations had also had similar values as each other with BEL having -0.92 and SAA having -0.7. LRD differed significantly from all other populations with a positive correlation of 0.62.

Heritability and genetic correlations vary based on environmental differences

We found that both heritability and genetic correlations of drought escape and drought avoidance differ significantly between the California populations and the Oregon populations. While the California populations had a similar heritability for flowering time

(all ~0.3), the Oregon populations differed significantly with SWC having a heritability of 0.61 se: 0.24 and LPD having a heritability of 0.06 se: 0.23. SWC is more of a coastal population while LPD is an inland population. The differences occurring not only between the California and Oregon populations but also within the Oregon populations suggest that heritability is variable across the range and does not associate with any geographic or climatic patterns. For $\delta^{13}C$, we found that the CA populations had no heritability while LPD had a heritability of 0.1 and SWC had 0.6 (Figure 3) This also suggests that heritability of drought avoidance in *Mimulus guttatus* varies across the range.

The correlation between $\delta^{13}C$ and flowering time seems to be associated with specific environmental differences and not necessarily by latitude. There is no correlation between the presence of magnitude of the genetic correlation between drought escape and avoidance with latitude. This lack of association is driven by the lowest latitudinal population (LRD), which does not fit the trend of the rest of the populations. LRD is the only population to exhibit a positive correlation between $\delta^{13}C$ and flowering time (table 2) LRD is also a unique population in terms of environment as it has the highest annual climate moisture deficit and annual heat-moisture index. This suggests that LRD is the driest environment of all the populations in our experiment. A positive correlation between flowering time and $\delta^{13}C$ would suggest that plants could evolve to both flower quickly and be efficient with their water usage - a potential benefit in a very dry environment. Alternatively, LRD is located in a temporarily flooded stream which could mean that it receives more water than the Sierra populations (SAA and BEL). The

positive association between drought avoidance and escape in LRD suggests that there are more underlying factors that determine drought resistant than initially considered.

Table 1. Heritability of morphological and phenotypic traits from all populations

Trait	Hereditary Estimate	Hereditary Estimate Std. Error	P-value	F-value	Degrees of Freedom
Flowertime	0.51	0.08	0.001*	41.05	1 and 232
Plant Height	0.85	0.06	0.001*	191.7	1 and 232
Branch Number	0.23	0.14	0.104	2.66	1 and 232
Leaf Number	0.28	0.07	0.001*	15.44	1 and 232
Corolla Length	0.67	0.07	0.001*	94.21	1 and 232
Corrolla Width	0.7	0.08	0.001*	84.24	1 and 232
Corolla Height	0.5	0.08	0.001*	39.47	1 and 232
Node	0.67	0.08	0.001*	88.75	1 and 232
Leaf Area	0.53	0.12	0.001*	20.9	1 and 242
Wet Leaf Mass	0.52	0.15	0.001*	12.39	1 and 242
Dry Leaf Mass	0.44	0.1	0.001*	20.65	1 and 242
Succulence	0.08	0.09	0.361	0.84	1 and 242
SLA	0.14	0.05	0.006	7.66	1 and 242
δ13C	0.27	0.1	0.006	7.75	1 and 235
δ13C%	-0.16	0.09	0.093	2.84	1 and 235
δ13N	0.06	0.06	0.295	1.1	1 and 223
δ13N%	0.27	0.09	0.003	9.3	1 and 223

Heritability less than zero refers to no heritability present. An asterisk refers to a p-value < 0.001.

Table 2: Phenotypic correlations between morphological and physiological traits

	flowertime	plant height	branch number	leaf number	corolla length	corolla width	corolla height	node	leaf area	wet leaf mass	dry leaf mass	succulence	SLA	d13C	d15N
flowertime	1	0.44**	0.16	0.6**	0.21*	0.25*	0.23*	0.82**	0.16	0.24*	0.13	0.05	0.01	0.13	-0.29*
plant height	0.44	1	0.41**	0.52**	0.64**	0.58**	0.53**	0.66**	0.62**	0.54**	0.57**	-0.05	-0.06	0.04	0.08
branch number	0.16	0.41**	1	0.75**	0.22*	0.24*	0.29*	0.33*	0.5**	0.43**	0.47**	-0.02	-0.15	-0.03	0.27*
leaf number	0.6	0.52**	0.75**	1	0.34**	0.28*	0.3*	0.72**	0.44**	0.41**	0.4**	-0.07	-0.08	-0.02	0.08
corolla length	0.21*	0.64**	0.22*	0.34**	1	0.66**	0.58**	0.43**	0.56**	0.46**	0.52**	-0.03	-0.07	-0.04	0.1
corolla width	0.25*	0.58**	0.24*	0.28*	0.66**	1	0.75**	0.4**	0.45**	0.43**	0.46**	-0.03	-0.16	0.06	0
corolla height	0.23*	0.53**	0.29*	0.3*	0.58**	0.75**	1	0.36**	0.39**	0.42**	0.42**	0.03	-0.1	0	0.05
node	0.82**	0.66**	0.33*	0.72**	0.43**	0.4**	0.36**	1	0.4	0.47	0.36**	0.02	-0.03	0.06	-0.08
leaf area	0.16	0.62**	0.5**	0.44**	0.56**	0.45**	0.39**	0.4**	1	0.84	0.9**	-0.11	-0.18*	0.02	0.33**
wet leaf mass	0.24*	0.54**	0.43**	0.41**	0.46**	0.43**	0.42**	0.47**	0.84**	1	0.85**	0.1	-0.23*	0.11	0.33**
dry leaf mass	0.13	0.57**	0.47**	0.4**	0.52**	0.46**	0.42**	0.36**	0.9**	0.85**	1	-0.18*	-0.34**	0.11	0.31**
succulence	0.05	-0.05	-0.02	-0.07	-0.03	-0.03	0.03	0.02	-0.11	0.1	-0.18*	1	0.5**	-0.02	0.04
SLA	0.01	-0.06	-0.15	-0.08	-0.07	-0.16	-0.1	-0.03	-0.18*	-0.23*	0.36**	0.5**	1	-0.12	-0.09
d13C	0.13	0.04	-0.03	-0.02	-0.04	0.06	0	0.06	0.02	0.11	0.11	-0.02	-0.12	1	0.08
d15N	-0.29*	0.08	0.27*	0.08	0.1	0	0.05	-0.08	0.33**	0.33**	0.31*	0.04	-0.09	0.08	1

Values with one asterisk represent p-value < 0.05 while values with two asterisks represent p-values < 0.001.

Table 3: Genetic correlations between traits

Population	Trait Combinations	Genetic Correlation	Covariance between traits	Covariance of 1st trait	Covariance of 2nd trait
All	Flowertime x Plant Height	0.71	7.15	12.18	8.4
All	Flowertime x δ13C	-0.1	-0.07	12.18	0.03
All	Flowertime x SLA	-0.34	-78.13	12.18	4353.87
All	Flowetime x Leaf Number	0.74	8.84	12.18	11.58
All	Plant Height x δ13C	-0.42	-0.22	8.4	0.03
All	Plant Height x SLA	-0.29	-56.03	8.4	4353.87
All	Plant Height x Leaf Number	0.95	9.37	8.4	11.58
All	δ13C x SLA	-0.17	-2.12	0.03	4353.87
All	δ13C x Leaf Number	-0.48	-0.3	0.03	11.58
All	SLA x Leaf Number	-0.48	-108.13	4353.87	11.58
All	Flowertime x Corolla Length	0.53	0.29	12.18	0.02
All	Flowertime x Corolla Width	0.39	0.32	12.18	0.06
All	Flowertime x Corolla Height	0.63	0.39	12.18	0.03
All	Flowertime x Node	0.87	2.59	12.18	0.73
All	Plant Height x Corolla Length	0.85	0.38	8.4	0.02
All	Plant Height x Corolla Width	0.67	0.46	8.4	0.06
All	Planth Height x Corolla Height	0.73	0.38	8.4	0.03
All	Plant Height x Node	0.86	2.12	8.4	0.73
All	δ13C x Corolla Length	-0.8	-0.02	0.03	0.02
All	δ13C x Corolla Width	-0.63	-0.03	0.03	0.06
All	δ13C x Corolla Height	-0.69	-0.02	0.03	0.03
All	δ13C x Node	-0.3	-0.05	0.03	0.73
All	SLA x Corolla Length	-0.31	-3.19	4353.87	0.02
All	SLA x Corolla Width	-0.25	-3.89	4353.87	0.06
All	SLA x Corolla Height	-0.25	-2.91	4353.87	0.03
All	SLA x Node	-0.51	-28.44	4353.87	0.73
All	Leaf Number x Corolla Length	0.75	0.39	11.58	0.02
All	Leaf Number x Corolla Width	0.53	0.43	11.58	0.06
All	Leaf Number x Corolla Height	0.66	0.4	11.58	0.03
All	Leaf Number x Node	0.97	2.82	11.58	0.73
All	Corolla Length x Corolla Width	0.8	0.03	0.02	0.06
All	Corolla Length x Corolla Height	0.75	0.02	0.02	0.03
All	Corolla Length x Node	0.71	0.09	0.02	0.73
All	Corolla Width x Corolla Height	0.9	0.03	0.06	0.03
All	Corolla Width x Node	0.6	0.12	0.06	0.73
All	Corolla Height x Node	0.74	0.11	0.03	0.73
BEL	Flowertime x δ13C	-0.92	0.13	6.82	-0.003
LPD	Flowertime x δ13C	-0.1	-0.01	0.59	0.02
LRD	Flowertime x δ13C	0.62	-0.2	15.65	-0.01
SAA	Flowertime x δ13C	-0.7	0.29	26.78	-0.01
SWC	Flowertime x δ13C	-0.02	-0.01	5.75	0.03

Genetic correlations and covariances between different combinations of traits as well as the heritability of each individual trait. Data pooled from all populations except for bottom five trait combinations that only looked at a comparison within a population. These five data points are labeled with their respective populations.

Figures

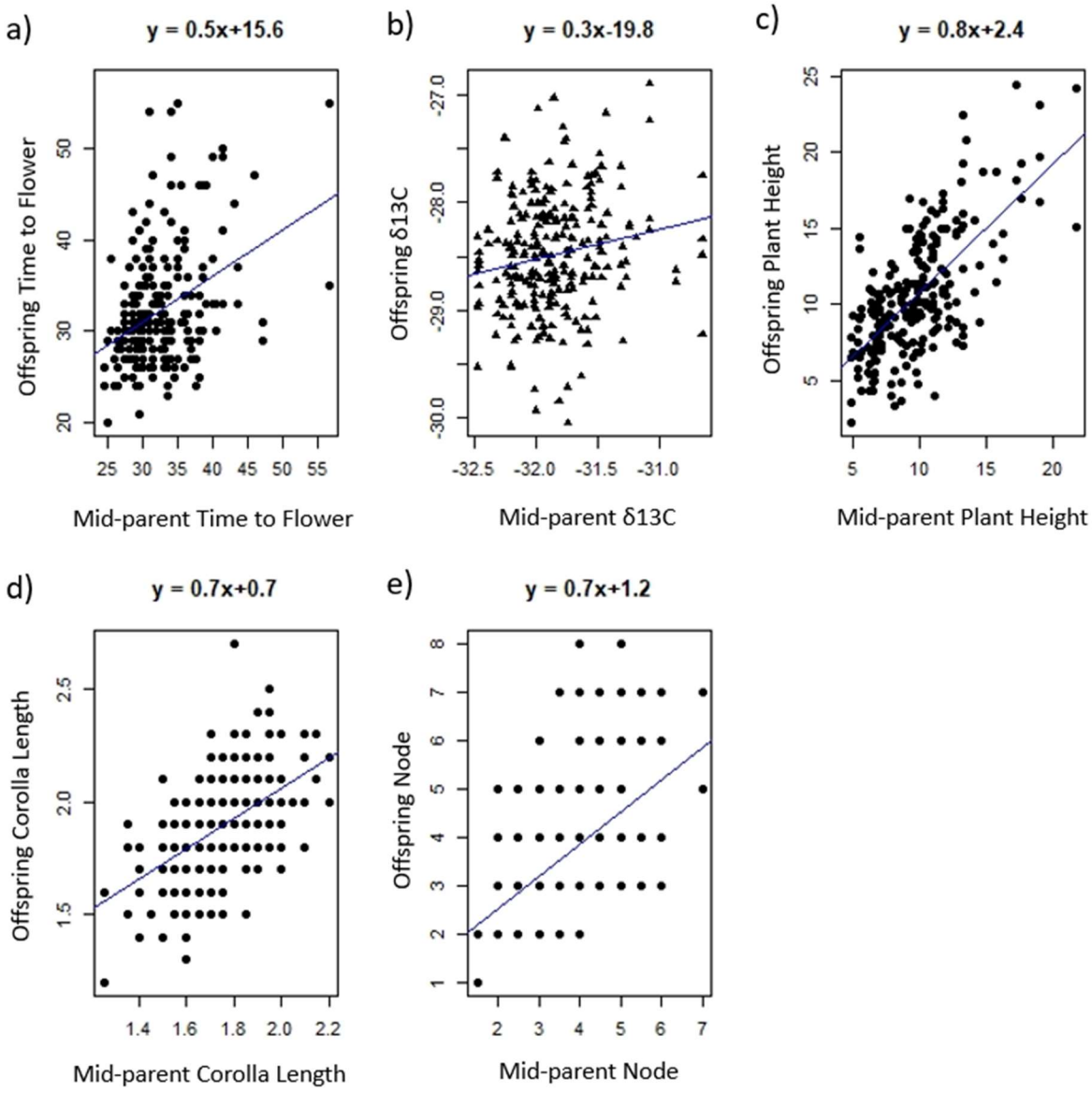

Figure 1. Linear regressions of mid-parent values from parental generation and offspring values from F_1 generation for flowering time (A), $\delta^{13}C$ (B), plant height (C), flowering node (D), and corolla length (E). These regressions pooled lines from all populations. The equation for linear regression is at the top of the graph. The slope of the line represents the heritability of the trait.

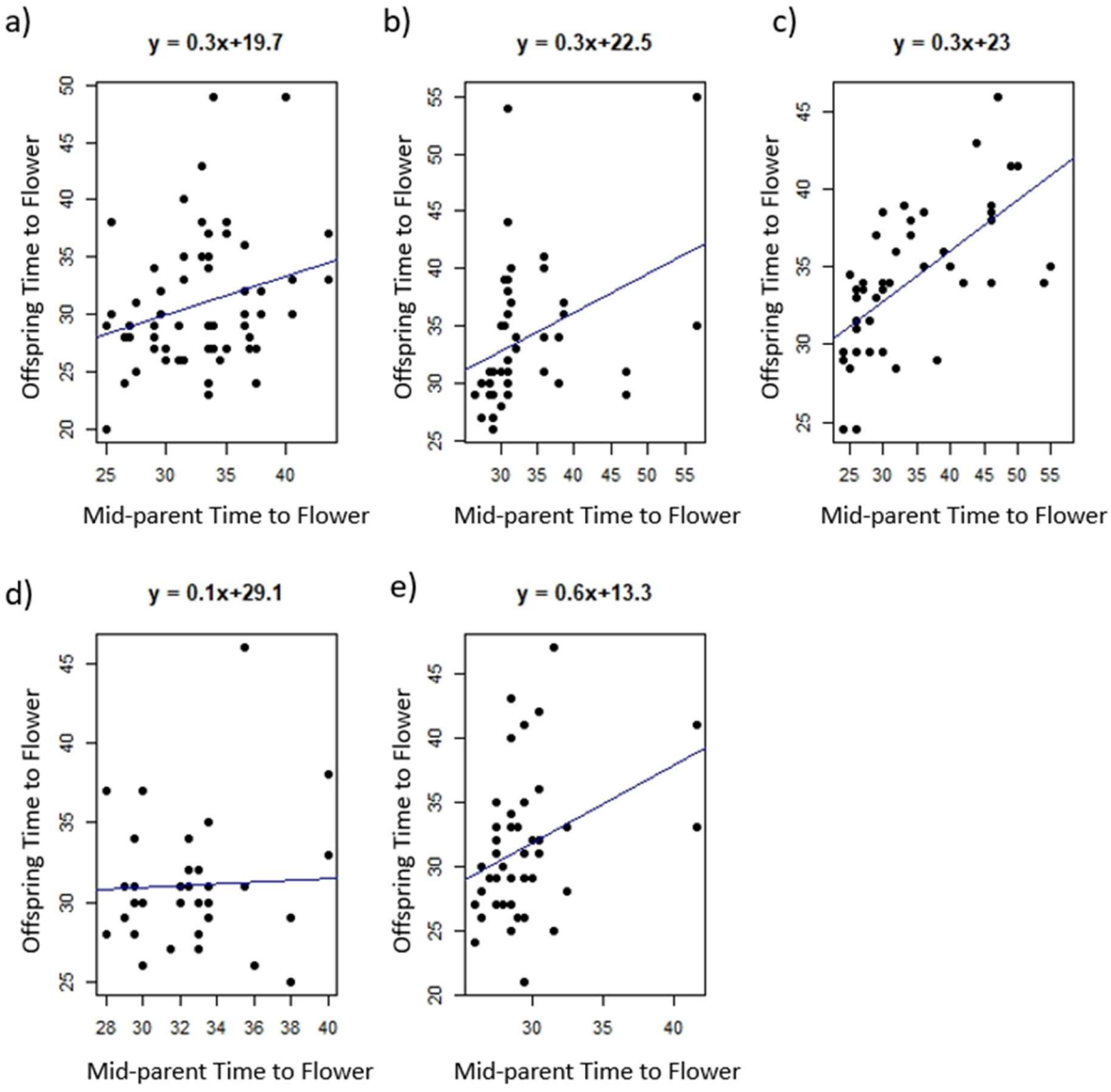

Figure 2. Linear regression between mid-parent flowering time and corresponding offspring's flowering time from the F_1 generation within BEL (A), LRD (B), SAA (C), LPD (D), and SWC (E). The equation for linear regression is at the top of the graph. The slope of the line represents the heritability of the trait.

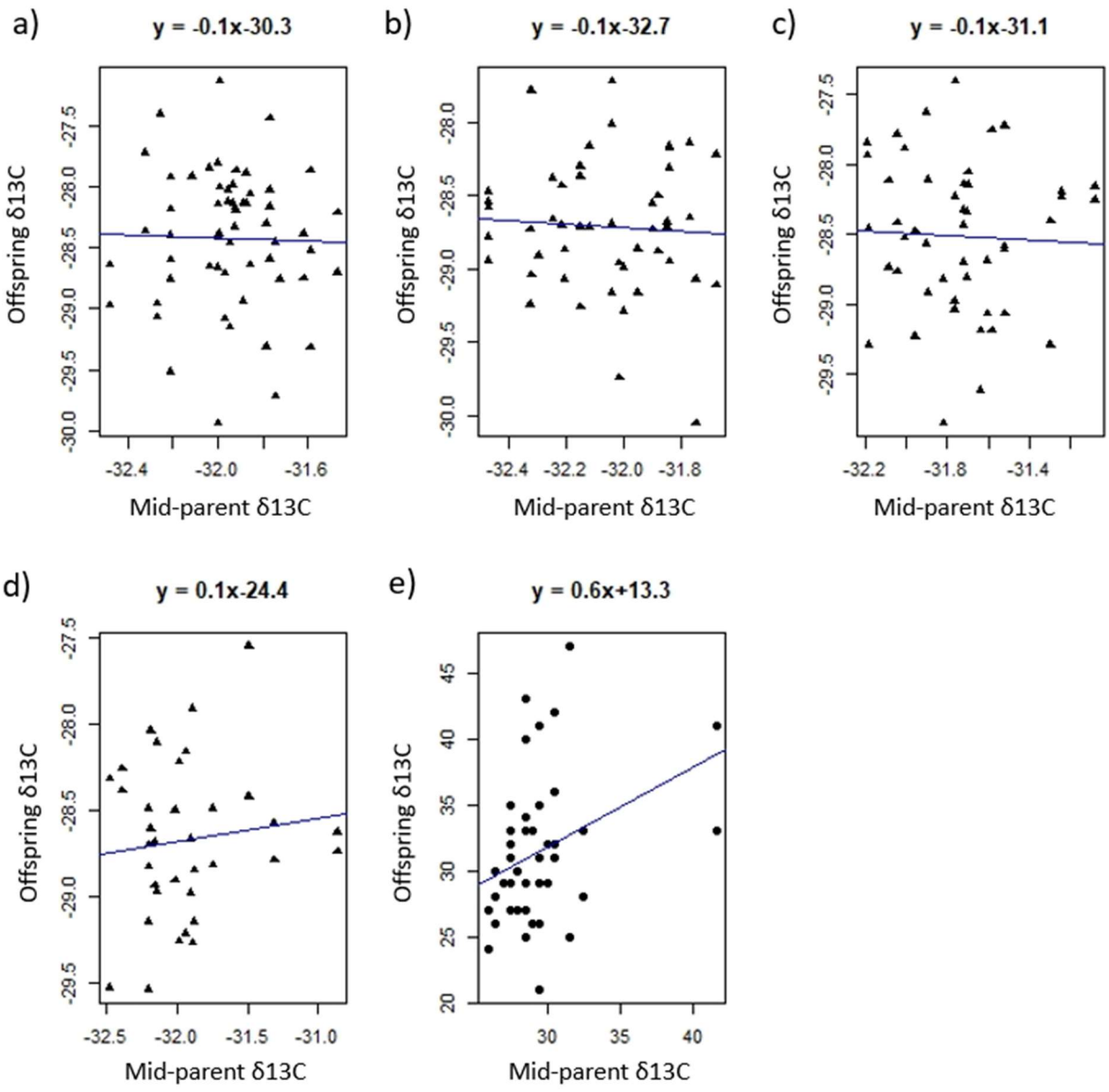

Figure 3. Linear regression between mid-parent $\delta^{13}C$ and the corresponding offspring's $\delta^{13}C$ content from the F_1 generation within BEL (A), LRD (B), SAA (C), LPD (D), and SWC (E). The equation for linear regression is at the top of the graph. The slope of the line represents the heritability of the trait.

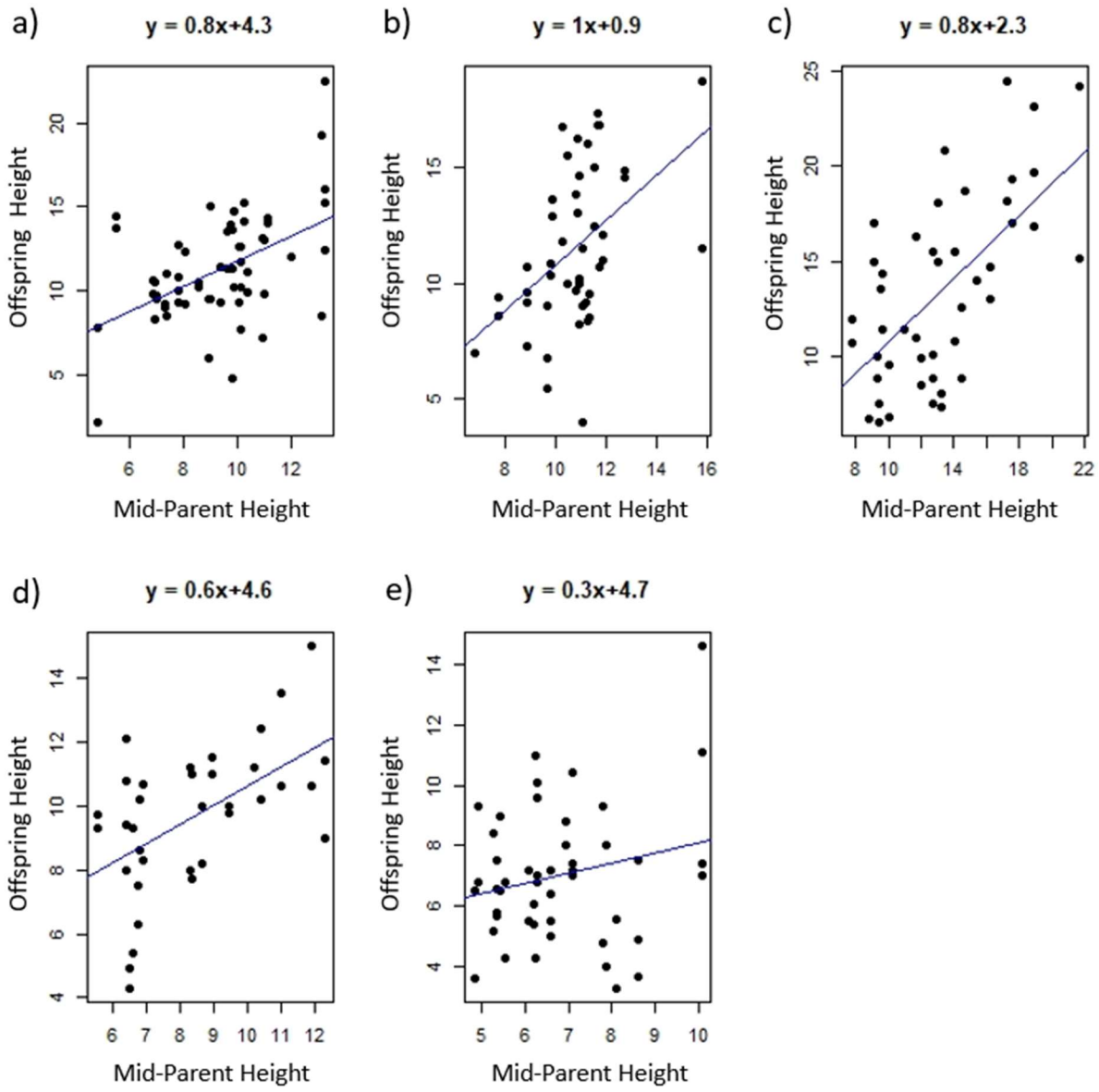

Figure 4. Linear regression between mid-parent plant height value and the offspring plant height from the F1 generation within BEL (A), LRD (B), SAA (C), LPD (D), and SWC (E). The equation for linear regression is at the top of the graph. The slope of the line represents the heritability of the trait.

Discussion

In this chapter, we find that most traits were heritable and were at least moderately phenotypically and genetically correlated with one another. However, the scale which we conducted these analyses mattered - that is, results when pooling populations were not necessarily the same as within each population. Below we discuss these results in the context of our expectations and results in other systems.

From previous literature, we expect morphological traits to exhibit higher heritability than physiological traits (Roff & Mousseau, 1987). This expectation held mostly true with our results. All morphological traits except for branch number were moderately to highly heritable (Table 1) and had higher heritability than physiological traits such as $\delta^{13}C$. Alternatively, of the traits we considered physiological traits, only $\delta^{13}C$ and $\delta^{15}N$ were heritable and had lower heritability compared to the morphological traits. Some traits, such as relative water content, we may not expect to be heritable as these traits are largely based on the environment of the plant (e.g. the amount of water deficit that the plant has experienced). The high heritability of the morphological traits suggest that these traits could all respond to future selection while physiological traits may be more constrained.

One of our most interesting results was observing variation in heritability between populations that appears to be structured by geography. This was most apparent in measure of $\delta^{13}C$ - our proxy for water use efficiency and drought avoidance. The California populations exhibited no heritability for $\delta^{13}C$ (Figure 3). The Oregon populations varied in their $\delta^{13}C$ heritability measurements with the LPD exhibiting low

heritability and SWC exhibiting a moderately high heritability. The lack of heritability in $\delta^{13}C$ within the California populations suggests that the California populations could have a higher plastic response to water deficits compared to the Oregon populations. This also matches the results of the dried down experiment in Chapter 2 where the California populations showed more plasticity in $\delta^{13}C$ based on the water-stress conditions.

An important caveat when discussing our quantitative genetic experiments and variation between populations in heritability and genetics correlations is our limited sample size There were some traits that exhibited a moderate to high level of heritability or genetic correlation that were not statistically significant. This was expected with the sample sizes of our populations and the limitations of conducting our experiments in a growth chamber. Future experiments should use >100 maternal lines within each population instead of ~30 unique lines we used for each of our populations.

While we saw no genetic or phenotypic correlation between drought escape and drought avoidance overall, there are significant genetic correlations present within populations. While the Oregon populations showed slight to no correlation in LPD (-0.1) and SWC (0.02), the California populations all showed significant correlations. The Sierra populations had a high genetic basis with BEL and SAA exhibiting -0.92 and -0.7 values while the LRD population exhibited the only positive genetic correlation with 0.62. This data may suggest that genetic correlations can evolve either greater or lower values based on long term patterns of selection corresponding to specific environments. The presence of a positive correlation in the LRD populations suggests that this population may have evolved to use both drought escape and drought avoidance to combat drought. One possible cause of the positive correlation in the LRD population

may be due to a possible third tradeoff with defense (Kooyers, Blackman, & Holeski, 2017). If this is the case, defense levels would be much lower in the LRD population compared to the other California and Oregon populations as this population is putting more of its resources into growth and reproduction. Future studies should also measure chemical and physical defense traits while measuring drought escape and drought avoidance traits to explore this three-way tradeoff. There could also be other underlying factors that could influence drought resistant traits that have yet to be considered (i.e. role of nitrogen limitation within drought responses). Further studies should focus on more physiological traits related to stomata such as instantaneous WUE and stomatal conductance to further look into the physiological tradeoff between drought escape and drought avoidance traits. Future studies should also investigate root traits to see how much water uptake is occurring during drought conditions and compare these values to drought escape and drought avoidance traits.

Chapter 4: Conclusions and Future Directions

The manipulative common garden experiment described in Chapter two and the quantitative genetic experiments in Chapter three revealed important conclusions regarding the inheritance and variation in important ecological traits. Chapter two described the variation for each trait within and among populations as well as determining whether there was plasticity in each trait that corresponded to water availability. Chapter three described the genetic basis for traits regarding the heritability of traits across populations and within populations as well as the presence of genetic correlations and their influence on phenotypic correlations. These experiments were most specifically targeted at understanding patterns of variation and inheritance in drought escape and avoidance strategies. We find that these strategies varied among populations, responded to differences in water availability, and had at least some element of heritability. The paragraphs below synergize our results from both chapters and discuss the importance of our results for understanding local adaptation and response to selection.

We found no significant variation in drought avoidance plasticity between different populations. All populations had increased drought avoidance response under water-stress conditions. However, we found significant variation in drought escape response/plasticity between the California and Oregon populations. The California populations showed significantly earlier flowering when under the water-stress conditions while the Oregon populations exhibited no significant difference between the control and dried-down treatment. This may be explained by the environments that the California and Oregon populations are found in. Since the California populations have historically experienced greater aridity and more severe drought conditions, their ability to have plasticity in both drought avoidance and drought escape is beneficial to surviving

the harsh conditions (Wang et al., 2016). On the other hand, the Oregon populations are found in a more temperate environment and experience less harsh drought conditions (Wang et al., 2016). Therefore, it may not be beneficial for them to have differences in flowering time based on water stress treatment. Also, another explanation could be that the Oregon population already flower earlier than the California populations. The earlier flowering in this population may already be enough to combat water-stress. Therefore, flowering earlier may not be much more beneficial as it would require and divert more resources toward quicker development and away from other physiological and morphological traits.

Like plasticity, we also saw variation in heritability. Overall, the heritability of all populations pooled together for flower time was moderate with 0.51, while d13C exhibited low heritability of 0.27. However, when we break it down by populations, we find variation between the Oregon and California populations. The narrow sense heritability calculation of WUE showed that the California populations had no heritability regarding $\delta^{13}C$ while the Oregon populations were heterogeneous with the SWC population having high heritability and LPD having low heritability. When examining flowering time, the results are a little more ambiguous. All three California populations exhibited the same heritability of 0.3 while the Oregon populations had more variation with LPD exhibiting low heritability and SWC exhibiting high heritability. These heritability measures tell us how much genetic variation there is between these populations (Falconer & Mackay, 2009). Heritability is also required for selection based on environment to occur. The fact that there is heterogeneity between the Oregon populations suggests that environmental conditions cause selection pressures which may

lead to changes in heritability or divergent selection of these drought resistant traits. Together these results suggest that flowering time is both heritable and responds to drought in all populations while $\delta^{13}C$ responds to drought in all populations but heritable in only in a single population. These results suggest there are limits on the drought escape and avoidance phenotypes that can evolve within each population.

While heritability is necessary for responses to selection to occur, responses to selection can be further limited by the presence of genetic correlations (Falconer & Mackay, 2009; Sgrò & Hoffmann, 2004). In order to see how genetic correlations may affect selection responses, we first looked at the presence of phenotypic correlations and then whether phenotypic correlations reflected genetic correlation. All significant phenotypic correlations resulting from when we pooled data from all populations measured in our manipulative drought experiment exhibited a genetic correlation when comparing the midparent values from the manipulative experiment to the offspring. Interestingly, the values of these genetic correlations were higher than the corresponding phenotypic correlations. The increased genetic correlation value compared to the phenotypic correlations could be a product of selection within the populations against the direction of the genetic correlation. That is, if a genetic correlation between flowering time and $\delta^{13}C$ is negative and selection is for both earlier flowering and higher water use efficiency, the phenotypic correlation would be less negative than the genetic correlation. Future phenotypic selection experiments in the field could be used to tested the direction of selection on each trait to determine whether this above hypothesis is correct.

We also found variation in genetic correlations between the Oregon and California populations. The Oregon populations have little to no genetic correlation

between flowering time and δ13C, while the California populations all had a significantly higher genetic correlations between these two traits. This may have resulted due to interactions with the environment. The more extreme drought conditions of the California populations may have caused selection for both drought escape and drought avoidance responses in independent populations. This provides a hypothesis of how local adaptation could be proceeding and that responses to environment can shape the presence of genetic correlations, type of correlation, and the influence that a genetic correlation has on the expression of traits (David L. Des Marais et al., 2013).

Considering the results of variation, heritability, and genetic correlations from our manipulative drought experiment, our results suggest that selection has shaped these traits across populations. The pattern of selection we expected was higher expression of drought resistant traits in more stressful conditions across in different environments. We see evidence of selection because of the variation of physiological and morphological traits between populations, especially regarding flowering time. We also see that this variation is associated with specific environmental differences as the California populations have more similarities to each other regarding genetic correlations, trait value, and heritability. The same can be said within the Oregon populations as well. However, we see major differences between the California and Oregon populations. This suggests that populations are locally adapting to their specific environments.

The results of our experiment and analysis are important in helping us predict how populations of *Mimulus guttatus* will respond to climate change. Climate change is on track to both bring warmer temperatures and drier seasons to the Western United States where the populations of *Mimulus guttatus* we used in this study were found (Cook

et al., 2018). The variability and differences we found in plasticity, heritability, and correlations between the populations can help us understand potential responses. Plasticity may be better suited in providing relief to short term extreme events such as a drought. California populations are more plastic in responses to drought, therefore they may be able to withstand relatively normal droughts in the future (Chevin & Hoffmann, 2017). However, plasticity of drought resistant traits has its limitations, therefore they may not be able to withstand drought outside of their historic conditions. For example, if a drought one year is too severe, the plastic response may not be able to withstand the extreme environment and may lead to the extirpation of that population. While plasticity is useful for withstanding short-term extreme events, it is not well suited in adapting to extreme droughts that may exceed any previous historic drought. Heritability is needed to adapt to long term extreme events. Oregon populations may be able to respond to selection more quickly since they have higher heritability of drought resistance traits relative to the California populations. The Oregon populations are also located in a temperate environment compared to a xeric environment where the California populations are located (Wang et al., 2016). The northern temperate environment would not be affected by climate change as much as a southern xeric environment, allowing the Oregon populations enough time to adapt and respond to climate change.

Before conclusions and predictions can be drawn on how certain populations will fare in response to climate change, more experimentation is needed. We still have little understanding on some of the underlying factors that may play a role in the expression of both drought escape and drought avoidance traits and how they correlate with other drought resistance strategies as well as morphology. Future studies should include more

populations from both Oregon and California located in a variety of environments ranging from dry to mesic populations and from coastal to inland locations. This larger range and variety of populations will help provide a more accurate depiction on the heritability and variation of both drought resistant traits and morphology and how this can help better understand the local adaptation behind these traits. Future experiments should also measure other traits such as mortality rates in response to different levels of drought to understand what populations are threatened by climate change. This would provide us the data needed to predict what extremes the plastic responses from both the California and Oregon populations can withstand and compare them to the expected future climates.

Finally, future experiments should also further explore genetic correlations with a QTL mapping experiment to discover the locations within the genome that correlate with each other. This type of experiment would provide a better look at the genetic correlations and determine whether genetic correlations are caused by genetically linked or controlled by pleiotropy. It can also be used to look at the genetic basis on how much variation in plasticity of drought resistant traits is present as well as the variation in constitutive differences in those same traits (Gutteling et al., 2007). All of these directions would provide a better understanding of how drought escape and drought avoidance has evolved in different environments and also provide more information that can help us predict the effects of climate change on plants that rely on these drought resistant strategies.

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