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Variation in soil-mediated drought response traits between sympatric species of *Mimulus*

A Thesis Presented

by

Anna Reitman

To the Keck Science Department

of

Claremont McKenna, Scripps, and Pitzer Colleges

In Partial Fulfillment of

The Degree of Bachelor of Arts

Senior Thesis in Environmental Science

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Table of Contents

Abstract	3
Acknowledgments	4
Introduction	5
Materials and Methods	11
Plant lines	11
Analysis of soil texture classes and organic inputs	11
Between treatment water capacity testing	12
Seed planting, germination, and incubation	12
Between treatment species survival in dry down	13
Species response to long-term, punctuated drought	13
Measuring drought response traits	14
Data analysis	14
Results	16
Sand shows significant influence on water capacity and resulting drought severity	16
<i>M. micranthus</i> is more resilient to frequent, mild droughts than <i>M. guttatus</i>	17
Analysis of numerous traits confirms phenotypic differences common to animal- vs. self-pollinated species	19
Discussion	22
Future experimental implications of sand texture as a mediator of drought intensity	22
Late stage phenological plasticity is more influential than early life history in conferring drought resilience via drought escape	23
Expression of drought avoidance may be confined to the harshest drought regimes	25
Conclusion	27
Works Cited	28

Abstract

The ‘time limitation hypothesis’ is an evolutionary theory regarding evolution in angiosperms. The theory attributes ephemeral drought stress to transitions from a perennial to annual life history and an animal- to self-pollinating mating style. The most influential edaphic parameter on water availability is soil texture (i.e. grain size) due to its controls on porosity. Adhesion weakens with coarseness allowing for expedited rates of water loss via evaporation and percolation. The potential implications of coarse soil textures on major transitions in angiosperms due to drought mediation as the ‘time-limitation hypothesis’ proposes has yet to be thoroughly examined. Here, we grew two sympatric species of *Mimulus* with distinct mating styles on varying sand textures with equal water inputs and measured their response to increasing texture-mediated drought. Texture was shown to moderate both water retention in the soils and the felt effects of drought by overlying plants. In terms of species response to intermittent droughts, the selfing species was more drought resilient and enacted drought escape behaviors where the animal-pollinator appeared to be more drought avoidant. These findings suggest a potential underlying causal link between soil texture and mating style transitions in angiosperms which is of interest given that historic drought events are expected to occur more frequently under climate change.

Acknowledgements

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Introduction

As sessile organisms, plants are particularly vulnerable to environmental stressors because they are incapable of moving to escape adverse conditions. Understanding plant resiliency to environmental stressors is therefore critical, as climate change is predicted to exacerbate abiotic stress through an increasing frequency of extreme weather events (Smith 2011; Ebi et al. 2021). One key axis of plant resilience to climate change is the relationship of a given plant to its soil environment. Soil systems dramatically impact plant fitness by modulating below-ground inputs such as water, pH, salinity, nutrient availability, mycorrhizal fungi and symbiotic microbes (Brandy and Weil 2007). These edaphic parameters can vary substantially over mere centimeters in natural environments (Brandy and Weil 2007). Such extreme heterogeneity means that soils are highly capable of moderating the intensity of climatic stressors, particularly drought, experienced by plants. Drought is defined in many ways (Kallis 2008), but for my purposes I will define it as a transient period of severe water scarcity above ambient conditions that adversely impacts plant health.

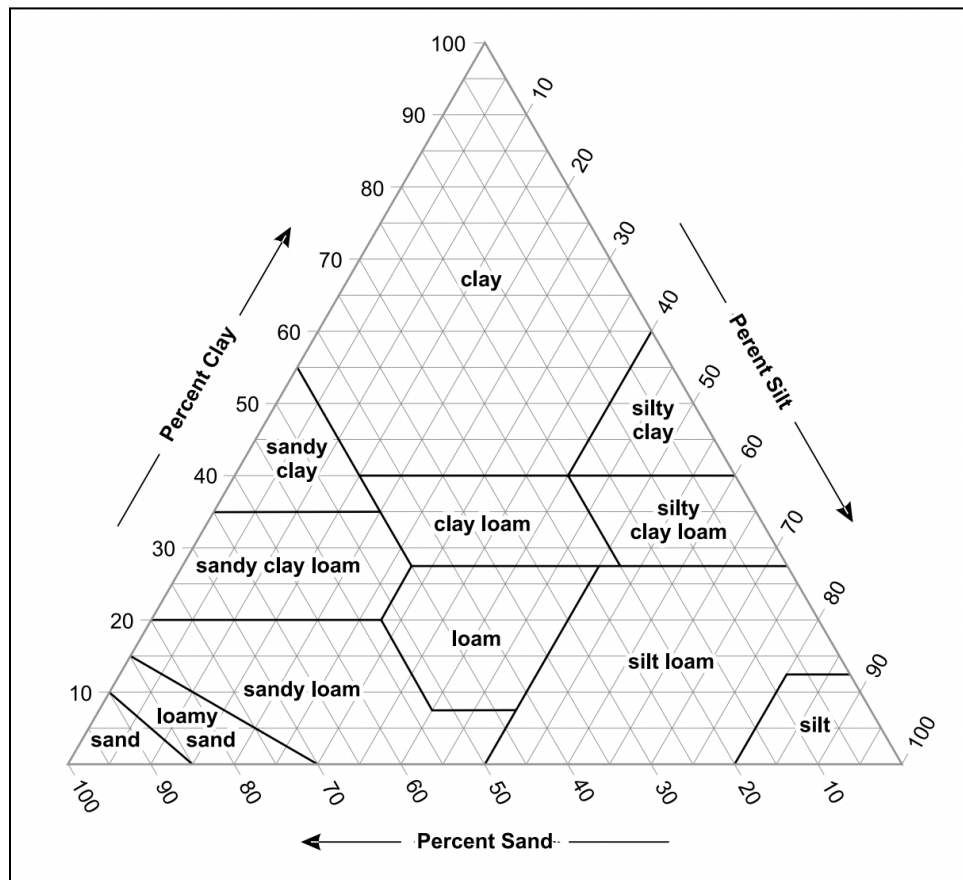


Fig 1. U.S. Department of Agriculture (USDA) soil texture classification triangle (Soil Science Division Staff 2017).

To investigate the relationship between drought intensity and plant health, a soil property one should primarily account for is texture. Soil texture classes can range from clay, at the finest end, all the way up to sand at the coarsest (Soil Science Division Staff 2017) with each class uniquely affecting the porosity, drainage, nutrient content, chemical reactivity, and water retention of a given soil (Fayos 1997; Rosenthal et al. 2005; Brandy and Weil 2007). All texture classes are defined according to a particular range of sand (2 – 0.5 mm grain size), silt (0.5 – 0.002 mm), and clay (> 0.002 mm) ratios (Fig. 1; Soil Science Division Staff 2017). The effects of climate-mediated droughts may be felt strongest in coarse (i.e. sandy) soils which exhibit increased rates of water loss due to their large pore spaces where the force of adhesion is relatively weak (Fig. 2; Brandy and Weil 2007). In fact, texture has been shown to impose greater controls on regional water retention than precipitation with coarser soils exhibiting significantly depressed moisture levels across the N. American Midwest (Rosenthal et al. 2005; Joshi and Mohanty 2010; Wang et al. 2017). The extent of sandy soil classes is relatively broad, increasing in coarseness from sandy loam, loamy sand, to sand; To achieve the standard of a sandy soil, however, the sand fraction must comprise at least 45% of the entire soil mass (Soil Science Division Staff 2017).

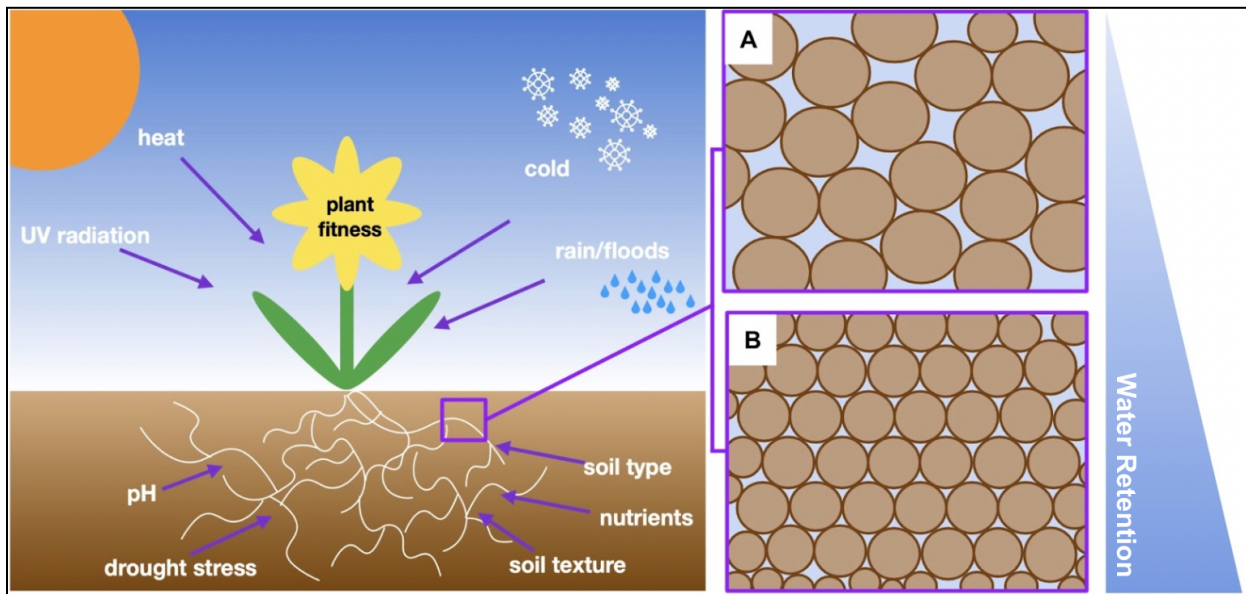


Fig 2. Overview of abiotic stressors in plants (left) and an illustrative schematic of the soil matrix with variable water retention capacity depending on soil texture (right). Panels A and B exhibit the grain-level porosity of a coarse sand (A) and fine sand (B) matrix.

Sandy soils tend to give off water relatively rapidly when compared to finer-grained soils or to soils rich in organic matter; but their inability to hold water is what allows them to exhibit enhanced water potential (Fayos 1997; Rosenthal et al. 2005). As soil water potential (Ψ) increases, ease of water extraction by plants also improves, which has been shown to decrease water use efficiency (WUE) in some deep-rooted desert plants (Rosenthal et al. 2005). However, the effect of Ψ may not be as favorable for shallow-, fibrous-rooted species whose root systems can only penetrate the top-most soil horizons where the effects of evaporation and percolation are most pronounced (Donovan and Ehleringer 1994). As such, the relatively rapid rate of water loss at shallow depths in coarse-textured soils puts pressures on plants living within this water-scare environment to evolve a drought response strategy (Ehleringer 1988; Donovan and Ehleringer 1994; Rosenthal et al. 2005).

Plants exhibit three main mechanisms of drought response: avoidance, tolerance, and escape. The advantage of each strategy is dependent on the environmental context (Table 1; Kooyers 2015). Drought avoidance behaviors are associated with mild, punctuated drought regimes in which plants may improve WUE by reducing transpiration (Kooyers 2015). Drought escape, however, is associated with changes in developmental timing, giving plants the ability to develop and complete reproduction before drought becomes lethal (Kooyers 2015). Because drought escape and avoidance are each associated with particular phenology and physiology, one should measure a variety of floral, vegetative, and life history traits in order to characterize a species' particular strategy (Kooyers 2015). Useful metrics are those that can capture a plant's developmental progress or zones of resource allocation (i.e. vegetative or floral biomass) because the strategies invoke discrepant distributions of energy costs. Drought escape emphasizes development traits such as first bud, flower, and fruit production whereas drought avoidance emphasizes reductions in transpiration through vegetative modifications such as lower specific leaf area (SLA) and greater succulence or rosette size (Kooyers 2015). The drought tolerance strategy is reminiscent of drought avoidance in that dehydration is also mitigated, but here it is achieved through osmotic adjustments and the production of protein stabilizing molecules (Kooyers 2015). Because drought tolerance pertains to molecular, biochemical modifications, this project will primarily focus on drought escape and avoidance seeing as these strategies result in more macro-scale adjustments and thereby can be measured in situ (Kooyers 2015).

Strategy	Characteristics	Traits	Associated Environment
Drought Escape**	Adjustments in developmental timing	Days to first bud, flower and fruit Height at first flower Seed set	Slow onset, end-of-season drought
Drought Avoidance**	Improved WUE Reduced transpiration	Leaf Area Succulence Rosette Diameter	Mild, punctuated droughts
Drought Tolerance	Osmotic adjustment Stabilizing protein production	Protein and mRNA production	Mild, punctuated droughts

Table 1. Overview of drought response strategies in angiosperms. This project focuses on the expression of drought escape and avoidance strategies.

The ‘time-limitation’ hypothesis is a theory of angiosperm evolution that implicates drought escape with the arisal of self-pollination. Specifically, the theory proposes that because selfing promotes faster vegetative and floral development, self-pollination should naturally arise in plants living in ephemeral environments as a means to ensure reproductive success (Snell and Aarssen 2005). This transition may come about in two ways. Firstly, selfing may be the consequence of direct selection because the transition from out-crossing to self-pollination has been shown to reduce floral and vegetative biomass as well as stigma-anther distance (i.e. herkogamy) while accelerating development rate relative to closely related outcrossing species (Ornduff 1969; Mazer et al. 2010; Snell and Aarssen 2005; Roels and Kelly 2011; Ivey and Carr 2012). Alternatively, selfing may be the product of indirect selection where drought initially induces faster floral development and smaller flower size which thereby reduces herkogamy and synchronizes anther dehiscence with stigma receptivity leading to the acquisition of selfing (Guerrant 1989; Snell and Aarssen 2005; Ivey and Carr 2012). Therefore, in the context of soil texture, self-pollinating plants may be not only better suited to dryer, coarser soils due to their temporal and developmental advantages, but may be evolutionarily favored as well.

We aim to address the hypothesis that selfing is inherently associated with coarse soils by measuring texture-mediated drought response traits in the common monkeyflower genus *Mimulus guttatus*. The *M. guttatus* species complex includes a group of diverse angiosperms that exhibit a variety of mating styles which makes them an ideal model organism for measuring drought response between mating systems. Their natural range also overlaps with the geographic

range of natural, climate-induced droughts suggesting a possible history of drought adaptation within their lineage (Lowry et al. 2008; Ivey and Carr 2012; Kooyers et al. 2015; Kooyers et al. 2021). Prior research on drought response in *M. guttatus* has largely been limited to reciprocal transplant experiments or common garden experiments using organic-rich soils with drought mediated via changing water inputs (e.g. Hall and Willis 2006; Wu et al. 2009; Ivey and Carr 2012; Kooyers et al. 2015; Mantel and Sweigart 2019; Kooyers et al. 2021). These studies have found that, under drought conditions, selfing species of *Mimulus* exhibit higher rates of survival and minimal reductions to seed set through faster floral and fruit development (Wu et al. 2009; Mantel and Sweigart 2019) indicative of a drought escape strategy; whereas annual ecotypes of *M. guttatus*, a mixed but primarily an animal-pollinator, may respond to drought by reducing flowering time, increasing vegetative biomass, or increasing WUE depending on standing genetic variation and environmental context (Ivey and Carr 2012; Kooyers 2015; Kooyers et al. 2015; Kooyer et al. 2021). The plasticity shown by both phenological and vegetative traits suggests the capability of both escape and avoidance strategies in *M. guttauts*. Generally, however, *Mimulus* exhibits phenotypic plasticity under drought (Ivey and Carr 2012; Anstett et al. 2021) with the drought resilience of natural populations correlating significantly to water availability in their native environment (Kooyers et al. 2015; Kooyers et al. 2021). While the mechanisms of drought response in *M. guttatus* are beginning to be understood, uncertainty remains regarding the role that texture may play in mediating drought and thereby directing evolution.

Here, I capture natural variation in drought response traits between two *M. guttatus* species complex members representing self- (*M. micranthus*) and bee- (*M. guttatus*) pollination modes. I manipulated drought intensity by increasing the degree of sand coarseness between treatments and exposing plants to mild, intermittent droughts over a six-week period (Fig. 3). I then quantified the phenotypic expression of drought escape and avoidance traits by measuring vegetative and floral phenotypes as well as seed production. My overarching goal was to understand how soil texture and pollination mode influence drought response in a native plant. First, I demonstrate that manipulating sand coarseness appropriately captures variable drought states. Then, I exposed species with different pollination modes to mild, intermittent droughts under variable sand textures. This design allowed me to investigate the role that mating style

plays in conferring drought resilience as well as parse out the role of genotype, environment, and any interactions on expression of drought escape and avoidance.

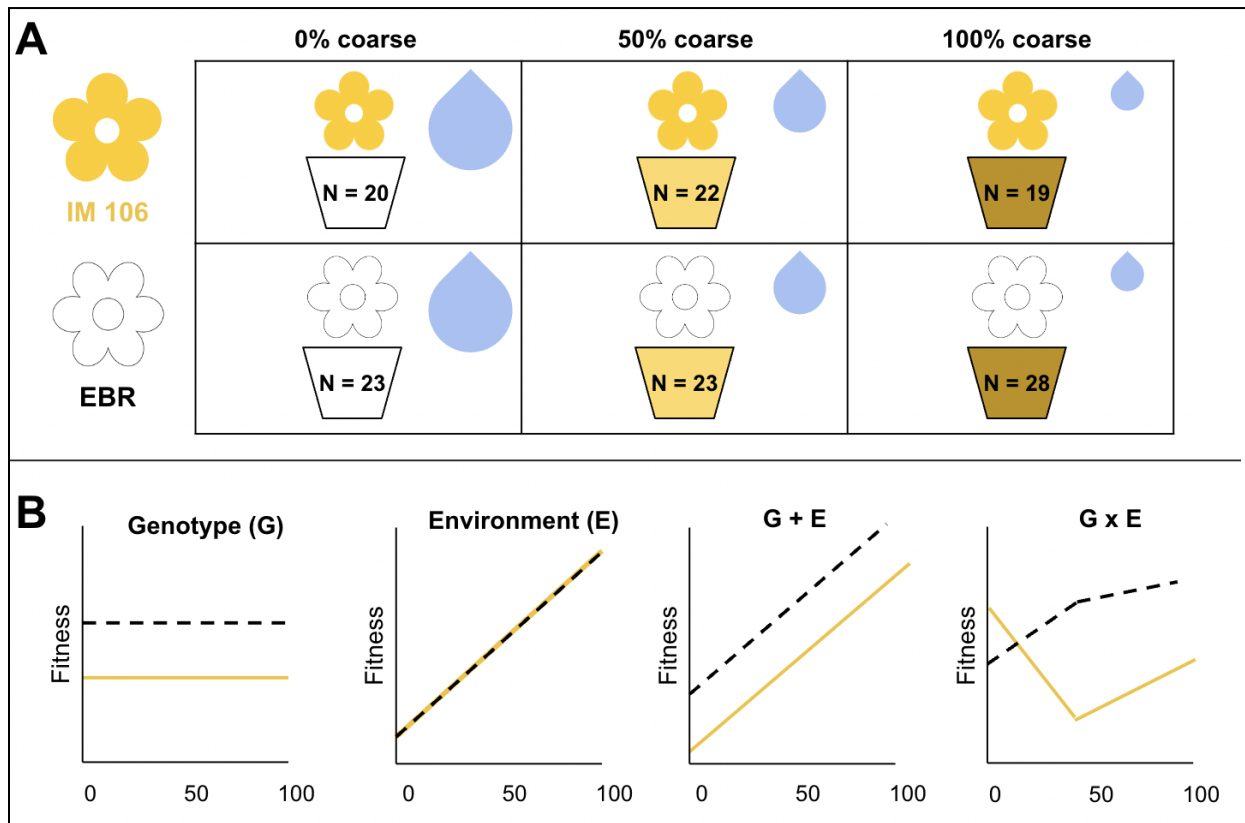


Fig 3. Experimental design (A) and predicted results (B). Predicted results depict possible effects according to species (G), treatment (E), species and treatment (G + E), and species by environment interactions (G x E).

Materials and Methods

Plant lines

The *Mimulus guttatus* species complex (common yellow monkeyflower) represents a suite of unique, phenotypically diverse species centrally located in North America (Vickery 1978; Wu et al. 2010). In addition to diverse life history, physiology, and developmental traits, members of this complex exhibit divergent mating systems including primarily self-pollinating, primarily outcrossing (i.e. animal-pollinated), and mixed (i.e. capable of outcrossing and selfing) mating styles (Wu et al. 2010). Their phenotypic and genetic diversity allows *M. guttatus* members to populate a wide range of climatic and edaphic habitats including alpine meadows, sandy coastlines, copper mining tails, serpentine soils, and geothermal crusts (Tilstone and MacNair 1997; Wu et al. 2010; Selby and Willis 2018; Kolis et al. 2022).

In this study, I measured the drought response traits of two *M. guttatus* annuals: *M. guttatus* and *M. micranthus*. Due to their disparate mating systems, one can use *M. guttatus* and *M. micranthus* to consider the importance of pollination mode on the arisal of texture-mediated drought tolerance in *Mimulus*. Evolutionarily, *M. micranthus* naturally derived from an ancestral population of *M. guttatus*; the two species are closely related and can intercross (Fenster and Ritland 1994). As such, measuring the drought response traits of these species offers the ability to perform between-genotype comparisons. I focused on two inbred lines for my experiment: *M. guttatus* and *M. micranthus*. The *M. guttatus* line (IM 106) from Iron Mountain, Oregon exhibits mixed mating (though is primarily bee-pollinated) and preferentially grows along wet, sandy seeps. IM 106 is a well-characterized inbred line that has undergone extensive phenotypic and genomic testing (Hall and Willis 2006; Wu et al. 2010; Roels and Kelly 2011; Mantel and Sweigart 2019). My *M. micranthus* experimental line (EBR) is from Mendocino, California where it grows on coastal, organic-rich mountain sides and almost exclusively self-pollinates.

Analysis of soil texture classes and organic inputs

For my sand treatments, I used Quikrete® Play Sand as the finer-grained (0% coarse) sand matrix and Quikrete® All Purpose Sand as the coarsest (100% coarse) sand matrix. I then hand-mixed equal parts of both the fine and coarse sand types in order to create an intermediate (50% coarse) sand treatment. Texture classifications for both sand types were obtained by hydrometer and performed by Dr. Colin Robins, after the methods outlined in Gee and Bauder (1986). Samples were sieved to 2 mm and then 100 g of each sample were soaked in 100 mL of

5% NaHMP (sodium hexametaphosphate solution), before mixing in a blender and dilution to 1L in a graduated cylinder. Fluid density was measured by hydrometer after agitation, at 44 sec and after 7.5 hrs. Based on temperature corrections and the settling times of sand, silt, and clay-sized particles, the textures of the soil samples were calculated (Gee and Bauder 1986).

Moisture content was determined gravimetrically, as the difference between air-dry mass and sample mass after heating overnight (12 hrs) at 105 °C. Organic matter content was determined gravimetrically via the loss on ignition method (Soil Survey Staff 2022), in which the oven dried sample from the moisture determination was combusted at 405 °C in a muffle furnace for four hours, and then re-weighed.

Between treatment water capacity testing

To test for significance in water availability between treatments over time, I measured dry basis soil moisture content (θ_d) for each sand treatment up to 72 hours after bottom watering to field capacity (Mantel and Sweigart 2019). To calculate θ_d , I filled 4" pots with 21 oz sand per treatment ($n = 20$). Before adding sand, I lined the pots with a coffee filter to prevent sediment from spilling out of the drainage holes. I then bottom watered the filled pots until the saturation point reached field capacity. After watering, I randomly assorted the pots in a complete block design (Wu et al. 2010) in the Pitzer College greenhouse and allowed the pots to dry under direct sunlight for 72 hours (July 1–4, 2022). Starting at 0 hours and then at each 24 hour interval, I measured the sand's wet mass (WM) by weighing the pot with sand and filter paper included. Afterwards, I randomly rearranged the pots in the block to minimize the potential effect of bench placement on the rate of water loss. After 72 hours in the greenhouse, I oven dried the sand contents of each pot at 105 °C for 24 hours. I then returned the samples to their original pot and dried filter and recorded dry mass (DM). I then calculated $\theta_d = (WM - DM) / (DM)$ using protocols established by Mantel and Sweigart (2019).

Seed planting, germination, and incubation

IM 106 and EBR seeds were scattered in separate 21" x 11" x 2" flats on moist Pro-Mix Premium Moisture Potting Mix (Day 0) and stratified in a growth chamber for 7 days in the dark at 4 °C. I then transferred the seedlings to gradually increasing long day photoperiod conditions until reaching 16 hours of light on Day 16 (22 °C days/18 °C nights). On Day 17 I began

gradually exposing the incipient sprouts to the greenhouse before permanently transferring the fledglings to the greenhouse on Day 20.

On Days 20 and 21 I transferred the first seedlings to individual pots filled with filter paper and wetted sand beginning with 13 replicates of each treatment per species. Once planted, I sprinkled the pots with vermiculite and misted water to discourage algae or bacterial growth. This group of thirteen replicates represented Experimental Set #1. Experimental Set #2 was planted on Days 26 and 27 following the same planting procedure as Set #1 with 19 to 23 replicates of each treatment planted per species. Immediately following their respective planting periods, both experimental groups were then placed in an incubation period for 7 days in which they were randomly assorted across the shaded portion of the greenhouse and constantly bottom watered via submergence in 20" x 10" x 2" flats without holes to encourage establishment on the sand. Following one week of establishment, each group's respective experimental protocols were initiated.

Between treatment species survival in dry down

To further validate significance in water retention between treatments, I performed a pseudo-pilot study wherein Experimental Set #1 plants experienced dry down conditions over a 72 hour period and proportional species survivorship between treatments was measured over at 24 hour intervals over 72 hours. On Day 28 incubation for Experimental Set #1 was terminated; all plants were moved to the sunny portion of the greenhouse and dry down was initiated. Proportional survivorship was then used to calculate the significance of drought intensity felt between treatments and drought resilience exhibited between species.

Species response to long-term, punctuated drought

To measure drought response traits and characterize the respective response strategies between IM 106 and EBR, I exposed Experimental Set #2 to frequent, punctuated droughts and recorded vegetative, floral, developmental, and geochemical trait expression. I elected to impose mild, punctuated droughts meaning volumetric water content (VWC) was kept at or above 10% and watering occurred approximately every 48 hours. If the average VWC of the 100% coarse sand pots reached 10% all the pots were immediately watered. Due to extreme variability in daily temperatures and evaporation rate over the trial period, VWC was monitored on a daily basis using the Acclima TDR-305N Soil Moisture Meter.

We transferred the plants from the incubation period to experimental conditions on Day 35 and mechanically removed all incipient buds. Pots were then randomly placed in a block formation on the sunny side of the greenhouse with placements randomly reassigned every 7 days. Fertilizer was added via bottom watering as needed, and BONIDE Eight ® Garden & Home Ready to Use pesticide was sprayed on a daily basis to prevent herbivory. I then measured and recorded drought response traits on a daily basis over a six-week period.

Measuring drought response traits

Life history, vegetative, and floral traits were recorded for Experimental Set #2 in order to capture genome-level and environmental-dependent phenotypic variation. In terms of life history, I recorded days to first bud, first flower, third flower, and first seed pod as well as the total number of days alive since germination. Flowers were considered open when the petals were fully reflexed and seed pods were considered mature once the first seed was visible within the pod. On the day of first flower I measured several vegetative and floral traits including plant height, rosette size, leaf number, corolla height, and corolla width. Rosette size, corolla height, and corolla width were measured at each of their widest points, and plant height was measured by manually drawing the plant up so as to record its true height. I also performed seed counts of the first and last pod produced for each plant able to produce a mature pod.

Additional leaf-level drought response metrics I measured to characterize drought response strategy included succulence and standard leaf area (SLA). I exclusively selected mature leaves that had developed after drought conditions were initiated to capture potential treatment effects. To measure leaf wet mass (WM), I mechanically removed each experimental leaf and immediately recorded its fresh weight. I then oven-dried the leaves at 50 °C for 48 hours and then re-weighed them to measure their dry mass (DM). I also calculated leaf area by capturing images of fresh leaves and measuring their surface area on ImageJ. I then calculated SLA as leaf area/DM and succulence as $(WM - DM) / \text{leaf area}$ (Kooyers et al. 2015).

Data analysis

We wanted to ensure there were significant differences in water storage capacity overtime between treatments to verify experimental power. To do so I measured water loss (θ_d) and conducted a two-way ANOVA with “hour” (fixed effect) and “treatment” (fixed effect) as main effects and “hour x treatment” as an interaction effect. Since there was significant variation

between all three effects, I performed a Tukey-Kramer honest significant difference (HSD) comparison. To calculate the significance of survivorship between species in the dry down experiment, I conducted a general linearized model (GLM) with a binomial distribution and a linked logit function. Here, my main effects were “treatment” (fixed effect), “genotype” (fixed effect), and “hour” (fixed effect). Due to the results of the GLM, I also performed a pairwise proportion test of survivorship at 72 hours between species. Additionally, I wanted to determine how traits were implicated in drought response for *M. guttatus* and *M. micranthus* by measuring life history, floral, and vegetative traits. For life history traits I conducted additional pairwise proportion tests and binomial GLMs with a linked logit function on the binary response variables of bud, first flower, and third flower production with “genotype” and “treatment” as main effects. To compare the expression of continuous phenotypic traits (days to first bud, days to first and third flowers, days to first fruit, corolla height and width, plant height, rosette size, leaf number, flower number, seed number, succulence, leaf area and SLA) under drought I performed two-way ANOVAs also with “genotype” and “treatment” as main effects and “genotype x treatment” as an interaction effect. Due to a lack of seed production in IM 106, I performed a one-way ANOVA on seed production in EBR with “treatment” as the main effect. These analyses were performed using R (R Core Team 2022) and RStudio (RStudio Team 2022).

Results

Sand shows significant influence on water capacity and resulting drought severity

Despite occupying similar soil texture classification classes, textural differences between the sand treatments were shown to successfully impact water retention. Results from the hydrometer testing designated the texture class of the Quikrete® Play Sand as “Sand” (Fig. 4; 91.27% sand, 0.0% silt, 8.73% clay) while the Quikrete® All Purpose Sand exhibited a slight silt fraction (Fig. 4; 89.27% sand, 2.0% silt, 8.73% clay) designating it as a “Loamy sand.” However, the proportion of organic matter and ambient moisture in both sand types fell below the 1% error limit meaning there was too little of either amendment to detect accurately.

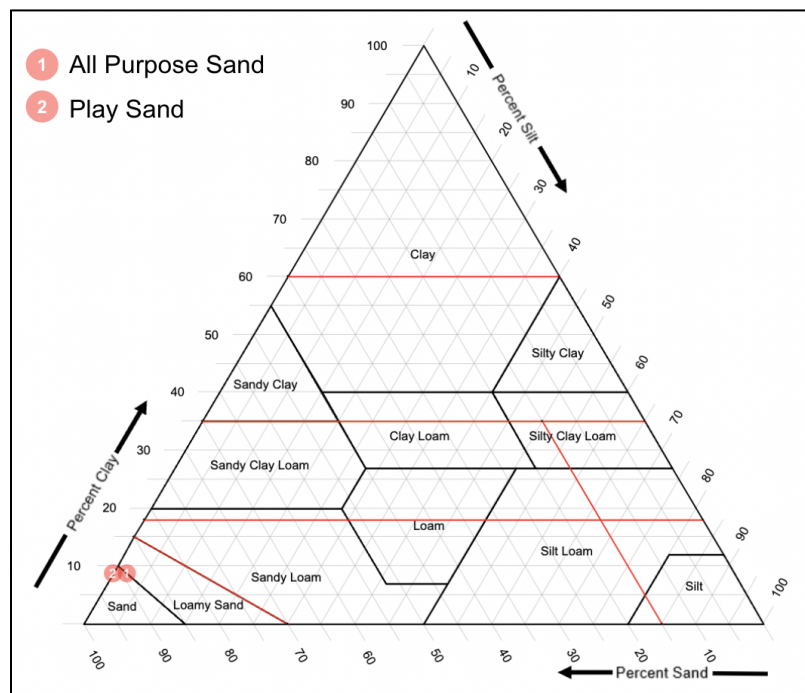


Fig. 4. USDA soil texture classification triangle with points input representing the texture classification of the 100% coarse (All Purpose) and 0% coarse (Play) sand treatments.

Although the All Purpose Sand falls under a finer soil texture classification, water capacity and survival testing confirmed experimental power of the original treatment designations. Empirical evidence from observations of each sand type can attest to the presence of a pronounced number of large sand grains (≥ 2 mm) in the All Purpose sand that likely contributed to its depressed water retention capabilities. Dry basis soil moisture content testing confirmed this supposition; moisture content was consistently lowest in the All Purpose Sand and greatest in the Play Sand in the water capacity experiment (Fig. 5; Tukey-Kramer HSD, all P

< 0.05). Differences in water retention were erased by 72 hours, however, at which point all treatments were practically completely dried down (Tukey-Kramer HSD, all $P > 0.05$). For this reason, I elected to impose mild, intermittent droughts so as to keep the conditions habitable.

In addition to moisture content, the dry down experiment also showed that texture significantly moderates the effects of drought felt by plants according to designated coarseness (i.e. 0%, 50%, and 100% coarse). Somewhat surprisingly, the dry down experiment did not capture significant differences in survivorship for the first 48 hours (Fig. 5); an insignificant number of *M. micranthus* and *M. guttatus* individuals were still alive past 24 ($100.0\% \pm 0.0$ and $97.4\% \pm 16.0$, respectively) and 48 hours ($97.4\% \pm 16.0$ and $94.9\% \pm 22.3$, respectively) across treatments. At 72 hours, however, marginal differences in survivorship according to treatment effects were observed (Fig. 5; GLM, $P < 0.05$). *M. micranthus* showed elevated survivorship in the 0% coarse treatment over the 50% and 100% coarse treatments 72 hours after initiating dry down (Pairwise Prop Test, $P < 0.05$) providing further validation that the All Purpose Sand enacts the severest drought regime. Overall, our soil and survival experiments indicate that texture controls water capacity and mediates the experience of drought for overlying plants.

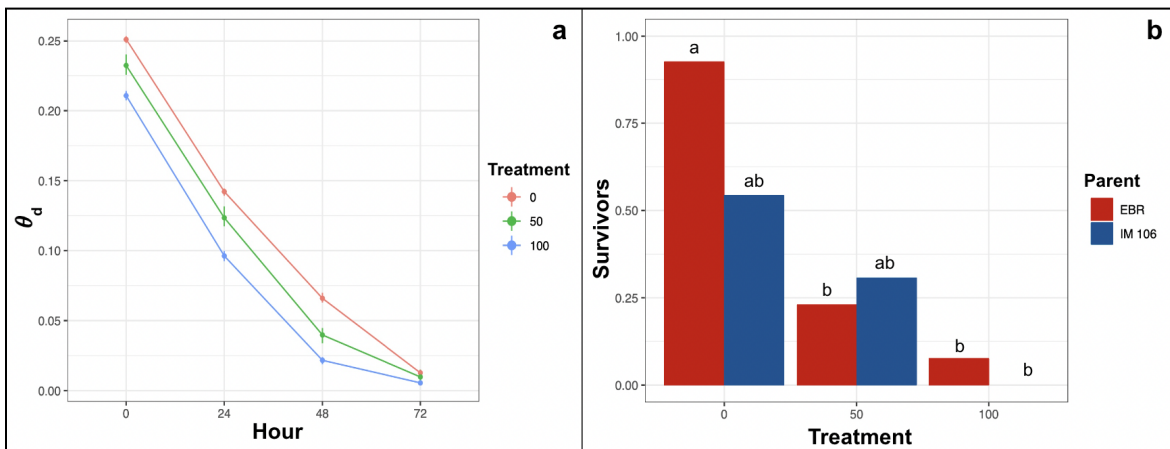


Fig. 5. (a) Mean θ_d (\pm 95% confidence intervals) shows volumetric water capacity is variable according to treatment coarseness as reflected volumetrically during dry down. (b) Proportion of surviving individuals 72 hours after initiating dry down with labeling to outline significance of genotype and treatment effects.

M. micranthus is more resilient to frequent, mild droughts than *M. guttatus*

Overall, *M. micranthus* was more resilient to drought than *M. guttatus* under the intermittent drought regime, but not under ephemeral drought. In the dry down experiment, a relatively equal number of *M. micranthus* and *M. guttatus* individuals within each treatment block survived to 72 hours after initiating dry down (Fig. 5). *M. micranthus* did not show greater

resilience to ephemeral drought stress which contradicts the ‘time limitation’ hypothesis (Snell and Aarssen, 2005). In the punctuated drought experiment, however, proportionally more *M. micranthus* individuals successfully produced buds, flowers, and fruit than *M. guttatus* across treatments (Fig. 6). Although there was a difference in the proportion of third flowers produced by *M. micranthus* in the 0% coarse (95.5% ± 21.3) versus the 100% coarse (52.2% ± 51.1) sand treatments (Pairwise Prop Test, $P < 0.05$), suggesting that the effects of drought may have been felt more intensely at later life history stages. But when *M. micranthus* did produce fruit, the species showed no significant reductions to seed set according to treatment (ANOVA, $P > 0.05$). Meanwhile, very few *M. guttatus* plants were able to reach later life history stages such as third flower and fruit production irregardless of treatment (Fig. 6; GLM, $P > 0.05$). Particularly after initial flowering, the resilience of *M. guttatus* to drought sharply dropped off (Fig. 6). The poor resilience and survivorship of *M. guttatus* under drought is expected due to the coastal, perpetually wet nature of the species’ natural habitat. Overall, greater resilience to frequent, mild drought was observed in the self-pollinator, *M. micranthus* as predicted given its developmental advantages, yet drought resilience progressively appeared to decrease over time in both species.

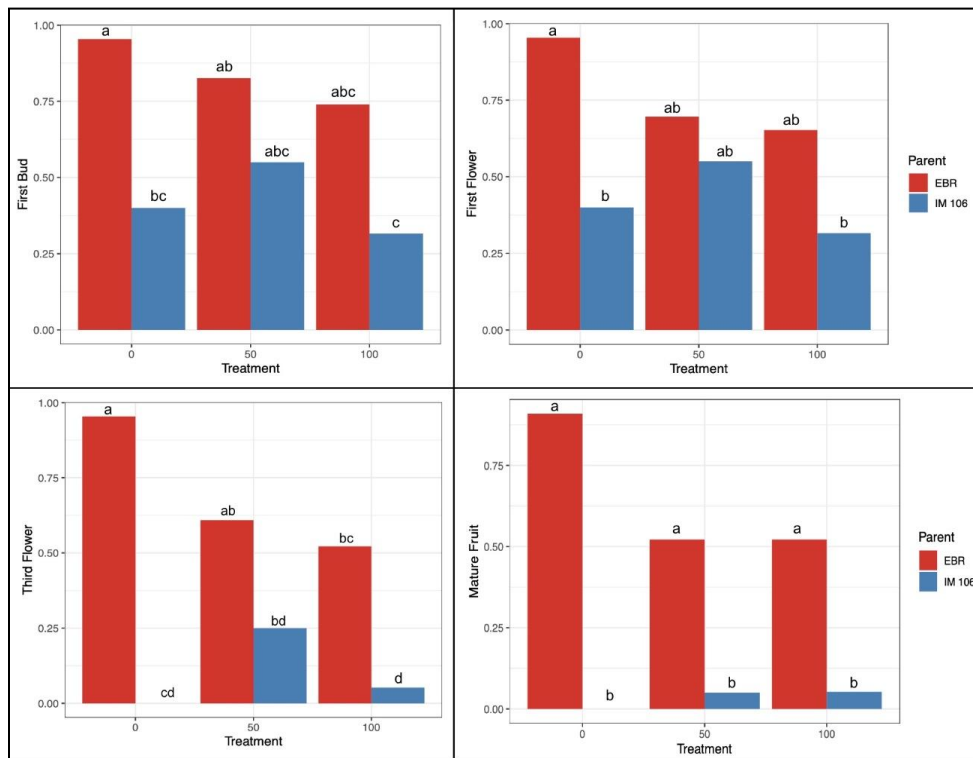


Fig. 6. Proportional expression of life history traits including bud, first flower, third flower and fruit production by treatment and species exhibit improved drought resilience in *M. micranthus* (EBR) under the finer sand treatment. Labeling illustrates significance by genotype and treatment effects.

Analysis of numerous traits confirms phenotypic differences common to animal- vs. self-pollinated species

M. guttatus and *M. micranthus* exhibit discrepant drought response strategies when experiencing mild, punctuated droughts. A principal component analysis (PCA) looked for patterns in the variation of composite phenological and physiological trait expression within treatment blocks in both species (Fig. 7). In the extracted principal components (PC), vegetative traits did not associate with floral traits or phenology. PC1, on the horizontal axis, corresponded with life history and flower size and explained 32.6% of variability in the dataset whereas vegetative phenotypes corresponded with PC2, on the vertical axis, and accounted for 19.7% of variability (Fig. 7). *M. guttatus* treatments primarily plotted along PC1 whereas *M. micranthus* groups plotted along PC2 illustrating their discrepant physiologies, phenologies and drought response pathways. While within-species treatments overlapped in trait expression, no overlap appeared between the 50% and 100% coarse *M. guttatus* groups with any *M. micranthus* treatments. Strikingly, marginal differences in expression of drought avoidance traits was observed in *M. micranthus* grown in the 0% coarse treatment (Fig. 7).

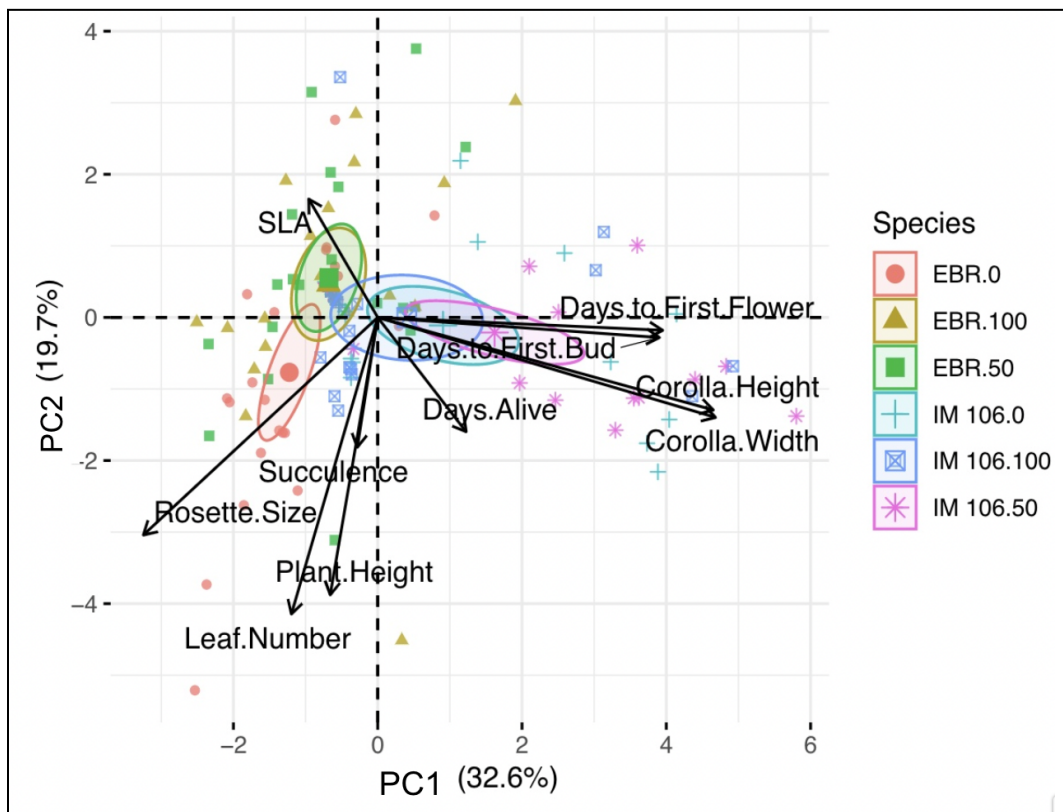


Fig. 7. Principal component analysis (PCA) of drought response traits underscores expression of vegetative traits by *M. micranthus* as well as floral traits and slowed phenology by *M. guttatus*.

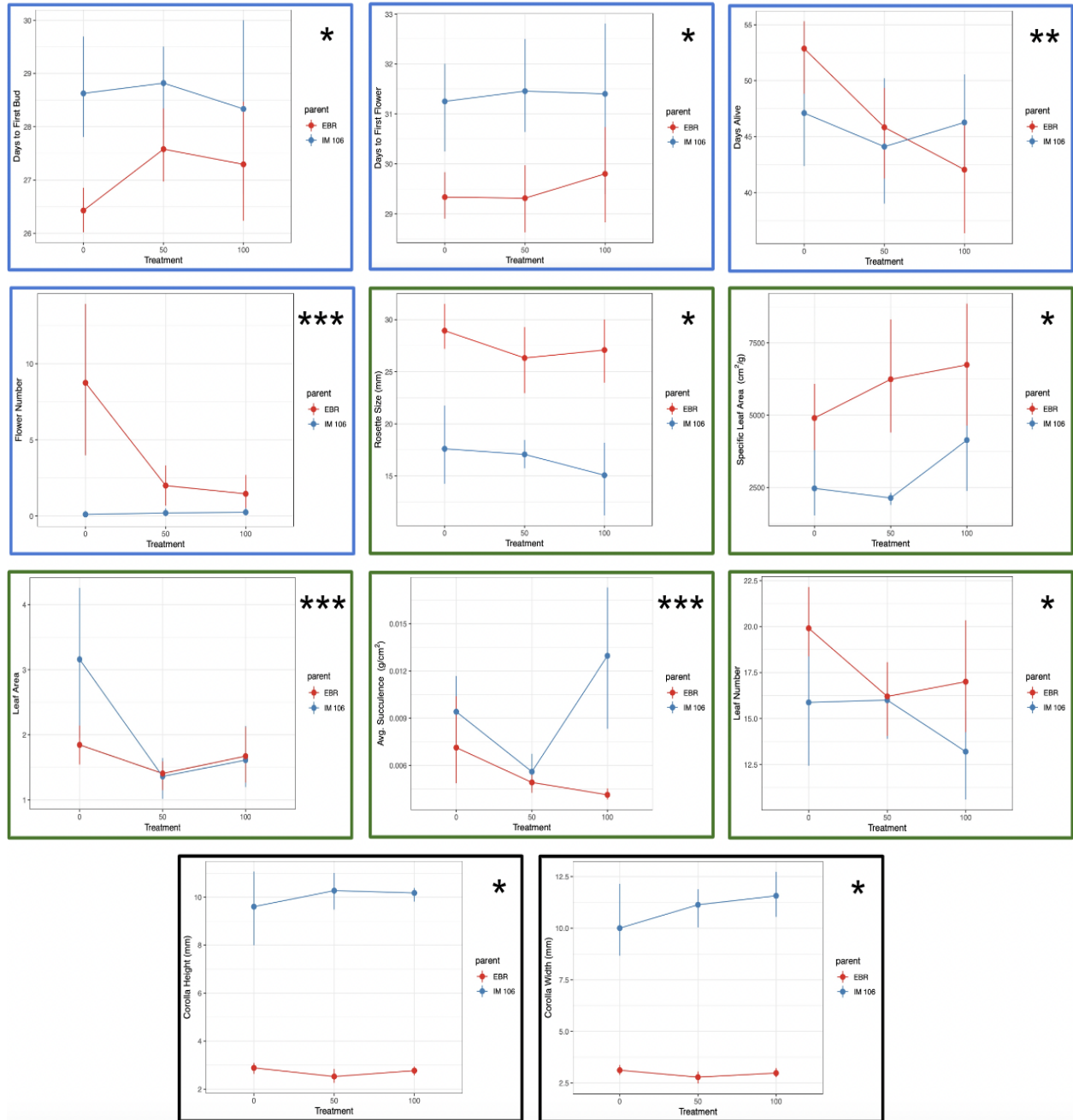


Fig. 8. Mean (\pm 95% confidence intervals) of continuous drought response traits capture significant differences by genotype (*), treatment (**), and mixed genotype:treatment interaction (***) effects. Drought escape traits are outlined in blue, drought avoidance traits are outlined in green, and traits not associated with a particular strategy are outlined in black.

Comparisons in expression of phenological and physiological traits according to species, treatment, and mixed effects show that *M. micranthus* invokes a drought escape strategy (Fig. 8). A fundamental effect of drought escape is the adoption of an accelerated life history in order to ensure reproduction (Snell and Aarssen 2005). In *M. micranthus*, a trend of decreasing lifespan

was observed according to the effect of treatment (ANOVA, $F = 3.59$, $P < 0.05$), yet the species showed no significant reductions to seed set (ANOVA, $P > 0.05$) or instances of mature fruit production (GLM, $P > 0.05$) under the same effects. Interestingly, however, the number of days to first bud and flower production in *M. micranthus* showed minimal plasticity (Fig. 8). It's possible that the effects of drought were not fully felt until later life history stages. Conversely, degrees of succulence (ANOVA, $F = 3.30$, $P < 0.05$) and SLA (ANOVA, $F = 12.30$, $P < 0.001$) meant WUE decreased in *M. micranthus* with increasing coarseness (Fig. 8). These behaviors depart from the principles of drought avoidance and likely helped facilitate *M. micranthus*' rapid development as required by drought escape (Fig. 8). The results of the PCA also show that *M. micranthus* is more drought resilient and less water use efficient given that this species plotted along PC2 meaning it exhibited larger rosettes (ANOVA, $F = 59.88$, $P < 0.001$) and higher leaf numbers (ANOVA, $F = 4.29$, $P < 0.05$) than *M. guttatus*.

M. guttatus, however, generally exhibited phenological and physiological signals of drought avoidance (Fig. 8). Notably, the species showed larger flowers than *M. micranthus* with greater corolla width and height (ANOVA, $F = 553.412$, $P < 0.001$ and $F = 671.50$, $P < 0.001$, respectively) as well as more days to first bud and flower production (ANOVA, $F = 14.92$, $P < 0.001$ and $F = 19.49$, $P < 0.001$) without an effect of treatment. The species plotted along PC1 which reflects its larger flower size and slower rate of development (Fig. 7). Additionally, the lifespan of *M. guttatus* also did not appear to vary according to treatment (Fig. 8). As it appears to exhibit no plasticity in lifespan or development rate as a result of varying drought intensity, it is unlikely that this *M. guttatus* line invokes drought escape. Indeed, similar limitations to phenological plasticity in other annual ecotypes of *M. guttatus* and *M. cardinalis*, an outcrossing species, have been reported (Kooyers et al. 2015; Vtipil and Sheth 2020; Anstett et al. 2021). Similarly, the IM 106 line appears to have resorted to drought avoidance in order to survive drought by improving WUE via dramatically increasing succulence and reducing leaf area between the 0% and 100% coarse sand treatment as predicted (Fig. 8; ANOVA, $F = 3.30$, $P < 0.05$; Tukey-Kramer HSD, $P < 0.05$). Yet despite *M. guttatus*' plasticity, it seems as though only drought escape was successful in being able to save *M. micranthus* from drought whereas drought avoidance was insufficient to prevent *M. guttatus* from senescing and producing very few flowers or fruit under a mild, punctuated drought regime (Fig. 6).

Discussion

Future experimental implications of sand texture as a mediator of drought intensity

Traditionally, artificial drought conditions in the garden or greenhouse are achieved by growing plants on organic soils and varying the volume and timing of water inputs according to desired drought intensity and regime (e.g. Hall and Willis 2006; Wu et al. 2009; Ivey and Carr 2012; Kooyers et al. 2015; Mantel and Sweigart 2019; Kooyers et al. 2021). While drier climates are a predicted effect of climate change (Smith 2011; Ebi et al. 2021), the extreme diversity of natural soils in terms of texture and other edaphic parameters means drought will not be felt equally by all plants. Here, sand texture rather than varying water inputs was used as the method of drought mediation to elucidate the potential role of soil texture in mediating the intensity of drought felt by plants. This experimental set up can also allow us to better understand how natural populations of *Mimulus* may respond to ephemeral and punctuated droughts in one of the most sensitive soil types.

While punctuated drought intensity was successfully mediated by texture, experimental shortcomings in the dry down experiment may have precluded my ability to capture the effect of genotype on drought resilience under this regime. In the punctuated drought experiment, I was able to successfully capture discrepancies in texture-mediated drought resilience and behaviors within the *M. guttatus* species complex. As such, the findings from the long-term experiment suggest that in natural habitats experiencing intermittent droughts, shallow, coarse soils may exhibit the severest degrees of water scarcity which may influence the phenology and physiology of any shallow-rooted, overlying species. However, conclusions regarding species response to ephemeral drought could not be extracted from the dry down experiment. My primary error in this experiment was the small sample sizes. Using small groups may have masked discrepant signals of drought resilience like those captured in the long-term experiment. However, the effect of treatment shown by *M. micranthus* was clear, even if the effect of genotype was not. This finding suggests the possibility that despite relatively equal VWC at 72 hours, the increased availability of water at earlier time points may have made individuals in the 0% coarse treatment more robust and thereby able to better withstand the dry conditions at 72 hours. As such, it is possible that texture may facilitate divergence events in populations growing on heterogeneous texture landscapes.

Despite observing significant differences in ephemeral and punctuated drought mediation between treatments, however, the experimental power of this study was ultimately limited by the relatively elevated coarseness of both sand types. Although the All Purpose Sand fell within a finer texture class than the Play Sand, the pronounced coarseness of both sand types meant all treatments lost water at an unsustainable rate in terms of experimental longevity. For my purposes, I was forced to impose mild, punctuated droughts over the long term to ensure that plants could survive to seed set. These environmental conditions favor the expression of drought avoidance behaviors and limited my experimental scope relative to drought studies in organic soils that were able to replicate slow-onset, dry down conditions (Kooyers 2015). To address this shortcoming, future experimental manipulations of texture-mediated drought may consider introducing organic matter or finer sand inputs into the coarse sand matrix to extend the duration of habitable drought conditions. In this case, results may better capture natural conditions where the soil environment gradually becomes drier over time as the growing season progresses from spring to summer. Additionally, when soils support overlying vegetation, plant tissues are naturally introduced into the soil matrix due to senescence and herbivory, so organic as well as bacterial or fungal amendments to coarse sand treatments may do better to capture the qualities of natural soil habitats as even small amounts of organic inputs can dramatically affect the water holding capacity of a given soil (Brady and Weil 2007).

Late stage phenological plasticity is more influential than early life history in conferring drought resilience via drought escape

The time-limitation hypothesis associates self-pollination with dry down conditions by suggesting that selfing is directly and indirectly favored via reductions in herkogamy and flower size as well as increasing synchronicity of stigma-anther maturation (Snell and Aarssen 2005). Furthermore, under the drought escape strategy selfing plants exhibit decreased floral and vegetative mass meaning they would be more capable of completing their life cycle prior to lethal drought onset (Ornduff 1969; Mazer et al. 2010; Roels and Kelly 2011; Ivey and Carr 2012). As such, drought escape should complement a selfing mating style as this drought response strategy affects the timing of life history traits such as days to first bud, flower, and fruit most influentially. However, in the PCA, *M. micranthus* plotted along the vegetative axis (PC2) and showed minimal correlation with the larger floral and phenological traits (PC1; Fig. 7). These patterns of trait expression correspond with those outlined in the “selfing syndrome:”

smaller flowers, greater vegetative mass, and faster floral and fruit development (Ritland and Ritland 1989; Snell and Aarssen 2005; Sicard and Lenhard 2011). While days to bud, flower, and fruit production are lower in *M. micranthus* due to genotype effects, I would still expect for time to bud, flower, and fruit production to decrease proportionally with drought intensity in this species due to the principles of drought escape (Kooyers 2015).

Plasticity in the acquisition of early life history stages by *M. micranthus* experiencing harsher drought may have been masked by the effects of late drought initiation. The discrepancies in drought resilience according to genotype effects in *M. guttatus* and *M. micranthus* are consistent with prior work that observed self-pollinating *Mimulus* species consistently outperforming out-crossing ecotypes of *M. guttatus* in terms of survival to seed set after drought onset (Wu et al. 2010; Mantel and Sweigart 2019). In this study, decreasing life span while maintaining stable fruit production was a strong phenological response shown by *M. micranthus* to successfully save itself from intensifying drought regimes. Such rapid development rate shown under elevated drought stress is indicative of the “live fast, die young” developmental strategy that is associated with drought escape (Wu et al. 2009). However, minimal plasticity in timing of bud and flower production was observed in *M. micranthus* individuals grown in coarser environments. During the incubation period for Experimental Set #2, some *M. guttatus* and *M. micranthus* individuals began forming buds which may have masked a potential plastic response in the execution of these earlier life stages. Support for this finding is shown in the dry down experiment where *M. micranthus* in the 0% coarse treatment exhibited greater rates of survival than individuals in coarser groups. Given that all treatment pots were shown to completely dry down within 72 hours in the water capacity experiment, it is likely that the effect of more ample water at earlier time points in the fine-grained treatment allowed for this group to more successfully endure extreme drought. Therefore, the same mechanism may have affected early plant robustness in the long-term experiment.

However, plasticity in developmental timing according to drought intensity in *M. micranthus* may have begun to be captured in later life stages, namely fruit production. Individuals in the 100% coarse treatment were first to produce viable seed pods, but not to a significant degree (Fig. 9). Additionally, Mantel and Sweigart (2019) reported marginally significant differences in timing of seed production but not bud or flowering time in *M. nasutus*, (a self-pollinator and closely related relative of *M. micranthus* and *M. guttatus*) under the effects

of gradual dry down. This finding is of interest given that in my study droughts occurred intermittently, so drought stress was held relatively consistent throughout the life cycle unlike dry down conditions where drought stress slowly escalates and becomes more influential later in life. But because drought conditions were imposed after budding began, future work should consider whether the plasticity of early life history events for *M. micranthus* increases when intermittent drought is initiated earlier in development.

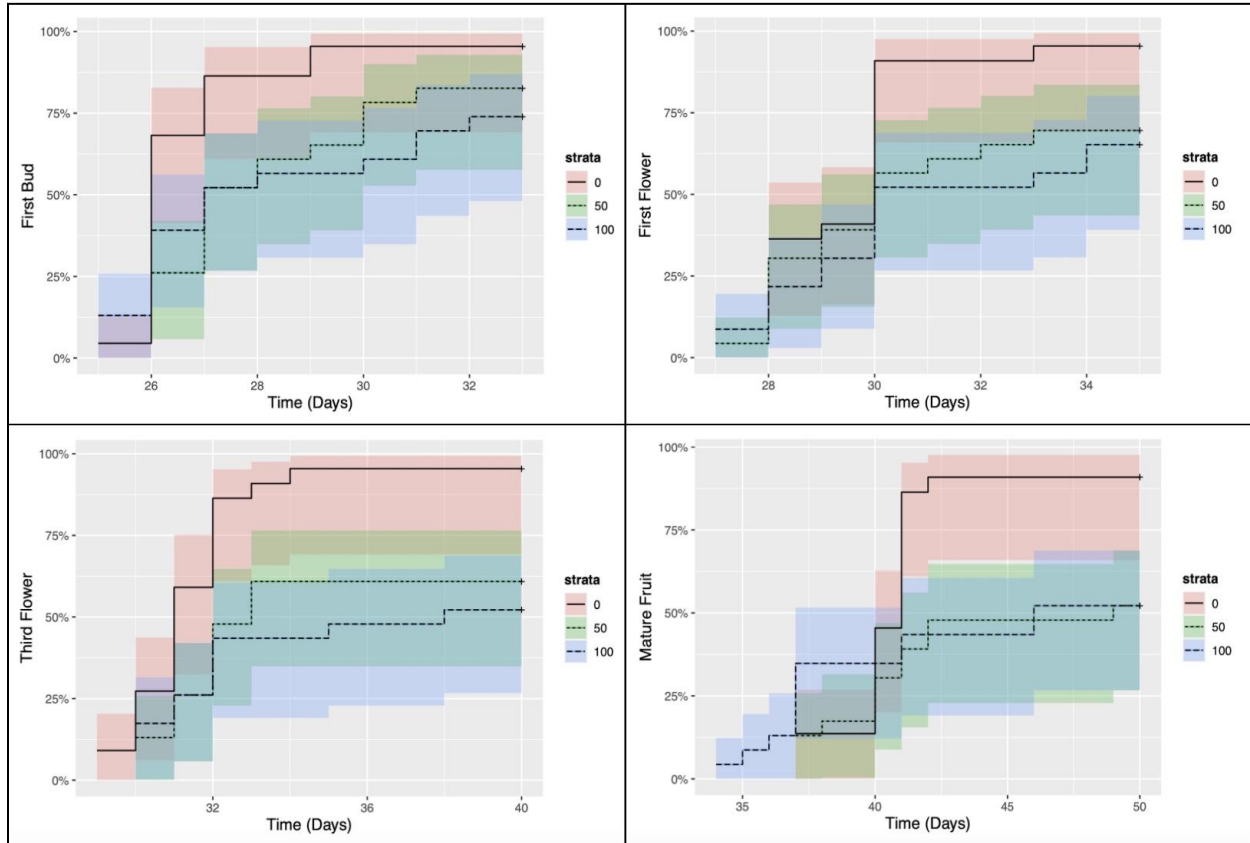


Fig. 9. Kaplan-Meier plots illustrate the proportion of *M. micranthus* individuals that produced a bud, first flower, third flower, and mature fruit over time according to treatment blocks. *M. micranthus* appears to exhibit relatively consistent developmental timing between treatments at multiple critical life history stages.

Expression of drought avoidance may be confined to the harshest drought regimes

The goal of the drought avoidance pathway is to increase water use efficiency (WUE) by lowering the rate of transpiration and increasing photosynthetic capacity via a variety of physiological pathways such as increasing succulence, leaf lobing, and reflectance while decreasing total leaf area, SLA, and stomatal density—however not all of these characteristics will necessarily occur in conjunction in every species (Kooyers 2015). Drought avoidance tends to be associated with environments under the influence of frequent, mild droughts. The nature of

my watering scheme thereby may have favored the expression of drought avoidance behaviors. Due to its mixed mating capacity and adaptation to moist environments, I expect that *M. guttatus* will exhibit signals of drought avoidance (Kooyers 2015). However, prior work has shown that climatic responses in *M. guttatus* have manifested as changes to traits found in both the drought avoidance and escape pathways (Kooyers et al. 2015; Kooyers et al. 2021).

Although *M. guttatus* showed low drought resilience, expression of leaf-level traits in the coarser treatments indicates the possible presence of a drought response threshold. Despite greater WUE mediated by leaf-level adjustments in the coarser treatments, this strategy was ultimately unsuccessful in saving *M. guttatus* from the effects of drought as many individuals failed to produce third flowers or mature fruit. However, succulence and leaf area were the only drought avoidance traits shown to mount a genotype by environment response in *M. guttatus*, meaning the expression of these traits varied depending on the environmental context (Saltz et al. 2018). Here, succulence was markedly greatest in the coarsest treatment, whereas leaf area was highest in the fine-grained sand. Given that there was no consistent relationship between these traits and treatment, very possibly this behavior may indicate the potential of a drought response threshold in this line of *M. guttatus*. Indeed, degrees of drought avoidance behaviors have been shown to vary in natural populations of *M. guttatus* according to standing genetic variation and climatic inputs (Kooyers et al. 2015). Experimental power to investigate genotype by environment interactions in drought avoidance may be improved if a true control were introduced where plants were grown on organic soils with ambient water inputs in order to compare expression of drought response phenotypes or to introduce a wider range of soil textures where plants are grown on clay, silt, and sand treatments to induce differences in *M. guttatus*' drought response by a broader array of environments.

Conclusion

Generally, the seasonal duration of habitable soil moisture conditions is likely to decrease as average daily temperatures increase, especially on coarse soils which I have shown to be already predisposed to rapid rates of water loss. As such, it is possible that species or populations adapted to coarse soils may be some of the first to exhibit evidence of water scarce adaptation directed by climate change. Our test of soil-mediated drought effects on *M. guttatus* and *M. micranthus*, two sympatric but reproductively unique species, captured a variety of genotype, treatment and mixed effects on phenotypic drought response. Plants in the finer-grained sands often outperformed individuals in the coarser treatments, but *M. micranthus* overall fared significantly better under drought than *M. guttatus*. Each species appeared to exhibit a unique drought response strategy consistent with established theory in angiosperm evolution implicating self-pollination with drought escape (Snell and Aarssen 2005; Ivey et al. 2016; Burnette and Eckhart 2021). Further testing on a broader range of *Mimulus* and other taxa should be undertaken in order to more specifically determine how soil texture will affect plants under climate change, especially on plants in natural environments and soils. But based on my findings it appears as though, between the additive effects of water loss and warmer climates, native plant populations may diverge further due the effect of drought on phenology and physiology. How these patterns will carry through offspring, however, is unclear and I am in the process of enacting a second generation study on seeds developed during this experiment in order to determine how maternal effects may direct species evolution. But greater steps should be taken to consider not only how other taxa respond to texture-mediated drought but also within the ecogeographic diverse distribution of natural *M. guttatus* and *M. micranthus* populations. Generally, however, my findings provide insight into the specific direction of angiosperm evolution grown on texturally heterogeneous soils under climate change.

Works Cited

- Anstett DN, Branch HA, Angert AL. 2021. Regional differences in rapid evolution during severe drought. *Evolution Letters*. 5:130–142.
- Brady NC, Weil RR. *The Nature and Properties of Soils*. 14th ed. New York (NY): Pearson.
- Burnette TE, Eckhart VM. 2021. Evolutionary divergence of potential drought adaptations between two subspecies of an annual plant: are trait combinations facilitated, independent, or constrained? *American Journal of Botany*. 108(2):309–319.
- Donovan LA, Ehleringer JR. 1994. Water stress and use of summer precipitation in a Great Basin shrub community. *Functional Ecology*. 8(3):289–297.
- Dudley LS, Mazer SJ, Galusky P. 2007. The joint evolution of mating system, floral traits and life history in *Clarkia* (*Onagraceae*): genetic constraints vs. independent evolution. *Journal of Evolutionary Biology*. 20(6):2200–2218.
- Ebi KL, Vanos J, Baldwin JW, Bell JE, Hondula DM, Errett NA, Hayes K, Reid CE, Saha S, Spector J, et al. 2021. Extreme weather and climate change: population health and health system implications. *Annual Review of Public Health*. 42:293–315.
- Ehleringer JR, Cooper TA. 1988. Correlations between carbon isotope ratio and microhabitat in desert plants. *Oecologia*. 76(4):562–566.
- Fayos CB. 1997. The roles of texture and structure in the water retention capacity of burnt Mediterranean soils with varying rainfall. *CATENA*. 31:219–236.
- Fenster CB, Ritland K. 1994. Evidence for natural selection on mating system in *Mimulus* (*Scrophulariaceae*). *International Journal of Plant Sciences*. 155:588–596.
- Fenster CB, Diggie PK, Barrett SCH, Ritland K. 1995. The genetics of floral development differentiating two species of *Mimulus* (*Scrophulariaceae*). *Heredity*. 74:258–266.
- Gee GW, Bauder JW. 1986. Particle-size analysis. In: Klute A, editor. *Methods of Soil Analysis: Part I*. 2nd ed. Madison (WI): Agronomy Monograph. p. 383–411.
- Hall MC, Willis JH. 2006. Divergent selection on flowering time contributes to local adaptation in *Mimulus guttatus* populations. *Evol*. 60:2466–2477.
- Ivey CT, Carr DE. 2012. Tests for the joint evolution of mating system and drought escape in *Mimulus*. *Annals of Botany*. 109:583–598.

- Joshi C, Mohanty BP. 2010. Physical controls of near-surface soil moisture across varying spatial scales in an agricultural landscape during SMEX02. *Water Resour. Res.* 46:W12503.
- Kallis G. 2008. Droughts. *Annu. Rev. Environ. Resour.* 2008. 33:85–118.
- Karron JD, Ivey CT, Mitchell RJ, Whitehead MR, Peakall R, Case AL. 2012. New perspectives on the evolution of plant mating systems. *Annals of Botany.* 109:493–503.
- Kolis KM, Berg CS, Nelson TC, Fishman L. 2022. Population genomic consequences of life-history and mating system adaptation to a geothermal soil mosaic in yellow monkeyflowers. *Evolution.* 76:765–781.
- Kooyers NJ. 2015. The evolution of drought escape and avoidance in natural herbaceous populations. *Plant Science.* 234:155–162.
- Kooyers NJ, Greenlee AB, Colicchio JM, Oh M, Blackman BK. 2015. Replicate altitudinal clines reveal that evolutionary flexibility underlies adaptation to drought stress in annual *Mimulus guttatus*. *The New Phytologist.* 206:152–165.
- Kooyers NJ, Morioka KA, Colicchio JM, Clark KS, Donofrio A, Estill SK, Pascualy CR, Anderson IC, Hagler M, Cho C, et al. 2021 Population responses to a historic drought across the range of the common monkeyflower (*Mimulus guttatus*). *American Journal of Botany.* 108(2):284–296.
- Lowry DB, Rockwood RC, Willis JH. 2008. Ecological reproductive isolation of coast and inland races of *Mimulus guttatus*. *Evolution.* 62(9):2196–2214.
- Mantel SJ, Sweigart AL. 2019. Divergence in drought-response traits between sympatric species of *Mimulus*. *Ecology and Evolution.* 9:10291–10304.
- Mazer SJ, Dudley LS, Hove AA, Emms SK, Verhoeven AS. 2010. Physiological performance in *Clarkia* sister taxa with contrasting mating systems: do early-flowering autogamous taxa avoid water stress relative to their pollinator-dependent counterparts? *International Journal of Plant Sciences.* 171:1029–1047.
- Ritland C, Ritland K. 1989. Variation of sex allocation among eight taxa of the *Mimulus guttatus* species complex (*Scrophulariaceae*). *American Journal of Botany.* 76(12):1731-1739.
- Roels SAB, Kelly JK. 2011. Rapid evolution caused by pollinator loss in *Mimulus guttatus*. *Evolution.* 65(9):2541–2552.

- Rosenthal DM, Ludwig F, Donovan LA. 2005. Plant responses to an edaphic gradient across an active sand dune/desert boundary in the Great Basin Desert. *International Journal of Plant Sciences*. 166:247–255.
- Saltz JB, Bell AM, Flint J, Gomulkiewicz R, Hughes KA, Keagy J. 2018. Why does the magnitude of genotype-by-environment interaction vary? *Ecology and Evolution*. 8:6342–6353.
- Sicard A, Lenhard M. 2011. The selfing syndrome: a model for studying the genetic and evolutionary basis of morphological adaptations in plants. *Annals of Botany*. 107:1433-1443.
- Smith MD. 2011. The ecological role of climate extremes: current understanding and future prospects. *Journal of Ecology*. 99:651–655.
- Snell R, Aarssen LW. 2005. Life history traits in selfing versus outcrossing annuals: exploring the ‘time-limitation’ hypothesis for the fitness benefit of self-pollination. *BMC Ecology*. 5:2.
- Soil Science Division Staff. 2017. Examination and Description of Soil Profiles. In: Ditzler C, Scheffe K, Monger HC, editors. *USDA Soil Survey Manual*. 18th ed. Washington D.C.: Government Printing Office. p. 83–233.
- Soil Survey Staff. 2022. Kellogg Soil Survey Laboratory methods manual. Soil Survey Investigations Report No. 42, Version 6.0. U.S. Department of Agriculture, Natural Resources Conservation Service.
- Tilstone GH, Macnair MR. 1997. Nickel tolerance and copper - nickel co - tolerance in *Mimulus guttatus* from copper mine and serpentine habitats. *Plant and Soil*. 191:173–180.
- Vtipil EE, Sheth SN. 2020. A resurrection study reveals limited evolution of phenology in response to recent climate change across the geographic range of the scarlet monkeyflower. *Ecology and Evolution*. 10:14165–14177.
- Wang T, Lui Q, Franz TE, Li R, Lang Y, Fiebrich CA. 2017. Spatial patterns of soil moisture from two regional monitoring networks in the United States. *Journal of Hydrology*. 552:578–585.
- Wu CA, Lowry DB, Nutter LI. 2010. Natural variation for drought-response traits in the *Mimulus guttatus* species complex. *Oecologia*. 162:23–33.
- Guerrant EO. 1989. Early maturity, small flowers and autogamy: a developmental connection? In: Bock JH, Linhart YB, editors. *The evolutionary ecology of plants*. 1st ed. Boulder (CO): Westview Press. p. 61–84.