Daisies in Distress: Growth Responses of *Lasthenia gracilis* to Simulated Drought over a Geographic Gradient

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ABSTRACT

Climate change impacts the severity and frequency of droughts in California. As a result, native plants will likely face changes in soil moisture as temperature and weather patterns shift. *Lasthenia gracilis*, commonly known as needle goldfields, is a species of native California flower with habitats ranging from northern to southern California. Therefore, this species is ideal for testing how a controlled variable affects a set of populations differing by latitude. Through a gridded greenhouse experiment, I measured the effects of water stress on the growth and reproductive fitness of this species of native California daisy to explore if there is variation in response between northern and southern populations. I also measured general survivorship, days to germination, germination rate, and days to flowering to further compare responses among the geographically distinct populations. The results of my research are expected to show that individuals from southern populations will be more resilient to drought conditions, by exhibiting slower growth rates and higher inflorescence counts than plants from northern populations exposed to the same degree of water stress. This higher drought fitness is expected to significantly correlate with higher numbers of inflorescences, a measure of reproductive fitness. Studying the effects of limited water availability on growth and reproductive ability of specific native California plants can provide insights into how climate change will impact important California ecosystems such as grasslands. This knowledge of ecological vulnerability is crucial when assessing and weighing the importance of mitigating the many detrimental effects of climate change.

KEYWORDS

water stress, adaptive differentiation, greenhouse experiment, climate change, phenology

INTRODUCTION

Anthropogenic climate change is causing an increase in global average temperatures, sea level rises, changes in precipitation patterns, and disturbances to species interactions (US Global Change Research Program 2009). California is experiencing an increase in the frequency, magnitude, and length of droughts with "chronic, long-term hydrological drought" looming at the end of this century (Mann & Gleick 2015, USGCRP 2017). Furthermore, global temperature increases of 1.8 °F have already been recorded, with business-as-usual scenarios predicting a global average increase of 9 °F by 2100 (USGCRP 2017). Both extended water stress and increasing temperatures have been correlated with earlier flowering, a potentially detrimental phenomenon to the community composition and ecosystem-level resilience of flowering grassland species (Suttle 2007, Crimmins 2009).

Water stress, especially the prolonged periods without precipitation associated with drought, can disrupt the phenology of plants (Suttle 2007). Phenology is the timing of various biological events in a life cycle such as flowering, breeding, and hibernation (Lieth 1974). Variability in phenology has been found to be most drastic in early-flowering plant families (Mazer 2012) and early-active species (Wainright 2012). Specifically in mid- to high-latitudes, increasingly-warming temperatures disproportionately affect the phenology of early-active plants due to higher temperature variability in spring months (Menzel 2006). Changes in phenology can have serious repercussions for both individual plant fitness and entire ecosystems (Mazer 2012). At an extreme, alterations to the phenology and reproductive ability of species can lead to phenological mismatches - asynchrony with pollinators or a loss of temporal overlap between mutualistic species (Rafferty 2015).

However, there is evidence of adaptive differentiation within a species in response to different amounts of precipitation in the environment (Sultan 1996, Rajakaruna 2002). For example, Sultan (1996) found that the offspring of *Polygonum persicaria* exposed to various environmental stressors, including low soil moisture, demonstrate wide plasticity in their growth responses due to higher provisioning of mass to seedlings, earlier germination time, and other individual parental compensations. Similarly, Rajakaruna (2002) recorded plasticity in the phenotypes of *Lasthenia californica* exposed to drought conditions and found them to be differentiated by race, indicating adaptive differentiation by population. This phenotypic plasticity, at the individual and population

level, could provide species with a wide geographic range with greater drought tolerance and increased resistance to competition caused by phenology shifts (Nicotra 2010). Through my research, I hope to determine if there is measurable plasticity in the growth and phenology, correlated with population, of an early-flowering native California annual exposed to controlled water stress, pointing to adaptive differentiation.

The purpose of this study is to determine how individuals from different populations of grassland plants respond to controlled water stress. I am studying this response using *Lasthenia gracilis* (DC.) Greene (Asteraceae), as a model organism. I chose this species because it is a flowering annual with a large geographic range and genetically-distinct populations (Calflora 2018, Rajakaruna & Bohm 1999). I hope to answer:

1) How are the growth rates and inflorescence counts of *L. gracilis* from different populations across California comparatively influenced by controlled water stress?
2) Are plants from populations that receive less precipitation historically more resilient to drought conditions, thus exhibiting slower growth rates and higher inflorescence counts than plants from historically wetter areas exposed to the same degree of water stress?
3) Is there a relationship between the number of days until the first flower and peak flowering time and drought stress that varies by population?
4) How is the length of longest leaf at first flower affected by water stress and how does this vary by population?

METHODS

Study species

Through this research, I investigated the effects of water stress on the growth of individuals from 6 populations of *Lasthenia gracilis*, a native California wildflower with a range of habitats from northern California to the southern border with Mexico (Calflora 2018). *Lasthenia* is a genus comprised of 21 known species and subspecies occupying a diverse set of habitats. These include coastal bluffs, open grasslands, oak woodlands, alkali flats, chaparral, pastures, roadsides, desert

habitats, and serpentine outcrops (Rajakaruna & Bohm 1999; Rajakaruna 2003). *Lasthenia gracilis* is an annual herbaceous species that is native to California with a typical bloom period from February to June (Calflora 2018). Due to hypothesized adaptive differentiation, the suitable temperature range, elevation, amount of precipitation, and morphological characteristics are extremely broad and varied. For instance, according to Calflora, the wet season is anywhere from 0 to 7 months with temperatures ranging from 32 °F to 98 °F (2018). Given this wide variation, I systematically chose the 6 populations to be represented in my drought study based on factors including climate moisture deficit and availability of seeds.

I chose *Lasthenia gracilis* as my model organism because it is a flowering annual with a large geographic range (Figure A1) and has genetically distinct populations. This makes it favorable for investigating adaptive differentiation in the context of drought conditions (Desrochers and Bohm 1995; Rajakaruna and Bohm 1999; Rajakaruna 2002). Furthermore, this species has relatively fast experimental germination (1-2 weeks) and short growth cycles (2 months) making it ideal to test my questions.

Study population selection

I chose study populations based on latitude, climate moisture deficit (CMD), and seed availability. I used second generation seeds collected from a previous phenological study conducted by Rachael Olliff-Yang (unpublished data). I selected populations with at least 50 seeds from the study and excluded populations with low germination rates. My populations represented a range from arid, southern habitats to wetter, northern habitats (Table 1, Figure A2).

Table 1. Summary of location and environmental condition details for chosen populations of *Lasthenia gracilis* used in the study. Temperature and precipitation values are averaged over the typical *Lasthenia* growing season (February – June).

Population	Latitude	Longitude	Average temp (°C)	Average precip (cm)	CMD (ave)
Anza Borrego (AB)	33.22204	-116.45735	15.06	24.4	95.1
Carrizo Plain 2 (CB)	35.07412	-119.66115	13.88	21.0	99.6
Henry Coe (HC)	37.17447	-121.51714	13.44	60.2	66.3
Pinnacles (PN)	36.48411	-121.1664	13.92	36.8	97.0
Tejon Mojave 3 (TC)	34.85118	-118.69142	14.72	31.2	91.0
Table Mountain (TM)	39.59691	-121.54249	15.02	105.8	63.5

Greenhouse experiment

To determine the relationship between population and growth response to water stress, I conducted a gridded greenhouse experiment at the Oxford Tract greenhouses at the University of California, Berkeley (UC Berkeley 2018). I controlled temperature, light, and pest exposure. The greenhouse was maintained at a temperature from 64-77 °F with a photoperiod of 12 hours of light from overhead high intensity discharge (HID) lights. Neither pesticides nor fertilizer was used during this experiment.

Experimental methods

Germination

To test the effects of 3 watering treatments on the growth and reproduction of *L. gracilis* from 6 populations, I created a quadruplicate set up per treatment with one extra seed per population, totaling planting 13 individuals from 6 populations (Figure A3). To do this, I germinated 50 seeds from 10 populations to compensate for variable germination rates between populations. For each population, I selected seeds most likely to be fertile based on color (dark brown to black) and fill (opaque) from 5 randomly chosen mother lines (Rachael Olliff-Yang, unpublished study). Next, I placed the seeds in petri dishes pre-moistened with 1-5 mL of deionized water. I then placed the dishes in a refrigerator at 2 degrees C until root tips emerged to mimic the cold, dark germination conditions characteristic of winter in California. I planted seeds from populations with the highest germination rates and greatest diversity in terms of latitude between populations (Table 2).

Table 2. Germination rates for 50 seeds per population planted on October 8, 2018.

Population	**AB**	**BP**	**IE**	**PN**	**HC**	**TC**	**TM**	**CB**	**FH**	**HH**
Germination Rate (10/25)	26%	30%	8%	72%	54%	78%	42%	30%	66%	26%
Germination Rate (10/26)	28%	42%	12%	72%	60%	84%	46%	32%	76%	38%

Planting

I transferred pre-germinated seeds into sterilized cones containing water-saturated potting mixture. I prepared containers by cleaning them with a 10% bleach solution and rinsing them with tap water (Figure A4). To prevent soil loss, I placed a jumbo cotton ball in the bottom of each cone and then filled them with Sunshine Growth 4 Aggregate Mix (Figure A5). To facilitate precise seedling transfer, I fully saturated soil by adding tap water, allowing the soil to settle, refilling each cone with around 1 cup of additional soil, and bringing the dry soil to saturation. I also employed bottom watering to keep the soil saturated for the first 17 days of growth, changing the water once a week to prevent algae growth. To prevent breakage due to pinching, I used tweezers to forklift one seed at a time into previously-created indents (~0.5 cm deep) in each cone. To randomize placement, I organized cones according to a random number generator (Figure A6) and labeled them with the population and seed number (Figure A7). To ensure contact between the root hairs and the soil, I gently pushed soil around the seed and moistened the area with 1-5 mL of water. I moved any large pieces of perlite away from the seed with tweezers to prevent dessication.

Water stress treatments

I established 3 watering treatments that ranged from saturated soil to extreme water stress, and exposed 4 individuals from each population to each treatment. My watering treatments were as follows:

- Low: 10 mL of tap water once per week
- Medium: 10 mL of tap water twice per week
- High: 25 ml of tap water twice per week, maintaining soils at saturation

To ensure that drought conditions began after the plants had set root and begun to grow, I began the watering treatments 17 days after planting the seeds. Up until this point, I employed bottom watering and moistened the top of each cone with ~5 mL of water once a day. I stopped moistening the top soil 3 days before the watering treatment began and removed the cone-tainers from bottom-watering tray on the day the watering treatments began. I continued watering according to this scheme until most individuals reached senescence and stopped producing flowers. The water stress experiment was initiated on October 26, 2018 and ran for 101 days.

Data collection

To quantify the effects of water stress on this native California wildflower, I recorded seedling growth metrics over 101 days. I chose growth rate (cm/day), inflorescence counts, days to flowering (days), peak flowering time (days since planting), and length of longest leaf at first flower (cm) because they represent growth, phenology, and reproductive fitness. I checked the plants at least two times per week for 12 weeks. To find the relationship between water stress and growth, I measured the distance from the top of the soil to the tallest part of the plant two times per week using a tape measurer. These heights divided by the total number of days during which the plant's height is positively increasing gave the growth rate for that plant. I also measured the length of the longest leaf at first flower on the day that a plant exhibits its first open flower, as a measure of relative size differences at the time of flowering.

To find the relationship between water stress and reproductive fitness and phenology, I collected inflorescence counts twice a week. Because *Lasthenia gracilis* belongs to the family Asteraceae, it produces a cyme-like head comprised of both disk and ray flowers (Keil 2017). To determine days to flowering, I recorded the date that each plant exhibited its first "open inflorescence", defined as having at least one open ray flower in an inflorescence (Figure A8). I then used this date to determine the number of days until flowering from the date of planting. I counted the number of open inflorescences on each plant every day until most populations, with the exception of PN and AB, had reached senescence, defined as the point when all inflorescences were counted dead. These measurements gave the inflorescence counts and the peak flowering time.

Data analysis

To determine the relationship of the growth and reproduction response variables with both population and water treatment, I employed various methods of statistical analyses in R (R Development Core Team 2014). For each response variable (growth rate, maximum number of inflorescences, length of longest leaf at first flower, flowering start date, flowering peak date, flowering end date, and flowering duration) I performed a Two-Way Analysis of Variance

(ANOVA) using R Studio. Due to my experimental design, the independent variables were not correlated, and I was able to perform the analysis using the following formulas:

- growth rate ~ population + water treatment
- maximum number of inflorescences ~ population + water treatment
- length of longest leaf at first flower ~ population + water treatment
- flowering start date ~ population + water treatment
- flowering peak date ~ population + water treatment
- flowering end date ~ population + water treatment
- flowering duration ~ population + water treatment

To visually compare the variation in growth responses to drought conditions between populations, I created ordered boxplots in R Studio. For my visualization method, I plotted the average of the growth rate on the Y axis for each group of four individuals from the same population exposed to the same water treatment on the X axis to look for clusters of like responses by population and trends. I repeated this ordination for the maximum number of inflorescences and length of longest leaf at first flower. To visually interpret the phenological responses, I created two horizontal boxplots of the start, peak, and end flowering dates grouped both by population and watering treatment. For all analyses, I removed the individuals TC.1, HC.12, and PN.12 because they failed to grow past germination and replaced them with the data for the 13th "extra" individual I planted for each population (TC.E, HC.E, and PN.E).

RESULTS

Effects of population x water on growth

Maximum height

I found that plant growth variables (height, growth rate for the first 51 days, length of longest leaf at first flower) showed significant population and water treatment effects (Table 3). The maximum heights were significantly affected by both population ($p = 0.040684$) and water treatment ($p = 0.0328$) (Table 4). Plants from population Table Mountain were the tallest on average across all watering treatments with the longest leaves at the first flower. Plants from Table

Mountain also demonstrated the greatest percent difference in average height and growth rate between the lowest and highest watering treatments (Figure 1). All populations with the exception of Tejon Mojave and Carrizo Plain consistently produced taller plants with increasing amounts of water.

Table 3. Means of growth responses by population and water treatment.

Population	Maximum height (cm)			Growth Rate over first 51 days (cm/day)			Length of longest leaf at first flower (cm)		
Water Treatment →	**Low**	**Med**	**High**	**Low**	**Med**	**High**	**Low**	**Med**	**High**
AB	10.075	10.675	14.175	0.1917	0.2036	0.2776	3.200	3.375	5.225
TC	10.050	7.550	10.300	0.1885	0.1380	0.1931	3.450	2.475	2.625
CB	10.200	10.175	9.975	0.1948	0.1969	0.1917	2.675	3.125	2.775
PN	10.000	9.325	12.750	0.1896	0.1776	0.2469	3.225	3.325	3.200
HC	10.525	11.225	12.650	0.2031	0.2161	0.2495	3.850	4.575	4.775
TM	8.875	11.325	16.150	0.1635	0.2094	0.3078	2.900	2.775	4.175

Table 4. The results of a Two-Way ANOVA that tested growth rate ~ population + water treatment
* denotes significant effect

Dependent Variable: Maximum Height

Source	Type III Sum of Squares	df	Mean Square	F value	Sig. (p)
Population	67.55	5	13.51	2.517	0.040684*
Water treatment	113.47	2	56.73	10.568	0.000137*

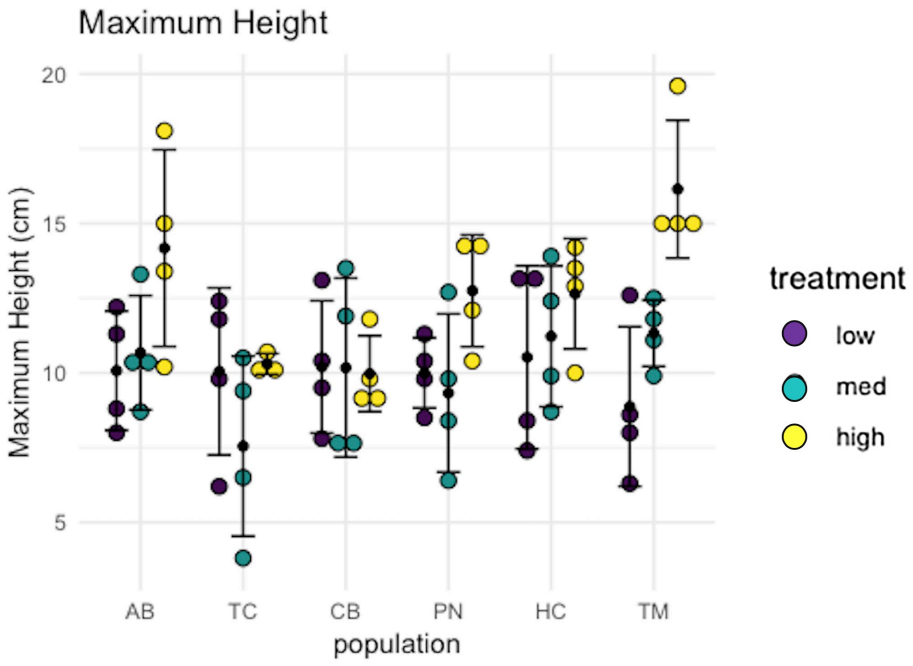

Figure 1. The average maximum heights ordered by population from south to north and sub-ordered by water treatment.

Growth rate over first 51 days

Differences in the growth rates over the first 51 days are significantly affected by water treatment ($p = 0.0328$), indicating that increasing amount of water is correlated with faster growth (Figure 2, Table 5). Within the population TC, plants exhibited the fastest growth when exposed to the highest watering treatment, however plants exposed to the lowest watering treatment grew faster than the medium watering treatment on average. Individuals from PN exhibited a similar trend. Individuals from CB exhibited similar growth rates across the three water treatments.

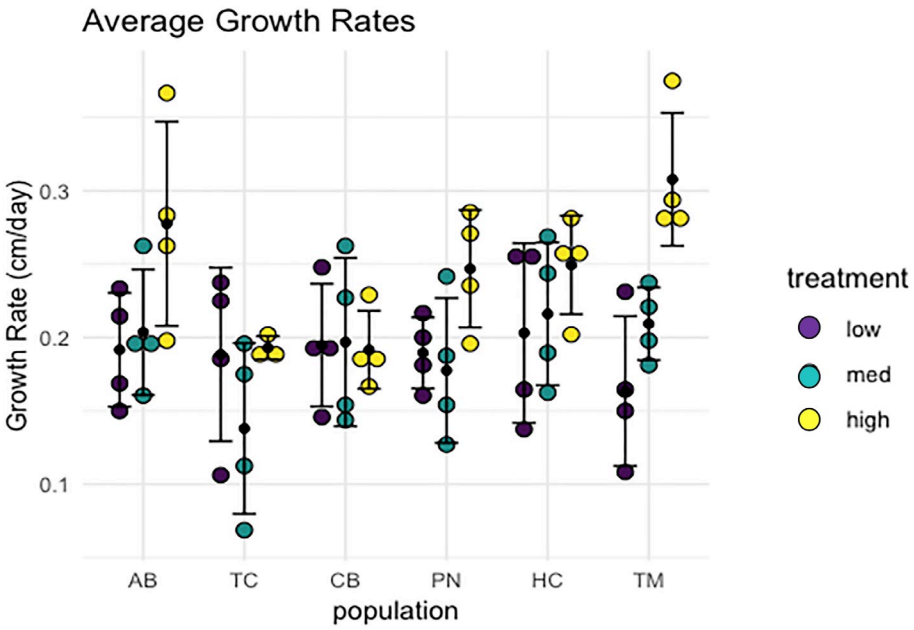

Figure 2. The average growth rates over the first 51 days ordered by population from south to north and sub-ordered by water treatment.

Table 5. The results of a Two-Way ANOVA that tested maximum height ~ population + water treatment.
* denotes significant effect

Dependent Variable: Growth Rate over the first 51 days

Source	Type III Sum of Squares	df	Mean Square	F value	Sig. (p)
Population	0.01051	5	0.002103	0.0937	0.4645
Water treatment	0.01636	2	0.008178	3.646	0.0328*

Length of longest leaf at first flower

Differences in the lengths of the longest leaf at first flower are significantly affected by population (p = 0.00526) (Table 6). The length of longest leaf did not exhibit differential response correlated with water treatment, indicating genetic differentiation for this trait (Figure 3). Plants from Anza Borrego exposed to the highest water treatment exhibited the longest leaves at first flower on average while plants from Tejon Mojave exposed to the medium water treatment exhibited the shortest leaves at first flower.

Table 6. The results of a Two-Way ANOVA that tested length of longest leaf ~ population + water treatment. * denotes significant effect

Dependent Variable: Length of Longest Leaf at First Flower

Source	Type III Sum of Squares	df	Mean Square	F value	Sig. (p)
Population	22.94	5	4.587	3.777	0.00526*
Water treatment	4.88	2	2.440	2.010	0.14395

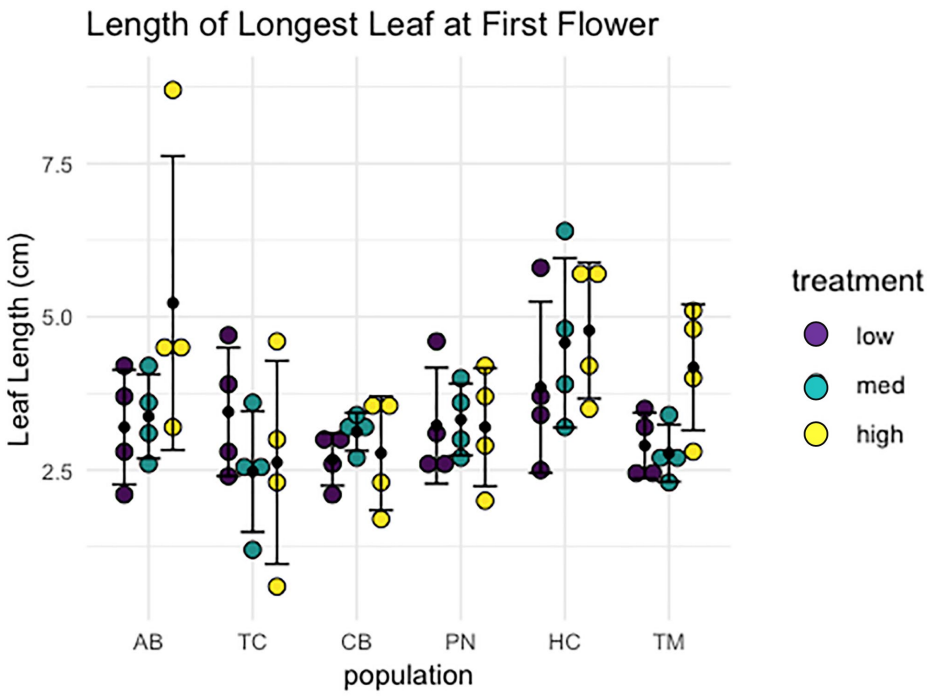

Figure 3. The average lengths of the longest leaf at the time of the first flower ordered by population from south to north and sub-ordered by water treatment.

Survivorship & Germination

Survivorship was high across all replicates with the exceptions of one replicate from Henry Coe (HC.12), Pinnacles (PN.12), and Tejon Mojave (TC.1) populations which did not grow past germination. I accidentally broke the stems of TC.6 and TC.7 during measurement on December 16, 2018, so I excluded these individuals from data analysis after this date.

Effects of population x water on reproductive fitness

Flowering

I found a significant positive relationship between amount of water and reproductive fitness. The maximum number of inflorescences are significantly affected by both population ($p < 0.0001$) and water treatment ($p = 0.000852$) (Table 7, Table 8). Within each population, individuals treated with the highest watering treatment yielded more inflorescences compared to individuals of the same population exposed to more drought stress, with the exception of Henry Coe. Consequently, plants in all populations except Henry Coe responded to drought conditions by producing fewer inflorescences (Figure 4).

Table 7. The results of a Two-Way ANOVA that tested maximum number of inflorescences ~ population + water treatment. * denotes significant effect

Dependent Variable: Maximum Number of Inflorescences

Source	Type III Sum of Squares	df	Mean Square	F value	Sig. (p)
Population	990.4	5	198.07	9.548	1.34e-06*
Water treatment	334.4	2	167.18	8.059	0.000852*

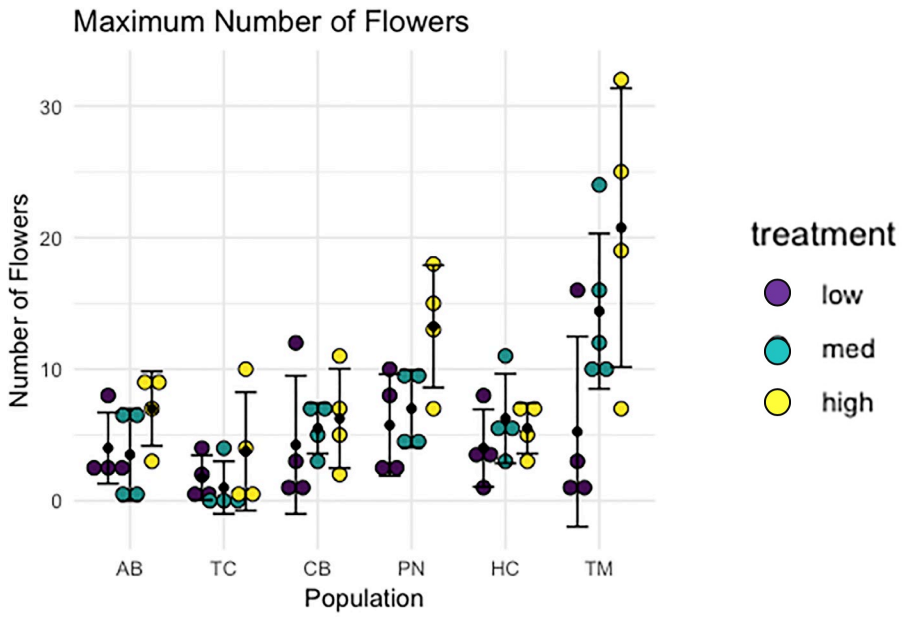

Figure 4. The maximum number of inflorescences ordered by population from south to north and sub-ordered by water treatment.

Table 8. Means of reproductive responses by population and water treatment.

Population	Maximum number of inflorescences			Flowering peak date (days since planting)			Flowering duration (days)		
Watering treatment →	**Low**	**Med**	**High**	**Low**	**Med**	**High**	**Low**	**Med**	**High**
AB	4	4	7	48.50	40.75	51.75	37.25	38.25	49.75
TC	2	1	4	40.50	44.50	55.25	31.75	19.25	29.75
CB	4	6	6	51.75	57.75	50.75	31.25	33.75	43.00
PN	6	7	13	51.50	55.25	56.00	38.00	41.50	58.25
HC	4	6	6	48.00	48.25	50.50	30.50	44.25	37.75
TM	5	14	21	44.50	50.20	62.25	34.75	45.20	53.25

A note on population effects on flowering

Desert populations (Anza Borrego, Pinnacles, and Carrizo Plain) continued flowering after the plants from other populations had reached senescence. Inflorescences produced after I had stopped watering were small, low on the plant, and often consisted of just a few open ray flowers poking through a bud (Figure A9). Since *Lasthenia gracilis* are annuals that produce seeds with limited longevity and short spread potential, they must set seed within their lifecycle for reproductive success (Hobbs & Mooney 1985). These small buds could be a strategy for reproductive success in the face of extreme drought conditions.

Phenology

I found that the phenology of *Lasthenia gracilis* (flowering start date, flowering end date, flowering duration, and peak flowering date) showed significant population and water treatment effects (Table 9). Population showed significant effects on flowering start date ($p = 0.010$), flowering end date ($p = 0.0176$), and flowering duration ($p = 0.00282$). Treatment showed significant effects on flowering end date ($p = 0.000536$), flowering duration ($p = 0.00496$), and peak flowering date ($p = 0.0213$). Flowers from all populations flowered later and longer when treated with more water (Figure 5). The peak flowering date was shifted 7 days later on average when comparing the highest and lowest treatments for all populations. The end flowering date was shifted 14 days later on average when comparing the highest and lowest treatments for all populations. The duration of flowering was shortened by 9 days on average when comparing the highest and lowest treatments for all populations. There is no discernible pattern of phenological shift ordered by latitudinal population (Figure 6).

Table 9. The results of the Two-Way ANOVAs that tested the effects of population + water treatment on the phenology of flowering.
* denotes significant effect

Dependent Variable	Source	Type III Sum of Squares	df	Mean Square	F value	Sig. (p)
Flowering Start Date	Population	333.3	5	66.66	3.369	0.010*
	Water treatment	80.6	2	40.30	2.037	0.140
Flowering End Date	Population	2139	5	427.8	3.021	0.017628*
	Water treatment	2454	2	1226.8	8.665	0.000536*
Flowering Duration	Population	2938	5	587.5	4.160	0.00282*
	Water treatment	1653	2	826.7	5.853	0.00496*
Peak Date	Population	652	5	130.39	1.740	0.1409
	Water treatment	619	2	309.71	4.133	0.0213*

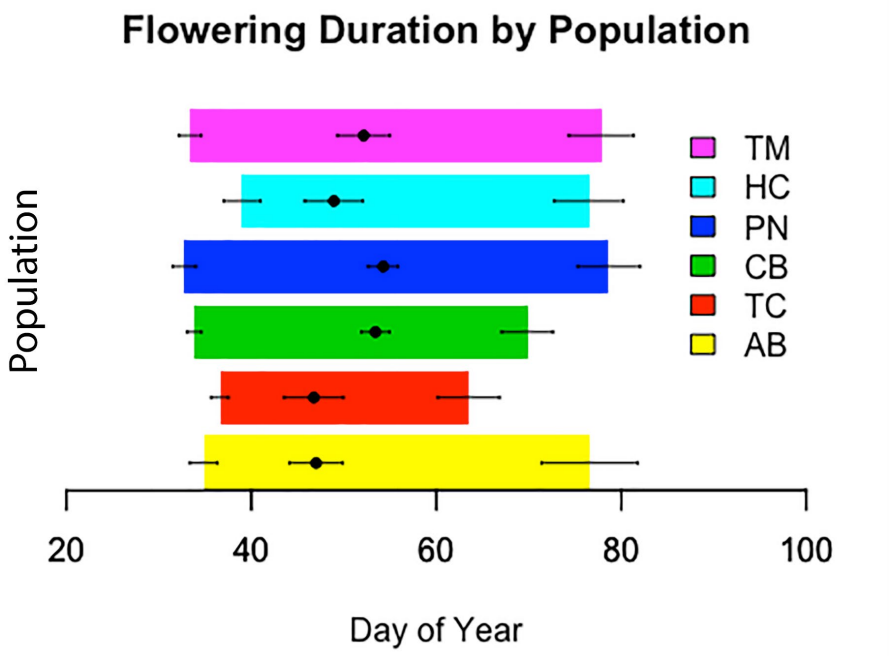

Figure 5. Average duration of flowering and peak flowering date for each population.

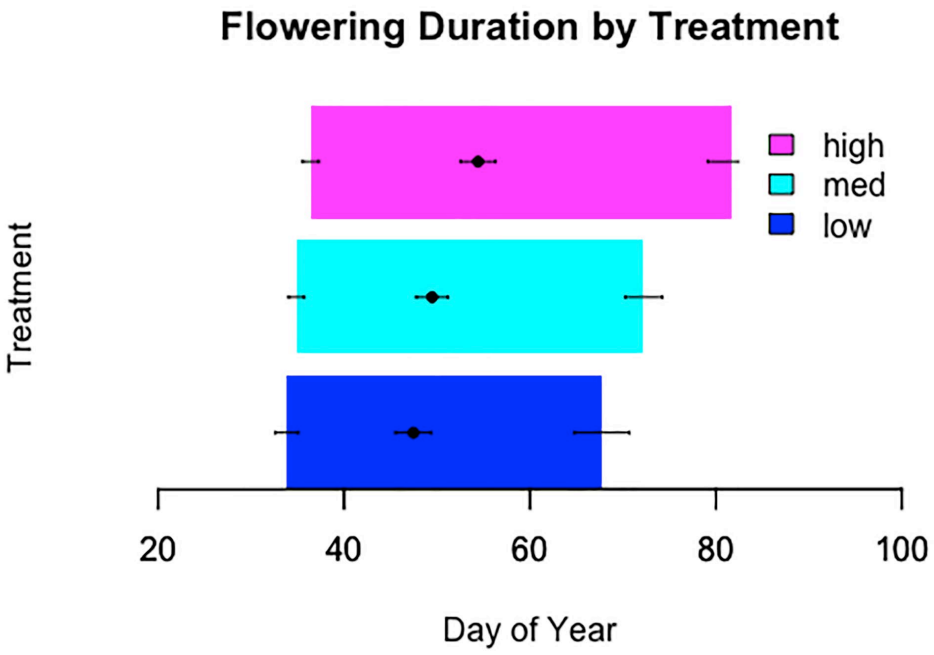

Figure 6. Average length of flowering and peak flowering date for each watering treatment.

DISCUSSION

Varied growth responses

I found that changing watering treatments produced significantly-varied growth responses in all populations, suggesting differentiated drought tolerance is grouped by geographic population. This is consistent with past studies that found that drought tolerance varies by population in *Lasthenia* (Rajakaruna et al. 2003). In my experiment, plants grew taller and faster with increasing amounts of water when compared to plants of the same population exposed to drought conditions, with the exception of plants from Tejon Mojave and Carrizo Plain. Previously, it has been proven that, "genetically based adaptive specialization occurs over relatively small spatial scales in edaphically heterogeneous environments" (Yost 2012). Consequently, because *Lasthenia gracilis* grows on a wide range of edaphic environments, the plasticity in growth responses fits a hypothesis of adaptive differentiation in this species (Rajakaruna & Bohm 1999).

Differences in the length of longest leaf at first flower were significantly affected by population but not water treatment, indicating genetic differentiation for this trait. This plasticity in response correlated with population follows a broader hypothesis of geographic speciation (Desrochers & Bohm 1995). In a past study by Rajakaruna, significant differences in size, flowering time, and number of flower heads were found to be correlated with population (Rajakaruna, Baldwin et al. 2003c). Similarly, I found that the maximum heights and lengths of longest leaf at first flower vary by population across the geographic range.

Flowering and phenology

Plants in all populations produced a significantly higher number of inflorescences when treated with more water, with the exception of Henry Coe. This behavior is consistent with resource cost hypotheses which highlight the inherent trade-off associated with the allocation of water for reproduction by asserting that this can be costly to vegetative growth and ultimately survival (Galen 1999). Namely, *Lasthenia gracilis* from all populations appear to allocate less water and energy to reproduction when resources are limited.

The severity of the negative impacts on reproductive output caused by drought stress is grouped by geographic population. Specifically, individuals from locations that receive lower amounts of precipitation on average (Anza Borrego, Tejon Mojave, Carrizo Plain, and Pinnacles) show less plasticity in reproductive response when comparing the number of flowers produced by plants grown under the lowest watering treatment with the number produced under the highest watering treatment within each populations (Table 1). For example, individuals from AB showed an average difference of 3 fewer inflorescences produced per plant when comparing those grown under the lowest watering treatment to those grown under the highest watering treatment. Individuals from TM, a site which receives about 4 times the amount of precipitation in the field compared with AB, showed an average difference of 16 fewer inflorescences per plant when comparing those treated with the lowest watering treatment to those treated with the highest watering treatment. Individuals from these historically drier locations (Anza Borrego, Tejon Mojave, Carrizo Plain, and Pinnacles) are able to reproduce to reach inflorescence numbers closer to their high watering treatment maximums, even when exposed to drought conditions. Since water use efficiency is a known drought adaptation, this trend in differential reproductive output correlated with population suggests that plants from these populations are more drought tolerant (Hendry 2005).

Plants from all populations flowered significantly earlier and for a shorter amount of time when exposed to drought conditions. These two strategies are both drought adaptations. For example, in desert plants, a lack of water has been shown to stimulate flowering of annuals (Rathcke & Lacey 1985). Phenotypically, this results in earlier flowering dates when annuals are exposed to drought conditions compared to when they are given ample water. However, there is no discernable pattern of stronger phenological shifts correlated with populations that have historically been exposed to less precipitation. As per previous geographic drought studies, I anticipated that plants from drier locations flower earlier and reach maturity faster (Table X.X, summary of flowering means, Rajakaruna et al. 2003).

Limitations

There are a few aspects of this project that could be improved. First, my growth measurement method was invasive as it required using my hands to pull the delicate plants up to the measuring

tape. This resulted in breakage of two plants and one leaf over the course of the greenhouse experiment. My method increased mortality but gave valuable growth response data including maximum height and growth rate. I had extra replicates to replace the affected individuals, but I would shift to a less invasive growth measure such as using above and below ground biomass to quantify growth response. Furthermore, the cones housing the plants were small, so soil got extremely stripped and sometimes caked by the end of the experiment. Planting in larger pots or implementing micro-tilling could solve this issue.

Future Directions

At any given site, the combination and interaction of countless environmental factors including soil type, temperature, precipitation, local biodiversity, and more have been shown to affect plant growth (Rajakaruna 2003, Dierig 2006, Rajakaruna 2003, Powell 2011). Through my research, I investigated the effect of water availability on the growth and reproduction of individuals from six geographically-distinct populations of *Lasthenia gracilis* chosen to represent a range of latitudes across California. Since many other factors besides simply the latitude of a site have been shown to affect plant growth, I see potential for a similar drought study to be conducted over various gradients including climate moisture deficit, elevation, temperature, as well as a comparative study of coastal versus inland plant responses (Figure 7).

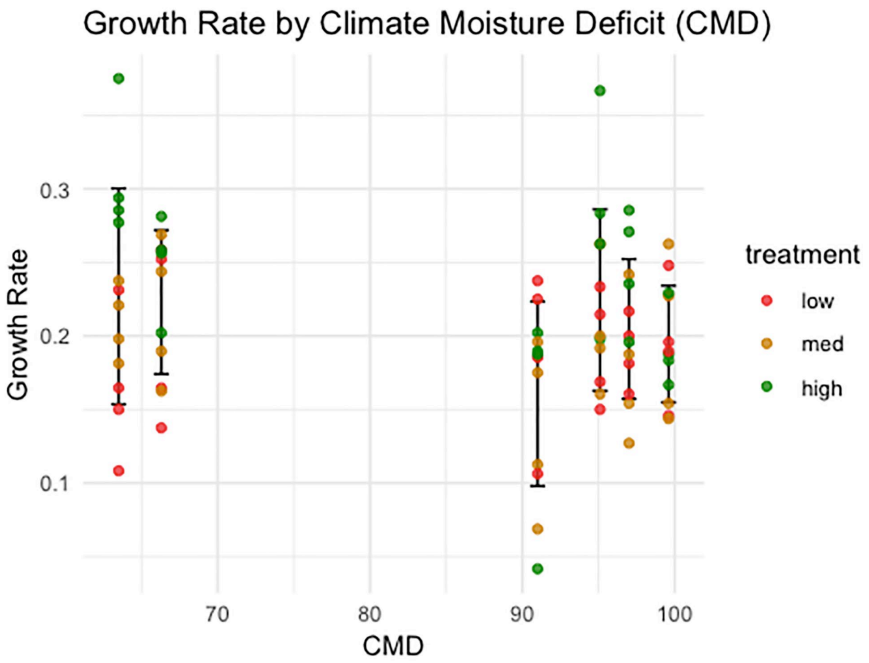

Figure 7. A preliminary graph showing growth rate responses grouped by climate moisture deficit of the site, rather than the latitude.

BROADER IMPLICATIONS

This research on the drought response of a species of native California wildflower has applications in management and ecosystem protection in the face of the changing climate. *Lasthenia gracilis* is already included in seed mixtures used for restoration projects and planted along highways by CalTrans because it is good for early cover (A. Montalvo pers. obs.) *Lasthenia* is also recommended for use in the rehabilitation of disturbed lands because it can tolerate a wide range of environments (Newton & Claassen 2003). Further understanding of the population-based limitations of these species can help fine-tune restoration plans based on changing climatic variables.

Additionally, knowledge of environmental preference by population can inform migration decisions. Assisted migration, or assisted colonization, is a process through which species that are at risk of extinction are introduced to a predicted more suitable environment (Gallagher 2014). As California faces a future of more frequent and intense droughts, translocating more drought tolerant lines in areas experiencing more drought could mitigate the possibility of entire loss of a species due to desiccation. Similarly, assisted gene flow is a conservation tactic in which more

resilient populations are crossbred with at-risk populations at a site (Aitken 2013). At its best, this process yields genetic resilience to environmental factors. However, there is also evidence for the inherent risk of outbreeding depression associated with the crossing of plants from populations insurmountable adaptive differences such as edaphic preferences (Montalvo 2017, Rajakaruna 2002). Knowledge of populations' adaptations to environmental factors such as water availability can help inform decisions to effectively implement conservation management tactics such as assisted migration and assisted gene flow in a vulnerable location.

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APPENDIX A: Maps & Photographs

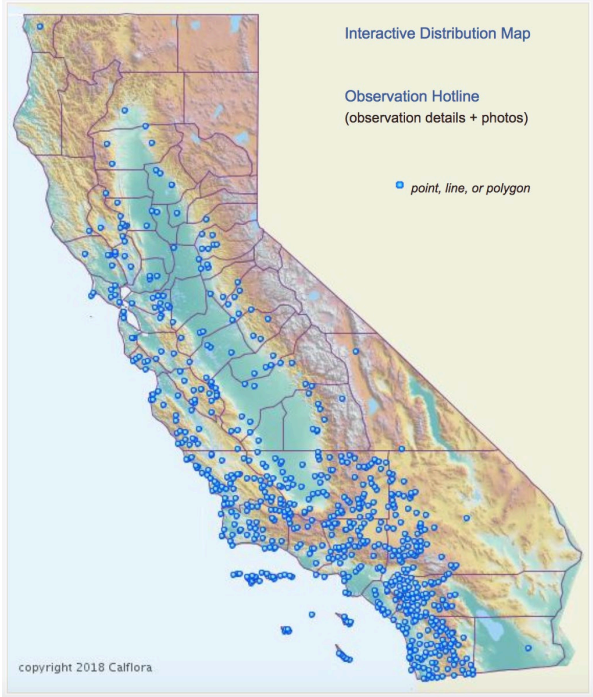

Figure A1. A map of the observations of *Lasthenia gracilis* in California (Calflora 2018).

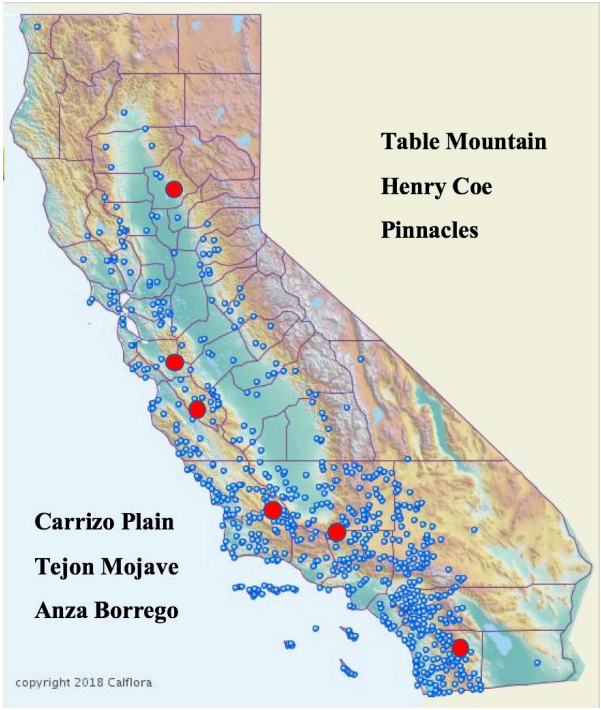

Figure A2. A map of the source locations of my 6 chosen populations of *Lasthenia gracilis* (Calfora 2018).

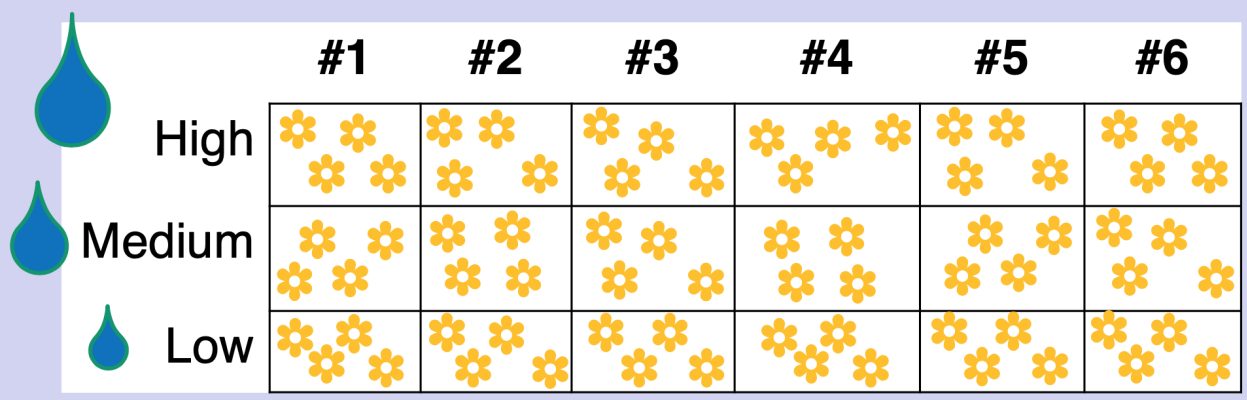

Figure A3. A diagram illustrating the experimental set-up for my greenhouse study. Four individuals from each population were exposed to one of three watering treatments. However, placement in the tray was randomized.

Figure A4. Cone-tainer sterilization and set-up. I rinsed soil from the cones used for Rachael Olliff-Yang's phenology experiment in a tub of water. Then I sanitized them in a tub of 1 part bleach:10 parts water and rinsed in a final tub of tap water.

Figure A5. Planting method. Emily Cox shown filling cones with Sunshine Growth 4 Aggregate Mix using a green shield to prevent excess soil from falling between the cones into the tray.

High	AB 1	26	CB 1	75	PN 1	23	HC 1	71	TC 1	24	TM 1	68		Extra 1 per pop		
High	AB 2	14	CB 2	41	PN 2	34	HC 2	32	TC 2	55	TM 2	43		27	AB	
High	AB 3	73	CB 3	33	PN 3	22	HC 3	17	TC 3	77	TM 3	37		61	CB	
High	AB 4	25	CB 4	58	PN 4	56	HC 4	15	TC 4	67	TM 4	18		38	PN	
Med	AB 5	36	CB 5	1	PN 5	13	HC 5	40	TC 5	9	TM 5	76		62	HC	
Med	AB 6	66	CB 6	39	PN 6	44	HC 6	30	TC 6	59	TM 6	53		2	TC	
Med	AB 7	12	CB 7	54	PN 7	3	HC 7	45	TC 7	60	TM 7	42		49	TM	
Med	AB 8	64	CB 8	21	PN 8	28	HC 8	7	TC 8	35	TM 8	8				
Low	AB 9	47	CB 9	11	PN 9	69	HC 9	51	TC 9	65	TM 9	70				
Low	AB 10	4	CB 10	5	PN 10	52	HC 10	78	TC 10	48	TM 10	74				
Low	AB 11	57	CB 11	6	PN 11	50	HC 11	72	TC 11	46	TM 11	20				
Low	AB 12	63	CB 12	29	PN 12	16	HC 12	10	TC 12	19	TM 12	31				
Planting Order																
	1 - CB.5	2 - TC.E	3 - PN.7	4 - AB.10	5 - CB.10	6 - CB.11	7 - HC.8	8 - TM.8	9 - TC.5	10 - HC.12	11 - CB.9	12 - AB.7	13 - PN.5	14 - AB.2		
	15 - HC.4	16 - PN.12	17 - HC.3	18 - TM.4	19 - TC.12	20 - TM.11	21 - CB.8	22 - PN.3	23 - PN.1	24 - TC.1	25 - AB.4	26 - AB.1	27 - AB.E	28 - PN.8		
	29 - CB.12	30 - HC.6	31 - TM.12	32 - HC.2	33 - CB.3	34 - PN.2	35 - TC.8	36 - AB.5	37 - TM.3	38 - PN.E	39 - CB.6	40 - HC.5	41 - CB.2	42 - TM.7		
	43 - TM.2	44 - PN.6	45 - HC.7	46 - TC.11	47 - AB.9	48 - TC.10	49 - TM.E	50 - PN.11	51 - HC.9	52 - PN.10	53 - TM.6	54 - CB.7	55 - TC.2	56 - PN.4		
	57 - AB.11	58 - CB.4	59 - TC.6	60 - TC.7	61 - CB.E	62 - HC.E	63 - AB.12	64 - AB.8	65 - TC.9	66 - AB.6	67 - TC.4	68 - TM.1	69 - PN.9	70 - TM.9		
	71 - HC.1	72 - HC.11	73 - AB.3	74 - TM.10	75 - CB.1	76 - TM.5	77 - TC.3	78 - HC.10								

Figure A6. Randomization of plant placement. A random number generator created a non-repeating list of numbers from 1-78. I assigned a number to each individual, ordered by population. I filled the tray from left to right, top to bottom according to this random order assigned.

Figure A7. Cones shown directly after planting one seed in each, labeled by population and individual.

Figure A8. An example of an "open inflorescence" as I define it with at least one open ray flower.

Figure A9. An example of a late-blooming ray flower pushing through an otherwise unopened bud in Populations from Henry Coe, Pinnacles, and Anza Borrego (left to right). Circled is an example of a late-blooming, technically "open" inflorescence, since a ray flower is poking through an otherwise unopened bud.