Rapid responses to drought in a rare California annual (San Francisco collinsia, Collinsia multicolor)

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Abstract

As climate change continues and the frequency and intensity of droughts become more prevalent in some regions, plant populations are facing greater ecological pressures. The objective of this study was to observe the response of a rare plant species to an extreme drought event associated with climate change. To study this response, we collected seeds from three populations of *Collinsia multicolor*(San Francisco collinsia, Plantaginaceae) found in central California both before and after the state's historic 2012- 2016 drought. We used a greenhouse study to examine contemporary evolution between the collection years, and included a drought treatment to study plasticity. We measured three traits that indicate life history, morphological, and physiological responses to drought, including flowering time, stomatal density, and chlorophyll fluorescence. In our two coastal populations, where interannual moisture variation is greatest, we observed evolution only in stomatal density, while we observed plasticity in all measured traits. In contrast, our driest inland population showed no response to the drought or to our watering treatments, which is consistent with other studies that have found less response to drought in pre-adapted populations. Overall, our results suggest that plasticity is favored in variable environments. However, they also highlight that the pace evolution may be insufficient to respond to current environmental change.

Introduction

As the frequency and severity of droughts associated with climate change increase, plants are challenged to react quickly in order to survive and reproduce (Karl and Trenberth 2003, Salinger 2005, Levine et al. 2008, Dai 2013, Xu et al. 2019, Metz et al. 2020). Therefore, plant populations need both evolutionary change and plasticity, which is a genotype's ability to develop differing phenotypes in response to the environment. While evolution increases a population's suitability to its habitat as it changes over time, plasticity enables plants to alter phenotypes in response to periodic and unpredictable changes. The challenges associated with drought have led plants to develop extensive life history, physiological, and morphological traits that enable them to cope with drought, including changes in flowering time, photosynthetic capacity, and stomatal density (Root et al. 2003, Jump et al. 2005, Franks et al. 2015, Carlson et al. 2016, Antunes et al. 2018, Chen et al. 2021, Román-Palacios and Wiens 2020, Woodmansee et al. 2021).

Early flowering time in plants that experience regular seasonal droughts, such as those found in Mediterranean climate regions like California, may enable them to strategically avoid the challenges of a more extreme drought year by flowering and setting seed rapidly before water becomes scarce (Jump and Penuelas 2005, Adler and Levine 2007, Angert et al. 2007, Levine et al. 2008, Jorgensen and Arathi 2013, Metz et al. 2020). In a study on the annual *Brassica rapa* (common mustard, Brassicaceae), plants following a drought had significantly earlier flowering than their ancestors from the same populations, confirming that flowering time was heritable and is subject to adaptive evolution over only a few generations (Franks et al. 2007). Similar responses were found by Lambrecht et al. (2020) in a California native annual, *Leptosiphon bicolor* (true babystars, Polemoniaceae), with one of three populations of

postdrought plants evolving towards earlier flowering than predrought plants, and all three populations exhibiting plasticity for earlier flowering in response to an experimental drought treatment. Additionally, results from a greenhouse study on the effects of drought on the California annual *Collinsia heterophylla* (Plantaginaceae), implied water stress may have an evolutionary effect (Jorgensen and Arathi 2013).

The rapid development of earlier flowering in response to drought survival comes at a cost, requiring greater physiological activity to support the accelerated growth rates (Qaderi et al. 2006, Osakabe et al. 2014, Antunes et al. 2018, Lambrecht et al. 2020, Metz et al. 2020, Hamann et al. 2021). To facilitate this rapid growth, plants may increase stomatal density, which is the number of leaf pores through which gases are exchanged (Magaña Ugante et al. 2020, Kosová et al. 2022, Magaña Ugante 2022). On the other hand, some plants experiencing drought reduce stomatal density to reduce water loss through the pores (Qaderi et al. 2006, Carlson et al. 2016, Antunes et al. 2018, Carlson et al. 2016, Metz et al. 2020, Anderson et al. 2021). Chlorophyll fluorescence, which is re-emitted light, is likewise a powerful tool to measure plant stress (Guidi et al. 2019). In cases of plants experiencing drought stress and the impacts on chlorophyll fluorescence, effective quantum yield (ΦPSII), or the measured proportion of light used in photosynthesis to reemitted light, ΦPSII values lowered, meaning more light was reemitted and photosynthesis was less efficient, indicating stress (Qaderi et al. 2006, Osakabe et al. 2014, Antunes et al. 2018).

In the past four decades, California has experienced four exceptional droughts, with the latest (between 2012 to 2016) being the most intense in the last ~ 1200 years (Griffin and Anchukaitis 2014, Hanak et al. 2016, Kooyers et al. 2021, Woodmansee et al. 2021). Additionally, due to its location in proximity to the Pacific Ocean, California's precipitation is greatly influenced by the El Niño southern oscillation (ENSO), which is an irregular climate phenomenon impacting global atmospheric circulation, making it difficult to predict annual precipitation (IPCC 2002, Kidd and Huffman 2011, Neelin et al. 2013, Dahlman and Lindsey 2019). Therefore, interannual precipitation is highly variable. At the height of the 2012 to 2016 drought, California received less than 50% of its average rainfall, while also experiencing above average temperatures (Hanak et al. 2016).

The goal of this study was to examine evidence of evolution and plasticity in a rare California annual wildflower during this recent historic drought. The study species, *Collinsia multicolor* (San Francisco Collinsia, Plantaginaceae), is an herbaceous annual wildflower listed by the California Native Plant Society (CNPS) as rare or endangered with a state and global rank of "imperiled". Like its close relatives, *C. sparsiflora* and *C. heterophylla*, *C. multicolor* uses autonomous-selfing, or self-fertilization, for reproduction (Baldwin et al. 2011). *Collinsia multicolor* seeds germinate with winter rains and plants bloom from late February or early March until the end of May. It grows in communities of closed-cone coniferous forests and northern coastal scrub, and is also sometimes found on serpentine soils. Historically, twenty-three populations of *C. multicolor* have been observed, but in the last twenty years, only about thirteen populations remain (Consortium of California Herbaria, 2021). For this study, we used seeds collected from three populations: two in coastal Santa Cruz County and one in the drier Santa Clara County, thereby including a precipitation gradient.

For this study, our specific questions were: **(A)** Is evolution occurring between generations of *C. multicolor* in response to drought? **(B)** Does *C. multicolor* exhibit plasticity in response to drought? and **(C)** Do the populations, which vary in precipitation, have different responses to drought? To answer these questions, we compared phenotypic traits of predrought (ancestral) and postdrought (descendant) plants grown simultaneously under different watering treatments. The differences between the generations indicated evolutionary change during the drought, while the differences between watering treatments indicated plasticity in response to water availability. We hypothesized treatments exposed to drought would show evolutionary and plastic responses toward earlier flowering time, lower ΦPSII values of chlorophyll fluorescence, and increased stomatal density. Between populations, we hypothesized the two coastal populations would have increased survival in the postdrought generations and greater changes in evolution and plasticity than the inland population as the latter is predisposed to a warmer drier climate making it less sensitive to drought.

Materials and Methods

Study System

Study sites. The *C. multicolor* populations used in this study were selected because they were regularly observed and had seed collections between 2009 and 2019. One of the populations is located near Anderson Dam (AD) in Santa Clara County, CA (37º 10´N, 121º 37´W) and grows along a serpentine outcrop that faces northwest. The other two populations are located in Santa Cruz County, CA, on the Swanton Pacific Ranch of California Polytechnic State University (Cal Poly). The north Swanton (NS, 37º 5´N, 122º 14´W) and south Swanton (SS, 37º 2´N, 122º 13´W) populations grow on fractured siliceous mudstone banks. The NS population faces east while the SS faces northwest. Seeds from AD were collected by Janell Hillman of Santa Clara Valley Water District and were stored at the California Botanic Garden (CABG, Claremont, CA). Seeds from NS and SS were collected by Jim West, Cal Poly botanist, and were stored at the University of California Santa Cruz Arboretum and Botanic Garden (UCSC). After collection, collected seeds were shipped to CABG or UCSC for long-term storage in barrier foil laminate pouches at freezing temperatures (-18 to -10 ºC).

The populations cover a range of moisture availability conditions in central California, with NS and SS being near the coast ~ 5 km apart from one another, and AD being ~ 55 km inland from NS and SS. From data collected over the past century, Santa Cruz County reported an average annual precipitation of 73.9 cm (1893–2021), while Santa Clara County reported an average of 52.2 cm (1906–2021) (WRCC 2022). During the 2012 to 2016 multiyear drought, average annual precipitation was reduced by 69.5% in Santa Cruz County and 65.9% in Santa Clara County (Fig. 1; WRCC 2022).

Experiment

Refresher Generation. Using a similar protocol to Franks et al. (2017 and 2018), a refresher generation (G_0) of seeds from the two collection years was grown under regular watering conditions to produce an

offspring (experimental, G_1) generation, to remove effects due to maternal resource variability or storage conditions. Two collection years were selected from each population to represent the predrought and postdrought generations. For NS and SS, the collection years were 2013 and 2017. For AD, the collection years were 2013 and 2018. In the fall of 2019, seeds were sown in SC-10 Ray Leach 20.96 cm cone-tainers (Stuewe and Sons, Corvallis, OR) and filled with Pro-Mix HP Mycorrhizae Growing Mix (Premier Tech Horticulture, Quakertown, PA). One seed was sown per cone-tainer, and these were randomly arranged in trays. For the refresher generation, 98 seeds were sown per year × population, for a total N = 588 seeds.

After sowing, seeds were stratified in a Percival Growth Chamber (PGC-15, Percival, 505 Research Drive, Perry, OK) for seven days with 12-h of light (~ 515 µmol m^{-2} s^{-1}) at 15°C, and 12-h of dark at 5°C. Following stratification, the trays were moved into a greenhouse with a 15-h day with a photosynthetic photon flux density ranging between ~ 320–1,100 µmol m^{-2} s^{-1} at 25ºC, and a 9-h night at 12ºC. Seed germination rates within each of the populations by years were similar, indicating no significant effect of seed storage viability within each population (overall mean = 63%; years within populations $0.36 < \chi^2 < 0.75$, $0.46 < P < 0.55$). As *Collinsia* are self-pollinating, G_0 set seed without pollination assistance (Lankinen et al. 2007, Baldwin et al. 2011, Jorgensen and Arathi 2013). Plants were allowed to self-pollinate to produce seeds for the experimental generation (G_1). Seeds were collected between February and April 2020.

Experimental generation. For the next phase of the study, the G_1 seeds were planted in summer 2020 using the same protocols as above. Additionally, to examine plasticity, plants in the G_1 generation were grown under one of two watering treatments: consistent precipitation (high watering) or drought conditions (low watering). For each watering treatment, two G_1 seeds were sown from each G_0 mother line. A total of 936 seeds were sown: 176 seeds for predrought AD, 156 seeds for postdrought AD; 156 seeds for predrought NS, 188 seeds for postdrought NS; 92 seeds for predrought SS, and 168 for postdrought SS. Due to the emergence of Covid-19 and the resulting restrictions, the growth chambers and controlled greenhouse facilities were unavailable during this phase of the experiment. As a result, the G_1 cells and trays were placed in a temperature-controlled refrigerator at the UCSC Arboretum. The refrigerator settings were manually changed to replicate the G_0 stratification temperature treatment, but the refrigerator light could not be altered so G_1 seeds were stratified in the dark. After seven days, the trays were placed in a greenhouse at the UCSC Arboretum. However, after 14 days, the G_1 plants had to be removed from the greenhouse due to an encroaching wildfire, and placed in an area outside the fire evacuation zone. The G_1 plants remained in the outdoor area for the rest of the experiment, where they grew in unfiltered natural light.

The experimental (G_1) generation was grown under regular daily watering conditions until 35 days after the sow date, at which time the watering treatments began. Plants under the high watering treatment were watered twice per week, while plants under the low watering treatment were watered once every two weeks. This watering plan was developed based on our preliminary greenhouse experiments to

imitate the natural period of declining precipitation that the field populations face in the spring, allowing us to begin drought treatments after germination but before flowering. Each cone-tainer was randomly placed into trays to avoid any effects of tray placement on the watering treatment. Each tray had both watering treatments, with one watering treatment on each half of the tray. A plastic divider was designed to maintain watering control between the two treatments within a tray.

Survival and Flowering Time

Germination and flowering date were monitored daily and recorded. Any plants that did not germinate or had died before flowering were recorded to assess survival across the populations, generations, and watering treatments. Flowering time was calculated as the difference between germination date (emergence of cotyledons) and flowering date (opening of first flower). The experiment was terminated 95 days after the sow date. A total of 432 of the 936 seeds planted had failed to germinate and 39 plants had failed to flower by that time.

Stomatal Density

To quantify stomatal density, an impression was made of the first new leaf initiated after flowering. With this impression method, the bottom of each leaf was coated in a thick layer of clear nail polish and left to dry for ~ 10 minutes. Clear packing tape was then used to peel off the impression of the leaf surface and affixed over a 20 × 20 mm grid on a microscope slide. Under a microscope, 5% of the total leaf peel was photographed at random, with the condition that stomata had to be more than 50% within the field of view. Stomata were counted for each of the images using the ImageJ application (Schneider et al. 2012) and used to determine the average stomatal density for each leaf.

Chlorophyll fluorescence

Chlorophyll fluorescence can be used to detect stress in plants by measuring the efficiency of PSII photochemistry, which executes the initial reaction of light capture and energy conversion of photosynthesis (Gremer et al. 2012, Antunes et al. 2018). Effective quantum yield (ΦPSII) measures the efficiency of light energy capture by the PSII reaction. Reduced ΦPSII values indicate a stress response in the plant, whereby they are not able to use absorbed light energy efficiently, thus re-emitting it (Genty et al. 1989). To measure ΦPSII, the first leaf that emerged after flowering began was measured using a Li-Cor Portable Photosynthesis System (Li-Cor 6400XTR, Li-Cor Biosciences, Lincoln, NE). Measurements were made on plants ~ 20 minutes after sun exposure, and they were, therefore, in a light-adapted state. The ΦPSII was estimated as ΦPSII = $((F_m' - F_s) / F_m')$, where F_m' and F_s are maximal and steady state fluorescence under actinic irradiance, respectively (Genty et al. 1989). During all measurements, flash duration was 1.02 seconds and set at an optimum flash intensity of ~ 875 μmol m^{-2} s^{-1}.

Statistical Analyses

To evaluate survival across populations, years, and treatment, a Pearson's χ^2 test with a $\alpha = 0.05$ level of significance was performed using the statistical software R (R Core Team 2021). To determine whether flowering time changed, a Cox proportional hazards model was used, and the data were evaluated with SPSS (v.28.0.0, IBM Corp., Armonk, N.Y., USA). This analysis compared flowering time across the predrought and postdrought years, populations, and watering treatments, as well as interaction terms. Evolution is detected by comparing years, while plasticity is detected by comparing watering treatments. A significant interaction between year and treatment indicates that the degree of plasticity changed during the drought. Since some plants die before flowering, the Cox Proportional Hazards model is necessary to use when determining flowering time since the model is semiparametric and does not assume a normal distribution (Muenchow 1986). This model generates a Wald χ^2 test, which is a one-tailed test, with a $\alpha = 0.10$. To determine whether chlorophyll fluorescence and stomatal density varied across populations, treatments or years, an ANOVA test with a $\alpha = 0.05$ level of significance was performed using R. When main effects and interaction terms showed a significant difference, Tukey multiple comparisons were used for pairwise analyses.

Results

Evolution and Plasticity in Response to Historic Drought

Survival rates. Two of the three populations showed a significant difference in survival between pre- and post-drought years. Postdrought plants from AD and NS had increased survival, in comparison with predrought plants ($\chi^2 = 8.74$, $df = 1$, $P = 0.003$; $\chi^2 = 3.73$, $df = 1$, $P = 0.05$; Fig. 2). These results suggest that changes in plant traits during the drought may have enhanced survival in these populations. In contrast, watering treatment did not impact plant survival in any of the populations (AD: $\chi^2 = 1.01$, $df = 1$, $P = 0.31$; NS: $\chi^2 = 0.05$, $df = 1$, $P = 0.82$; SS: $\chi^2 = 0.02$, $df = 1$, $P = 0.88$).

Flowering time. None of the populations expressed significant evolution toward earlier flowering. Although flowering time did not change significantly, each of the three populations flowered at different times, suggesting differences exist among populations for this species (Wald $\chi^2 = 6.77$, $df = 2$, $P = 0.03$; Table 1a). AD, which is the driest of the populations, flowered earliest, flowering 0.26 days earlier than SS and 2.72 days earlier than NS, which flowered the latest (AD:SS Wald $\chi^2 = 1.33$, $df = 1$, $P = 0.25$; AD:NS Wald $\chi^2 = 0.14$, $df = 1$, $P = 0.71$; NS:SS Wald $\chi^2 = 6.60$, $df = 1$, $P = 0.01$). Additionally, in the low watering treatment, two of the three populations showed significant plasticity by flowering earlier in comparison to the high watering treatment. The low watering treatment caused NS to flower 2.3 days earlier and SS to flower 3.0 days earlier (Table 1b, Fig. 3a). The NS population also expressed a significant year × treatment interaction ($P = 0.05$, Table 1b, Fig. 3a), indicating there was evolution toward increased plasticity in response to the drought. Further pairwise analyses comparing populations also revealed NS and SS responded differently in flowering time from one another (pairwise comparison, Wald $\chi^2 < 6.60$, $df = 1$, $P = 0.01$).

Table 1

(a) Analysis of flowering time for evolution and plasticity across populations, years, and treatments. (b) Analyses within each population for evolution (year) and plasticity (treatment) effects. For Cox-proportional hazards models, α = 0.10 is the level of significance. *P < 0.05; **P < 0.01; ***P < 0.001.

(a)	Effect	Wald χ^2	*df*	*P*
	Population	6.77	2	**0.03***
	Year	1.63	1	0.20
	Treatment	20.71	1	**< 0.001****
	Population × Year	0.22	2	0.90
	Population × Treatment	0.78	2	0.38
	Year × Treatment	2.83	1	0.24
	Population × Year × Treatment	1.68	2	0.43

(b) Population	Effect	Wald χ^2	*df*	*P*
	Year	$1.11e^{-04}$	1	1.00
Anderson Dam	Treatment	0.14	1	0.87
	Year × Treatment	0.30	1	0.70
	Year	1.05	1	0.31
North Swanton	Treatment	12.59	1	**< 0.001****
	Year × Treatment	3.71	1	**0.05***
	Year	1.18	1	0.29
South Swanton	Treatment	17.02	1	**< 0.001****
	Year × Treatment	0.65	1	0.42

Stomatal density. Of the three populations, only one demonstrated evolution in stomatal density between pre- and postdrought years. Stomatal density in NS increased by 8.9/mm^2 between years (Table 2a, Fig. 3b). However, SS did express a significant interaction of year × treatments (P = 0.03, Table 2, Fig. 3b), suggesting that there was evolution for increased plasticity in plants in the postdrought years. No significant effect of treatments was found, ruling out our hypothesis of flexibility owing to drought-induced stomatal density reduction. Interestingly, stomatal density across all three populations was not statistically different in spite of the different climates in which they are found (P = 0.08, Table 2).

Table 2
Analysis of stomatal density and chlorophyll fluorescence for evolution and plasticity. Values shown are from an ANOVA. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

(a)		Stomatal density			Chlorophyll fluorescence		
	Effect	**F**	***df***	***P***	**F**	***df***	***P***
	Population	2.52	2	0.08	0.43	2	0.65
	Year	4.65	1	**0.03***	0.40	1	0.53
	Treatment	2.22	1	0.14	37.28	1	**< 0.001*****
	Population × Year	3.93	2	**0.02***	0.11	2	0.90
	Population × Treatment	1.92	2	0.15	1.47	2	0.23
	Year × Treatment	3.73	1	**0.05***	0.02	1	0.88
	Population × Year × Treatment	0.32	2	0.73	0.65	2	0.53

(b) Population		Stomatal density			Chlorophyll fluorescence		
	Effect	**F**	***df***	***P***	**F**	***df***	***P***
	Year	0.01	1	0.94	0.10	1	0.75
Anderson Dam	Treatment	1.36	1	0.25	1.25	1	0.27
	Year × Treatment	0.38	1	0.54	1.30	1	0.27
	Year	9.87	1	**< 0.001*****	0.08	1	0.78
North Swanton	Treatment	1.65	1	0.20	16.42	1	**< 0.001*****
	Year × Treatment	0.39	1	0.53	0.07	1	0.79
	Year	0.07	1	0.80	0.31	1	0.58
South Swanton	Treatment	2.48	1	0.19	19.80	1	**< 0.001*****
	Year × Treatment	4.92	1	**0.03***	0.00	1	0.99

Chlorophyll fluorescence. Chlorophyll fluorescence did not vary across years for any population (Table 2, Fig. 3c). However, two of the three populations demonstrated a significant difference in fluorescence between watering treatments, indicating plasticity in photosynthetic efficiency. Between the high and low watering treatments, NS had a significant decrease of 0.05 ΦPSII and SS had a decrease of 0.08 ΦPSII (Table 2b, Fig. 3c). These results indicate that plants in these populations were less efficient at capturing and converting light energy at low water availability. In contrast, AD, which received the least natural rainfall of the populations, was unaffected by the watering treatments.

Discussion

Our resurrection study, in which we grew seeds collected before and after California's recent historic drought, revealed diverse ways that populations of a native plant species may respond to drought. Two of our study populations experienced a significant increase in survival between the pre- and post-drought generations, suggesting changes in these populations during the drought. However, of our measured traits, evolution in stomatal density was observed in only one population. We did observe plasticity in all of our measured traits in two of the populations, with earlier flowering and increased stomatal density in the low watering treatment, along with evolution of increased plasticity. However, only one of these populations showed an increase in survival after the drought. In spite of any trait changes, plants in the two populations exhibiting plasticity experienced lower photosynthetic efficiency in the low watering treatment, suggesting they continued to experience stress under drought conditions. Finally, one of the populations demonstrating increased survival after the drought exhibited no plasticity or evolution in any of our measured traits, suggesting that the traits we measured did not fully capture the changes that occurred in these populations during this drought.

The limited evolution in the traits we measured may reflect the relative stability of our *C. multicolor* populations. Apparent stability of traits can occur in a variety of situations, including 1) the populations might have pre-existing adaption to drought, making them less sensitive to moisture fluctuations; 2) variation in our measured traits may not be driven by climatic factors; 3) changes are too small to be detected due to poor data resolution; 4) the populations fail to respond to current climate change (Parmesan and Yohe 2003). Several other studies have observed that evolution in response to drought is less likely for populations that already experience dry conditions (Franks et al. 2007; Lambrecht et al. 2020). For example, a resurrection study comparing three populations of the California annual, *Leptosiphon bicolor* (true babystars, Polemoniaceae), in response to drought, the two driest populations showed no evolution in flowering time, suggesting evolution occurs when drought produces conditions that are more distinct from those prior to the onset of drought (Lambrecht et al. 2020). Similar results were found in a study comparing two populations of *Brassica rapa*, where evolution in flowering time was not observed in the drier population (Franks et al. 2007).

Although there was no evolution of flowering time, the two coastal populations (SS and NS) exhibited plasticity by flowering earlier in the low watering treatment. The drier inland population (AD) did not exhibit any significant flowering time plasticity, despite the fact that all three populations experienced a similar reduction in precipitation from their average annual precipitation during the 2012 to 2016 drought. Previous studies have hypothesized that plasticity in flowering time is favored in environments that experience more interannual variation, such as those found in our coastal populations (Kenney et al. 2014; Austen et al. 2017; Lambrecht et al. 2020). Notably, the drier inland population showed no trait changes for any of our measured traits between generations or in response to our watering treatments. As coastal conditions have more variable inter-annual precipitation than inland conditions, which are more consistently dry, this difference between the two coastal populations and the inland population suggests that the environments of these populations may play a more meaningful role in their responses

to drought. Additionally, one of the coastal populations showed evolution toward increased plasticity in flowering time. These results suggest that flowering time is responsive to climatic factors, but that plasticity may be preferential as part of a drought avoidance strategy to survive when conditions change between years (Kenney et al. 2014, Austen et al. 2017, Lambrecht et al. 2020).

Populations may also exhibit trait stability when their response to environmental change is slow or is too small to be detected by poor data resolution (Paremsan and Yohe 2003). In a review by Jump and Puenuelas (2005), numerous plant species were cited as having no evolutionary response to climate change, despite significant phenotypic variation in the source populations. Therefore, a slow response to drought may have played a role in our populations of *C. multicolor.* Additionally, we cannot ignore that the unforeseen modifications to our experimental protocols due to Covid-19 and wildfires may have impacted our results. While some of our flowering time results are consistent with those of some other resurrection studies, we had a much lower rate of germination in our experimental generation, particularly for the dry inland population, as compared to both our trial experiment and our refresher generation. The uncontrolled light environment and imprecise temperature control during incubation of the experimental generation may have impacted its overall germination and survival. Additionally, the lack of sunlight during the wildfire may have impacted growth. Therefore, although we could attribute the lack of trait change in our driest population to pre-adaptation to drought, it may alternatively due to lack of data precision.

In contrast to our flowering time results, we did observe evolution in stomatal density. One of our coastal populations had higher stomatal density after the drought, while the other showed evolution toward increased plasticity, whereby post-drought plants had higher stomatal density in the low watering treatment. By increasing stomatal density, plants can support rapid rates of photosynthesis, assisting in rapid development, as other drought studies have shown (Osakabe et al. 2014, Franks et al. 2015, Kooyers et al. 2021, Chen et al. 2021).

None of our populations showed an increase in photosynthetic efficiency, as indicated by the effective quantum yield of photosystem II (ΦPSII) during the drought. Additionally, our coastal populations showed a decline in photosynthetic efficiency in our low watering treatment. Therefore, drought still posed a significant stress on these populations, in spite of any trait changes that may have occurred during the drought. However, this response to increase fluorescence in order to retain water as a strategy during drought aligns with our expectations and is supported by previous studies that suggest increased chlorophyll fluorescence as a trait that is an important coping mechanism in response to drought. (Gremer et al. 2012, Antunes et al. 2018).

In conclusion, the findings from this research suggests plant populations within a species may respond differently to exceptional droughts. In particular, our results shed light on how precipitation availability affects quick responses across populations, even when populations are within close proximity to one another and within a similar precipitation gradient. As significant drought episodes and interannual precipitation changes are anticipated to become more common, we need to understand how populations

and species respond to climate change through multiyear studies spanning species' ranges and across a broader diversity of taxa.

Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contribution

Both authors contributed to study conception and design. SS initiated the experiment, and also collected and analyzed all data. SS wrote the initial draft of this manuscript, which has been edited by SL.

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Figures

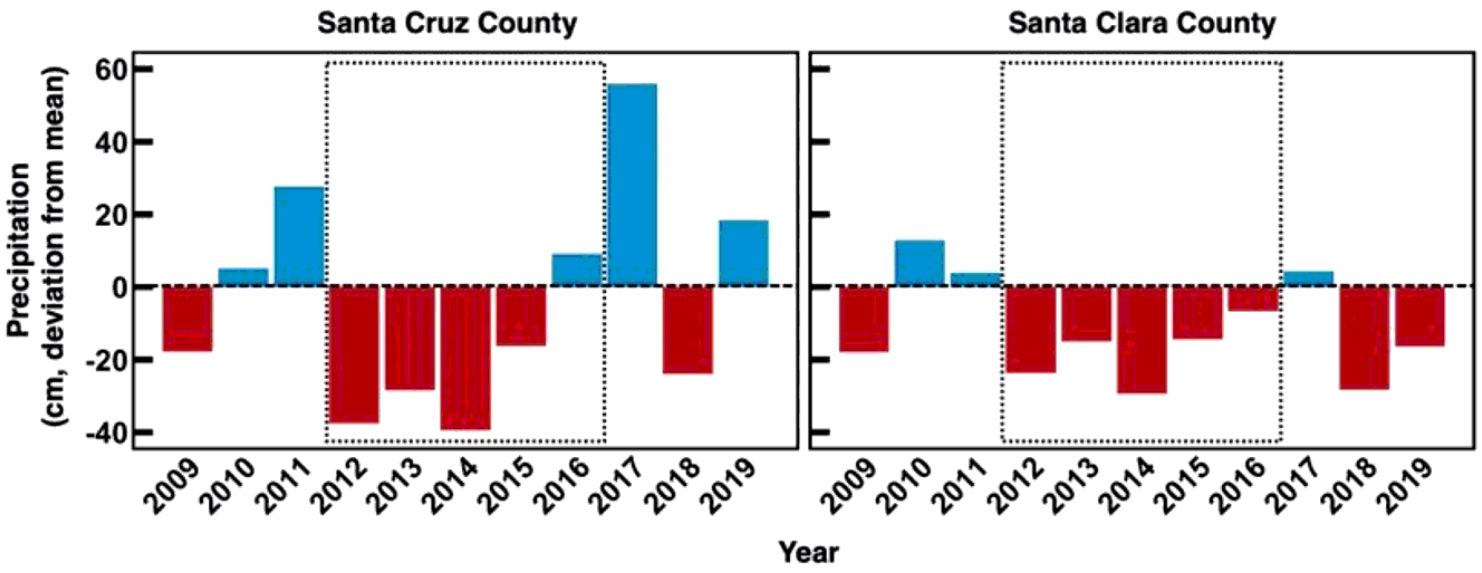

Figure 1

Precipitation in Santa Cruz and Santa Clara Counties from 2009 to 2019 (WRCC 2022). Long-term average precipitation is represented by the horizontal dashed line: 73.9 cm for Santa Cruz County (1893

– 2021) and 52.2 cm for Santa Clara County (1906 – 2021). Bars represent deviation from the average. The dotted lines highlight the years during the 2012 to 2016 drought.

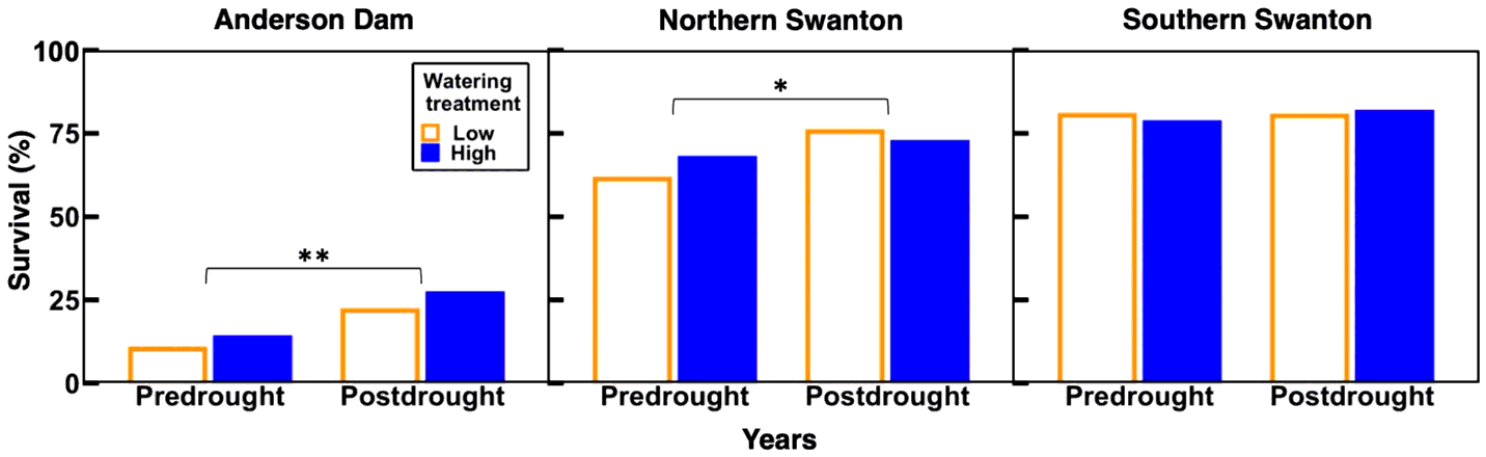

Figure 2

Survivorship across all populations and years. Values are survival (%) for both the predrought and postdrought generations of each population exposed to the two watering treatments. Filled blue bars represent the well-watered treatment and open orange bars represent the drought treatment. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

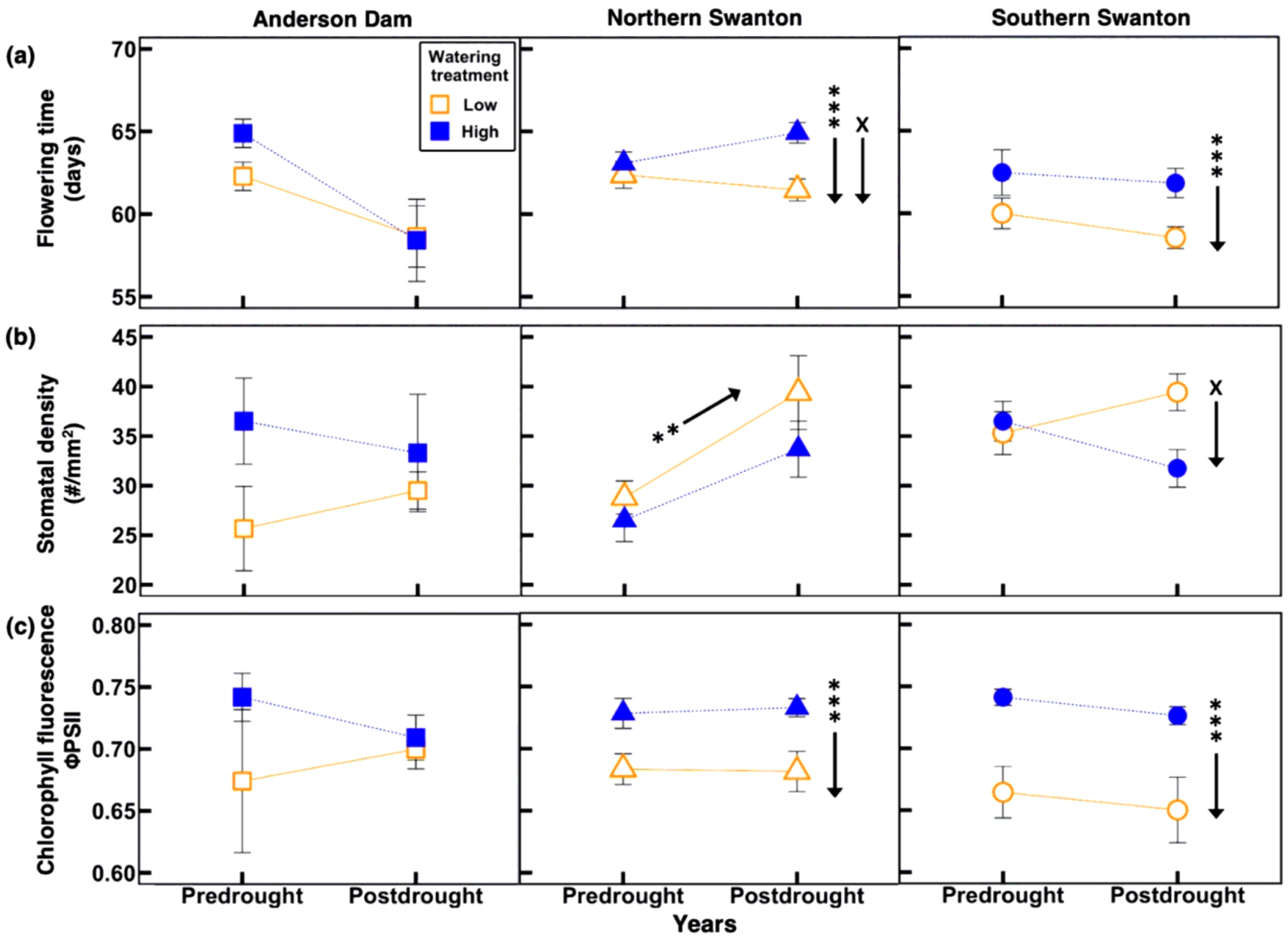

Figure 3

Evolution and plasticity of flowering time (a), stomatal density (b), and chlorophyll fluorescence (c). Values shown are mean ± 1 SE. Populations are shown from left to right in alphabetical order and measured traits are shown from top to bottom. Predrought and postdrought years are shown on the *x*-axis. Filled blue symbols represent the well-watered treatment and open orange symbols represent the drought treatment. Vertical arrows signify significant treatment effects, indicating plasticity, while horizontal arrows signify significant differences between years, suggesting evolution. The X arrow indicates a significant treatment ´ year interaction ($P < 0.05$) within a population. *$P < 0.05$; ** $P < 0.01$; ***$P < 0.001$.