Germination responses to experimental rainfall timing identify potential vulnerability to climate change across a clade of California wildflowers

Samantha J. Worthy[1*], Arquel Miller[1], Sarah R. Ashlock[1], Eda Ceviker[1], Julin N. Maloof[2], Sharon Y. Strauss[1,3], Johanna Schmitt[1,3], Jennifer R. Gremer[1,3]

[1]Department of Evolution and Ecology, University of California, Davis, CA, USA

[2]Department of Plant Biology, University of California, Davis, CA, USA

[3]Center for Population Biology, University of California, Davis, CA, USA

[*]Corresponding Author: Samantha J. Worthy; 2320 Storer Hall, University of California, Davis, One Shields Avenue, Davis, CA, 95616; E-mail: sjworthy@ucdavis.edu

Abstract

1. The timing of germination, driven by seasonal cues, is critical for the life cycle of plants. Variation among species in germination responses can reflect evolutionary processes and adaptation to local climate and can reveal vulnerability to changing conditions. Climate change is altering the timing of precipitation and associated temperatures, which may interact with germination cueing to affect the timing, quantity, and speed of germination. Germination responses to climate change can then have consequences for individual fitness, population dynamics, and species distributions.
2. Here we assessed responses to the timing of germination-triggering rains and corresponding temperatures for 11 species across the *Streptanthus* (*s.l.*) clade of Brassicaceae. To do so, we experimentally manipulated the onset date of rainfall events and measured effects on germination fraction and rate. We also evaluated how responses varied with phylogeny. We then explored the possible consequences of these responses to contemporary shifts in precipitation timing.
3. Later onset rains and cooler temperatures significantly decreased germination rates for all species. Germination fractions decreased with later rains and cooler temperatures for all species except three *Caulanthus* species. We found that six species are likely already experiencing significant decreases in germination fractions and/or germination rates with observed climate change, which has shifted the timing of rainfall towards the cooler, winter months in California. Species' germination responses to the timing of rainfall and seasonal

temperatures were phylogenetically constrained, with *Caulanthus* species appearing less sensitive.

4. *Synthesis*. Across the *Streptanthus* clade, later onset of seasonal rains during cooler temperatures decreases germination fractions and rates. Contemporary shifts toward later rainfall offset the effects of ongoing climate warming and may already be negatively affecting germination in several species. Species' germination responses may have evolved along the phylogeny, with *Caulanthus* species, from drier and more variable climates, found to be less sensitive to the timing of rainfall events or their associated temperatures than species from wetter, less variable environments.

Key Words: germination cues, germination responses, rainfall onset timing, *Streptanthus* clade

Introduction

The timing of germination is critical to seedling establishment, seasonal phenology, and ultimately plant fitness (Kalisz, 1986; Donohue et al., 2010; Gremer et al., 2020). Germination timing is driven by climatic cues including precipitation and temperature (Grubb, 1977; Baskin and Baskin, 2014). Climate change is already altering germination conditions through increasing temperatures and shifting precipitation regimes, leading to potential mismatches between formerly adaptive germination cues and both current and future environmental conditions (McNamara et al., 2011; Parmesan and Hanley, 2015; Bernhardt et al., 2020). Shifts in climate may affect the seasonal timing of germination, the proportion of viable seeds that germinate (germination fraction), and/or the speed at which germination proceeds (germination rate). The degree to which germination cues are constrained by evolutionary history could, in part, determine closely related species' germination responses to future climates with resulting impacts on individual fitness and population dynamics (Arène et al., 2017; Fang et al., 2017; Fernández-Pascual et al., 2021).

Germination timing determines the abiotic and biotic environments seedlings will experience, influencing performance during one of the most vulnerable plant life stages (Donohue et al., 2010; Matías et al., 2011; Gremer et al., 2016). To time germination with favorable conditions, germination cues may evolve such that seeds respond to partially or wholly reliable cues signaling favorable conditions for germination and establishment (e.g., plasticity; Cohen, 1967, Donohue et al., 2010, Bonamour et al., 2019; Gremer et al., 2016; Gremer, 2023). Precipitation and temperature are critical cues for germination (Probert, 2000; Finch-Savage and Leubner-Metzger, 2006;

Burghardt et al., 2015; Barga et al., 2017; Puglia et al., 2018), in particular, the timing of precipitation and how it interacts with temperature associated with rain events (Went, 1949; Levine et al., 2008; Kimball et al., 2010; Walck et al., 2011; Mayfield et al., 2014; Huang et al., 2016). For example, a global-scale meta-analysis of 661 alpine species revealed their strong propensity to germinate in response to warm, wet conditions (Fernández-Pascual et al., 2021), which signal the onset of the summer growing season.

In variable and changing environments, seeds may not germinate in a given year, either because appropriate conditions or cues are unavailable, or because seeds may remain dormant even in the presence of those cues. Seed dormancy can act to distribute germination through time, spreading the risk of germinating into unfavorable conditions and acting as a bet hedging strategy (Cohen, 1966; Hoyle et al., 2015; Gremer and Venable, 2014). Bet-hedging through germination is often found in unpredictable environments like deserts, where environmental cues are unreliable (Venable, 2007; Gremer and Venable, 2014). Thus, we may expect seeds from more variable environments to have lower germination fractions that are less sensitive to seasonal cues and conditions. If seeds fail to germinate because of the absence of appropriate cues and conditions or due to seed dormancy, they must survive in the soil seedbank in order to germinate in future conditions.

Variation among species' germination strategies and responses to environmental cues for germination suggests that species may differ in vulnerability to climate change (Kimball et al., 2011; Liu et al., 2013). Changes in environmental conditions and cues under climate change affect germination timing, fractions, and rates (Lopéz et al., 2020;

96 ller et al., 2021). In addition to rising temperatures, climate change has also shifted the
97 timing of germination-triggering rains (Kimball et al., 2010; Levine et al., 2011; Mayfield
98 et al., 2014; Pathak et al., 2018; Luković et al., 2021) both of which will affect
99 germination conditions. For species with winter growing seasons, rainfall events arriving
100 later in the season may occur under cooler temperatures, affecting germination fractions
101 and rates (Kimball et al., 2010, Kimball et al., 2011; Huang et al., 2016). Earlier
102 germination often leads to longer growing seasons and higher fitness (Donohue et al.,
103 2010; Kimball et al., 2011; Levine et al., 2011), so later onset of rains may adversely
104 affect fitness and population dynamics. Moreover, climate change may create
105 mismatches between germination cues and conditions, such that seeds may not
106 germinate at all if they do not receive appropriate germination cues or germinate outside
107 favorable environmental conditions (Donohue et al., 2010; Walck et al., 2011).
108 Depending on how increasing temperatures interact with rainfall timing, climate change
109 may either slow or accelerate the rate at which germination proceeds, with implications
110 for phenology and seedling establishment. Together, climate change induced
111 disruptions to both temperature and precipitation have implications for germination
112 processes (Kimball et al., 2010; Walck et al., 2011).
113 Indeed, climate change is already altering both temperature and precipitation
114 patterns and forecasts indicate continued change in coming decades (Wright et al.,
115 2016; Pathak et al., 2018; Swain et al., 2018). For example, the IPCC (2021) reports
116 global increases in temperature, shifts in the timing of precipitation, and increased
117 variability in both temperature and precipitation, along with more frequent and intense
118 extreme climatic events. Previous research has shown that plants in Mediterranean

climates are responsive to temperature and the timing of precipitation. In these climates seeds typically germinate in cooler, wet conditions that arrive in the fall or early winter, after a hot, dry summer (Levine et al., 2011; Gremer et al., 2020a). Studies have also noted the potential for strictly cold temperature-cued germination in Mediterranean climates associated with winter rainfall and species with winter growing seasons (Levine et al., 2008; Levine et al., 2011; Mayfield et al., 2014). Contemporary climate change has already shifted patterns of seasonal precipitation in Mediterranean climates (Giorgi and Lionello, 2008; Walck et al., 2011; Barredo et al., 2018). For example, the timing of first seasonal rains in California, USA, has shifted to later in the fall and winter growing season, resulting in less fall precipitation and more concentrated precipitation during the colder months (Luković et al., 2021). Conversely, Swain et al. (2018) report an increased likelihood of extreme dryness during rainy seasons of the future, highlighting the unpredictability of the timing and quantity of precipitation. However, much less is known about how these shifts influence germination of closely-related native plants, which has implications for population dynamics, species' persistence, and species' distributions in the face of ongoing change.

The *Streptanthus* (*s.l.*) clade of Brassicaceae and its allies are an ideal system to ask how shifting rainfall onset will alter germination patterns. The clade has desert origins and diversified as it moved northward (e.g., Cacho et al., 2021), as did other taxa comprising the Madro-tertiary geoflora (Axelrod, 1958). *Streptanthus* and *Caulanthus* species have also diversified across a range of typically drier environments in the Mediterranean climate of California (Cacho and Strauss, 2014; Cacho et al., 2021). Prior work in this system has shown that as these species spread from the

142 desert, many of their traits diversified along the phylogeny, with closer relatives having
143 more similar traits (Cacho and Strauss, 2014; Christie and Strauss, 2018; Pearse et al.,
144 2020). For example, Pearse et al. (2020) found phylogenetic signal in water inflection
145 point, the water availability level at which a species' fitness increases most rapidly,
146 suggesting species have diverged from drought-adapted ancestral species.

147 To investigate how the timing of rainfall affects germination fraction and rate of
148 *Streptanthus* and *Caulanthus* species across the *Streptanthus* (*s.l.*) clade, we
149 experimentally varied the date of rainfall onset and asked three main questions, 1) How
150 does variation in the timing of germination-triggering precipitation and corresponding
151 seasonal temperature affect germination fraction and rate?, 2) What are the possible
152 effects of contemporary shifts in precipitation timing on germination fraction and rate
153 and do species vary in their sensitivity to these shifts?, and 3) How have germination
154 responses to seasonal conditions diversified across these closely related species? We
155 hypothesized that species would germinate at higher fractions and faster rates with
156 earlier rainfall events that occur during warmer fall temperatures because earlier
157 germination maximizes the growing season leading to higher fitness, and because
158 germination is expected to be faster under warmer temperatures. As a corollary, we
159 hypothesized that species would show decreased germination fractions and rates if
160 contemporary shifts in rainfall timing occur at cooler temperatures. We expected these
161 germination responses, unless there is germination cuing towards colder temperatures
162 associated with winter growing season of these species. We also expected that species
163 would vary in their sensitivity to germination conditions due to divergent climatic
164 conditions they have evolved under and adapted to. In particular, we hypothesized that

patterns of germination fraction and rate would differ among species based on climate of origin. *Caulanthus* species occupy drier, more variable environments, and should be less affected by the timing of germination-triggering rains and corresponding temperatures than *Streptanthus* species, adapted to wetter, less variable environments (Golodets et al., 2013; Figure 1). Correspondingly, we also expected to see lower germination fractions in *Caulanthus* species as they are expected to be less responsive to the timing of germination-triggering rain events owing to variability and unpredictability of precipitation in their local environments.

Materials and Methods

Study System

To address these questions, we used 13 populations from 11 species spanning the *Streptanthus clade* (*s.l.*; Thelypodieae Brassicaceae), which includes nonmonophyletic genera *Streptanthus* and *Caulanthus* (Table 1; Cacho et al., 2014). As a group, these species span the latitudinal range of the California Floristic Province and typically inhabit relatively barren, dry substrates ranging from sandy deserts to rocky and serpentine outcrops (Figure 1A). The clade spans habitats with a range of mean temperature and precipitation, and also a range of variability in these measures (Figure 1B, Table 1; Table S1). All species and populations in this study experience the Mediterranean climate in California, in which the winter growing season begins with germination-triggering rain events in the fall or early winter and ends with the onset of summer drought. Interannual variation in the onset of germination-triggering rains is also high across California (Gremer et al., 2020; Luković et al., 2021).

188

189 *Experimental Design*

190 To assess species differences in germination responses to natural and climate
191 change-induced variation in the timing of germination-triggering rains, we experimentally
192 imposed seven rainfall onset events throughout the germination season. Specifically,
193 seven distinct germination cohorts were created by simulating germination-triggering
194 rain events every two weeks during the fall and winter of 2020: September 17, October
195 2, October 16, October 30, November 13, November 27, and December 11, 2020,
196 which encompasses natural interannual variation in the arrival of rains, as well as shifts
197 observed with contemporary climate change (Gremer et al., 2020, Luković et al., 2021).
198 This experiment was conducted in a "screenhouse" with a clear plastic roof that allowed
199 for controlled watering, but exposed seeds to seasonal temperature fluctuations and
200 changes in ambient light.

201 Seeds were collected as maternal families from field locations in 2019 and
202 pooled for this experiment (Table S2). For two species (*C. anceps* and *C. inflatus*) we
203 included seeds from two sites. For each cohort, individual seeds were sown into 107 mL
204 cone-tainer pots (Stuewe and Sons SC7) filled with a mix of 2/3 UC Davis potting soil
205 (1:1:1 parts sand, compost, peat moss with dolomite) and ⅓ coarse 16 grit sand. For
206 each species, except *C. coulteri*, 16 seeds were sown in each of three replicate blocks
207 for a total of 48 seeds sown per cohort. For *C. coulteri*, for which we had limited seed,
208 four seeds were sown per replicate block, 12 total seeds sown per cohort. Pots with
209 seeds were randomly assigned to one of three blocks, which were arranged across
210 three screenhouse benches.

We simulated germination-triggering rain events every two weeks by bottom-watering pots to saturation before sowing, then intensely misting pots for one week after sowing (Table S3). Soil moisture conditions following the germination-triggering rain events were maintained by lower levels of watering (Table S3). This procedure was repeated for each cohort with the watering amount reduced as the experiment progressed because of cooler seasonal temperatures. One week after the last cohort was sown, all cones were subjected to maintenance watering until germination surveys ceased on April 16, 2021.

Germination surveys were conducted daily in which every cone was censused and the germination date for each individual was recorded. By January 11, 2021, germination had slowed, and germination surveys were reduced to twice weekly (Mondays and Fridays) and then further reduced on February 1, 2021, to once per week (Mondays). All surveys and watering were completely stopped on April 16, 2021, after no germination had been observed in the previous two weeks.

To simulate the dry, Mediterranean California summer, all cones with ungerminated seeds were allowed to dry out and kept in the screenhouse to expose seeds to natural drying and hot temperatures over the summer. The following fall (September 2021), these pots were re-randomized into new blocks, bottom-watered, and then received one week of high-frequency watering, as in the previous year, starting on September 15, 2021, to simulate the beginning of the next growing season (year 2). Germination surveys were then conducted daily until November 5, 2021, when they were reduced to three-times weekly (Monday, Wednesday, Friday). On November

233 22, 2021, surveys decreased to once per week (Monday) and were stopped on
234 December 27, 2021, after a two-week period without any germination.
235
236 *Phylogeny*
237 Full methods for phylogeny estimation can be found in Cacho et al. (2014). In
238 brief, the phylogenetic hypothesis was generated using six single copy nuclear genes,
239 three identified specifically for this group in combination with three traditionally used
240 nuclear regions (phyA, ITS, PEPC), and two chloroplast regions (trnL, trnH-psbA). The
241 hypothesis was based on Bayesian MCMC runs consisting of three 50-million-
242 generation independent runs with sampling every 5000 generations (details in Cacho et
243 al., 2014). For the two species with two populations, additional populations were added
244 to the phylogeny at the same node with the same edge length as the other population of
245 the species using the phytools (Revell, 2012) and ape (Paradis and Schliep, 2019)
246 packages in R programming language (R Core Team 2021).
247
248 *Temperature Estimates*
249 Thermochron DS1921G iButtons were deployed with each cohort, in each of the
250 three racks, into soil-filled blank cones to measure soil temperature. These sensors
251 recorded the soil temperature every hour. We determined the temperature each seed
252 experienced prior to germination by calculating the mean temperature between the day
253 of rainfall onset and day of germination from the iButton data (Figure 2; Figure 3B).
254 These values were then used in models of germination rate. In models of germination
255 fraction, the average was taken of the mean temperatures experienced by each seed in

each block, within each cohort. This gave a block-level estimate of mean temperature experienced by seeds in each block between rainfall onset date and germination date.

Data Analysis

Germination Fraction

Germination fraction was calculated at the block level as the number of germinated seeds divided by the total number of seeds sown. To evaluate the relationship between germination fraction and the timing of rainfall onset and seasonal temperature, we used grouped logistic regressions. Regressions were built for each species, with groups comprising the seeds sown for each block (3), and successes indicating germination, with block included as a random effect. Both linear and quadratic models were fit for models of the timing of rainfall onset and likelihood ratio tests were used to determine the significance of factors in the models. Only linear model results are presented as their results were not distinguishable from quadratic models. Marginal means were estimated for each species using the emmeans package (Lenth, 2022) for use in *post hoc* comparisons.

We also tested whether species differ in their germination responsiveness to the timing of rainfall onset. A Pearson's Chi-squared test with three contingencies (Year 1, Year 2, Never) was used to evaluate germination timing. We performed *post hoc* analyses using the chisq.posthoc.test package (Ebbert, 2019) in R with a Bonferroni p-value adjustment.

Germination Rate

Germination rate was calculated for each seed as the reciprocal of the number of days between germination date and rainfall onset date (Bewley et al., 2013). Models were built to evaluate the relationship between germination rate and the timing of rainfall onset, as well as the temperature each seed experienced during the season. For each species, both linear and quadratic models of the timing of rainfall onset were fit and likelihood ratio tests were used to determine the best fit model for each species. Only linear model results are presented as they were not distinguishable from quadratic models. A linear mixed effects model was also built, using the lme4 package (Bates et al., 2015), with an interaction term between mean temperature each seed experienced and species along with cohort included as fixed effects and block included as a random effect. Marginal means were estimated for each species using the emmeans package (Lenth, 2022) for use in *post hoc* comparisons.

Potential Effects of Climate Change

We sought to understand how climate change induced shifts in precipitation timing affect germination responses. Specifically, we compared germination responses (fractions and rates) on dates that represent mean historical and contemporary rainfall onset. According to Luković et al. (2021), historical onset of precipitation in California was October 1 based on years 1960 to1989, which has shifted approximately 27 days to October 28 as the contemporary onset of precipitation (over the last 30 years). These dates align closely with the rainfall onset dates of October 2 (historical date) and October 30 (contemporary date) in this study. We compared germination fractions of historical and contemporary rainfall onset dates using species-specific generalized

linear models with binomial error and a logit link function. The relationship between germination rate and rainfall onset date was evaluated using species-specific linear models. Marginal means were estimated, and comparisons between historical and contemporary rainfall onset dates were made using the emmeans package (Lenth, 2022) with p-values adjusted using the Tukey method for multiple comparisons.

Phylogenetic Analyses of Species' Responses

We explored germination responses to rainfall onset date and seasonal temperatures across the phylogeny to understand if these responses are constrained by evolutionary history. We extracted the slopes from the relationships between germination fraction and rate in response to rainfall onset and temperature for each species and tested for phylogenetic signals in slopes. Estimates of phylogenetic signals allow us to understand the degree to which phylogenetic relatedness explains similarity in species' germination responses and how these responses have evolved as these species spread across, and diversified into, different environments. Using the R package phytools (Revell, 2012), we estimated phylogenetic signals using Pagel's λ. We tested the null hypothesis that Pagel's $\lambda = 0$, where a value equal to 0 indicates low phylogenetic signal, evolution of germination responses is independent of phylogeny, and a value equal to 1 indicates high phylogenetic signal, differences in species' responses are proportional to their shared history (Pagel, 1999). Intermediate values of Pagel's λ suggest phylogenetic relatedness is not the only factor contributing to the observed patterns.

Results

Throughout the experiment, temperatures decreased through time such that seeds in earlier rainfall onset events experienced warmer temperatures than seeds in later rainfall onset events (Figure 3A). In general, later rainfall onset had a negative effect on both germination fraction (Figure 4, Table 2) and germination rate (Figure 5, Table 3), though responses varied among species. Germination fraction significantly decreased with later onset of rains for all but three species (*C. amplexicaulis*, *C. anceps*, and *C. coulteri*) (Figure 4). Germination rate significantly decreased for all species with later onset of rains (Figure 5). Germination fraction and rate were sensitive to temperatures seeds experienced following germination-triggering rain events. Germination fraction significantly decreased for all species but *C. amplexicaulis*, *C. anceps*, and *C. coulteri* as the mean temperature experienced by seeds decreased (Figure 6; Table 4). Germination rate significantly decreased for all species as the mean temperature experienced by seeds decreased (Figure 7; Table 5).

To understand how climate change induced shifts in precipitation timing affect germination responses, we compared historical and contemporary timings of rainfall onset. We found that some species are likely already experiencing significant decreases in germination fractions and/or germination rates with observed climate change. Germination fraction significantly decreased between historical and contemporary rainfall timing for *S. diversifolius*, *S. glandulosus*, *S. tortuosus*, and one of the populations of *C. inflatus* (CAIN4; Figure 4; Table S4). Germination rate significantly decreased for *S. diversifolius*, *S. insignis*, *S. polygaloides*, *S. tortuosus*, and the other population of *C. inflatus* (CAIN3; Figure 5; Table S5).

348 We estimated phylogenetic signals of germination responses to rainfall onset date and
349 seasonal temperatures to evaluate if these responses are constrained by evolutionary
350 history (Figure 8). Slopes of relationships between germination fraction, the timing of
351 rainfall onset (λ = 0.87, P = 0.05), and the mean temperature seeds experienced (λ =
352 0.84, P = 0.08) showed moderate, but non-significant phylogenetic signal. Slopes of
353 relationships between germination rate and the timing of rainfall onset (λ = 0.99, P <
354 0.005) and between germination rate and the mean temperature seeds experienced (λ
355 = 0.99, P < 0.05) showed significant phylogenetic signal.
356 We also investigated whether species differed in their germination
357 responsiveness to the timing of rainfall onset to evaluate potential differences in
358 species' sensitivity to germination cues and germination strategies. Using a Pearson's
359 Chi-squared test with three contingencies of germination timing (Year 1, Year 2, Never),
360 we found that species differed in the fates of their seeds across the study (germination
361 in year 1, fall year 2, or never; chi-squared = 1006.1, df = 24, p < 0.0001; Figure 3A).
362 *Post hoc* analysis revealed significant associations between species and a variety of the
363 germination timing categories (Table S6). Most species germinated much less in the
364 second year (Figure 3A; Table S6). However, both populations of *C. anceps*, a desert
365 dwelling species, had higher germination in year 2 than in year 1. *S. glandulosus* had
366 low germination in the later rainfall onset events of year 1, which then germinated in
367 high fractions in the early season rainfall event of year 2 (Figure 3A: 27-Nov, 11-Dec).
368 *C. amplexicaulis*, a mid- high elevation lower latitude species, differed from other
369 species as it was not as sensitive to the timing of rainfall onset or temperature, with
370 significantly lower germination in both years (Figure 3A; Table S6).

Discussion

Here, we used experimentally imposed rainfall onset events to assess the impact of the timing of germination-triggering rains and corresponding temperatures on germination fraction and rate for closely related species that radiated into different climate niches within California's Mediterranean climate. Our results indicate that later onset of seasonal rains during cooler seasonal temperatures decreases germination fractions and rates, with variable responses among species. Further, some species may already be experiencing negative effects of contemporary shifts in the timing of rainfall. While species varied in germination responses to the timing of rainfall and corresponding temperatures, we found phylogenetic signal among species' responses, evidence that the seasonal germination niche may be constrained by phylogenetic relationships. Lastly, we found species differences in responsiveness to environmental cues affecting germination timing: *Caulanthus* species from drier (*C. anceps*) and more variable (*C. amplexicaulis* and *C. coulteri*) sites were found to be less sensitive to the timing of rainfall onset events or their associated temperatures than species from wetter, cooler environments.

In systems with winter growing seasons, earlier onset of seasonal rains typically corresponds with warmer temperatures and may also lead to a longer growing season. Thus, earlier rains may drive higher and faster germination simply due to thermal requirements for germination or may be an adaptive response in which seeds are tuned to early conditions for germination in order to take advantage of a longer growing season. On the other hand, there may be advantages to germinating later in the season

394 during cooler temperatures that may indicate that reliable cool, wet weather has arrived
395 (Mayfield et al., 2014). In the current study, germination rate significantly decreased for
396 all species with later onset of precipitation that occurred under cooler temperatures.
397 Germination fraction also showed this pattern for most species. Huang et al. (2016)
398 reported similar results in a Sonoran Desert plant community finding longer thermal
399 times to germination (slower germination rates) and lower germination percentages
400 when germination occurred later in the season under less favorable conditions. They
401 also found greater germination when temperatures were high during rainfall (Huang et
402 al., 2016), as was found during this study. In their system and in ours, most of the
403 variability in growing season length comes from the onset of germination-triggering
404 rains, which start the growing season, instead of the timing of the onset of dry
405 conditions that end it (Kimball et al., 2011; Luković et al., 2021). Thus, earlier
406 germination, under warmer temperatures, is the main avenue to increase growing
407 season length. Research on the alpine plant *Wahlenbergia ceracea* also found
408 decreased germination, both rate and fraction, with lower temperatures and suggested
409 that the temperature window of ideal conditions for germination is narrow (Notarnicola et
410 al., 2022). Together, our findings and previous research highlight how important the
411 interaction of precipitation timing and temperature is for driving germination patterns.
412 However, understanding the adaptive nature of these responses depends on their
413 relationship with fitness.

414 A growing body of literature has found that germination cues interact to drive
415 climate change effects on germination timing, fractions, and rates (Levine et al., 2008;
416 Kimbell et al., 2010; Levine et al., 2011; Huang et al., 2016). Our findings here further

417 stress the importance of considering a combination of germination cues when
418 investigating climate change impacts on phenology (Donohue et al., 2010). Results here
419 also support the idea that changes in the timing of rainfall onset in concert with
420 temperatures following these rain events may have stronger effects on population
421 dynamics than changes in total rainfall or temperature alone (Levine et al., 2008;
422 Kimball et al., 2010; Levine et al., 2011).

423 While the timing of precipitation onset has shifted later since 1960 in California
424 (Luković et al., 2021), average temperature has not followed the same trend as sharply
425 as precipitation (Rapacciuolo et al., 2014; Wright et al., 2016; Pathak et al., 2018),
426 suggesting a recent and continuing mismatch between the timing of precipitation and
427 the temperature following rain events relative to historic patterns. Here, we found that
428 species in this study show decreases in germination fractions and rates when we
429 compare historic and contemporary onsets of precipitation (Figures 4-5; Tables 4-5).
430 Two species, *S. diversifolius* and *S. tortuosus* show significant decreases in both
431 germination fraction and rate when making this comparison, suggesting they may
432 already be experiencing more extreme effects of shifting precipitation timing than other
433 species. This highlights that the severity of impacts caused by climate change may be
434 species-specific, with each species differentially affected, calling for species-level
435 restoration and conservation plans (Barga et al., 2017; Finch-Savage and Footitt, 2017).

436 Species in this study showed variation in relationships between germination
437 responses and environmental cues for germination, but phylogenetic signal suggest
438 these responses may have evolved as these species diverged. Slopes of relationships
439 between germination fraction, germination rate, the timing of rainfall onset, and

temperatures experienced all had moderate to high phylogenetic signal, suggesting that adaptation of germination to climate may be constrained by evolutionary history. Previous work has also found strong phylogenetic signal in germination cues (Arène et al., 2017; Fernández-Pascual et al., 2021; Baskin et al., 2022). In the *Streptanthus* (*s.l.*) clade, research has linked divergence and spread of these species to edaphic and climatic adaptations, including soil nutrients and precipitation quantity (Cacho and Strauss, 2014; Christie and Strauss, 2018; Pearse et al., 2020). In this study, we found that many of the *Caulanthus* species have much shallower slopes for their relationships between germination fraction, timing of rainfall onset, and temperatures experienced than *Streptanthus* species (Figure 8). Lower latitude *Caulanthus* species have typically evolved in drier, warmer, and more variable habitats than higher latitude *Streptanthus* species (Christie and Strauss, 2018, Fig. 1), potentially contributing to divergence in their germination sensitivities and strategies. Germination fraction is an important demographic parameter and fitness component where variation among species may indicate differences in sensitivity of species to environmental cues for germination with implications for the success of germination and populations in future climate scenarios. With continued climate change, seeds will either germinate under suboptimal conditions based on formerly adaptive germination cues, shift germination timing by tracking optimal conditions (Catelotti et al., 2020), adapt to respond to new combinations of germination cues (Donohue et al., 2010; Walck et al., 2011), or use a combination of these strategies (Gremer et al., 2016; Gremer et al., 2020a).

Species can have different germination strategies including using germination cues to germinate during appropriate conditions, not germinating in a given year if

germination conditions or cues are unavailable, or seeds remaining dormant in the soil even if appropriate germination cues are given. Species in this study encompassed all of these germination strategies where the majority of species were quite responsive to the interaction of precipitation and temperature germination cues. *S. glandulosus* showed the steepest decrease in germination fractions with later onset of rains and with decreasing temperatures suggesting it may be more sensitive than the other species to environmental conditions necessary for germination and have a narrower seasonal germination niche. Indeed, *S. glandulosus* seeds in later rainfall onset events that did not get the appropriate conditions for germination in the first year had high germination in the second year. Secondary dormancy can be an evolved strategy to prevent germination under unfavorable conditions, which could be occurring in these *S. glandulosus* seeds from later rainfall onset events and then released under favorable conditions in the early rainfall event of year 2. Other studies have also found support for secondary dormancy in seeds when environmental conditions are no longer suitable for germination (Probert, 2000; Walck et al., 2011; Finch-Savage and Footitt, 2017). For example, Hawkins et al. (2017) found that seeds of *Bromus tectorum* cycled into secondary dormancy when soil water potentials and temperatures were below the lower limit of appropriate conditions for germination. In contrast, three of the *Caulanthus* species, *C. amplexicaulis*, *C. anceps*, and *C. coulteri*, appear to favor a bet-hedging strategy with similar low germination fractions regardless of the timing of rainfall onset or corresponding temperatures that may indicate dormancy fractions. These species inhabit a variety of habitats in lower latitudes (Figure 1A) where environmental conditions can be harsh and temporally unpredictable (Figure 1B), conditions previously

linked to bet-hedging germination strategy (Cohen, 1966; Venable, 2007; Gremer and Venable, 2014).

In this study we quantified germination responses to the timing of rainfall onset and corresponding temperatures, though other seasonal cues may be at work that were not considered here (Baskin and Baskin, 2014). Overall, our findings shed further light on the germination niche and its species-specific sensitivity to changing climatic conditions and environmental cues for germination (Barga et al., 2017). We also draw attention to the importance of considering multiple environmental cues (Bernhardt et al., 2020) and how climate change is creating mismatches between cues with effects on germination processes that should scale-up to impact population persistence and species dynamics. We do acknowledge the need for additional investigations of the role of other potential germination cues, such daylength and chilling hours in future studies. The data generated here will prove useful in studies seeking to generate process-based predictions of germination under future climate conditions (Hamann et al., 2021), vital studies needed to further our understanding of how climate factors drive germination and contribute to demographic variation.

Authors' Contributions

J.R.G, J.S., S.Y.S. and J.N.M conceived and designed the study. A.M, E.C., S.R.A, J.S performed the experiment, with help from J.R.G. and S.Y.S in data collection. S.J.W., J.R.G., S.Y.S., and J.S designed the data analyses. S.J.W. and J.R.G. analyzed the data with assistance from J.S., S.Y.S, A.M., S.R.A., and E.C. S.J.W., J.R.G. A.M., and

J.S. wrote the manuscript with contributions from all other authors. All authors contributed to development of ideas, analyses, and interpretation of results.

Data Availability Statement

Data and code are currently available on GitHub at https://github.com/StreptanthusDimensions/Germination.Timing. A Zenodo doi will be obtained for the GitHub material upon manuscript acceptance.

References

1. Arène, F., Affre, L., Doxa, A., & Saatkamp, A. (2017). Temperature but not moisture response of germination shows phylogenetic constraints while both interact with seed mass and lifespan. *Seed Science Research, 27,* 110-120.
2. Axelrod, D. I. (1958). Evolution of the madro-tertiary geoflora. *The Botanical Review, 24,* 433-509.
3. Barga, S., Dilts, T. E., & Leger, E. A. (2017). Climate variability affects the germination strategies exhibited by arid land plants. *Oecologia, 185,* 437-452.
4. Barredo, J. I., Mauri, A., Caudullo, G., & Dosio, A. (2018). *Pure and Applied Geophysics, 175,* 3955-3971.
5. Baskin, C. C., & Baskin, J. M. (2014). *Seeds: ecology, biogeography, and evolution of dormancy and germination*, 2nd ed. San Diego, Academic Press.
6. Baskin, C. C., Baskin, J. M., Hu, X. W., & Zhang, C. H. (2022). Width of the temperature range for seed germination of herbaceous plant species in temperate eastern North American: life cycles, seasons and temperature variation and implication for climate warming. *Seed Science Research, 32,* 13-22.
7. Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software, 67,* 1-48.
8. Bernhardt, J. R., O'Connor, M. I., Sunday, J. M., & Gonzalez, A. (2020). Life in fluctuating environments. *Philosophical Transactions of the Royal Society, B, Biological Sciences, 375,* 20190454.
9. Bewley, J. D., Bradford, K. J., Hilhorst, H. W. M., & Nonogaki H. (2013). *Seeds: physiology of development, germination and dormancy*, 3rd ed. New York, Springer.

10. Bonamour, S., Chevin, L.-M., Charmantier, A., & Teplitsky, C. (2019). Phenotypic plasticity in response to climate change: the importance of cue variation. *Philosophical Transactions of the Royal Society, B, Biological Sciences, 374,* 20180178.
11. Burghardt, L. T., Metcalf, C. J. E., Wilczek, A. M., Schmitt, J., & Donohue, K. (2015). Modeling the influence of genetic and environmental variation on the expression of plant life cycles across landscapes. *The American Naturalist, 185,* 212-227.
12. Cacho, N. I., & Strauss, S. Y. (2014). Occupation of bare habitats, an evolutionary precursor to soil specialization in plants. *Proceedings of the National Academy of Sciences, USA, 111,* 15132-15137.
13. Cacho, N. I., Burrell, A. M., Pepper, A. E., & Strauss, S. Y. (2014). Novel nuclear markers inform the systematics and the evolution of serpentine use in Streptanthus and allies (Thelypodieae, Brassicaceae). *Molecular Phylogenetics and Evolution, 72,* 71-81.
14. Cacho, N. I., McIntyre, P. J., Kliebenstein, D. J., & Strauss, S. Y. (2021). Genome size evolution is associated with climate seasonality and glucosinolates, but not life history, soil nutrients or range size, across a clade of mustards. *Annals of Botany, 127,* 887-902.
15. Catelotti, K., Bino, G., & Offord, C. A. (2020). Thermal germination niches of *Persoonia* species and projected spatiotemporal shifts under a changing climate. *Diversity and Distributions, 26,* 589-609.

16. Christie, K., & Strauss, S. Y. (2018). Along the speciation continuum: Quantifying intrinsic and extrinsic isolation barriers across five million years of evolutionary divergence in California jewelflowers. *Evolution, 72,* 1063-1079.
17. Cohen, D. (1966). Optimizing reproduction in a randomly varying environment. *Journal of Theoretical Biology, 12,* 119-129.
18. Cohen, D. (1967). Optimizing reproduction in a randomly varying environment when a correlation may exist between the conditions at the time a choice has to be made and the subsequent outcome. *Journal of Theoretical Biology, 16,* 1-14.
19. Donohue, K., de Casas, R. R., Burghardt, L., Kovach, K., & Willis, C. G. (2010). Germination, postgermination adaptation, and species ecological ranges. *Annual Review of Ecology, Evolution, and Systematics*, *41*, 293-319.
20. Ebbert, D. (2019). chisq.posthoc.test: a post hoc analysis for Pearson's chi-squared test for count data. R package version 0.1.2.
21. Fang, X.-W., Zhang, J.-J., Xu, D.-H., Pang, J., Gao, T.-P., Zhang, C.-H., Li, F.-M., & Turner, N. C. (2017). Seed germination of *Caragana* species from different regions is strongly driven by environmental cues and not phylogenetic signals. *Scientific Reports, 7,* 11248.
22. Fernández-Pascual, E., Carta, A., Mondoni, A., Cavieres, L. A., Rosbakh, S., Venn, S., … Jiménez-Alfaro, B. (2021). The seed germination spectrum of alpine plants: a global meta-analysis. *New Phytologist, 229,* 3573-3586.
23. Finch-Savage, W. E., & Leubner-Metzger, G. (2006). Seed dormancy and the control of germination. *New Phytologist, 171,* 501-523.

24. Finch-Savage, W. E., & Footitt, S. (2017). Seed dormancy cycling and the regulation of dormancy mechanisms to time germination in variable field environments. *Journal of Experimental Botany, 68,* 843-856.
25. Flint, L. E., & Flint, A. L. (2014). California basin characterization model: A dataset of historical and future hydrologic response to climate change. U.S. Geological Survey Data Release, https://doi.org/10.5066/F76T0JPB.
26. Giorgi, F., & Lionello, P. (2008). Climate change projections for the Mediterranean region. *Global and Planetary Change, 63,* 90-104.
27. Golodets, C., Sternberg, M., Kigel, J., Boeken, B., Henkin, Z., Seligman, N. G., & Ungar, E. D. (2013). From desert to Mediterranean rangelands: will increasing drought and inter-annual rainfall variability affect herbaceous annual primary productivity? *Climate Change, 119,* 785-798.
28. Gremer, J. R. (2023). Looking to the past to understand the future: linking evolutionary modes of response with functional and life history traits in variable environments. *New Phytologist, 237,* 751-757.
29. Gremer, J. R., & Venable, D. L. (2014). Bet hedging in desert winter annual plants: optimal germination strategies in a variable environment. *Ecology Letters, 17,* 380-387.
30. Gremer, J. R., Kimball, S., & Venable, D. L. (2016). Within-and among-year germination in Sonoran Desert winter annuals: bet hedging and predictive germination in a variable environment. *Ecology Letters, 19,* 1209-1218.
31. Gremer, J. R., Chiono, A., Suglia, E., Bontrager, M., Okafor, L., & Schmitt, J. (2020). Variation in the seasonal germination niche across an elevational gradient: the role

of germination cueing in current and future climates. *American Journal of Botany, 107,* 350-363.

32. Gremer, J. R., Wilcox, C. J., Chiono, A., Suglia, E., & Schmitt, J. (2020a). Germination timing and chilling exposure create contingency in life history and influence fitness in the native wildflower *Streptanthus tortuosus. Journal of Ecology, 108,* 239-255.
33. Grubb, P. J. (1977). The maintenance of species-richness in plant communities: the importance of the regeneration niche. *Biological Review, 52,* 107-145.
34. Hamann, E., Denney, D., Day, S., Lombardi, E., Jameel, M. I., MacTavish, R., & Anderson, J. T. (2021). Plant eco-evolutionary responses to climate change: emerging directions. *Plant Science, 304,* 110737.
35. Hawkins, K. K., Allen, P. S., & Meyer, S. E. (2017). Secondary dormancy induction and release in *Bromus tectorum* seeds: the role of temperature, water potential and hydrothermal time. *Seed Science Research, 27,* 12-25.
36. Hoyle, G. L., Steadman, K. J., Good, R. B., McIntosh, E. J., Galea, L. M. E., & Nicotra, A. B. (2015). Seed germination strategies: an evolutionary trajectory independent of vegetative functional traits. *Frontiers in Plant Sciences, 6,* 731.
37. Huang, Z., Liu, S., Bradford, K. J., Huxman, T. E., & Venable, D. L. (2016). *Ecology, 97,* 250-261.
38. Iler, A. M., CaraDonna, P. J., Forrest, J. R. K., & Post, E. (2021). Demographic consequences of phenological shifts in response to climate change. *Annual Review of Ecology, Evolution, and Systematics, 52,* 221-245.

39. IPCC. (2021). Climate Change 2021: The Physical Science Basis. *Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge, United Kingdom and New York, NY, USA: Cambridge University Press.

40. Kalisz, S. (1986). Variable selection on the timing of germination in *Collinsia verna* (Scrophulariaceae). *Evolution, 40,* 479-491.

41. Kimball, S., Angert, A. L., Huxman, T. E., & Venable, D. L. (2010). Contemporary climate change in the Sonoran Desert favors cold-adapted species. *Global Change Biology, 16,* 1555-1565.

42. Kimball, S., Angert, A. L., Huxman, T. E., & Venable, D. L. (2011). Differences in the timing of germination and reproduction relate to growth physiology and population dynamics of Sonoran Desert winter annuals. *American Journal of Botany, 98,* 1773-1781.

43. Lenth, R. V. (2022). emmeans: estimated marginal means, aka least-squares means. R package version 1.7.3.

44. Levine, J. M., McEachern, A. K., & Cowan, C. (2008). Rainfall effects on rare annual plants. *Journal of Ecology, 96,* 795-806.

45. Levine, J. M., McEachern, A. K., & Cowan, C. (2011). Seasonal timing of first rain storms affects rare plant population dynamics. *Ecology, 92,* 2236-2247.

46. Liu, K., Baskin, J. M., Baskin, C. C., Bu, H., Du, G., Ma, M. (2013). Effect of diurnal fluctuating versus constant temperatures on germination of 445 species from the Eastern Tibet Plateau. *PloS ONE, 8,* e69364.

47. López, R. P., Hayes, M., Maidana-Tuco, Y., & Naoki, K. (2020). Summer annual plants exhibit germination responses to an aridity gradient consistent with both delayed germination and phenotypic plasticity. *Plant Ecology, 221,* 1233-1242.
48. Luković, J., Chiang, J. C. H., Blagojević, D., & Sekulić, A. (2021). A later onset of the rainy season in California. *Geophysical Research Letters, 48,* e2020GL090350.
49. Matías, L., Gómez-Aparicio, L., Zamora, R., & Castro, J. (2011). Effects of resource availability on plant recruitment at the community level in a Mediterranean mountain ecosystem. *Perspectives in plant ecology, evolution, and systematics, 13,* 277-285.
50. Mayfield, M. M., Dwyer, J. M., Main, A., & Levine, J. M. (2014). The germination strategies of widespread annual plants are unrelated to regional climate. *Global Ecology and Biogeography, 23,* 1430-1439.
51. McNamara, J. M., Barta, Z., Klaassen M., & Bauer, S. (2011). Cues and the optimal timing of activities under environmental changes. *Ecology Letters, 14,* 1183-1190.
52. Notarnicola, R. F., Nicotra, A. B., Kruuk, L. E. B., & Arnold, P. A. (2022). Effects of warming temperatures on germination responses and trade-offs between seed traits in an alpine plant. *Journal of Ecology, 111,* 62-76.
53. Pagel, M. (1999). Inferring the historical patterns of biological evolution. *Nature, 401,* 887-884.
54. Paradis, E., & Schliep, K. (2019). ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics, 35,* 526-528.
55. Parmesan, C., & Hanley, M. E. (2015). Plants and climate change: complexities and surprises. *Annals of Botany, 116,* 849-864.

56. Pathak, T. B., Maskey, M. L., Dahlberg, J. A., Kearns, F., Bali, K. M., & Zaccaria, D. (2018). Climate change trends and impacts on California agriculture: a detailed review. *Agronomy, 8,* 25.

57. Pearse, I. S., Aguilar, J. M., & Strauss, S. Y. (2020). Life-history plasticity and water-use trade-offs associated with drought resistance in a clade of California jewelflowers. *American Naturalist, 195,* 691-704.

58. Probert, R. J. (2000). The role of temperature in the regulation of seed dormancy and germination. In: Fenner, M. (ed) *Seeds: the ecology of regeneration in plant communities*. Wallingford: CAB International, 261-292.

59. Puglia, G., Carta, A., Bizzoca, R., Toorop, P., Spampinato, G., & Raccuia, S. A. (2018). Seed dormancy and control of germination in *Sisymbrella dentata* (L.). O.E. Schulz (Brassicaceae). *Plant Biology, 20,* 879-885.

60. R Core Team (2021). R: a language and environment for statistical computing. *R Foundation for Statistical Computing*, Vienna, Austria.

61. Rapacciuolo, G., Maher, S. P., Schneider, A. C., Hammond, T. T., Jabis, M. D., Walsh, R. E., … Beissinger, S. R. (2014). Beyond a warming fingerprint: individualistic biogeographic responses to heterogeneous climate change in California. *Global Change Biology, 20,* 2841-2855.

62. Revell, L. J. (2012). phytools: an R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution, 3,* 217-223.

63. Swain, D. L., Langenbrunner, B., Neelin, J. D., & Hall, A. (2018). Increasing precipitation volatility in twenty-first-century California. *Nature Climate Change, 8,* 427-433.

64. Venable, D. L. (2007). Bet hedging in a guild of desert annuals. *Ecology, 88,* 1086-1090.

65. Walck, J. L., Hidayati, S. N., Dixon, K. W., Thompson, K., & Poschlod, P. (2011). Climate change and plant regeneration from seed. *Global Change Biology, 17,* 2145-2161.

66. Went, F. W. (1949). Ecology of desert plants. II. The effect of rain and temperature on germination and growth. *Ecology, 30,* 1-13.

67. Wright, A. N., Schwartz, M. W., Hijmans, R. J., & Shaffer, H. B. (2016). Advances in climate models from CMIP3 to CMIP5 do not change predictions of future habitat suitability for California reptiles and amphibians. *Climate Change, 134,* 579-591.

705 **Table 1.** Species (11) and populations (13) included in this study from the *Caulanthus*
706 and *Streptanthus* genera of Brassicaceae. Average germination season (September –
707 December) climate data from 1991 – 2015 are also reported for each species' location:
708 precipitation (PPT, mm), maximum temperature (Tmax, °C), and minimum temperature
709 (Tmin, °C). Climate data sourced from Flint and Flint (2014).

Species	**Abbreviation**	**PPT**	**Tmax**	**Tmin**
Caulanthus amplexicaulis	CAAM	39.90	21.44	8.76
Caulanthus anceps	CAAN1	16.42	22.98	5.93
Caulanthus anceps	CAAN2	19.00	21.92	4.92
Caulanthus coulteri	CACO	21.74	18.71	8.22
Caulanthus inflatus	CAIN3	19.97	21.97	5.77
Caulanthus inflatus	CAIN4	18.07	21.03	9.04
Streptanthus breweri	STBR	66.99	21.71	8.45
Streptanthus diversifolius	STDI	58.07	20.61	8.08
Streptanthus drepanoides	STDR	100.18	21.66	9.61
Streptanthus glandulosus	STGL	124.75	18.45	8.29
Streptanthus insignis	STIN	30.53	22.27	5.47
Streptanthus polygaloides	STPO	142.42	19.82	7.93
Streptanthus tortuosus	STTO	50.76	22.11	8.25

710

711 **Table 2.** Results from grouped logistic regressions with germination fraction as the
712 response variable and rainfall onset date as the predictor variable. Coefficient estimates
713 are reported for the Intercept and Rainfall Onset Date terms from the models along with
714 the p-value associated with the Rainfall Onset Date term. P-values in bold are
715 significant.

Species	Intercept	Rainfall Onset Date	P-value
CAAM	-1.22	-0.11	0.26
CAAN1	-1.29	-0.08	0.33
CAAN2	-1.91	0.03	0.68
CACO	0.64	-0.12	0.36
CAIN3	1.96	-0.49	**< 0.0001**
CAIN4	0.99	-0.42	**< 0.0001**
STBR	2.20	-0.37	**< 0.0001**
STDI	1.33	-0.40	**< 0.0001**
STDR	1.52	-0.36	**< 0.0001**
STGL	2.00	-0.59	**< 0.0001**
STIN	0.53	-0.33	**< 0.0001**
STPO	1.96	-0.25	**< 0.0001**
STTO	1.75	-0.24	**< 0.0001**

716

717 **Table 3.** Results from linear regressions with germination rate as the response variable
718 and rainfall onset date as the predictor variable. Coefficient estimates are reported for
719 the Intercept and Rainfall Onset Date terms from the models along with the p-value
720 associated with the Rainfall Onset Date term. Adjusted R^2 values for the models are
721 also given. P-values in bold are significant.

Species	Intercept	Rainfall Onset Date	P-value	R^2
CAAM	0.11	-0.009	**0.04**	0.07
CAAN1	0.26	-0.06	**< 0.0001**	0.50
CAAN2	0.25	-0.03	**< 0.0001**	0.41
CACO	0.26	-0.03	**< 0.0001**	0.50
CAIN3	0.23	-0.02	**< 0.0001**	0.25
CAIN4	0.21	-0.02	**< 0.0001**	0.27
STBR	0.15	-0.01	**< 0.0001**	0.25
STDI	0.37	-0.04	**< 0.0001**	0.63
STDR	0.12	-0.004	**< 0.0001**	0.02
STGL	0.18	-0.01	**< 0.0001**	0.18
STIN	0.28	-0.03	**< 0.0001**	0.56
STPO	0.25	-0.03	**< 0.0001**	0.56
STTO	0.36	-0.04	**< 0.0001**	0.75

722

723 **Table 4.** Results from grouped logistic regressions with germination fraction as the
724 response variable, mean temperature seeds experienced as the predictor variable, and
725 block included as a random effect. Least-squares means were estimated for each
726 species and are reported along with 95% confidence intervals (CI) used to evaluate
727 significance. CI values in bold are significant.

Species	Temperature	CI
CAAM	0.05	-0.02 – 0.13
CAAN1	0.05	-0.02 – 0.11
CAAN2	-0.01	-0.08 – 0.05
CACO	0.06	-0.05 – 0.17
CAIN3	0.19	**0.14 – 0.24**
CAIN4	0.18	**0.13 – 0.24**
STBR	0.17	**0.11 – 0.22**
STDI	0.17	**0.13 – 0.22**
STDR	0.16	**0.10 – 0.22**
STGL	0.27	**0.21 – 0.33**
STIN	0.15	**0.10 – 0.20**
STPO	0.11	**0.06 – 0.17**
STTO	0.10	**0.05 – 0.15**

728

729 **Table 5.** Results from linear mixed-effects model with germination rates as the
730 response variable, mean temperature seeds experienced, species, their interaction, and
731 cohort as fixed effects, and block included as a random effect. Least-squares means
732 were estimated for each species and are reported along with 95% confidence intervals
733 (CI) used to evaluate significance. CI values in bold are significant.

Species	Temperature	CI
CAAM	0.01	**0.01 – 0.02**
CAAN1	0.02	**0.02 – 0.02**
CAAN2	0.02	**0.02 – 0.02**
CACO	0.02	**0.02 – 0.02**
CAIN3	0.02	**0.01 – 0.02**
CAIN4	0.01	**0.01 – 0.02**
STBR	0.01	**0.01 – 0.02**
STDI	0.02	**0.02 – 0.03**
STDR	0.01	**0.01 – 0.01**
STGL	0.01	**0.01 – 0.02**
STIN	0.02	**0.02 – 0.02**
STOP	0.02	**0.02 – 0.02**
STTO	0.02	**0.02 – 0.03**

734

Figure 1. Location and climate for study species. (A) Seeds were collected from 13 populations of 11 species across California. See Table 1 for species abbreviations. (B) Principal component analysis of average germination season (September - December) climate for 1991-2015 for each location. First two principal components (PCs) illustrated on the x and y axes. Climate variables include precipitation (PPT), minimum and maximum temperature (Tmin, Tmax, respectively), as well as variability in precipitation (coefficient of variation, PPT_CV) and temperature (standard deviation of Tmin and Tmax, Tmin_SD and Tmax_SD respectively). Data source was Flint and Flint (2014).

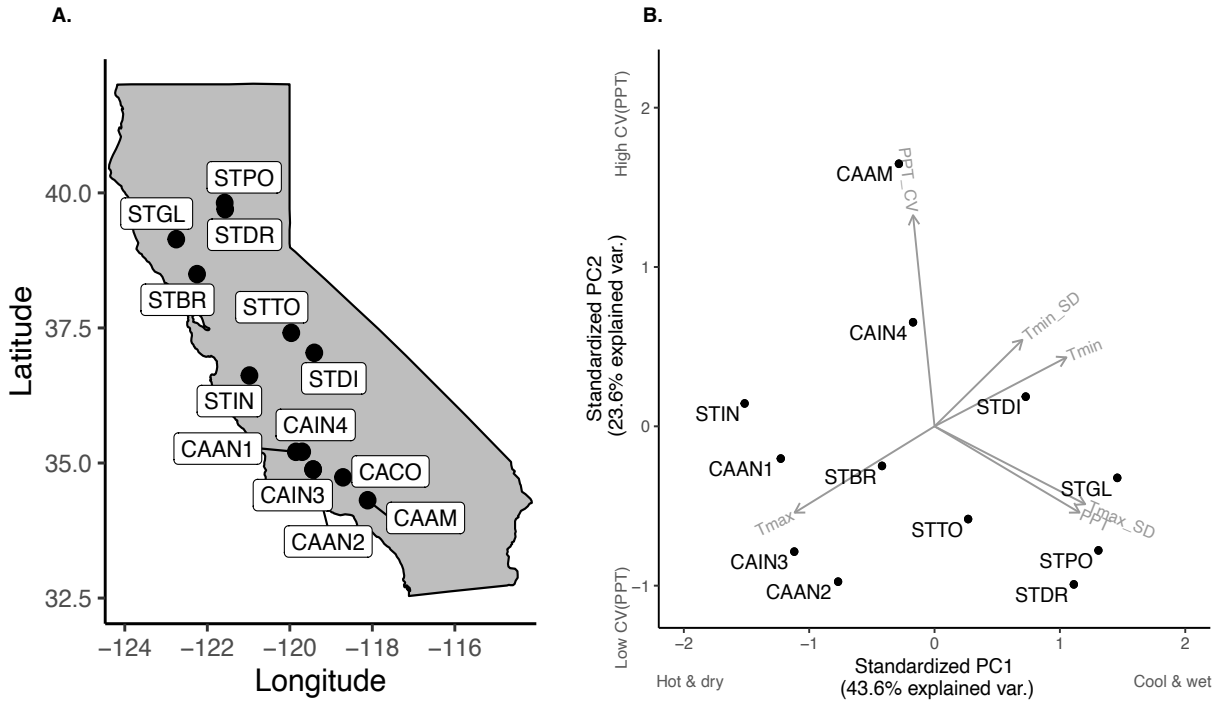

Figure 2. Mean and standard deviation of daily temperatures (°C) from the first rainfall onset date (September 17, 2020) to the last day of observed germination (January 25, 2021) in year one of the experiment. Dashed lines represent each rainfall onset date for each of seven cohorts with colors corresponding to those in Fig. 3A. The solid black line corresponds to the date of historic onset of rainfall (October 1) and the solid gray line represents the 27 day shift to the contemporary date of rainfall onset (October 28) in California according to Luković et al. (2021).

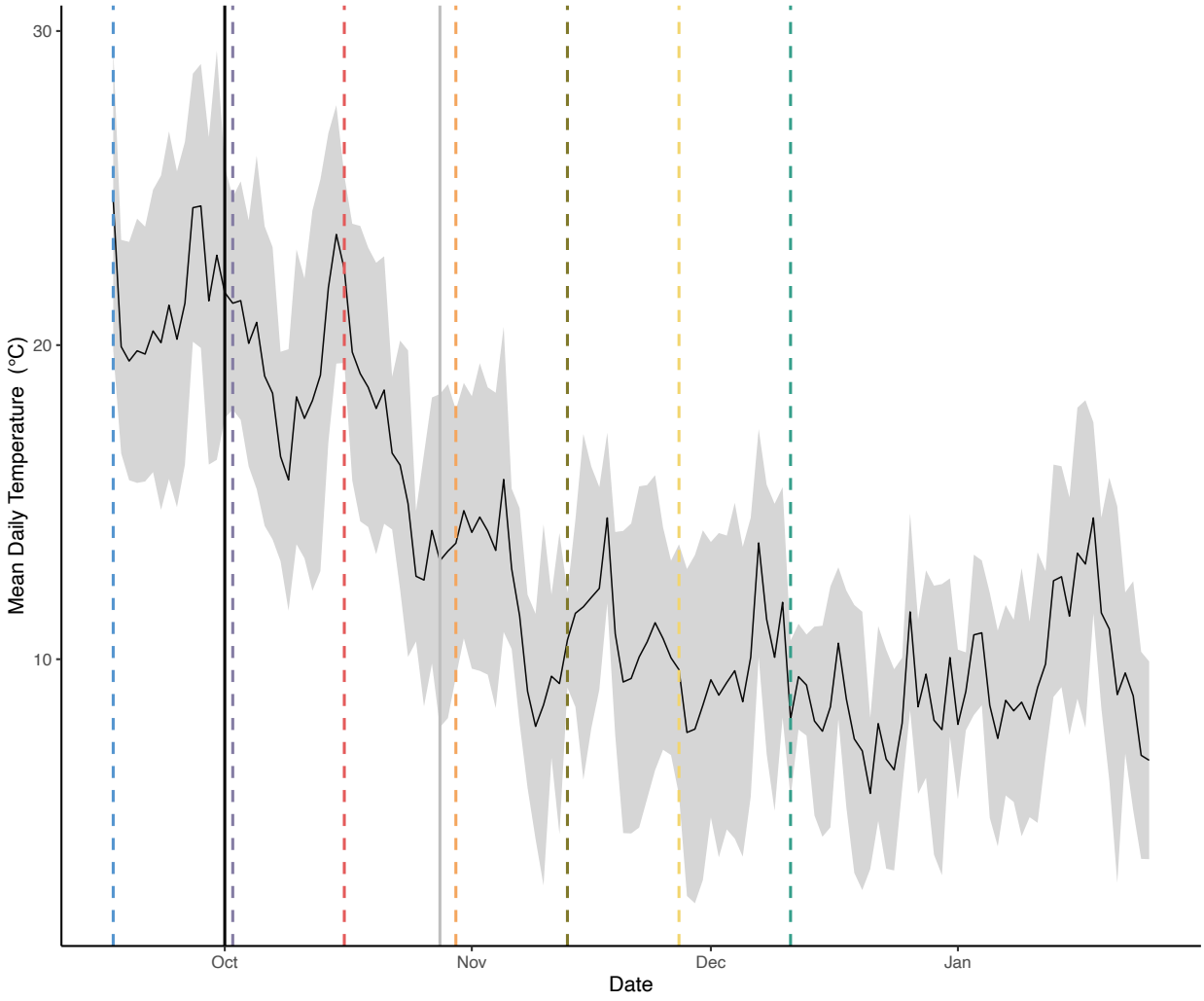

Figure 3. Germination fractions and temperatures seeds experienced during the study. (A) Distribution of mean temperature seeds in each cohort experienced between their rainfall onset date, displayed on the y-axis, and germination date. Seeds that did not germinate during year one of the experiment (September 17, 2020, to April 16, 2021) were re-watered on September 15, 2021, and germination was followed until December 6, 2021. (B) Amount of total germination fraction for each species during year one versus year two of the study at each rainfall onset date.

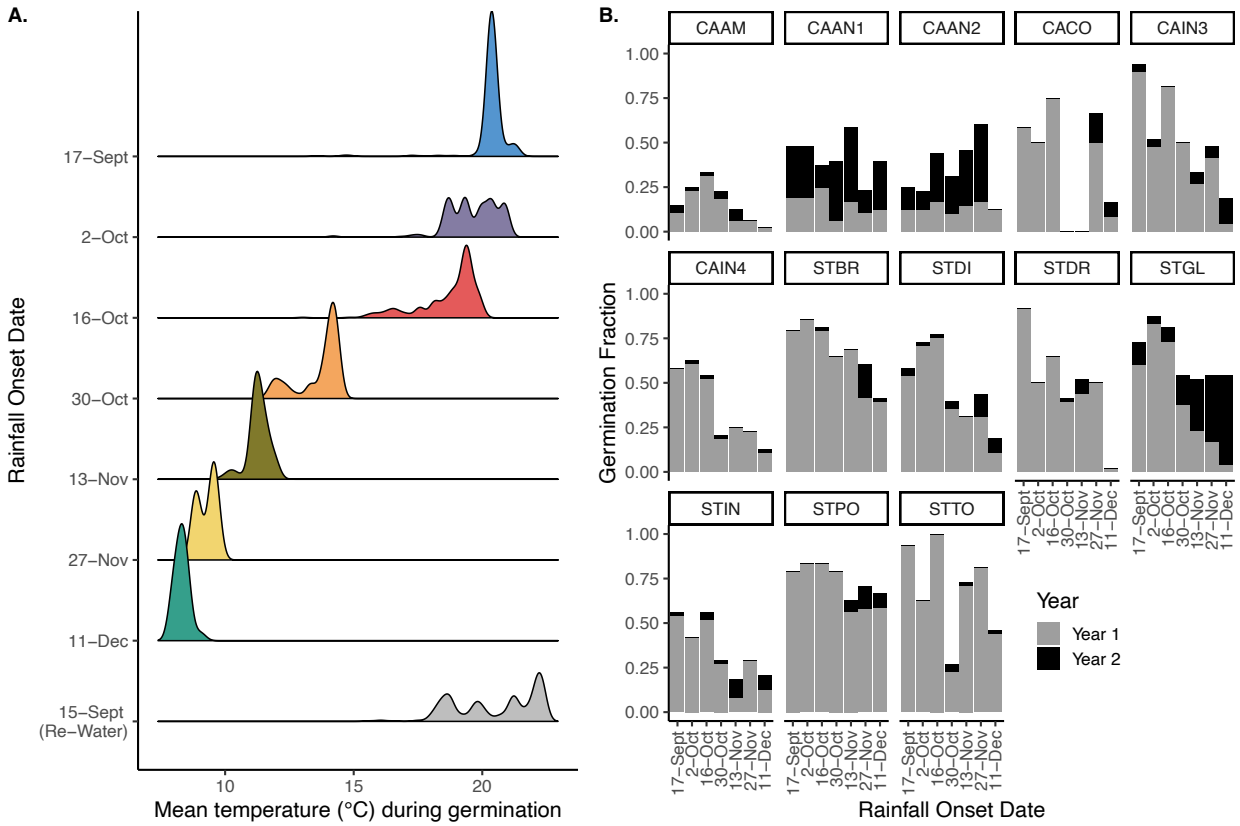

Figure 4. Relationships between germination fraction and rainfall onset date and comparison of germination fraction between 2-Oct (historical onset of rainfall) and 30-Oct (contemporary onset of rainfall). Plots where regression lines are present had significant relationships between germination fraction and rainfall onset date (back transformed from logit scale). For all significant relationships, germination fraction decreased as rainfall onset occurred later in the season. Points represent germination fraction for each cohort (mean ± 1 SE, back transformed from logit scale). In plots where points and SE bars are red, a significant decrease in germination fraction was found between the historical onset date of rainfall (2-Oct) and the contemporary onset date of rainfall (30-Oct).

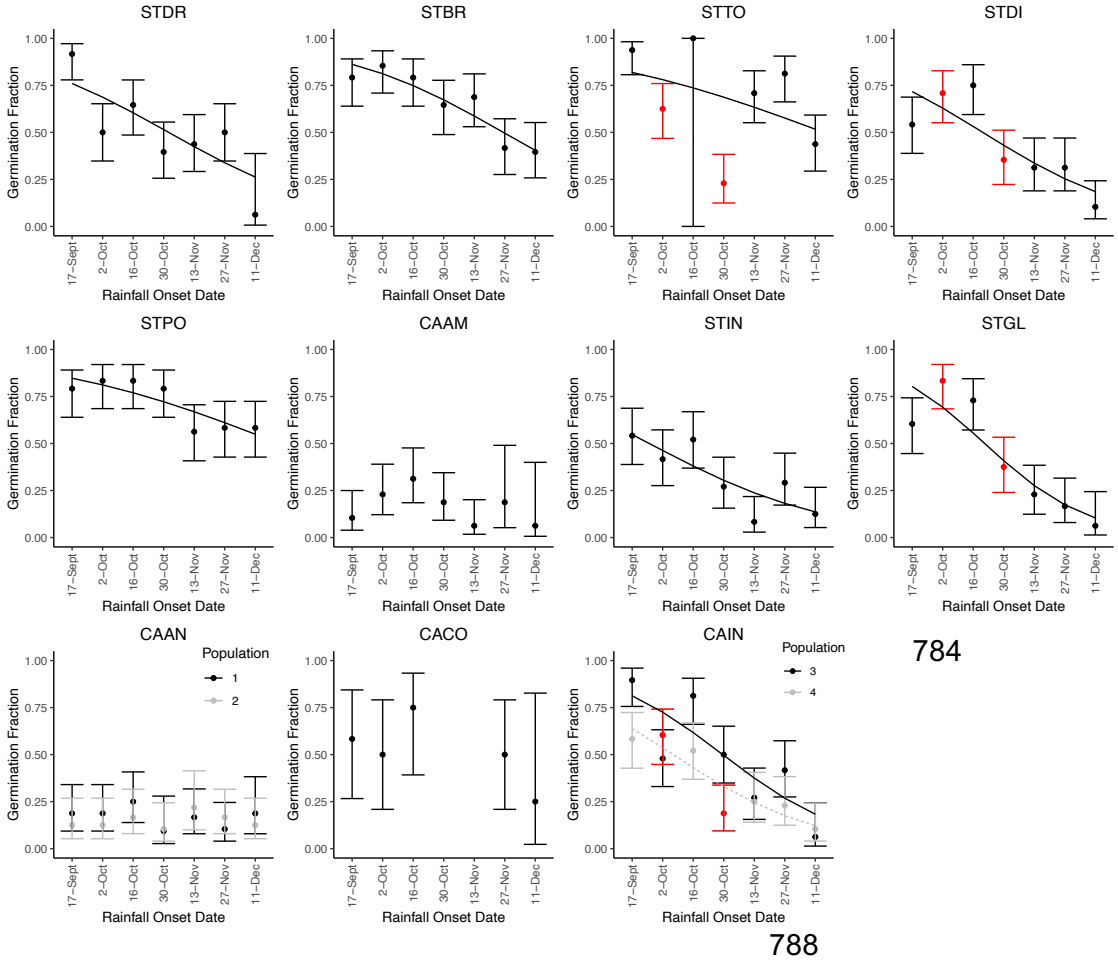

Figure 5. Relationships between germination rate (1/days to germination) and rainfall onset date and comparison of germination rate between 2-Oct (historical onset of rainfall) and 30-Oct (contemporary onset of rainfall). Regression lines represent significant, negative relationships between germination rate and rainfall onset date, where germination rate decreased as rainfall onset occurred later in the season. Points represent germination rate for each cohort (mean ± 1 SE). In plots where points and SE bars are red, a significant decrease in germination rate was found between the historical onset date of rainfall (2-Oct) and the contemporary onset date of rainfall (30-Oct).

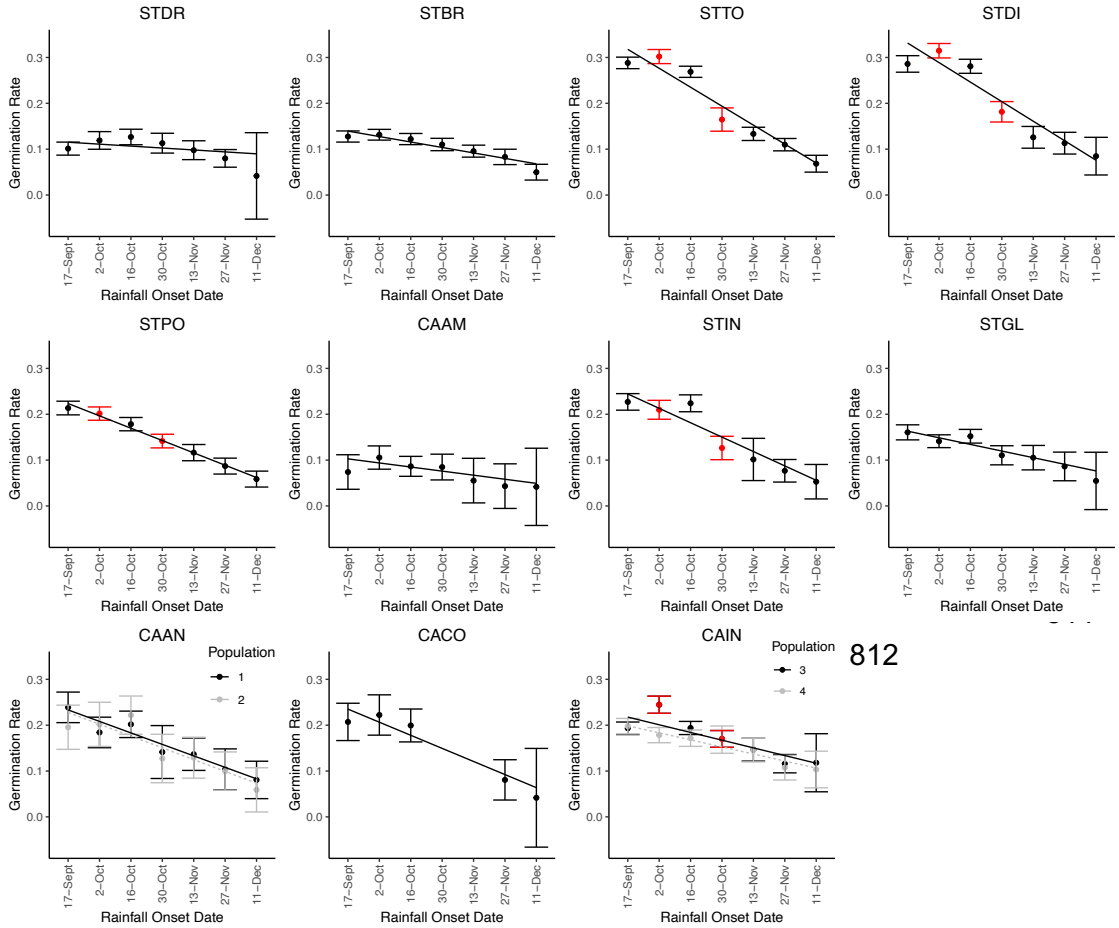

Figure 6. Relationships between germination fraction and mean temperature seeds experienced between their rainfall onset date and germination date. Regression lines represent significant, positive relationships between germination fraction and mean temperature seeds experienced (back transformed from logit scale).

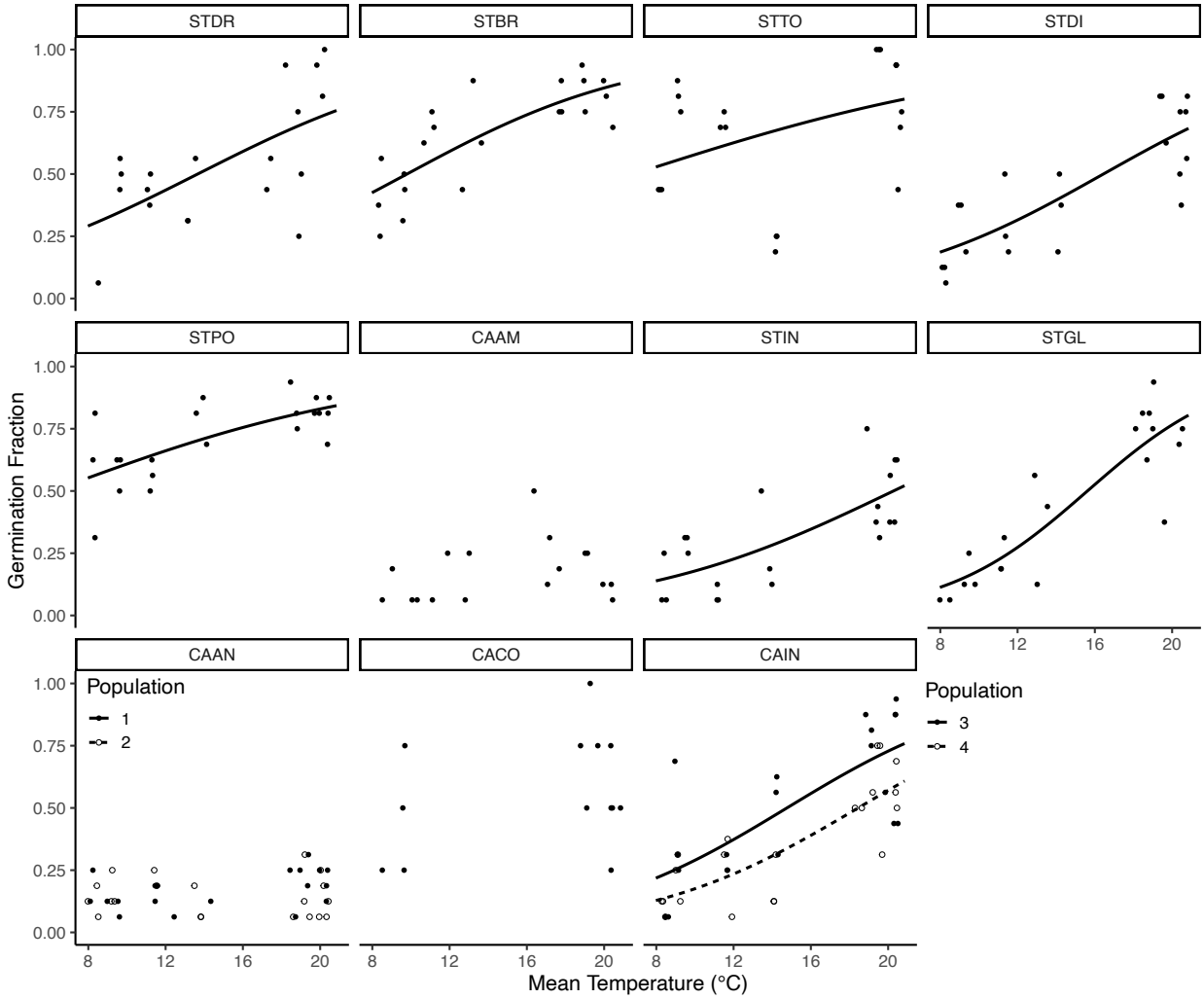

Figure 7. Relationships between germination rate and mean temperature seeds experienced between their rainfall onset date and germination date. Regression lines represent significant, positive relationships between germination rate (1/days to germination) and mean temperature seeds experienced.

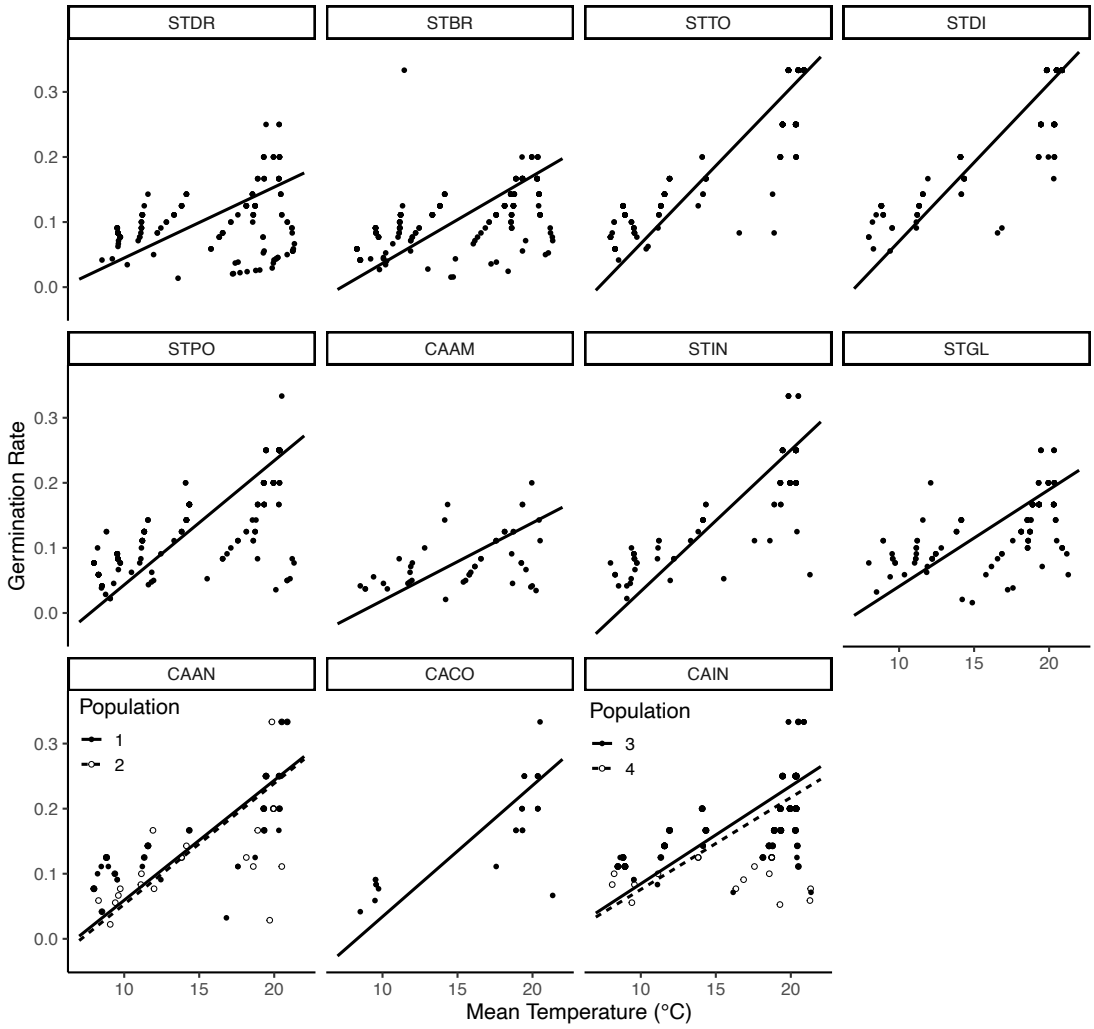
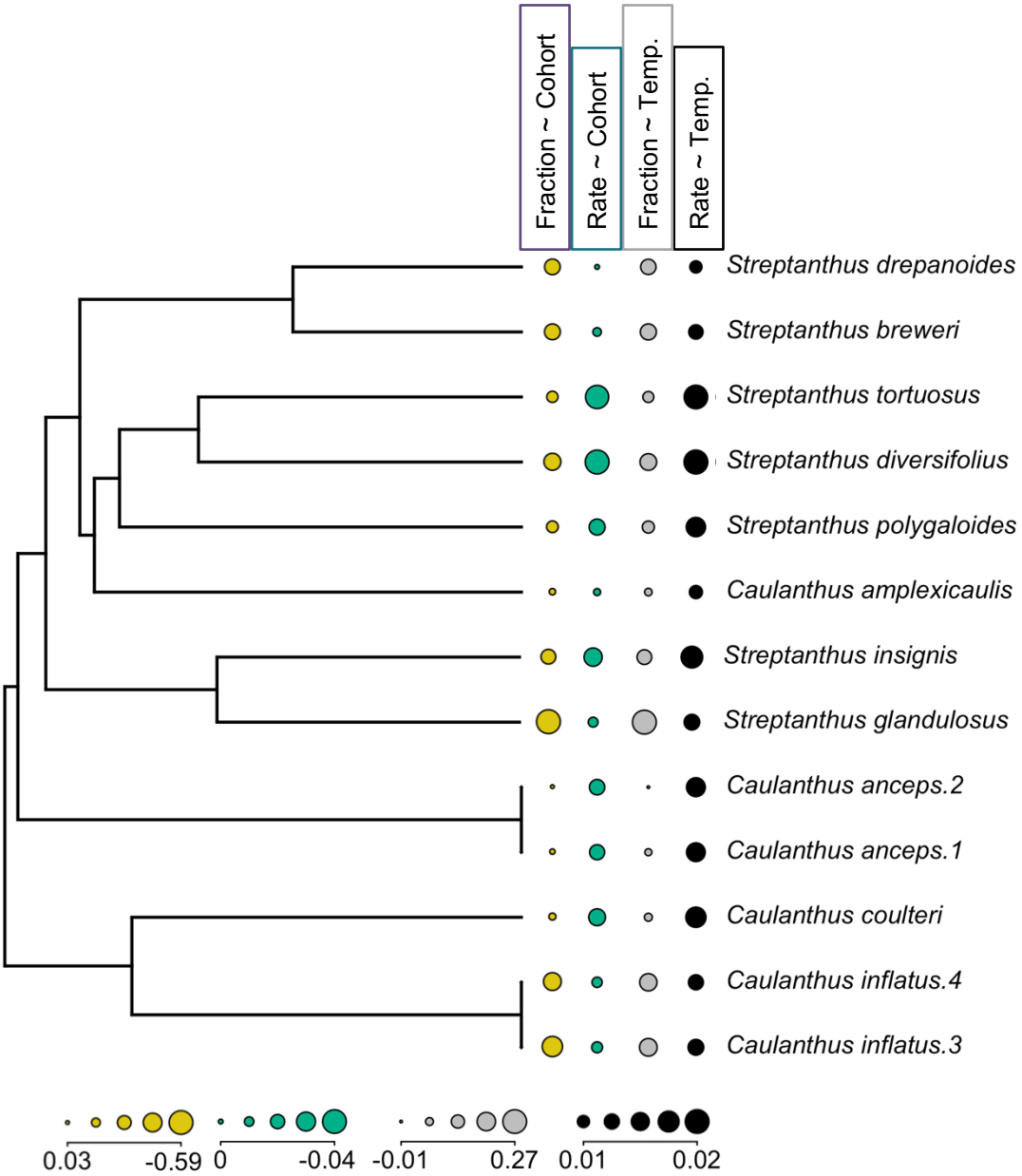
Figure 8. Slopes of the relationships evaluated in this study displayed across the phylogeny. Yellow dots display the slopes of the relationship between germination fraction and rainfall onset date (Fraction ~ Cohort) (Fig. 4). All slopes are negative except for *Caulanthus anceps.2* (0.03). Green dots display the slopes of the negative relationships between germination rate and rainfall onset date (Rate ~ Cohort) (Fig. 5). Gray dots display the slopes of the relationship between germination fraction and mean temperature experienced by seeds (Fig. 6). All slopes are positive except for *Caulanthus anceps.2* (-0.01). Black dots display the positive slopes of the relationships between germination rate and mean temperature experienced by seeds (Fig. 7).