Rapid evolution in response to climate-change-induced drought and its demographic and genetic controls

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Abstract

Populations often vary in their evolutionary responses to a shared environmental perturbation. A key hurdle in building more predictive models of rapid evolution is understanding this variation – why do some populations and traits evolve while others do not? However, studies documenting rapid evolution usually lack the demographic and genetic data needed to understand varied evolutionary responses. We combined long-term demographic and environmental data, estimates of quantitative genetic variance components, and a resurrection experiment to gain mechanistic insights into variation in evolutionary responses of five traits in two populations of a California endemic plant that recently experienced a severe multiyear drought. Earlier flowering phenology evolved in only one of the two populations, though both populations experienced similar precipitation patterns and demographic declines during drought and were estimated to have similar narrow-sense heritability of flowering phenology. However, demographic data indicated that seed input in years prior to the drought was 125% higher in the non-evolving population compared to the evolving population, suggesting that recruitment from the seedbank may have constrained evolution in the non-evolving population. Gene flow through time via seed banks may be an important, underappreciated control on rapid evolution in response to extreme environmental perturbations.

Keywords: adaptation; evolutionary rescue; Clarkia xantiana; seed bank; genetic constraints

Introduction

Extreme environmental perturbations offer excellent opportunities to examine evolution in natural populations on ecological timescales [1]. Strong episodes of environmental change have caused rapid evolution in lizards [2,3], finches [4], and plants [5]. Beyond offering fundamental insights into the evolutionary process, this and related work speak to the potential for natural populations to evolve in response to anthropogenic forcings like climate change, and to the importance of adaptation for the persistence of populations [6–8]. However, it remains poorly understood as to why rapid evolution often fails to occur in some populations despite the same environmental episode resulting in evolution in other populations.

Resurrection experiments are increasingly used to directly examine evolutionary change before and after an environmental event predicted to exert strong selection on populations [5,9]. The power of resurrection experiments lies in their ability to precisely quantify short-term evolutionary responses, rather than only making predictions of the trajectory of evolution, while controlling for confounding environmental effects on phenotypes. In short, the resurrection approach entails rearing different generations of a population contemporaneously in a common environment to directly quantify phenotypic evolution. Though only a modest number of resurrection studies have been published to date [10], such experiments with plants have sometimes demonstrated rapid evolution in response to periods of drought [5,11,12].

Just as important as these positive results, however, are instances in which resurrection experiments do *not* find evidence of rapid evolution. Populations of a species can vary in their responses to the same climatic fluctuation [12,13], and even extreme climatic anomalies can result in no discernible phenotypic evolution of putatively important traits [14]. What underlies this variation in populations' evolutionary responses to extreme events? While the resurrection

61 approach is a powerful method to determine if phenotypic evolution has occurred, it does not
62 typically provide insight into the demographic and genetic controls on evolutionary change.
63 A more comprehensive understanding of the causes and consequences of rapid evolution
64 can be gained by coupling evolutionary analyses with long-term demographic and quantitative
65 genetic investigations. Sharp declines in population size due to environmental perturbations will
66 reduce the efficacy of selection and increase the influence of stochastic processes like genetic
67 drift [15,16]. Such demographic, environmental, or genetic stochasticity may stymie adaptation,
68 potentially causing population extinction before 'evolutionary rescue' can occur [17–19]. Models
69 and experiments have further shown that evolutionary rescue is less probable when the
70 environment changes suddenly compared to when the change is gradual [20]. As such, the rate
71 and severity of demographic decline can provide insight into instances where some populations
72 fail to evolve in response to an extreme event that drove rapid evolution in other conspecific
73 populations. These demographic costs may be especially important if the extreme event occurs
74 across multiple generations [21].
75 For organisms with dormancy, long-term outcomes of selection can also be affected by
76 the presence of a germ bank (seed or egg bank). Populations that often experience significant
77 environmental fluctuations may evolve mechanisms that confer dormancy as a bet-hedging
78 strategy [22]. While germ banks may buffer populations from extinction [23,24], they may also
79 constrain evolutionary responses to environmental change due to gene flow among generations
80 [25,26]. This "temporal migration" can slow the rate of adaptive evolution [27,28]. Alleles
81 favored prior to a selective event will be 'reintroduced' to the population from the germ bank
82 during and after the event. If those same alleles are selected against during the event, this gene
83 flow can retard adaptation [but see 29]. Moreover, the environmental and demographic history of

84 populations just prior to an extreme event likely affects the magnitude of gene flow from the
85 past. For example, high fecundity of generations immediately prior to an extreme environmental
86 event would increase input to the seed bank. Therefore, historical demographic data should
87 provide insight into the influence of germ banks on population responses to selection.

88 Strong environmental perturbations typically exert selection on traits, but the response to
89 selection will depend upon the presence of sufficient additive genetic variance for traits
90 underlying fitness variation [30,31]. A population's response to selection will be directly
91 proportional to a given trait's narrow-sense heritability, $h^2 = V_A/V_p$ [32]. In the context of
92 resurrection experiments, measuring the additive genetic variance of ecologically-important traits
93 may help us understand why traits do or do not evolve in different populations exposed to similar
94 selective regimes. While tests of rapid evolution are increasingly common, few studies have
95 simultaneously examined quantitative genetic variation and its potential role in facilitating or
96 hindering adaptation (but see Franks et al. 2007). Furthermore, resurrection experiments usually
97 examine cohorts from before and at the end of an extreme event [but see 33]. A key question is
98 how long evolutionary changes that occur in response to these events persist beyond them.
99 Including later cohorts in a resurrection experiment allows us to assess the temporal stability of
100 evolutionary responses and the extent to which dormancy might delay responses to selection.
101 When paired with associated demographic data from natural populations, such a design gives
102 inference to how rapid evolution (or its absence) influences a populations' longer-term
103 phenotypic and demographic trajectory.

104 The Southwest of the United States is in the midst of the most severe multi-decadal
105 drought (i.e., megadrought) in recorded history (at least since 800 C.E.) [34,35]. Recent analyses
106 have shown that anthropogenic climate change has been a key driver and accounts for an

107 estimated 42% of the soil moisture anomaly [35,36]. We examined evolutionary responses to the
108 most severe episode of this climate anomaly, which began in the latter half of 2011 and ended in
109 late 2015 in our study area. Our work focused on a well-studied plant, *Clarkia xantiana* ssp.
110 *xantiana*, which is endemic to Southern California, U.S.A. We used a resurrection experiment to
111 test whether traits likely to mediate drought adaptation exhibited rapid evolution and whether
112 genetic or demographic constraints modulated responses.
113 First, we monitored environmental and demographic variation over 12 years (2006 –
114 2017), spanning a period from six years prior to drought until two years after the drought ended.
115 Those data provided insight into the severity and rate of environmental and demographic change,
116 as well as the magnitude of input to the seed bank in years just prior to the drought episode.
117 Second, to test for rapid evolution in response to drought, we used a resurrection experiment
118 with individuals from three time points — prior to the prolonged drought (2011), at the end of
119 the drought (2015), and two years later, after more average precipitation resumed (2017). This
120 latter sample allowed us to examine whether any evolutionary responses persisted, increased, or
121 evolved back toward the original population mean. We measured a suite of traits that prior
122 studies have shown to confer drought escape and/or avoidance. Last, we also conducted crosses
123 to generate a pedigreed population to estimate the additive genetic variance and narrow-sense
124 heritability for each of these traits in each population. We predicted that traits exhibiting high
125 heritability would be more likely to respond to selection while those exhibiting low heritability
126 may not have evolved.

Material and Methods

Study System

Clarkia xantiana ssp. *xantiana* A. Gray (Onagraceae) is a predominantly outcrossing winter annual native to the southern Sierra Nevada foothills and Transverse Ranges of California, USA [37]. In this Mediterranean climate, the bulk of precipitation occurs during winter and early spring. Plants germinate November - March during the rainy season, begin flowering in May, and set seed in late June. In this study, we focus on two populations, KYE and S22. KYE occupies oak woodland habitat on granite-derived soils characteristic of the more mesic, western portion of *C. x. xantiana*'s distribution (35.6240674°, -118.5156798°). S22 is located near the subspecies' eastern geographic range limit and occupies a more xeric, higher elevation site in pine woodland on metasedimentary-derived soils (35.83996°, -118.450386°). These metasedimentary-derived soils occur along the Kern Canyon Fault, a ca. 150km fault that parallels the Kern River through the Southern Sierra [38,39]. [Site identifiers are 57x (KYE) and 22x (S22) for consistency with previous and future *C. x. xantiana* studies).]

Climatic and demographic data

We obtained growing season precipitation data (November - June) for years 2006-2017 from long-term weather monitoring stations (HOBO Onset) at each site. Data on population size and seed production were collected as part of a long-term demographic study of 36 populations across the range of *C. x. xantiana*. As a proxy for *C. x. xantiana* population size at each site in years 2006-2017, we obtained counts of the number of fruiting plants at each site, as estimated

from censusing 58-129 0.5 m^2 quadrats across the extent of each site in late June / early July of each year. We report these data as the average number of fruiting plants per plot. We also recorded the number of fruits per plant on a subset of these plants (mean of 132 plants per site per year). We obtained estimates of the number of seeds per fruit from fruits collected from across each population (mean of 27 fruits per site per year). We report the estimated seed input per plot as the product of the average number of fruiting plants per plot, the average number of fruits per plant, and the average number of seeds per fruit.

Seed collection and refresher generation

We used seeds collected as part of the aforementioned long-term demographic study on *C. x. xantiana*. Seeds were collected from a haphazard sampling of plants (dozens to hundreds of plants, depending on field conditions) across the spatial extent of sites KYE and S22 in late June of years 2011 (pre-drought), 2015 (end of drought), and 2017 (after two years of more average precipitation following the drought). All seeds from a given dam constitute a maternal family. To standardize maternal environmental effects, we grew plants from each year together in a greenhouse for one generation to produce seeds for the resurrection experiment. This step is essential because 1) maternal environmental effects from each time point could confound inferences of phenotypic evolution, and 2) the differing lengths of seed storage for each generation could affect the subsequent expression of traits (e.g., longer germination times for older seeds) [10].

For each of the three generations of each population (six “cohorts”), we grew 1-5 plants from each of 20 haphazardly selected maternal families (N = 219 plants) in a fully randomized design in the greenhouse in spring 2018. Most (66%) of maternal families were represented by

two plants; representation of families varied due to unequal germination. Within each cohort, half the plants were randomly assigned as sires and each sire was mated to two unique dams (with all plants serving as dams) to produce a pedigreed population for the measurement round of the experiment. We also grew individuals from a third site, Mill Creek, during this generation prior to the resurrection experiment, but dropped this site from the subsequent experiment for feasibility; data from the Mill Creek refresher generation did not suggest evolution of any of the three measured traits (growth rate, days to flowering, SLA; Fig. S1).

Measurement generation

With the seeds produced from crosses, we grew the six offspring cohorts in a fully randomized design in the greenhouse in spring 2019 to assess phenotypic changes across generations for each population. Of the original 20 maternal families planted for each year cohort in the refresher generation, 16-20 were represented (as sire and/or dam) in this pedigreed population of offspring. We sowed six seeds for each of 22-28 dams from each parental cohort in plug trays with germination mix in the growth chamber and scored germination for 33 days; there were 83-122 germinants per cohort (Table S1). Up to four seedlings per dam (haphazardly chosen) were transplanted into individual 656 mL Deepots (Stuewe & Sons, Tangent, OR) filled with a 1:1 mix of sand and potting soil. Pots were arranged in a completely randomized design in the greenhouse on a 16/8 hr light schedule and watered as needed. Final sample sizes varied due to unequal seed availability and germination among cohorts (n = 65 - 97 individuals per year cohort for post-germination traits; Table S1).

Trait measurements

We measured five traits that have previously been shown to be related to drought escape and/or avoidance: days to germination, growth rate, days to flowering, specific leaf area (SLA), and leaf succulence. Faster germination, growth, or flowering phenology may allow plants to take advantage of the relatively mesic early growing season and complete their life cycle before the onset of drier conditions [5,11,40], though delayed germination may also be selected for in arid environments [e.g., 41]. Lower SLA and increased leaf succulence can increase water use efficiency, and evolution in these physiological traits may represent drought avoidance adaptations [40,42]. We also measured two fitness proxies: total number of flowers produced and shoot (aboveground) biomass.

Days to germination was the number of days elapsed between sowing and cotyledon emergence. Growth rate was measured as the number of leaves produced per day, measured for plants 41-43 days post-germination (i.e., growth rate was calculated as the number of leaves at measurement divided by the plant age in days at measurement). One fully-expanded leaf was collected from each plant 64-75 days post-germination; we recorded fresh and dry weight for that leaf and used ImageJ [43] to measure leaf area from a photo of the fresh leaf. SLA was calculated as $mm^2\ mg^{-1}$; leaf succulence was calculated as leaf wet weight / leaf dry weight. Days to flowering was measured as the days elapsed between seed sowing and the opening of a plant's first flower. Measuring flowering time in such a way, as opposed to the days elapsed between germination and flowering, incorporates variability in time to germination and is more directly relevant to phenological timing as measured in wild populations; both traits showed a similar pattern across years (Table S1). Total number of flowers produced was measured when all flowers had opened on a plant. Shoot biomass was collected at the end of the experiment and

plants were dried before weighing. Two pairs of traits were strongly correlated [number of flowers produced and shoot biomass (r = 0.72); SLA and leaf succulence (r = 0.81); Fig. S2]. We report on number of flowers produced and specific leaf area below, as flowers produced is a more direct proxy for plant fitness than shoot biomass, and SLA is a more commonly-reported trait than leaf succulence. We include descriptive statistics for shoot biomass and leaf succulence in Table S1.

Statistical analyses

All statistical analyses were conducted in R version 4.1.2 [44]. Data were organized and summarized using the tidyr [45] and dplyr [46] packages and plotted using ggplot2 [47]. All data and code necessary to reproduce these analyses are uploaded with the submission and will be archived at FigShare upon acceptance.

Phenotypic evolution

We used linear mixed models [package lme4 [48]] to test for differences among year cohorts in the measured phenotypic traits (days to germination, growth rate, flowering date, SLA, and flower number). We analyzed populations separately because our main interest was in differences among year cohorts, not populations, and we had limited power to detect interactions between population and year. All models took the general form of *response ~ year + transplant age + (1|sire/dam)*. Transplant age (days elapsed between germination and transplanting from germination tray to full pot) was included as a covariate to account for differing seedling ages when transplanted to pots. Sire, and dam nested within sire, were included as random effects to account for non-independence amongst related individuals. For days to germination, dam

233 average seed weight was included as a covariate and transplant age was not. Only seeds that
234 germinated were included in days to germination analyses (germination rates for cohorts ranged
235 from 0.61 to 0.75). Days to germination and SLA were log transformed prior to analysis to better
236 meet assumptions of homoscedasticity and normality of model residuals. We tested whether
237 inclusion of terms improved model fit with Wald λ^2 tests using Type II SS [Anova.mermod()
238 function in the car package [49]]. If year was a significant predictor at $\alpha = 0.05$, we followed up
239 with pairwise Tukey contrasts between year cohorts using the emmeans package [50].
240

241 ## Genetic variance components

242 We estimated quantitative genetic variance components for all traits in both populations
243 using an animal model implemented in the MCMCglmm package [51]. In short, the animal
244 model approach comprises a linear mixed effects model with an individual's breeding (i.e.,
245 additive genetic) value modeled as a random effect [52,53]. A pedigree of the population
246 provides an expectation of how breeding values should covary between individuals, and thus
247 allows for an estimate of a trait's additive genetic variance in that population. MCMCglmm uses
248 a Markov Chain Monte Carlo approach in a Bayesian framework to approximate the posterior
249 distribution of quantitative genetic variance components.
250 Days to germination and SLA were log transformed prior to analysis. Each model
251 included year and transplant age as fixed effects (except for the model of days to germination,
252 where we used dam seed weight instead of transplant age) and animal as a random effect (in
253 MCMCglmm, individuals are "animals" and this model form is used to estimate additive genetic
254 variance). We used weakly informative priors for each model: an inverse-Gamma prior for
255 residual variance (V=1, nu=1) and a parameter-expanded prior for the random effect (V=1, nu=1,

alpha.mu=0, alpha.V=1000). We ran each model for 800,000 iterations with a burn-in of 15,000 and thinning of 250. The effective sampling sizes for random effects ranged from 2,038-3,442. To test for maternal environmental effects, we also built models with *dam* as a random effect in addition to *animal* and compared these models to the model without *dam* using deviance information criterion (DIC). If including the *dam* term improved model fit (i.e., smaller DIC value), we used this model including maternal environmental effects to estimate variance components [53]. We used parameter-expanded priors because these often result in better mixing of the MCMC chains and allow more flexibility in the shape of the prior [54]. Using a weakly informative prior without parameter expansion (V=1, nu = 0.002 for both residual variance and random effects) resulted in qualitatively similar patterns in h^2 overall, though estimates of h^2 for days to germination and growth rate for the S22 population, and growth rate and SLA for the KYE population, were higher compared to results using parameter expanded priors (Fig. S3).

Following de Villemereuil [54] we obtained estimates of additive genetic variance (V_A), residual variance (V_R), and the variance explained by fixed effects (V_F) from the model, and calculated $h^2 = V_A / (V_A + V_R + V_F)$; we report the mean of the posterior distribution of h^2 as our estimate. We considered a trait to exhibit significant heritability if its 95% credible interval for h^2 did not touch zero (in MCMCglmm, variance estimates cannot go below zero). MCMCglmm diagnostic plots and posterior distribution of model parameters are included in SI: MCMCglmm.

Results

Climate and demography

Both sites experienced a period of substantially reduced precipitation in years 2012-2015 (Fig 1). For KYE, growing season precipitation was reduced by 45% compared to 2006-2011; for S22, precipitation was reduced 57%. Precipitation increased in 2016 and 2017 at both sites, with particularly high precipitation in 2017.

Overall, population densities were higher at KYE, the site nearer the range center, relative to S22, which is located near the range edge. Reduced precipitation during the 2012-2015 drought period had strong effects on population abundance at both sites (Fig. 1). At KYE, fruiting plant estimates during this drought period were 94% lower than pre-drought estimates (0.5 and 7.7 fruiting plants per m^2, respectively), though the pre-drought average was largely influenced by high abundances in 2010 and 2011 (Fig. 1). At S22, plant densities during the drought period were 83% lower than pre-drought estimates (0.4 and 2.3 fruiting plants per m^2, respectively). In the two years post-drought, fruiting plants remained few at KYE. At S22, the number of fruiting plants somewhat rebounded in 2017.

Seed input prior to the drought was roughly twice as high at KYE relative to S22 (yearly mean of1198 and 646 seeds per m^2, respectively). Seed input was especially high at KYE in the two years preceding the drought, 2010-2011. Seed input was similar at KYE and S22 during the 2012-2015 drought period (92 and 76 seeds per m^2, respectively; Fig. 1). In the two years post-drought, patterns of seed input mirrored patterns of plant density, with input remaining low at KYE but trending upward at S22 in 2017.

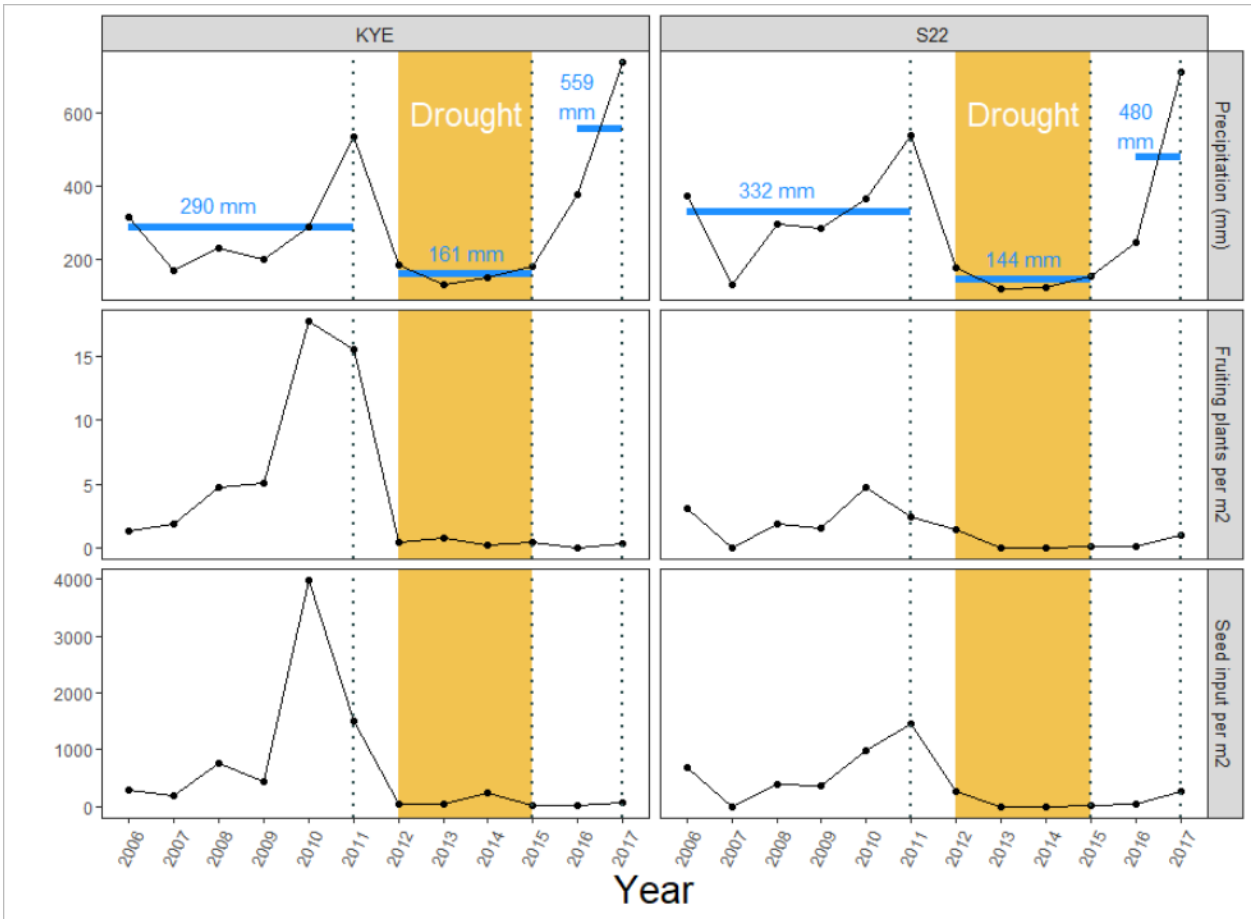

Figure 1. Environmental and demographic trends in two populations (KYE, S22) of the California annual plant *Clarkia xantiana ssp. xantiana* from 2006-2017. Precipitation represents the cumulative growing season precipitation for a given year (i.e., precipitation for year 2011 was the cumulative precipitation from November 2010 – June 2011). Blue horizontal bars in top panels mark the mean precipitation across three periods – pre-drought, during drought, and post-drought, with the drought period (2012-2015) highlighted in gold. Seeds were collected in July of each year, at the end of the growing season.

Phenotypic evolution

For S22, days to flowering differed among year cohorts ($P = 0.004$; Fig. 2; Table S2). Days to flowering decreased 1.6 days from 2011 to 2015, and another 1.2 days from 2015 to 2017. Pairwise Tukey tests identified the 2011 / 2017 contrast in days to flowering as significant (69.4 vs. 66.4 days, respectively; $P = 0.006$). Phenotypic variance in days to flowering also decreased across year cohorts, from 28.6 in 2011, to 21.3 in 2015, to 14.3 in 2017 (Table S1). There was also a trend in S22, albeit not significant, for growth rate to increase over this timeline, which may have contributed to earlier flowering time. There was no discernible evidence of phenotypic evolution of other traits for S22, nor any traits in the KYE population.

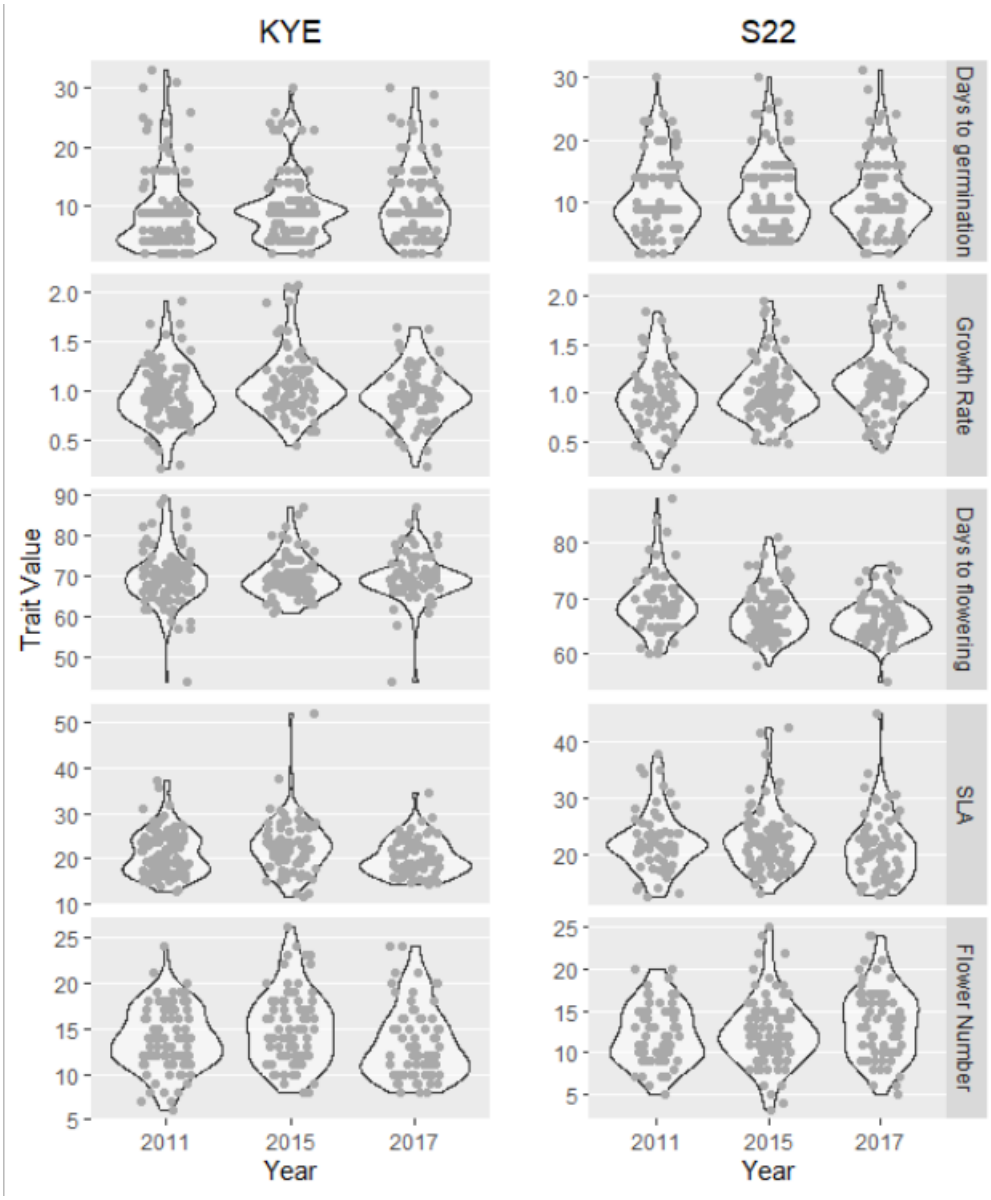

Figure 2. Measured phenotypes across the three sampling years for populations KYE and S22. Each panel shows raw data (jittered horizontally) and violin (kernel density) plots. Days to germination was measured as days elapsed between sowing and emergence; growth rate was measured as leaves produced per day; days to flowering was measured as days elapsed between sowing and opening of the first flower; SLA was calculated as $mm^2\ mg^{-1}$; flower number was measured as the total number of flowers an individual produced in its lifetime.

Additive genetic variance

There was evidence of significant additive genetic variance (V_A) for days to germination, flowering date, and flower number in both populations (Fig. 3; Table S3) The h^2 estimates for these traits ranged from 0.32 – 0.59. There was also evidence of significant V_A for growth rate in the S22 population, but not in the KYE population. We did not find evidence that V_A for SLA was significantly different from zero in either population. For KYE, growth rate and SLA models included a *dam* term (representing a maternal environmental effect; see Materials and Methods).

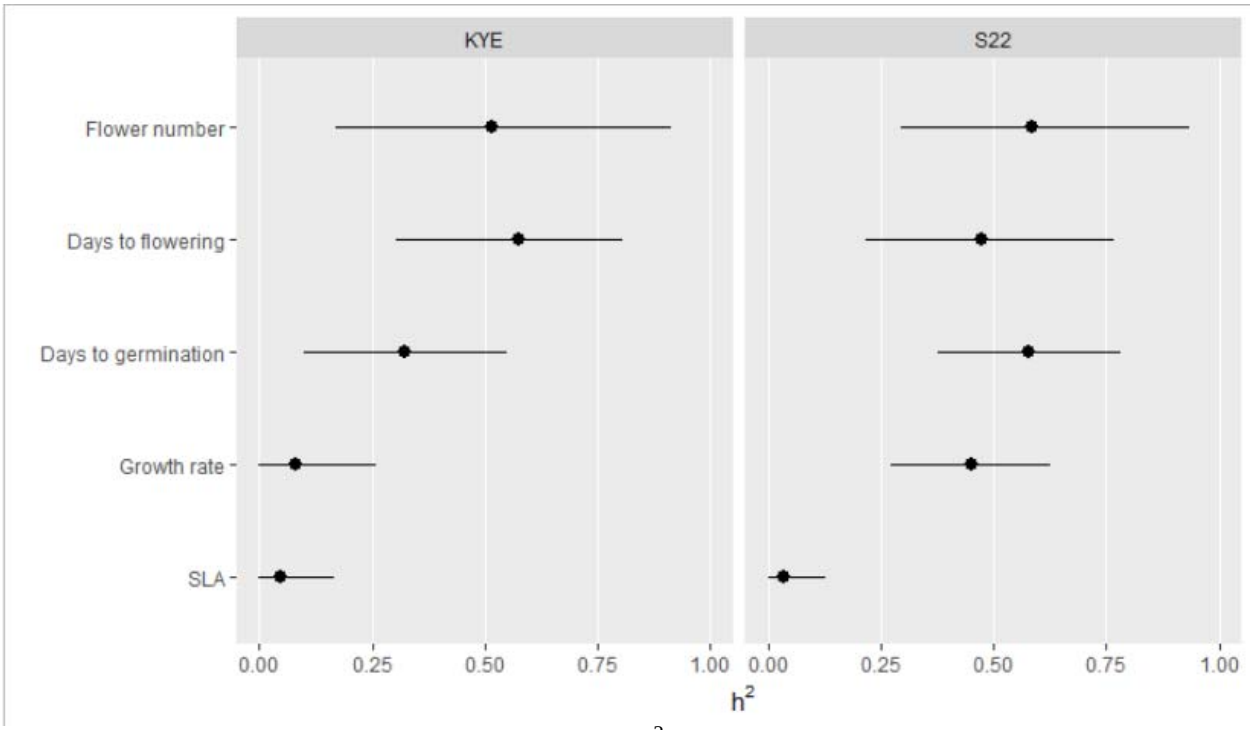

Figure 3. Estimates of narrow-sense heritability (h^2) for traits, with 95% credible intervals. Estimates of additive genetic variance for each trait are in Table S3.

Discussion

Extreme environmental perturbations offer an important opportunity to examine rapid evolution in natural populations. We leveraged a resurrection approach to test for rapid evolution in two populations of a California annual plant in response to a multi-year drought where growing season precipitation decreased by ca. 50% compared to the previous six-year period. We found evidence of evolution in flowering phenology in one of the populations (S22), such that earlier flowering had evolved by the end of the drought and continued to evolve after the drought ended. We did not find evidence of phenotypic evolution in any other measured trait at S22, and in no traits in the other population, KYE. We detected substantial additive genetic variance in three traits in both populations, including flowering phenology, suggesting that responses to selection for those traits were not constrained by limited genetic variation. Our long-term demographic observations showed that inputs to the seed bank in the two years prior to the drought were 125% higher in KYE than S22, suggesting that the probability of rapid evolutionary responses to drought may have been influenced by the magnitude of gene flow through time.

Why does rapid evolution occur in some populations but not others? One hypothesis is that a lack of quantitative genetic variation in a trait will limit its response to even intense episodes of selection. Given that there was no observable phenotypic evolution of phenology (or any other trait) in KYE, this hypothesis would predict low amounts of V_A for phenology in this population. However, we found substantial V_A and high heritability for flowering phenology in both populations. Thus, these results do not support genetic variation limiting phenological evolution in KYE (with the caveat that estimates of h^2 are environment-dependent and could differ between the greenhouse and field). Other studies have similarly found that rapid evolution

365 occurred in some but not all studied populations [12,13]. However, in most studies, estimates of
366 V_A are not available to evaluate whether genetic constraints might be important (but see Franks et
367 al. 2007). It is also worth noting that phenotypic variance in flowering phenology in S22
368 decreased 50% over the timespan of our study, consistent with a strong episode of selection.
369 Though we did not have the statistical power to precisely estimate V_A for individual year cohorts,
370 this decrease in V_P should largely reflect a decrease in V_A, because environmental variation
371 should have been relatively constant across cohorts grown contemporaneously in the greenhouse.
372 Thus, the erosion of genetic variation due to this drought could constrain future responses to
373 other episodes of selection on phenology.

374 A more likely explanation for the lack of phenological evolution in KYE is that gene
375 flow in space or time might reintroduce alleles that are maladapted to conditions during an
376 extreme event. Spatial gene flow among populations could potentially oppose allele frequency
377 changes favored by selection. However, over the course of this four-year drought, gene flow
378 among populations is unlikely to be strong enough to influence evolutionary change, given that
379 our focal populations are isolated from other populations by at least one kilometer and this
380 species lacks adaptations for long-distance dispersal. For many plant species, however, gene
381 flow also occurs through time as individuals emerge from a seed bank and mate [25,26]. For
382 annual plants, seed banks thus result in overlapping, instead of discrete generations. If older
383 generations were input to the seed bank during a period when the nature of selection differed,
384 these “stored genotypes” could prevent or slow adaptation to the contemporary environment
385 [27].

386 Though it was long assumed that *Clarkia* species had little if any seed storage [e.g., 55],
387 recent work has challenged this assumption for *C. x. xantiana* [e.g., 56]. Though the seed bank is

388 relatively short-lived when seeds are exposed near the soil surface, with ~ 90% of seeds
389 germinating or dying within three years (M. Geber et al., unpubl. data), seeds may remain viable
390 for at least 11 years when buried deeper (Moeller, unpubl. data). Thus a population's seed
391 production in the years prior to the drought likely affected its genotypic composition in later
392 years, with the magnitude of that effect presumably proportional to seed input. There were
393 substantial differences in pre-drought seed input between our two studied populations. Seed
394 input at KYE in the two years pre-drought (2010-2011) was more than twice that at S22 (two
395 year total of 5,479 vs. 2,429 seeds per m^2, respectively). Furthermore, field experiments showed
396 that KYE seeds were twice as likely as S22 seeds to remain viable but ungerminated after three
397 years on the soil surface (ca. 10% vs. 5%, respectively; M. Geber et al., unpubl. data). High rates
398 of gene flow from pre-drought generations at KYE thus may have constrained rapid evolution of
399 phenology during the drought in this population. This hypothesis is further supported by the
400 observation that at Mill Creek (MC), the third population grown during the refresher generation,
401 pre-drought seed input was even higher than at KYE (2010-2011 total of 8,101 seeds per m^2) and
402 no traits exhibited signs of phenotypic evolution (Fig. S1). The seed bank could also help explain
403 our observation that S22 continued to evolve more accelerated flowering phenology after the
404 drought ended and selection was presumably relaxed. This may simply reflect the gradual
405 turnover of the seed bank – i.e., the influence of pre-drought generations on the genotypic
406 composition of the sampled population will decrease with time.

407 The hypotheses above are not exhaustive, and it is worth exploring alternative
408 explanations for the lack of evolutionary change in phenology at KYE. The demographic cost of
409 a selective episode could lead to reduced efficacy of selection and increased influence of
410 stochastic processes. We observed large demographic declines in both populations due to the

411 drought, which likely increased the influence of genetic drift. However, the fact that both
412 precipitation and population density during the drought are similar for these two populations
413 leaves us still with the conundrum of why phenology evolved in S22 but not KYE. It is possible
414 that there was simply no or weak selection on phenology in KYE. This could be because the
415 drought was less severe at that site or because KYE was already adapted due to historical
416 selection on phenology. Though these scenarios are possible, the population's large demographic
417 decline during the drought suggests a severe environmental perturbation similar to that at S22,
418 and any "pre" adaptation, if present, was not sufficient to avoid large demographic
419 consequences. It is also possible that advanced phenology simply did not confer fitness benefits
420 during drought at KYE, though this would be contrary to both general trends in plants [40] and
421 the well documented relationship between early flowering time and adaptation to aridity in *C. x.*
422 *xantiana* and close relatives [37,41,57–60].

423 The evolution of earlier flowering observed at S22 is consistent with post-drought
424 resurrection studies in *Brassica rapa* [5,33], and likely reflects a strategy of drought escape as
425 documented in many other plant species [reviewed in 40,61,62]. Though the evolution of more
426 rapid phenology during drier periods is not ubiquitous [see 12,14], there are several reasonable
427 hypotheses for why flowering phenology is more likely to rapidly evolve relative to other traits.
428 Phenology is often influenced by alleles of major effect [e.g., 61], which may enable it to
429 respond quickly to abrupt environmental changes relative to more highly polygenic traits.
430 Experimental evolution studies have shown that adaptation to abrupt environmental change often
431 involves few mutations of large effect [61,63,64], whereas adaptation to gradual change may
432 involve more loci of smaller effect [64]. Furthermore, past work on *C. x. xantiana* has shown that
433 flowering phenology has the highest Q_{ST} of six ecologically-important traits examined across its

range [57]. This result suggests that flowering phenology is often the target of spatially-variable selection in this system and readily evolves. The evolutionary lability of flowering phenology could also be due to its direct tie to assortative mating [65] — early flowering individuals tend to mate with other early flowering individuals. Theoretical work has shown that when there is directional selection on flowering phenology, positive assortative mating via flowering time can increase genetic variation, and consequently the rate of phenotypic evolution of phenology compared to scenarios with random mating [66,67].

A population's response to environmental change will be determined by the interplay of demography, genetics, selection, and stochastic processes. Here, we have shown that flowering phenology rapidly evolved during and after a climate change-induced drought in one population. Whereas, in a second population we observed no sign of rapid evolution despite similar environmental stress, demographic decline, and the presence of substantial additive genetic variance in measured traits. In both populations, our results are consistent with the hypothesis that gene flow through time (via seed banks) either slowed or prevented rapid evolution. While the potential for seed banks to influence evolutionary processes is well known, there is little consideration of this phenomenon as it relates to adaptation in response to extreme environmental events, which are increasing in frequency with climate change. Our results suggest the somewhat unintuitive notion that, given the existence of a seed bank, higher fecundity of populations prior to an extreme event could actually serve to retard responses to selection during the event. As we seek to build more predictive models of rapid evolution in natural populations, the synthesis of demographic, life history, and quantitative genetic data will be invaluable for understanding where and when rapid evolution occurs.

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References

1. Grant PR, Grant BR, Huey RB, Johnson MTJ, Knoll AH, Schmitt J. 2017 Evolution caused by extreme events. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **372**. (doi:10.1098/rstb.2016.0146)

2. Donihue CM, Herrel A, Fabre A-C, Kamath A, Geneva AJ, Schoener TW, Kolbe JJ, Losos JB. 2018 Hurricane-induced selection on the morphology of an island lizard. *Nature* **560**, 88–91.

3. Campbell-Staton SC, Cheviron ZA, Rochette N, Catchen J, Losos JB, Edwards SV. 2017 Winter storms drive rapid phenotypic, regulatory, and genomic shifts in the green anole lizard. *Science* **357**, 495–498.

4. Grant PR, Grant BR. 2002 Unpredictable evolution in a 30-year study of Darwin's finches. *Science* **296**, 707–711.

5. Franks SJ, Sim S, Weis AE. 2007 Rapid evolution of flowering time by an annual plant in response to a climate fluctuation. *Proc. Natl. Acad. Sci. U. S. A.* **104**, 1278–1282.

6. Shaw RG, Etterson JR. 2012 Rapid climate change and the rate of adaptation: insight from experimental quantitative genetics. *New Phytol.* **195**, 752–765.

7. Peschel AR, Boehm EL, Shaw RG. 2021 Estimating the capacity of Chamaecrista fasciculata for adaptation to change in precipitation. *Evolution* **75**, 73–85.

8. Geber MA, Dawson TE. 1993 Evolutionary responses of plants to global change. In *Biotic interactions and global change* (ed Kareiva, PM and Kingsolver, JG and Huey, RB), pp. 179–197. Sunderland, Mass.: Sinauer.

9. Etterson JR, Franks SJ, Mazer SJ, Shaw RG, Gorden NLS, Schneider HE, Weber JJ, Winkler KJ, Weis AE. 2016 Project Baseline: An unprecedented resource to study plant evolution across space and time. *Am. J. Bot.* **103**, 164–173.

10. Franks SJ, Hamann E, Weis AE. 2018 Using the resurrection approach to understand contemporary evolution in changing environments. *Evol. Appl.* **11**, 17–28.

11. Dickman EE, Pennington LK, Franks SJ, Sexton JP. 2019 Evidence for adaptive responses to historic drought across a native plant species range. *Evol. Appl.* (doi:10.1111/eva.12803)

12. Anstett DN, Branch HA, Angert AL. 2021 Regional differences in rapid evolution during severe drought. *Evol. Lett.* (doi:10.1002/evl3.218)

13. Wooliver R, Tittes SB, Sheth SN. 2020 A resurrection study reveals limited evolution of thermal performance in response to recent climate change across the geographic range of the scarlet monkeyflower. *Evolution* (doi:10.1111/evo.14041)

14. Vtipil EE, Sheth SN. 2020 A resurrection study reveals limited evolution of phenology in

response to recent climate change across the geographic range of the scarlet monkeyflower. *Ecol. Evol.* **10**, 14165–14177.

15. Haldane JBS. 1957 The cost of natural selection. *J. Genet.* **55**, 511.

16. Gomulkiewicz R, Houle D. 2009 Demographic and genetic constraints on evolution. *Am. Nat.* **174**, E218-29.

17. Bell G. 2017 Evolutionary Rescue. *Annu. Rev. Ecol. Evol. Syst.* **48**, 605–627.

18. Lynch M, Lande R. 1993 Evolution and extinction in response to environmental change. In *Biotic interactions and global change* (ed Kareiva, PM and Kingsolver, JG and Huey, RB), pp. 234–250. Sunderland, Mass.: Sinauer.

19. Hufbauer RA, Szűcs M, Kasyon E, Youngberg C, Koontz MJ, Richards C, Tuff T, Melbourne BA. 2015 Three types of rescue can avert extinction in a changing environment. *Proc. Natl. Acad. Sci. U. S. A.* **112**, 10557–10562.

20. Lindsey HA, Gallie J, Taylor S, Kerr B. 2013 Evolutionary rescue from extinction is contingent on a lower rate of environmental change. *Nature* **494**, 463–467.

21. Holt RD. 2004 Temporal variation can facilitate niche evolution in harsh sink environments. *Am. Nat.* **164**, 187–200.

22. Evans MEK, Dennehy JJ. 2005 Germ banking: bet-hedging and variable release from egg and seed dormancy. *Q. Rev. Biol.* **80**, 431–451.

23. Kalisz S, McPeek MA. 1992 Demography of an age-structured annual: Resampled projection matrices, elasticity analyses, and seed bank effects. *Ecology* **73**, 1082–1093.

24. Evans MEK, Ferrière R, Kane MJ, Venable DL. 2007 Bet hedging via seed banking in desert evening primroses (Oenothera, Onagraceae): demographic evidence from natural populations. *Am. Nat.* **169**, 184–194.

25. Gottlieb LD. 1974 Genetic Stability in a Peripheral Isolate of Stephanomeria exigua ssp. coronaria That Fluctuates in Population Size. *Genetics* **76**, 551–556.

26. Epling C, Lewis H, Ball FM. 1960 The Breeding Group and Seed Storage: A Study in Population Dynamics. *Evolution* **14**, 238–255.

27. Templeton AR, Levin DA. 1979 Evolutionary Consequences of Seed Pools. *Am. Nat.* **114**, 232–249.

28. Hairston NG Jr, De Stasio BT Jr. 1988 Rate of evolution slowed by a dormant propagule pool. *Nature* **336**, 239–242.

29. Levin DA. 1990 The Seed Bank as a Source of Genetic Novelty in Plants. *Am. Nat.* **135**, 563–572.

30. Blows MW, Hoffmann AA. 2005 A reassessment of genetic limits to evolutionary change. *Ecology* **86**, 1371–1384.

31. Barton N, Partridge L. 2000 Limits to natural selection. *Bioessays* **22**, 1075–1084.

32. Falconer DS. 1996 *Introduction to quantitative genetics*. 4th edn. Essex, England: Pearson Education.

33. Hamann E, Weis AE, Franks SJ. 2018 Two decades of evolutionary changes in Brassica rapa in response to fluctuations in precipitation and severe drought. *Evolution* (doi:10.1111/evo.13631)

34. Cook BI, Ault TR, Smerdon JE. 2015 Unprecedented 21st century drought risk in the American Southwest and Central Plains. *Sci Adv* **1**, e1400082.

35. Williams AP, Cook BI, Smerdon JE. 2022 Rapid intensification of the emerging southwestern North American megadrought in 2020–2021. *Nat. Clim. Chang.* **12**, 232–234.

36. Williams AP, Cook ER, Smerdon JE, Cook BI, Abatzoglou JT, Bolles K, Baek SH, Badger AM, Livneh B. 2020 Large contribution from anthropogenic warming to an emerging North American megadrought. *Science* **368**, 314–318.

37. Eckhart VM, Geber MA. 1999 Character variation and geographic range in Clarkia xantiana (Onagraceae): breeding system and phenology distinguish two common subspecies. *Madrono*

38. Webb RW. 1936 Kern Canyon Fault, Southern Sierra Nevada. *J. Geol.* **44**, 631–638.

39. Brossy CC *et al.* 2012 Map of the late Quaternary active Kern Canyon and Breckenridge faults, southern Sierra Nevada, California. *Geosphere* **8**, 581–591.

40. Kooyers NJ. 2015 The evolution of drought escape and avoidance in natural herbaceous populations. *Plant Sci.* **234**, 155–162.

41. Burnette TE, Eckhart VM. 2021 Evolutionary divergence of potential drought adaptations between two subspecies of an annual plant: Are trait combinations facilitated, independent, or constrained? *Am. J. Bot.* (doi:10.1002/ajb2.1607)

42. Ogburn RM, Edwards EJ. 2012 Quantifying succulence: a rapid, physiologically meaningful metric of plant water storage. *Plant Cell Environ.* **35**, 1533–1542.

43. Schneider CA, Rasband WS, Eliceiri KW. 2012 NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* **9**, 671–675.

44. R Foundation For Statistical Computing. 2021 *R: A language and environmnet for statistical computing*.

45. Wickham H, Girlich M. 2022 tidyr: Tidy Messy Data. See https://tidyr.tidyverse.org,

593 https://github.com/tidyverse/tidyr.

594 46. Wickham H, François R, Henry L, Müller K. 2022 dplyr: A Grammar of Data
595 Manipulation. See https://dplyr.tidyverse.org, https://github.com/tidyverse/dplyr.

596 47. Wickham H. 2009 *ggplot2: Elegant Graphics for Data Analysis*. New York: Springer-
597 Verlag.

598 48. Bates D, Mächler M, Bolker B, Walker S. 2015 Fitting Linear Mixed-Effects Models Using
599 lme4. *Journal of Statistical Software*. **67**, 1–48. (doi:10.18637/jss.v067.i01)

600 49. Fox J, Weisberg S. 2011 *An R Companion to Applied Regression*. Thousand Oaks, CA:
601 Sage.

602 50. Lenth R. 2018 Emmeans: Estimated marginal means, aka least-squares means. *R Package*
603 *Version* **1**.

604 51. Hadfield JD. 2010 MCMC Methods for Multi-Response Generalized Linear Mixed Models:
605 The MCMCglmm R Package. *J. Stat. Softw.* **33**, 1–22.

606 52. Kruuk LEB. 2004 Estimating genetic parameters in natural populations using the 'animal
607 model.' *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **359**, 873–890.

608 53. Wilson AJ, Réale D, Clements MN, Morrissey MM, Postma E, Walling CA, Kruuk LEB,
609 Nussey DH. 2010 An ecologist's guide to the animal model. *J. Anim. Ecol.* **79**, 13–26.

610 54. de Villemereuil P. 2018 Quantitative genetic methods depending on the nature of the
611 phenotypic trait. *Ann. N. Y. Acad. Sci.* **1422**, 29–47.

612 55. Lewis H. 1962 Catastrophic Selection as a Factor in Speciation. *Evolution* **16**, 257–271.

613 56. Eckhart VM, Geber MA, Morris WF, Fabio ES, Tiffin P, Moeller DA. 2011 The geography
614 of demography: long-term demographic studies and species distribution models reveal a
615 species border limited by adaptation. *Am. Nat.* **178**, S26-43.

616 57. Gould B, Moeller DA, Eckhart VM, Tiffin P, Fabio E, Geber MA. 2014 Local adaptation
617 and range boundary formation in response to complex environmental gradients across the
618 geographical range of Clarkia xantiana ssp . xantiana. *J. Ecol.* **102**, 95–107.

619 58. Eckhart VM, Geber MA, McGuire CM. 2004 Experimental studies of adaptation in Clarkia
620 xantiana. I. Sources of trait variation across a subspecies border. *Evolution* **58**, 59–70.

621 59. Anderson JT, Eckhart VM, Geber MA. 2015 Experimental studies of adaptation in Clarkia
622 xantiana. III. Phenotypic selection across a subspecies border. *Evolution* **69**, 2249–2261.

623 60. Mazer SJ, Dudley LS, Hove AA, Emms SK, Verhoeven AS. 2010 Physiological
624 Performance in Clarkia Sister Taxa with Contrasting Mating Systems: Do Early-Flowering
625 Autogamous Taxa Avoid Water Stress Relative to Their Pollinator-Dependent

626 Counterparts? *Int. J. Plant Sci.* **171**, 1029–1047.

627 61. Fulgione A *et al.* 2022 Parallel reduction in flowering time from de novo mutations enable
628 evolutionary rescue in colonizing lineages. *Nat. Commun.* **13**, 1461.

629 62. Metz J, Lampei C, Bäumler L, Bocherens H, Dittberner H, Henneberg L, de Meaux J,
630 Tielbörger K. 2020 Rapid adaptive evolution to drought in a subset of plant traits in a large-
631 scale climate change experiment. *Ecol. Lett.* (doi:10.1111/ele.13596)

632 63. Lenski RE, Travisano M. 1994 Dynamics of adaptation and diversification: a 10,000-
633 generation experiment with bacterial populations. *Proc. Natl. Acad. Sci. U. S. A.* **91**, 6808–
634 6814.

635 64. Collins S, de Meaux J. 2009 Adaptation to different rates of environmental change in
636 Chlamydomonas. *Evolution* **63**, 2952–2965.

637 65. Franks SJ, Weis AE. 2009 Climate change alters reproductive isolation and potential gene
638 flow in an annual plant. *Evol. Appl.* **2**, 481–488.

639 66. Fox GA. 2003 Assortative mating and plant phenology: evolutionary and practical
640 consequences. *Evol. Ecol. Res.* **5**, 1–18.

641 67. Godineau C, Ronce O, Devaux C. 2021 Assortative mating can help adaptation of flowering
642 time to a changing climate: Insights from a polygenic model. *J. Evol. Biol.*
643 (doi:10.1111/jeb.13786)