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Authors for correspondence:
John W. Benning
e-mail: jwbenning@gmail.com
David A. Moeller
e-mail: moeller@umn.edu

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Rapid evolution during climate change: demographic and genetic constraints on adaptation to severe drought

John W. Benning[1,2], Alexai Faulkner[2] and David A. Moeller[2]

[1]Department of Botany, University of Wyoming, Laramie, WY 82071, USA
[2]Department of Plant and Microbial Biology, University of Minnesota, Saint Paul, MN 55455, USA

JWB, 0000-0002-2583-2503; DAM, 0000-0002-6202-9912

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? We combined long-term demographic and environmental data, estimates of quantitative genetic variance components, a resurrection experiment and individual-based evolutionary simulations to gain mechanistic insights into contrasting evolutionary responses to a severe multi-year drought. We examined five traits in two populations of a native California plant, *Clarkia xantiana*, at three time points over 7 years. Earlier flowering phenology evolved in only one of the two populations, though both populations experienced similar drought severity and demographic declines and were estimated to have considerable additive genetic variance for flowering phenology. Pairing demographic and experimental data with evolutionary simulations suggested that while seed banks in both populations likely constrained evolutionary responses, a stronger seed bank in the non-evolving population resulted in evolutionary stasis. Gene flow through time via germ banks may be an important, underappreciated control on rapid evolution in response to extreme environmental perturbations.

1. Introduction

Extreme environmental perturbations offer excellent opportunities to examine evolution in natural populations on ecological time scales [1]. Rapid evolution in response to strong episodes of environmental change has been documented in many taxa, including 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 nearby populations.

Resurrection experiments are increasingly used to directly examine evolutionary change resulting from 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 [12–14],

and even extreme climatic anomalies can result in no discernible phenotypic evolution of putatively important traits [15]. What underlies this variation in populations' evolutionary responses to extreme events? While the resurrection approach is a powerful method to determine if phenotypic evolution has occurred, it does not typically provide insight into the demographic and genetic controls on evolutionary change.

A more comprehensive understanding of the causes and consequences of rapid evolution can be gained by coupling evolutionary analyses with long-term demographic and quantitative genetic investigations. Sharp declines in population size due to environmental perturbations will reduce the efficacy of selection and increase the influence of stochastic processes like genetic drift [16,17]. Such demographic, environmental, or genetic stochasticity may stymie adaptation, potentially causing population extinction before 'evolutionary rescue' can occur [18–20]. Models and experiments have further shown that evolutionary rescue is less probable when the environment changes suddenly compared to when the change is gradual [21]. As such, the rate and severity of demographic decline can provide insight into instances where some populations fail to evolve in response to an extreme event that drove rapid evolution in other conspecific populations.

For organisms with dormancy, outcomes of selection can also be affected by the presence of a germ bank (seed or egg bank). Populations that often experience significant environmental fluctuations may evolve mechanisms that confer dormancy as a bet-hedging strategy [22]. For annual plants, seed banks thus result in overlapping, instead of discrete generations. While germ banks may buffer populations from extinction [23,24], they may also constrain evolutionary responses to environmental change due to gene flow among generations [25,26]. This 'temporal migration' can slow the rate of adaptive evolution [27,28]. Alleles favoured prior to a selective event will be 'reintroduced' to the population from the germ bank during and after the event. If those same alleles are selected against during the event, this gene flow can retard adaptation (but see [29,30]). Moreover, the environmental and demographic history of populations just prior to an extreme event likely affects the magnitude of gene flow from the past. For example, high fecundity of generations immediately prior to an extreme environmental event would increase input to the seed bank. Therefore, historical demographic data can also provide insight into the influence of germ banks on population responses to selection.

Strong environmental perturbations typically exert selection on traits, but the same perturbation may still result in selection regimes that vary among sites [5]. The response to any selection pressure will depend upon the presence of sufficient additive genetic variance for traits underlying fitness variation [31,32]. A population's response to selection will be directly proportional to a given trait's narrow-sense heritability, $h^2 = V_A/V_p$ [33]. In the context of resurrection experiments, measuring the additive genetic variance of ecologically important traits may help us understand why traits do or do not evolve in different populations exposed to similar selective regimes. While tests of rapid evolution are increasingly common, few studies have simultaneously examined quantitative genetic variation and its potential role in facilitating or hindering adaptation (but see [5]).

Furthermore, resurrection experiments usually examine cohorts from before and at the end of an extreme event (but see [34]). A key question is how long evolutionary changes that occur in response to these events persist beyond them. Including later cohorts in a resurrection experiment allows us to assess the temporal stability of evolutionary responses and the extent to which dormancy might delay responses to selection. When paired with associated demographic data from natural populations, such a design gives inference to how rapid evolution (or its absence) influences a populations' longer term phenotypic and demographic trajectory.

The Southwest of the United States is in the midst of the most severe multi-decadal drought (i.e. megadrought) in recorded history (at least since 800 CE) [35,36]. Recent analyses have shown that anthropogenic climate change has been a key driver of this drought and accounts for an estimated 42% of the soil moisture anomaly [36,37]. We examined evolutionary responses to the most severe episode of this climate anomaly, which began in the latter half of 2011 and ended in late 2015 in our study area. Our work focused on a well-studied annual plant, *Clarkia xantiana* ssp. *xantiana*, which is endemic to Southern California, USA. We used a resurrection experiment to test whether traits likely to mediate drought adaptation exhibited rapid evolution and whether genetic or demographic constraints modulated responses.

We monitored environmental and demographic variation over 12 years (2006–2017), spanning a period from 6 years prior to drought until 2 years after the drought ended. Those data provided insight into the severity and rate of environmental and demographic change, as well as the magnitude of input to the seed bank in years just prior to the drought episode. To test for rapid evolution in response to drought, we used a resurrection experiment with pedigreed individuals from three time points: prior to the prolonged drought (2011), at the end of the drought (2015) and 2 years later, after more average precipitation resumed (2017). This latter sample allowed us to examine whether any evolutionary responses persisted, increased or evolved back toward the original population mean. We measured a suite of traits that prior studies have shown to confer drought escape and/or avoidance, and estimated additive genetic variance for each of these traits in each population. Lastly, we leveraged these demographic and experimental data in the construction and analysis of an individual-based evolutionary simulation model to test the hypothesis that a seed bank influenced the evolutionary responses of these populations to extreme drought.

2. Material and methods

(a) 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 [38]. In this Mediterranean climate, the bulk of precipitation occurs during winter and early spring [39]. 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's eastern geographical 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, an approximately 150 km fault that parallels the Kern River through the Southern Sierra [40,41]. Site identifiers are 57x (KYE) and 22x (S22), for consistency with previous and future *C. x. xantiana* studies. Though it was long assumed that *Clarkia* species had little if any seed storage (e.g. [42]), recent work has shown that *C. x. xantiana* populations can harbour substantial seed banks [39,43].

(b) 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. Demographic data were collected as part of a long-term study of 36 populations across the range of *C. x. xantiana* in years 2006–2017 (detailed in electronic supplementary material, A). Using a population projection model approach, we combined estimates of seed input in each year with estimates of seed bank vital rates (survival and germination) for KYE and S22 from [43] to estimate the age distribution of germinants in each population in each year (assuming a maximum seed age of 3 years). Siegmund *et al*. [43] estimated these vital rates for the two populations in our study as seed survival rate (KYE = 0.66; S22 = 0.61) and germination rate (KYE = 0.13; S22 = 0.27). The multi-year field experiments used to estimate these vital rates are detailed in [43] and [39].

(c) 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 2 years of more average precipitation following the drought). All seeds from a given dam constitute a maternal family. Because *C. x. xantiana* is a highly outcrossing species [44] with multiple paternity (D.A.M. 2006–2017, unpublished data), each maternal family sampled from the field was a collection of full and half-sibs. Seeds were stored at room temperature in plastic boxes containing silica desiccant, with maternal families each in their own coin envelope. To standardize maternal environmental effects, we grew plants from each year together in a greenhouse for one 'refresher' generation to produce seeds for the resurrection experiment [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 total) 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, specific leaf area (SLA); electronic supplementary material, figure S4).

(d) 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 refresher cohort in plug trays with germination mix in the growth chamber and scored germination for 33 days. There were 83–122 germinants per cohort (electronic supplementary material, table S2); germination rates ranged from 0.61 to 0.75 (electronic supplementary material, 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 h 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; electronic supplementary material, table S2).

(e) 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, 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 [5,11,45], though delayed germination may also be selected for in arid environments (e.g. [46]). Lower SLA and increased leaf succulence can increase water use efficiency, and evolution in these physiological traits may represent drought avoidance adaptations [45,47]. 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 [48] 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 were 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 (electronic supplementary material, table S2). 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 ($r = 0.81$); electronic supplementary material, figure S5]. We report on number of flowers produced and SLA 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 the electronic supplementary material, table S2. Our analyses of invisible fraction bias (*sensu* [49]) indicated that any bias in estimation of trait means due to unequal seed survival among genotypes was likely minimal (electronic supplementary material, B).

(f) Statistical analyses for resurrection experiment

All statistical analyses were conducted in R v. 4.1.2 [50]. Data were organized and summarized using the tidyr [51] and dplyr [52] packages and plotted using ggplot2 [53].

(i) Phenotypic evolution

We used linear mixed models (package lme4 [54]) to test for differences among year cohorts in the measured phenotypic traits (days to germination, growth rate, flowering date, SLA and flower number). We analysed 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 $response \sim year + transplant\ \ age + (1 \mid sire/dam)$. 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 among related individuals. For days to germination, dam average seed weight was included as a covariate and transplant age was not. Only seeds that germinated were included in days to germination analyses to avoid any confounding of dormancy with seed viability. Days to germination and SLA were log transformed prior to analysis to better meet assumptions of homoscedasticity and normality of model residuals. We tested whether inclusion of terms improved model fit with Wald λ^2 tests using Type II SS (Anova.mermod() function in the car package [55]). If year was a significant predictor at $\alpha = 0.05$, we followed up with pairwise Tukey contrasts between year cohorts using the emmeans package [56]. We computed estimated marginal means for each year cohort using emmeans.

(ii) Genetic variance components

We estimated quantitative genetic variance components for all traits in both populations using an animal model implemented in the MCMCglmm package [57]. In short, the animal model approach comprises a linear mixed effects model with an individual's breeding (i.e. additive genetic) value modelled as a random effect [58,59]. A pedigree of the population provides an expectation of how breeding values should covary between individuals and thus allows for an estimate of a trait's additive genetic variance in that population. MCMCglmm uses a Markov chain Monte Carlo approach in a Bayesian framework to approximate the posterior distribution of quantitative genetic variance components (analyses detailed in electronic supplementary material, C). Following [60], 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 0.001 (in MCMCglmm, variance estimates must be greater than zero). MCMCglmm diagnostic plots and posterior distribution of model parameters are included in electronic supplementary material: MCMCglmm.

(g) Testing seed bank effects on evolutionary responses

We used an individual-based, genomically explicit evolutionary simulation model, paired with approximate Bayesian computation (ABC), to test two key hypotheses: (1) seed banks influenced the degree of evolutionary change in our populations, and (2) the selective environment returned to its pre-drought state after drought. Using the SLiM modelling framework [61], we built models tracking the demography and evolution of a population of diploid, hermaphroditic individuals that experience an environmental perturbation (e.g. drought). For each of our two populations, we asked which of four candidate models (table 1) best fit our observed evolutionary and demographic data. Models varied in whether or not they had seed banks, and whether or not the selective environment returned to historical conditions once the perturbation ended.

(i) Model structure

We focused this investigation on one trait, flowering phenology, which displayed contrasting evolutionary responses in our two populations (Results). In our models, individual female fitness (here, the probability of surviving to set seed) was determined by the deviation of an individual's phenotype (here, flowering phenology, which is under stabilizing selection and controlled by multiple QTL) from the optimal phenotype; the optimal phenotype is assumed to change based on current environmental conditions. We focused on female fitness (i.e. siring success is not affected by an individual's phenotype) because earlier work in *C. x. xantiana* has demonstrated strong selection on flowering phenology via female fitness in arid conditions [62] but we have no such tests of selection via male fitness. After a long burn-in period with a relatively stable environment (and thus phenotypic optimum), the model environment and population size were explicitly tied to our observed 12 year dataset. There was an environmental perturbation (e.g. drought) in year 7 that changes the phenotypic optimum, and modelled population sizes in each year were proportional to the observed census sizes of natural populations in each year. Full model details are in the electronic supplementary material, D.

We ran 20 000 simulations of each model (table 1) for model comparison via ABC (see *Overview of ABC* in electronic supplementary material, D). Models were run separately for each population because model demography was explicitly tied to observed census sizes in each population (see above). In the models without seed banks (Models A and B), seed survival was set to 0.0 and seed germination was set to 1.0, while the change in optimum was allowed to vary between −20.0 and 0.0 (table 1) across the 20 000 simulations. For models with seed banks (Models C and D), seed survival and germination rates were also allowed to vary between 0.05 and 0.75, which encompasses the range of values for these parameters estimated across 20 *C. x. xantiana* populations in [43] (range of posterior mode for germination = [0.11, 0.27]; seed survival [0.45, 0.72]). In models A and C, the phenotypic optimum returned to its pre-drought level after the drought period ended, but in models B and D, the phenotypic optimum did not change in the post-drought period.

(ii) Model comparison and parameter estimation

Posterior probabilities of our four candidate models were calculated via ABC (abc package [63]) based on the distance between observed and simulated summary statistics (see *Overview of ABC* in the electronic supplementary material, D). ABC is a statistical method used to estimate model parameters and choose among a set of candidate models when likelihood calculations are intractable (reviewed in [64]). The small number of simulations that went extinct (KYE: 1.3% of simulations; S22: 2.3%) were discarded, as summary statistics could not be calculated. Our three summary statistics were (i) the difference in mean phenotype between 2011 and 2015 (which we measured in the resurrection experiment), (ii) the difference in mean phenotype between 2015 and 2017 (measured in the resurrection experiment) and (iii) the ratio of survival rates between the drought (averaging 2012–2015) and pre-drought (averaging 2006–2011) periods (measured in the field). The observed values of the first two summary statistics were calculated using the estimated marginal means from the linear mixed effects models described above. For the observed value of the third summary statistic, because survival in the model is only affected by the focal trait but in the field is likely affected by multiple traits, we assumed that one-third of the decrease in survival during drought in natural populations was due to the focal trait (flowering phenology). Assuming instead that one-quarter or one-half of the decrease in survival was due to the focal

Table 1. Description and parameter prior distributions for the four candidate models. Values for varying parameters were drawn from uniform distributions (e.g. U[x, x]).

	description	parameters		
		seed survival rate	seed germination rate	change in optimum
Model A	no seed bank, with the trait optimum changing at the beginning of the drought, then returning to the historical optimum after drought	0.0	1.0	U[−20.0, 0.0]
Model B	no seed bank, with the trait optimum changing at the beginning of the drought and staying the same post-drought	0.0	1.0	U[−20.0, 0.0]
Model C	with seed bank, with the trait optimum changing at the beginning of the drought, then returning to the historical optimum after drought	U[0.05, 0.75]	U[0.05, 0.75]	U[−20.0, 0.0]
Model D	with seed bank, with the trait optimum changing at the beginning of the drought and staying the same post-drought	U[0.05, 0.75]	U[0.05, 0.75]	U[−20.0, 0.0]

trait produced qualitatively and quantitatively similar seed bank parameter estimates (electronic supplementary material, figure S6). We used the rejection method with a tolerance rate of 0.01 to calculate posterior probabilities for each model and used the resulting Bayes factors to select the best model for each population. We ran an additional 20 000 simulations of the selected model for each population to aid in parameter estimation. Parameter posterior distributions were estimated using localized linear regression and a tolerance rate of 0.01. Graphical posterior predictive checks were performed using 500 simulations run with parameter values drawn from these estimated parameter posterior distributions.

(iii) What underlies contrasting evolutionary responses between populations?

To estimate the individual effects of aboveground demography (seed input), seed bank vital rates (survival and germination) and selection environment (change in phenotypic optimum during drought) on the evolutionary stasis of KYE relative to S22 (Results), we compared the posterior predictive checks described above to additional batches of simulations (500 simulations each) as follows:

1. Aboveground demographic effect: seed bank (survival and germination) and optimum change parameters estimated for KYE, but with demographic history of S22.
2. Seed bank effect: optimum change estimated for KYE, with KYE demographic history, but with seed bank parameters estimated for S22.
3. Selection environment effect: seed bank parameters estimated for KYE, with KYE demographic history, but with optimum change parameter estimated for S22.

3. Results

(a) Climate and demography

Both sites experienced a period of substantially reduced precipitation in years 2012–2015 (figure 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, KYE, which is nearer the range centre, had higher density and total population size than S22, which is located near the range edge (average density across 12 years: 4.03 and 1.36 fruiting plants per m^2, respectively; average population size: 282 704 and 9166 fruiting plants). Reduced precipitation during the 2012–2015 drought period had strong demographic effects at both sites (figure 1). At both sites, survival rates of plants in permanent plots decreased over 85 per cent during drought relative to pre-drought averages (KYE survival rates: pre-drought = 0.34, drought = 0.05; S22 pre-drought = 0.35, drought = 0.02). 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 (figure 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 2 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 of 1198 and 646 seeds per m^2, respectively). Seed input was especially high at KYE in the 2 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; figure 1). In the 2 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. The decreased germination and increased seed survival rates of KYE (estimated in [43]) along with high seed input resulted in KYE having a more substantial seed bank than S22, with estimated mean germinant age *ca*. 20% higher than S22 (1.27 versus 1.04 mean germinant age, respectively; figure 1). In both populations, low seed input during drought resulted in increased germinant age during that period due to older seeds comprising a larger proportion of the seed bank (figure 1; electronic supplementary material, figure S9).

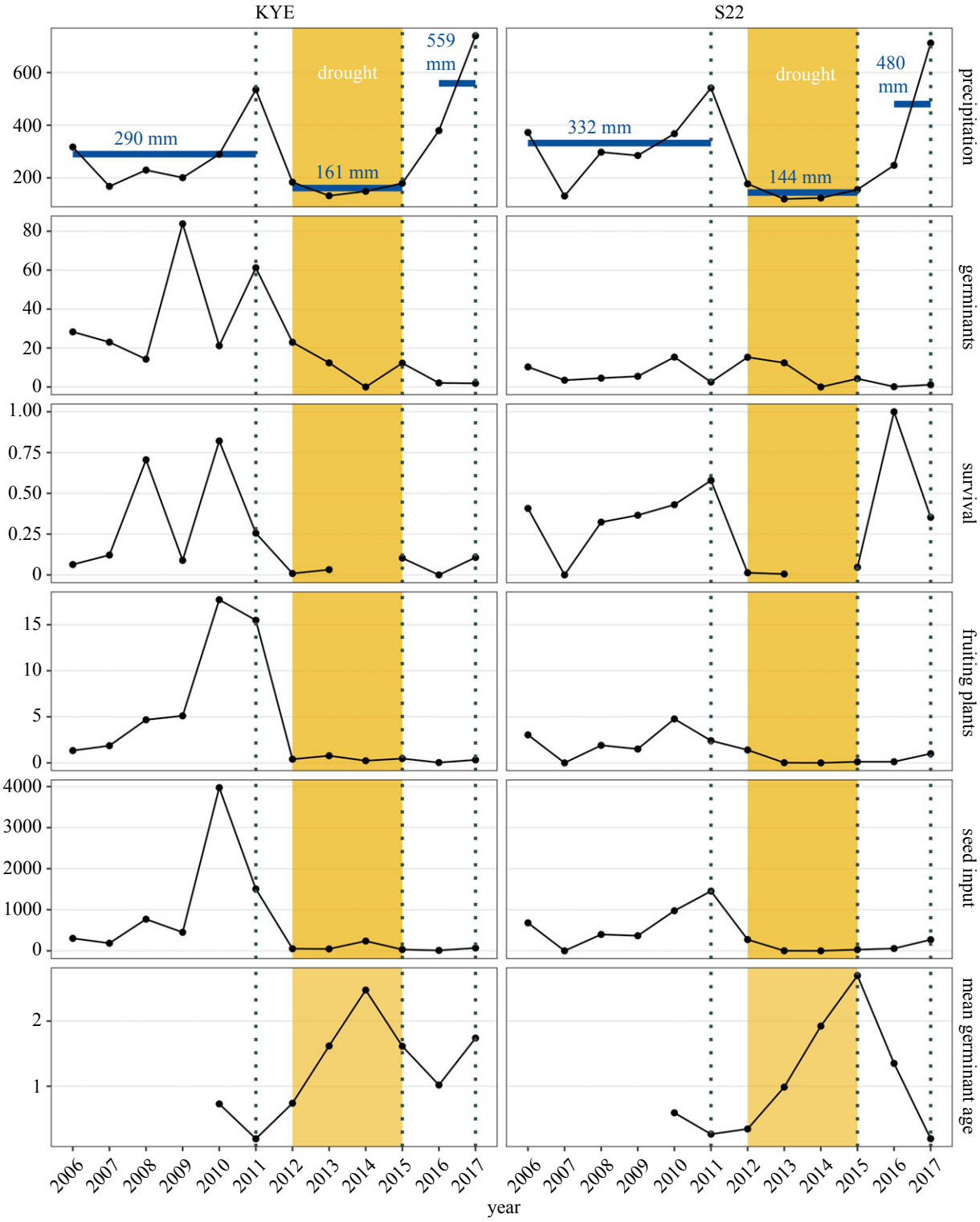

Figure 1. Environmental and demographic trends in two populations (KYE, S22) of the California annual plant *Clarkia xantiana ssp. xantiana* from 2006 to 2017. Dotted vertical lines mark cohorts collected for the resurrection experiment. Precipitation represents the cumulative growing season precipitation, in mm, for a given year (i.e. precipitation for year 2011 was the cumulative precipitation from November 2010 to 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. Germinants, fruiting plants and seed input are per m^2. Survival rate is based on censuses of germinants and fruiting plants in permanent plots in each population; no survival data are presented for 2014 because zero germinants were recorded in both populations. Mean germinant age in each year is estimated based on seed input in years prior and seed bank vital rates from [43].

(b) Phenotypic evolution

For S22, days to flowering differed among year cohorts ($p = 0.005$; figure 2; electronic supplementary material, table S3). Days to flowering decreased 1.6 days from 2011 to 2015, and another 1.4 days from 2015 to 2017. Pairwise Tukey tests identified the 2011 / 2017 contrast in days to flowering as significant (69.4 versus 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 (electronic supplementary material, table S2). There was also a trend in S22, albeit not significant, for growth rate to increase over this timeline, which may have

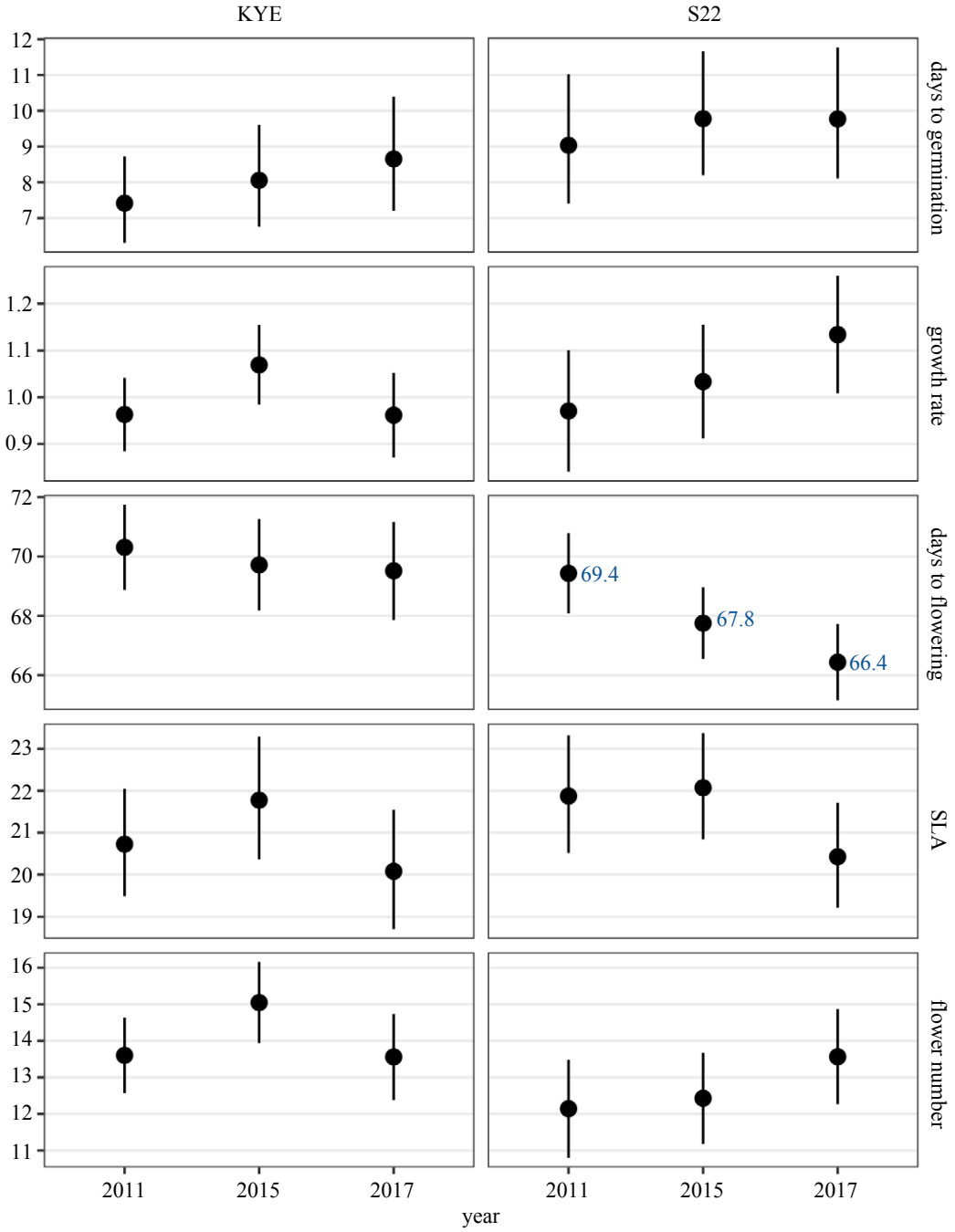

Figure 2. Measured phenotypes across the three sampling years for populations KYE and S22. Each panel shows estimated marginal means with 95% CI's estimated from the model *trait*~*year* + *transplant age* + *(1|sire/dam)*. Days to germination was measured as days elapsed between sowing and emergence; 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. Raw data are shown in the electronic supplementary material, figure S7.

contributed to earlier flowering time. There was no statistically significant evidence of phenotypic evolution of other traits for S22, nor any traits in the KYE population.

(c) 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 (figure 3; electronic supplementary material, tables S4 and S5) 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.

(d) Testing seed bank effects on evolutionary responses

For both populations, the model with the highest posterior probability was Model D (population with a seed bank, with post-drought phenotypic optimum equal to optimum during drought) (electronic supplementary material, table S6). Posterior probability of Model D was much higher than all other models for KYE (Bayes factors all greater than 12; electronic supplementary material, table S6). For S22, the contrast of Model D with the second best model, Model B (population *without* a seed bank, with post-drought

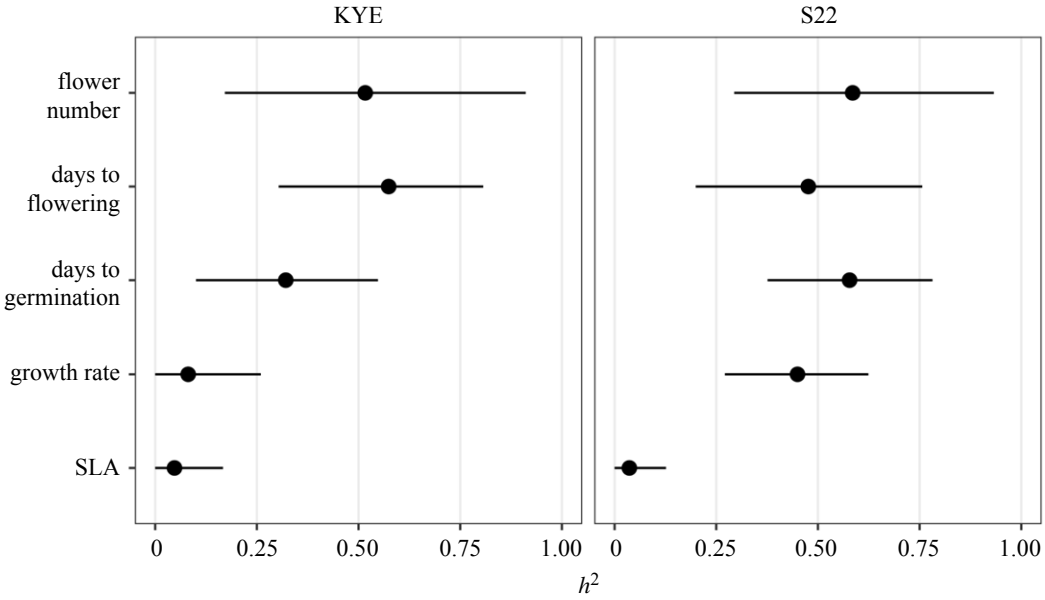

Figure 3. Estimates of narrow-sense heritability (h^2) for traits, with 95% credible intervals. Estimates of additive genetic variance for each trait are in the electronic supplementary material, table S4.

phenotypic optimum equal to optimum during drought), was weaker (Bayes factor = 2.3), indicating less support for an influential seed bank in that population compared to KYE. Overall, without a seed bank, populations experiencing a strong demographic decline due to large changes in phenotypic optima saw rapid phenotypic evolution toward the new optimum (electronic supplementary material, figure S10a). However, with a seed bank, even large changes in optima usually resulted in only modest phenotypic evolution like that observed in our populations. These constrained evolutionary responses were caused by gene flow from prior generations via the seed bank.

We can concisely describe the strength of a population's seed bank as a combination of the seed germination and survival rates, $\phi = (1 - g)s$. Thus, ϕ ('seed bank strength' in figure 4) describes the expected proportion of seeds available for germination in year t that will be available for germination in year $t + 1$, with larger values of ϕ indicating a stronger seed bank. The strength of the seed bank at KYE was estimated to be more than five-fold higher than that of S22 (0.47 versus 0.07) due to a higher seed survival rate and a lower germination rate (figure 4*a*). KYE was also estimated to have experienced a 15% smaller change in optimum during drought compared to S22 (−6.1 versus −7.2 days; figure 4*a*). Observed evolutionary and demographic responses of both populations fell within the distributions of posterior predictive checks (figure 4*b*).

KYE's mating population had a consistently higher mean age than S22's because of its stronger seed bank (figure 4*c*). However, mean age for both populations increased during the drought period due to low fecundity and increased representation of older generations. The increased gene flow from earlier generations in KYE led to the mean phenotype of its mating pool responding much more slowly to the environmental perturbation than S22 (figure 4*c*; electronic supplementary material, figure S10b). Further simulation runs indicated that the difference in evolutionary responses was overwhelmingly due to differences in seed bank vital rates, as opposed to differences in aboveground demography or selective environments (figure 4*d*). Simulations run with the selective environment and aboveground demography of KYE, but with the seed bank rates of S22, produced evolutionary change nearly equal to that observed in simulations run with full S22 parameters.

4. Discussion

Extreme environmental perturbations offer an opportunity to examine rapid evolution in natural populations. We used a resurrection experiment to test whether native plant populations rapidly evolved in response to a multi-year drought despite severe demographic declines. In one population (S22), earlier flowering evolved by the end of the drought and continued to evolve in the same direction after the drought ended. However, no other traits diverged at S22, and all traits exhibited stasis in the second population, KYE. We detected substantial additive genetic variance for multiple traits in both populations, including flowering phenology, suggesting that responses to selection were not constrained by limited genetic variation. Evolutionary simulations integrated with our demographic and experimental data suggested that seed banks constrained evolutionary responses in both populations. In KYE, the seed bank had a stronger effect resulting in evolutionary stasis; whereas in S22, the seed bank had a weaker effect, which facilitated closer tracking of changing phenotypic optima.

Why does rapid evolution occur in some populations but not others? One hypothesis is that a lack of quantitative genetic variation limits adaptive responses even when selection is intense. However, we found substantial V_A and high heritability for flowering phenology in both populations (with the caveat that estimates of h^2 are environment-dependent and could differ between the greenhouse and field). In fact, KYE contained twice the additive genetic variance in flowering time compared to S22. Thus, these results are inconsistent with the hypothesis that genetic variation prevented rapid evolution. Other studies have similarly found that rapid evolution occurred in some but not all studied populations [12–14]. However, estimates of V_A are rarely quantified in

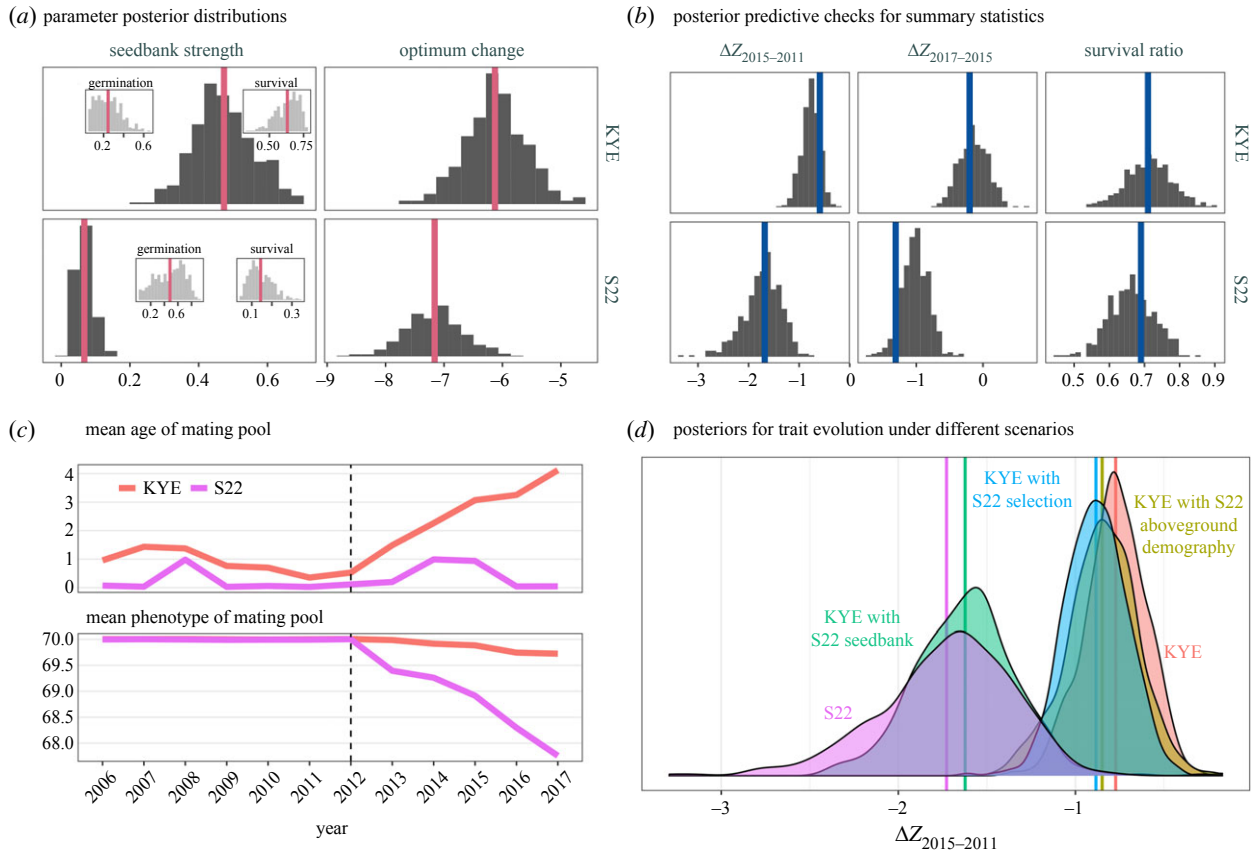

Figure 4. Results of evolutionary simulations and ABC analyses for the best-fitting model, model D (table 1). (*a*) Seed bank strength is greater, and change in phenotypic optimum is smaller, in KYE than S22. Estimated posterior distributions for model parameters (germination rate, seed survival rate and change in trait optimum during drought). Red vertical lines mark the posterior means. (*b*) Observed data are in accord with model simulations. Posterior predictive checks for the three summary statistics used in ABC analyses (change in trait mean 2015–2011, change in trait mean 2017–2015 and ratio of survival during drought to survival pre-drought). Blue vertical lines mark observed summary statistics from empirical data. (*c*) The stronger seed bank of KYE leads to increased representation of older generations and slower phenotypic evolution. Mean age and phenotype of mating pools in both populations over the 12 focal years during the simulation. Dashed vertical line marks the year when the trait optimum changed. (*d*) Differences in seed bank strength drive contrasting evolutionary responses between KYE and S22. Distributions of the summary statistic $\Delta Z_{2015-2011}$ (change in trait mean 2015–2011) under varying model scenarios. Coloured vertical lines mark posterior means for each scenario.

this context and thus the importance of genetic constraints is poorly understood (but see [5]). It is also worth noting that phenotypic variance in flowering phenology in S22 decreased 50% over our study, consistent with a strong episode of selection (electronic supplementary material, tables S2 and S5). Thus, the erosion of genetic variation due to this drought could constrain future responses to selection on phenology.

A more likely explanation for the lack of phenological evolution in KYE is that gene flow from the seed bank reintroduced alleles that were maladaptive during the drought. Previous field observations and experiments had demonstrated that both populations harboured seed banks but differed in their seed bank vital rates. We used individual-based simulations to ask whether these seed bank differences could explain the populations' contrasting evolutionary responses to drought. Models suggested that decreased germination and increased seed survival rates resulted in a seed bank at KYE that strongly constrained phenotypic evolution. The recruitment and mating of older individuals from the seed bank essentially caused maladaptive gene flow through time (figure 4*c*). A third population grown during the refresher generation likewise showed no significant evolutionary response to the drought (electronic supplementary material, figure S4) and was estimated in [43] to have seed bank vital rates similar to KYE (low germination and high seed survival). The seed bank also constrained phenotypic evolution in the evolving population, S22, but to a lesser extent due to a relatively weaker seed bank.

In general, theory has outpaced empirical work on the evolutionary consequences of germ banks in natural populations (but see [25,26,28]). Future resurrection studies could explicitly sample populations across a range of germ bank vital rates to further dissect germ bank effects on evolutionary responses. Wholly lacking are experimental investigations, where germ banks are manipulated in natural populations prior to (natural or experimentally imposed) environmental change. Such work is needed to test predictions arising from theory regarding the balance of negative (adaptation lag) and positive (increase in genetic variance) genetic effects of germ banks on adaptation [30]. Of course, evolutionary effects of germ banks will be influenced by, and potentially influence, their more well-studied demographic effects [22,39,43,65,66]. An especially fruitful approach would integrate experiments with models exploring the interplay between demographic and evolutionary rescue during environmental change across short and long time scales.

The hypotheses tested above are not exhaustive, and it is worth exploring alternative explanations for the lack of evolutionary change in phenology at KYE. Trait plasticity could

allow a population to weather an environmental perturbation with little or no evolutionary change [67]. However, plastic responses in flowering time were nearly identical for our two populations (electronic supplementary material, figure S11), suggesting that greater plasticity in KYE cannot explain its evolutionary stasis. Spatial gene flow among populations could potentially oppose allele frequency changes favoured by selection. However, gene flow is unlikely to be strong enough over this short time window to prevent evolutionary change, especially given that our populations are isolated from others (greater than 1 km) and lack adaptations for long-distance dispersal. It is also possible that advanced phenology simply did not confer fitness benefits during drought at KYE, though this would be contrary to both general trends in plants [45] and the well-documented relationship between early flowering time and adaptation to aridity in *C. xantiana* and close relatives [38,46,62,68–70]. It is also interesting that, in contrast with expectations, empirical and simulation results suggested that phenotypic optima did not return to their pre-drought level after the drought ended. This insight is consistent with the observation that population demography did not rebound after drought as one might expect (figure 1). Although the causes of this trend are unclear, it could be related to the timing of precipitation (e.g. most precipitation fell early in the season, which seems to promote germination but not survival in *C. x. xantiana*; D.A.M. & J.W.B. 2006–2017, personal observation).

The evolution of earlier flowering at S22 is consistent with post-drought resurrection studies in *Brassica rapa* [5,34] and probably reflects a strategy of drought escape (reviewed in [45,71,72]). Past work on *C. x. xantiana* has shown that Q_{ST} for flowering phenology is more than twice as high (Q_{ST} = 0.7) than Q_{ST} for five other ecologically important traits [68]. This result suggests that flowering phenology is often the target of spatially variable selection and readily evolves, as has been frequently observed in many systems [5,45,73,74]. Earlier flowering may evolve more readily than other traits under selection if its genetic architecture is comparatively simple (e.g. [71]), which can quicken responses to selection relative to more highly polygenic traits. The evolutionary lability of flowering phenology could also be due to its direct tie to assortative mating [75]—early flowering individuals tend to mate with other early flowering individuals. Theoretical work has shown that when there is directional selection on flowering time, positive assortative mating can increase genetic variation and the rate of phenotypic evolution compared to scenarios with random mating [76,77].

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 severe drought in one population. In a second population, we observed no sign of rapid evolution despite similar environmental stress, demographic decline and even greater additive genetic variance in phenology. In both populations, our results are consistent with the hypothesis that gene flow through time via seed banks either slowed or prevented rapid evolution. Our results are inconsistent with the hypotheses that plasticity or limited genetic variation constrained responses to selection. While rapid evolution in response to environmental perturbations has been repeatedly demonstrated, few studies have tackled the problem of why evolutionary stasis is so commonly observed across studies and systems. Our results emphasize that studies of adaptation in plant populations experiencing environmental change should consider both above and belowground processes affecting population demography and fitness. Extensions of our model could incorporate assortative mating, asymmetry in fitness functions [78] and genetic correlations to further our understanding of the genetic and ecological controls on rapid adaptation. Future empirical work should attempt to experimentally test key theoretical predictions and consider both the demographic and evolutionary effects of germ banks. As we seek to build more predictive models of the evolutionary process, the synthesis of demographic, environmental and quantitative genetic data with empirical and modelling approaches will be invaluable for understanding where and when rapid evolution is likely to occur.

Data accessibility. All data and code is archived at Figshare [79].

Supplementary material is available online [80].

Authors' contributions. J.W.B.: conceptualization, data curation, formal analysis, investigation, methodology, project administration, supervision, visualization and writing—original draft; A.F.: conceptualization, funding acquisition, investigation, methodology, project administration, supervision and writing—review and editing; D.A.M.: conceptualization, funding acquisition, investigation, methodology, project administration, supervision and writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

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