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RESEARCH ARTICLE

# Resurrected seeds from herbarium specimens reveal rapid evolution of drought resistance in a selfing annual

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## Abstract

**Premise:** Increased aridity and drought associated with climate change are exerting unprecedented selection pressures on plant populations. Whether populations can rapidly adapt, and which life history traits might confer increased fitness under drought, remain outstanding questions.

**Methods:** We utilized a resurrection ecology approach, leveraging dormant seeds from herbarium collections to assess whether populations of *Plantago patagonica* from the semi-arid Colorado Plateau have rapidly evolved in response to approximately ten years of intense drought in the region. We quantified multiple traits associated with drought escape and drought resistance and assessed the survival of ancestors and descendants under simulated drought.

**Results:** Descendant populations displayed a significant shift in resource allocation, in which they invested less in reproductive tissues and relatively more in both above- and below-ground vegetative tissues. Plants with greater leaf biomass survived longer under terminal drought; moreover, even after accounting for the effect of increased leaf biomass, descendant seedlings survived drought longer than their ancestors.

**Conclusions:** Our results document rapid adaptive evolution in response to climate change in a selfing annual and suggest that shifts in tissue allocation strategies may underlie adaptive responses to drought in arid or semi-arid environments. This work also illustrates a novel approach, documenting that under specific circumstances, seeds from herbarium specimens may provide an untapped source of dormant propagules for future resurrection experiments.

KEYWORDS

climate change, drought, herbarium collections, life history strategy, *Plantago patagonica* (Plataginaceae), rapid evolution, resurrection ecology

Global climate change is applying novel selection pressures (Anderson, 2016; Anderson and Song, 2020; Hamann et al., 2021), challenging populations to adapt, respond plastically, or migrate (Nogués-Bravo et al., 2018). Strong and continued selection pressure associated with climate change may exceed the efficacy of plastic responses (Jump and Penuelas, 2005; Stotz et al., 2021) and the pace of climate change may render effective migration biologically impossible (Christmas et al., 2016; González-Varo et al., 2021; Fricke et al., 2022). Unprecedented abiotic change may reduce species' ranges, decrease their genetic diversity and adaptive capacity, and cause extirpations or extinctions (Exposito-Alonso et al., 2022). Strong selection associated with decreased precipitation and increased potential evapotranspiration represents one of the chief challenges that climate change may pose to natural populations (Siepielski et al., 2017; Caruso et al., 2020). In the southwestern United States, climate change is driving precipitation variability and increased aridity (Seager et al., 2007; Cook et al., 2015) resulting in a century- to millennial-scale megadrought characterized by cumulative soil moisture deficits (Kogan and Guo, 2015; Williams et al., 2022). As the intensity of

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drought events are predicted to increase across much of the globe (Schipper et al., 2020; Legg, 2021), the pressing question remains – can adaptive evolution keep pace with rapid environmental change (Shaw and Etterson, 2012)?

Despite the long-held view that evolution occurs gradually over long timescales, evidence for rapid evolution (i.e., evolution across one to tens of generations) is now common (e.g., Thompson 1998; Hairston et al., 2005; Ellner 2013). Perhaps one of the most emblematic instances of rapid evolution is that of Darwin's finches, in which body and beak size rapidly tracked food availability over a 30-year period characterized by El Niño and La Niña oscillations (Grant and Grant, 2002). Several noteworthy examples in plants include the rapid evolution of copper tolerance in *Agrostis* in response to industrial pollution (Wu et al., 1975), the evolution of either decreased (Cheptou et al., 2008 in *Crepis* [Asteraceae]) or increased (Williams et al., 2016 in *Arabidopsis* [Brassicaceae]) seed dispersal in response to urbanization and habitat fragmentation, and the evolution of either increased selfing (Bodbyl Roels and Kelly, 2011 in *Mimulus* [Phrymaceae]) or increased investment in pollinator attraction (Bishop et al., 2023 in *Ipomoea* [Convolvulaceae]) in response to shifting pollinator dynamics. Given the profound effects climate change has natural populations, understanding the capacity of organisms to adapt to novel biotic and abiotic conditions has become a priority for many evolutionary biologists, ecologists, and conservationists (Catullo et al., 2019; Nadeau and Urban, 2019). A variety of studies in plants have documented rapid adaptive shifts in response to increasing aridity after only a few generations (e.g., Franks et al., 2007; Nguyen et al., 2016; Metz et al., 2020; Sun et al., 2020); however, those specifically focusing on the evolution of life-history traits and life-history strategies have thus far been relatively few (Grainger and Levine, 2022).

One increasingly popular method to explore the outcome of evolution in real time is through a resurrection approach, in which dormant propagules are revived and compared with descendants from the same populations under common conditions (Franks et al., 2018; Nogués-Bravo et al., 2018; Weider et al., 2018). By experimentally holding environmental conditions constant, such as in common gardens, observed phenotypic differences in descendants relative to their ancestors should reflect underlying genetic differences and thus evolutionary change (Franks et al., 2018). This approach approximates an "evolutionary time machine" by allowing insight into the genotypic and phenotypic composition of populations at different timepoints, both before and after selection (Orsini et al., 2013) imposed by environmental perturbations (e.g., Dickman et al., 2019; Vtipil and Sheth, 2020; Anstett et al., 2021; Kooyers et al., 2021). Resurrections typically relies on serendipitously collected propagules including seeds that have been collected as part of broader research programs (e.g., Franks et al., 2007; Wooliver et al., 2020), those stored in natural (Summers et al., 2018; Blum et al., 2021) or archival seedbanks (e.g., Cheptou et al., 2008; Everingham et al., 2021; Rauschkolb et al., 2022), or seeds collected specifically for use in resurrections, such as those from Project Baseline (Etterson et al., 2016).

Following earlier work examining the evolution of ozone resistance in *Plantago major* L. [Plantaginaceae] (Davison and Reiling, 1995) and the seminal work of Franks et al. (2007), documenting the evolution of earlier flowering time in *Brassica rapa* L. [Brassicaceae] in response to a multi-year drought in California, an increasing number of resurrection experiments have been conducted in plants (see Franks et al., 2018 for a partial list). Due to their sessile nature, plants are immediately subject to novel abiotic conditions associated with climate change, and thus are ideal study systems to explore if and how adaptation can maintain fitness in a changing world. Unfortunately, the typical availability of plant seeds for resurrection experiments may be incompatible with specific research questions or local logistic considerations. Herbarium collections provide a massive reservoir of dormant plant seeds from myriad locations across the globe, and several authors have suggested using dormant seeds from herbarium collections in resurrection studies (Heberling and Isaac, 2017; Meineke and Davies, 2019), yet to our knowledge no resurrection studies to date have utilized seeds from herbaria.

We employ a resurrection approach, utilizing dormant seeds from herbarium collections of *Plantago patagonica* Jacq., together with descendant individuals collected from the same populations, to understand how an annual plant species in the southwestern United States has responded to intense drought in the region. We use a series of greenhouse common garden experiments to explore the evolution of traits and life-history strategies associated with drought resistance. Specifically, we are interested in understanding whether adaptive evolution in a selfing annual can occur rapidly in response to climate change, and whether phenotypic shifts are consistent with adaptation to drought or directly confer increased fitness under drought. Lastly, we explore the feasibility of utilizing seeds from herbarium specimens in resurrection studies.

## MATERIALS AND METHODS

### Study system and seed sampling

The annual herb *Plantago patagonica* Jacq. (Plantaginaceae) occurs in sandy soils and open sites throughout much of the United States (Freeman et al., 2019). In Arizona it occupies deserts and desert grasslands at 365 to 2070 m in elevation. It germinates in the late winter or early spring, flowers in the spring, and senesces by late spring or early summer (Huisinga and Ayers, 1999). The species is an obligate inbreeder with cleistogamous flowers (Sharma et al., 1992). Among populations from the Colorado Plateau, seed and seedling traits show evidence of local adaptation across a natural aridity gradient (Christie et al., 2022), suggesting that abiotic selection has shaped trait variation.

We sampled ancestral genotypes of *P. patagonica* by collecting seeds from five herbarium sheets collected from five locations in northern Arizona (Figure 1) between 2003 and 2014 (Table 1). Individual herbarium sheets contained between two and seven individual plants (Table 1). We revisited these sites in fall 2019 and collected mature seeds from 11 to 25 individuals per population. Herbarium-collected seeds thus represent "pre-drought" genotypes, and field-collected seeds represent "post-drought" genotypes collected 5 to 16 years later. Multiple consecutive years of drought between 2000 and 2021 have contributed to cumulative soil moisture deficits and a resulting mega-drought in the southwestern United States (Williams et al., 2022). Specifically, our five focal *P. patagonica* populations have experienced increased springtime temperatures (+6.8% mean) and decreased springtime precipitation (−10.7% mean) compared to historical averages (Table 1; Appendix S1, Figure S1), resulting in drier springtime conditions (a mean 6.6% decrease in the Hogg's climatic moisture index; Wang et al., 2016).

## Greenhouse refresher generation

To minimize maternal effects (Donohue, 2009) and storage effects (Rogalski, 2015), we grew ancestral herbarium-derived seeds and descendant field-collected seeds under shared environmental conditions for one generation prior to conducting subsequent experiments (Figure 2). Seventy-two percent (72%) of all ancestral seeds and 88% of descendant seeds germinated at the onset of the refresher generation. We grew plants in a benign greenhouse environment (Appendix S2), and allowed them to autonomously self-fertilize, produce seeds, and senesce. Given that *P. patagonica* is an obligate inbreeder with largely cleistogamous flowers, we expect very little inadvertent gene flow in the greenhouse.

## Calculating heritabilities

Using the refresher generation, we created multiple maternal families originating from individual seeds from ancestral plants (i.e., herbarium plants; Table 1). We subsequently grew multiple individuals from each maternal family from each population in randomized designs in greenhouse experiments. We utilized the resulting phenotypic data (see Results section), to perform sibling analyses testing for the presence of meaningful genetic variation that evolution might act on (Conner and Hartl, 2004). Our approach conceptually mirrors a conventional ANOVA in which we determine whether there is significantly greater variance between compared to within maternal families. For each phenotypic trait in each ancestral population (and under each experimental treatment if multiple treatments were applied), we leveraged replicated phenotypic measures from the same genotype (i.e., selfed offspring) to calculate broad-sense heritability ($H^2$) following the methods of Schmidt et al. (2019). $H^2$ captures the effects of dominance and epistasis, and thus is important to the evolutionary potential of highly selfing species (Conner and Hartl, 2004). We were unable to calculate $H^2$ for root to shoot ratios due to inadequate replication of genotypes within families.

## Resurrection experiments

Following the refresher generation, we conducted three experiments in the greenhouse to explore evolutionary responses of *P. patagonica* to increased aridity and drought associated with climate change (Figure 2).

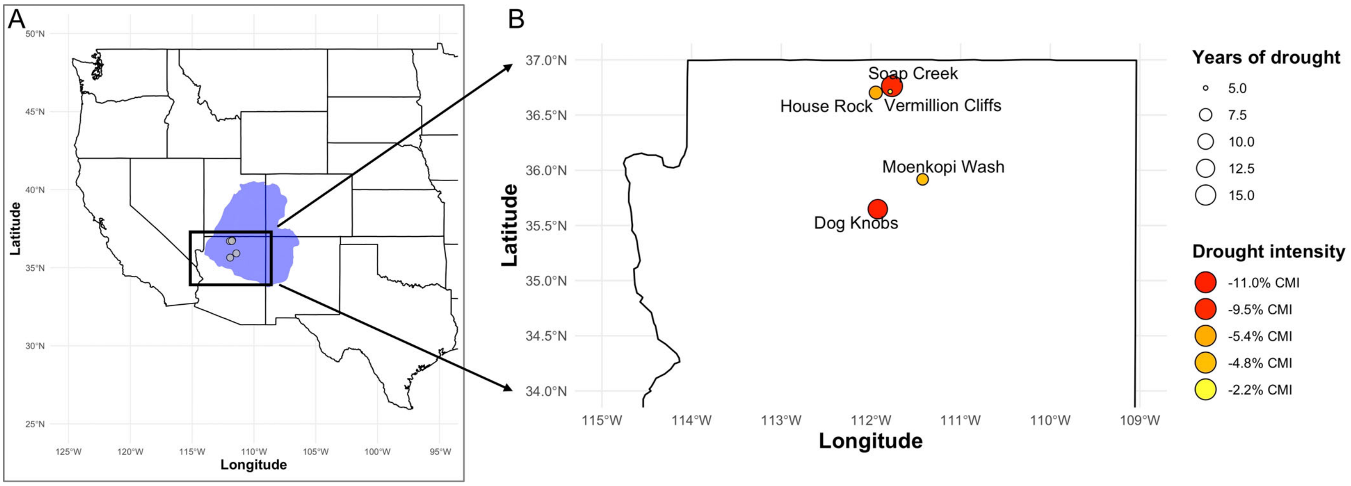

**FIGURE 1** Map of collection locations showing (A) five *Plantago patagonica* herbarium collections made from the southern Colorado Plateau (blue) in northern Arizona; (B) a close-up of five collection locations symbolized by the number of years between the original herbarium collections and our re-collections in 2019, and the average reduction in the springtime climatic moisture index (CMI) between pre-drought ancestral conditions and post-drought conditions experienced by descendants.

**TABLE 1** Summary of *Plantago patagonica* populations from northern Arizona utilized in this study. Changes in springtime climate conditions were calculated by comparing historical averages (1920 – year of herbarium collection) to contemporary averages (year of herbarium collection – 2019) using data from ClimateNA (Wang et al., 2016).

| Population | Herbarium collection | Collection year | Re-collection year | Elevation (meters) | Change in mean springtime temperature | Change in springtime precipitation | Change in springtime climatic moisture index (CMI) | Herbarium plants sampled | Contemporary plants sampled | n maternal families generated after refresher (herbarium) | n maternal families generated after refresher (field) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Dog Knobs | *K. Christie* 738 (ASC) | 2005 | 2019 | 2024 | +11.5% | −14.9% | −11.0% | 4 | 25 | 15 | 29 |
| Soap Creek | *D. Atwood* 29270 (ASC) | 2003 | 2019 | 1392 | +5.5% | −16.3% | −9.5% | 3 | 19 | 29 | 29 |
| House Rock | *W. Fertig* 26439 (ASC) | 2011 | 2019 | 1528 | +5.7% | −9.9% | −5.4% | 7 | 11 | 18 | 25 |
| Moenkopi Wash | *R. Crawford* 450 (ASC) | 2012 | 2019 | 1269 | +7.7% | −8.9% | −4.8% | 2 | 22 | 28 | 28 |
| Vermillion Cliffs | *W. Fertig* 29450 (ASU) | 2014 | 2019 | 1349 | +3.8% | −3.5% | −2.2% | 3 | 21 | 28 | 29 |

## Quantifying vegetative vs. reproductive allocation, and phenology

At the end of the refresher generation, we randomly selected 180 seeds from each population ($n = 1800$ in total), ideally six seeds from each of 30 maternal lines per population, or more seeds from fewer maternal lines based on availability (Table 1). We germinated seeds under benign conditions for four weeks and allowed seedings to establish for an additional three weeks (Appendix S2). At this point we initiated three watering treatments of differing frequencies ("wet," watered to saturation every 7 days; "medium," watered to saturation every 10.5 days; "dry," watered to saturation every 14 days). We quantified germination timing of all individual seeds, days to first flower, seed number, seed mass, leaf mass, and reproductive effort (the proportion of total above-ground biomass devoted to seed mass). During the 10-week experiment, watering treatments were effective in differentially altering the gravimetric water content of the potting soil (Appendix S1, Figure S2).

## Quantifying resource allocation to roots

Root traits, including root biomass, can directly confer increased fitness under drought (Harrison and LaForgia, 2019; Garbowski et al., 2020; Larson et al., 2020). To explore drought strategies associated with shifts in below-ground resource allocation, we conducted this experiment using 480 total seeds, and typically 24 maternal lines per population (Table 1). We germinated and established seedlings under benign conditions (Appendix S2), imposed two watering treatments ("wet," watered every 7 days; "dry," watered every 14 days) for four weeks, and then waited one additional week before harvesting plants. We harvested the entire plants, washed all debris from the roots, dried plants at 70°C for 72 hours, and weighed root biomass and leaf biomass to calculate root to shoot ratios.

## Quantifying drought resistance in seedlings

*P. patagonica* behaves as a spring annual in northern Arizona (Huisinga and Ayers, 1999), and we expect decreased springtime precipitation and drought in the region (Seager et al., 2007; Coats et al., 2013; Cook et al., 2015; Appendix S1, Figure S1) to exert strong selection pressure on seedling establishment. To determine if descendants were more resistant to drought than their ancestors, we conducted a terminal drought experiment imposed at the seedling stage. We randomly selected 144 seeds (6 seeds from each of typically 24 maternal lines) from each of the populations ($n = 1440$ seeds in total). We lightly scarified seeds and planted them in 72-cell germination trays ($4\,\text{cm} \times 4\,\text{cm} \times 5\,\text{cm}$ cells, $80\,\text{cm}^3$) in potting soil covered with a thin layer of sand, randomizing both

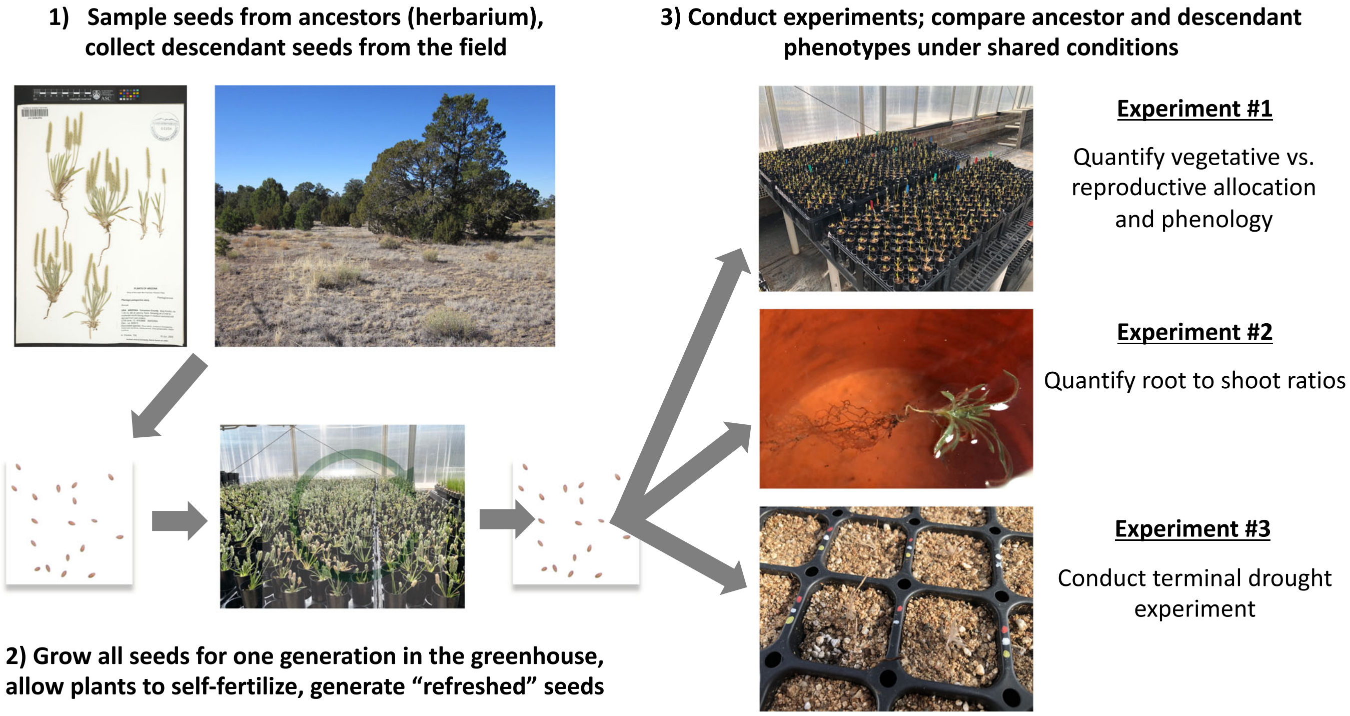

**FIGURE 2** Experimental overview using "pre-drought" genotypes collected from herbarium specimens and "post-drought" genotypes collected in the field from the same populations following a period of intense drought.

populations and maternal lines across rows in the trays. We misted trays daily for four weeks, allowing the seeds to germinate. After four weeks of benign conditions, we halted daily watering, simulating a scenario in which seedlings germinated under favorable conditions, and then experienced a prolonged period of intense drought. We monitored the seedlings daily for four weeks and recorded survival time. As in Experiment #1 (Figure 2), we also measured days to germination.

Reduced specific leaf area (SLA) is often associated with reduced transpiration and represents one mechanism by which plants may conserve water and tolerate drought (Harrison and LaForgia, 2019). Just prior to the onset of the terminal drought, we collected a single leaf (either the 2nd or 3rd true leaf) from each seedling that was at least 5 mm tall, and calculated its fresh area using the *Leafscan* app for iOS (Anderson and Rosas-Anderson, 2017). We then dried the leaves at 70°C for 24 hours and measured dried leaf mass, and calculated SLA as leaf area (mm$^2$) divided by leaf dry mass (mg). We assessed overall changes in SLA across generations and included SLA as a predictor variable in the mortality analysis.

## Statistical analyses

We utilized a regression approach to determine whether focal traits differed between ancestors and descendants (i.e., whether the phenotype had evolved). In general, we fit linear mixed models using the *lme4* package (Bates et al., 2015) in R (R Core Team, 2021), with fixed effects for generation and population (for which there were five levels; Gelman and Hill, 2006; Bolker, 2015), a population by generation interaction effect, and random effects accounting for repeated measures, such as individuals from the same maternal line or individuals growing in the same experimental racks. When applicable, we also included fixed effects for watering treatment and a treatment by generation interaction. If there were no significant interactions ($p < 0.05$) between generation and population or between treatment and population, we re-fit the model excluding the interaction term(s). We used the *emmeans* function from the *emmeans* package (Lenth, 2021) to generate least squared means and 95% confidence intervals. We accounted for multiple testing using the *p.adjust* function in R using the Benjamini-Hochberg false discovery rate ("fdr") criterion (Benjamini and Hochberg, 1995). We further describe each specific model in Appendix S2.

## Visualizing overall phenotypic trajectories across generations

We employed non-metric multidimensional scaling (NMDS) via the *metaMDS* function from the *vegan* package (Oksanen et al., 2022) to visualize the evolutionary trajectories of populations in multivariate trait space. NMDS ordinations were based on nine phenotypic (non-fitness) traits (Table 2).

**TABLE 2** Summary of predicted trait means and 95% confidence intervals for ancestors and descendants. *P*-values indicate significance of the effect of generation in each model. Bolded values are significant ($p < 0.05$). Adjusted $p$-values for multiple testing ($n = 10$) are based on the false discovery rate. Sub-sampling $p$-values (**$p < 0.01$; ***$p < 0.001$) reflect significance of binomial tests assessing deviation from the expectation that ancestors and descendants should show increased (or decreased) mean trait values in half of the randomization trials.

| Trait | Ancestor mean (95% CI) | Descendant mean (95% CI) | *p*-value | Adjusted *p*-value | Sub-sampling *p*-value |
|---|---|---|---|---|---|
| Germination timing (Exp. #1) | 11.6 days (10.9–12.3) | 11.4 days (10.8–12.1) | 0.15 | 0.21 | NS ($p = 0.16$) |
| Germination timing (Exp. #3) | 9.77 days (8.78–10.9) | 10.09 days (9.04–11.3) | 0.55 | 0.69 | *(**$p = 0.02$**) |
| Flowering phenology | 97.2 days (94.7–99.8) | 98.4 days (95.9–100.8) | 0.69 | 0.77 | *(**$p = 0.04$**) |
| Seed number | 13.6 (12.5–14.7) | 12.2 (11.1–13.4) | **0.0003** | **0.003** | *** |
| Seed mass | 10.64 mg (9.33–12.0) | 8.93 mg (7.64–10.2) | **0.008** | **0.016** | *** |
| Leaf mass | 23.3 mg (21.7–24.8) | 24.7 mg (23.1–26.2) | **0.003** | **0.01** | ** |
| Reproductive effort | 0.325 (0.298–0.352) | 0.294 (0.267–0.320) | **0.006** | **0.015** | *** |
| Root:shoot ratio | 0.379 (0.333–0.426) | 0.444 (0.399–0.489) | **0.015** | **0.025** | *** |
| Specific leaf area | 21.7 (20.7–22.7) | 21.6 (20.5–22.6) | 0.8 | 0.8 | NS ($p = 0.68$) |
| Seedling survival | 17.8 days (17.2–18.3) | 18.8 days (18.2–19.4) | **0.002** | **0.01** | *** |

## Assessing potentially unbalanced sampling across generations

Our initial sampling of ancestral maternal lines was limited compared to that of descendants (Table 1), and this initial bottleneck may have resulted in reduced trait variance observed in ancestors. To address this possibility, we conducted a Levene's test using the *leveneTest* function from the *car* package (Fox et al., 2019) to test for homogeneity of variances for each trait. Insignificant differences in trait variance between generations would suggest that individuals from herbarium specimens (and more precisely, their selfed offspring following the refresher generation) did not represent biased sampling relative to contemporary populations sampled in the field (Table 1). There was almost no evidence for heterogeneity of variances across generations, as only a single trait displayed a significant difference (and not in the direction consistent with reduced variance in ancestors; Appendix S3, Table S1). Thus, in the obligately selfing *P. patagonica*, and following a refresher generation, sampling 2–7 (grand)maternal lines from herbarium collections did not capture a reduced subset of trait variance compared to sampling 11–25 (grand)maternal lines from contemporary populations in the field (Table 1). Accordingly, we expect trait differences across generations to reflect biological differences as opposed to sampling artifacts.

Secondly, we employed a randomization procedure (Appendix S2) to determine if results based on sub-sampling fewer descendant lines were in concordance with overall results based on the full dataset. Briefly, for each descendant population, we sampled at random the same number of maternal lines present in the herbarium ancestors (e.g., four grand-maternal lines from Dog Knobs; Table 1). Next, using all individuals that originated from ancestral and the sub-sampled descendant (grand)maternal lines (all ancestral and descendant lines were represented by an equivalent number of individuals in the experiments) we calculated the mean values of focal traits for both generations. We repeated this sub-sampling procedure 30 times for each population ($n = 150$ total) and tallied the number of iterations in which descendants showed higher (or lower) trait means compared to their ancestors. We employed a binomial test (*binom.test* function in R) to determine if descendant trait values were more frequently higher (or lower) than expected by chance. This method did not account for random effects.

# RESULTS

## Heritabilities of focal traits

In the five ancestral *Plantago patagonica* populations, average broad-sense heritability ($H^2$) ranged from 0.13 to 0.32 for the nine traits we assessed (Appendix S3, Table S2). Some individual populations showed heritabilities of zero for some traits under our greenhouse conditions; however, all traits showed appreciable $H^2$ when averaged across the five populations (Appendix S3, Table S2), indicating that sufficient genetic variation existed within ancestral populations at large to fuel evolutionary change following selection. As expected, heritability for the fitness trait (days to death) was relatively lower than the other traits (zero for most populations; Appendix S3, Table S2), potentially due to the previous depletion of additive genetic variance following selection (Mousseau and Roff, 1987; Kruuk et al., 2000) associated with drought.

## Allocation to vegetation vs. reproduction, and phenology

When grown under shared environmental conditions, descendants produced fewer seeds than their ancestors

(12.2 vs. 13.6 on average; $p < 0.001$; Table 2; Appendix S1, Figure S3C); however, there were also significant population by generation interactions (Appendix S1, Figure S4; Appendix S3, Table S3). Results for seed mass mirrored those for seed number, as descendants invested less in seed mass than their ancestors ($p = 0.008$), typically producing 10.65 mg of seeds per plant compared to 8.93 mg (Table 2; Appendix S1, Figure S3D). Only a single population of descendants produced greater seed mass than its ancestors (Appendix S1, Figure S5). Across all watering treatments, descendants invested significantly more in leaf biomass ($p = 0.003$) compared to their ancestors (24.7 mg compared to 23.3 mg on average; Table 2; Appendix S1, Figure S3E). There was variation in responses of individual populations evidenced by significant population by generation interactions (Appendix S3, Table S3), with two of the five descendant populations investing relatively less in leaf tissue compared to their ancestors (Appendix S1, Figure S6). When jointly considering resource allocation trade-offs or the proportion of above-ground biomass allocated to reproduction, descendants invested relatively less in reproductive tissue and thus relatively more in vegetative tissue, compared to their ancestors ($p = 0.006$; Figure 3A; Appendix S1, Figure S3F). This trend was consistent in four of the five populations (Appendix S1, Figure S7). We also observed a significant effect of treatment ($p = 0.004$), with plants growing in the "dry" treatment investing relatively less in reproduction (and thus relatively more in leaf tissue) compared to those in the "wet" treatment (Figure 3A). Seed mass significantly varied in relation to leaf mass, typically peaking at intermediate leaf masses ($p < 0.001$ for quadratic leaf mass term), and was lower in descendants compared to ancestors ($p = 0.03$) across all of the watering treatments (Figure 3B).

We observed no differences in germination timing or flowering phenology across generations. In Experiment #1, both ancestors and descendants germinated in approximately 11.5 days on average ($p = 0.63$; Table 2; Appendix S1, Figures S3A, S8). In Experiment #3, there was a qualitative trend for later germination in ancestors (10.09 vs. 9.77 days on average), although this effect was not significant ($p = 0.55$; Table 2; Appendix S1, Figure S9). Despite another qualitative trend for delayed flowering time in descendants (four of the five descendant populations flowered later than their ancestors; Appendix S1, Figure S10), there was no significant difference in flowering phenology across

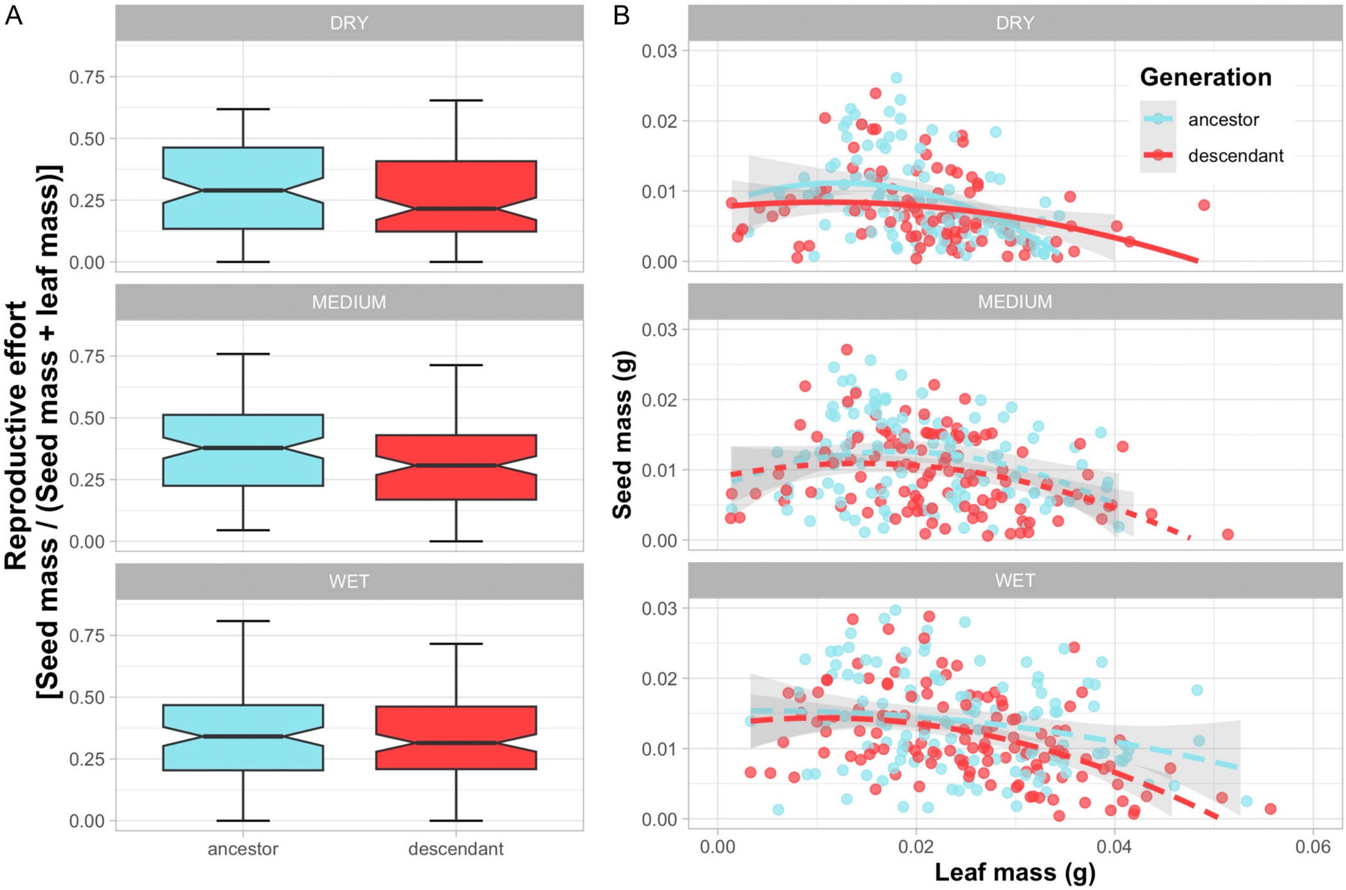

**FIGURE 3** Tissue allocation strategies in *P. patagonica* ancestors (blue) and descendants (red). (A) Reproductive effort (the proportion of above-ground biomass allocated to reproduction) was lower in descendants than in ancestors. (B) Descendants showed lower seed masses across all three watering treatments, indicative of a shift in life-history strategy associated with greater allocation to vegetative biomass at the cost of reduced fecundity.

generations ($p = 0.69$; Table 2; Appendix S1, Figure S3C; Appendix S3, Table S3). We observed a trend in which plants in the "dry" treatment were predicted to flower 0.9 days earlier than those in the "medium" treatment and 3.5 days earlier than those in the "wet" treatment, yet the effect of watering frequency was not significant in the full model ($p = 0.33$).

Pairwise correlations among the six traits we assessed in Experiment #1 ranged from −0.71 to 0.90 (Appendix S1, Figure S11), and all pairwise correlations (other than the single comparison between germination timing and seed mass) were statistically significant ($p < 0.001$).

## Resource allocation to roots

Overall, descendants invested relatively more in roots compared to their ancestors ($p < 0.01$; Figure 4; Appendix S1, Figure S3G), and four of the five descendant populations allocated relatively more to roots compared to their ancestors (Appendix S1, Figure S12). Plants growing in the "dry" treatment showed higher root to shoot ratios compared to those in the "wet" treatment ($p < 0.001$; Figure 4). Descendants tended to invest relatively more in roots in the "dry" treatment (Figure 4), suggesting greater plasticity and ability to optimize tissue allocation strategies based on moisture availability; however, this interaction term was not statistically significant ($p = 0.09$).

## Drought resistance in seedlings

### Fitness under drought

Descendant seedlings survived experimental drought for approximately 5% longer than their ancestors (18.8 days compared 17.8 days on average; $p = 0.002$; Table 2; Appendix S1, Figure S3I). There were no significant population by generation interactions, as all descendant populations responded similarly, surviving between 0.3 and 1.2 days longer than their ancestors (Appendix S1, Figure S13). The Cox survival model corroborated that descendants survived experimental drought better than their ancestors ($p = 0.03$). Survival curves show that increased drought resilience became pronounced 18 days after the onset of drought (Figure 5). There was also a significant effect of vegetative biomass on survival time ($p < 0.001$) as plants with larger leaves survived longer. Earlier-germinating plants also survived longer than later-germinating plants ($p = 0.03$). Ancestors and descendants showed no difference in specific leaf area ($p = 0.80$; Table 2; Appendix S1, Figures S3H, S14), and SLA was not significant in predicting survival time in the terminal drought experiment ($p = 0.16$; model not shown).

## Multivariate evolutionary trajectories

Four of the five *P. patagonica* populations evolved in a consistent direction, adopting a life-history strategy

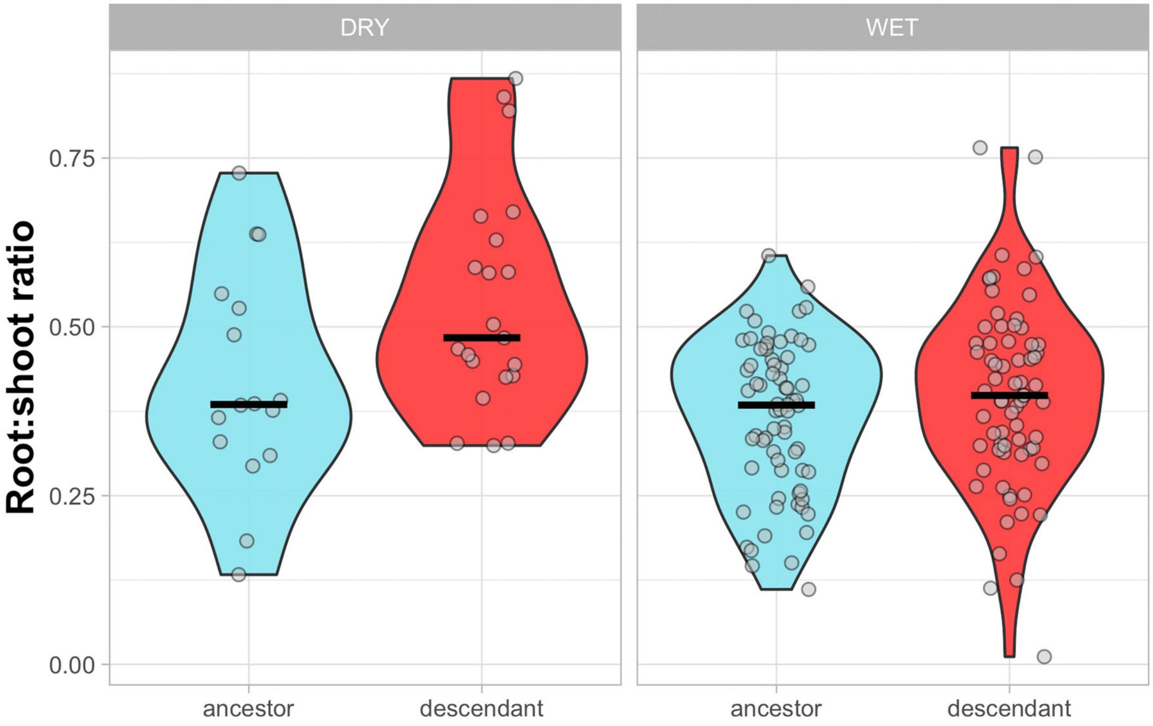

**FIGURE 4** Density plots of root to shoot ratios with horizontal bars depicting median values. Overall, descendants showed higher root to shoot ratios than their ancestors, indicating a relatively greater investment in below-ground vegetative tissues. This effect was more pronounced in the dry treatment, although the generation by watering treatment interaction was not significant, potentially due to reduced sample size associated with dry treatment mortality.

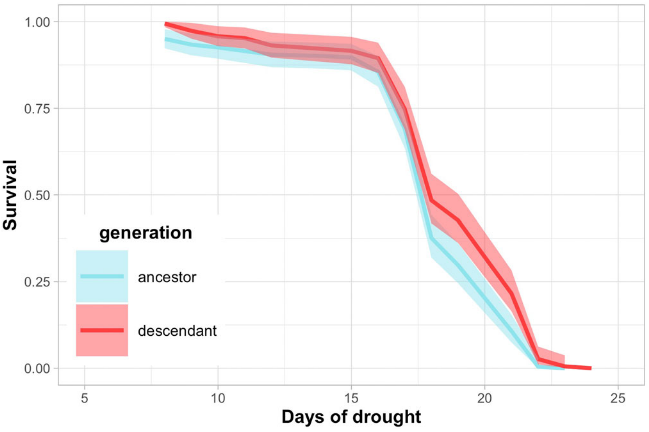

**FIGURE 5** Survival curves for *Plantago patagonica* seedlings subjected to terminal drought. Lines represent model predictions and shaded areas represent 95% confidence intervals. Descendants survived drought significantly longer than their ancestors, with this effect becoming pronounced after 17–18 days of drought, as indicated by the divergence of confidence intervals.

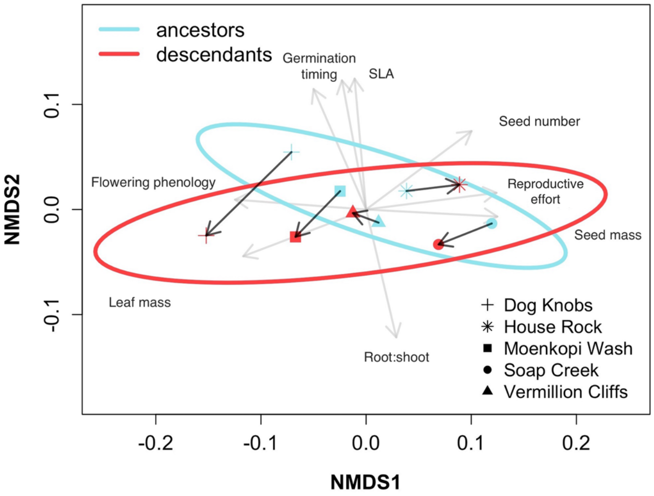

**FIGURE 6** Non-metric multidimensional scaling (NMDS) plot based on nine phenotypic traits showing the evolutionary trajectories of five *P. patagonica* populations following 5–16 years of intense drought in northern Arizona. Black arrows show multivariate trait shifts of individual populations; gray arrows depict trait loadings; ellipses depict 95% confidence intervals around phenotypic compositions of ancestors (blue) and descendants (red). Four of the five populations evolved in a consistent direction, adopting a life-history strategy characterized by greater allocation to vegetative tissues, at the cost of reduced fecundity.

characterized by increased allocation to vegetative resources and reduced seed production (Figure 6).

## Sub-sampling descendant individuals

There was a strong concordance between results from the full models using all the available data and those derived from randomizations based on equivalently sized subsets of grandmaternal lines from both generations for all traits that showed evolutionary change in response to drought (Table 2; Appendix S1, Figure S15). The sub-sampling procedure was slightly sensitive to qualitative trends in the data; producing significant $p$-values ($p < 0.05$) for germination timing and flowering phenology (Table 2), indicating that descendants had delayed phenologies in a greater number of randomizations than expected by chance. The test showed no tendency for false positives when qualitative trends were absent (e.g., germination timing in Experiment #1 and specific leaf area; Table 2).

# DISCUSSION

In the southwestern United States climate change has contributed to prolonged drought, increased aridity, and extreme cumulative soil moisture deficits over the past two decades (Williams et al., 2022). Key components of drought—precipitation and potential evapotranspiration (Siepielski et al., 2017)—are believed to exert particularly strong selection pressures on natural plant populations (Caruso et al., 2020). We leveraged seeds from herbarium collections within a resurrection ecology framework (Franks et al., 2018) to address a crucial question in ecology and evolution—can natural populations keep pace with climate change via rapid adaptation (Shaw and Etterson, 2012; Nogués-Bravo et al., 2018)? In the annual plant *Plantago patagonica*, six of the nine traits we assessed showed significant evolutionary change following 5 to 16 years of drought. We found strong evidence for greater investment in above-ground and below-ground vegetative biomass, but no evidence of phenological acceleration. These findings contrast those of many other resurrection studies, in which drought results in the evolution of rapid development and a relatively greater investment in reproductive tissues. Four of the five populations evolved along consistent phenotypic trajectories, and descendant seedlings from all five populations showed prolonged survival under drought compared to their ancestors, illustrating repeatable outcomes of natural selection imposed by climate change.

## Drought escape through phenological acceleration

A rich literature documents advanced phenology in response to climate change (e.g., Fitter and Fitter, 2002; Calinger et al., 2013; CaraDonna et al., 2014; Wadgymar et al., 2017; Menzel et al., 2020; Rafferty et al., 2020) and the evolution of earlier flowering times has been an almost ubiquitous result in resurrection studies that have examined phenology (e.g., Franks et al., 2007; Rhoné et al., 2010; Vigouroux et al., 2011; Nevo et al., 2012; Van Dijk and Hautekèete, 2014; Thomann et al., 2015; Dickman et al., 2019; Lambrecht et al., 2020; Valencia-Montoya et al., 2021; Cheptou et al., 2022; Johnson et al., 2022; Rauschkolb et al., 2022; Bishop et al., 2023).

Similarly, more rapid germination can also act to expedite the life cycle and maximize fitness in a growing season constrained by drought (Dickman et al., 2019). In contrast to these many studies, we found no evidence for accelerated germination (Table 2; Appendix S1, Figure S3a) or earlier flowering in post-drought descendants (Table 2; Appendix S1, Figure S3b). Compared to Mediterranean environments in which drought can manifest as a truncated growing season in which accelerated flowering may be beneficial (e.g., Lambrecht et al., 2020), early flowering may not increase fitness under the continuous aridity and depleted soil moisture associated with drought in the arid southwestern United States (Williams et al., 2022). Interestingly, we found no effect of watering frequency on flowering time, suggesting that phenology may be canalized in *P. patagonica*, or that genetic correlations with other traits may be constraining its evolution (Vtipil and Sheth, 2020). Here, days to flowering was strongly positively correlated with leaf mass (Appendix S1, Figure S11), thus the fitness benefit associated with larger leaves under drought (see the following section) may have acted to constrain the evolution of earlier flowering.

Although statistically non-significant, we observed a qualitative trend for delayed flowering time in descendants in three of the five populations, and one descendant population flowered significantly later than its ancestors (Appendix S1, Figure S10). Two separate resurrections in *Mimulus* have documented a delay in flowering time in response to drought, coupled with associated shifts towards a dehydration avoidance strategy mediated by vegetative traits (Anstett et al., 2021; Kooyers et al., 2021). Specifically, *M. guttatus* Fisch. ex DC. populations from California that suffered the greatest drought intensities evolved to flower later, but also accumulated more vegetative biomass, which could bestow greater water-use efficiency or might otherwise prolong survival under drought (Kooyers et al., 2021). This explanation seems plausible in *P. patagonica* as well, as descendants invested more in leaf biomass (see the following section), and thus seem to have evolved increased drought resilience due to tissue allocation shifts as opposed to phenological shifts.

## Drought resilience via altered life history strategies

Most of the significant phenotypic responses in post-drought *P. patagonica* descendants were related to tissue allocation traits, including reduced investment in seeds (Table 2; Appendix S1, Figure S3) and increased investment in leaf tissue (Table 2; Appendix S1, Figure S3e). In contrast to our findings, common garden experiments often reveal a genetic basis for higher reproductive effort in plants from arid environments (e.g., Aronson et al., 1993; Berger et al., 2017; Ryan and Cleland, 2021), and several resurrection experiments have also documented the evolution of greater reproductive allocation in response to drying conditions (Sultan et al., 2013; Dickman et al., 2019; Metz et al., 2020). Increased reproductive effort (and thus decreased allocation to vegetative tissues) may manifest at dry sites as a by-product of reduced competition (Aronson et al., 1993; Bonser, 2013; Metz et al., 2020). Conversely, in sparsely vegetated sites of the Colorado Plateau, annual plants may face relatively stronger selection pressures associated with drought compared to those associated with competition, as plants that do not survive, regardless of the potential effects of competition on fecundity, simply will not reproduce. Thus, the environmental context in which plants live likely affects the fundamental trade-off between allocating resource to reproduction or vegetation (Appendix S1, Figure S11).

Previous work in contemporary populations of *P. patagonica* has shown that seedlings with larger leaves survive terminal drought for longer than those with smaller leaves (Christie et al., 2022). Descendant populations of *P. patagonica* have shifted their resource allocation strategies towards greater vegetative investment at the cost of fecundity (Figure 3), as such a strategy may prioritize survival under increasingly arid conditions associated with prolonged drought in the region. Larger leaves can be directly associated with morphologies associated with dehydration avoidance (Kooyers et al., 2021), or they may be correlated with other traits such root length or root biomass (Figure 4), traits that directly confer increased fitness under drought (Harrison and LaForgia, 2019; Garbowski et al., 2020; Larson et al., 2020; Welles and Funk, 2021). Given that there is no evidence of perenniality in *P. patagonica* such that a greater investment in leaf tissue might result in greater fecundity in subsequent growing seasons, one might argue that descendants producing fewer seeds than their ancestors are simply maladapted to present-day conditions. This argument, however, fails to recognize that increased leaf mass was associated with increased survival under terminal drought, and individuals that succumb to drought as seedlings, regardless of their potential fecundity, simply do not reproduce.

## Is observed phenotypic change adaptive?

Not all evolved traits directly underlie fitness differences across generations (Sekor and Franks, 2018; Kooyers et al., 2021), as trait shifts may be explained by drift (Gay et al., 2022), unmeasured transgenerational effects, or other epigenetic mechanisms (Rey et al., 2016). Given these uncertainties, investigators have typically relied on theory, corroborating evidence from natural environmental gradients (Metz et al., 2020; Wooliver et al., 2020), selection analyses (Sekor and Franks, 2018; Metz et al., 2020), or $Q_{ST}$-$F_{ST}$ comparisons (Valencia-Montoya et al., 2021; Rauschkolb et al., 2022) to determine if evolved traits represent adaptations. Relatively fewer resurrection studies have directly quantified fitness of ancestors and descendants under present day conditions or those simulating a novel environment (e.g., Sultan et al., 2013; Bustos-Segura et al., 2014;

Valencia-Montoya et al., 2021). One of the most conclusive findings of our study emerged from our assessment of survival time under terminal experimental drought, in which descendant seedlings showed significantly prolonged survival than their ancestral counterparts (Figure 5). Based on theory we might posit that increased vegetative allocation and increased root to shoot ratios (Figure 5) are likely adaptive for *P. patagonica* populations facing drought, yet plant size was also directly associated with survival in the terminal drought experiment, supporting the hypothesis that increased vegetative allocation *per se* (Figures 3, 4) is adaptive for *P. patagonica* populations under water-limited conditions. Moreover, descendants outlived their ancestors, even after accounting for the effect of increased leaf biomass, suggesting that they also possessed additional unmeasured traits that further promoted drought resilience. In contrast to many of the phenotypic traits that showed population by generation interactions (Appendix S3, Table S2), all five descendant populations showed increased fitness compared to their ancestors (Appendix S1, Figure S13), regardless of the number of years or the magnitude of climate change since collection (Table 1).

Finally, while we lacked sufficient population sampling to include the magnitude environmental change experienced by descendants in our models, anecdotally, the two populations that have experienced the greatest decrease in springtime moisture availability (Dog Knobs and Soap Creek; Table 1) also showed the greatest change in reproductive-vegetative trade-offs (Appendix S1, Figure S3), the greatest shift in root to shoot ratios (Appendix S1, Figure S3g), and among the greatest increases in survival times under drought (Appendix S1, Figure S13). This concordance, while circumstantial, suggests a direct link between evolved phenotypes and increased fitness under drought. Overall, four of the five populations evolved along consistent trajectories in multivariate trait space (Figure 6). These repeated evolutionary responses suggest that populations are adapting in consistent and predictable ways (Johnson et al., 2022). The House Rock population, which is ecologically similar to the other populations, has experienced a moderate degree of environmental change (Table 1), and showed no evidence of an invisible fraction problem (Appendix S1, Figure S17). Unfortunately, we can offer little insight into why it evolved along an opposite trajectory (Figure 6), showing decreased leaf production and earlier flowering, other than to re-iterate that this response is simply another possible evolutionary strategy of dealing with selection pressures imposed by drought.

## Are seeds from herbarium specimens legitimate propagules for resurrection studies?

The power of museum collections for evaluating the impact of global climate change on biodiversity (Meineke et al., 2019) and the tempo of evolutionary processes (Kreiner et al., 2022) is well-established. Several authors have suggested that seeds from herbarium collections might contribute to our understanding of biological processes through their use in resurrection studies (Heberling and Isaac, 2017; Meineke and Davies, 2019), yet to our knowledge this possibility has not yet been pursued, perhaps due to potential pitfalls associated with biased sampling in resurrection experiments (Etterson et al., 2016). These potential limitations are two-pronged: first, effective resurrection experiments require a representative sample of standing genetic variation of both ancestral and descendant generations (Franks et al., 2018). Secondly, non-random mortality following storage, which can occur if focal phenotypes are genetically correlated with the likelihood of surviving storage, may bias estimates of ancestral phenotypic distributions and thus skew estimates of evolutionary change (Franks et al., 2018; Weis, 2018).

*P. patagonica* may serve as a best-case scenario for determining whether herbarium collections are tractable sources of seeds for resurrection studies due to several specific attributes. Due to its diminutive size, often up to 10 to 15 or more individuals are mounted on individual herbarium sheets (Appendix S1, Figure S16), allowing greater sampling per herbarium sheet than larger individuals. Additionally, its selfing mating system, extended seed viability, and its occupation of arid habitats and inclusion in herbaria with reduced threats of insect damage and thus reduced need for pesticide treatments which can further degrade seed viability (Abeli et al., 2020), all to further contribute to experimental amenability. In a pilot experiment, we were unable to germinate seeds from herbarium sheets of either *Draba cuneifolia* Nutt. Ex Torr. & Gray [Brassicaceae]or *Mimulus rubellus* A.Gray across similar timespans of up to 15 years. While these are also annual species occurring in similarly arid environments as *P. patagonica*, they possess substantially smaller seeds, perhaps a trait associated with reduced viability under long-term storage.

As *P. patagonica* is an obligately selfing annual (Sharma et al., 1992), typically occurring in isolated patches (personal observations, K.C.), we expect it to show high levels of inbreeding and relatively low genetic diversity within populations (Koelling et al., 2011; Jullien et al., 2019; Toczydlowski and Waller, 2019; Huang et al., 2021), such that few individuals should capture much of the population-level variation present. While we are unaware of any studies documenting patterns of genetic diversity within and between populations of *P. patagonica*, a survey of genetic diversity in 63 populations of the ecologically similar and mixed-breeding *P. ovata* Forssk. found that all populations showed positive $F_{IS}$ values (Shryock et al., 2021), indicating an over-abundance of homozygotes consistent with reduced within-population genetic diversity.

We implemented several precautions to assess potential bias in our results stemming from non-representative sampling. First, we quantified broad-sense heritability ($H^2$) using sibling analysis and found significant genetic variation among ancestral families generated from herbarium seeds from each population. Had we not found positive mean heritability values (i.e., appreciable differences between maternal families within populations), regardless of whether evolution might still occur via some type of epigenetic mechanism, the amplification of seeds during the refresher generation may have acted to artificially inflate the power of our subsequent analyses. Second, we assessed whether sampling relatively few (grand)maternal lines via herbarium specimens resulted in reduced trait variance compared to sampling many more (grand)maternal lines from the field (Table 1). While the number of individual plants on herbarium sheets with seeds that germinated was modest (2–7; 3.8 on average; Table 1), only one of nine traits showed significant heterogeneity of variances across generations (and here descendants and not ancestors showed reduced variance; Appendix S3, Table S1), suggesting that our sampling of ancestral individuals did not capture a reduced subset of the variation present in populations. Strong selection in subsequent descendant generations however, and an associated reduction in trait variation among survivors may have acted to mask biased sampling of ancestral generations, so this test alone may be insufficient. Results from randomized subsampling using equivalent numbers of ancestral and descendant (grand)maternal lines, corroborated model-based findings for the evolution of life-history shifts and increased survival under drought (Appendix S1, Figure S15), supporting the validity of our overall findings.

Another potentially serious pitfall for many resurrection studies relates to the "invisible fraction" in which unequal rates of survivorship following storage and revival can bias estimates of evolutionary change (Franks et al., 2018). This issue is well-described by Weis (2018), but to briefly summarize, high germination rates of stored ancestral seeds mitigate potential problems. Here in *P. patagonica*, 72% of all ancestral seeds collected from herbarium specimens germinated compared to 88% of descendant seeds. Species with relatively high germination rates from herbarium specimens should not be plagued by fatal invisible fraction problems; however, given a high degree of variation in germination success among populations, such as observed here (Appendix S1, Figure S17), we cannot entirely discount the possibility. Additionally, the oldest populations had the lowest germination rates (Appendix S1, Figure S17), suggesting that there is a limit to the sampling horizon of seeds stored on herbarium sheets, such that assaying evolutionary change over many decades may be impossible using this approach.

Reduced germination in ancestors is only a substantial limitation if there is a strong correlation between emergence likelihood and other focal traits (Weis, 2018). Situations in which there could be an invisible fraction problem based on mildly reduced germination (such as observed here) have left most empiricists with somewhat unsatisfying alternatives. We could estimate that 70% to 90% germination results in minimal invisible fraction bias (e.g., Vtipil and Sheth, 2020), or we could use the simulations in Weis (2018) to back-calculate whether descendant traits means are substantially different from ancestors given various genetic correlations and germination rates. The most elegant solution, proposed by Weis (2018), is to leverage relatedness information among individuals that germinated (i.e., non-missing family members) to create a shared parameter model to precisely quantify the extent of the invisible fraction problem (Steinsland et al., 2014; Espeland, 2020; Hofman et al., 2022 [preprint]). This approach is computationally intensive even for a single trait, and to our knowledge has been infrequently applied outside of the field of applied mathematics. Given the recent interest in resurrection studies at large and the pending release of stored seeds from Project Baseline (Etterson et al., 2016), we advocate for the creation of R package implementing shared parameter models to assist members of the botanical, evolution, and ecology community in quantifying the invisible fraction problem in future resurrection studies.

## CONCLUSIONS

This work adds to a growing body of literature documenting rapid evolution in response to global climate change. Over the course of 5–16 years within the ongoing 2000–2021 megadrought in the southwestern United States, multiple populations of the selfing annual *Plantago patagonica* showed rapid and repeatable evolutionary change. Notably, descendants evolved to survive terminal drought longer than their ancestors, a pattern potentially explained by a greater investment in leaf and root biomass. These findings suggest that some plant populations from arid regions have thus far kept pace with the unprecedented selective pressures imposed by climate change via adaptive responses. Conversely, several traits did not evolve, and multiple traits were significantly correlated, suggesting limits to adaptive capacity and/or genetic constraints along ecologically important axes of trait variation. Museum collections can provide genotypic snapshots of the past and thus the ability to create evolutionary time machines capable of illuminating the outcome of natural selection in real-time. In many situations, seeds stored on herbarium specimens will be simply inappropriate for resurrection studies; however, our work suggests that archived herbarium collections may be valuable seed sources for future resurrection studies in some specific instances and for some specific study systems, particularly for the 10–15% of seed plants that predominantly reproduce via self-fertilization (Goodwillie et al., 2005).

## AUTHOR CONTRIBUTIONS

K.C., L.M.H., and D.B.L. designed the study; K.C. and N.R.P. performed the research; K.C. conducted analyses; K.C. wrote the first draft of manuscript; K.C., L.M.H., and D.B.L. contributed to the final draft.

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## COMPETING INTERESTS

The authors declare no conflicts of interest.

## DATA AVAILABILITY STATEMENT

Data are available at the Dryad Digital Repository: https://doi.org/10.5061/dryad.b5mkkwhkg (Christie, 2023).

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## REFERENCES

Abeli, T., S. Dalrymple, S. Godefroid, A. Mondoni, J. V. Müller, G. Rossi, and S. Orsenigo. 2020. Ex situ collections and their potential for the restoration of extinct plants. *Conservation Biology* 34: 303–313.

Anderson, C. J., and P. J. Rosas-Anderson. 2017. Leafscan (version 1.3.21). [Mobile application software]. Website: https://itunes.apple.com/app/id1254892230

Anderson, J. T. 2016. Plant fitness in a rapidly changing world. *New Phytologist* 210: 81–87.

Anderson, J. T., and B.-H. Song. 2020. Plant adaptation to climate change—Where are we? *Journal of Systematics and Evolution* 58: 533–545.

Anstett, D. N., H. A. Branch, and A. L. Angert. 2021. Regional differences in rapid evolution during severe drought. *Evolution letters* 5: 130–142.

Aronson, J., J. Kigel, and A. Shmida. 1993. Reproductive allocation strategies in desert and Mediterranean populations of annual plants grown with and without water stress. *Oecologia* 93: 336–342.

Bates, D., M. Maechler, B. M. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* 67: 1–48.

Benjamini, Y., and Y. Hochberg. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society Series B* 57: 289–300.

Berger, J. D., D. Shrestha, and C. Ludwig. 2017. Reproductive strategies in Mediterranean legumes: Trade-offs between phenology, seed size and vigor within and between wild and domesticated *Lupinus* species collected along aridity gradients. *Frontiers in Plant Science* 8: 548.

Bishop, S. G., S.-M. Chang, and R. S. Baucom. 2023. Not just flowering time: A resurrection approach shows floral attraction traits are changing over time. *Evolution Letters* 7: 88–98.

Blum, M. J., C. J. Saunders, J. S. McLachlan, J. Summers, C. Craft, and J. D. Herrick. 2021. A century-long record of plant evolution reconstructed from a coastal marsh seed bank. *Evolution Letters* 5: 422–431.

Bodbyl Roels, S. A., and J. K. Kelly. 2011. Rapid evolution caused by pollinator loss in *Mimulus guttatus*. *Evolution* 65: 2541–2552.

Bolker, B. M. 2015. Linear and generalized linear mixed models. *In* G. Fox, S. Negrete-Yankelevich, & V. Sosa [eds.], Ecological statistics: Contemporary theory and application, 309–333. Oxford University Press, Oxford, UK.

Bonser, S. P. 2013. High reproductive efficiency as an adaptive strategy in competitive environments. *Functional Ecology* 27: 876–885.

Bustos-Segura, C., J. Fornoni, and J. Núñez-Farfán. 2014. Evolutionary changes in plant tolerance against herbivory through a resurrection experiment. *Journal of Evolutionary Biology* 27: 488–496.

Calinger, K. M., S. Queenborough, and P. S. Curtis. 2013. Herbarium specimens reveal the footprint of climate change on flowering trends across north-central North America. *Ecology Letters* 16: 1037–1044.

CaraDonna, P. J., A. M. Iler, and D. W. Inouye. 2014. Shifts in flowering phenology reshape a subalpine plant community. *Proceedings of the National Academy of Sciences, USA* 111: 4916–4921.

Caruso, C. M., H. Maherali, and R. A. Martin. 2020. A meta-analysis of natural selection on plant functional traits. *International Journal of Plant Sciences* 181: 44–55.

Catullo, R. A., J. Llewelyn, B. L. Phillips, and C. C. Moritz. 2019. The potential for rapid evolution under anthropogenic climate change. *Current Biology* 29: R996–R1007.

Cheptou, P.-O., O. Carrue, S. Rouifed, and A. Cantarel. 2008. Rapid evolution of seed dispersal in an urban environment in the weed *Crepis sancta*. *Proceedings of the National Academy of Sciences, USA* 105: 3796–3799.

Cheptou, P., E. Imbert, and M. Thomann. 2022. Rapid evolution of selfing syndrome traits in *Viola arvensis* revealed by resurrection ecology. *American Journal of Botany* 109: 1838–1846.

Christie, K. 2023. Data from: Resurrected seeds from herbarium specimens reveal rapid evolution of drought resistance in a selfing annual (Version 4) [Data set]. *Dryad*. https://doi.org/10.5061/DRYAD.B5MKKWHKG

Christie, K., N. R. Pierson, D. B. Lowry, and L. M. Holeski. 2022. Local adaptation of seed and seedling traits along a natural aridity gradient may both predict and constrain adaptive responses to climate change. *American Journal of Botany* 109: 1529–1544.

Christmas, M. J., M. F. Breed, and A. J. Lowe. 2016. Constraints to and conservation implications for climate change adaptation in plants. *Conservation Genetics* 17: 305–320.

Coats, S., J. E. Smerdon, R. Seager, B. I. Cook, and J. F. González-Rouco. 2013. Megadroughts in southwestern North America in ECHO-G millennial simulations and their comparison to proxy drought reconstructions. *Journal of Climate* 26: 7635–7649.

Conner, J. K., and D. L. Hartl. 2004. A primer of ecological genetics. Sinauer Associates, Sunderland, Massachusetts, USA.

Cook, B. I., T. R. Ault, and J. E. Smerdon. 2015. Unprecedented 21st century drought risk in the American Southwest and Central Plains. *Science Advances* 1: e1400082.

Davison, A. W., and K. Reiling. 1995. A rapid change in ozone resistance of *Plantago major* after summers with high ozone concentrations. *New Phytologist* 131: 337–344.

Dickman, E. E., L. K. Pennington, S. J. Franks, and J. P. Sexton. 2019. Evidence for adaptive responses to historic drought across a native plant species range. *Evolutionary Applications* 12: 1569–1582.

Donohue, K. 2009. Completing the cycle: Maternal effects as the missing link in plant life histories. *Philosophical Transactions of the Royal Society, B, Biological Sciences* 364: 1059–1074.

Ellner, S. P. 2013. Rapid evolution: from genes to communities, and back again? *Functional Ecology* 27: 1087–1099.

Espeland, L. F. 2020. A shared parameter model accounting for non-ignorable missing data due to dropout-modelling of blood pressure based on the HUNT Study. Master's Thesis. Norwegian University of Science and Technology, Department of Mathematical Sciences, Trondheim, Norway.

Etterson, J. R., S. J. Franks, S. J. Mazer, R. G. Shaw, N. L. S. Gorden, H. E. Schneider, J. J. Weber, et al. 2016. Project Baseline: An unprecedented resource to study plant evolution across space and time. *American Journal of Botany* 103: 164–173.

Everingham, S. E., C. A. Offord, M. E. Sabot, and A. T. Moles. 2021. Time-traveling seeds reveal that plant regeneration and growth traits are responding to climate change. *Ecology* 102: e03272.

Exposito-Alonso, M., T. R. Booker, L. Czech, L. Gillespie, S. Hateley, C. C. Kyriazis, P. L. Lang, et al. 2022. Genetic diversity loss in the Anthropocene. *Science* 377: 1431–1435.

Fitter, A. H., and R. S. R. Fitter. 2002. Rapid changes in flowering time in British plants. *Science* 296: 1689–1691.

Fox, J., S. Weisberg, B. Price, D. Adler, D. Bates, G. Baud-Bovy, and B. Bolker. 2019. car: Companion to applied regression. R package, version 3.0-2. Website: https://CRAN.R-project.org/package=car [accessed 17 March 2020].

Franks, S. J., E. Hamann, and A. E. Weis. 2018. Using the resurrection approach to understand contemporary evolution in changing environments. *Evolutionary Applications* 11: 17–28.

Franks, S. J., S. Sim, and A. E. Weis. 2007. Rapid evolution of flowering time by an annual plant in response to a climate fluctuation. *Proceedings of the National Academy of Sciences, USA* 104: 1278–1282.

Freeman, C. C., R. K. Rabeler, and W. J. Elisens. 2019. Plantaginaceae. *In* Flora of North America Editorial Committee [eds.], Flora of North America, north of Mexico, 17, 11-323. Oxford University Press, New York, New York, USA.

Fricke, E. C., A. Ordonez, H. S. Rogers, and J.-C. Svenning. 2022. The effects of defaunation on plants' capacity to track climate change. *Science* 375: 210–214.

Garbowski, M., B. Avera, J. H. Bertram, J. S. Courkamp, J. Gray, K. M. Hein, R. Lawrence, et al. 2020. Getting to the root of restoration: Considering root traits for improved restoration outcomes under drought and competition. *Restoration Ecology* 28: 1384–1395.

Gay, L., J. Dhinaut, M. Jullien, R. Vitalis, M. Navascués, V. Ranwez, and J. Ronfort. 2022. Evolution of flowering time in a selfing annual plant: Roles of adaptation and genetic drift. *Ecology and Evolution* 12: e8555.

Gelman, A., and J. Hill. 2006. Data analysis using regression and multilevel/hierarchical models. Cambridge University Press, Cambridge, UK.

González-Varo, J. P., B. Rumeu, J. Albrecht, J. M. Arroyo, R. S. Bueno, T. Burgos, L. P. da Silva, et al. 2021. Limited potential for bird migration to disperse plants to cooler latitudes. *Nature* 595: 75–79.

Goodwillie, C., S. Kalisz, and C. G. Eckert. 2005. The evolutionary enigma of mixed mating systems in plants: occurrence, theoretical explanations, and empirical evidence. *Annual Review of Ecology, Evolution, and Systematics* 36: 47–79.

Grainger, T. N., and J. M. Levine. 2022. Rapid evolution of life-history traits in response to warming, predation and competition: A meta-analysis. *Ecology Letters* 25: 541–554.

Grant, P. R., and B. R. Grant. 2002. Unpredictable evolution in a 30-year study of Darwin's finches. *Science* 296: 707–711.

Hairston Jr., N. G., S. P. Ellner, M. A. Geber, T. Yoshida, and J. A. Fox. 2005. Rapid evolution and the convergence of ecological and evolutionary time. *Ecology Letters* 8: 1114–1127.

Hamann, E., D. Denney, S. Day, E. Lombardi, M. I. Jameel, R. MacTavish, and J. T. Anderson. 2021. Plant eco-evolutionary responses to climate change: Emerging directions. *Plant Science* 304: 110737.

Harrison, S., and M. LaForgia. 2019. Seedling traits predict drought-induced mortality linked to diversity loss. *Proceedings of the National Academy of Sciences* 116: 5576–5581.

Heberling, J. M., and B. L. Isaac. 2017. Herbarium specimens as exaptations: New uses for old collections. *American Journal of Botany* 104: 963–965.

Hofman, A. C., L. Espeland, I. Steinsland, and E. M. Ingeström. 2022. A shared parameter model for systolic blood pressure accounting for data missing not at random in the HUNT Study. arXiv:2203.16602v1 [stat.AP] Website: https://arxiv.org/abs/2203.16602 [preprint].

Huang, R., Z.-D. Zhang, Y. Wang, and Y.-Q. Wang. 2021. Genetic variation and genetic structure within metapopulations of two closely related selfing and outcrossing *Zingiber* species (Zingiberaceae). *AoB Plants* 13: plaa065.

Huisinga, K. D., and T. J. Ayers. 1999. Vascular plants of Arizona: Plantaginaceae Plantain Family. *Journal of the Arizona-Nevada Academy of Science* 32: 62–76.

Johnson, S. E., E. Hamann, and S. J. Franks. 2022. Rapid, parallel evolution of field mustard (*Brassica rapa*) under experimental drought. *Evolution* 76: 262–274.

Jullien, M., M. Navascués, J. Ronfort, K. Loridon, and L. Gay. 2019. Structure of multilocus genetic diversity in predominantly selfing populations. *Heredity* 123: 176–191.

Jump, A. S., and J. Penuelas. 2005. Running to stand still: Adaptation and the response of plants to rapid climate change. *Ecology Letters* 8: 1010–1020.

Koelling, V. A., J. L. Hamrick, and R. Mauricio. 2011. Genetic diversity and structure in two species of *Leavenworthia* with self-incompatible and self-compatible populations. *Heredity* 106: 310–318.

Kogan, F., and W. Guo. 2015. 2006–2015 mega-drought in the western USA and its monitoring from space data. *Geomatics, Natural Hazards and Risk* 6: 651–668.

Kooyers, N. J., K. A. Morioka, J. M. Colicchio, K. S. Clark, A. Donofrio, S. K. Estill, C. R. Pascualy, et al. 2021. Population responses to a historic drought across the range of the common monkeyflower (*Mimulus guttatus*). *American Journal of Botany* 108: 284–296.

Kreiner, J. M., S. M. Latorre, H. A. Burbano, J. R. Stinchcombe, S. P. Otto, D. Weigel, and S. I. Wright. 2022. Rapid weed adaptation and range expansion in response to agriculture over the past two centuries. *Science* 378: 1079–1085.

Kruuk, L. E., T. H. Clutton-Brock, J. Slate, J. M. Pemberton, S. Brotherstone, and F. E. Guinness. 2000. Heritability of fitness in a wild mammal population. *Proceedings of the National Academy of Sciences, USA* 97: 698–703.

Lambrecht, S. C., A. K. Gujral, L. J. Renshaw, and L. T. Rosengreen. 2020. Evolutionary and plastic changes in a native annual plant after a historic drought. *Ecology and Evolution* 10: 4570–4582.

Larson, J. E., B. L. Anacker, S. Wanous, and J. L. Funk. 2020. Ecological strategies begin at germination: Traits, plasticity and survival in the first 4 days of plant life. *Functional Ecology* 34: 968–979.

Legg, S. 2021. IPCC, 2021: Climate change 2021-the physical science basis. Contribution of working group 1 to the sixth assessment report of the [United Nations] Intergovernmental Panel on Climate Change. *Interaction* 49: 44–45.

Lenth, R. V. 2021. emmeans: Estimated marginal means, aka least-squares means. R package version 1.6. 1. Website: https://github.com/rvlenth/emmeans

Meineke, E. K., and T. J. Davies. 2019. Museum specimens provide novel insights into changing plant–herbivore interactions. *Philosophical Transactions of the Royal Society, B, Biological Sciences* 374: 20170393.

Meineke, E. K., T. J. Davies, B. H. Daru, and C. C. Davis. 2019. Biological collections for understanding biodiversity in the Anthropocene. *Philosophical Transactions of the Royal Society, B, Biological Sciences* 374: 20170386.

Menzel, A., Y. Yuan, M. Matiu, T. Sparks, H. Scheifinger, R. Gehrig, and N. Estrella. 2020. Climate change fingerprints in recent European plant phenology. *Global Change Biology* 26: 2599–2612.

Metz, J., C. Lampei, L. Bäumler, H. Bocherens, H. Dittberner, L. Henneberg, J. de Meaux, and K. Tielbörger. 2020. Rapid adaptive evolution to drought in a subset of plant traits in a large-scale climate change experiment. *Ecology Letters* 23: 1643–1653.

Mousseau, T. A., and D. A. Roff. 1987. Natural selection and the heritability of fitness components. *Heredity* 59: 181–197.

Nadeau, C. P., and M. C. Urban. 2019. Eco-evolution on the edge during climate change. *Ecography* 42: 1280–1297.

Nevo, E., Y.-B. Fu, T. Pavlicek, S. Khalifa, M. Tavasi, and A. Beiles. 2012. Evolution of wild cereals during 28 years of global warming in Israel. *Proceedings of the National Academy of Sciences, USA* 109: 3412–3415.

Nguyen, M. A., A. E. Ortega, K. Q. Nguyen, S. Kimball, M. L. Goulden, and J. L. Funk. 2016. Evolutionary responses of invasive grass species to variation in precipitation and soil nitrogen. *Journal of Ecology* 104: 979–986.

Nogués-Bravo, D., F. Rodríguez-Sánchez, L. Orsini, E. de Boer, R. Jansson, H. Morlon, D. A. Fordham, and S. T. Jackson. 2018. Cracking the code of biodiversity responses to past climate change. *Trends in Ecology & Evolution* 33: 765–776.

Oksanen, J., F. G. Blanchet, R. Kindt, P. Legendre, P. R. Minchin, R. B. O'hara, G. L. Simpson, et al. 2022. Package 'vegan'. Community ecology package, ordination, diversity and dissimilarities, version 2.6, 2 April 2022. Website: https://github.com/vegandevs/vegan

Orsini, L., K. Schwenk, L. De Meester, J. K. Colbourne, M. E. Pfrender, and L. J. Weider. 2013. The evolutionary time machine: Using dormant propagules to forecast how populations can adapt to changing environments. *Trends in Ecology & Evolution* 28: 274–282.

R Core Team. 2021. R: A language and environment for statistical computing. R Foundataion for Statistical computing, Vienna, Austria. Website: http://www.R-project.org

Rafferty, N. E., J. M. Diez, and C. D. Bertelsen. 2020. Changing climate drives divergent and nonlinear shifts in flowering phenology across elevations. *Current Biology* 30: 432–441.

Rauschkolb, R., Z. Li, S. Godefroid, L. Dixon, W. Durka, M. Májeková, O. Bossdorf, et al. 2022. Evolution of plant drought strategies and herbivore tolerance after two decades of climate change. *New Phytologist* 235: 773–785.

Rey, O., E. Danchin, M. Mirouze, C. Loot, and S. Blanchet. 2016. Adaptation to global change: A transposable element–epigenetics perspective. *Trends in Ecology & Evolution* 31: 514–526.

Rhoné, B., R. Vitalis, I. Goldringer, and I. Bonnin. 2010. Evolution of flowering time in experimental wheat populations: A comprehensive approach to detect genetic signatures of natural selection. *Evolution: International Journal of Organic Evolution* 64: 2110–2125.

Rogalski, M. A. 2015. Tainted resurrection: metal pollution is linked with reduced hatching and high juvenile mortality in *Daphnia* egg banks. *Ecology* 96: 1166–1173.

Ryan, E. M., and E. E. Cleland. 2021. Clinal variation in phenological traits and fitness responses to drought across the native range of California poppy. *Climate Change Ecology* 2: 100021.

Schipper, A. M., J. P. Hilbers, J. R. Meijer, L. H. Antão, A. Benítez-López, M. M. de Jonge, L. H. Leemans, et al. 2020. Projecting terrestrial biodiversity intactness with GLOBIO 4. *Global Change Biology* 26: 760–771.

Schmidt, P., J. Hartung, J. Bennewitz, and H.-P. Piepho. 2019. Heritability in plant breeding on a genotype-difference basis. *Genetics* 212: 991–1008.

Seager, R., M. Ting, I. Held, Y. Kushnir, J. Lu, G. Vecchi, H.-P. Huang, et al. 2007. Model projections of an imminent transition to a more arid climate in southwestern North America. *Science* 316: 1181–1184.

Sekor, M. R., and S. J. Franks. 2018. An experimentally introduced population of *Brassica rapa* (Brassicaceae). 2. Rapid evolution of phenotypic traits. *Plant Ecology and Evolution* 151: 293–302.

Sharma, N., P. Koul, and A. K. Koul. 1992. Reproductive biology of *Plantago*: Shift from cross-to self-pollination. *Annals of Botany* 69: 7–11.

Shaw, R. G., and J. R. Etterson. 2012. Rapid climate change and the rate of adaptation: Insight from experimental quantitative genetics. *New Phytologist* 195: 752–765.

Shryock, D. F., L. K. Washburn, L. A. DeFalco, and T. C. Esque. 2021. Harnessing landscape genomics to identify future climate resilient genotypes in a desert annual. *Molecular Ecology* 30: 698–717.

Siepielski, A. M., M. B. Morrissey, M. Buoro, S. M. Carlson, C. M. Caruso, S. M. Clegg, T. Coulson, et al. 2017. Precipitation drives global variation in natural selection. *Science* 355: 959–962.

Steinsland, I., C. T. Larsen, A. Roulin, and H. Jensen. 2014. Quantitative genetic modeling and inference in the presence of nonignorable missing data. *Evolution* 68: 1735–1747.

Stotz, G. C., C. Salgado-Luarte, V. M. Escobedo, F. Valladares, and E. Gianoli. 2021. Global trends in phenotypic plasticity of plants. *Ecology Letters* 24: 2267–2281.

Sultan, S. E., T. Horgan-Kobelski, L. M. Nichols, C. E. Riggs, and R. K. Waples. 2013. A resurrection study reveals rapid adaptive evolution within populations of an invasive plant. *Evolutionary Applications* 6: 266–278.

Summers, J. L., B. Bernik, C. J. Saunders, J. S. McLachlan, and M. J. Blum. 2018. A century of genetic variation inferred from a persistent soil-stored seed bank. *Evolutionary Applications* 11: 1715–1731.

Sun, Y., O. Bossdorf, R. D. Grados, Z. Liao, and H. Müller-Schärer. 2020. Rapid genomic and phenotypic change in response to climate warming in a widespread plant invader. *Global Change Biology* 26: 6511–6522.

Thomann, M., E. Imbert, R. C. Engstrand, and P.-O. Cheptou. 2015. Contemporary evolution of plant reproductive strategies under global change is revealed by stored seeds. *Journal of Evolutionary Biology* 28: 766–778.

Thompson, J. N. 1998. Rapid evolution as an ecological process. *Trends in Ecology & Evolution* 13: 329–332.

Toczydlowski, R. H., and D. M. Waller. 2019. Drift happens: Molecular genetic diversity and differentiation among populations of jewelweed (*Impatiens capensis* Meerb.) reflect fragmentation of floodplain forests. *Molecular Ecology* 28: 2459–2475.

Valencia-Montoya, W. A., E. Flaven, J. Pouzadoux, E. Imbert, and P.-O. Cheptou. 2021. Rapid divergent evolution of an annual plant across a latitudinal gradient revealed by seed resurrection. *Evolution* 75: 2759–2772.

Van Dijk, H., and N.-C. Hautekèete. 2014. Evidence of genetic change in the flowering phenology of sea beets along a latitudinal cline within two decades. *Journal of Evolutionary Biology* 27: 1572–1581.

Vigouroux, Y., C. Mariac, S. De Mita, J.-L. Pham, B. Gérard, I. Kapran, F. Sagnard, et al. 2011. Selection for earlier flowering crop associated with climatic variations in the Sahel. *PLoS One* 6: e19563.

Vtipil, E. E., and S. N. Sheth. 2020. A resurrection study reveals limited evolution of phenology in response to recent climate change across the geographic range of the scarlet monkeyflower. *Ecology and Evolution* 10: 14165–14177.

Wadgymar, S. M., D. B. Lowry, B. A. Gould, C. N. Byron, R. M. Mactavish, and J. T. Anderson. 2017. Identifying targets and agents of selection: innovative methods to evaluate the processes that contribute to local adaptation. *Methods in Ecology and Evolution* 8: 738–749.

Wang, T., A. Hamann, D. Spittlehouse, and C. Carroll. 2016. Locally downscaled and spatially customizable climate data for historical and future periods for North America. *PLoS One* 11: e0156720.

Weider, L. J., P. D. Jeyasingh, and D. Frisch. 2018. Evolutionary aspects of resurrection ecology: Progress, scope, and applications—An overview. *Evolutionary Applications* 11: 3–10.

Weis, A. E. 2018. Detecting the "invisible fraction" bias in resurrection experiments. *Evolutionary Applications* 11: 88–95.

Welles, S. R., and J. L. Funk. 2021. Patterns of intraspecific trait variation along an aridity gradient suggest both drought escape and drought tolerance strategies in an invasive herb. *Annals of Botany* 127: 461–471.

Williams, A. P., B. I. Cook, and J. E. Smerdon. 2022. Rapid intensification of the emerging southwestern North American megadrought in 2020–2021. *Nature Climate Change* 12: 232–234.

Williams, J. L., B. E. Kendall, and J. M. Levine. 2016. Rapid evolution accelerates plant population spread in fragmented experimental landscapes. *Science* 353: 482–485.

Wooliver, R., S. B. Tittes, and S. N. Sheth. 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* 74: 1699–1710.

Wu, L., A. D. Bradshaw, and D. A. Thurman. 1975. Potential for evolution of heavy metal tolerance in plants. III. *The rapid evolution of copper tolerance in Agrostis stolonifera. Heredity* 34: 165–187.

## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**Appendix S1**. Supporting figures.

**Appendix S2**. Supporting materials and methods.

**Appendix S3**. Supporting tables.

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