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Bet hedging is not sufficient to explain intraspecific variation in germination patterns of a winter annual plant

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Abstract

Bet hedging consists of life history strategies that buffer against environmental variability by trading off immediate and long-term fitness. Delayed germination in annual plants is a classic example of bet hedging, and is often invoked to explain low germination fractions. We examined whether bet hedging explains low and variable germination fractions among 20 populations of the winter annual plant *Clarkia xantiana* ssp. *xantiana* that experience substantial variation in reproductive success among years. Leveraging 15 years of demographic monitoring and 3 years of field germination experiments, we assessed the fitness consequences of seed banks and compared optimal germination fractions from a density-independent bet-hedging model to observed germination fractions. We did not find consistent evidence of bet hedging or the expected trade-off between arithmetic and geometric mean fitness, though delayed germination increased long-term fitness in 7 of 20 populations. Optimal germination fractions were 2 to 5 times higher than observed germination fractions, and among-population variation in germination fractions were not correlated with risks across the life cycle. Our comprehensive test suggests that bet hedging is insufficient to explain the observed germination patterns. Understanding variation in germination strategies will likely require integrating bet hedging with complementary forces shaping the evolution of delayed germination.

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Introduction

Organisms across the tree of life exhibit life-history strategies that allow persistence in the face of environmental uncertainty. For annual plants, interannual variation in reproductive success driven by environmental variation can favor the evolution of delayed germination that establishes soil seed banks. Seed banks not only buffer plant populations against environmental change and stochasticity (Eager et al. 2014; Paniw et al. 2017), but also increase effective population size (Nunney 2002; Waples 2006), and maintain genetic diversity (McCue and Holtsford 1998). Theory thus suggests that seed banks have key ecological and evolutionary consequences (Evans and Dennehy 2005).

Evolutionary ecologists have long interpreted delayed germination, caused by persistent or variable seed dormancy, as a bet hedging strategy (Bulmer 1984; Cohen 1966; Ellner 1985*a*,*b*; Philippi and Seger 1989; Simons 2011). Bet hedging increases geometric mean fitness by reducing variability in reproductive success, even if it decreases the arithmetic mean fitness (Seger and Brockman 1987). At the level of individuals, this trade-off between fitness mean and variance is the product of a single genotype that expresses phenotypic variance (Philippi and Seger 1989; Seger and Brockman 1987). For example, a genotype that produces seeds with varying levels of dormancy may have lower fitness in years when all seedlings successfully set seed because only a fraction of the bet hedging genotype's seeds contribute to next year's population. However, geometric mean fitness is multiplicative and thus sensitive to variability in reproductive success between years. A seed bank prevents the bet hedging genotype's extinction if there is any chance of complete reproductive failure. Genotypes without delayed germination would be lost. The value of delayed germination also depends on how safe the seed bank is; if seed mortality is high, there is a greater risk to remaining in the soil (Cohen 1966). Ultimately, the individual-level advantage of bet hedging translates to the population-level by increasing long-term population growth rates and persistence.

Some empirical studies suggest that delayed germination, relative to a strategy with com-

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plete germination, meets the criteria for bet hedging (Clauss 1999; Evans et al. 2007; Gremer and Venable 2014; Kalisz and McPeek 1993). Specifically, these studies identify the following population-level patterns: (1) reduced arithmetic mean fitness but (2) lower variance in fitness (Clauss 1999), (3) higher long-term stochastic population growth rate (Kalisz and McPeek 1993), or all three at once (Evans et al. 2007; Gremer and Venable 2014). Some degree of delayed germination should be favored when there is a nonzero probability of complete reproductive failure (Cohen 1966). Species exhibit substantial intraspecific variation in germination fractions (e.g., Fernández-Pascual et al. 2013; Gremer et al. 2020; Torres-Martínez et al. 2017), and determining the fitness consequences of this variation remains an open area of inquiry.

If delayed germination functions as a bet hedging strategy that maximizes geometric mean fitness, the optimal germination fraction in a population is expected to have evolved in response to that population's seed mortality and temporal variability in reproductive success (Cohen 1966; Franch-Gras et al. 2017; Pinceel et al. 2021). A strong test of whether germination fractions are optimally adaptive would be to compare observed and optimal germination fractions, taking into account the complete life-history (Childs et al. 2010; Simons 2011). For example, interspecific comparisons of a winter annual plant community in the Sonoran Desert demonstrated the adaptive value of delayed germination; lower germination fractions were accurately predicted for species whose seeds experienced low mortality and whose seedlings had high variability in reproductive success (Gremer and Venable 2014). However, we are not aware of studies focused on whether intraspecific (among-population) differences in delayed germination reflect variation in the fitness consequences of germination. Such a test would provide evidence that delayed germination functions as bet hedging and determine the extent to which observed germination fractions maximize fitness (categories V and IV in Simons 2011).

Populations of the winter annual, *Clarkia xantiana* ssp. *xantiana*, in the southern Sierra Nevada are distributed across a complex landscape of varying elevation, slope, aspect, precipitation, and temperature (Fig. 1A; Eckhart et al. 2011; Gould et al. 2014). Despite early work that suggested the species lacked a seed bank (Lewis 1962), multiple lines of evidence now support the presence

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and relevance of a seed bank in populations of *C. xantiana* ssp. *xantiana*. In field experiments, seeds can germinate at least up to three years after being buried in bags (Eckhart et al. 2011) or pots (M. A. Geber, unpublished data). Fifteen years of surveys suggest that the seed bank allows some populations to persist exclusively as seeds for as long as 4 consecutive years (Fig. 1B). Seeds can also remain viable for up to 11 years when buried in bags 30 cm below the soil surface (D. A. Moeller, unpublished data). *Clarkia xantiana* ssp. *xantiana* seeds lack morphological adaptations for dispersal (Knies et al. 2004), and the species' small-scale spatial distribution is consistent with dispersal limitation (Kramer et al. 2011). We thus expect a limited role for dispersal to complement delayed germination under temporal variability (Venable and Brown 1988), and little seed dispersal among populations during this study.

A previous study of *C. xantiana* ssp. *xantiana* population dynamics identified a decrease in population growth rate from west to east across the species' distribution in the southern Sierra Nevada, CA (Eckhart et al. 2011). Germination rate of first-year seeds increased from west to east (Eckhart et al. 2011). Variability in rainfall during the growing season shows a similar pattern as germination, from less variable (and wetter) in the west to more variable (and drier) in the east (Eckhart et al. 2011). Demographic observations (Eckhart et al. 2011) and transplant experiments demonstrate that seed set can exhibit dramatic interannual variation associated with rainfall [e.g., 30-fold between a wet and dry year in Geber and Eckhart (2005)].

Here, we test whether observed germination fractions and life-history patterns in *Clarkia xantiana* ssp. *xantiana* are consistent with predictions made by bet hedging models. We combine 15 years of observations on reproductive success and 3 years of seed burial experiments from 20 populations to address the following questions. (1) Does delayed germination and the formation of a seed bank meet the criteria for bet hedging? Specifically, for each population, we test whether delayed germination decreases arithmetic mean fitness, reduces the variability in fitness, and increases the long-term stochastic population growth rate. Next, we tested whether the observed germination fractions are likely to be adaptive. (2) For each population, does the optimal germination fraction predicted by bet hedging models match observed germination fraction? We

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find that life-history patterns are not entirely consistent with bet hedging expectations. We thus examine the relationship between germination fraction and risk, both by seeds before germination and by seedlings after germination. Under bet hedging, we expect a negative correlation between germination fraction and risk, so we specifically ask the following questions: (3) Is there a negative correlation between germination fraction and seed survival across populations? (4) Is there a negative correlation between germination fraction and variability in per-capita reproductive success across populations?

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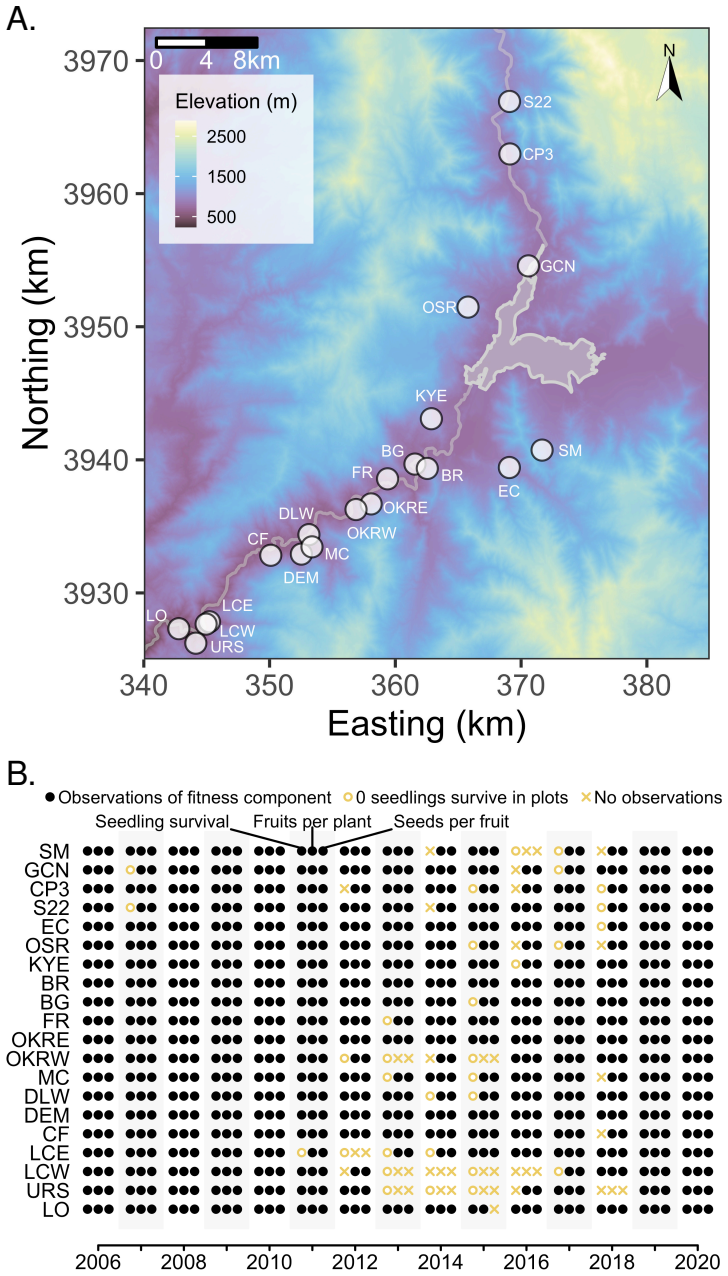

Figure 1: Map of the populations, and summary of aboveground observations of demography. (A) Elevation map of study populations. (B) Graphical summary of 15 years of aboveground observations at study populations. Open circles indicate that no seedlings survived in permanent plots; Xs indicate that no seedlings or plants were observed in surveys. Populations are arrayed from west (bottom) to east (top).

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Methods

Clarkia xantiana ssp. *xantiana* life history

Clarkia xantiana ssp. *xantiana* is a winter annual that germinates with late fall and winter rains, and sets seeds during the summer drought, in California's Mediterranean climate. In our study region, the Kern River Canyon and Valley (Kern and Tulare Counties, California, U.S.A.), germination happens from November through March. Seedlings grow in winter and spring, and surviving plants flower in late spring and early summer, late April into mid-June. Pollinated fruits set seed in the early summer, June to July, and fruits subsequently dry out and gradually split open. Most seeds appear to be shed from fruits within 3-4 months after production, but can remain on the plant for more than a year. Seeds are small ($<$ 1 mm in width) and have no structures to aid in aerial or other dispersal.

We represent the *Clarkia xantiana* ssp. *xantiana* life-history in terms of transitions from October of year t to October of year $t+1$. Transitions are the product of seed survival and germination, and aboveground seedling survival to fruiting, fruit production, and seeds per fruit. For this study, we assume that the new and old seeds differ in their survival rates in the seed bank, but do not include additional age structure and assume germination of new and old seeds is the same. We also assume that all plants experience the same vital rates upon germination. We describe population growth rate by the following equation:

$$\lambda = g_1 Y(t) s_0 s_1 + (1 - g_1) s_2 s_3. \tag{1}$$

Germination is given by g_1. Seed survival from seed production in June/July to the first October is s_0. Seed survival from the first October to germination in January/February is s_1. Survival of ungerminated seeds from January/February to the next October is s_2. Seed survival from October to the second germination opportunity the following January/February is s_3. Per-capita reproductive success in year t, $Y(t)$ is the product of seedling survival to fruiting, fruits per plant,

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and seeds per fruit. All parameters are summarized in Table 1.

Creating the dataset

We used field surveys and experiments to assemble observations of above- and below-ground demography for 20 populations of *Clarkia xantiana* ssp. *xantiana* across its range (Table 2, S1). A subset of the demographic data has been used to test hypotheses about geographic variation in population growth rate and species distributions (Eckhart et al. 2011; Pironon et al. 2018). Here, we used field surveys to collect data on seedling survival, fruit production, and seed set. We also conducted field experiments to observe emergence of seedlings and seeds remaining intact in the soil seed bank. We used the data from the surveys and experiments to estimate the demographic parameters that describe the life cycle (Equation 1). Ultimately, we used these estimates to calculate per-capita reproductive success, seed survival, and germination to test predictions of bet hedging models.

Field surveys for aboveground components of demography

We conducted field surveys of seedlings, fruiting plants, fruits per plant, and seeds per fruit at two spatial scales (Figure 2A; Eckhart et al. 2011). First, in October 2005, we established 30 1×0.5 m^2 permanent plots at each of the 20 study populations. The permanent plots were arrayed across four to six transects per site, and each plot was 2.5 m apart along a transect. Permanent plots were used for annual surveys of seedlings, fruiting plants, and fruits per plant. Second, additional, haphazardly distributed 1×0.5 m^2 plots were used each year to supplement estimates of fruits per plant from permanent plots, and to identify plants for fruit collection. By collecting fruits from plants outside the permanent plots, we did not affect seed input into the permanent plots.

To estimate the survival of seedlings to fruiting plants, we counted seedlings (n_{ijk}) and fruiting plants (y_{ijk}) in each permanent plot each year from 2006–2020. Seedlings and fruiting plants were counted in January/February and June, respectively, in plot i, year j, and population k.

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Of more than 8000 observations, there were fewer seedlings than fruiting plants in approximately 5% of observations; 50% of these had 1 fewer seedling than fruiting plant (Table S5). There are at least two possible sources of undercounts of seedlings. An observer might miss small seedlings that were present at the January/February seedling census, or additional seedlings emerged after the census. We assume that we did not under- or over-count fruiting plants because plants stand out from the background vegetation in June. To account for the undercount of seedlings, we recoded the data so that the count of seedlings was equal to the number of fruiting plants observed later in the season.

To determine the number of fruits per plant, we counted the number of fruits per plant on up to 15 plants in each of the permanent plots from 2007–2020, and on additional plants in the haphazardly distributed plots from 2006–2020 (Figure 2A). We combined counts from plants in permanent and haphazardly distributed plots, because the latter often sampled a broader distribution of plant sizes and combining them allowed us to better estimate fruit number per plant in years with relatively few plants in permanent plots.

From 2006–2012, we counted the number of undamaged fruits on a plant. We then took the damaged fruits on a plant and visually stacked them end to end to estimate how many additional undamaged fruits that was equivalent to (e.g., two half fruits corresponded to one undamaged fruit). We used this as our count (y_{ijk}^{TFE}) of total fruit equivalents on plant i, in year j, and in population k. From 2013–2020, we separately recorded the number of undamaged (y_{ijk}^{UF}) and damaged (y_{ijk}^{DF}) fruits on a plant.

From 2006–2020, we counted the number of seeds in one undamaged fruit (y_{ijk}^{US}) collected from each of 20-30 plants in the haphazardly distributed plots. Our counts corresponded to fruit i, in year j, and in population k. From 2013–2020, we also counted the number of seeds in one damaged fruit (y_{ijk}^{DS}) collected from each of 20-30 plants in the haphazardly distributed plots.

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Field experiments for belowground components of demography

We conducted a field experiment to estimate seed persistence from fall (October) to winter (January/February), emergence in the winter, and seed persistence from winter to fall (Fig. 2B). At each population, we buried seeds in mesh bags in the fall, counted intact seeds and seedlings in a subset of bags in the winter, and then retrieved those bags the following fall to count intact seeds and conduct a two-stage lab trial to assay viability of intact seeds. Seed persistence and emergence do not incorporate loss of seed viability because seeds that are intact in the field may not be viable. We thus combine the field and lab experiments to estimate seed survival and germination, which do account for loss of viability.

The experiment consisted of three rounds starting in October 2005, 2006, or 2007. For each round, we collected seeds at each population in summer before the round started. For each population, we pooled and distributed seeds across 5×5-cm nylon mesh bags (100 seeds/bag). In October, we returned the bags to the population at which the which seeds were collected, staked one bag near each permanent plot (Methods: Field surveys for aboveground demography) and covered the bags with soil.

In Round 1, we placed 30 bags at each population in October 2005. We unearthed a first set of 10 bags in January 2006 to count the number of intact seeds (y) and the number of seedlings (y_g) (Age 0 in Fig. 2B). We returned the bags to the ground until October 2006, when we retrieved bags to the lab to count intact seeds (y) and test seed viability (see below). In the second year of Round 1, we counted intact seeds and seedlings in a second set of 10 bags unearthed in January 2007 (Age 1 in Fig. 2B). We again returned these bags to the ground until October 2007, when we retrieved these 10 bags to count intact seeds and test seed viability. In the third year of Round 1, a third set of 10 bags was unearthed in January 2008 to count intact seeds and seedlings (Age 2 in Fig. 2B), and brought to the lab in October 2008 for seed counts and viability tests.

The experiment was repeated in all populations two more times. Round 2 started in October 2006 with 20 bags per population, and 10 bags were dug up in the first and second year (2007 and

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2008, respectively). Round 3 started in October 2007 with 10 bags per population, and 10 bags each were dug up after one year (2008). We thus made three sets of observations associated with age 0 seeds (brought to the lab after one year in the field), two sets of observations associated with age 1 seeds (brought to the lab after two years in the field), and one set of observations associated with age 2 seeds (brought to the lab after three years in the field).

In October of each experimental year, the seeds remaining intact in the subset of bags that were brought to the lab were counted and tested for viability in a two-stage trial (Fig. 2B). We placed up to 15 seeds from each bag on moist filter paper in a disposable cup; over a 10-day span, we counted and removed germinants every two days. Because we conducted 2-3 tests of 15 seeds each per bag, we summed the number of seeds tested ($n_{\mathrm{g}}^{\mathrm{viab}}$) and germinating ($y_{\mathrm{g}}^{\mathrm{viab}}$) to summarize the trials and successes.

After 10 days, up to 10 remaining ungerminated seeds were sliced in half and individually placed into 96-well plates filled with a solution of tetrazolium chloride, which stains viable tissue red. We covered the plates with foil. Each 96-well plate contained seed from at least one bag per population of a given seed-age class. We counted viable seeds every 2 days for 10 days. For each bag, we summed the number of seeds tested ($n_{\mathrm{v}}^{\mathrm{viab}}$) and staining ($y_{\mathrm{v}}^{\mathrm{viab}}$) to summarize the trials and successes.

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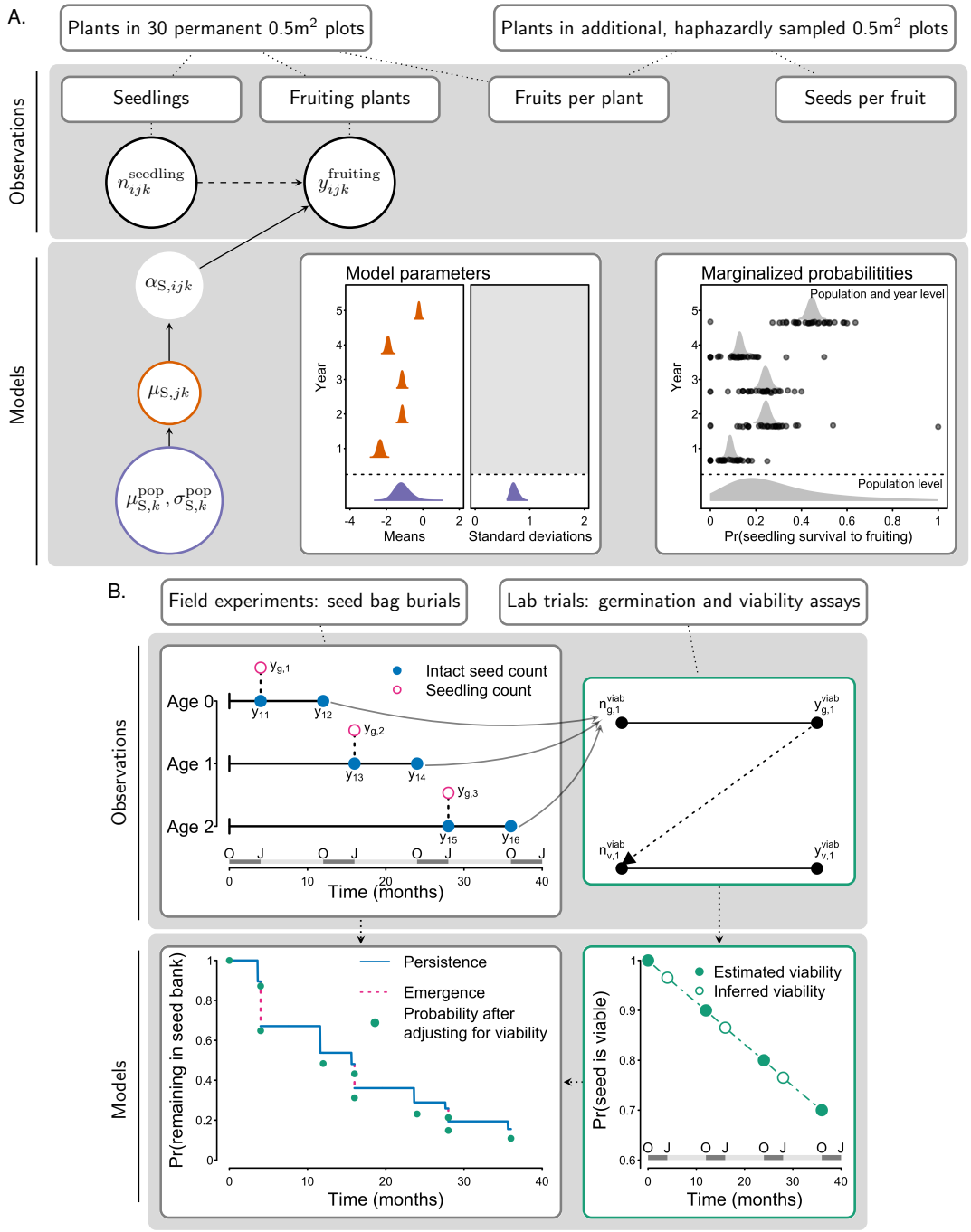

Figure 2: Graphical summary of the observations, models, and parameters used to estimate per-capita reproductive success, germination, and seed survival. (A) A graphical representation of the relationship between the structure of observations and the data. A directed acyclic graph for the model of seedling survival to fruiting, with colors corresponding to the simulated example in the plots showing the relationship between model parameters, marginalized probabilities, and data. (B) A graphical representation of the field seed bag experiments and lab viability trials. The experiments are related to estimates of seed survival, germination, and viability.

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Statistical models

We used observational and experimental data from 20 populations to estimate the demographic parameters that describe the life cycle (Fig. 2). To calculate variation in per-capita reproductive success for the study populations, we obtained annual estimates for seedling survival to fruiting, fruits per plant, and seeds per fruit from the field surveys. Because our goal was to compare patterns of seed bank dynamics among populations, we obtained population-level estimates for germination and seed survival from the seed bag burial experiment. We refer readers to Appendix S2 for a description of the statistical models, directed acyclic graphs, and for the mathematical expressions for the posterior proportional to the joint distribution for all the models.

Aboveground components of demography

We used a hierarchical, Bayesian approach to fit models to observations of seedling survival, fruits per plant, and seeds per fruit. As an example, we describe the structure of the model for seedling survival to fruiting, which is essentially a generalized linear mixed model with a binomial likelihood and a logit link (Fig. 2A). We use directed acyclic graphs (DAGs) to illustrate the relationship between the observations, the model, and parameters of interest. In the field, we counted seedlings ($n_{ijk}^{\text{seedlings}}$) and fruiting plants ($y_{ijk}^{\text{fruiting}}$) in plot i, year j, and population k. These quantities are outlined in black in the DAG and are shown as black points in the corresponding graphs. The model uses a binomial likelihood and relates the data to a probability of survival, α_{S}. This parameter is logit-transformed and links the year-level distribution, outlined in orange, to the observations. Parameters for the year-level distribution are annual estimates of the mean, which are drawn from the population-level distribution, outlined in purple. We write the model using hierarchical centering to account for the structure of our observations and for computational efficiency (Evans et al. 2010; Ogle and Barber 2020), but it is equivalent to a random effects structure in which years are nested within populations.

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The models for fruits per plant and seeds per fruit have a similar hierarchical structure but use Poisson likelihoods and a log link (Appendix S2.2.2). We separately modeled observations of total fruit equivalents per plant for 2006–2012 and total fruits per plant for 2013–2020. In years with observations of total fruits per plant, we also estimated the proportion of fruits that were undamaged vs. damaged. We estimated undamaged seeds per fruit for 2006–2020, and combined those estimates with counts of damaged seeds per fruit to infer the proportion of seeds that were lost to herbivory for 2013–2020. For each set of observations, we fit separate models to each population so that the resulting annual estimates were partially pooled towards the population-level mean. To make the two sets of observations for fruits per plant compatible, we used the proportion of fruits per plant that were damaged and the proportion of seeds lost to herbivory on a damaged fruit to calculate total fruit equivalents per plant from 2013–2020.

We chose to fit hierarchical, Bayesian models to our data for several reasons. First, hierarchical models perform well for making inferences about annual variation in demography (Metcalf et al. 2015). Second, the study period included substantial variation in sample size (Tables S2-S4, S6-S9), including years in which we did not observe plants in permanent plots even when they were present in the broader population (Fig. 1B). Hierarchical models for seedling survival introduce partial pooling, which allows us to account for sampling variation in fitting the model rather than post-hoc. Third, our approach makes it straightforward to quantify uncertainty associated with annual estimates of components of reproductive success. Fourth, estimating germination and seed survival from the seed bag experiment required combining three datasets (see below), a process that is a strength of Bayesian methods (Hobbs and Hooten 2015).

Belowground components of demography

Estimating seed survival and germination from the seed experiment required combining datasets. Here, we describe and graphically illustrate the model that we fit to observations from field experiments (Fig. 2B). The model we fit to the observational data jointly accounts for loss of seeds from the seed bank through mortality and germination. Germination occurs once a year in the

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winter, and is estimated from the seeds that germinate each year. Mortality occurs throughout the year, and is estimated from the seeds that remain intact. In Fig. 2B, the model describes the stairstep shape of the curve in the lower left panel. In practice, we fit a survival function that is the product of discrete germination and mortality hazards (Klein and Moeschberger 2003).

Separately, we obtained viability of seeds using the two-stage lab trials. Each lab trial consisted of two binomial experiments that measured (1) germination of intact seeds and then (2) viability of seeds that did not germinate. We combined these estimates to infer viability in each population and year. The lab trials involved destructive sampling, and we only conducted them when bags were retrieved in October (filled points in lower right panel of Fig. 2B). We inferred the viability of intact seeds in January by assuming that seeds lost viability at a constant rate (exponential decay). Further, we interpolated between estimates by assuming that viability changed at a constant rate between years, and that all seeds were viable at the start of the experiment (open points in lower right panel of Fig. 2B).

Finally, because plants set seed in July but the field experiments with seed bags did not start until October, we did not have direct observations to inform estimates s_0, the probability of seed survival from seed production in July to four months later in October. To infer seed survival during this part of the life cycle, we combined data from the field surveys and seed bag experiments (Elderd and Miller 2016). We assumed that the seedlings emerging in permanent plots in 2008 were primarily from seeds produced in permanent plots in the previous two years, 2006 and 2007, that survived to and germinated in 2008. We ignored contributions from older seeds, assuming for simplicity that they make up a small proportion of seedlings. We used counts of fruiting plants in the permanent plots, and estimates of seed set per fruiting plant, to calculate the average seed set per transect in 2006 and 2007. We then linked seed set, and estimates of seed survival and germination from the seed bag burial experiment, to the average number of seedlings observed in permanent plots. Once we joined these observations, we inferred s_0 as the proportion of seeds lost between seed set in July and October.

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Model statements, implementation, and fitting

We show the expressions for the posterior proportional to the joint distribution, and corresponding directed acyclic graphs, for all models in Appendix S2. Prior choice is described in Appendix S3, and Table S10 shows all parameters with associated priors. We prepared data for analysis using the tidyverse (Wickham and RStudio 2021) and tidybayes (Kay and Mastny 2020) packages in R version 3.6.2 (R Core Team 2019). We wrote, fit all models, and estimated posterior distributions using JAGS 4.10 with rjags (Plummer et al. 2019). We used the MCMCvis package to work with the model output, check chains for convergence, and recover posterior distributions (Youngflesh et al. 2021). We randomly generated initial conditions for all parameters with a prior by drawing from the corresponding probability distribution in R before passing the initial values to rjags. We ran three chains for 45,000 iterations. The first 10,000 iterations were for adaptation, the next 15,000 iterations were discarded as burn-in, and we sampled the following 15,000 iterations. We assessed convergence of the MCMC samples with visual inspection of trace plots, by calculating the Brooks-Gelman-Rubin diagnostic, $\hat{R}$, and by calculating the Heidelberg-Welch diagnostic (Hobbs and Hooten 2015; Elderd and Miller 2016).

Computing vital rates

In the following sections, we describe how we used estimates from the statistical models to obtain the parameters that describe the *Clarkia xantiana* ssp. *xantiana* life-history. The calculations summarized here are described in detail in Appendix S4.

Per-capita reproductive success

We calculated annual per-capita reproductive success as the number of seeds produced per seedling each year, on average (Gremer and Venable 2014; Venable 2007). In other words, it is the product of the annual mean probabilities of seedling survival to fruiting, fruits per plant, and seeds per fruit. We calculated the posterior mode of annual estimates for each of these

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parameters in each year (the orange distribution in Fig. 2A) and multiplied them to obtain the per-capita reproductive success in that year.

To compute per-capita reproductive success, we used 15 years of observations from each of 20 populations. Our observations throughout the study period include missing data that reflects natural variability in population size or the spatial distribution of plants at study populations (Fig. 1B). We accounted for missing data while calculating per-capita reproductive success. In some years (n=3), we observed no seedlings or fruiting plants in permanent plots or in additional plots distributed haphazardly across the population, while in other years (n=8) we observed seedlings but no fruiting plants at the population. We assumed that this reflects a true absence of fruiting plants in that year and that there was no seed set in these years, so we set fruits per plant and seeds per fruit to 0. In one year at one population, we observed a single fruiting plant with 3 fruits, from which we did not collect seeds. For this estimate, we substituted the population average of seeds per fruit. Finally, there were years (n=11) when there were no plants in permanent plots but we found plants elsewhere throughout the population. We had no information about seedling survival in these years, and so used the population's average for seedling survival to fruiting.

Belowground vital rates

Estimates from the seed bag burial experiment describe persistence, the probability that a seed remains intact in the seed bank, and emergence, the probability that an intact seed becomes a seedling. To estimate seed survival and germination, which account for loss of seed viability in our estimates of seed survival and germination, we combined information from the seed bag burial experiment and the lab trials (Table S11). First, we estimated the probability that seeds persist, or remain intact, in the seed bank (Fig. 2B). We combined estimates for persistence with the viability estimates to calculate seed survival, the probability that seeds remains intact and viable in the seed bank. Similarly, we combined estimates for emergence with viability to calculate germination, the probability that viable, intact seeds become seedlings. We used the

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seed survival (s_1, s_2, s_3) and germination (g_1) probabilities to test predictions from bet hedging theory. Because seed survival from seed set in July to October (s_0) implicitly included loss of seed viability, we did not adjust these estimates.

Analysis

Demographic test of bet hedging

We used estimates for the vital rate components to test whether delayed germination is an adaptive bet hedging trait in *Clarkia xantiana* ssp. *xantiana*. The life-history described by equation 1 incorporates a seed bank. Specifically, populations form a seed bank by delaying germination (i.e. $g_1 < 1$). Immediate germination ($g_1 = 1$) eliminates the seed bank, in which case equation 1 reduces to

$$\lambda = \frac{N(t+1)}{N(t)} = Y(t)s_0s_1. \tag{2}$$

Per capita reproductive success, $Y(t)$, is calculated as the product of seedling survival to fruiting, fruits per plant, and seeds per fruit. We tested whether delayed germination ($g_1 < 1$) functions as bet hedging by eliminating the seed bank (Eq. 2). If delayed germination is consistent with bet hedging, we expect eliminating the seed bank to increase arithmetic mean fitness, increase the variability in fitness, and decrease the long-term stochastic population growth rate (Clauss 1999; Evans et al. 2007).

To calculate the arithmetic mean population growth rate, we calculated an average environment growth rate, λ_a (Evans et al. 2007). We assumed that each of the 15 values for per-capita reproductive success, $Y(t)$, are equally likely. We obtained values for the average population growth rate with the field estimates of germination as well as with the seed bank eliminated ($g_1 = 1$). In each case, we used the posterior modes of the parameters in equations 1 or 2.

To calculate temporal variability in population growth rate, we drew 1,000 samples from the 15 years of per-capita reproductive success estimates with replacement. We paired these resampled years of estimates with the population-level values for germination and seed survival

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rates to calculate annual population growth rates. For both the case with and the case without a seed bank, we calculated the variance of the sequence of population growth rates.

To calculate the long-term stochastic population growth rate, we used the same sequence of population growth rates that we used to calculate temporal variability in fitness. We calculated the long-term stochastic population growth rate with the field estimates of germination, as well as with the seed bank eliminated ($g_1 = 1$). We used the following equation to calculate the stochastic population growth rate

$$\lambda_s = \exp\left(\frac{\sum \log(\frac{N(t)}{N(t-1)})}{1000}\right) = \exp\left(\frac{\sum \log(\lambda)}{1000}\right). \tag{3}$$

To examine the effect of uncertainty about parameter estimates on our results, we used the full posterior distribution for parameter estimates to calculate the arithmetic mean growth rate, temporal variability in population growth rate, and long-term stochastic population growth rate (Appendix S5.1).

Density-independent model for germination fractions

We calculated the optimal germination fraction for the observed variation in reproductive success and seed survival. For each population, we used a sequence of 1,000 resampled values for per-capita reproductive success, $Y(t)$, and the observed seed survival probabilities, s_0, s_1, s_2, and s_3, to calculate population growth rates at each germination fraction, G, along an evenly spaced grid of values from 0 and 1. Temporal variation was incorporated into the model by resampling per-capita reproductive success, $Y(t)$. The optimal germination fraction is the value of G that maximizes the geometric mean of the population growth rate. We found the optimal germination fraction by using a one-dimensional optimization algorithm to find the optimal G between 0 and 1 (Brent 1973). For each population, we replicated the optimization 50 times; each time, we drew a new sequence of years, $Y(t)$ and recalculated G. To summarize the results for each population, we calculated the median and 95% percentile intervals of these replicates. To

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evaluate the relationship between the optimal and observed germination fractions, we calculated the Pearson correlation coefficient between the median of the optimal G and the posterior mode of g_1.

To assess the influence of parameter uncertainty on optimal germination fractions, we examined how optimal G varied when we sampled from the posterior distribution of each parameter in the population model (Appendix S5.2). For each sample, we found the optimal germination fraction, G, for 50 replicates.

Correlation between germination and seed survival

We tested whether observed germination, g_1 was negatively correlated with seed survival, $s_2 s_3$. We calculated the probability that seeds which do not germinate in January remain in the seed bank until the following January. We obtained the posterior distribution for the correlation between germination and seed survival by calculating the sample correlation of g_1 and $s_2 s_3$ at each iteration of the MCMC output.

Correlation between germination and variability in per-capita reproductive success

We tested whether observed germination, g_1 was negatively correlated with the temporal variability in per-capita reproductive success for each population. We estimated variability by sampling the posterior distribution of reproductive success for each year and calculating the geometric SD of per capita reproductive success as exp(SD (log (per capita reproductive success+0.5))). We obtained the sample correlation of germination and geometric SD of per capita reproductive success at each iteration of the MCMC output.

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Results

Demographic test of bet hedging

To determine whether the seed bank meets the criteria for bet hedging, we compared the arithmetic mean population growth rate, variance in population growth rate, and long-term stochastic population growth rate with and without a seed bank. The arithmetic mean growth rate was greater without a seed bank than with a seed bank (Fig. 3A). The variability in population growth rates was also greater without a seed bank than with a seed bank (Fig. 3B). However, the long-term stochastic population growth rate was not always higher with a seed bank (Fig. 3C); the stochastic population growth rate was only greater with a seed bank in 7 out of 20 populations. These results were robust to uncertainty in parameter estimates (Fig. S7).

Observed germination fractions are lower than predicted by a density-independent model

To evaluate the density-independent model, we compared observed germination to predicted germination optima (Fig. 4). Optimal germination fractions were less than 1 in 13 out of 20 populations (Fig. 4). Optimal and observed germination fractions were uncorrelated (Fig. 4; r=-0.158, p=0.507). Predictions from the density-independent model were higher than observed germination fractions. These results were robust to uncertainty in parameter estimates, in most populations, but parameter uncertainty produced a wide range of optimal germination fractions for GCN and FR (Fig. S8).

Germination and seed survival are uncorrelated

To assess the relationship between germination and risk experienced by seeds that remain in the seed bank, we calculated the correlation between germination fraction and seed survival. We did

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not observe a correlation between germination and seed survival in the seed bank (Fig. 5A). The 95% credible interval for the posterior distribution of the correlation between germination and seed survival overlapped 0.

Germination and variability in per-capita reproductive success are uncorrelated

To assess the relationship between germination and risk experienced after germination, we calculated the correlation between germination fraction and geometric standard deviation in per-capita reproductive success. The correlation between germination and geometric standard deviation in per-capita reproductive success was negative (Fig. 5B). However, the 95% credible interval for the posterior distribution of the correlation overlapped 0, indicating that there was not strong support for a non-zero correlation between germination and variability in reproductive success.

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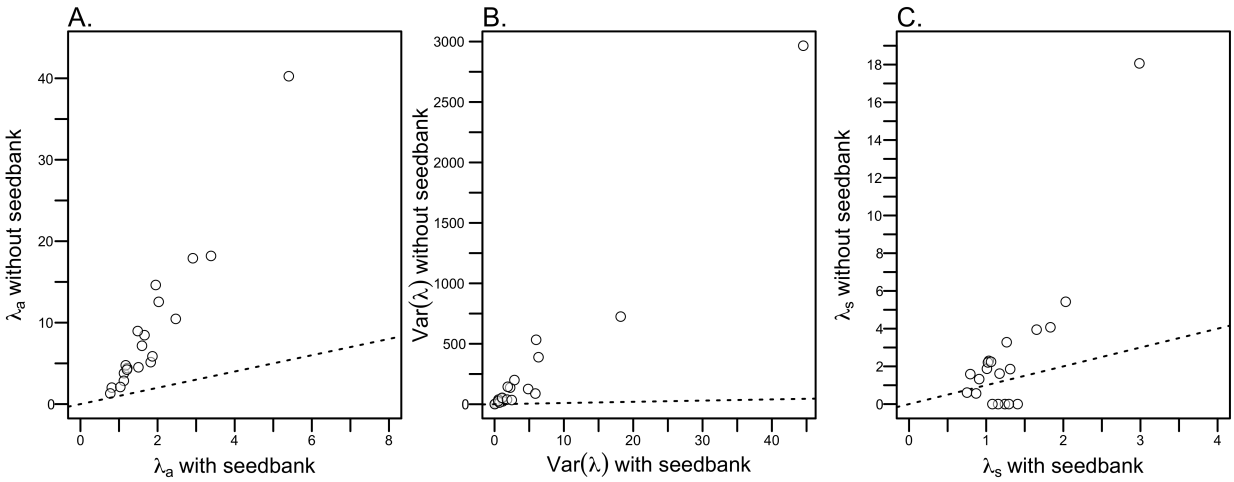

Figure 3: Test of the demographic patterns expected with bet hedging. (A) Plot of the arithmetic population growth rate without a seed bank against arithmetic population growth with a seed bank. (B) Plots of the variance in annual population growth rate without a seed bank against the variance in population growth rate with a seed bank. (C) Plot of the long-term stochastic population growth rate without a seed bank against the long-term stochastic growth rate without a seed bank. In all plots, the dotted line is the 1:1 line.

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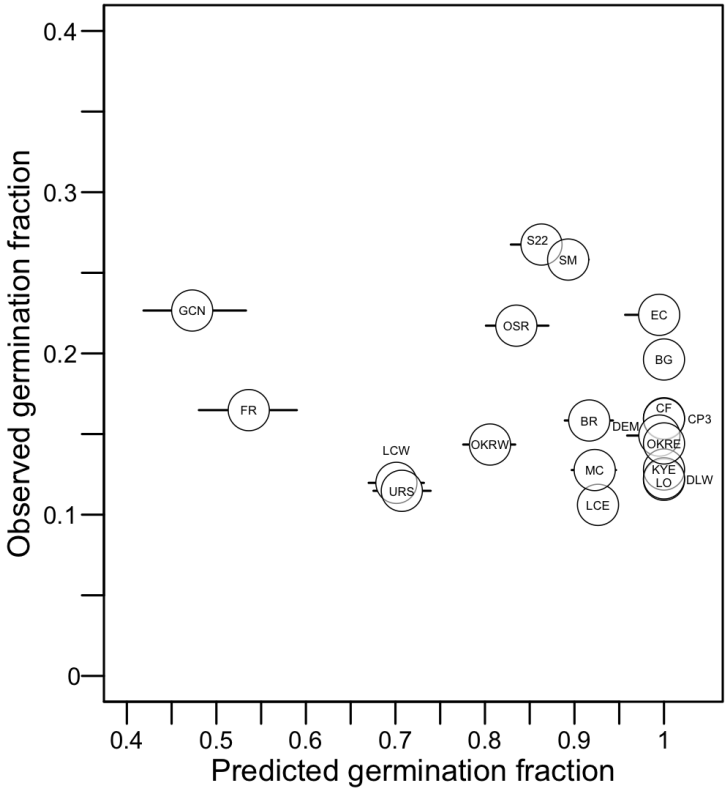

Figure 4: Comparison of observed and predicted, optimal germination fractions from a density-independent model of bet hedging. For each population, the observed germination fraction, g_1, is estimate from the model for seed bank vital rates. Each point is the population-specific mode of the posterior of g_1 for a model fit to data from seed bag experiments from 2005-2008 plotted against the predicted, optimal germination fractions. For each population, we found the optimal germination fraction for a density-independent population model. We ran 1000 replicates in which we resampled the annual estimates of per-capita reproductive success. Values for predicted germination fractions are the medians of these replicates, and the error bars are the 95% percentile intervals.

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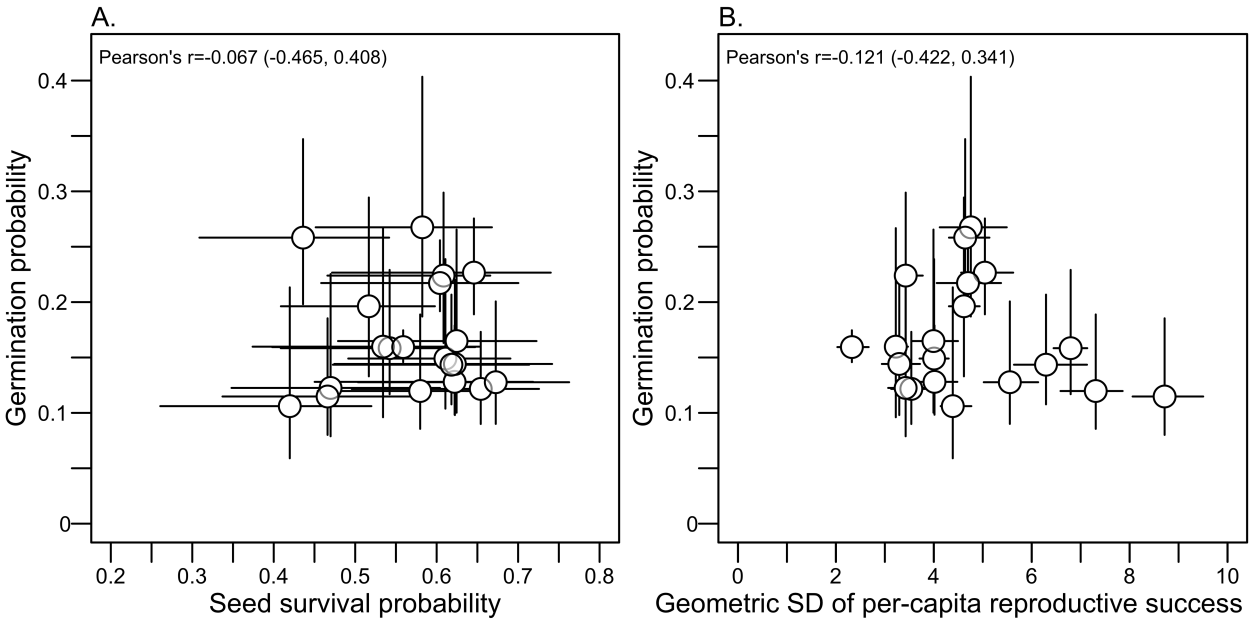

Figure 5: Relationship between germination and seed survival, and between germination and the geometric standard deviation of per-capita reproductive success. (A) The observed germination probability, g_1, plotted against probability of seed survival, $s_2 s_3$. (B) Correlation between observed germination probability, g_1, and the geometric standard deviation of per-capita reproductive success, a measure of the temporal temporal variability in per-capita reproductive success. In both panels, points are the posterior modes; error bars are the 68% highest posterior density intervals (under a normal distribution, 68% of the distribution is within ± 1 standard deviation).

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Discussion

We used an extensive demographic dataset to conduct an unusually comprehensive test of whether bet hedging explained germination patterns among populations of *Clarkia xantiana* ssp. *xantiana*. All 20 populations in our study exhibit delayed germination. However, we found weak support for the expected trade-off between arithmetic and geometric mean fitness, mixed support that delayed germination acts as bet hedging, and no evidence that observed germination fractions are adaptive under a density-independent bet hedging model. Observed germination fractions were also uncorrelated with risk experienced by seeds that remain in the seed bank or by plants after germination. Collectively, we interpret our results to suggest that delayed germination acting as bet hedging alone is insufficient to explain germination patterns among our study populations.

Demographic test of bet hedging

To determine if delayed germination functions as bet hedging in each population, we tested for a trade-off between arithmetic and geometric mean population growth rate mediated by reduced variability in population growth rate (Cohen 1966; Philippi and Seger 1989). We observed average germination fractions below 0.3 in all populations. However, our demographic analysis failed to demonstrate the expected trade-off between mean and stochastic population growth rate, despite 15 years of observations of reproductive success (Table 3). We evaluated a strategy with the observed germination fraction against a strategy with no seed bank (Evans et al. 2007). Delayed germination reduced average population growth rate (Fig. 3A) and variance in reproductive success (Fig. 3B). But for most populations, delayed germination did not increase long-term stochastic population growth rate (Fig. 3C).

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Observed germination fractions are lower than predicted by bet hedging models

To complement the demographic test of bet hedging, we calculated the optimal germination fractions that maximize each population's growth rate (Childs et al. 2010; Simons 2011). We derived these optimal germination fractions by parameterizing density-independent population models with estimates of seed survival and reproductive success (Gremer and Venable 2014). Some delayed germination was favored by the observed levels of seed mortality and temporal variability in reproductive success in 13 populations (Fig. 4; Table 3). Relative to the demographic test, the optimal germination fractions thus provide slightly more support for the idea that delayed germination acts as bet hedging. However, even when we predicted optimal germination fractions less than one, these were still much higher than germination fractions observed in the field. We may have underestimated germination if we missed seedlings that died before, or if there was additional germination after, our annual census of seed bags. But the predicted germination fractions are 2 to 5 times the observed fractions, and we think it is unlikely that we underestimated germination to this extent. We also did not find the expected positive correlation between observed and predicted germination fractions. Jointly, we interpret these results to suggest that even when delayed germination is favored, the observed germination fractions are lower than would be adaptive under density-independent bet hedging alone. Our results also parallel the findings in Gremer and Venable (2014) that density-independent models tend to predict higher germination fractions than observed in the field.

Germination and risk across the life cycle

Under bet hedging, we expected that seeds from populations that experienced a greater degree of risk in the seed bank would have lower germination fractions (Cohen 1966; Gremer and Venable 2014; Venable 2007). While high mortality risk in the soil seed bank should select against delayed germination, we did not find support for the expected relationship among germination and seed survival (Fig. 5A; Table 3). Some populations with low seed survival also exhibited low

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germination (e.g., FR, BR, SM), while some populations with high seed survival also had high germination (e.g., S22, CP3).

High variability in per-capita reproductive success should also select for delayed germination (Cohen 1966). However, in our study populations, variability in reproductive success was uncorrelated with germination (Fig. 5B; Table 3). We observed similar germination fractions (approximately 0.1) for populations with very different levels of variability in reproductive success (similar germination probabilities for a range of geometric standard deviations from 3-9 in Fig. 5B). Lack of support for this prediction is consistent with other results in our study. Populations with low variability in reproductive success and low germination were often the same populations that did not experience complete reproductive failure (Fig. 1B), for which stochastic population growth rates were higher without a seed bank, and for which we predicted high optimal germination fractions (e.g., OKRE, CP3 in Fig. 4).

Temporal variability in reproductive success

Delayed germination decreases arithmetic mean fitness, and the variance in fitness, because it dampens the effect of years with low per-capita reproductive success. To meet the criteria for bet hedging, delayed germination should also increase geometric mean fitness; whether it does so depends strongly on the minimum reproductive success or probability of reproductive failure (Childs et al. 2010; Cohen 1966; Evans et al. 2007). At the extreme, if there is no risk of reproductive failure, a strategy with delayed germination should always have lower geometric mean fitness than one with full germination. All populations in which stochastic population growth rate without a seed bank is lower than with a seed bank (URS, LCW, LCE, OKRW, FR, GCN, SM; Fig. 3C) either experienced reproductive failure or had no seedlings survive in permanent plots in at least one year (Fig. 1B). In contrast, populations in which stochastic population growth rate without a seed bank is higher than with a seed bank include those populations that either had some plants survive in permanent plots (LO) or populations in which plants set seed in all years. Although our demographic observations were exceptionally broad, 15 years of observations may

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have been insufficient to encounter reproductive failure in some populations. Our measurements may thus be conservative for testing predictions of bet hedging theory.

At the same time, California is experiencing an ongoing drought and the 2005-2020 study period included precipitation anomalies with severe ecological impacts (Cook et al. 2015; Prugh et al. 2018; Williams et al. 2022). Studies of bet hedging through delayed germination often assume precipitation variability is a primary driver of variability in fitness (e.g., Clauss and Venable 2000; Philippi 1993*b*; Tielbörger et al. 2012; Venable 2007). If this were the case in *C. xantiana* ssp. *xantiana*, our study should have had a high chance of observing its effects on reproductive success. Instead, it is possible that precipitation alone may not be enough to explain variation in reproductive success in some populations.

Seed mortality across the life cycle

While seed mortality in the seed bank after seeds have had the opportunity to germinate selects against delayed germination, seed mortality before seeds have had the opportunity to germinate favors the evolution of delayed germination (Gremer and Venable 2014). Seed mortality after the germination opportunity is a risk borne by seeds that remain in the seed bank. In contrast, seed mortality between seed production and the germination opportunity discounts reproductive success. It may thus be safer for a seed to remain in the seed bank if there is substantial seed mortality between seed set and the opportunity to germinate.

We conducted a follow-up analysis that shows the optimal germination fractions we predicted are more sensitive to estimates of seed survival before than after germination (Appendix S5.3). Optimal germination fractions could thus be lower than we predicted if we overestimated seed survival before germination (s_0 or s_1). To estimate survival from seed production in June/July to burial in October, s_0, we combined observations from surveys and field experiments. We may have overestimated survival if our approach failed to fully capture mortality due to seed predation. In addition, the seed bag burial experiments could have overestimated seed survival from October to January, s_1, if deep burial of seeds is a major source of loss from the seed bank, as bags

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prevent seeds from mixing into the soil. However, the experiments may underestimate survival if seed densities in bags are high enough to promote the growth of pathogenic fungi (Van Mourik et al. 2005). These caveats could also affect estimates of seed survival after germination (s_2 or s_3), but the optimal germination fraction is not as sensitive to these parameters.

Intra- and interspecific interactions shape optimal germination fractions

In this study, we considered a density-independent model of bet hedging, which is particularly sensitive to variability in reproductive success resulting from complete reproductive failure (Cohen 1966). However, density-dependence can also affect the value of delaying germination because competitors may alter reproductive success; years that would otherwise be good for growth and reproduction may become less favorable if there is strong competition (Ellner 1985*a*,*b*). Although our estimates of per-capita reproductive success implicitly incorporate the effects of density (Ellner 1985*b*), we did not explicitly model density-dependence in reproductive success. Optimal germination fractions may thus be lower than we predicted in this study if we were to calculate evolutionary stable strategies that account for competition (e.g., Gremer and Venable 2014).

More broadly, competitive and facilitative interactions with intra- and inter-specific plant neighbors, as well as with pollinators, herbivores, and seed predators, could all modify the temporal variability of reproductive success. Reproductive success in *C. xantiana* ssp. *xantiana* is affected by plant neighbors (James and Geber 2021), mammalian herbivores (Benning et al. 2019), and insect pollinators (Moeller 2004). If these interactions amplify variability in per-capita reproductive success, they could also favor lower germination fractions than those we predicted here (Brown and Venable 1991). Crucially, we would need to measure and model the temporal variability in the effect of these interactions in order to understand their impact on the evolution of delayed germination.

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Phenotypic plasticity in germination

To test bet hedging theory, we estimated fixed, population-level germination fractions with field experiments in which we collected and buried seeds in the same population. While we assumed that the germination fractions reflected genetic differentiation among populations, germination phenotypes are influenced by seed genotype, maternal genotype, and offspring or maternal environment (Clauss and Venable 2000; Lampei et al. 2017; Philippi 1993*a*; Tielbörger and Petrů 2010; Tielbörger et al. 2012). We could not partition the relative contribution of these influences in this study but, in general, germination phenotypes of *C. xantiana* ssp. *xantiana* do exhibit plasticity. In the field, germination varies interannually with rainfall (Geber and Eckhart 2005) and among microsites (James et al. 2020). In the lab, germination responds to water potential and temperature (I. Vergara and V. M. Eckhart, *unpublished data*). If germination reflects a response to environmental cues such as these, the distribution of those cues in the study years would determine the observed germination fractions (Clauss and Venable 2000). Studies that experimentally partition phenotypic variation in germination phenotypes of *C. xantiana* ssp. *xantiana* would be extremely valuable in complementing the present work.

Our results suggest that variation in germination fractions among populations of *Clarkia xantiana* ssp. *xantiana* is unlikely to be explained exclusively by bet hedging. Instead, we hypothesize that germination strategies are likely shaped by the combined influence of bet hedging and plasticity. Bet hedging assumes that the reproductive success is unpredictable at the time of germination (Cohen 1966). If germination responds to environmental cues that also predict reproductive success, plasticity should evolve in accordance to the correlation between the cue and fitness; such adaptive germination plasticity is termed predictive germination (Cohen 1967; Venable and Lawlor 1980). Empirical studies suggest that germination strategies may often be a mix of bet hedging and predictive germination (Clauss and Venable 2000; Evans et al. 2007; Gremer et al. 2016; Simons 2014). More generally, strategies are expected to combine bet hedging and plasticity in proportion to the uncertainty and predictability in the environment (Donaldson-Matasci et al.

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2013; Tufto 2015).

To incorporate predictive germination into our bet hedging model, we could build on the approach taken by Gremer et al. (2016). Briefly, we would estimate annual germination fractions and retain the observed correlation between germination and reproductive success when calculating population growth rates. Estimating the correlation between germination and reproductive success would require more data than we have with the three years of seed bag burial experiments. While it is beyond the scope of the present study, examining how bet hedging and plasticity jointly contribute to the evolution of delayed germination in *C. xantiana* ssp. *xantiana* would be an excellent task for future work.

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Author contributions

GS and MAG conceived of the ideas and analysis, using data collected by MAG, VME, and DAM. GS analyzed the data with input from MAG; GS wrote the manuscript with input from MAG. All authors contributed critically to the drafts.

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Tables

Table 1: Vital rate components of the structured population model.

Component	Description	Data contributing to quantity
SEED SURVIVAL		
s_0	Probability that a seed produced in July of year t is intact and viable in October of year t	Seed bag burial experiment, viability trials, seedling counts in permanent plots, fruiting plant counts in permanent plots, fruit per plant counts, seeds per fruit counts
s_1	Probability that a seed survives from October of year t to January of year $t+1$, for seeds produced in year t	Seed bag burial experiment, viability trials
s_2	Probability that a seed survives from January of year $t+1$ to October of year $t+1$, for seeds produced in year t	Seed bag burial experiment, viability trials
s_3	Probability that a seed survives from October of year $t+1$ to January of year $t+2$, for seeds produced in year t	Seed bag burial experiment, viability trials
GERMINATION		
g_1	Probability of germination for a seed that has survived to January of year $t+1$, for seeds produced in year t	Seed bag burial experiment, viability trials
PER-CAPITA REPRODUCTIVE SUCCESS		
σ	Probability of seedling survival to fruiting, from a January/February census through reproduction in June/July	Seedling count in permanent plots, fruiting plant count in permanent plots
F	Number of fruits per fruiting plant	Fruit counts on plants in permanent plots, fruit counts on plants in additional plots, seeds per fruit counts on plants in additional plots
ϕ	Number of seeds per fruit	Seeds per fruit counts on plants in additional plots

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Table 2: Summary of observations and experiments

Parameter data	Description	Time span
Seed vital rates	—	—
Seed survival and germination	Seed bag burial	2005-2008
Seed viability	Viability trials	2005-2008
Seedling survival	—	—
Seedling survival to fruiting	Field surveys	2006-2020
Fruits per plant	—	—
Total fruit equivalents per plant	Field surveys	2006-2012
Undamaged and damaged fruits per plant	Field surveys	2013-2020
Total fruit equivalents per plant	Extra plots	2006-2012
Undamaged and damaged fruits per plant	Extra plots	2013-2020
Seeds per fruit	—	—
Seeds per undamaged fruit	Lab counts	2006-2020
Seeds per damaged fruit	Lab counts	2013-2020

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Table 3: Summary of key results for tests of bet hedging.

Demographic test of bet hedging	Summary
Reproductive success accounting for complete reproductive failure	
1. $\lambda_a(\text{noSB}) > \lambda_a(\text{SB})$	20/20 populations
2. $\text{Var}\big(\lambda(\text{noSB})\big) > \text{Var}\big(\lambda(\text{SB})\big)$	20/20 populations
3. $\lambda_s(\text{noSB}) < \lambda_s(\text{SB})$	7/20 populations
Predicted vs. observed germination	**Summary**
Germination fractions less than 1	13/20 populations
Life history components	**Posterior mode (95% credible interval)**
Correlation between germination and seed survival	$\rho_{g,s_2s_3} = -0.067\ (-0.465, 0.408)$
Correlation between germination and geometric standard deviation of per-capita reproductive success	$\rho_{g,\text{GSD}} = -0.121\ (-0.422, 0.341)$

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Figure legends

Figure 1: Map of the populations, and summary of aboveground observations of demography. (A) Elevation map of study populations. (B) Graphical summary of 15 years of aboveground observations at study populations. Open circles indicate that no seedlings survived in permanent plots; Xs indicate that no seedlings or plants were observed in surveys. Populations are arrayed from west (bottom) to east (top).

Figure 2: Graphical summary of the observations, models, and parameters used to estimate per-capita reproductive success, germination, and seed survival. (A) A graphical representation of the relationship between the structure of observations and the data. A directed acyclic graph for the model of seedling survival to fruiting, with colors corresponding to the simulated example in the plots showing the relationship between model parameters, marginalized probabilities, and data. (B) A graphical representation of the field seed bag experiments and lab viability trials. The experiments are related to estimates of seed survival, germination, and viability.

Figure 3: Test of the demographic patterns expected with bet hedging. (A) Plot of the arithmetic population growth rate without a seed bank against arithmetic population growth with a seed bank. (B) Plots of the variance in annual population growth rate without a seed bank against the variance in population growth rate with a seed bank. (C) Plot of the long-term stochastic population growth rate without a seed bank against the long-term stochastic growth rate without a seed bank. In all plots, the dotted line is the 1:1 line.

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Figure 4: Comparison of observed and predicted, optimal germination fractions from a density-independent model of bet hedging. For each population, the observed germination fraction, g_1, is estimate from the model for seed bank vital rates. Each point is the population-specific mode of the posterior of g_1 for a model fit to data from seed bag experiments from 2005-2008 plotted against the predicted, optimal germination fractions. For each population, we found the optimal germination fraction for a density-independent population model. We ran 1000 replicates in which we resampled the annual estimates of per-capita reproductive success. Values for predicted germination fractions are the medians of these replicates, and the error bars are the 95% percentile intervals.

Figure 5: Relationship between germination and seed survival, and between germination and the geometric standard deviation of per-capita reproductive success. (A) The observed germination probability, g_1, plotted against probability of seed survival, $s_2 s_3$. (B) Correlation between observed germination probability, g_1, and the geometric standard deviation of per-capita reproductive success, a measure of the temporal temporal variability in per-capita reproductive success. In both panels, points are the posterior modes; error bars are the 68% highest posterior density intervals (under a normal distribution, 68% of the distribution is within ± 1 standard deviation).