LETTER

Within-and among-year germination in Sonoran Desert winter annuals: bet hedging and predictive germination in a variable environment

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Abstract

In variable environments, organisms must have strategies to ensure fitness as conditions change. For plants, germination can time emergence with favourable conditions for later growth and reproduction (predictive germination), spread the risk of unfavourable conditions (bet hedging) or both (integrated strategies). Here we explored the adaptive value of within- and among-year germination timing for 12 species of Sonoran Desert winter annual plants. We parameterised models with long-term demographic data to predict optimal germination fractions and compared them to observed germination. At both temporal scales we found that bet hedging is beneficial and that predicted optimal strategies corresponded well with observed germination. We also found substantial fitness benefits to varying germination timing, suggesting some degree of predictive germination in nature. However, predictive germination was imperfect, calling for some degree of bet hedging. Together, our results suggest that desert winter annuals have integrated strategies combining both predictive plasticity and bet hedging.

Keywords

Bet hedging, density dependence, desert annuals, dormancy, evolutionarily stable strategies, integrated strategies, population dynamic models, predictive plasticity, seed bank, within-year germination.

Ecology Letters (2016) **19**: 1209–1218

INTRODUCTION

In variable environments, it can be difficult for organisms to time life history functions, such as growth and reproduction, to coincide with favourable conditions. Faced with such difficulties, life histories may evolve to anticipate conditions through phenotypic plasticity or to spread the risk of uncertainty through time or space (Seger & Brockmann 1987; Philippi & Seger 1989; Simons 2011). While these two strategies are often studied in isolation, integrated strategies that incorporate elements of both phenotypic plasticity and bet hedging may also evolve (Cohen 1967; Donaldson-Matasci *et al.* 2013; Aikens & Roach 2014; Simons 2014; Botero *et al.* 2015). Rigorous empirical tests of bet hedging are rare, and even fewer studies assess both phenotypic plasticity and bet hedging simultaneously (Simons 2014). Here, we use data from long-term demographic monitoring to evaluate the role of bet hedging and plasticity for both within- and among-year germination fractions for 12 species of Sonoran Desert winter annual plants.

Germination is a critical transition at which a plant leaves the relative safety of the seed stage and enters the highly vulnerable seedling stage. As such, germination timing can have powerful effects on performance and is expected to be under strong selection. This is particularly true for annual plants whose seeds provide the only link to future years. In variable environments, traits may evolve to either enhance germination responses to cues predicting favourable conditions or spread the risk of germinating into unfavourable conditions through germinating at multiple times (Cohen 1967; Seger & Brockmann 1987; Philippi & Seger 1989; Clauss & Venable 2000; Simons 2014). In the first case (predictive plasticity or predictive germination), variation in germination timing is expected to correspond with fitness benefits (Cohen 1967). The second case describes biological bet hedging, in which mean fitness is sacrificed to reduce variance in fitness (Cohen 1966; Seger & Brockmann 1987; Philippi & Seger 1989; Simons 2011, 2014). Under bet hedging, lower germination fractions (proportions of seeds that germinate in any given year) reduce variation in fitness through time, hence increasing geometric mean fitness, but may not always maximise fitness in the short term. The utility of these different strategies depends on the predictability of the environment as well as the time scale of environmental variability experienced relative to generation time. It also may be advantageous to integrate both predictive germination and bet hedging (Cohen 1967; Donaldson-Matasci *et al.* 2013; Aikens & Roach 2014; Botero *et al.* 2015), such that more, but not all, seeds germinate in response to environmental cues that correlate imperfectly to favourable conditions for growth and reproduction.

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Germination timing is typically studied at one of two time scales: across years or growing seasons and within growing seasons (Turkington *et al.* 2005; Donohue *et al.* 2010). A robust literature on delaying germination for one or more years has demonstrated significant benefits for escaping predation, competition and variability in abiotic conditions (Cohen 1966, 1967; Bulmer 1984; Ellner 1985a,b, 1987; Venable & Brown 1988; Tielbörger & Valleriani 2005; Venable 2007; Gremer & Venable 2014). Germination timing can have similar advantages at the within-season time scale. For instance earlier germination provides more time to acquire resources as well as the opportunity to preempt resources that would otherwise be used by competitors (Lortie & Turkington 2002; Tielbörger & Prasse 2009; Donohue *et al.* 2010; Mercer *et al.* 2011). However, early germination may expose plants to greater risk of unfavourable conditions that may occur earlier in the growing season (Petru *et al.* 2006; Donohue *et al.* 2010; Mercer *et al.* 2011). Germinating at multiple times during the season can spread risk, analogously to among-year germination delay (Simons 2009).

Here, we explored the fitness consequences of within-season germination timing and how it interacts with among-year germination timing. In the Sonoran Desert, weather patterns are highly variable, both among and within growing seasons (Venable & Pake 1999; Davidowitz 2002; Huxman *et al.* 2004). Species differ in their response to this variability, in terms of among-year germination fractions as well as germination phenology within seasons (Venable & Pake 1999; Venable 2007; Kimball *et al.* 2011; Gremer & Venable 2014). Previous work in this system demonstrated that germination delay across years acts as a bet hedging strategy, allowing plants to spread the risk of unfavourable conditions among years and reduce variance in fitness (Pake & Venable 1996; Clauss & Venable 2000; Venable 2007; Gremer & Venable 2014). Furthermore, intraspecific competition impacts the fitness consequences of delayed germination (Gremer & Venable 2014). However, it remains unclear whether plants adaptively spread germination within growing seasons too. If so, we might expect both among-year delay and within-year diversification of germination timing to be used to reduce risk, with each of these strategies possibly lessening the need for the other (Simons 2014). Alternatively, if reliable cues are available, plants may shift within- and among-year timing of germination in an attempt to coordinate with variable timing of favourable conditions. Lastly, we may find evidence of both predictive germination and bet hedging at either or both temporal scales. In this study, we parameterised adaptive dynamic models with 32 years of demographic data on germinant performance and 25 years of data on seed bank dynamics for 12 abundant species in a Sonoran Desert winter annual community to (1) predict evolutionarily stable strategies (ESSs) for within- and among-year germination fractions and compare them to observed patterns and (2) evaluate the roles of predictive germination

Table 1 Mean germination timing and annual germination fractions for 12 species of Sonoran Desert winter annual plants

Species	Family	Abbrev.	Mean germination date	SE	Annual germination fraction	SE	Years with > 1 cohort
Erodium cicutarium (L.) L'Hér. ex Aiton	Geraniaceae	ERCI	7-Dec	5.72	0.66	0.01	15
Eriophyllum lanosum A. Gray	Asteraceae	ERLA	22-Dec	6.24	0.33	0.02	21
Erodium texanum A. Gray	Geraniaceae	ERTE	11-Dec	6.38	0.69	0.02	13
Evax multicaulis DC *(Evax verna)*	Asteraceae	EVMU	31-Dec	8.38	0.09	0.01	18
Monoptilon bellioides (A. Gray) H.M. Hall	Asteraceae	MOBE	16-Dec	7.26	0.67	0.02	18
Pectocarya heterocarpa I. M. Johnst	Boraginaceae	PEHE	18-Dec	6.11	0.30	0.02	14
Pectocarya recurvata I. M. Johnst	Boraginaceae	PERE	17-Dec	4.99	0.43	0.01	21
Plantago insularis Forssk *(Plantago ovata)*	Plantaginaceae	PLIN	12-Dec	7.42	0.54	0.02	21
Plantago patagonica Jacq.	Plantaginaceae	PLPA	15-Dec	6.61	0.48	0.02	21
Schismus barbatus (Loefl. ex L.) Thell.	Poaceae	SCBA	13-Dec	7.01	0.19	0.01	21
Stylocline micropoides A. Gray	Asteraceae	STMI	20-Dec	7.44	0.39	0.02	18
Vulpia octoflora (Walter) Rydb. *(Festuca octoflora)*	Poaceae	VUOC	17-Dec	7.64	0.31	0.02	14

Authorities are listed below species names. We use nomenclature consistent with previous work in the system; current synonymy is indicated by names in parentheses. Data for within-year germination date and years with > 1 cohort only includes years in which there was more than one germination cohort across all species (i.e., there was more than one germination triggering event in that season; maximum of 21 years). Means for annual germination fractions were calculated from annual data from 1990 to 2014. SE = one standard error of the mean; Abbrev. = Abbreviations.

and bet hedging within and across seasons. Together, these analyses provide valuable insight into the evolution of germination timing in a variable environment, and into the deployment of strategies at different time scales.

MATERIALS AND METHODS

Study system and field sampling

Detailed demographic data on this winter annual community have been collected since 1982 at the University of Arizona's Desert Laboratory at Tumamoc Hill in Tucson, AZ, USA (32°13′ N, 111°0′ W, 723 m above sea level) which has been protected from grazing since 1906 (Venable & Pake 1999; Clauss & Venable 2000; Venable & Kimball 2013; details in Appendix S1). At this site, Sonoran Desert winter annuals germinate with autumn and winter rains, usually between October and January of each year. Flowering occurs in early spring, and plants complete their life cycle before the onset of the fore-summer heat and drought in May (Venable & Pake 1999; Clauss & Venable 2000). In every year of the study, plots were visited *c*. 7–10 days after every autumn and winter rain event to record germination, which gives adequate time for seeds to respond to rain events (99% of emergence occurs within 10 days of germination triggering rain; U. Basinger and D.L. Venable, *personal communication*). Seeds generally germinate in discrete 3–8 day episodes following rainfall events which may be separated by a week or up to a few months (Huxman *et al.* 2004; Kimball *et al.* 2012). These discrete germination events correspond to germination 'cohorts'. Individual seedlings were mapped upon germination and followed until death to quantify lifetime survival and reproduction. Here, we use this detailed per-capita data on germination phenology, survival to reproduction (l), average number of seeds produced by plants that survived to reproduction (b), and lifetime fecundity (lb, the product of the previous two metrics) from 1982 to 2014. In this study, we focused on 12 usually abundant species in the winter annual community for which we have good demographic data across the sampling period (Table 1).

Within-year germination timing was quantified as the proportion of early vs. late germinating seeds, i.e., number of seeds germinating early in the season divided by the total number of seeds that germinated all season (hereafter p_e, the proportion of current year seedlings that germinated in the first cohort) and the number of seeds germinating after the first cohort ($1-p_e$). Lifetime fecundity (lb) was calculated separately for early and late cohorts in each year. Among-year germination fractions were estimated from a long-term study of seed bank dynamics that was initiated in the 1989/1990 field season (see Venable & Pake 1999; Venable 2007; and Appendix S1 for details). To study seed bank dynamics, seeds were separated from soil cores that were collected after all germination had occurred, usually in February, but before new seeds were produced in March or April. The fraction of seeds that germinated (among-year germination fraction, G) was determined from the density of seeds that germinated in the long-term plots (N) and the density of seeds that did not germinate as determined from the seed bank samples (S), giving the among-year germination fraction as $G = N/(N+S)$. Note that, while this measure of germination

fractions is measured at the population level, within-individual variation in germination has been observed to be consistent with these patterns (X.Y. Ge and D.L. Venable, *personal communication*). This measure does not account for possible mortality after germination but before emergence aboveground, a source of bias that we expect to be low since seeds are usually at, or very near, the soil surface at the time of germination (Pake & Venable 1996).

Density-dependent model for within- and among-year germination delay

We used an adaptive dynamics approach to find evolutionarily stable germination strategies (ESSs) in empirically parameterised density-dependent population models. Density-dependent models have been shown to better predict empirically observed among-year germination delay (Ellner 1987; Gremer & Venable 2014). Evolving germination phenotypes in our models consist of among-year germination fraction (G) and the proportion germinating early within seasons (p_e). The models calculate the finite rate of increase, λ, of a mutant germination phenotype in competition with a resident germination phenotype using the following equations parameterised with the long-term demographic data:

Resident:

$$n_r(t+1) = n_r(t)G_r p_{er} Y_e(t)S_n + n_r(t)G_r(1-p_{er})Y_l(t)S_n \\ + n_r(t)(1-G_r)WS_o \tag{1}$$

Mutant:

$$n_m(t+1) = n_m(t)G_m p_{em} Y_e(t)S_n + n_m(t)G_m(1-p_{em})Y_l(t)S_n \\ + n_m(t)(1-G_m)WS_o \tag{2}$$

Where

$$Y_e(t) = \frac{K_e(t)}{1 + aG_r p_{er} n_r(t)} \tag{3}$$

and

$$Y_l(t) = \frac{K_l(t)}{1 + aG_r n_r(t)} \tag{4}$$

and

$$\lambda = \frac{n(t+1)}{n(t)} \tag{5}$$

The first terms of eqns 1 and 2 describe growth due to the fraction of seeds germinating early (p_e) and the second terms refer to growth from the proportion of seeds that germinate late ($1-p_e$), while the third term describes seeds that did not germinate ($1-G$). Subscripts r and m refer to resident and mutant strategies, e and l describe early and late germinants respectively. Summer and winter seed survival rates (S and W) were estimated using the long-term seed bank study; S_n describes survival of newly produced seeds through their first summer until the following germination season, S_o refers to survival of older seeds (> 1 year old) during this same time period from reproductive season until germination season, W denotes the survival

of ungerminated seeds during the winter growing season, i.e., from the germination season until the reproductive season (see Appendix S1 and Gremer & Venable 2014 for details).

Y describes the per capita seed yield of each cohort. K is low-density fecundity (i.e., lb at low density) and a is the competition parameter. Ideally, we would estimate K and a for each of the > 700 species × year × cohort (early vs. late) combinations using the long-term demographic data, but natural variation in density among our plots for each cohort was not sufficient for such an analysis for many year and species combinations. In a previous paper, we estimated a and K for each species/year combination that pooled germination cohorts (Gremer & Venable 2014). Here we used the previously calculated species mean values for a. To estimate K for early and late cohorts, we used the previously determined estimates of K for each species/year combination with cohorts pooled (Gremer & Venable 2014), then we determined the ratio of this K(t) to observed average l(t)b(t) (pooled across germination cohorts for that species/year combination), and calculated the mean (across years) of those ratios for each species. We then multiplied the observed l(t)b(t) of early and late cohorts in each species/year combination by the mean of those ratios. For years with only one germination cohort, we assigned the same yield to both early and late cohorts. Because we assume the mutant strategy is at low density (n_m(t)→ 0), all competition is due to germinating residents. We parameterised competition such that early germinants compete only with other early germinants but late germinants compete with all individuals, which seemed reasonable due to the phenology of germination and growth in our system (Kimball *et al.* 2011). We explored other parameterisations, for example those that included competition only within cohorts (i.e., early vs. early, late vs. late), or competition with all cohorts (i.e., the same denominator in eqns 3 and 4), but results were qualitatively similar and did not improve fits with observed patterns.

Growth rates of mutant and resident phenotypes were projected for 1500 years (holding the mutant at low density) and the geometric mean growth rate of the mutant strategy was compared to that of the resident strategy. If the mutant strategy had higher fitness, the population was assumed to evolve to that value (a new resident strategy was set to the value of the old mutant strategy) and a new mutant strategy was tested. This was repeated until a value was reached that resisted invasion by further mutation (the ESS). Among-year germination fraction (G) as well as within-year germination fractions (p_e) were allowed to evolve. Because previous work did not provide evidence for temporal autocorrelation in yield values (Gremer & Venable 2014), we incorporated environmental stochasticity by randomly choosing each year from the empirically estimated combinations of K_e and K_l. In our search algorithm, we first mutated values of G until a mutant G strategy failed to invade. From this point, we then mutated values of p_e until no mutant p_e strategy could invade; from there, the algorithm returned to testing for invasion of alternate G phenotypes. This process was iterated until values of both G and p_e were reached that resisted invasion by any p_e or G mutant strategy. The entire search algorithm was replicated 9 times with different start values for G and p_e without

substantial effects on the ESS values. We then compared these ESS values to mean G and p_e observed in the long-term data for each species.

Fixed vs. plastic strategies

The ESS analyses above identified optimal values for constant within- and among-year germination fractions, but actual germination fractions in the field vary. To test whether observed variation in within-year and among-year germination fractions is adaptive, we compared the long-term fitness of fixed (i.e., constant) vs. variable germination strategies (testing each as the resident and then as the mutant strategy). This analysis was conducted separately for p_e and G. For each analysis, the fixed G or p_e strategy was set to the observed long-term mean from the field data. We also explored using the ESS germination strategy as the fixed strategy, but the results were qualitatively similar and are not presented. To create the variable strategy, we used observed combinations of K_e, K_l, and germination fractions (p_e or G) that occurred together in the different years of the long-term data set. This correctly represents observed plasticity in germination with its consequent yields. Stochasticity was incorporated by randomly selecting from these sets of values in the simulations. For these analyses, we only used data for years with more than one cohort. For each species, we competed a variable mutant strategy against a fixed resident strategy, as well as a fixed mutant strategy vs. a variable resident strategy for 1500 years, and evaluated whether mutant strategies could invade (i.e. mutant geometric mean λ > 1).

To evaluate how successful variation in germination fractions is at synchronising germination with fitness opportunities, we also created a mutant 'correctly-predicting' germination phenotype. For p_e, this correctly-predicting phenotype always allocated all germination into the cohort that, in retrospect, had the highest yield each year (e.g., if $Y_e(t)$ > $Y_l(t)$ then $p_e = 1$, if not, $p_e = 0$). For G, the correctly-predicting mutant phenotype had complete germination ($G = 1$) if the sum of the increase due to early and late germinants was greater than survival in the seedbank ($p_e Y_e(t)S_n + (1-p_e) Y_l(t)S_n > WS_o$), otherwise $G = 0$. By comparing these different germination phenotypes, we could evaluate the benefit of observed variation in germination to that of no variation and to correctly-predicting germination. If observed variation in germination timing is adaptive, we expected that variable germination phenotypes would invade fixed strategies. However, if observed germination timing is not able to completely synchronise germination with favourable fitness opportunities, we expected the correctly-predicting variable strategy to have higher fitness than the observed variable strategy.

Observed variation in germination timing in relation to fitness

Another way to assess the adaptive value of variable germination is to directly compare observed germination patterns to fitness results. To do this for within-year germination timing, we calculated the log fitness ratio, $\ln\left(\frac{l_t b_t(\text{early}) + 0.5}{l_t b_t(\text{late}) + 0.5}\right)$, and compared it to the proportion germinating early. Positive values

for this log fitness ratio indicate that early germinants had higher fitness than later germinants. Negative values indicate that later germinants had higher fitness.

If variation in germination timing synchronises emergence with favourable conditions for survival and reproduction, then years with higher early germination fractions should also have higher log fitness ratios. The same should be true for variation in among-year germination fraction (G) and per capita fecundity ($l(t)b(t)$). Across species, we expected that those with high average p_e should have higher mean log fitness ratios and vice versa. We tested these predictions using linear regression between log fitness ratios and p_e, and between lb and G for each species separately. G and p_e were arcsine-square root transformed prior to analysis, lb values were log-transformed [$\ln(lb + 1)$]. We then compared species mean p_e against species mean log fitness ratios, using only data for years in which there was more than one germination triggering rain event (i.e., number of cohorts was greater than 1). Finally, to explore the relationship between patterns of within-year timing (p_e) and among-year germination fractions (G), we used linear regression on species means.

RESULTS

Optimal trait values for within- and among-year germination fractions

For all species, predicted and observed among-year germination fractions (G) were less than one (Fig. 1a), and most within-year fractions (proportion early, p_e) were less than one but greater than zero (Fig. 1b; Appendix S3-1). *Pectocarya heterocarpa* (PEHE) was the only species with an optimal ESS within-year germination fraction (p_e) at one. Predicted ESS germination strategies corresponded well with observed mean germination fractions in the field (G: $r = 0.652$, $P = 0.022$; p_e: $r = 0.888$, $P = 0.0001$), though predicted values were often higher than observed.

Comparison of fixed vs. variable strategies

For all species, mutant strategies exhibiting the field-observed variation in p_e could invade a fixed resident strategy, suggesting that plasticity of within-year germination fraction is adaptive (Table 2). A mutant fixed p_e was able to invade variable resident strategies in three species, *Eriophyllum lanosum* (ERLA), *Evax multicaulis* (EVMU) and *Stylocline micropoides* (STMI). Either strategy has an intrinsic invasion advantage by virtue of being different and rare in a density-dependent world (Metcalf *et al.* 2015). In these three species, the advantage of rarity was sufficient to outbalance any fitness benefits of adaptive plasticity. For ERLA and STMI, mutants had higher invasion fitness with variable strategies than they had with fixed strategies, while for EVMU, the fixed strategy had slightly higher invasion fitness than the variable observed strategy. For the rest of the species, our results suggest that variable strategies consistently invade but resist invasion by fixed strategies. However, observed variation in within-year germination timing is imperfect, since the correctly-predicting mutant always had still higher invasion fitness and could invade either the observed variable or mean fixed p_e strategies (Table 2). For most species, invasion fitness of the correctly-predicting mutant was always higher when competing against a fixed strategy than when competing against a variable resident strategy, further evidence that observed variable germination is adaptive.

Mutant strategies with the observed among-year variation in G could invade resident fixed strategies in all species except the two *Erodium* species (ERCI, ERTE) and *Vulpia octoflora* (VUOC; Table 2). For those three species, mutant-fixed strategies could invade resident variable strategies, thus fixed strategies outperformed variable strategies as both mutant and resident. Consistent with results for p_e, we saw an advantage of the rare phenotype for *Evax* (EVMU) and *Stylocline* (STMI) such that the mutants with observed variation invaded fixed residents and vice versa. In addition to
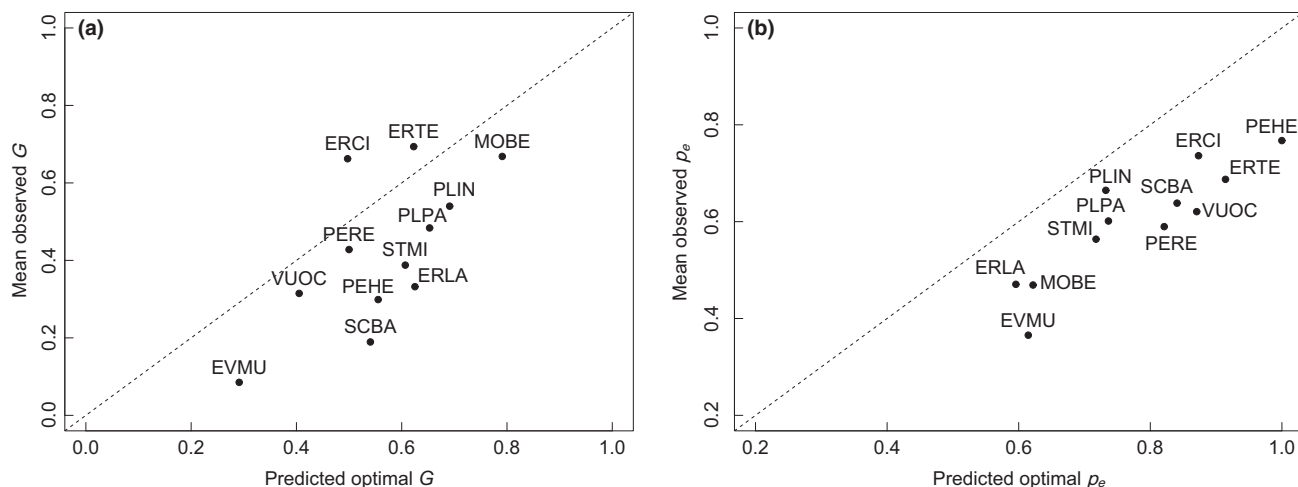

Figure 1 Observed germination fractions in relation to predicted evolutionarily stable strategies (ESSs) for all 12 species for (a) among-year germination fractions (G) and, (b) proportion of germinants in the early cohort (p_e). See Table 1 for species abbreviations. Dashed line indicates 1:1 line for reference. Values below this line indicate that predicted values are higher than observed and vice-versa.

Table 2 Invasion fitness (geometric mean λ) for mutants germination strategies, including: a mutant strategy with the variation in germination fractions observed during the long-term study (variable) competing against a resident with a fixed strategy; a fixed mutant strategy competing against a variable resident (fixed mutant); a 'correctly-predicting' mutant strategy competing with a fixed resident strategy; a correctly-predicting mutant competing against a variable resident (with observed variation). See Table 1 for species abbreviations. Fixed strategies were set to the long-term mean germination fractions. Values above 1 are in bold and indicate that the mutant strategy has higher fitness and can invade the resident population

Species	Variable mutant vs. fixed resident	Fixed mutant vs. variable resident	Correctly-predicting mutant vs. fixed resident	Correctly-predicting mutant vs. variable resident
Within-year germination (p_e)				
ERCI	**1.075**	0.933	**1.320**	**1.220**
ERLA	**1.043**	**1.010**	**1.160**	**1.134**
ERTE	**1.030**	0.973	**1.275**	**1.244**
EVMU	**1.021**	**1.035**	**1.123**	**1.151**
MOBE	**1.159**	0.906	**1.438**	**1.264**
PEHE	**1.045**	0.968	**1.094**	**1.043**
PERE	**1.128**	0.909	**1.230**	**1.088**
PLIN	**1.101**	0.943	**1.343**	**1.276**
PLPA	**1.080**	0.975	**1.239**	**1.207**
SCBA	**1.055**	0.978	**1.172**	**1.137**
STMI	**1.055**	**1.042**	**1.240**	**1.277**
VUOC	**1.018**	0.993	**1.094**	**1.066**
Among-year germination (G)				
ERCI	0.256	**1.509**	**1.568**	**2.380**
ERLA	**1.259**	0.883	**1.878**	**1.531**
ERTE	0.144	**1.608**	**1.599**	**2.601**
EVMU	**1.029**	**1.132**	**1.951**	**2.343**
MOBE	**1.446**	0.712	**1.526**	**1.065**
PEHE	**1.553**	0.698	**2.087**	**1.316**
PERE	**1.193**	0.905	**1.650**	**1.457**
PLIN	**1.137**	0.978	**1.504**	**1.486**
PLPA	**1.151**	0.918	**1.648**	**1.488**
SCBA	**1.066**	**1.001**	**2.349**	**2.351**
STMI	**1.207**	**1.006**	**1.785**	**1.776**
VUOC	0.737	**1.318**	**1.756**	**2.412**

these two species, observed variable and fixed strategies had invasion fitness just greater than one for *Schismus barbatus* (SCBA). For all species, correctly-predicting mutants had fitness much larger than one, and much greater than invasion fitness for correctly-predicting p_e mutants, when competing against resident strategies with fixed G or observed variability in G.

Observed variation in germination timing in relation to fitness

Relationships between p_e and relative fitness of cohorts (log fitness ratios) in different years were positive (though not always significantly so) for all 12 species yielding a highly significant sign test across species ($P = 0.0002$; Fig. 2, Table S2-1). While there was a lot of scatter around the positive slopes within species, species averages (across years) for p_e were strongly correlated with average species log fitness ratios (Fig. 3a; $F_{1,10} = 24.81$, $P = 0.0006$, adjusted $R^2 = 0.68$).

Similarly, relationships between among-year germination fractions (G) and per-capita fecundity were all positive (sign test across species, $P = 0.0002$), and often significant on a species by species basis ($P < 0.05$ for 7 species and $P = 0.08$ for an eighth; Fig. 4, Table S2-1). Our results also suggest a positive, but not significant, trend for within-year germination

timing to correspond with among-year germination fractions, such that species that germinate early (i.e., have high p_e) tend to have high among-year germination fractions (G; Fig. 3b, $F_{1,10} = 1.52$, $P = 0.25$, adjusted $R^2 = 0.05$).

DISCUSSION

For annual plants inhabiting variable environments, germination timing is of paramount importance because it sets the context for future growth and reproduction. Here we explored the adaptive value of within- and among-year germination timing for 12 co-occurring species in a winter annual community and identified optimal strategies for germination delay in a variable environment. Optimal among-year germination fractions, G, were always less than one which suggests that spreading germination across multiple years is beneficial for all species (Fig. 1a). These patterns are consistent with previous studies in this system demonstrating that among-year germination delay acts as a bet hedging strategy (Venable 2007; Gremer & Venable 2014). The ESS early germination fraction, p_e, was less than one for all but one species (Fig. 1b, S3-1), and always greater than zero, suggesting that spreading germination across multiple cohorts within a season is also adaptive for most species in this variable environment.
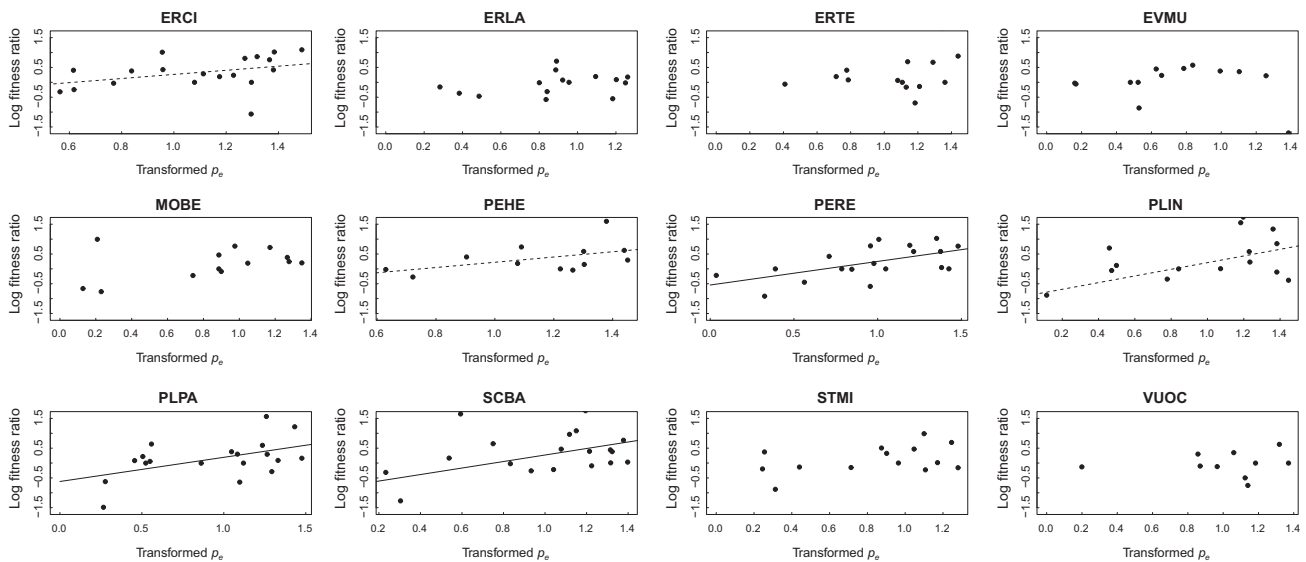
Figure 2 Relative germination timing (proportion of early germinants, p_e) vs. the log fitness ratios for each species. Positive relationships indicate more early germination in years that early germinants had higher fitness relative to late germinants, and more late germination in years with higher fitness for late germinants (i.e., predictive plasticity in germination timing). Each panel is a different species, see Table 1 for species abbreviations. Solid lines indicate significant relationships ($P < 0.05$), dashed lines indicate marginally significant relationships ($P < 0.12$). Values for p_e were arcsine-square root transformed prior to analysis.
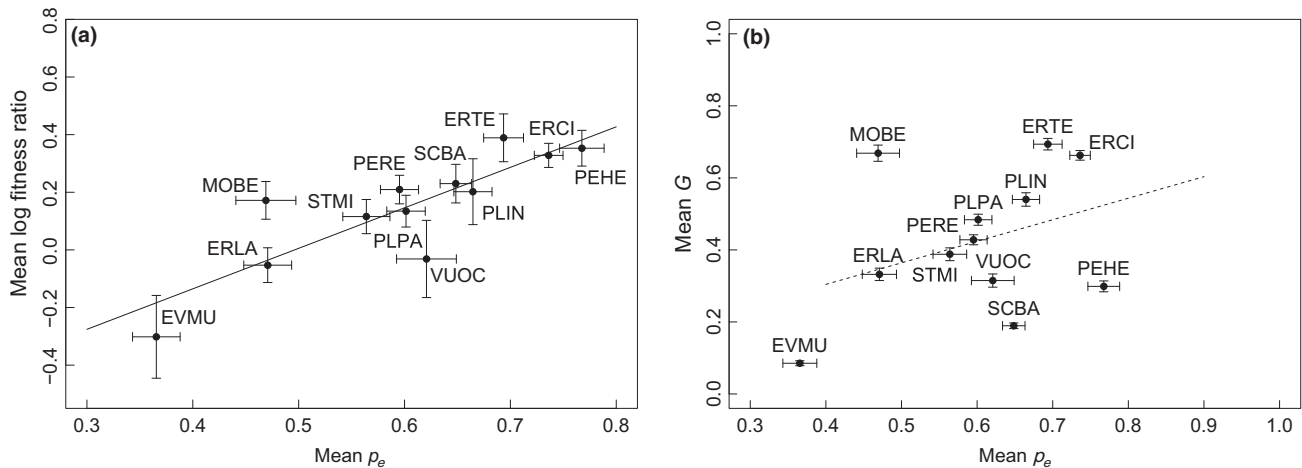

Figure 3 Mean relative germination timing (proportion of early germinants, p_e) vs. (a) mean relative fitness (log fitness ratios) and (b) mean annual germination fractions (G). Each point represents a species, see Table 1 for species abbreviations. Error bars represent one standard error of the mean. (a) The solid line represents the significant linear regression line ($P = 0.003$), (b) the dashed line represents the non-significant linear regression line ($P = 0.25$).

Many variable environments have components that are more or less predictable, which can favour integrated strategies that incorporate both plasticity and bet hedging (Cohen 1967; Simons 2011; Donaldson-Matasci *et al.* 2013; Simons 2014). Both within- and among-year germination timing vary in real populations (Clauss & Venable 2000), and we tested whether such variation was adaptive by competing plastic against fixed strategies with constant germination fractions. Generally we found that strategies with the empirically observed variation in germination timing had higher fitness and were able to invade fixed strategies. However, observed variable strategies always had lower fitness than a 'correctly-predicting' mutant strategy that was created with the benefit

of hindsight. Notably, invasion fitness for correctly-predicting among-year germination strategies was much higher than that for within-year germination, suggesting that plasticity in G has a stronger effect on fitness than plasticity in p_e. The value of G has a greater impact on fitness than the value of p_e in general, as can be seen in the shallower slopes of fitness topographies in the direction of the p_e axis as compared to the G axis (Appendix S3-1). These analyses suggest that both bet hedging and plasticity may be more important at the among-year temporal scale. Of course, we only considered observed variation in within-year timing and likely selection has removed additional variation, e.g., germination too early or late in the season to coincide with opportunities for growth
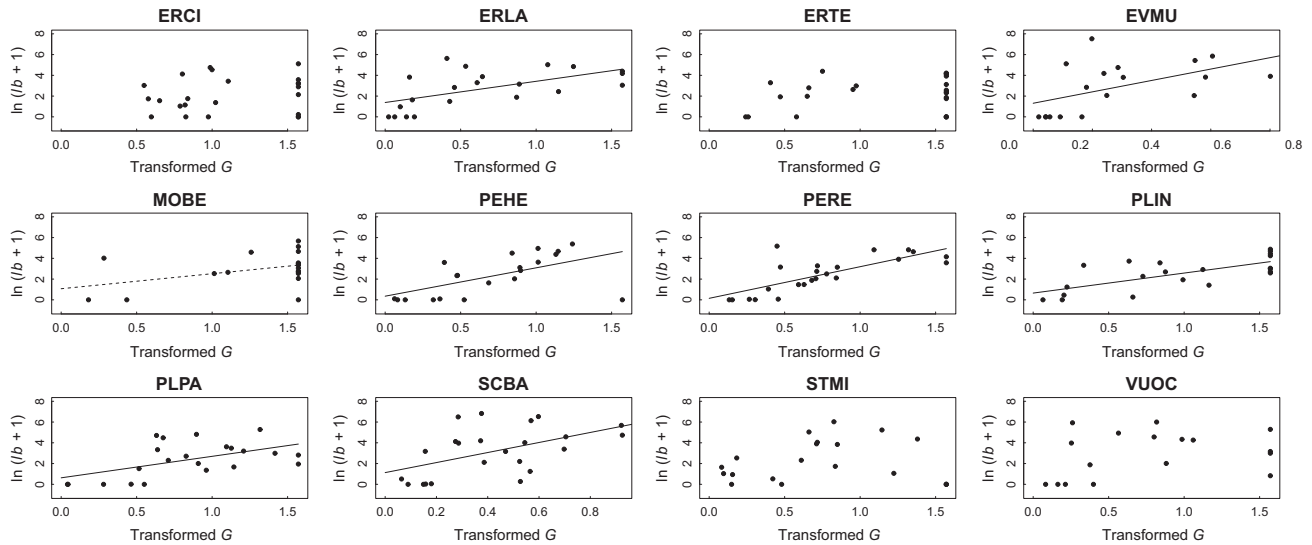
Figure 4 Among-year germination fractions (G; arcsine-square root transformed) vs. per-capita fecundity (lb, log-transformed) for each species. Positive relationships indicate that years of higher germination fractions were also years of higher fitness. Each panel is a different species, see Table 1 for species abbreviations. Solid lines indicate significant relationships ($P < 0.05$), dashed lines indicate marginally significant relationships ($P < 0.12$).

and reproduction. We also found considerable scatter around the positive relationships between germination fraction and fitness (Figs 2 and 4). This is not surprising since weather in the Sonoran Desert exhibits high within- and among-year variation, likely making successful evolutionary response to such cues difficult. Together, these patterns suggest that, while variation in germination fractions is beneficial, some degree of bet hedging is also advantageous. The joint expression of bet hedging and plasticity is likely to be common and, indeed, plastic responses to environmental cues such as temperature, precipitation or resource availability have been often observed in conjunction with bet hedging traits in plants (Clauss & Venable 2000; Evans *et al.* 2007; Gremer *et al.* 2012; Simons 2014).

While observed variation in within-year germination timing for a given species did not always correlate with increased fitness, species average p_e corresponded well with species average fitness benefits (Fig. 3a). These patterns suggest that species differences in within-year germination timing are adaptive and that germination strategies correspond well with species differences in post-germination traits that enhance fitness. Previous work in this system has identified post-germination traits that mediate responses to both abiotic conditions, such as temperature and precipitation, and to competition. For example species with germination physiologies conferring high germination fractions and fast germination rates tend to have more stress-tolerant vegetative physiologies with higher water-use efficiency and low relative growth rates (Kimball *et al.* 2011; Huang *et al.* 2016). These stress-tolerant species are better able to utilise small pulses of precipitation and withstand dry periods in this water-limited environment (Angert *et al.* 2010; Huxman *et al.* 2013; Huang *et al.* 2016); they also respond better to competition under drier conditions (Gremer *et al.* 2013). The opposite is true for species with low and slow germination and physiologies that enable high growth rates and rapid responses to larger, sustained precipitation pulses and provide a competitive advantage under high soil moisture.

Thus, in this system, post-germination traits are linked to germination timing and seem to influence the evolution of germination strategies. Such patterns have been predicted by life history theory (Templeton & Levin 1979; Brown & Venable 1986), but until recently, few studies have shown such fitness correlations between germination and post-germination traits (Donohue *et al.* 2010; Huang *et al.* 2016).

Our model predicted within- and among- year germination fractions that corresponded quite well with mean observed germination strategies in the field. However, predicted optima were often higher than observed. Why the mismatch? Perhaps it results from some model assumptions not being met. For instance our model assumes that plants can germinate every year and in every cohort, while there may be years and cohorts that do not have requisite temperature and moisture combinations for germination (Baskin & Baskin 1998). Also, our data did not permit separate empirical parameterisation of competition coefficients for early and late cohorts. Experiments to quantify the impact of germination timing on competition would permit a more refined analysis. Uncertainty in seed bank survival was not included in our model, but that would further decrease the value of delaying germination, moving predicted optima further from observed patterns and not improving the mismatch. Finally, thirty years is still just a snapshot of the evolutionary history of these species and studies of ecological processes have shown that variability usually increases with time due to the addition of more processes at longer time scales (Halley 1996). Germination strategies may have evolved to hedge against variability not captured in the time frame of our study. It would be interesting to explore what types of higher order temporal variation would lead to the cautious patterns we observed.

Alternately, genetic, developmental or physiological constraints may limit the evolution of life history traits such as delayed germination (Antonovics and van Tienderen 1991;

Burns *et al.* 2010). Ultimately, germination fractions do not evolve, but rather it is the physiological and morphological mechanisms that control germination responses that respond to selection. Life-history models in which germination fractions emerge from the interaction of such mechanisms with environmental variation, such as those being developed for *Arabidopsis thaliana* (Burghardt *et al.* 2015; Donohue *et al.* 2015; Springthorpe & Penfield 2015) would significantly advance our understanding of the evolution of dormancy strategies in variable environments. Also, such pathways can affect other functions, such as flowering, and can be subject to correlated and possibly conflicting selection (Chiang *et al.* 2009, 2013). In addition, past selection may limit the ability to reach evolutionary optima. Lampei and Tielbörger (2010) demonstrated that evolvability (and heritability) of germination fractions were significantly different for two desert annuals with different selection histories.

In a variable environment, organisms must have strategies to deal with unpredictable changes in conditions. Such variation can stem from abiotic sources, such as the high variability in the timing and amount of precipitation observed in the Sonoran Desert (Davidowitz 2002). Biotic sources of variation, such as competition, can also have strong effects on the evolution of life history timing (Ellner 1987; Metcalf *et al.* 2015). Even in the absence of environmental variation, competition can increase the adaptive value of delaying germination among years (Ellner 1987). Competition may also increase the value of spreading germination timing within years, more so with increased environmental stochasticity (Metcalf *et al.* 2015). Our results also suggest that density dependence influences the evolution of germination fractions. In three species (*Eriophyllum*, *Evax* and *Stylocline*) we observed a low-density advantage for mutants with divergent germination strategies. Together, these patterns suggest that germination strategies are strongly influenced by both environmental variation and competition, and that understanding the evolution of germination timing in variable environments requires integrating the effects of both within- and among-season germination timing as well as the roles of plasticity and bet hedging.

DATA ACCESSIBILITY STATEMENT

The data supporting our results are publicly available at http://www.eebweb.arizona.edu/faculty/venable/LTREB.htm.

AUTHORSHIP

JRG and DLV conceived of and designed the study. JRG, SK and DLV developed research and analyses on within-year germination timing. JRG and DLV developed modelling approaches and comparisons; JRG performed analyses in collaboration with DLV. JRG wrote the manuscript, DLV and SK contributed to manuscript content and revisions.

ACKNOWLEDGEMENTS

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