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Playing smart vs. playing safe: the joint expression of phenotypic plasticity and potential bet hedging across and within thermal environments

A. M. SIMONS

Department of Biology, Carleton University, Ottawa, ON, Canada

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Abstract

Adaptive phenotypic plasticity evolves when cues reliably predict fitness consequences of life-history decisions, whereas bet hedging evolves when environments are unpredictable. These modes of response should be jointly expressed, because environmental variance is composed of both predictable and unpredictable components. However, little attention has been paid to the joint expression of plasticity and bet hedging. Here, I examine the simultaneous expression of plasticity in germination rate and two potential bet-hedging traits – germination fraction and within-season diversification in timing of germination – in seeds from multiple seed families of five geographically distant populations of *Lobelia inflata* (L.) subjected to a thermal gradient. Populations differ in germination plasticity to temperature, in total germination fraction and in the expression of potential diversification in the timing of germination. The observation of a negative partial correlation between the expression of plasticity and germination variance (potential diversification), and a positive correlation between plasticity and germination fraction is suggestive of a trade-off between modes of response to environmental variance. If the observed correlations are indicative of those between adaptive plasticity and bet hedging, we expect an optimal balance to exist and differ among populations. I discuss the challenges involved in testing whether the balance between plasticity and bet hedging depends on the relative predictability of environmental variance.

Introduction

> In times of profound change, the learners inherit the earth, whereas the learned find themselves beautifully equipped to deal with a world that no longer exists.
>
> Eric Hoffer

Adaptations are typically characterized as traits that enhance reproductive success within a particular ecological setting. However, natural environments are variable, and adaptations are more comprehensively characterized as traits that have enhanced reproductive success across the array of environments encountered. Two principal classes of adaptation to environmental change – besides evolutionary tracking (Hendry & Kinnison, 1999; Barrett & Schluter, 2008; Bell & Gonzalez, 2009), in which selection acts to maximize fitness within each sequential environment – have been described: adaptive phenotypic plasticity (Schmalhausen, 1949; Bradshaw, 1984; Via & Lande, 1985; Schlichting & Pigliucci, 1998; van Kleunen & Fischer, 2005; Chown *et al.*, 2007; Charmantier *et al.*, 2008; Visser, 2008) – which may include nongenetic inheritance (Bonduriansky *et al.*, 2012) – and bet hedging (Seger & Brockmann, 1987; Philippi & Seger, 1989).

Adaptive phenotypic plasticity evolves if the relationship between phenotypic expression and fitness across environments is predictable, and if not limited by constraints (van Kleunen & Fischer, 2005; Caruso *et al.*, 2006). Bet-hedging (Slatkin, 1974) traits maximize long-term (geometric mean) fitness through the reduction in fitness variance among generations; their evolu-

Correspondence: Andrew M. Simons, Department of Biology, Carleton University, 1125 Colonel By Drive, Ottawa, ON K1S 5B6, Canada.
Tel.: 613 520 2600 x.3869; fax: 613 520 3539;
e-mail: andrew.simons@carleton.ca

tion is expected under a variety of circumstances (Simons, 2002), including if the relationship between phenotype and fitness cannot be predicted at the time a developmental, behavioural or life-history 'decision' is made (Seger & Brockmann, 1987; Philippi & Seger, 1989; Simons, 2011). Environmental variance has both predictable and unpredictable components; thus, the concurrent evolution of plasticity and bet hedging is expected (Crean & Marshall, 2009; Simons, 2011; Donaldson-Matasci *et al.*, 2013). However, although adaptive plasticity is well documented and there has been a recent surge of interest in bet hedging (Venable, 2007; Simons, 2009; Ratcliff & Denison, 2010; Rovira-Graells *et al.*, 2012; Starrfelt & Kokko, 2012; Fehrmann *et al.*, 2013; Gremer & Venable, 2014), they have been studied in isolation. Thus, little is known about the relationship or joint evolution of these two modes of response (Crean & Marshall, 2009; Donaldson-Matasci *et al.*, 2013).

The timing of seed germination provides an example of a trait in which the evolution of both phenotypic plasticity and bet hedging is expected (Cohen, 1967; Clauss & Venable, 2000; Simons, 2009). Reproductive success in plants can depend primarily on seedling establishment, yet both natural selection acting on timing of seed germination and the timing of seed germination are notoriously variable in the field (Pake & Venable, 1996; Simons & Johnston, 2000; Imbert, 2002; Castellanos *et al.*, 2008; Wagmann *et al.*, 2010; Alberto *et al.*, 2011; Mercer *et al.*, 2011; Hoyle *et al.*, 2013). Variance in the timing of germination among seeds of the same plant is attributable to several sources. It may be a nonadaptive, direct effect of plasticity expressed under variable environments. Adaptive explanations for the expression of variation in germination timing take two main forms: first, it may result from phenotypic plasticity to variable cues for appropriate germination environments. The evolution of adaptive phenotypic plasticity is expected only if environmental cues can be perceived by seeds, and if these cues reliably predict germination conditions that lead to successful establishment. Second, variance may be apparently random, originating from unknown causes or microplasticity unrelated to environmental cues (Simons & Johnston, 2006), or resulting from inherent differences among seeds. Although imperfect or 'noisy' control of germination timing may account for this random germination timing, the degree of variance expressed may be under selection as a diversification bet-hedging strategy when environments are unpredictable because cues are either unavailable or unreliable (Simons & Johnston, 1997).

Photoperiod, temperature and moisture may act as cues, but no cue is perfectly reliable. For example, photoperiod provides reliable information about time of year, but environmental conditions may vary dramatically from year to year. Furthermore, successful establishment requires appropriate conditions well beyond the moment of germination. Thus, natural environments in which seeds must germinate are characterized by both predictable and unpredictable components, and the total observed variance in timing of germination should be a result of both phenotypic plasticity to cues and diversification bet hedging. To the extent that adaptive plasticity to a particular cue is an effective response, within-population variance in germination timing should be manifested primarily as norms of reaction. If, on the other hand, plasticity is an ineffectual response, variance in germination timing should be manifested primarily as diversification.

Here, I study the joint expression of phenotypic plasticity and two forms of potential bet hedging in *Lobelia inflata* (Campanulaceae), a short-lived, obligately self-fertilizing monocarpic perennial (i.e. semelparous) herb. This species shows exceptionally high within-parent variance in time to germination within a season under the constant temperature conditions of a growth chamber (Simons & Johnston, 2006), but also exhibits plasticity in germination rate across temperatures (Simons & Wagner, 2007). Germination delay among seasons also occurs within *L. inflata* parents, with almost 100% nongerminating seeds then germinating in a subsequent season (Simons & Johnston, 2006). Seeds of growth chamber produced offspring of parents collected from five geographically distant populations from across eastern North America were tested for germination fraction and timing along a thermogradient incubator.

This design, in which seeds of many individuals ('genotypes') are replicated across ecologically relevant temperature environments, allows direct assessment of the relationship among three traits: germination fraction (Fig. 1) – the subject of Cohen's (1966) classic bet-hedging model in which dormancy (i.e. nongerminating fraction) is expected to evolve in proportion to the probability of encountering a 'bad' year; norms of reac-

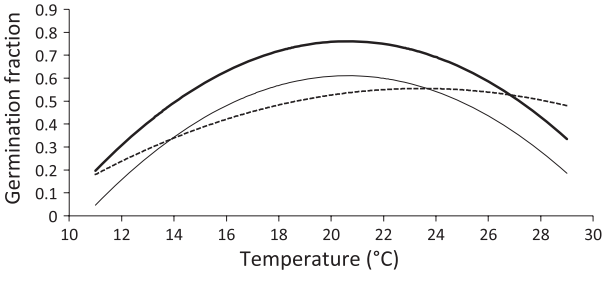

Fig. 1 Conceptual illustration of germination fraction and plasticity in germination fraction across temperatures. The heavy solid line shows higher overall germination fraction than either the thin solid, or dashed line. The two solid lines show high (and equal) plasticity to temperature, and the dashed line shows low plasticity measured as the cumulative change in absolute value of the quadratic predictor over the observed temperature range.

tion in germination fraction (Fig. 1), which are expected to evolve by natural selection to the extent that temperature provides a reliable predictor of success; and the expression of total variance in within-season timing of germination (Fig. 2), which has been shown to be advantageous when germination success is unpredictable within seasons (Simons, 2009). Because the plasticity under study here is the phenotypic outcome (germination fraction) of particular temperatures rather than of the response of individuals to changing or variable temperatures, I refer to these developmental reaction norms simply as phenotypic plasticity rather than as thermal performance curves (Kingsolver *et al.*, 2004).

The aim here is to assess the relationship between potential adaptive plasticity and bet hedging; it is beyond the scope of this study to assess the adaptive significance of the particular norms of reaction and putative diversification bet hedging observed in the five populations. Although I do not assume that phenotypic plasticity in germination fraction is adaptive or that variance expression in timing of germination is diversification, I do assume a functional relationship between them; that plasticity and variance expression as measured are positively related to the potential for evolution of these traits. If the total variation expressed by individuals – through plasticity and within-temperature variance – is constrained, and plasticity and diversification are alternative strategies, their optimal balance should depend on the relative predictability of cues. Thus, the central prediction of the balance hypothesis is that the strength of norms of reaction to temperature in germination rate should be negatively correlated with expression of total variance in time to germination. Because dormancy and within-season germination variance are both putative bet-hedging responses to environmental unpredictability, a secondary prediction is that nongerminating fraction (dormancy, assuming low seed mortality; see below) and variance in timing of germination will be positively correlated.

Materials and methods

I collected flowering stalks (racemes) with mature capsules from *L. inflata* at five locations in eastern North America: Martock, Nova Scotia (MTK); Petawawa Research Forest, Ontario (PET); Harvard Forest, Massachusetts (HFR); Pymatuning Laboratory of Ecology, Pennsylvania (PLE); and Daniel Boone National Forest, Kentucky (KTY). Seeds from 68 field-collected parents (16 from each of PET, HFR, PLE, KTY, and four from MTK) were propagated in growth chambers, and seeds from two replicate offspring of each seed parent were then used for the study on the thermogradient incubator.

The thermogradient incubator plate consists of a block of aluminium measuring 115 × 42 × 4.5 cm. Cold water from a cooling water bath is circulated in counter-current through duplicate holes drilled through the width of the block at one end, and heated water in counter-current through duplicate holes in the opposite end. The entire thermogradient plate surface was lined with moistened filter paper, and the gradient was covered with a Plexiglas lid to retain moisture and to promote thermal stability. The distribution of temperature along the length the gradient was initially established using temperature probes until the thermogradient stabilized. The relationship between distance on the gradient and temperature was determined in three trials using five temperature probes and is described by the linear equation, Temperature (°C) = 4.797 + 0.230*Position (cm); $r^2 = 0.999$. No variation in temperature was detected across the width of the plate at a measurement resolution of 0.5 °C. Twenty-five temperature positions from 5 to 29 °C (i.e. 1 °C spacing) were established along the thermal gradient. *L. inflata* does not require a period of cold

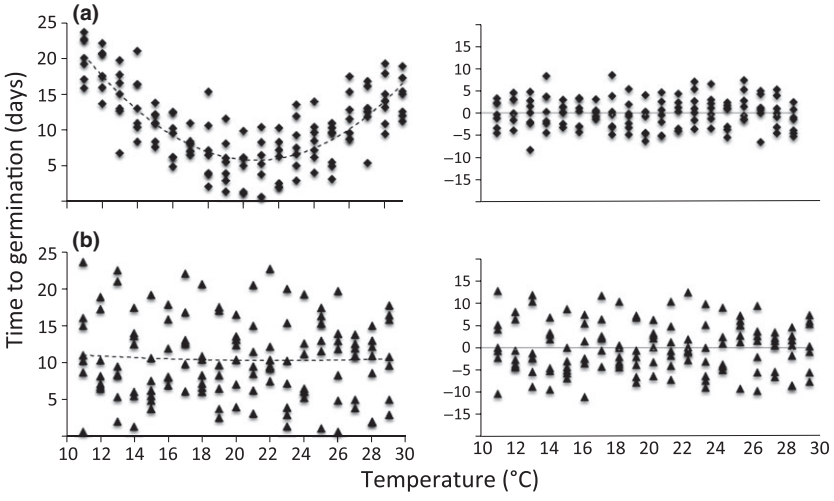

Fig. 2 Conceptual illustration of two measures of potential diversification using variance in germination fraction across and within temperatures. The total variance in time to germination expressed across temperatures is identical in (a) and (b). However, the residual variance around a best-fit quadratic curve in case (a) is lower than that in case (b). See text for explanation and interpretation of diversification bet hedging.

stratification for germination (Simons & Johnston, 2000); thus, for consistency, all were allowed an afterripening interval, but none were stratified. Two seeds from each of 68 replicated seed parents of the five populations were placed on the filter paper lining the thermogradient plate at each temperature position, for a total of 3400 seeds. Seeds were placed in a line within each temperature at marked positions randomly assigned with respect to population and parental individual, and a unique randomization was used for each temperature. The gradient was monitored at five fixed points throughout the germination trials to confirm thermal stability.

Seed germination was assessed as visible protrusion of the radicle under a 10× magnifier. The date and position of each germinating seed was recorded (the observer was blind to both population and genotype) at a resolution of 24 h, and germinating seeds were discarded at that time. Cumulative germination distributions tend to be sigmoidal, with an asymptote that converges on germination fraction – 'extent of germination' *sensu* McNair *et al.* (2012) – or proportion of seeds capable of germinating under the given conditions. Because both germination fraction and time to germination are focal traits in this study, it was important to allow all germinable seeds the opportunity to germinate. Therefore, the observation period included the peak germination period for all temperatures and was only terminated once there was evidence the asymptote had been reached. The study was thus terminated at day 60 after no new germination event had been observed at any temperature for eight consecutive days (Fig. S1).

Temperatures between 11 and 29 °C (inclusive) were used in analyses because, experiment-wide, only one seed germinated at any temperature below this range, and > 1 seed was found to germinate at all temperatures within this range (Data available from the Dryad Digital Repository: doi:10.5061/dryad.sr148). Germination fractions, total plasticity, standard deviation in time to germination and optimal temperature for germination were calculated for seeds of each replicate seed parent. Germination fractions were transformed as arcsin ($p^{0.5}$). To obtain total plasticity in germination fraction, a quadratic function was fitted to the data for seeds of each individual seed parent over the observed temperature range, and plasticity was calculated as the cumulative change in absolute value of the quadratic predictor. Thus, if an individual seed parent shows little plasticity to temperature in germination fraction of its seeds, the cumulative change in the best-fit quadratic function will be close to zero.

The measurement of putative diversification across and within temperatures is less straightforward. There is no clear adaptive significance of plasticity in time to germination in response to temperature (e.g. a longer delay to germination at cool temperatures) because current temperature offers no obvious information on the likelihood of successful germination if delayed to some time in the future. Thus, plasticity in time to germination within a season may be interpreted as a direct physiological, nonadaptive plastic response to temperature. However, environmental variance that has little ecologically relevant information content may still generate diversification (Bradshaw, 1984; Simons & Johnston, 2006), and we must consider the possibility that it still acts as a diversification mechanism (Simons & Johnston, 1997). This is why I conservatively use both variation in time to germination across temperatures and variation in time to germination within temperatures (i.e. residuals around this plasticity) as potential diversification. Potential diversification in time to germination was calculated in two ways: (1) as the standard deviation in time to germination observed for each individual seed parent across all temperatures (i.e. regardless of temperature) and (2) as the standard deviation in residuals around the best-fit quadratic function for each individual seed parent; that is, variation within temperatures and controlling for variation among temperatures (this concept is illustrated in Fig. 2). Optimal temperature for germination for each seed parent was estimated as the temperature (between 11 and 29 °C) at which the first derivative of the best-fit quadratic function for transformed germination fraction is zero. Individual seed parents with a total of two or fewer germinating seeds over all temperatures were eliminated from analyses involving quadratic fitting. For use in one analysis (see family mean correlations, below), family mean values were calculated as the mean of replicate individual seed parents for each of the above response variables.

Comparisons in germination patterns through time are best made using 'time-to-event' or survival analyses because germination patterns are characterized by serially autocorrelated and right-censored data (McNair *et al.*, 2012). It is important to note that, in the present study, the focal traits are point estimates of germination fraction (i.e. extent of germination) and variation in time to germination, which are explicitly defined as restricted to only those seeds germinating under each particular temperature environment (i.e. 'within-season'). The quadratic functions are fit to series of these independent point estimates (for each replicate seed parent) over all temperatures rather than to a temporal pattern of germination and thus contain no serial autocorrelation.

Differences among populations and genotypes in each of the response variables (germination fraction, total plasticity, standard deviation in germination time, optimal temperature for germination) were first analysed using nested ANOVAS, where both population and genotype nested within population were treated as random effects. Variance components were obtained using restricted maximum-likelihood methods.

To test the main hypotheses on the associations between germination fraction, plasticity and potential diversification, multivariate partial correlations were

performed. These were first performed at the level of individual seed parents for the entire data set. Because these overall correlations may be caused by correlations among or within groups, partial correlations among the three focal traits were then calculated both at the among- and within-population levels. The t-statistics for estimating significance levels for partial correlation coefficients were calculated as:

$$t = r_{ij}\sqrt{\frac{n-2-k}{1-r_{ij}^2}}$$

Where r_{ij} is the partial correlation between i and j, with k control variables (Morrison, 1976). Family mean correlations – although imperfect estimators of genetic correlations and life-history trade-offs – were available and were thus calculated (both bivariate and partial) within the five populations for completeness where sample size allowed.

Results

Germination fraction showed strong phenotypic plasticity to temperature across the entire dataset (Fig. 3) and was better explained as a quadratic ($r^2 = 0.54$) than linear function ($r^2 = 0.35$; AICc relative model likelihood < 0.0001) of the continuous temperature gradient. Across the dataset, germination occurred most rapidly at high temperatures (26–29 °C) but reached a higher germination fraction at medium temperatures (20–25 °C) than at either high (26–29 °C) or low (11–19 °C) temperatures (Fig. S1).

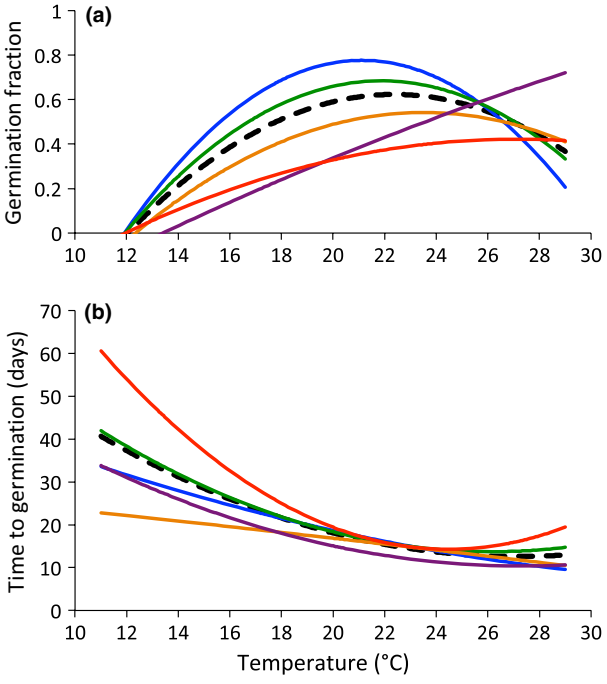

Fig. 3 Plasticity in seed germination fraction (a) and time to germination (b) to temperature in *Lobelia inflata*. Seeds derived from two offspring of 68 seed parents from five eastern North American populations were placed at each temperature position along a thermogradient incubator, for a total of 3400 seeds. Quadratic functions were fit for the entire data set (dashed) and for each population (HFR, blue; KTY, green; MTK, orange; PET, purple; PLE, red). For quadratic predictors, fit at the genotype level, see Fig. S2.

Population differentiation

Hierarchical ANOVA (Table 1) reveals significant differences among populations in germination fraction and total plasticity in germination fraction (Fig. 3), standard deviation in time to germination, and optimal temperature for germination. Although variation in plasticity among individual seed parents is evident (Fig. S2), there is no evidence of genetic variance within populations for any of these traits (Table 1). Seed parents from Kentucky (KTY) and Massachusetts (HFR) generally differ most from those from Ontario (PET) and Pennsylvania (PLE) in germination characters (Table 2). Differences among populations in variance in time to germination were confirmed with a Levene's test ($F_{4,594} = 6.38$; $P < 0.0001$).

Phenotypic correlations

Across the entire dataset (i.e. combining populations), partial phenotypic correlations for all three trait-pair combinations (Table 3) are significant, including a negative correlation between total plasticity and standard deviation in time to germination. The strong correlation between germination fraction and total plasticity exists also as a bivariate correlation (which does not control for the third factor), but bivariate correlations between the other two pairs of germination characters are nonsignificant (Table 3).

Partial correlations between optimal temperature for germination and the focal germination traits were also calculated. No partial correlation is detected between

Table 1 Hierarchical ANOVA for differentiation in four seed traits among and within five eastern North American populations. Both population and genotype within population are random effects; variance components (%Var) are based on maximum-likelihood methods.

Trait	Source	MS	d.f.	*F*	*P*	%Var
Germination fraction	Population	0.9424	4	11.0358	< 0.0001	25.57
	Genotype	0.08539	63	1.0868	0.3674	2.99
Total plasticity	Population	1.15513	4	5.7343	0.0006	24.44
	Genotype	0.20715	46	1.2260	0.2965	15.20
SD time to germination	Population	41.8462	4	2.8614	0.0314	15.59
	Genotype	15.2838	46	1.4051	0.1819	19.47
Optimal °C	Population	97.7814	4	13.4725	< 0.0001	47.99
	Genotype	7.62182	46	1.4686	0.1520	14.88

Table 2 *Post hoc* tests for differences among means of five populations in germination traits of *Lobelia inflata*. Populations not sharing a letter are significantly different after correcting for multiple comparisons (Tukey's HSD). For population abbreviations, see Materials and methods.

		Germination fraction (transformed)			Total plasticity			SD time to germination			Optimal °C			
Pop	°Lat	Mean	HSD		Mean	HSD		Mean	HSD		Mean	HSD		
KTY	36.6	0.65	A		1.04	A	B	9.06	A		22.93			C
PLE	41.6	0.28		B	0.59		B	9.76	A	B	26.57	A	B	
HFR	42.5	0.58	A		1.35	A		8.88	A	B	21.42			C
MTK	44.9	0.46	A	B	0.99	A	B	6.77	A	B	23.38		B	C
PET	46.0	0.27		B	0.75		B	4.91		B	28.08	A		

Table 3 Multivariate partial correlations (above diagonal) for the three focal seed germination traits ($n = 76$ for each correlation) of *Lobelia inflata* collected from five populations and germinated on a common garden thermogradient incubator. Bivariate correlations (below diagonal) are included for comparison.

	Germination fraction (transformed)	Total plasticity	SD time to germination
Germination fraction (transformed)	–	0.7571 $P < 0.0001$ d.f. = 73	0.3164 $P = 0.0057$ d.f. = 73
Total plasticity	0.7288 $P < 0.0001$ d.f. = 74	–	−0.3301 $P = 0.0038$ d.f. = 73
SD time to germination	0.1078 $P = 0.3540$ d.f. = 74	−0.1461 $P = 0.2078$ d.f. = 74	–

optimal temperature and transformed germination fraction ($r = 0.090$; $P = 0.444$), but total plasticity declines with optimal temperature ($r = -0.5466$; $P < 0.0001$), as does *SD* time to germination ($r = -0.2539$; $P = 0.029$) after controlling for the effects of other variables.

Although a sample size of 5 (d.f. = 2 for partial correlation) for analyses using the five population means lacks sufficient power to detect significance (at $\alpha = 0.05$) even with high correlation coefficients, the partial correlations (Table 4) show no evidence of incongruence with those using all individual data. Partial correlations among the three focal germination characters calculated for each population (Table 5) are again broadly consistent with combined and population mean correlations. All populations show a significant partial correlation between germination fraction and total plasticity (i.e. controlling for variation in time to germination), and a negative partial correlation between total plasticity and variation in time to germination. When the standard deviation of the residuals around norms of reaction is used instead (Table 5, below diagonal), the partial correlation coefficients are consistent with, but weaker than those using overall standard deviation. The elimination of individuals with < 3 seeds germinating has the potential to bias the results. However, analyses performed after eliminating individuals with < 2, < 4 and < 5 seeds revealed no qualitative differences in results.

Table 4 Among-population partial correlations for the three focal seed germination traits ($n = 5$ for each correlation) germinated on a common garden thermogradient incubator.

	Germination fraction (transformed)	Total plasticity	SD time to germination
Germination fraction (transformed)	–	0.8611 $P = 0.1389$ d.f.=2	0.5138 $P = 0.4862$ d.f. = 2
Total plasticity		–	−0.3708 $P = 0.6292$ d.f. = 2

Family mean correlations can be calculated only for genotypes in which both replicate individual seed parents had ≥ 3 seeds germinate, and were estimable for three populations (HFR, KTY, PLE). The correlation between germination fraction and total plasticity is significant for all three populations regardless of whether *SD* germination time or residual *SD* germination time is used as the control variable (Table 6). Although all six estimates of partial correlation coefficients between plasticity and *SD* in time to germination are negative, none are significantly different from zero when estimated using the reduced sample size of family means (Table 6).

Discussion

Phenotypic plasticity and bet-hedging strategies can enhance fitness under environmental variation, yet little is known about the relationship in expression between these two modes of response. Seed germination characteristics are ideally suited to the study of this relationship because they are known to exhibit both plasticity and high variance (Pake & Venable, 1996; Simons & Johnston, 2000; Imbert, 2002; Castellanos *et al.*, 2008; Wagmann *et al.*, 2010; Alberto *et al.*, 2011; Mercer *et al.*, 2011; Hoyle *et al.*, 2013).

Table 5 Within-population partial correlations. Correlations involving SD time to germination above, and those involving SD of residual variation around the best-fit quadratic function for plasticity below diagonal.

	Germination fraction (transformed)	Total plasticity	SD time to germination
HFR			
Germination fraction (transformed)	–	0.7183 $P = 0.0002$ d.f. = 20	0.2089 $P = 0.3508$ d.f. = 20
Total plasticity	0.6994 $P = 0.0002$ d.f. = 20	–	−0.4240 $P = 0.0492$ d.f. = 20
SD residual time to germination	0.0083 $P = 0.9707$ d.f. = 20	−0.1922 $P = 0.3902$ d.f. = 20	
KTY			
Germination fraction (transformed)	–	0.6553 $P = 0.0003$ d.f. = 24	0.4553 $P = 0.0194$ d.f. = 24
Total plasticity	0.5822 $P = 0.0018$ d.f. = 24	–	−0.4366 $P = 0.0257$ d.f. = 24
SD residual time to germination	0.1844 $P = 0.3672$ d.f. = 24	−0.1567 $P = 0.4446$ d.f. = 24	
MTK			
Germination fraction (transformed)	–	0.9728 $P = 0.0272$ d.f. = 2	0.3169 $P = 0.6831$ d.f. = 2
Total plasticity	0.9656 $P = 0.0344$ d.f. = 2	–	−0.4470 $P = 0.553$ d.f. = 2
SD residual time to germination	0.5735 $P = 0.4265$ d.f. = 2	−0.7352 $P = 0.2648$ d.f. = 2	
PET			
Germination fraction (transformed)	–	0.6622 $P = 0.037$ d.f. = 8	0.1513 $P = 0.6765$ d.f. = 8
Total plasticity	0.6770 $P = 0.0315$ d.f. = 8	–	0.0503 $P = 0.8902$ d.f. = 8
SD residual time to germination	0.0150 $P = 0.9672$ d.f. = 8	−0.0887 $P = 0.8075$ d.f. = 8	
PLE			
Germination fraction (transformed)	–	0.9366 $P = 0.0002$ d.f. = 7	0.4991 $P = 0.1714$ d.f. = 7
Total plasticity	0.9154 $P = 0.0005$ d.f. = 7	–	−0.4518 $P = 0.2221$ d.f. = 7
SD residual time to germination	0.0364 $P = 0.972$ d.f. = 7	−0.1383 $P = 0.8939$ d.f. = 7	

Table 6 Family mean partial correlations. Correlations involving SD time to germination above, and those involving SD of residual variation around the best-fit quadratic function for plasticity below diagonal. Family mean correlations were inestimable for two populations (MTK, PET).

	Family mean germination fraction (transformed)	Family mean total plasticity	Family mean SD time to germination
HFR			
Family mean germination fraction (transformed)	–	0.8942 $P < 0.0001$ d.f. = 12	0.4650 $P = 0.2072$ d.f. = 7
Family mean total plasticity	0.8690 $P < 0.0001$ d.f. = 12	–	−0.5800 $P = 0.1016$ d.f. = 7
Family mean SD residual time to germination	0.2340 $P = 0.5478$ d.f. = 7	−0.3680 $P = 0.3298$ d.f. = 7	
KTY			
Family mean germination fraction (transformed)	–	0.5837 $P = 0.0284$ d.f. = 12	0.0611 $P = 0.8584$ d.f. = 9
Family mean total plasticity	0.5390 $P = 0.0467$ d.f. = 12	–	−0.4025 $P = 0.2197$ d.f. = 9
Family mean SD residual time to germination	−0.2892 $P = 0.3884$ d.f. = 9	−0.1414 $P = 0.6784$ d.f. = 9	
PLE			
Family mean germination fraction (transformed)	–	0.9727 $P < 0.0001$ d.f. = 7	0.7966 $P = 0.2034$ d.f. = 2
Family mean total plasticity	0.9715 $P < 0.0001$ d.f. = 7	–	−0.7479 $P = 0.2521$ d.f. = 2
Family mean SD residual time to germination	0.7235 $P = 0.2765$ d.f. = 2	−0.7466 $P = 0.2534$ d.f. = 2	

This study focuses on correlations among germination traits; however, mean trait differences among populations are also of interest and are reported: results obtained using seeds of replicated seed parent genotypes observed on a thermogradient plate (i.e. continuous common garden) indicate genetic population differentiation with respect to important life-history characters including germination fraction, plasticity to temperature, potential diversification and optimal temperature for germination. As might be anticipated, the northernmost populations that can experience short growing seasons generally show more restrictive germination behaviour than the more southern populations in that germination fractions are lower and higher temperatures are required for peak germination. Although mean total plasticity is lower in the northern populations that show high potential bet hedging in the form of germination fractions, the potential for diversification

(*SD* in time to germination) is significantly higher for the Kentucky than the Petawawa population. However, testing the adaptive significance of these observed differences in trait values across populations would be a difficult task for reasons outlined below and is beyond the scope of this study.

Across the entire dataset, seed parents that show strong norms of reaction to temperature in germination also show low total variance (and within-temperature variance) in germination behaviour, suggesting weaker potential for diversification bet hedging. Observation of the predicted phenotypic correlations between plasticity and both potential forms of bet hedging – nongerminating fraction and within-season variance in time to germination – support the hypothesis that plasticity and diversification are alternative responses to environmental variation. The argument could be advanced that such relationships might be generated by statistical effects. First, the use of partial correlation coefficients suggests otherwise; for example, a strong negative correlation exists between plasticity and potential diversification after correcting for germination fraction. Although variation in norms of reaction exists (Fig. S2), total plasticity may be constrained by total germination fraction. Second, a statistical effect does not necessarily imply lack of biological significance. For example, higher germination fractions may directly influence the expression of diversification bet hedging within a season for statistical reasons, but with no loss of biological meaning (Simons, 2007).

Differences among populations measured in a (continuous) common garden provide evidence for evolutionary divergence, but it does not follow that the observed correlations among germination traits are genetically based. The family mean correlations are suggestive of a genetic basis to the relationship between plasticity and variance expression, but a more comprehensive quantitative genetic design, or a study of correlated selection, is needed to infer a trade-off between these two modes of response.

No interpretation of results is intended beyond the existence of population differentiation and the relationship between the expression of plasticity and potential diversification. The results reported here cannot be used to draw inferences about adaptation to environmental variance in the particular populations studied. Making inferences about the adaptive significance of the differences among populations in particular ratios of plasticity and diversification would be a difficult task: environmental variance would not only have to be quantified, but it would have to be partitioned into predictable and unpredictable components. Particularly onerous is the partitioning of predictability and unpredictability from an organism's perspective: how a particular environmental change is perceived by an organism and, once perceived, whether the appropriate phenotypic adjustment can be made; that is, whether a predictable relationship between phenotype and fitness exists and the phenotype can be expressed. Furthermore, differences in plasticity exist among populations, but plasticity assumed to be adaptive may often be neutral or maladaptive (Caruso *et al.*, 2006). Only if the balance between plasticity and variance expression (i.e. not necessarily adaptive) reflects that between adaptive plasticity and bet-hedging diversification can we expect an optimal balance to exist, and possibly differ among populations.

The relationship between phenotypic plasticity and both forms of bet hedging supports the central 'balance' hypothesis, but the negative relationship between the two forms of putative bet hedging – dormancy fraction and potential diversification – does not provide support for the secondary hypothesis: populations with higher germination fractions (i.e. lower dormancy, assuming mortality is low) express more potential diversification after correcting for possible confounding effects of third variable. Although this result does not lend support to the secondary hypothesis, several alternative viable biological hypotheses may be proposed and subjected to further test. Germination fraction may have a statistical effect on potential diversification because offspring number can directly affect variance (Simons, 2007). Also, an optimal level of bet-hedging expression for a given extent of unpredictability may lead to a positive correlation (Simons, 2009); dormancy and diversification in timing of germination within years may act in a compensatory manner, rendering their joint expression redundant. Furthermore, dormancy fraction and diversification, although both are responses to unpredictability, may not evolve in concert: dormancy fraction is a response to unpredictability occurring among years (Cohen, 1966; Venable, 2007), whereas diversification is a response to within-season unpredictability (Simons, 2009), and the relative importance of unpredictability may differ at these two temporal scales. Finally, a recent comprehensive study of Sonoran Desert winter annuals provides strong evidence that density dependence (i.e. competition) contributes to the adaptive significance of germination delay (Gremer & Venable, 2014).

This approach is meant to isolate the study of response to temperature and does not assume that other cues (or combinations of cues) are unimportant. Germination characters are expected to show phenotypic plasticity to environmental variables experienced both by seed parents prior to dispersal and directly by seeds following dispersal (Schmitt *et al.*, 1992; Lacey, 1996; Donohue *et al.*, 2005) such as photoperiod, humidity and temperature, which may be adaptive if such environmental variables provide reliable cues about the conditions that newly germinated seedlings are likely to encounter. Studies of cues such as photoperiod and moisture are needed to ask whether the results obtained here apply generally to the relationship between plasticity and diversification.

In summary, I have demonstrated a negative phenotypic correlation between plasticity and potential bet-hedging traits. This suggests the possibility of a trade-off between these modes of response to environmental variance, where the balance would depend on the extent to which this variance is predictable or unpredictable. However, considerable obstacles, including the partitioning of environmental variance into unpredictable and predictable components from an organism's perspective, still hinder a test of this adaptive balance hypothesis.

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Supporting information

Additional Supporting Information may be found in the online version of this article:
Figure S1 Cumulative germination over time for the entire dataset, grouped by four temperature bins of equal number of germinating seeds.
Figure S2 Plasticity in seed germination fraction to temperature in *Lobelia inflata*.

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