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ENVIRONMENTAL AND GENETIC SOURCES OF DIVERSIFICATION IN THE TIMING OF SEED GERMINATION: IMPLICATIONS FOR THE EVOLUTION OF BET HEDGING

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Abstract.—Environmental variation that is not predictably related to cues is expected to drive the evolution of bet-hedging strategies. The high variance observed in the timing of seed germination has led to it being the most cited diversification strategy in the theoretical bet-hedging literature. Despite this theoretical focus, virtually nothing is known about the mechanisms responsible for the generation of individual-level diversification. Here we report analyses of sources of variation in timing of germination within seasons, germination fraction over two generations and three sequential seasons, and the genetic correlation structure of these traits using almost 10,000 seeds from more than 100 genotypes of the monocarpic perennial *Lobelia inflata*. Microenvironmental analysis of time to germination suggests that extreme sensitivity to environmental gradients, or microplasticity, even within a homogeneous growth chamber, may act as an effective individual-level diversification mechanism and explains more than 30% of variance in time to germination. The heritability of within-season timing of germination was low ($h^2 = 0.07$) but significant under homogeneous conditions. Consistent with individual-level diversification, this low h^2 was attributable not to low additive genetic variance, but to an unusually high coefficient of residual variation in time to germination. Despite high power to detect additive genetic variance in within-season diversification, it was low and indistinguishable from zero. Restricted maximum likelihood detected significant genetic variation for germination fraction ($h^2 = 0.18$) under homogeneous conditions. Unexpectedly, this heritability was positive when measured within a generation by sibling analysis and negative when measured across generations by offspring-on-parent regression. The consistency of dormancy fraction over multiple delays, a major premise of Cohen's classic model, was supported by a strong genetic correlation ($r = 0.468$) observed for a cohort's germination fraction over two seasons. We discuss implications of the results for the evolution of bet hedging and highlight the need for further empirical study of the causal components of diversification.

Key words.—Diversification bet hedging, dormancy, environmental unpredictability, heritability, microplasticity, phenotypic plasticity, seed germination.

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Cole's (1954) renowned result—showing that the fitness of an annual and a perennial plant are equal given that the annual produces just one seed more than the perennial—prompted the development of life-history theory by underscoring the evolutionary importance of age(stage)-dependent survival. Mortality at the seed and seedling stage is expected to be much higher than at later stages, especially for annual plants that produce a large number of small seeds (Harper 1977). From the perspective of life-history evolution, the importance of the timing of germination is clear: upon germination, a plant steps from the least to the most vulnerable stage of its life cycle. Thus, the timing of seed germination is expected to be under strong selection. Despite the expectation of strong selection, germination timing is notable because of its high variance expressed both as among-season delays and as within-season variation. Variance in seed germination behavior has thus been the focus of intense study in agricultural settings—with the aim of its control—and by evolutionary biologists in an attempt to understand its evolutionary implications.

Intermediate dormancy fractions can maximize population growth rate under environmental unpredictability (Cohen 1966): the dormant fraction of a population's seeds allows survival through poor growing seasons. Cohen's (1966) model led directly to the development of bet-hedging theory, with diversification in the timing of seed germination as its archetypal trait. A diversification bet-hedging strategy implies that variance is expressed by individuals, not as genetic polymorphisms or as population-level variation expressed among individuals (Seger and Brockmann 1987; Philippi and Seger 1989). The evolution of such traits thus requires that trait diversification, rather than the trait itself, respond to selection. Despite considerable sophistication in bet-hedging theory, empirical knowledge of the potential for selection to shape diversification traits is lacking. This paper considers diversification as a target of natural selection and advocates further empirical work on the topic.

An explanation of seed trait diversification as bet hedging requires not only that diversification be an individual-level character, but also that variation in seed traits underlies fitness variance. The two most commonly studied seed traits are seed size and the timing of germination, and both have been shown to have important fitness consequences. Seed size affects fecundity (Kalisz 1989) and survival (Galen and Stanton 1991; Simons and Johnston 2000a), the timing of germination influences fecundity (Baskin and Baskin 1972; Marks and Prince 1981; Kalisz 1986; Biere 1991b; Galen and Stanton 1991; Shitaka and Hirose 1993; Donohue et al. 2005) and survival (Baskin and Baskin 1972; Marks and Prince 1981; Biere 1991b; Simons and Johnston 2000a), and seed size has an effect on the timing of germination (Schaal 1980; Roach 1986; Winn 1988; Kalisz 1989; Zammit and Zedler 1990; Biere 1991b; Platenkamp and Shaw 1993; Simons and Johnston 2000a).

Seed trait variation observed among taxa may represent coadapted syndromes involving several seed traits (Venable

and Brown 1988; Kalisz 1989; Rees 1997). Among species, seed size varies by over 10 orders of magnitude (Westoby et al. 1992). Much seed trait variation also resides within species (Schaal 1980; Stanton 1984; Thompson 1984; Kalisz 1986; Michaels et al. 1988; Zammit and Zedler 1990; Biere 1991a; Wolfe 1995; Galloway 2002; Imbert 2002), even in crop plants that have been subjected to artificial selection to control this variance (references in Silvertown 1984). This was neither expected on theoretical grounds (e.g., Smith and Fretwell 1974) nor recognized until the 1980s (Michaels et al. 1988; Westoby et al. 1992).

Variance in time to germination and hatching asynchrony have mostly been discussed in the context of among-season delays, or dormancy (Cohen 1966; Silvertown 1984; Philippi 1993; Nilsson et al. 1994; Evans and Cabin 1995; Andersson and Milberg 1998; Clauss and Venable 2000; Easterling and Ellner 2000), but considerable asynchrony within a season also exists (e.g., Kalisz 1986; Biere 1991a; Clauss and Venable 2000; Simons and Johnston 2000a; Galloway 2002). Because the timing of germination is closely associated with fitness, explaining variance in this trait is of broad evolutionary interest.

An overriding proportion of the within-population variance in both seed size and germination behavior has been explained by parental effects (Thompson 1984; Antonovics and Schmitt 1986; Mazer 1987; Schwaegerle and Levin 1990; Biere 1991a; Platenkamp and Shaw 1993), within-parent effects such as positional or developmental differences (Stanton 1984; Thompson 1984; Roach 1986; Wolfe 1992, 1995; Vallius 2000) and microenvironmental conditions (Galen and Stanton 1991; Shitaka and Hirose 1993; Baskin et al. 1994; Horvitz and Schemske 1994; Galloway 2002) that may affect both the level of dormancy and ultimate release from dormancy (Benech-Arnold et al. 2000). Other possible sources of diversity in both seed size and germination timing include variable inbreeding coefficients (Johnston 1992), developmental instability (Simons and Johnston 1997), adaptive phenotypic plasticity (Harper 1977), and genetic variation (Meyer and Pendleton 2000).

Despite the expectation of strong selection on life-history traits in general, significant additive genetic variation (average $h^2 = 0.27$) is often found for this category of traits under laboratory conditions (Mousseau and Roff 1987). The timing of germination, however, is exceptional in that significant heritabilities are rarely detected or are very low (Schaal 1980; Kalisz 1986; Mazer 1987; Schwaegerle and Levin 1990; Biere 1991a; Wolfe 1995), even when measured under homogenous conditions that may inflate heritabilities (Simons and Roff 1994). For agronomists, within-season germination variance causes problems such as preharvest sprouting; even when major genetic factors influencing germination are identified, epistatic effects and environmental conditions influence the expression of these genetic factors (Holdsworth et al. 2001). Low heritability, however, is a predicted outcome of selection for diversification. Because heritability is the ratio of additive genetic variance to total phenotypic variance, the evolution of high phenotypic variance as an individual strategy caused by a high residual component of variance directly implies low heritability.

A hypothesis at the core of diversification bet-hedging theory is that diversification is an individual-level strategy and not simply a population-level effect; therefore, the premise is that it is the variance in seed germination timing, rather than the mean, that is acted on directly by selection and is the evolutionarily relevant trait. Diversification bet-hedging strategies are selected for under conditions of environmental unpredictability, and this has been widely cited in the theoretical literature as an explanation for seed trait variance (e.g., Cohen 1966; Westoby 1981; Venable 1985; Venable and Brown 1988; Evans and Cabin 1995; Simons and Johnston 1997). In monocarpic plants, persistence depends critically on seed and seedling survival among seasons, whereas polycarpic plants can avoid risk by spreading reproduction over multiple seasons. As predicted on these grounds, stronger seed banks tend to be produced by short-lived species (Rees 1997), and seed heteromorphisms are found more commonly in monocarpic than polycarpic plants (Imbert 2002).

The goals of this study are to assess the extent to which putative diversification strategies—both within and among seasons—occur at the individual level; to establish possible mechanisms that underlie the generation of seed trait diversification; to assess the quantitative genetic basis of time to germination, variation in time to germination, and dormancy fraction; to evaluate a major tenet of Cohen's (1966) classic dormancy bet-hedging model, that a parent's dormant seed fraction in a given season will germinate at a similar rate under similar conditions in a future season; and to assess the genetic architecture (correlation structure) linking among- and within-season bet-hedging traits.

In a series of experiments on the monocarpic perennial *Lobelia inflata* (Campanulaceae) performed under controlled conditions, we determined the timing of germination within seasons for close to 10,000 individual offspring derived from more than 100 replicated genotypes over two generations and further assessed the germination fraction over multiple seasons for all seeds of the second generation. It should be understood that we use the term ''season'' to indicate an uninterrupted time period in the growth chamber that is meant to mimic a natural growing season under conditions that satisfy the germination requirements of nondormant seeds. If diversification traits are shaped by natural selection, it is predicted that variable dormancy and within-season diversification will be expressed within sibships. However, because variable germination expression within sibships cannot be explained by genetic variance, other mechanisms must be explored. Therefore, we investigate the extent to which plasticity to microenvironmental variation within a growth chamber, or microplasticity, acts as a putative mechanism generating diversification among genetically identical siblings. This study was performed under homogeneous conditions; thus, microenvironmental effects contributing to residual variance are interpreted as minimum values.

Because bet-hedging diversification is, by definition, trait variance expressed within rather than among genotypes, diversification traits will be characterized by low heritabilities. Heritability is the ratio of additive genetic to total phenotypic variance, and low heritability may be caused either by low additive genetic variance or by high total phenotypic variance. Clearly, the expectation for a diversification trait is that it should be characterized by especially high within-geno-

type, or residual, variance. We thus assessed the coefficient of residual variation. The environmental component of variance was further controlled through a seed-sampling scheme that reduced positional effects within seed parents. We thereby constructed a strong test of the existence of diversification within individuals by increasing the potential to reject the hypothesis of zero heritability for time to germination. The effect of this experimental design on the detection of among-genotype differences in germination diversification, however, is less straightforward. If diversification is generated in part by genetic variance in plant architecture (e.g., number of branches), then this sampling design would fail to capture some among-genotype diversification. On the other hand, if variance in plant architecture excluded by this design is largely environmentally generated, the detection of genetic variance for diversification would be enhanced. In any case, the environmental component of variation was reduced by this design, and heritability values should be interpreted accordingly (see Discussion).

An analysis of the genetic architecture underlying germination traits is also of interest because it is unknown whether seed dormancy and diversification within seasons share a genetic basis, or whether they are alternative strategies maintained by antagonistic pleiotropy. Because the genotypes were drawn from three different populations, genetic population differentiation with respect to time to germination, diversification in time to germination, and germination fraction were also analyzed.

Materials and Methods

Lobelia inflata (L.) is a wide-ranging monocarpic perennial plant inhabiting disturbed sites from Nova Scotia southward to Georgia and westward to Minnesota. Features of *L. inflata* lend themselves particularly well to the present study. This species is completely self-fertilizing and, because homozygosity increases rapidly over few generations of selfing, recombination does not lead to genetic variance among offspring of a single parent. Given the low heritability of seed traits in nature, the probability of detecting genetic differences was maximized because interpretations of differences within plants were not confounded by possible genetic differences that would otherwise be caused by recombination or differences among fruits or inflorescences caused by differences either in paternity or developmental constraints on maternal allocation among fruits. Furthermore, both maternal resource allocation based on the genetic quality of her offspring (Temme 1986) and the degree of inbreeding (Kalisz 1989) may be ruled out as sources of variation in seed traits.

The experiment consisted of two generations of seed germination in which the timing of germination was recorded for seeds within each of many genotypes. Germination was observed under growth-chamber conditions for both generations; in contrast to the generation 1 experiment, which used field-collected seeds, the generation 2 experiments used seeds produced in the growth chamber by the generation 1 plants. This allowed for offspring-on-parent regressions across the two generations and sibling analyses within the second generation. The two generations thus differed in design in several respects.

Generation 1

Mature seed collected from 100 individuals growing at Martock (MTK), Nova Scotia, were used in generation 1 of the growth-chamber germination experiment. Forty seeds from each of the 100 individuals, (hereafter referred to as ''genotypes'') were randomly selected by emptying all seeds collected from a genotype onto a glass plate that had been marked with a grid and removing all but the required number of seeds remaining at the centermost position using an aspirator. These remaining seeds were placed (dry) onto small hole-punched discs of filter paper until the start of the experiment. This is the only time at which seeds were directly contacted. Damage or scarification of seeds was avoided because transfer using forceps was effected using static electricity only; forceps were not closed on a seed. To avoid confounding genotypic and common environmental effects, each 90-mm Petri dish contained a single seed of every genotype. Because it is possible that a seed's position within a Petri dish would affect germination, a genotype's position within a plate was assigned randomly to one of the 100 positions, and a novel randomization pattern was used for each plate. Because each genotype was represented by 40 seeds, 40 Petri dishes were prepared. These plates were assembled in four batches of 10 plates staggered in time to avoid synchronous peak germination of the 4000 seeds.

All seeds for a given batch were placed (dry) on the hole-punched discs of filter paper and then were moistened simultaneously with distilled water before transfer to their correct randomized positions on the Petri plates. Because light exposure prior to stratification can affect germination behavior (Simons and Johnston 2000a), we ensured that every seed experienced equal light exposure during handling: seeds spent 5 h under fluorescent light, including the time taken to allocate seeds to their positions, before all were stratified in darkness at 5°C for 30 days. Seeds were then placed in the growth chamber under a 14-h photoperiod and thermoperiod of 21°C/14°C for germination. In the growth chamber, 10 plates were placed in each of four white doubled trays, covered by a dome lid. The filter paper within each Petri dish was kept moist by watering ad libitum with distilled water using an eye dropper. To further avoid desiccation, trays were lined with paper towel and contained standing water that had previously been boiled. Each of the 4000 seeds was checked for germination every second day under a dissecting microscope. The microscope was set up directly outside the growth chamber, and plates were removed in groups of five at a time to minimize the effect of the germination check procedure. A seed was judged to have germinated upon protrusion of the radicle, whereupon the date of germination, position, and genotype were recorded. Seeds were removed upon germination.

Generation 2

The generation 2 design reflects a balance between maximizing the number of genotypes in common with generation 1 for offspring-on-parent analyses and the need to include replicated genotypes from MTK as well as from two other populations, Mt-St. Hilaire (MSH) and Harvard Forest (HFR), to accommodate sibling and population analyses. The

major differences between the two generations are; (1) 30 rather than 40 seeds per genotype were used in germination trials; (2) seeds were obtained from two replicate individuals of each genotype; (3) only seed from the first two fruits were used (to minimize within-maternal positional effects); and (4) nongermination of seeds was attributed either to dormancy or to mortality by restratifying the seeds and following subsequent germination over two more seasons, followed by viability tests.

Eighty-four MTK genotypes from generation 1 are represented in generation 2. The 16 genotypes were eliminated either through mortality at the seedling stage or were not used because of insufficient viable seed production. Of the 84 genotypes, 50 were replicated using seeds that were siblings of those used in generation one. Seed was collected from MSH and HFR during the same autumn as the MTK collection was made, and seed for the germination experiment was produced after a generation under the same growth-chamber conditions used for MTK. Fifteen replicated genotypes from both MSH and HFR were produced following the same methods as for the MTK population. One replicate of one MTK genotype died prior to seed production, yielding a total of 79 genotypes (49 MTK, 15 MSH, 15 HFR) for use in sibling analyses.

Differences in after-ripening duration can influence germination traits. Even under controlled conditions, individual seed parents initiate reproduction asynchronously (Simons and Johnston 2000b). Therefore, minimizing the effect of after-ripening has to be balanced with minimizing the effect of asynchronous maturation of individual seed parents. Influence of after-ripening due to asynchronous seed maturation was minimized in three ways. First, the duration of afterripening (dark, dry storage in the lab) was very long (8 months) compared to the slight differences (2–3 weeks) among seed parents in time of maturation of first two fruits. Second, the confounding effect of after-ripening and genotype was minimized by the use of replicate sibling seed parents. Third, any remaining differences in after-ripening were moderated by the synchronizing effect of a stratification period of fixed duration prior to the beginning of germination trials.

Sibling seeds of those tested in generation 1 were used to found the seed parents for generation 2 for use in the offspring-on-parent analysis. Parental effects specific to individual seeds (e.g., positional effects) used in generation 1 would thus not be present in offspring of their siblings. However, because siblings may be assumed to be nearly genetically identical, offspring of siblings would be genetically identical to direct offspring. About 20 sibling seeds of each genotype produced in generation one were placed in Petri plates on moistened filter paper and placed in a cold room at 5°C. The plates were protected from desiccation by sealing with parafilm. The seeds were transferred after 43 days to a growth room. Eight germinated seeds of each genotype were transferred to cell packs containing ProMix (Dorval, Quebec, Canada) at a density of two seeds per cell under a changing photoperiod schedule. One seedling was removed after 10 days if both survived the transplant. Photoperiod was set to mimic the growing season of the regions of origin of the seeds. Beginning with 13-h light, photoperiod was gradually increased to 15-h by day 84 and was reduced again to 14-h by day 104. Seeds were harvested from the bolted genotypes as they ripened, and the first and second fruits on the main inflorescence were stored separately from the remaining fruits.

Seed plates were assembled for generation 2 in a similar manner as for generation 1, but in two separate germination trials. The first trial consisted of all 79 replicated genotypes from all three populations. Because each genotype was represented by two replicated individuals, two sets of 30 plates, each with 79 positions, were assembled. Thus, each position on a Petri dish was occupied by a single seed of each genotype, and this position was randomized separately for each plate. The second trial consisted of the 35 remaining unreplicated MTK genotypes. Thirty plates, each containing a single seed from each of the 35 genotypes, were thus assembled, again randomizing genotype position within the plates.

Germination trials were conducted under the same conditions as for generation 1. Seeds were removed from the growth chamber after germination on a plate had ceased and were placed once again at 5°C for restratification. Plates were again checked for germination immediately upon removal from the first restratification treatment (some germination occurred during restratification) and were checked for germination. A third stratification was then conducted at 5°C for 30 days, and seeds were examined for germination. Finally, to determine whether the remaining seeds were viable, triphenyl tetrazolium chloride (TTC) viability tests were performed on all nongerminating seeds.

Because no appropriate TTC staining technique has been described for this small-seeded species, viability trials on reserve seeds were first performed. Three techniques were tried using seeds imbibed on moistened filter paper in Petri dishes: (1) seed coat intact; (2) seed bisected longitudinally; and (3) seed chipped and seed coat peeled back slightly at end opposite hypocotyl. In all cases, the filter paper was blotted dry and remoistened with a 1% TTC solution prior to the cutting treatment. TTC was added liberally immediately following cutting treatment, the Petri plates were placed in trays containing water in a growth chamber, and seeds were observed after both 4 h and 24 h. Seeds were deemed viable if the embryo was obviously red without dissection or if the embryo of a dissected seed appeared red. The chipping/peeling treatment, allowing 24 h for the staining reaction, showed highest viability. Assuming equal true viability within each treatment, the chipping/peeling treatment was judged the most appropriate. All remaining nongerminated seeds were left in their original Petri dishes and were tested for viability using the chipping/peeling treatment described above.

Statistical Analyses

Time to germination at the level of individual observations tends to be skewed, with a high frequency of observations occurring early. Tests of normality were performed using the Shapiro-Wilk W statistic for $N < 2000$, and using Kolmogorov-Smirnov-Lilliefors (KSL) D statistic for $N > 2000$. The entire dataset did not conform to a normal distribution (KSL test: $D = 0.101$, $P = 0.001$, $N = 7556$); nor did generation 1 (KSL test: $D = 0.171$, $P = 0.001$, $N = 3250$) or

TABLE 1. Characteristics of datasets used for analyses of overall germination distributions, sibling analyses using restricted maximum likelihood (REML), and offspring-on-parent (O-P) analyses of heritability for the three germination traits under study.

Germination trait	Analysis	Unit of observation and sample size	Dataset	Number of seeds in dataset
	general trait distributions	all individuals per 130 genotypes	both generations	9790
Germination fraction	ANOVA REML	two sibship means per 79 genotypes	generation 2	4720
	ANCOVA (O-P)	one genotype mean per 84 genotypes per two generations	both generations	7350
Time to germination	ANOVA REML	all individuals per two sibships per 79 genotypes	generation 2	3775
	ANCOVA (O-P)	all individuals per 84 genotypes per two generations	both generations	5688
Diversification in time to germination	ANOVA REML	two sibship means per 79 genotypes	generation 2	3775
	ANCOVA (O-P)	one genotype mean per 84 genotypes per two generations	both generations	5688

generation 2 for either trial 1 (KSL test: $D = 0.069$, $P = 0.001$, $N = 3775$) or trial 2 (Shapiro-Wilk test: $W = 0.925$, $P < 0.001$, $N = 531$). Two standard transformations (Zar 1999) were assessed; $g' = \log(g + 1)$ and $g' = (g + 0.5)^{0.5}$. The means of sibship transformed individual values used to calculate heritabilities and genetic correlations through ANOVA (generation 2, trial 1), deviated slightly from a normal distribution for log-transformed germination time (Shapiro-Wilk test: $W = 0.956$, $P = 0.025$; $N = 79$), but did not differ from a normal distribution for square-root-transformed germination times (Shapiro-Wilk test: $W = 0.968$, $P = 0.161$, $N = 79$). Therefore, results based on the square-root transformation are reported throughout, but we also note where log transformation yields a qualitatively different result.

Germination and viability fractions p are proportional measurements and were transformed as arcsin ($p^{0.5}$). Arcsine-transformed germination fractions did not differ significantly from a normal distribution (Shapiro-Wilk test: $W = 0.967$, $P = 0.152$, $N = 79$).

Because mean time to germination and variance in time to germination are positively related, a measure of diversification in time to germination was obtained by computing the residuals from regressions of variance on mean using transformed ($[g + 0.5]^{0.5}$, above) individual times to germination for both variance and mean. Residuals, observed at the level of two sibships per genotype (Table 1), were used because the coefficient of variation overcorrects for mean, resulting in a spurious negative relationship. In separate-slopes ANCOVAs, no differences in residual variance-mean regression slopes for the two MTK germination trials within both generations (interaction generation 1: $P = 0.599$; generation 2: $P = 0.170$) or for the three populations (interaction: $P = 0.358$) were observed. However, to avoid the possibility of generating erroneous results caused by even weak differences in relationships between mean and residual variation among trials or populations, the residuals from the variance-mean ANCOVA models were used rather than the residuals from the simple regression of variance on mean. The complete independence of these residuals with mean transformed germination time both at the level of the dataset and within trials and populations was verified ($r = 0$, $F = 0$, $P = 1$).

The two germination trials of generation 2 were conducted at different times. Differences between these two trials were accounted for in the offspring-on-parent analyses by computing regression slopes based on an ANCOVA model that included the nominal variable trial, the parental value as the continuous covariate, as well as their interaction term to predict offspring value. For heritability analyses based on siblings in generation 2, heritabilities and their 95% confidence intervals were computed using restricted maximum likelihood (REML) estimates of variance components from random effects models including population and genotype nested within population. Significance of variance components was assessed through likelihood-ratio testing (LRT), wherein the test statistic for a particular effect is given by twice the difference in the value of the log-likelihood score (2∂LL) associated with eliminating the random effect from the model. The LRT is approximated by a chi-square distribution and, because a more complex hierarchical model necessarily improves with the addition of an effect, significance is established using a one-tailed test with one degree of freedom (Littell et al. 1996; Shaw and Geyer 1997).

Genetic correlations were estimated based on family-mean correlations because diversification in time to germination and germination fractions were measured at the sibling-group level. Again, diversification in time to germination within seasons given by the residuals of the regression of transformed variance on transformed mean calculated independently for trial and population.

The unit of observation used in particular ANOVAs and ANCOVAs depends on whether traits are individual level (timing of germination) or sibling-group level (germination fraction, germination variance), as well as on whether replicated or unreplicated genotypes are available. The principle followed was to use the finest unit of observation available. For example, data from individual seeds were used in analyses of time to germination, whereas across-generation analyses of germination fraction were performed on genotype means. For clarity, an overview of datasets used in each analysis is given in Table 1.

RESULTS

General Germination Characteristics

At the level of the whole dataset, variance in time to germination exists among seasons (Fig. 1): both directly ger-

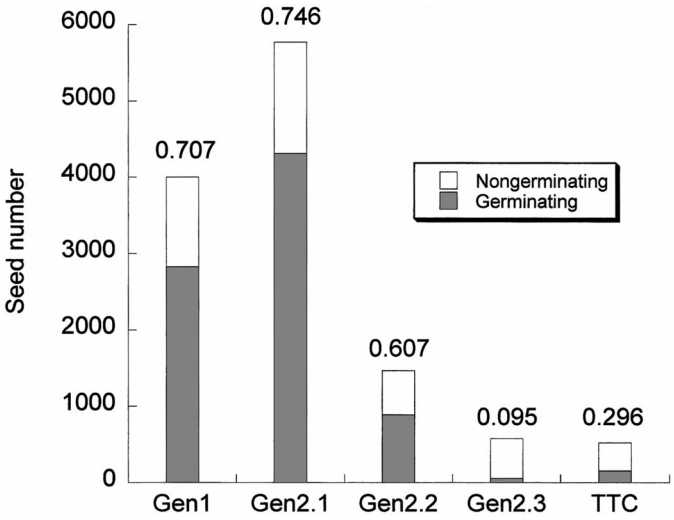

FIG. 1. Germination of seeds of *Lobelia inflata* over two generations and over multiple seasons. Seeds of generation 1 (Gen1) were collected from mature individuals growing wild at Martock, Nova Scotia, and were tested over one growing season in a growth chamber. Parental plants of seeds of generation 2 were produced in the growth chamber. In generation 2, germinability was tested over three seasons (Gen2.1, 2.2, and 2.3) following stratification, and the remaining nongerminating seeds were tested for viability (TTC). Germination or viability fractions are given above each column. See text for separate results of the two germination trials in generation 2.

minating and nongerminating seeds occurred, and nongerminating seeds germinated after further stratification. The germination fraction following one stratification (i.e., during the first season) was 0.707 in generation 1 and 0.746 in generation 2 (Fig. 1). Much variation also existed within seasons at the level of the whole dataset (Fig. 2). Within a season, the mean time to germination was 16.6 ± 8.4 days in generation 1 and 27.94 ± 13.11 days overall in generation 2 (Fig. 2).

This variance observed at the dataset level was indeed produced by variance occurring at the individual level: both variable germination fraction and substantial variance in time to germination within a season were found among seeds within individuals (Fig. 3): extreme examples included seeds from the first two fruits within a single individual that germinated in the first, second, and third season, and during the first season over a range of 52 days.

The main germination trial (trial 1, generation 2) data suggest that characteristics of the three populations were similar (Table 2), with a germination fraction of 0.800 ($N = 4720$) and mean time to germination of 28.4 ± 13.3 days. However, seeds from MSH appeared to germinate slightly earlier within a season and showed slightly higher germination fraction (Table 2). In this trial, a further 0.632 of the seeds that had not germinated in the first season germinated during the second after restratification, and 0.379 of the remaining seeds either germinated or were still viable during the third season. Seeds of trial 2 of generation 2, which were included to increase the number of MTK genotypes common to both generations, showed lower germination fractions yet seeds that germinated did so earlier, on average, during the growing season (Table 2). In this second trial, MTK's second season germination rate was similar to that in trial 1, and 0.338 ($N = 228$) of the seeds remaining after two seasons either germinated or were viable in a third season. Only 521 seeds of the total 5770 for generation 2 did not germinate during the course of the experiments, for a total germination rate of 0.910. The overall viability of seeds, as judged by the cumulative germination over three stratification treatments and viability tests of the remaining seeds, was 0.936.

Growth Chamber Positional Effects

The position of each seed within Petri dishes, the position of Petri dishes within trays, and the position of trays within the growth chamber were recorded, allowing for an analysis of the plastic response of seed germination to microenvironment over these spatial scales. Because their positions were randomized within Petri dishes, genotype did not confound environmental effects. A three-factor random effects ANOVA demonstrated that the positional effects, including genotype's seed randomized on Petri dishes, the position of Petri plates in trays, and the location of trays inside the

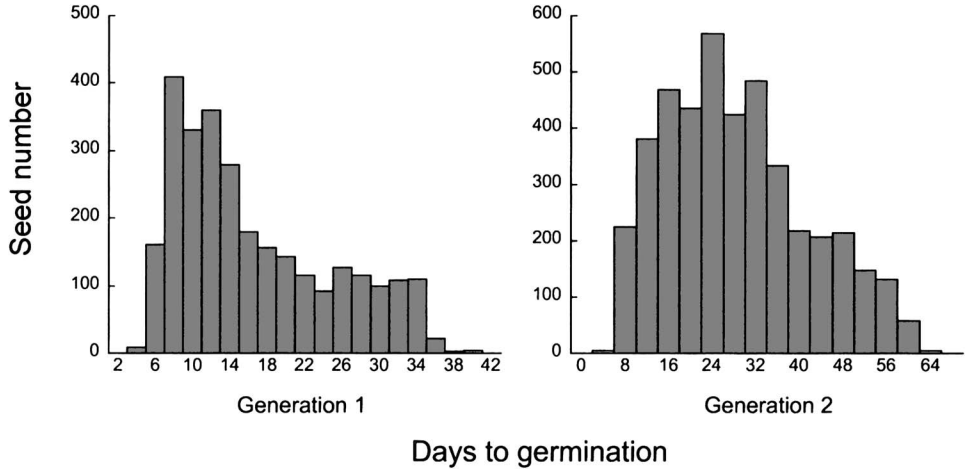

FIG. 2. Timing of germination of seeds of *Lobelia inflata* within a single growing season tested for two generations under growth-chamber conditions.

growth chamber, explained a high proportion of variation in timing of germination (Table 3). The only nonsignificant main effect was the plate effect, but the interactions of plate and both tray and seed position were significant (Table 3).

Quantitative Genetic Analyses

Germination fraction

In a random effects ANOVA with two observations of arcsine-square-root transformed germination fraction per genotype nested within population, a LRT found a significant heritability of germination fraction ($h^2 = 0.18$), whereas no significant difference was found among populations (Table 4). Pooling populations in a one-way random effects REML analysis increased the heritability slightly ($h^2 = 0.20$). Because one-way analyses are not amenable to LRTs, significance was established using traditional expected mean squares (EMS) methods ($P = 0.0359$, $F = 1.51$, df = 78) for this pooled population test. To explore possible differences in genetic variation within populations, post hoc tests were performed on individual populations. The population with the largest sample of replicated genotypes ($N = 49$; MTK) showed a significant heritability ($h^2 = 0.26$, $P = 0.0344$, $F = 1.70$, df = 48). No significant genetic variation for germination fraction was detected within the two populations with low sample size (HFR: $h^2 = 0.00$, $P = 0.829$, $F = 0.597$, df = 14), although the limited data suggest a high heritability for the trait at MSH ($h^2 = 0.33$, $P = 0.099$, $F = 1.992$, df = 14). Significance for post hoc tests was calculated based on EMS, and heritabilities were identical to two decimal places using LS and REML methods.

In an offspring-on-parent ANCOVA model with separate slopes for the two germination trials (interaction: $P = 0.045$), the germinability of field-collected seeds from generation 1 and that of their growth-chamber-produced offspring was significantly, but negatively, related (Table 5): genotypes with a relatively low germination fraction in one generation showed high germinability in the next. As post hoc confirmation, simple offspring-on-parent regressions were performed separately for the two trials and were consistent with above results (trial 1: $h^2 = -0.796 \pm 0.362$, $r^2 = 0.128$, $P = 0.035$, $N = 35$; trial 2: $h^2 = -0.062 \pm 0.164$, $r^2 = 0.003$, $P = 0.708$, $N = 49$). This across-generation negative relationship in germinability was maintained through two seasons: a highly significant negative family-mean correlation was found between transformed germination fraction in generation 1 and in the second season of generation 2 ($r = -0.283$, $P = 0.0091$, $N = 84$): if a genotype exhibited strong dormancy, its offspring germinated at a disproportionately high rate not only in the first season (see negative heritability, above), but also following a second stratification. The correlation coefficient was negative for both germination trials (trial 1: $r = -0.233$, $P = 0.107$, $N = 49$; trial 2: $r = -0.380$, $P = 0.024$, $N = 35$). Again, results were qualitatively identical using untransformed germination fractions ($r = -0.302$, $P = 0.0052$, $N = 84$).

Mean timing of germination within seasons

Although seed germination timing within seasons exhibited high variation (above), the comparatively low proportion of variation occurring among genotypes ($h^2 = 0.07$; Fig. 3a) was significantly greater than zero by a LRT (Table 4) in a random effects nested ANOVA estimated using REML with 30 seeds from two individuals nested within 79 genotypes nested within their respective populations. Although seed

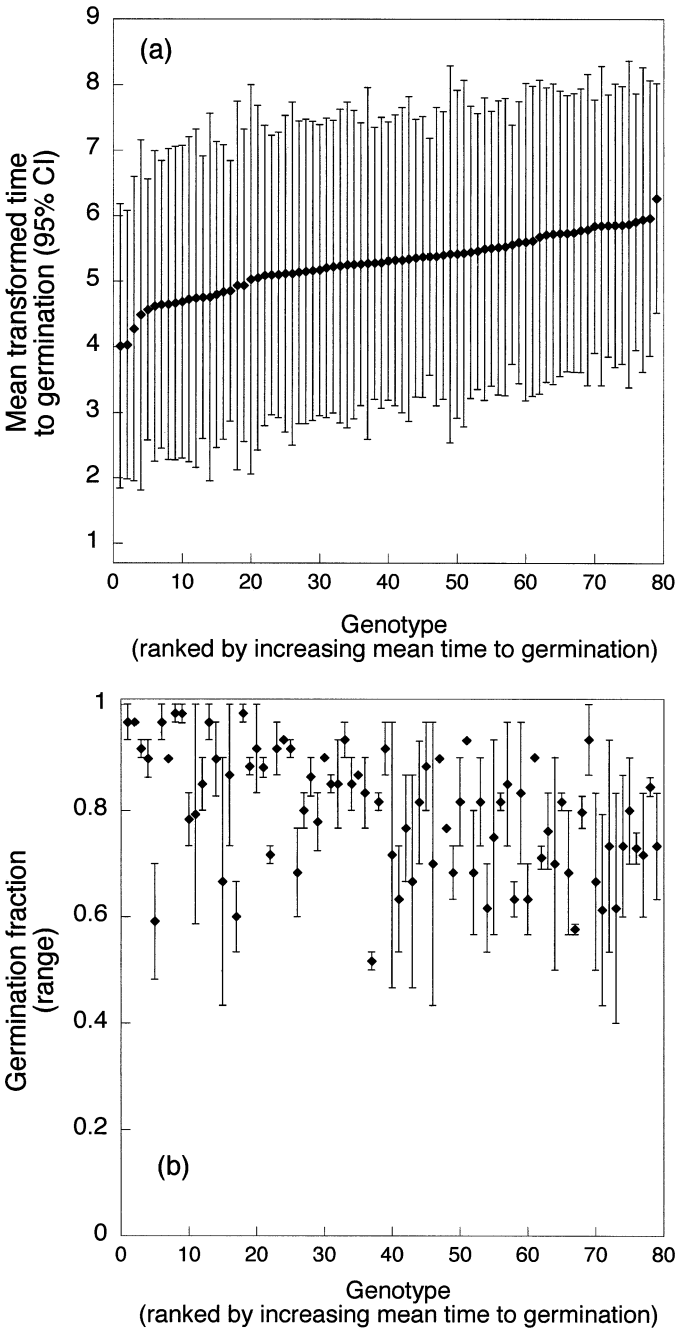

FIG. 3. The expression of germination variation within and among genotypes of *Lobelia inflata*. Diversification in transformed ($g' = [g + 0.5]^{0.5}$) time to germination within a season (a) and germination fractions (b) for 79 genotypes are both presented in order of increasing genotype mean time to germination. Error bars represent 95% confidence intervals for time to germination (a), and the minimum and maximum germination fractions for sibling groups of each genotype (b). Data are from the main germination trial (generation 2, season 1, trial 1).

TABLE 2. General germination characteristics of three populations used in generation 2, trial 1. Populations are Martock, Nova Scotia (MTK); Mt-St. Hilaire, Quebec (MSH); and Harvard Forest, Massachusetts (HFR). Time to germination (T_G) is the within-season timing, whereas germination fractions are proportions germinating after one (G_1), two (G_2), or three (G_3) sequential stratification treatments. The proportion of viable, nongerminating seeds remaining after three germination trials (TTC) was determined using a tetrazolium viability assay.

Trial	Population	T_G (days)	G_1	G_2	G_3	TTC	N
1	MTK	28.87 ± 13.16	0.795	0.559	0.086	0.280	2931
	MSH	26.78 ± 13.59	0.843	0.752	0.029	0.265	895
	HFR	28.67 ± 13.40	0.774	0.766	0.191	0.579	894
2	MTK	24.54 ± 11.02	0.506	0.561	0.097	0.267	1050

TABLE 3. Microenvironmental effects on time to germination within a homogeneous growth chamber. Seeds from 79 genotypes were randomly allocated to 79 positions within each Petri plate, and Petri plates were placed in each of 10 locations within each of six trays. Germination data are from the main germination trial (3775 seeds germinating in trial 1, generation 2). The model, which explains time to germination as microplasticity in response to unmeasured gradients within a growth chamber, accounted for 31.7% of the total variance in time to germination.

Source	df	MS	F	% Variance explained
Tray	5	73.53	9.24	7.20***
Plate	9	8.32	0.97	0.00
Position	78	6.21	3.21	6.02***
Tray × plate	45	8.24	7.29	7.46***
Plate × position	702	1.96	1.73	10.76***
Tray × position	390	1.17	1.03	0.30

*** $P < 0.0001$.

families differed in time to germination, no difference in mean time to germination among populations was observed (Table 4). Lumping populations in a reduced model had no effect on heritability estimation. Because time to germination was observed at the level of the individual seed, the power of analyses was greatly enhanced compared to that for the sib-group-level observation of germination fraction and within-season variation in timing of germination. Post hoc analysis performed at the population level using traditional EMS revealed no significant genetic variation in time to germination among MTK genotypes ($h^2 = 0.020$, $P = 0.108$, $F = 1.42$, df = 48), highly significant genetic variation among the 15 genotypes from MSH ($h^2 = 0.212$, $P = 0.004$, $F = 4.29$, df = 14), and low and nonsignificant genetic variation among genotypes from HFR ($h^2 = 0.050$, $P = 0.166$, $F = 1.68$, df = 14).

The alternative approach to heritability estimation using offspring and parents was consistent with the sibling analyses: the covariance analysis using two trials of the 84 MTK genotypes common to both generations showed no significant resemblance between offspring and midparent values in time to germination (Table 5).

Low heritability can be caused by high residual variance (Houle 1992; Simons and Roff 1994) rather than by low additive genetic variance. To obtain meaningful comparative values, the degree of additive genetic and residual variance can be standardized by trait mean to yield the coefficient of additive genetic variation in time to germination,

$$CV_A = \frac{100\sqrt{V_A}}{\bar{X}} = 11.6, \quad (1)$$

and the coefficient of residual variation

$$CV_R = \frac{100\sqrt{V_R}}{\bar{X}} = 45.4. \quad (2)$$

Variation in timing of germination within seasons

Diversification of the timing of germination, which is quantified as the residual values from the relationship between mean and variance in square-root-transformed time to germination, did not show significant variation among genotypes in an ANOVA using replicate individuals' residual values as observations nested within populations (Table 4). Power analysis showed that a heritability of <10% would have been detected with the sample size used. A sample size of >50,000 genotypes would be required to distinguish the observed ef-

TABLE 4. Restricted maximum likelihood estimates of variance components of three germination traits from three populations of *Lobelia inflata*. The genotype component of variance (Geno) estimates the heritability (h^2; see Materials and Methods), and the population component (Pop) estimates variance among populations (V_{pop}). Analyses were performed on transformed germination data (see Materials and Methods). Genotype was nested within population of origin for all analyses and, because observations are available at the seed level for the analysis of time to germination, data from sibling seeds of replicate individuals (Ind) were nested within genotypes. Likelihood-ratio tests were employed to establish significance of random effects based on the test statistic 2∂LL. Standard errors on variance components are reported, but true confidence limits are asymmetric.

Trait	Effect	2∂LL	Variance component (SE)	% Variance explained
Germination fraction	geno(pop)	2.73	0.00804 (0.00583)	$h^2 = 17.86$*
	pop	0.716	0.00180 (0.00345)	$V_{pop} = 4.00$
	residual	—	0.0352	78.14
Time to germination	ind(geno, pop)	—	0.14745 (0.0326)	9.24
	geno(pop)	409.9	0.11003 (0.0377)	$h^2 = 6.91$***
	pop	0.00	0.0000 (0)	$V_{pop} = 0.00$
	residual	—	1.3362	83.86
Germination variation	geno(pop)	0.00117	0.00053 (0.015)	$h^2 = 0.37$
	pop	1.0972	0.00526 (0.00906)	$V_{pop} = 3.74$
	residual	—	0.1348	95.88

* $P < 0.05$; *** $P < 0.001$ by likelihood-ratio test.

TABLE 5. Offspring-on-parent ANCOVA estimates of heritabilites (h^2) of germination characteristics in a population of *Lobelia inflata* from Martock, Nova Scotia. Analyses were performed on transformed germination data (see Materials and Methods).

Trait	h^2 (SE)	P	N
Germination fraction	−0.492 (0.180)	0.0194	84
Mean time to germination	0.006 (0.0948)	0.946	83
Germination variation	−0.259 (0.2071)	0.215	83

fect size from zero. The analysis suggested a marginal effect of population for log-transformed ($V_{pop} = 0.0657$; $2\partial LL = 2.655$, $P = 0.0516$), but not for square-root-transformed (Table 4) residual variance. The within-population heritabilities of residual germination variance also suggested no genetic variation for the diversification trait at MTK ($h^2 = 0.000$, $P = 0.594$, $F = 0.933$, df = 48), MSH ($h^2 = 0.026$, $P = 0.458$, $F = 1.054$, df = 14), or HFR ($h^2 = 0.004$, $P = 0.492$, $F = 1.008$, df = 14).

Parental seeds from the field and their offspring produced in the growth chamber in generation 2 showed a weak negative nonsignificant resemblance in residual germination variation (calculated separately for each trial) using midparent values for all available (both replicated and unreplicated) genotypes in an ANCOVA (Table 5). This negative resemblance between generations was stronger when only the 49 replicated genotypes from generation 2 and their midparent values were included ($h^2 = -0.376 \pm 0.181$, $F = 4.30$, $P = 0.0436$, $N = 49$).

Trait correlations

Family mean correlations among the three germination characteristics under study—germination fraction, within-season time to germination, and variation in time to germination—were used to assess the common genetic basis underlying these traits. A significant correlation was found between germination fraction and within-season residual germination variation (Table 6). This relationship was alternatively tested as an ANCOVA with germination fraction dependent on residual germination variation ($P = 0.0244$, $F = 5.2$, df = 105) including separate slopes for trial and population ($P < 0.001$, $F = 21.66$, df = 3) and the interaction term ($P = 0.981$, $F = 0.0595$, df = 3). Further exploratory analyses using residual germination variation revealed that this relationship between germination fraction and within-season residual germination variation was weaker for the MTK population in trial 1 ($r = 0.149$, $P = 0.308$) than in trial 2 ($r = 0.383$, $P = 0.025$), and weaker for the MSH population ($r = 0.085$, $P = 0.764$) than for the HFR population ($r = 0.455$, $P = 0.089$). Significance values are reported only for interest in these post hoc analyses using subsets of the data.

Transformed germination fraction over the first two seasons showed a strong family mean correlation (Table 6); a genotype's nongerminated seeds after one germination trial showed a similar germination frequency following a second stratification treatment. No significant genetic correlation was found, however, for the first to the third season nor the second to third (Table 6). Results were qualitatively identical using untransformed germination fractions.

TABLE 6. Family-mean pairwise genetic correlations among germination traits of *Lobelia inflata*. G_1, germination fraction in first season; T_G, mean time to germination; Var_G, variation in time to germination; G_2, germination fraction in second season; G_3, germination fraction in third season. Analyses were performed on transformed germination data, and Var_G values are residuals from relationship between transformed mean and variance (see Materials and Methods).

	T_G	Var_G	G_2	G_3
G_1	−0.054	0.207*	0.468***	−0.020
T_G	—	0†	0.0359	−0.073
Var_G		—	0.150	−0.086
G_2			—	0.120

* $P < 0.05$; *** $P < 0.001$.
† Forced to zero through use of residuals from mean-variance relationship.

DISCUSSION

Traits that appear to be suboptimal over short time scales may be optimal when environments are increasingly variable over longer time scales (Simons 2002). Monocarpic plants offer an ideal model to test bet-hedging theory because lifetime reproductive success can be measured within a discrete time period (Simons and Johnston 2003). If parental plants evolved to program all progeny seeds to germinate synchronously on the date that, on average, leads to the greatest reproductive success, expected (or arithmetic mean) fitness would be maximized. However, a single generation in which that date proves to be inappropriate would lead to the elimination of the lineage. Observed seed germination variance is often explained as a diversification bet-hedging strategy whereby individuals minimize the likelihood of complete reproductive failure by paying a cost in the form of a reduction in expected fitness. Explaining seed trait variance has been, and continues to be, a major concern to those interested in life-history evolution because of the close association between seed traits and fitness (e.g., Cohen 1966; Janzen 1969; Smith and Fretwell 1974; Harper 1977; Marks and Prince 1981; Venable 1985; Kalisz 1986; Michaels et al. 1988; Venable and Brown 1988; Westoby et al. 1992; Philippi 1993; Rees 1997; Simons and Johnston 2000a; Galloway 2002; Donohue et al. 2005; Evans and Dennehy 2005).

The hypothesis that germination timing variance, rather than mean germination timing, is an evolutionarily relevant trait would account for the ineffectual attempts to eliminate within-season variation in germination timing: selected early or late germinators would merely be a random sample from a diversified germination schedule of a genotype. Even in agricultural settings, diversification may be a desirable trait: Peruvian farmers are known to exploit existing seed-size variation as a means of increasing yield under variable soil moisture conditions (Zimmerer 2003). Knowledge of the genetic basis of dormancy resulting in among-season germination variance is rudimentary (Foley and Fennimore 1998), and our understanding of the genetic basis of within-season germination variance, and its association with dormancy, is even more deficient.

The present work is an attempt to understand the roles of

microenvironmental and genetic variance in generating seed germination variance, both within and among growing seasons, by following the germination patterns of individual seeds of known genotype of origin over two generations and multiple seasons under highly controlled conditions. It is important to understand that results were affected by performing germination tests under homogeneous conditions that may inflate heritabilities (Simons and Roff 1994) and alter genetic correlations (Simons and Roff 1996), and in generation 2 by using seeds from only the first two capsules. This design reflects the unusual focus in this study on diversification, which is expressed as the within-genotype component of variance. An essential feature of the experimental design, therefore, was to ensure that an inability to reject the null hypothesis of $h^2 = 0$ for time to germination cannot be explained by an inflated environmental component of variance. However, this design may have altered the detection of among-genotype differences in diversification in time to germination: heritability is underestimated if excluded sources of variance associated with plant architecture, such as branching patterns, are generated by additive genetic variance. However, if architectural variation among individuals reflects phenotypic plasticity, any resulting germination variance would have further increased the within-genotype, environmental component of variance. The heritability analyses are thus best conservatively interpreted as tests for the presence of additive genetic variance.

The evolution of diversification implies high variance in time to germination occurring at the within-genotype level. The observed levels of within-genotype germination variance (measured as CV_R; below) were remarkably high, especially given that seeds within sibships were genetically identical, and germination trials were conducted under growth-chamber conditions that were ostensibly homogeneous. Furthermore, the use of seeds from only the first two capsules produced in generation 2 should have reduced this residual component of germination variance because fruit size and position within a plant are important sources of germination variance (Simons and Johnston 2000a). The production of diversification in the timing of seed germination among genetically identical progeny thus requires explanation and may be accomplished through intrinsic differences among seeds generated during development or by identical seeds through plastic germination responses. The boundary between plasticity and developmental noise becomes indistinct when environmental gradients occur on small scales because noise includes plasticity resulting from unknown sources (Bradshaw 1965). Such microplasticity can result in the randomization of phenotypic expression and may thus act as a diversification mechanism (Simons and Johnston 1997). We emphasize that environmental effects found within a growth chamber are likely to be underestimates of the potential diversification resulting from microplasticity under a more heterogeneous field environment.

Although the growth-chamber environment was homogeneous relative to natural environments, microenvironmental gradients existed. Because the germination behavior of every seed—each at a known position within Petri plates within trays—was followed, the effect of gradients on germination characteristics could be analyzed. The high proportion (31.7%) of total variance in time to germination explained by position (Table 3) indicates that germination behavior was highly sensitive to environmental gradients occurring within the growth chamber. For example, seeds within tray 3 took longer to germinate (31.3; backtransformed to days) than those in tray 5 (22.4 days). Furthermore, apparent environmental gradients existed within Petri dishes: position 36 was consistently associated with late germination (36.4 days) and position 11 with rapid germination (21.0 days). The strong interaction effects (Table 3) further indicate the existence of complex microplasticity. Although the nature of the gradients is unknown, possibilities include moisture, light, and their interaction. Under field conditions, the range of these gradients is expected to be much greater. Therefore, these gradients, compounded by additional sources of microenvironmental variance in the field, such as pH and soil texture, might be expected to further amplify phenotypic variance in the timing of germination.

Extensive variation in among-season delay also existed within sibships (Fig. 3b). However, analyses performed on seeds produced in generation 2 under growth-chamber conditions indicate significant covariance of germination fraction among siblings. Nonzero heritability offers evidence for the presence of additive genetic variance and thus that germination fraction can respond to selection; however, because of the homogeneous conditions and sampling design, the value of the measured heritability is suspect. The negative across-generation heritability indicates that the progeny of genotypes expressing high germinability express low germinability. The fact that the germination pattern of the dormant subsample following stratification corroborates this negative heritability lends credence to the relationship.

Although no support for mechanisms underlying this negative heritability can be offered at present, two possibilities exist. First, genotype-by-environment interaction could have produced this result. However, this mechanism is supported only if the environments experienced by generations 1 and 2 were different and the environments experienced by the two germination seasons of generation 2 were not different, because a positive genetic correlation existed among seasons. The protocol difference between the two generations (the use of seeds from only the first two capsules from each plant in generation 2; field-collected seeds used in generation 1) may have decreased heritability by increasing environmental variance, but it cannot explain the negative heritability. A second explanation for this unexpected result is that an alternating germination strategy has evolved. Hypothetically, for example, the phenotypic expression of genetic factors influencing germinability might be context dependent, where the context is itself the degree of parental germinability. To our knowledge, no theoretical work has examined the fitness consequences of alternating germination strategies.

If diversification is an evolved strategy, it must occur at the individual level. Our results clearly demonstrate that variance in the timing of seed germination occurs within genotypes and is not merely a population-level phenomenon caused by differences among genotypes (Fig. 3a). Under the conditions of low environmental variation characterizing this study, no genetic variation in within-season germination diversification was detected; thus, there is no evidence that

selection can modify the expression of within-season germination diversification directly. Although statistical power of this analysis was reduced because the unit of observation was sibling groups of seeds (individuals have no variance), a power analysis showed that a heritability of less than 10% would have been detected with the sample size used. Furthermore, the observed effect size was so low that a sample size of over 50,000 genotypes would have been required to distinguish it statistically from zero at $\alpha = 0.05$. Given the low effect size and that the experiments were conducted under laboratory conditions, our results constitute strong evidence for low heritability of within-season germination variation. Again, only if genetic variation underlying plant architecture, which was omitted in our sampling design, contributes to genetic variance in germination characteristics would our heritabilities be underestimated. Genetic variation is commonly found in fitness traits expected to be under directional selection (Mousseau and Roff 1987); therefore, it would be difficult to claim that the lack of genetic variance found here for germination diversification indicates the erosion of genetic variance through a history of strong selection.

Diversification should be characterized by high phenotypic variance expressed within genotypes; low heritability offers only an incomplete measure of this phenomenon. Because heritability is the fraction of total phenotypic variance explained by additive genetic variance, low heritabilities do not necessarily reflect a lack of additive genetic variance (Houle 1992), but can be caused by high residual variance (Houle 1992; Simons and Roff 1994). The coefficient of genetic variation in time to germination ($CV_A = 11.6$) was relatively high, but the coefficient of residual variation ($CV_R = 45.4$) was higher even than the highest CV_R of 39.02 reported in Houle's (1992) demonstration of the potential importance of residual variation in driving the low heritabilities of fitness traits. The finding of an exceptionally high residual component of variance (and low heritability) even after eliminating known and important environmental sources of germination variance constitutes strong support for the claim that diversification exists as a within-individual trait. It should also be noted that we included only seeds germinating within a growing season in this calculation; the additional contribution to variance from dormant seeds could not be included because time unit measurements for within and among seasons cannot be meaningfully related.

The significant genetic (family-mean) correlation between germination fraction in the first and second seasons lends support to the underlying assumption of Cohen's (1966) classic dormancy model that the dormant seed fraction will germinate in a future season at a similar rate under appropriate conditions; this finding also demonstrates that dormancy fraction is an individual characteristic. The significant genetic correlation between germination fraction and within-season germination diversification is suggestive of a genetic tradeoff: genotypes that spread risk among seasons do not spread risk as strongly within a single season. Low within-season variance in time to germination may be a direct result of the reduced number of seeds germinating from genotypes of high dormancy. This would be an interesting case of direct biological implications of a statistical phenomenon and suggests that seed number may be under direct selection for its effect on the expression of diversification bet hedging (Simons 2007).

The phenomenon of high germination variance has engendered theoretical work describing the fitness advantages associated with such variance occurring both within a single growing season and as dormancy among seasons. Advantages occur under variability in seedling density over space (Geritz 1995), when sibling competition is high (Nilsson et al. 1994), and as bet hedging under temporal environmental unpredictability. Critical tests of bet-hedging theory are rarely attempted because of the inherent difficulties associated with the time requirements of such studies, with the quantification of environmental variance, and with the empirical assessment of bet-hedging strategies (Simons and Johnston 2003). Knowledge of the fitness effects of environmental variability, however, is fundamental to a thorough grasp of the basic concept of optimality (Simons 2002). The present work provides evidence that germination diversification may result from microplasticity, that is, contingency of trait expression upon microenvironmental variation that acts as a randomization mechanism (Simons and Johnston 1997), and thus contributes to an understanding of the causal components of diversification. Mechanisms underlying diversification as well as the evolutionary significance of diversification require greater empirical attention.

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