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VARIATION IN SEED TRAITS OF *LOBELIA INFLATA* (CAMPANULACEAE): SOURCES AND FITNESS CONSEQUENCES[1]

ANDREW M. SIMONS[2] AND MARK O. JOHNSTON

Department of Biology, Life Sciences Centre, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J1

Seed germination constitutes an important event in the life cycle of plants. Two related seed traits affect fitness: seed size and the timing of seed germination. In three sets of experiments, we (1) partition the sources of seed-size variance in *Lobelia inflata* into components attributable to fruit size, relative fruit position, and parental identity; (2) examine the influence of pregermination conditions and seed size on time to germination; and (3) assess the fitness consequences of seed size and germination timing under seminatural, harsh conditions. Seed-size variance is attributable to both parental identity and fruit position within an individual. Distal fruits produce larger but fewer seeds. No significant correlation exists between fruit size and seed size, but a trade-off is found between the number and size of seeds contained in a fruit after correcting for fruit size. The timing of germination is influenced by seed size, light conditions before winter, and winter duration. Germination timing influences survival, and despite small seed size in this species (2×10^{-5} g/seed), seed size has a persistent and significant association with both final plant size and the probability of survival to autumn.

Key words: Campanulaceae; fitness; fruit size; germination; *Lobelia inflata*; rosette; seed number; seed size; survival.

Seed traits, through their pronounced and multiple fitness effects (Janzen, 1969; Harper, 1977), are a critical element in the ecology and evolution of plant life histories. Two seed traits closely related to fitness are size and germination timing. Whereas seed-size variation within species may be slight compared to the 10^{11}-fold variation found among species (Westoby, Jurado, and Leishman, 1992), it is nonetheless significant (Schaal, 1980; Thompson, 1984; Michaels et al., 1988). Seed size is one element of a coevolving complex of traits (Venable and Brown, 1988; Rees, 1997) including seed dormancy, dispersal, plant mass, longevity, niche specialization, and competition among species, all constrained by phylogeny. Because individual seed traits do not evolve independently (Venable and Brown, 1988), conclusions about the fitness consequences of seed-size variance cannot be generalized among species. Thus, although seed-size variance may be lower within than among species, the fitness consequences of within-species variation are more tractable than for an among-species study (Venable et al., 1998). The present study focuses on within-species sources of variation in seed size, as well as on its associated ecological and evolutionary importance.

Plants that have produced the highest number of seeds surviving to successfully reproduce are the best represented in the present population. Preventing runaway evolution of high seed number is a balance between seed number and the probability of survival of an individual seed as determined by resource investment per seed (Smith and Fretwell, 1974). The most commonly cited among-species advantages of large seed size through greater reserves are drought resistance, early shade tolerance, and other direct effects of larger initial seedling size (Westoby, Jurado, and Leishman, 1992). In general, the correlation between seed size and seedling size disappears a few days after germination because of a negative correlation between seed size and relative growth rate (Westoby, Jurado, and Leishman, 1992, and references therein), presumably driven by resource allocation to other functions such as stress tolerance (Armstrong and Westoby, 1993). In addition, it has been demonstrated that within-species seed-size variance is associated with a variety of fitness-related traits such as both the probability and timing of germination (Schaal, 1980; Roach, 1986; Winn, 1988; Biere, 1991b; Platenkamp and Shaw, 1993).

Smith and Fretwell's (1974) model predicts a single optimal seed size, but the assumptions underlying the model may often be violated. For example, the optimal investment per seed may be time dependent, changing with the parent's reproductive value. Seeds go from being the least to the most vulnerable stage of the plant life cycle upon germination and, although large seeds may have higher fitness under given conditions (Biere, 1991b; Galen and Stanton, 1991), even the largest seed may exhibit low fitness if the timing of germination is inappropriate. Under environmental uncertainty, the timing of germination both within a season (Marks and Prince, 1981; León, 1985; Silvertown, 1985; Venable, 1989; Biere, 1991b; Simons and Johnston, 1997) and among seasons (Cohen, 1966; Philippi, 1993; Pake and Venable, 1995) may have important fitness consequences, and selection can favor diversification at both temporal scales. Density may also have pronounced effects on selection for germination time (Miller, Winn, and Schemske, 1994); germination variance among sibs may result from density-dependent selection (Geritz, 1995).

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[2] Author for correspondence, current address: Department of Genetics, Box 7614, North Carolina State University, Raleigh, North Carolina 27695-7614 USA (amsimons@unity.ncsu.edu).

Indeed, much within-species variation exists in both seed size (Schaal, 1980; Stanton, 1984; Thompson, 1984; Michaels et al., 1988; Biere, 1991a; Wolfe, 1995) and timing of germination (Marks and Prince, 1981; Kalisz, 1986; Biere, 1991a), even in agricultural species with restricted genetic variation (Silvertown, 1984), but few studies explicitly examine the sources of within-individual seed polymorphism in natural populations. Furthermore, studies tend to be restricted to either seed-size or germination variance and rarely reveal their joint fitness consequences.

This study aims to determine sources of among- and within-individual variation in both seed size and time to germination, and to discover the fitness consequences of this variation in *Lobelia inflata* (Campanulaceae). Seed traits are somewhat exceptional in that their heritabilities are typically extremely low (Schaal, 1980; Kalisz, 1986; Mazer, 1987; Schwaegerle and Levin, 1990; Biere, 1991a; Wolfe, 1995), with phenotypic variance occurring at the parental (Thompson, 1984; Antonovics and Schmitt, 1986; Mazer, 1987; Schwaegerle and Levin, 1990; Biere, 1991a; Platenkamp and Shaw, 1993) and within-individual (Stanton, 1984; Thompson, 1984; Roach, 1986; Wolfe, 1992) levels. Thus, although we use seeds from different populations and individuals within these populations, we do not consider genetic differentiation at either of these two levels. Rather, we opt for a design allowing for detailed discrimination of seed size at the within-plant level and, to a lesser degree, among individuals.

Lobelia inflata is particularly well suited for such a study for a number of reasons. First, its seeds are toward the small extreme of the size spectrum, so the direct advantage of initial size, inasmuch as size is an indication of reserves, should diminish very rapidly through the growing season. Second, the stamens form a ‘‘tube’’ that completely surrounds the stigma, preventing outcrossing. In wholly self-fertilizing species, parents are completely homozygous, and all seeds derived from a single plant may be assumed to be genetically identical. Thus, the effect of mating system on genetic load found in some species (Wolfe, 1995) can be ruled out as an explanation of fitness differences among siblings. Furthermore, whereas seed-size variance may result from differential energy allocation to individual offspring based on genetic quality for outcrossing species (Lloyd, 1980; Temme, 1986), this cannot occur in species with a history of complete selfing. Also owing to a history of complete self-fertilization, the within-plant seed-size variance due to positional or fruit-specific traits is not clouded by genetic differences among seeds. We have further attempted to control sources of germination and fitness variance by germinating the seeds under controlled homogeneous conditions, by ensuring that none of the variance is due to differential emergence from a seed bank (Kalisz, 1986) and by growing the seedlings in low and even densities.

MATERIALS AND METHODS

Lobelia inflata L. may act as a summer annual, facultative biennial (Bowden, 1959), or monocarpic perennial (bolting in the first year is very rare in Nova Scotia [personal observation]), depending on its location, and exhibits characters typical of residents of disturbed habitats. Individuals are exclusively self-fertilizing and produce thousands of tiny ($\sim 2.3 \times 10^{-5}$ g) seeds, which have no specialized dispersal mechanism, late in the season. Seeds may germinate at any time during the growing season (Baskin and Baskin, 1992), but only when exposed to light (Muenscher, 1936; Baskin and Baskin, 1992; personal observation). Mature fruits were collected during the autumn of 1994 from natural populations in Quebec, Nova Scotia, and Massachusetts. The experiments below examined (1) the contribution of parent, fruit position, and fruit size to the variance in seed size; (2) the effects of seed size and environmental conditions (light exposure prior to winter, and duration of cold) on the timing of germination; and (3) the effects of seed size and the timing of germination within one growing season on fitness traits.

Sources of seed-size variance—Total fruit number per plant varies from one to several hundred for *L. inflata* in nature (personal observation). In order to generate a wide range of plant sizes under growth-chamber conditions, stratified seeds (31 d in darkness at 5°C) from the three populations were introduced into the growth chamber under a diminishing photoperiod. The photoperiod schedule mimicked that of June to November, starting at 15.62 h light and ending with 10.0 h light. To ensure that different seeds would experience sufficient differences in season length to produce a range of rosette sizes, natural germination variance was supplemented by the introduction of two batches of seeds separated by 36 d. Plants that bolted at this point were not used in the experiment. After a 30-d vernalization treatment at 5°C in the dark, rosettes were introduced to constant, long-day conditions (15.25 h L/8.75 h D, 19°C/13°C) in the growth chamber to promote bolting. Fruits were allowed to mature fully, whereupon the first two and every second subsequent fruit on the main stem and every second fruit on each branch (if branching occurred) were collected. A wide range of plant sizes was obtained: fruit production of mature plants ranged from six to 52 fruits.

Diameter was recorded for 174 fruits from 12 parents using NIH Image, a public domain analysis system (http://rsb.info.nih.gov/nih-image/), and fruit position was noted. The chronological order of fruit production is indicated by position along the main raceme: older fruits are more basal. Because the temporal order of fruits on branches cannot be inferred from position, only the fruits on the main stem (151 of the 174 fruits) were included in analyses of positional effects. Although final fruit number ranged from six to 52, these numbers represent the total reproductive capacity of fully mature experimental plants. For this reason, fruit position is most meaningful when expressed as its relative position rather than its absolute position: the sixth fruit on a plant bearing a total of six, for example, should be compared to the 52nd on one bearing 52. For fruits on the main inflorescence, the relative position of a fruit was calculated as the chronological order of the fruit divided by the total number of fruits. Prior to analyses, relative position was transformed by taking the arcsine of its square root (Zar, 1984, p. 239). The diameter of 20 randomly selected, dry seeds was measured to the nearest 0.005 mm from every collected fruit from both main stems and branches (except for one fruit containing only five seeds) for a total of 3465 seeds measured. The relationship between fruit size and seed number was linearized by cubing fruit diameter, and the cube of fruit diameter was used in all analyses involving these two variables. We are principally interested in among- and within-parent seed-size variance and, although the 12 seed parents were from three populations (three from Nova Scotia, seven from Quebec, two from Massachusetts), population was not included as an effect in the analysis (see discussion).

Sources of variance in time to germination—Germination variance in *L. inflata* may be attributable to, among other factors, seed size, variable conditions before winter, and variability in winter conditions. The range of conditions experienced by seeds in nature is not known in enough detail to enable a meaningful evaluation of their relative

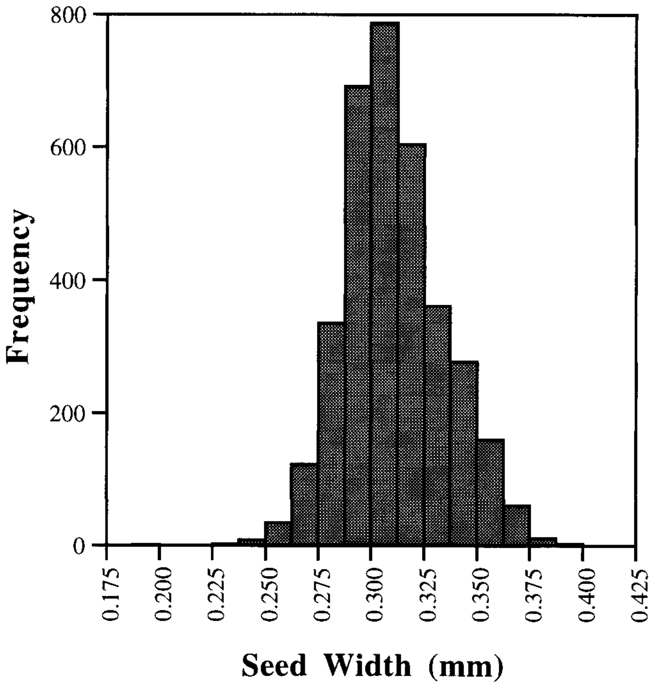

Fig. 1. The frequency of seed sizes, measured as the diameter of dry seeds. The 3465 seeds include seeds from 174 fruits, from both the main stem and branches, of 12 individuals.

effects on germination variance, so these three sources of germination variance were evaluated in three separate experiments. The purpose was to isolate potential sources of germination-time variance in *L. inflata.*

Seed size—To obtain a relationship between seed size and time to germination, seeds were randomly selected from 15 individuals from the Massachusetts population. Three germination trials, each consisting of 18 seeds from all 15 individuals, were established. For each germination trial, the first ten of the 18 seeds per individual were measured using the computer digital analysis system and, consequently, only these seeds were available for analyses involving seed size. One seed from each of the 15 parents was placed on moistened filter paper in each of 18 petri dishes, and this was repeated for the three germination trials. The plates were stored in darkness at 5°C for 9 d prior to germination in a growth chamber set at 24°/14°C and a natural spring photoperiod (15 h L/9 h D). The seeds were checked for germination (protrusion of the radicle) every 2 d under a dissecting microscope. Germinated seeds from the first trial were used to form the fitness variance experiment described below.

Light exposure—Although it is known that seeds of *L. inflata* require light for germination (Muenscher, 1936; Baskin and Baskin, 1992), it is not known whether light conditions at the onset of winter dormancy, which presumably could be used as an assessment of post-dispersal microhabitat conditions, also influence germination. Approximately 25 dried seeds from the Massachusetts population were placed on filter paper in each of four replicate petri plates for each of five light treatments. The plates were then moistened and seeds were immediately exposed to a mixture of cool white and incandescent lighting in a growth chamber for 0, 5, 10, 30, or 120 min. Plates were sealed with parafilm and wrapped in foil directly after light exposure and were transferred to 5°C for 21 d. Seed plates were then transferred to the growth chamber and were arranged in a randomized block design with all five treatments represented within each of four blocks. Germination occurred under a 24°/14°C and 15 h L/9 h D regime and was scored every 2nd d. The time to germination response variable shows positive skew and was $\log_e$ transformed (Zar, 1984, p. 238) prior to all analyses.

TABLE 1. Sources of variation in seed size and time to germination. The ANOVAs depict two separate experiments (see text). For the effects of light exposure on time to germination see text, and for the effects of seed size on time to germination see Fig. 3b.

Trait	Source of variation	df	SS	*F*	*P*
Seed size	parent	11	1.055	39.0	<0.001
	fruit (parent)	146	0.359	15.2	<0.001
Time to germination	"winter" duration	1	1.631	21.6	<0.001
	population	3	0.543	62.1	0.003
	interaction	3	0.009	0.038	0.989

Stratification duration—The effect of variable stratification on germination propensity was assayed by exposing seeds to six different 5°C "winter" durations: 111, 91, 68, 47, 24, and 0 d. Each treatment consisted of five replicate plates of 20 seeds from each of four populations (three from Nova Scotia, one from Massachusetts) for a total of 2400 seeds. Dry seeds were placed in small petri plates on moistened filter paper, and the plates were sealed and placed in uninterrupted darkness for the length of the stratification treatment. To reduce the possible confounding effect of seed age and stratification treatment, the termination rather than the initiation of all stratification treatments coincided. One plate from each population and treatment was randomly positioned within each of five blocks in a growth chamber under a 24°/14°C and 15 h L/9 h D regime, and plates were checked every 2nd d for germination. This study concentrates on within-season germination variance; inferences about the nongerminating seeds are not made.

Sources of fitness variance—The first replicate from the germination experiment was placed in the growth chamber on 18 May so that the timing of germination would coincide with exterior natural spring conditions. Seeds from this replicate were transferred individually upon germination (23 May–20 June) to Promix-filled plastic cell packs (Kord 606) and placed outside where they experienced natural temperature fluctuations. One seed derived from each of the 15 seed parents from the Massachusetts population (see above, *Seed size* section) was randomly allocated to a position within each of ten blocks. Because the cell packs were aboveground in a wood frame they were subject to desiccation; rainfall was therefore supplemented with watering. Survival, rosette size, and bolting status were assessed on 9 August and again on 7 November. Rosette size in this species is efficiently measured by the length of the longest leaf, as shown by a least-squares regression forced through the origin: dry mass (in grams) = 5.4×10^{-7} [length of longest leaf (in millimetres)]^3; $r^2 = 0.94$; $N = 37$; $F = 5240$; $P < 0.001$. All analyses were performed using SAS (SAS, 1989).

RESULTS

Sources of seed-size variance—A histogram showing the frequency distribution of the 3465 seeds measured for this experiment is given in Fig. 1. The first analysis examines the relative importance of parental effects and the effects of fruit within parents to the total variance in seed size. Seed-size variance is attributable both to parental origin and to fruits within individuals (Table 1; random effects ANOVA in PROC GLM; significance of *F* for parental effect tested using MS of the nested effect as the denominator). This model accounts for 75.5% of the variance in seed size. PROC VARCOMP using the same model reveals that parental origin accounts for 57.4%,

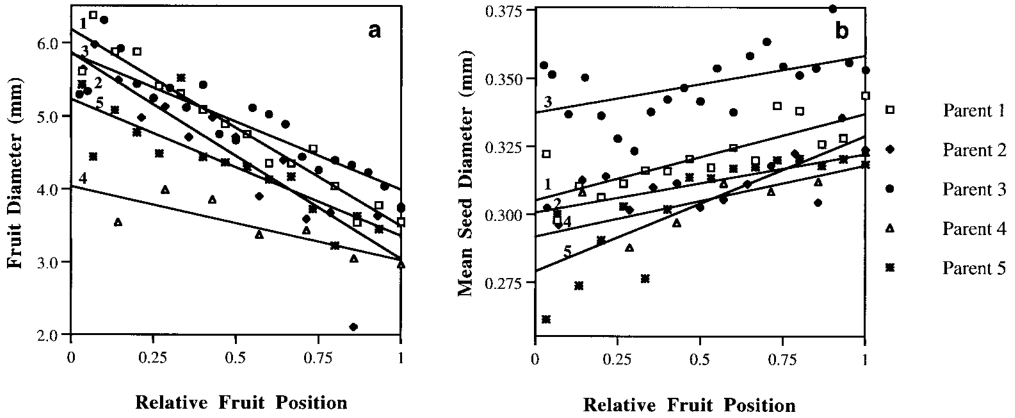

Fig. 2. Regressions of (a) fruit size and (b) mean seed size on relative fruit position for fruits produced on the main stem. For clarity, five individuals representative of the analyses based on all 12 are included in the figure. Each individual's regression line is labeled by parent number.

and fruit position within parents accounts for 18.1% of the total seed-size variance. There is no indication that these parental effects are a consequence of larger parents producing larger seeds: there is no evident relationship between a plant's total fruit number and the mean size of its seeds ($r = -0.081$; $P = 0.802$), although it should be noted that this analysis is based on only 12 observations.

The above analyses reveal differences in seed size among fruits and, because fruit sizes change with position on a plant, the relationship between seed size and fruit size and position was investigated further. The Pearson product-moment correlation between fruit diameter and transformed relative fruit position shows a strong decreasing relationship ($r = -0.681$; $P < 0.001$; $N = 151$; Fig. 2a); fruits produced later on a plant are smaller. Only fruits on the main stem were included in this analysis (see Materials and Methods), and fruits on the main stem are larger than those on branches (separate variances $T = 2.68$; $P = 0.010$; df = 50). The correlation between fruit diameter and mean seed size, using all fruits for which seed size was measured, is nonsignificant and slightly negative (Table 2): seed size is not simply a function of fruit size. Seed size increases through the season (Fig. 2b), however, as shown by the correlation between transformed relative fruit position and seed size (Table 2). Multiple regression indicates that fruit position, after controlling for fruit diameter, is a significant predictor of seed size ($F = 6.63$; $P = 0.011$), whereas the independent effect of fruit size is not ($F = 0.22$; $P = 0.642$).

The total seed number per fruit was determined on a subsample of 57 fruits that ranged from 2.1 to 6.1 mm in diameter. The mean number of seeds contained by a single fruit was 166 and ranged from 5 to 521. Seed number was significantly correlated with fruit volume (Table 2). The presence of a phenotypic trade-off between seed size and number within a fruit was detected by the partial correlation between size and number after controlling for fruit diameter ($r = -0.565$; $P < 0.001$): a fruit of a given size may contain few large or many small seeds.

TABLE 2. Pearson product-moment correlations among seed and fruit traits. Values given are correlation coefficient, significance level, and sample size. See text for independent relationships among traits and explanations of measurements.

Trait	Seed number per fruit	Fruit size	Relative fruit position
Mean seed size	−0.533	−0.107	0.245
	<0.001	0.158	0.002
	56	174	151
Seed number per fruit		0.915	−0.526
		<0.001	<0.001
		57	56
Fruit size			−0.681
			<0.001
			151

Sources of variance in time to germination—*Seed size*—Of the 810 seeds used in the experiment, 410 germinated. Only ten of 18 seeds per individual per trial (450 seeds) were measured and, of the 410 seeds that germinated, 225 were of known size. Exactly 50% of the 450 measured seeds germinated in the seed-size germination experiment and, although the mean diameters of the germinators and nongerminators (0.300 vs. 0.286 mm) differed by <5% (Fig. 3a), this difference is highly significant: Logistic regression predicting probability of germination shows an effect of seed size (Wald $\chi^2 = 33.7$; $P < 0.001$), and this relationship holds within each of the three cohorts (cohort 1: $\chi^2 = 5.49$, $P < 0.019$; cohort 2: $\chi^2 = 22.8$, $P < 0.001$; cohort 3: $\chi^2 = 17.1$, $P < 0.001$): larger seeds have a higher probability of germinating. For the seeds that germinated (and were measured), the Pearson product-moment correlation (Fig. 3b) between seed size and log-transformed time to germination is highly significant ($N = 225$; $r = 0.455$; $P < 0.001$), indicating that large seeds germinate comparatively early. A separate-slopes model confirms that seed size within cohorts is a significant ($P < 0.001$) predictor of time to germination.

Light exposure—Time to germination is influenced significantly ($P = 0.010$) by light exposure prior to cold exposure in a mixed-model ANOVA including a random block effect. However, individuals within plates were necessarily from the same light treatment, and it is possible that, although light treatments were represented in

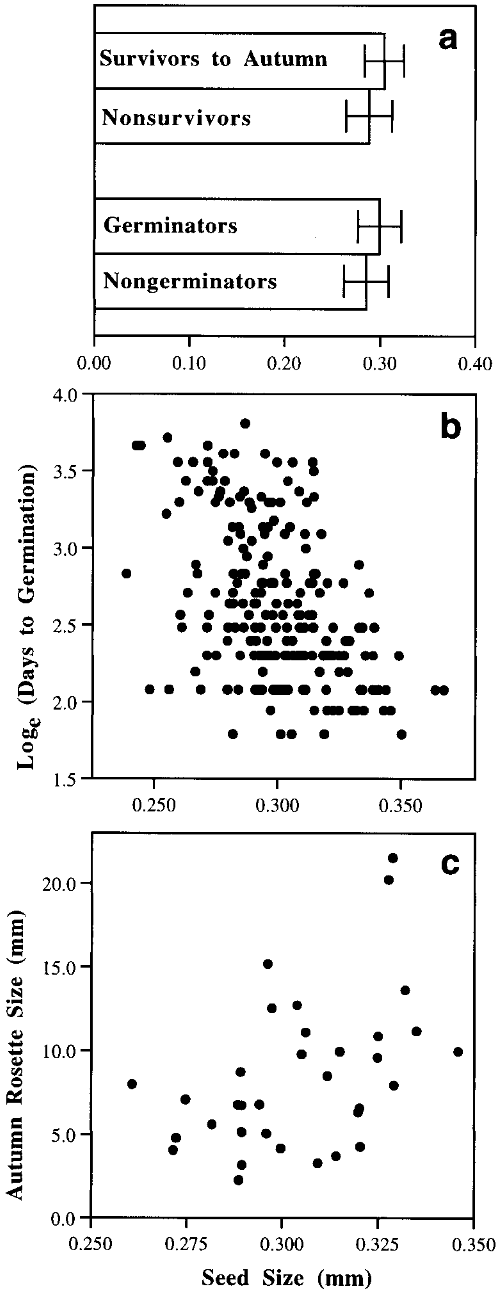

Fig. 3. The relationships between seed size, as measured by maximum dry width, on germination and fitness traits for one growing season. Error bars represent SD. (a) Rosette survival to late autumn (7 November) under seminatural, harsh conditions is based on only those seeds from the third germination trial that germinated (N = 74) (see Materials and Methods); germination is based on all measured seeds from three germination trials (N = 450). (b) Seeds that were measured and germinated (N = 225) are represented in this relationship between time to germination and seed size. (c) Rosette size, as measured by the length of the longest leaf on 7 November, and seed size (N = 34).

each of four blocks, the effect of petri plate is confounded with light treatment within each block. A one-way ANOVA using plate means instead of individual germination values shows a marginally nonsignificant effect of light treatment (F = 3.93; P = 0.063) with the reduced sample size. A Spearman rank correlation, between length of light exposure and the log of the mean time to germination of all seeds within a plate, is strong and negative (N = 20; r = −0.57; P = 0.010), confirming the results based on individual germination dates: longer exposure to light prior to cold exposure had the effect of decreasing the time to germination, with mean time to germination varying from 10.0 d for the 0-min treatment to 8.1 d for the 120-min treatment.

Stratification duration—A mixed-model ANOVA was used in the analysis of stratification effects on time to germination, with cold treatment and block declared as random effects. Again, because seeds from a population were grouped together in petri dishes, it is possible that the effect of petri dish is confounded with population, and the ANOVA model uses plate means instead of individual germination data. The effect of site of origin includes environmentally generated parental effects and is not intended to address the issue of population differentiation (see Discussion). Variation in the timing of germination is attributable to duration of cold treatment and to site of origin, but the interaction between treatment and population is nonsignificant (Table 1). Seeds experiencing a longer "winter" germinate sooner when exposed to springlike conditions.

Sources of fitness variance—Fitness, as measured by survival and rosette size after one growing season, may be influenced by seed size, the timing of germination, or both. We first consider the fitness effects of seed size alone. Plants surviving to autumn (9 August) and late autumn (7 November) were derived from larger seeds (Fig. 3a) than were plants that died post germination (9 August: separate variance T = 2.69; P = 0.009; df = 70.6) (7 November: separate variance T = 3.05; P = 0.003; df = 72). Seed size is a significant predictor of survival in simple logistic regression models for both autumn (P = 0.008) and late autumn (P = 0.003). Of the survivors to autumn, final plant size as measured by the length of the longest leaf is correlated with initial seed size (r = 0.440; P = 0.006; N = 37), with the relationship remaining essentially unchanged using total leaf area or survivors to late autumn (Fig. 3c).

Bolting in the first year is comparatively rare in nature for the populations under study (personal observation). For completeness, and because flowering obviously has important fitness consequences, we include results on bolting behavior observed in the fitness experiment. No plants in the fitness experiment had bolted by August 9, and the seven individuals that subsequently bolted were from larger rosettes than were nonbolters (separate variance T = 6.23; P < 0.001; df = 7.6). Bolters originated from slightly larger seeds (0.310 mm) than did nonbolters (0.300 mm), but only four plants bolted for which seed size was measured, and this difference is not significant (T = 1.43; P = 0.236).

Not every seed was measured (see Materials and Methods); however, data for both measured and unmeasured germinating seeds could be used to test for relationships between time to germination and the various fitness measures. The difference in germination date between survivors and nonsurvivors is significant both to autumn (T = 2.11; P = 0.037) and to late autumn (T = 2.79; P =

0.006). Time to germination significantly affects the probability of survival to autumn ($P = 0.038$) and late autumn ($P = 0.007$) in logistic regression analyses. Seed size and time to germination, however, are correlated variables. It is possible, then, that the association between time to germination and survival, for example, is indirect and is driven solely by direct effects of seed size on fitness. Results of a multiple logistic regression indicate that, although the model is significant overall (Wald $\chi^2 = 7.712$; $P = 0.021$), there are no independent effects on survival to autumn for either date of germination (Wald $\chi^2 = 0.681$; $P = 0.409$) or seed size (Wald $\chi^2 = 2.211$; $P = 0.137$). Likewise, survival to late autumn is attributable to neither the independent effect of time to germination (Wald $\chi^2 = 2.513$; $P = 0.113$) nor seed size (Wald $\chi^2 = 1.544$; $P = 0.214$), although the model explains a highly significant proportion of variation in survival (Wald $\chi^2 = 10.729$; $P = 0.005$)

The timing of germination could influence final rosette size because early germinators, if they survive the hazardous early spring, use a greater proportion of the growing season than would later germinators. Variation in plant size in autumn ($r^2 = 0.254$; df = 49; $P < 0.001$) and late autumn ($r^2 = 0.119$; df = 46; $P = 0.016$) is attributable to transformed time to germination. As for the analyses of survival, the independent effects of time to germination and seed size on final plant size are tested by using multiple regression. In a multiple regression model, plant size in autumn is predicted ($r^2 = 0.316$; $P = 0.002$) by date of germination ($P = 0.019$) and not by initial seed size ($P = 0.225$). The multiple regression remains significant for late autumn rosette size ($r^2 = 0.233$; $P = 0.016$), but the relative contribution of the two predictors in this model changes from 9 August to 7 November. The independent effects of neither germination time ($P = 0.301$) nor seed size ($P = 0.065$), however, are significant on 7 November.

Because many seeds from each of 15 parents were used in the experiment, we could address the question of whether the association between seed traits and fitness results from trait variance among seeds produced by a parent, or from fitness differences among parents, possibly through differences in genetic load. Multiple logistic regression shows that parent identity and seed width account for a significant portion of the variance in survival (Wald $\chi^2 = 10.20$; $P = 0.006$), but that seed size within parents has a stronger association with survival (Wald $\chi^2 = 3.59$; $P = 0.058$) than does seed size variation among parents (Wald $\chi^2 = 1.99$; $P = 0.158$). Also, seed size within parents contributes more strongly ($F = 2.10$; $P = 0.107$) than does the among-parent contribution ($F = 1.19$; $P = 0.365$) in the relationship between seed size and final rosette size. It is therefore unlikely that the variance in seed size and fitness traits observed in these experiments can be explained by differences in genetic load.

DISCUSSION

Seed traits have profound effects on fitness, yet seed-trait variation exists at many scales. As a consequence, explaining seed-trait variance has been vigorously pursued in the fields of both plant reproductive biology and life history theory. Both seed size and time to germination exhibit substantial variability in *Lobelia inflata,* even under the controlled conditions of a growth chamber. In this series of experiments we attempt to attribute this variance to its sources and describe its consequences.

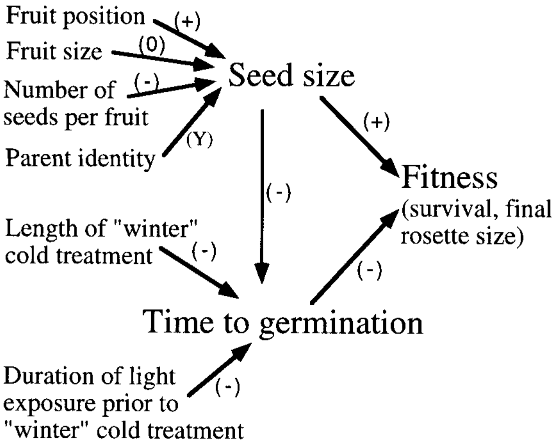

Fig. 4. Summary of the multiple influences on seed traits and fitness. The bracketed symbols indicate the sign of the relationship (+, −, or 0) or the presence of a significant effect (*Y*). These total effects include both direct and indirect effects. The relationship among fruit size, relative fruit position, and seed size is qualitatively unaltered when only direct effects are considered (see text).

Sources and consequences of seed trait variance—We found that fitness, measured as survival and final rosette size after one season, is associated with seed size and time to germination, which, in turn, are influenced by a number of factors (Fig. 4). The most important source of seed-size variance is found among parents. Because the aim of the present study is primarily to determine within-individual and other nongenetic sources of variance in seed traits, it is possible that the among-parent effects on seed size observed in this study include within-population genetic variance and genetic population differentiation, especially because homogeneous growth conditions can lead to elevated expression of genetic variance (Simons and Roff, 1994). However, a negligible proportion of total phenotypic variance in seed size is typically attributable to additive genetic variance (Schaal, 1980; Kalisz, 1986; Mazer, 1987; Schwaegerle and Levin, 1990; Biere, 1991a; Wolfe, 1995), and parental effects would likely be stronger had the experimental plants been grown under natural conditions. Our finding that 57% of variance in seed size is of parental origin is consistent with the finding of Schwaegerle and Levin (1990) that 56% of seed-size variance is extranuclear, and additive genetic effects are negligible. Nonetheless, the observed among-parent effects should not be interpreted as strict parental effects.

Seed-size variation among fruits within individuals is also substantial. The size of plant reproductive structures, because of architectural and resource constraints, may diminish the later in the season they are produced (Wolfe, 1992). Fruit size diminishes through time in the present experiment, but seed size increases in later produced, smaller fruits. We find that the size of a fruit randomly selected from the population, however, is not an appropriate predictor of seed size. Rather, it is the relative position of a fruit on an inflorescence that can be used to predict seed size. Whereas diminishing seed size may be explained on the basis of allometry and by architectural constraints, the present result suggests the need for a model of the life-history consequences of seed-size variation among fruits. Annuals, biennials, and monocarpic perennials such as *L. inflata* are regarded as semelparous, but the temporal separation of individual fruits may be viewed as iteroparity on the within-season scale if reproductive characters have some degree of independence among fruits (Lloyd, 1980). Producing many small seeds may reduce risk through the interactive effects of dispersal (Venable and Brown, 1988), but the optimal ratio of seed size to seed number might well change among fruits within an individual when season length dictates the total number of fruits that can be produced, and the season length is unpredictable. There is thus a need for a theoretical treatment of changes in the optimal seed size-to-number ratio through the season, incorporating relationships among seed size and survival (as well as how this relationship changes through the season) when the time available for growth and reproduction is unpredictable.

Seed characteristics are largely parental in origin (Westoby, 1981), and differences among genetically identical seeds within an individual may be selected for. The experimental plants were grown under constant conditions and did not experience photoperiod and temperature limitations that might have otherwise obscured an observation of increasing seed size through time. It is possible, then, that under constraining natural conditions seed size would diminish through time. This does not detract from the present finding; increasing seed size might be an underlying strategy that is successfully realized only in diminishing the magnitude of seed-size reduction. The optimal balance between seed size and number within a fruit may change within a plant through the season, but a model of such changes through the season should be based on seed sizes obtained under natural conditions.

The presence of trade-offs among traits closely related to fitness is fundamental to life-history theory (Roff, 1992). Such trade-offs are most appropriately assessed as negative genetic correlations (antagonistic pleiotropy) or as a negatively correlated response to selection (Reznick, 1985, 1992). The correlations reported here are restricted to phenotypic correlations and, although phenotypic correlations are more stable than are genetic correlations across environments (Simons and Roff, 1996), they may result from environmental or maternal effects common to seeds within parental individuals. Although costs of reproduction may be masked by the use of the phenotypic correlation (Reznick, 1985), it would be difficult to argue that the observed negative phenotypic correlation between seed size and number after controlling for fruit size is driven by an environmental correlation, because an environmental correlation would be expected to result in a positive association between seed size and number.

Nongerminating seeds used in the germination trials tended to be small. This experiment is principally concerned with the sources and consequences of germination variance within a season, and it is not known whether the seeds that failed to germinate were inviable or dormant. Small seed size, however, has been associated with failure to germinate in the year of production after correcting for viability (Platenkamp and Shaw, 1993; Andersson, 1996), and this relationship holds among species (Rees, 1997). The within-season germination variance is also consistent with such an interpretation in *L. inflata* and other species (Platenkamp and Shaw, 1993): of the seeds that germinate, small seeds germinate late.

The results of the prestratification light treatment experiment and the stratification experiment demonstrate the extreme sensitivity of seeds of *L. inflata* to environmental conditions that may serve as germination cues. Seeds are shed in the autumn prior to snowfall, and a seed's ability to assess microhabitat conditions before the onset of dormancy would be advantageous. The delayed germination in response to low light observed in this experiment might thus be viewed speculatively as adaptive, but, if nothing more, the results indicate that light microhabitats are a source of germination variance. Similarly, the stratification experiment attests to the sensitivity of seed behavior to microclimates.

Seed size was associated with fitness both directly and through its correlated effect on time to germination. Remarkably, although seeds are very small in this species, the advantage of relatively large seed size is manifested both through an increased probability of seedling survival and through a larger rosette size at the end of the season. Whereas survival is directly related to fitness, rosette size after one season, used here as a surrogate for fecundity, is an indirect measure. We assume that autumn rosette size and fecundity in the subsequent season are positively correlated.

A relationship between seed size and fitness could be generated by variation in genetic load among seeds. The relationship between seed size and seedling survival, as well as between seed size and final rosette size, however, is found among seeds that are wholly or nearly genetically identical. Furthermore, in two closely related species, *Lobelia cardinalis* and *L. siphilitica*, no inbreeding depression was found for either seed number per fruit or seed mass (Johnston, 1992).

Although, overall, large seeds germinate early, residual variation in this relationship means that some small seeds germinate early. The observed increase in relative influence of initial seed size on rosette size as the season progressed indicates differential mortality of rosettes originating from smaller, although early-germinating, seeds. Seeds that germinate early have the advantage of a longer effective season for growth and reproduction. This is offset, though, by vulnerability of newly germinated seeds to frost or other insults. The optimal date of germination is unpredictable from one season to the next, and in a season with no late-spring killing frost the relative advantage of early germination might be high. Therefore, the relative importance of date of germination and seed size observed in one experiment should not be interpreted as general for any species.

The potential adaptive significance of seed trait variance—Within-genotype phenotypic variance is advantageous if the optimal phenotype varies unpredictably among seasons (Bull, 1987); seed size and germination variance may be an adaptive diversification strategy. Although heritabilities of seed traits are low, it is possible that the extent of variance itself has a genetic basis (Biere, 1991a). Alternatively, within-genotype seed-size variance may simply reflect developmental or resource constraints (Wolfe, 1992). Determination of the mechanisms through which seed trait variance is generated, however, cannot discriminate between adaptive and nonadaptive explanations because developmental noise may be maintained as a bet-hedging strategy (Simons and Johnston, 1997). It is almost inconceivable that if a single seed type were optimal that selection would not have eroded some of the variance in seed size or the extreme sensitivity of germination behavior to intrinsic and extrinsic factors observed in the present set of experiments. Variance in germination time resulting from slight differences in light conditions experienced by seeds prior to ''winter,'' for example, is striking. In these experiments, germination occurred in a growth chamber where the environmental variance must be slight compared to that characterizing field conditions and, presumably, many other unmeasured environmental variables would also influence germination. The present results thus suggest the need for further research focusing directly on selection for within-plant diversification in response to factors such as density-dependent survival and temporal environmental uncertainty, both within and among years.

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