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EVOLUTION IN RESPONSE TO ENVIRONMENTAL UNPREDICTABILITY IN INDIAN TOBACCO, LOBELIA INFLATA (CAMPANULACEAE)

by

Andrew M.B. Simons

Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy

at

Dalhousie University
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by Andrew Simons

in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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To Ellyn

no blames attached

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GENERAL ABSTRACT

Organisms interact with an environment that is variable over time scales of seconds to millions of years. Although evolution in response to predictable events may be more tractable, unpredictable environmental events also have evolutionary consequences. Under unpredictable environments, so-called "bet-hedging" strategies are expected to evolve. There are two ways in which bet hedging may occur: through the evolution of trait diversification, and through the evolution of conservative traits. These two forms of bet hedging are illustrated by two stages in the life cycle of Indian tobacco, Lobelia inflata: variation in seed germination behaviour is an oft-cited form of diversification, and the "decision" to reproduce is a potential conservative trait.

The timing of germination is found to be influenced by seed size and environmental conditions. Both seed size and the timing of germination within a growing season are found to affect fitness. Little is known, though, about the mechanistic basis of seed trait diversification. The heritability of the diversification in time to germination is found to be nonsignificant. Based on empirical evidence for seemingly random but strong environmental control of germination, I argue that diversification bet-hedging might arise through the evolution of extreme sensitivity of the timing of germination to environmental stimuli, possibly through selection for developmental instability.

L. inflata, with a single opportunity to reproduce, should evolve conservative "rules" governing the timing of reproduction (bolting) if the optimal date changes from year to year. I find that the rules influencing bolting are plastic; they change as the summer progresses. Although the heritability of the reproductive decision is high, no genetic variation for the observed plasticity is detected. Bolting behaviour in the field appears to be suboptimal during the year of the experiment: this behaviour is better accommodated by an empirically-based model if unpredictability in season length is included. Bolting behaviour might thus have evolved as a conservative bet-hedging strategy. Plant survival is dependent not only on season length, but also on other forces of destruction such as herbivores. A bet-hedging model is developed that offers an explanation for plants' ability to compensate for tissue lost to destruction, and some empirical evidence in support of this model is presented.

A major unresolved issue in evolutionary biology is whether the effects of selection operating within populations accumulate to produce the trends observed over macroevolutionary time. I suggest that bet-hedging theory is relevant to evolution over longer time scales, and that recognizing this helps resolve part of the ongoing debate over the continuity between micro- and macroevolution.

LIST OF ABBREVIATIONS AND SYMBOLS

α	rosette size parameter
$\underline{a}$	probability of destruction
$\underline{b}$	fraction of resources available to initial reproduction
β	probabalistic bolting behaviour
χ^2	chi-squared statistic
d	day
DI	developmental instability
$\underline{df}$	degrees of freedom
e	~ 2.71828
$\underline{F}$	F-test statistic
FA	fluctuating asymmetry
FN	fruit number
g	gram
GR	growth rate
h	hour
$\underline{h}^2$	heritability
$\underline{l}$	survival
$\underline{\mu}$	intercept
m	fecundity
mm	millimetre
$\underline{N}$	sample size
ϕ	season length
P	petri plate
$\underline{P}$	probability
p	proportion or probability
$\underline{r}$	correlation coefficient
Ros	rosette size
S	position
$\underline{S}$	expected fitness
$\underline{S}_{GM}$	geometric mean fitness
T	tray
t	time
$\underline{w}$	fitness
$\underline{y}$	size

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PREFACE

At the time of printing of this thesis, manuscripts based on the first, second and sixth chapters have been published, and permission to include these publications has been obtained from the journal offices of American Journal of Botany, and Oikos. The University of Chicago grants permission for the use by the author of material published in The American Naturalist, provided that the following copyright notice appears:
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GENERAL INTRODUCTION

Natural selection, within the confines of constraints, maximizes fitness; heritable traits conferring relatively high fitness on their bearers rise in frequency in a population over generations. Natural selection maximizes fitness only with respect to a given set of conditions, and environments are variable over all time scales. Some environmental events are predictable, such as diurnal fluctuations or seasonal patterns, and other events are unpredictable. Ideally, under environmental unpredictability, the organism would respond by assuming the appropriate phenotype, through adaptive plasticity (Bradshaw 1965; Via and Lande 1985; Schlichting 1986), for the particular environment experienced.

Obviously, there are limits to the evolution of adaptive plasticity; otherwise, all organisms would be well suited to all environments. Limits to adaptive plasticity are imposed, for example, when a developmental "decision" must be made before it is possible to predict what the consequences of this decision will be (because the environment is temporally unpredictable), or when it is impossible or too costly for an individual to exhibit the amplitude of response appropriate for all temporal environments. Unpredictability can occur from one generation to the next (in other words, it is "coarse-grained"), and a trait that increases the probability of survival (for example) in one generation may be detrimental in the following generation.

Fitness is determined by a multiplicative process: the total number of descendants left by an individual after $\underline{n}$ generations depends on the product of the number surviving to reproduce in each generation. The geometric mean (the $\underline{n}$th root of the product of $\underline{n}$ values) is thus the appropriate measure of fitness over generations (Gillespie 1977). The idea that natural selection maximizes the geometric mean fitness over generations is generally attributed to Dempster's (1955) work on the maintenance of genetic variance when selection intensity varies with time. The geometric mean is strongly influenced by low values, and the more variable a set of values, the lower its geometric mean compared to its arithmetic mean. Selection, then, should act to reduce the variance in fitness over generations, even if this entails the "sacrifice" of the expected fitness within any given generation.

The geometric-mean principle and the concept of bet hedging (Slatkin 1974) are tightly linked. Although the term "bet hedging" has been used in a broader sense, bet-hedging strategies in the strict sense are defined in terms of the sacrifice of expected fitness for the geometric mean (Seger and Brockmann 1987): only those traits that reduce the expected within-generation fitness by according a higher geometric mean fitness qualify as bet-hedging traits. Bet-hedging strategies can take one of two forms; diversification or conservative. A genotype that spreads its risk by producing structures with high phenotypic variance is practicing diversification bet hedging, and a genotype that minimizes the risk of low fitness by "playing it safe" rather than by taking a risk with an associated higher expected return is a conservative bet hedger.

Two sayings frequently used as metaphors for the different types of bet-hedging strategies are "Don't put all your eggs in one basket," and "A bird in the hand is worth two in the bush" (Seger and Brockmann 1987; Philippi and Seger 1989). Both convey advice that seems (to most post-adolescents) to be wise: play it safe. The concept of risk aversion may seem a rational choice in human conduct, but there is little empirical evidence for the effects of environmental variability on trait evolution. This results partially from the fact that incorporating temporal variability into evolutionary studies inherently involves greater time expenditure than would simply measuring selection at one instant or over a short time scale.

The appropriateness of a plant species for the study of bet hedging is contingent upon the identification of characters that are at least interpretable as bet hedging traits; an expression of within-individual variability, or a life-history "decision" that must be made without reliable information of the conditions which will bear on the success of this decision. A second preference in the choice of organism is for one that reproduces only once in its lifetime (semelparous or monocarpic). With only one chance to reproduce, the intensity of selection against a semelparous organism that makes the "wrong" decision would be great. Furthermore, evaluations of fitness, fundamental to life-history analyses, are not confounded in semelparous organisms by the possibility of tradeoffs between early and late reproduction. An organism that reproduces by seed, and has no alternative modes of reproduction, would also greatly simplify analyses.

The study organism that met these several requirements is *Lobelia inflata*. *L. inflata* occurs locally and flowers only once but may act facultatively as an annual, biennial, or monocarpic perennial depending on environmental conditions. I am concerned with two potential bet-hedging traits in this species: variance in the timing of seed germination as a diversification strategy, and the timing of reproduction as a conservative strategy. Diversification in germination of seeds produced by an individual may occur both within a single growing season or, through dormancy, among years. Similarly, reproductive behaviour may be studied as a delay within a single growing season, or as a delay among years.

The optimal behaviour with respect to both of these traits depends on environmental parameters that change from year to year. A seed gains potential expected fitness by germinating very early in the spring, because it makes full use of the growing season. This seed, however, has an increased probability of death or damage if it germinates before the final frost of the spring. The optimal timing of germination within a season cannot be predicted, because of variance in the frost-free period among years (Fig. 0.1a). The variance in frost-free period as well as unpredictability in the overall quality of the growing season, as measured by the accumulation of degree-days above 5°C (Fig. 0.1b), are expected to affect the evolution of dormancy among seasons.

Seeds of Lobelia inflata germinate and produce a rosette, a prostrate cluster of hardy leaves capable of surviving winter. In response to internal and external cues, a rosette will enter the reproductive phase through a process known as "bolting." The irreversible

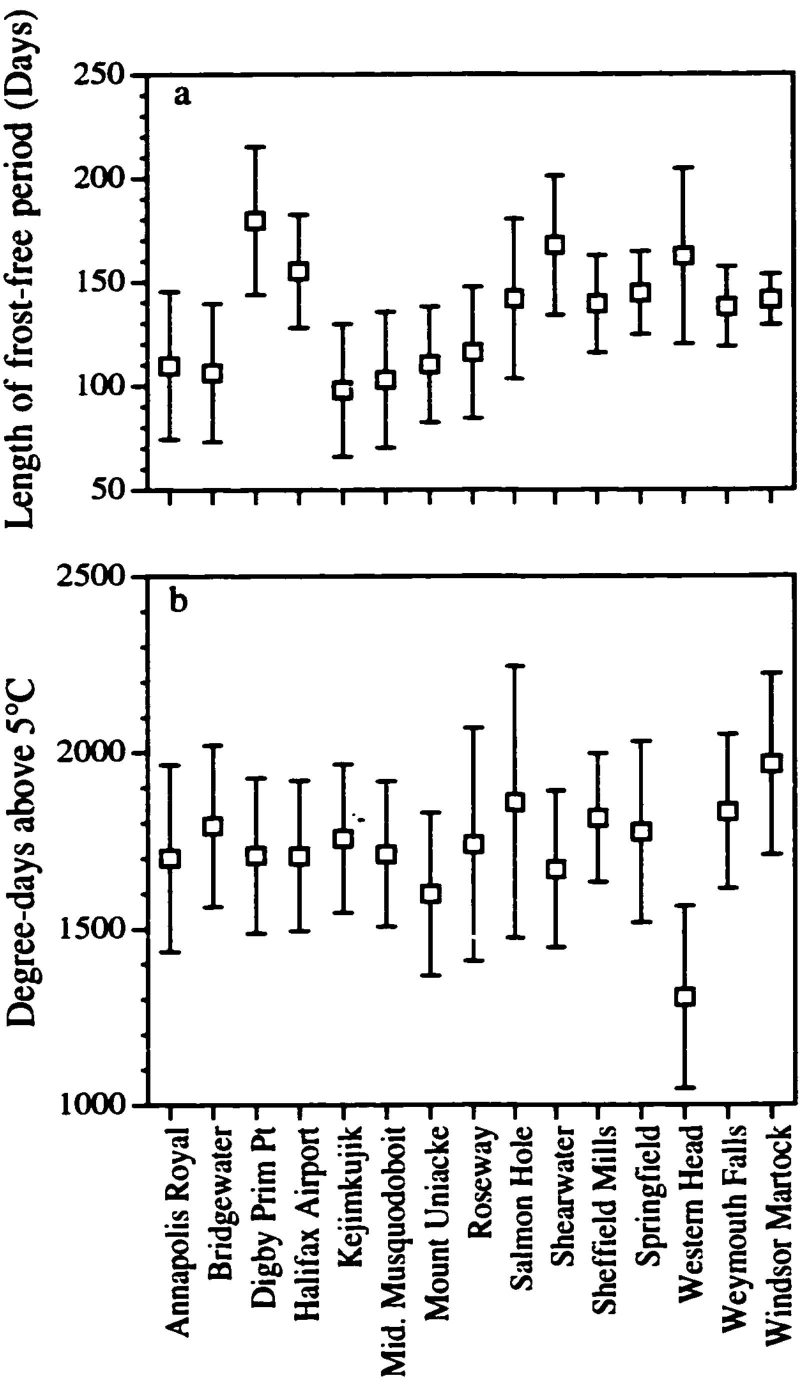


Figure 0.1. Means and 95% confidence intervals for (a) the duration of the frost-free period and (b) the accumulation of degree-days > 5°C observed over the past 30 years at 15 stations across Nova Scotia, Canada.

bolting event leads to the development of a flowering stalk, and renders the plant vulnerable to frost and herbivore damage. The development of this flowering stalk requires appropriate growing conditions. Again, the time remaining for growth and the quality of the remaining growing season are unpredictable (Fig. 0.1) at the time bolting occurs. A rosette reaching an appropriate developmental state to bolt, but toward the end of the season, would be practicing conservative bet hedging by delaying bolting until early the next year rather than risking low or zero reproductive success if the autumn were to be shorter than average. The individual thereby maximizes its geometric mean fitness, even if its expected fitness would have been maximized by bolting immediately.

The first three chapters deal with seed traits. When temporal variability is explicitly included in evolutionary models, seed germination, perhaps because of its extensive variance and its multiple and clear connections with fitness, has been used widely as an illustration of diversification bet hedging. In the first chapter, based on results from five separate experiments, I explore the nongenetic sources of variation in seed size and in germination variance, and test the effect of these traits on survival and final rosette size in autumn, two important fitness measures. Seed-size variance is measured on about 3500 seeds from 174 fruits of known position on 12 individuals. In three germination experiments, the effect of seed size, light conditions before winter, and the duration of winter conditions on germination timing is tested.

The mechanism whereby a single individual produces variable phenotypes such as seeds is largely unexplored. In the second chapter, I develop a theoretical framework for the consideration of developmental instability as such an underlying mechanism. Developmental instability, measured as the fluctuating asymmetry (FA) of paired characters, has formerly been exclusively interpreted in a negative light. The proposal that developmental instability might generate germination diversification, a simulation model showing the fitness consequences of within-season germination variance, and suggestions for empirical tests are presented.

The typically low heritabilities of seed traits, measured both through sib analysis and selection experiments, suggest that seed trait variance might itself be under selection. Having found much intraindividual variance in seed germination in the first chapter, and having suggested a mechanism underlying this variance, I attempt in the third chapter to determine the quantitative genetic basis of variance in seed traits. I measure the germination variance of seeds from 84 genotypes over two generations, and also study the importance of small environmental differences in determining the timing of germination. In addition, the fluctuating asymmetry of the seedlings' cotyledons is measured for the prospect of gaining some insight into the role of developmental instability in the generation of seed trait diversification.

Whereas seed germination is a classical example of diversification, at least in the theoretical literature, there is no equivalent model trait for conservative bet hedging. Rosette bolting, the transition from the vegetative to flowering phase, marks a stage in the life cycle of L. inflata at which conservative bet hedging might occur. The costs and payoffs associated with the bolting decision may be quite complex, and depend on the time of year, the size of the rosette, and the probability of overwinter survival. In chapter four, sources of variance in bolting behaviour are tested over two generations in a growth chamber experiment. For both generations, three cohorts of newly germinated seeds are introduced into diminishing photoperiod conditions designed to replicate the natural season. In addition to determining the heritable basis of bolting and the plasticity of bolting behaviour, a goal of this work is to determine the "rules" followed by rosettes in making their reproductive decisions.

Chapter five relates the findings of the fourth chapter to their implications in nature: I attempt to address whether bolting "rules" are appropriate under field conditions. It was first necessary to obtain measures of natural overwinter mortality, mortality within the growing season, size-specific growth rates, the relationship between rosette size and fecundity, and the fitness consequences of variation in bolting time within a growing season for plants naturally occurring in the field. Because bolting in naturally occurring plants is restricted to a few weeks early in the summer, the consequences of a greater range of bolting times was studied by transplanting newly bolted rosettes from the growth chamber into the field. Results from these two chapters are then used in a model to ask whether the bolting "decisions" being made maximize fitness.

An individual, once it has bolted, is vulnerable not only to the onset of harsh conditions, but also to grazing or trampling. Some plants, including L. inflata, show the ability to "compensate" for tissue that has been lost to herbivory. The idea that fitness is increased through partial destruction, based on observations of compensation, has been a controversial one. A model and empirical results are presented in the sixth chapter that suggest that such an ability is not evidence that destruction increases fitness; it has evolved at an evolutionary cost. This cost takes the form of a tradeoff between the allocation of reserves between an initial flowering episode and a second episode in case the first is destroyed.

Bet-hedging strategies, such as the allocation to reproduction following partial destruction studied in the sixth chapter, evolve in response to reversals in adaptive trends from one generation to the next. In the concluding chapter I show that environmental unpredictability, from minor disturbances to major extinctions, may result in bet-hedging. This chapter proposes that bet-hedging theory applies to evolution over all time scales. It is hoped that this argument partially resolves a controversy over the continuity between micro- and macroevolution.

CHAPTER 1

Variation in seed traits of Lobelia inflata (Campanulaceae): sources and fitness consequences

Abstract—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, I (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.

INTRODUCTION

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 et al. 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 et al. 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 et al. 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 et al. 1994); germination variance among sibs may result from density-dependent selection (Geritz 1995).

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 I use seeds from different populations and individuals within these populations, I do not consider genetic differentiation at either of these two levels. Rather, I opt for a design allowing for detailed discrimination of seed size at the within-plant level and, to a lesser degree, among individuals.

L. 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. I 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 (~ 2.3×10^{-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 on fitness traits within one growing season.

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 terminating 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 6 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. I am principally interested in among- and within-parent seed-size variance and, although the 12 seed parents were from three populations (3 from Nova Scotia, 7 from Quebec, 2 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 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 10 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 second day. The time to germination response variable shows positive skew and was $\log_e$ transformed (Zar 1984, p. 238) prior to all analyses.

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 to 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 methods for Sources of variance in time to germination--Seed size section, above) 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 (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. 1.1. 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 1.1; 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%, 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.

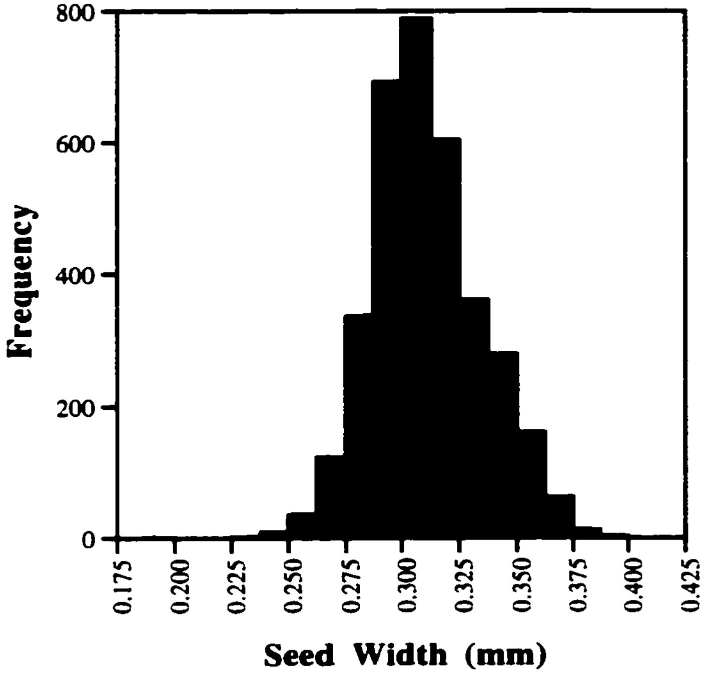


Figure 1.1. The frequency of seed sizes, measured as the diameter of dry seeds. The 3465 seeds include seeds from 174 fruits, from both the main stem and branches, of 12 individuals.

Table 1.1. 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. 1.3b.

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

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. 1.2a); 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 1.2): seed size is not simply a function of fruit size. Seed size increases through the season (Fig. 1.2b), however, as shown by the correlation between transformed relative fruit position and seed size (Table 1.2). 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 1.2). 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.

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

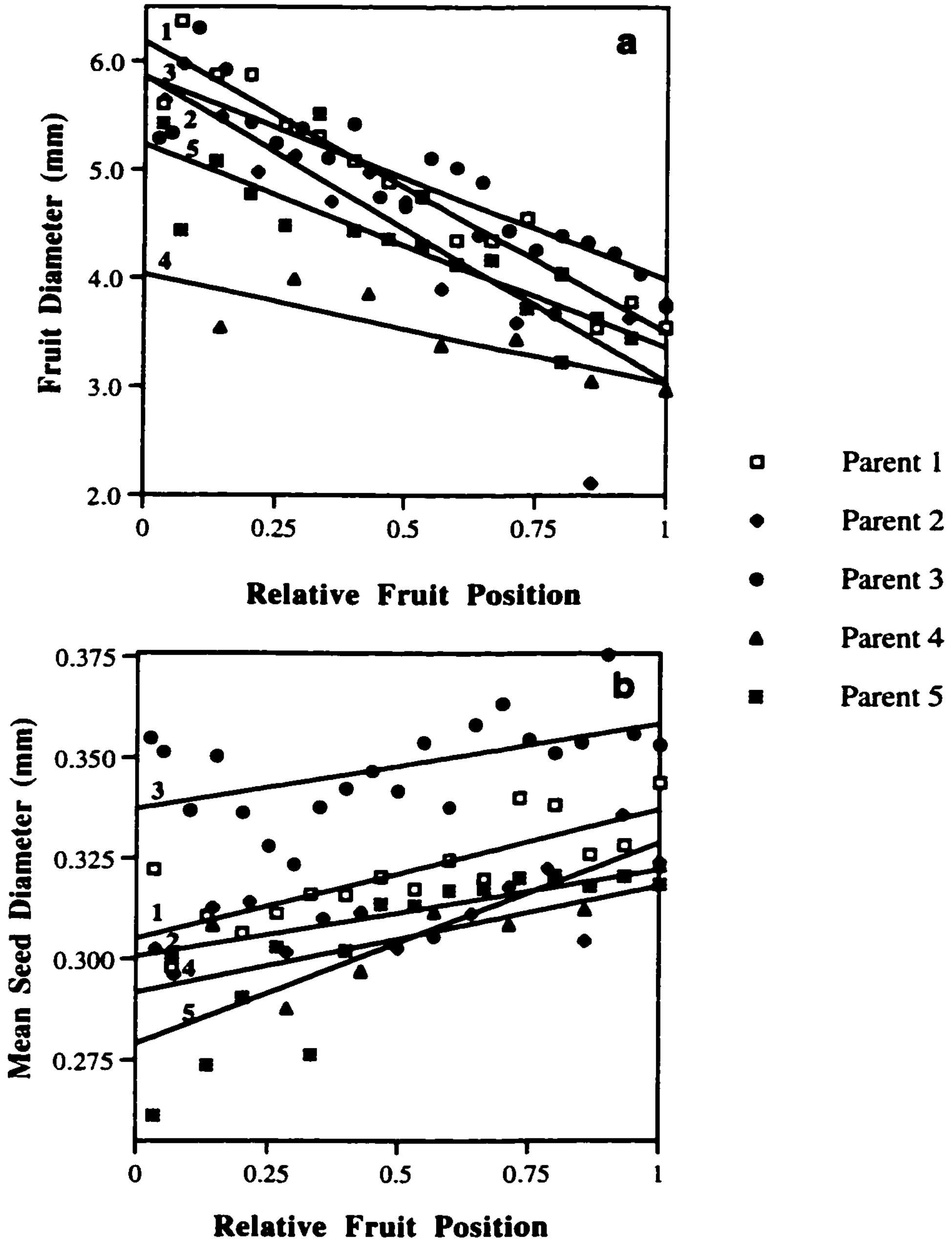


Figure 1.2. Regressions of (a) fruit size and (b) mean seed size on relative fruit position for fruits produced on the main stem. For clarity, five individuals representative of the analyses based on all 12 are included in the figure. Each individual's regression line is labeled by parent number.

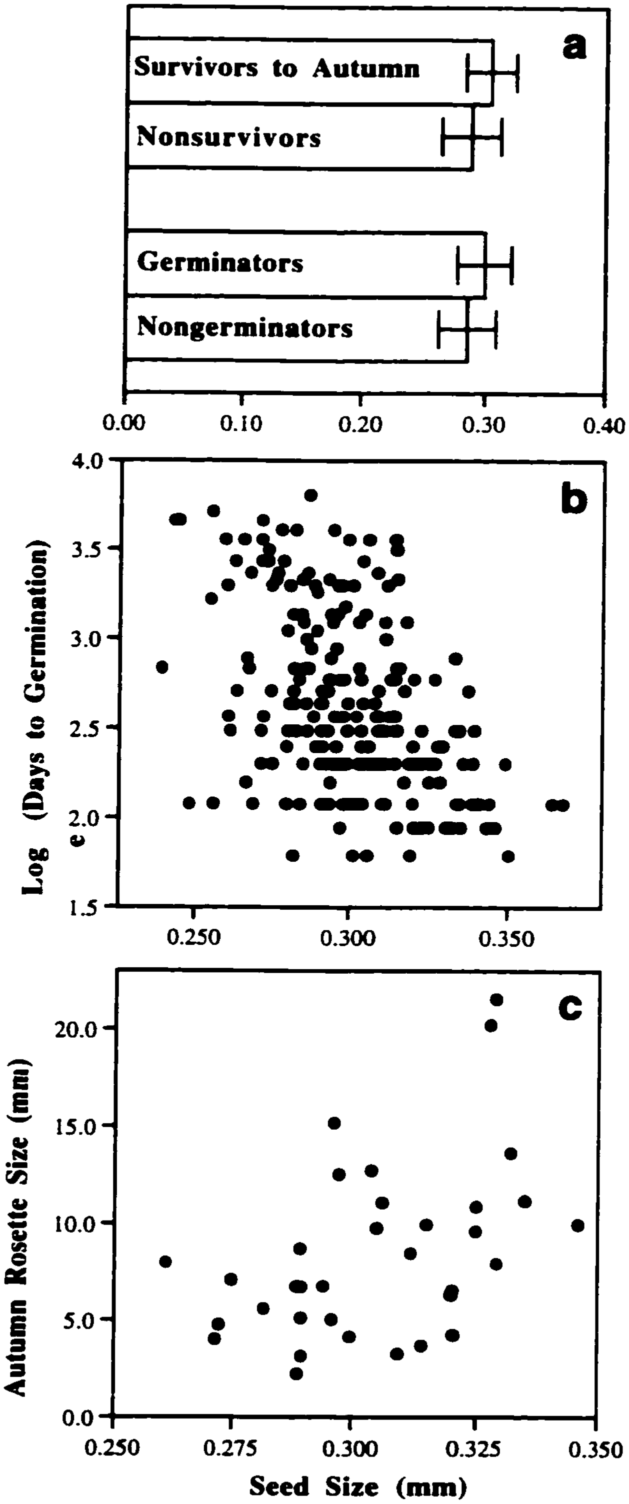
Table 1.2. Pearson product-moment correlations among seed and fruit traits. Values given are correlation coefficient, significance level, and sample size. See text for independent relationships among traits and explanations of measurements.

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

germinators and nongerminators (0.300 vs. 0.286 mm) differed by < 5% (Fig. 1.3a), this difference is highly significant: Logistic regression predicting probability of germination shows an effect of seed size (Wald $\chi^2 = 33.7$; $\underline{P} < 0.001$), and this relationship holds within each of the three cohorts (cohort 1: $\chi^2 = 5.49$, $\underline{P} < 0.019$; cohort 2: $\chi^2 = 22.8$, $\underline{P} < 0.001$; cohort 3: $\chi^2 = 17.1$, $\underline{P} < 0.001$): larger seeds have a higher probability of germinating. For the seeds that germinated (and were measured), the Pearson product-moment correlation (Fig. 1.3b) between seed size and log-transformed time to germination is highly significant ($\underline{N} = 225$; $\underline{r} = 0.455$; $\underline{P} < 0.001$), indicating that large seeds germinate comparatively early. A separate-slopes model confirms that seed size within cohorts is a significant ($\underline{P} < 0.001$) predictor of time to germination.

Light exposure—Time to germination is influenced significantly ($\underline{P} = 0.010$) 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 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 ($\underline{F} = 3.93$; $\underline{P} = 0.063$) 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 ($\underline{N} = 20$; $\underline{r} = -0.57$; $\underline{P} = 0.010$), 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.

Figure 1.3. 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).



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 1.1). 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. I 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. 1.3a) than were plants that died post germination (9 August: separate variance $\underline{T} = 2.69$; $\underline{P} = 0.009$; $\underline{df} = 70.6$) (7 November: separate variance $\underline{T} = 3.05$; $\underline{P} = 0.003$; $\underline{df} = 72$). Seed size is a significant predictor of survival in simple logistic regression models for both autumn ($\underline{P} = 0.008$) and late autumn ($\underline{P} = 0.003$). Of the survivors to autumn, final plant size as measured by the length of the longest leaf is correlated with initial seed size ($\underline{r} = 0.440$; $\underline{P} = 0.006$; $\underline{N} = 37$), with the relationship remaining essentially unchanged using total leaf area or survivors to late autumn (Fig. 1.3c).

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, I 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 $\underline{T} = 6.23$; $\underline{P} < 0.001$; $\underline{df} = 7.6$). 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 ($\underline{T} = 1.43$; $\underline{P} = 0.236$).

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 ($\underline{T} = 2.11$; $\underline{P} = 0.037$) and to late autumn ($\underline{T} = 2.79$; $\underline{P} = 0.006$). Time to germination significantly affects the probability of survival to autumn ($\underline{P} = 0.038$) and late autumn ($\underline{P} = 0.007$) 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 $\chi^2 = 7.712$; $\underline{P} = 0.021$), there are no independent effects on survival to autumn for either date of germination (Wald $\chi^2 = 0.681$; $\underline{P} = 0.409$) or seed size (Wald $\chi^2 = 2.211$; $\underline{P} = 0.137$). Likewise, survival to late autumn is attributable to neither the independent effect of time to germination (Wald $\chi^2 = 2.513$; $\underline{P} = 0.113$) nor seed size (Wald $\chi^2 = 1.544$; $\underline{P} = 0.214$), although the model explains a highly significant proportion of variation in survival (Wald $\chi^2 = 10.729$; $\underline{P} = 0.005$)

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 ($\underline{r}^2 = 0.254$; $\underline{df} = 49$; $\underline{P} < 0.001$) and late autumn ($\underline{r}^2 = 0.119$; $\underline{df} = 46$; $\underline{P} = 0.016$) 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 ($\underline{r}^2 = 0.316$; $\underline{P} = 0.002$) by date of germination ($\underline{P} = 0.019$) and not by initial seed size ($\underline{P} = 0.225$). The multiple regression remains significant for late autumn rosette size ($\underline{r}^2 = 0.233$; $\underline{P} = 0.016$), but the relative contribution of the two predictors in this model change from 9 August to 7 November. The independent effects of neither germination time ($\underline{P} = 0.301$) nor seed size ($\underline{P} = 0.065$), however, are significant on 7 November.

Because many seeds from each of 15 parents were used in the experiment, I 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 $\chi^2 = 10.20$; $\underline{P} = 0.006$), but that seed size within parents has a stronger association with survival (Wald $\chi^2 = 3.59$; $\underline{P} = 0.058$) than does seed size variation among parents (Wald $\chi^2 = 1.99$; $\underline{P} = 0.158$). Also, seed size within parents contributes more strongly ($\underline{F} = 2.10$; $\underline{P} = 0.107$) than does the among-parent contribution ($\underline{F} = 1.19$; $\underline{P} = 0.365$) 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 I attempt to attribute this variance to its sources and describe its consequences.

Sources and consequences of seed trait variance

I 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, is influenced by a number of factors (Fig. 1.4). 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. I 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

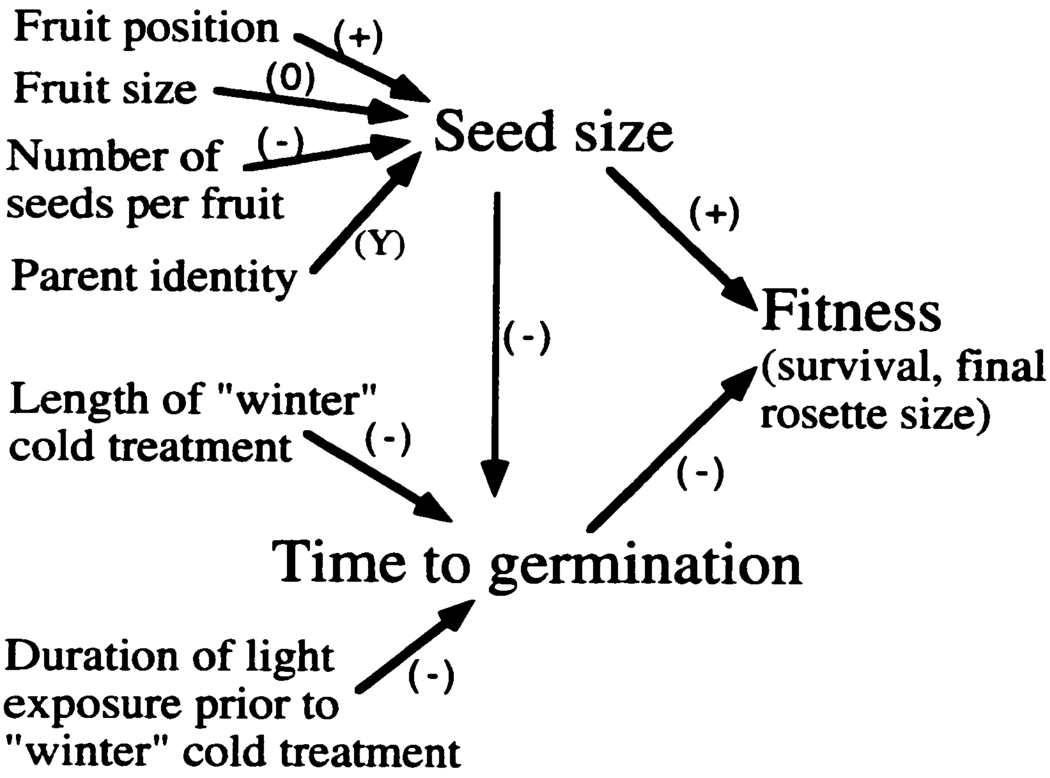


Figure 1.4. Summary of the multiple influences on seed traits and fitness. The bracketed symbols indicate the sign of the relationship (+,-, or 0) or the presence of a significant effect (Y). These total effects include both direct and indirect effects, and do not represent path coefficients. The relationship among fruit size, relative fruit position, and seed size is qualitatively unaltered when only direct effects are considered (see text).

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. I 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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CHAPTER 2

Developmental Instability as a Bet-Hedging Strategy

In temporally varying environments selection will often act, at the expense of expected fitness in any given generation, so as to maximize geometric mean fitness across a number of generations (Gillespie 1977). Such "bet hedging" (Slatkin 1974) may occur in two ways (Seger and Brockmann 1987). In conservative bet hedging a genotype minimizes the risk of a very low fitness bout by producing a narrowly unimodal distribution of "safe" trait values. In contrast, a genotype practicing diversification bet hedging spreads the risk by producing traits with increased variance (see Philippi and Seger 1989 for a full discussion). A classical example of such diversification both in theoretical and empirical studies is that of seed germination behaviour (Cohen 1966; Janzen 1977; Venable and Lawlor 1980; Cooper and Kaplan 1982; Bulmer 1984; Ellner 1985; León 1985; Venable 1985; Kalisz 1986; Bull 1987; Venable and Brown 1988; Biere 1991), but diversification or multiple strategies (Lloyd 1984) could be equally relevant to other processes such as insect diapause (Tuljapurkar and Istock 1993). Little is known, however, about how such within-genotype diversification might be produced. Here I propose that diversification bet hedging may be attained through a mechanism traditionally considered to be exclusively detrimental: developmental instability. I discuss the plausibility of this perspective, present testable hypotheses that emerge directly if bet hedging is achieved through instability of development, and suggest appropriate tests of these hypotheses. In this paper I discuss the mechanisms underlying diversification bet hedging although these mechanisms should apply to other, nonbet-hedging situations in which within-genotype trait variance is shown to be adaptive (see Geritz 1995 for density-dependent selection of seeds under spatial heterogeneity).

There exists considerable confusion surrounding the use of terms related to the degree of variability inherent to developmental processes (Zakharov 1992). The relative fidelity of development to a program is known variously as developmental stability (Mather 1953; Waddington 1957; Parsons 1992; Møller 1995), homeorhesis (Waddington 1957), homeostasis (Lerner 1954; Orzack 1985; Parsons 1992; Møller 1995), buffering capacity

(Van Valen 1962), and developmental canalization (Mather 1953; Thoday 1958; Waddington 1960; Levin 1988). The mechanisms that these terms describe all have the effect of decreasing trait variance. Developmental instability, environmental sensitivity (Jinks and Pooni 1988), and phenotypic plasticity (Bradshaw 1965; Via and Lande 1985; Schlichting 1986) are all means by which trait variance is increased. There is considerable overlap in the definitions of some of these terms, and their usage may vary by author (Zakharov 1992).

Phenotypic variance expressed among individuals of a genotype is usually attributed to two general sources: plasticity and noise. Plasticity is measured as the phenotypic expression of genotypes across an environmental gradient, whereas developmental noise is assumed to result from random errors of development, but in fact is variance resulting from all sources unknown. Developmental noise, then, may include plasticity in response to environmental variables that have not been identified (Bradshaw 1965). There is ample evidence for the existence of genetic variation for plasticity (Bradshaw 1965; Perkins and Jinks 1971; Schlichting and Levin 1986; Scheiner et al. 1991; Oyama 1994). Although phenotypic plasticity is the variable expression of a genotype under differing environmental circumstances, it is quite possible to have well-canalized reaction norms, and the degree of plasticity is a genotype-level property. There is no reason to suppose that, like plasticity, developmental noise could not be maintained at some optimal level by stabilizing selection.

The genetic basis of developmental stability is not yet well established (see Clarke 1993 for a discussion of competing hypotheses). Developmental instability may be assessed through the occurrence of phenodeviants or, in (normally) bilaterally symmetrical organisms, as the fluctuating asymmetry (FA) of paired characters (Mather 1953; Waddington 1957; Palmer and Strobeck 1986; Parsons 1992; Polak and Trivers 1994; Møller 1995), and trait stability has been found to have a genetic basis (Mather 1953; Thoday 1958; Waddington 1960; Kaufman et al. 1977; Scheiner et al. 1991). It is probable, however, that developmental noise would be much reduced under the homogeneous conditions typically used in breeding experiments; the fine-grained environmental variability of field conditions has the effect of increasing the environmental component of phenotypic variance of quantitative traits (Simons and Roff 1994). Fluctuating asymmetry is a weak indicator of developmental instability, and heritability estimates of FA are likely to severely underestimate the heritability of developmental instability (Whitlock 1996). Any among-genotype differences in the stability of phenotypic expression could be acted on by natural selection and, analogously to norms of reaction, the degree of instability would be considered a property of the genotype. It is apparent that trait variance in the case of plasticity is capable of adaptive evolution; the set of conditions under which fitness would be a function of developmental stability is less evident.

The prevailing view of developmental stability is one in which the production of variation is interpreted as an inferiority: the inability of a genotype to control the effects of the environment in the development of the phenotype. Møller's (1995) statement, "Fluctuating asymmetry (FA) is an epigenetic measure of the lack of developmental homeostasis and thus a measure of the inability of individuals to cope with genetic and environmental stress," and the assertion of Stearns *et al*. (1995) that "stabilizing selection should favor improved canalization, and the degree of a trait's canalization should be positively correlated with its impact on fitness" typify a conviction well-founded in results of recent research (Polak and Trivers 1994). It has been recognized, though, that by an indiscriminate acceptance of this interpretation of instability, other interpretations are ignored (Kaplan and Cooper 1984). Simply because it has been demonstrated that stress may lead to instability which, in turn, leads to asymmetry does not *a priori* imply that selection cannot favour instability. Circumstances under which selection favours high phenotypic variance have been described (Wourms 1972; Real 1980; Venable and Lawlor 1980; Crump 1981; Kaplan and Cooper 1984; Bull 1987; Geritz 1995), and I suggest that developmental instability may be maintained in a character by the direct action of selection or, indirectly, by selection on a correlated character. In some cases, then, fluctuating asymmetry might well be explained as a correlated response to selection for developmental instability in an associated trait.

In an unpredictable environment, elevated variance in traits closely related to fitness (such as hatching or germination timing) can lead to low temporal variance in fitness (Gillespie 1977). Instability would be interpreted as a bet-hedging strategy (Slatkin 1974; Philippi and Seger 1989) if it reduces the intergenerational variance in mean fitness of a genotype (thus increasing its long-term geometric mean fitness) by producing a diversity of phenotypes within a generation (Slatkin 1974; see Orzack 1985 for situations under which fitness homeostasis would not be expected to evolve). If the intergenerational environmental variance is high enough compared to the within-generation stabilizing selection on a trait such as germination time, a bet-hedging strategy is expected to evolve (Bull 1987).

Whereas the theoretical treatment of bet hedging has progressed rapidly, empirical validation of theory has been slower, perhaps impeded by difficulties related to the comparison of variances and by the practical difficulties associated with obtaining sample sizes large enough to estimate trait variances rather than simple trait values. A trait extensively cited as a possible bet-hedging trait, and the one used here as a model character for the purpose of discussion, is seed heteromorphism; a single genotype produces two or more seed types that behave differently under similar environmental conditions. The mechanism whereby a single genotype may produce seeds that differ in their germination behaviours is not well understood, and possibilities presented in the literature include individual genetic bases for seeds at different positions (in the case of seed dimorphism), differential timing of seed development and thus stage of maturation of seeds within plants (Silvertown 1984), change in developmental constraints within plants through time (Wolfe 1995), and the resource status or size of individual plants (Venable 1992; Philippi 1993). Developmental instability could also explain diversified germination behaviour of seeds produced by a single genotype, and would be selected for to the extent to which bet hedging was appropriate.

Considering annual plant species with seed banks, Cohen (1966) modeled germination under various regimes of temporal uncertainty, and found that selection should favour a lower germination fraction per generation when the probability of success was less predictable. In addition to germination variance associated with seed banks, developmental instability is equally relevant to variance in germination timing within a season or generation under coarse-grained environmental uncertainty, the type modeled by León (1985) and Venable (1989), and discussed by Silvertown (1985) and Marks and Prince (1981). Size and germination are largely controlled by the parent plant, and germination strategies should evolve to maximize parental fitness (Westoby 1981; Silvertown 1984). Marks and Prince (1981) showed that for Lactuca serriola (L.), plants produced by seeds that germinate later in a season suffer a fecundity cost in relation to early germinators, but these plants may act as insurance against the complete loss of the early cohort characterized by high mortality. In the cricket Gryllus pennsylvanicus, delayed hatching has the same result; late hatchers develop quickly and become small adults (Carrière et al. 1996). Within-season diversification, then, analogously to among-season germination diversification through the production of a seed bank, promotes the avoidance of unpredictable bouts of detrimental conditions within a season for at least some of the seeds produced by an individual: a plant producing seeds that germinate simultaneously has a high fitness if the time happens to be "right" (no killing frost occurs after germination, for example) relative to a plant whose seeds germinate less synchronously. If the timing is "wrong," though, the synchronous genotype's relative fitness could be close to zero. The mathematics describing within-season diversification bet hedging are very involved (León 1985), and have rarely been attempted. Here I present results of a model simulating the fitness consequences of seed germination diversification strategies within a season (Fig. 2.1). Because natural selection acts on geometric-mean fitness, the cost of a season of low

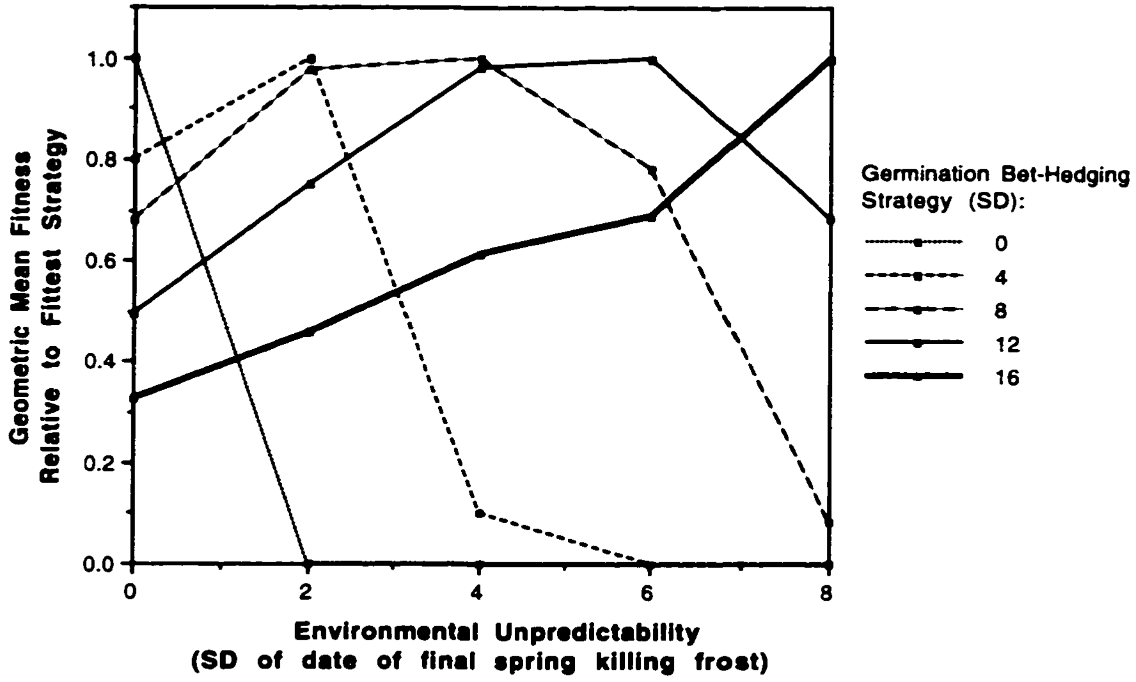

Figure 2.1. Results of a model comparing long-term fitness of five germination strategies differing in within-season diversification, under five levels of environmental unpredictability. This model shows that the level of unpredictability characterizing the environment determines which of the bet-hedging strategies has the highest relative geometric mean fitness: the optimum germination variance increases with increasing unpredictability of final spring frost, and only in the case of zero unpredictability is synchronized germination selected for. The length of any particular growing season was randomly assigned from a normal distribution with a mean of 100 days and standard deviation given by the level of unpredictability. The mean germination date was fixed, regardless of diversification strategy, and was equal to the mean date of final spring frost. The germination date of an individual seed was drawn randomly from a normal distribution with a standard deviation given by the diversification strategy of its parent. Seeds germinating before the final frost at the beginning of the season did not survive, and were assigned a fitness of zero; seeds germinating after the final frost survived, but the fitness of late germinators was discounted in proportion to the relative time "wasted" by not germinating immediately after the final frost. The geometric mean fitness was calculated over thirty seasons, and each of the 25 fitness estimates represents the average from twenty runs of the simulation program. Fitnesses were scaled to a maximum of one for each level of environmental unpredictability.

fitness is higher than the benefit of a correspondingly good season, and platykurtic distributions of seed germination time are expected to evolve in response to unpredictable environments. Therefore, under environmental unpredictability, individuals producing seeds whose germination timing is highly unstable and thus more variable would be at a selective advantage.

PREDICTIONS AND TESTS

Under the traditional interpretation, directional selection acts to minimize developmental instability, especially in fitness characters (Stearns et al. 1995). When a particular level of instability benefits a genotype through an increase in its geometric mean fitness, stabilizing selection acts on developmental instability. If a bet-hedging strategy can be attained through developmental instability by an increase in the variance in seed germination behaviour, and if this instability is expressed pleiotropically in another trait such as cotyledon asymmetry, then a correlation between these two traits is produced. Four main predictions follow directly in cases where instability of a bet-hedging trait can be observed as fluctuating asymmetry. First, when additive genetic variation exists for the variable expression of a bet-hedging trait within a population, genotypes exhibiting high variance will also show high FA of an associated bilateral trait. Second, populations expressing comparatively high diversification in a bet-hedging trait will also show high FA in that or a developmentally related trait compared to a population expressing relatively low diversification. The third prediction arising from the hypothesis that bet hedging may be generated by developmental instability is that populations inhabiting relatively unpredictable environments should show both high diversification (germination variance, for example) and FA. Fourth, because bet hedging is predicted to be more prevalent in short-lived than long-lived species (Seger and Brockmann 1987, p195), annual species are expected to show higher FA in traits related to bet hedging than are either biennials or perennials.

Tests of the above predictions demand that a bet-hedging trait be identified, and that a second, developmentally related trait, be available for the direct measurement of instability. It should be highlighted here that such an observation would not be indicative of the presence of bet hedging; such an inference would require a study of the fitness consequences of the generation of trait variance. Given the efficacy of the trait to act as a bet-hedging strategy, though, selection for developmental instability would be a possible mechanism underlying the evolution of diversification.

Experiments intended expressly for the purpose of testing the association between bet-hedging traits and instability should be designed to identify this association within genotypes, and should exclude the conflation of unintended causes of this association. To test the within-population, among-genotype association of germination variance and cotyledon asymmetry, for example, an appropriate design would partition the among-seed, within-genotype variance from other sources including block effects and maternal effects. The design would be much simplified by using a completely self-fertilizing species because genetic identity among seeds of a single parent may be assumed. Partitioning of these effects could be achieved by first growing several parental plants of each genotype at randomized positions within blocks in a controlled environment. With the seed produced by these parental plants, a number of germination blocks (petri dishes, for example) could be established, and one seed from every parental plant of every genotype randomly allocated to a position within each block. Replication, in a design where observations consist of a measure of variance, may be accomplished by including sufficient numbers of blocks so that each block may be preassigned to one of two (for example) replicate treatments. As an alternative to replication, bootstrapping techniques could be used to obtain sample variances of the coefficients of variation of germination time for each genotype. Subsequent analysis on measurements of time to germination and cotyledon FA could then attribute the covariance of these two traits to its genetic, maternal, and environmental sources.

A selection experiment could also be designed to test the association between developmental instability and a potential bet-hedging trait. Again using the example of cotyledon asymmetry and CV of germination time; high asymmetry, low asymmetry, and control lines could be established and one could determine the correlated response in the variability of germination time.

The viability of these predictions and tests rests on four assumptions. First, that the observed variance is an effective bet-hedging trait. Because a bet-hedging trait is one which maximizes the long-term geometric mean fitness, theoretical support for the existence of such traits is far more advanced than is empirical support. A second assumption is that developmental instability can produce a diversification of phenotypic expression in the bet-hedging trait. A further complication may arise if other causes of diversification behaviour obscure the diversification generated by instability. Third, it is necessary that developmental instability producing the diversification can be independently assessed by a known index of instability such as FA. Cases in which the diversification trait is itself amenable to FA measurement would not be as difficult as ones in which the diversification trait is more remotely associated with the trait amenable to FA measurement. The presence of strong genetic correlations among the means of traits does not imply that FA will be correlated. There is little empirical support for among-trait correlations of fluctuating asymmetry (Palmer and Strobeck 1986) but, because the aim of these studies was usually to compare instability of different kinds of traits or to test whether instability is an organism-wide phenomenon, the traits chosen may have been more developmentally distinct than traits most useful for testing the association between instability and bet hedging. Because FA is only a weak indicator of instability, even when strong among-trait genetic correlations for developmental instability exist, the expected correlations involving FA are much lower (Whitlock 1996). Clearly, investigations of genetic correlations of instability among traits are needed.

Finally, the predictions and tests, as well as the proposition itself that developmental instability is a possible bet-hedging mechanism, rest on the assumption that variation in developmental instability is explained partially by additive genetic variation. In a recent meta-analysis, Møller and Thornhill (1997) concluded that FA has a relatively low (0.27) but significant heritability. Far from closing the issue of FA heritability, this meta-analysis provoked several harsh solicited commentaries disclaiming the validity of its findings (Markow and Clarke 1997; Palmer and Strobeck 1997). The one point on which there seemed to be agreement, though, is that there is a lack of heritability analyses in the literature. Negative results in a test for the association between developmental instability and trait diversification could imply either that instability is not a bet-hedging strategy, or that one or more of the above assumptions do not hold.

SUMMARY

Developmental instability and bet hedging have been regarded as disparate fields of study. Diversification bet-hedging strategies are said to have evolved if a genotype's intergenerational fitness is increased, to the detriment of short term expected fitness, by risk spreading. A trait of low developmental stability is more sensitive to the effects of the environment than is a trait of higher developmental stability, and thus exhibits more developmental "noise." This noise would be advantageous if it has the effect of increasing the phenotypic variance of a bet-hedging trait.

I argue that, given the appropriateness of variance (in timing of germination, for example) as a bet-hedging strategy, developmental instability is a possible and unappreciated mechanism by which it evolves. The extensive interpretation of fluctuating asymmetry as the "inability" of a genotype to direct its development is appropriate given the results of much recent research. Such interpretations should be made with caution if the asymmetrical character is closely associated with another that may exhibit instability as a bet-hedging strategy. Studies specifically designed to assess the genetic basis of instability in characters such as seed germination behaviour would greatly enhance our understanding of the potential for the evolution of instability as a bet-hedging strategy: developmental instability may not only be the inability to stabilize, but the ability to destabilize development.

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CHAPTER 3

Noise, quiescence, and the misbehaviour of seeds

Abstract—Seeds provide the means for a plant to avoid conditions that would be fatal to any other stage of the life cycle, and the timing of seed germination should thus be under strong selection. Germination behaviour, however, is typified by high variance, both within species and within genotypes. Diversification in germination behaviour, both within and among seasons, is expected to evolve in response to unpredictable environments. The extremely low heritability of seed traits combined with ineffectual attempts at controlling germination timing through artificial selection suggests that the variance of, in addition to the mean time to germination might be a relevant evolutionary seed trait. The goals of this experiment are to determine whether there is a quantitative genetic basis for the time to germination and the diversification in time to germination, as well as to explain the residual variation in terms of seed size variance, microenvironmental effects, and the possible influence of developmental noise in Lobelia inflata. It is found that the heritability of neither time to germination within a season nor among seasons is different from zero; however, a significant negative genetic correlation between the germination fraction in the first season of one generation and the second season of the subsequent generation implies that genotypes may alternate germinability among generations. The heritability of the diversification in time to germination is found to be nonsignificant. The fractions of seeds germinating during the first season and delaying germination until the second season are similar. Genotypes with a high proportion germination within the first season also show a high proportion germination in the second season. The timing of germination, even under the highly controlled conditions characterizing this experiment, is found to be sensitive to microenvironmental differences. Developmental instability, as measured by fluctuating asymmetry of cotyledons, is linked to differences in germination timing, and the evolutionary implications of this association are discussed. I suggest that diversification bet-hedging might arise through the evolution of extreme sensitivity of the timing of germination to environmental stimuli.

INTRODUCTION

The evolution of life history traits depends on the strength of selection acting on the traits and on the composition of expressed phenotypic variation. The seed stage of a plant's life cycle is expected to be especially responsive to selection because it is typified by low survivorship (Harper 1977). Furthermore, individual fitness, in terms of both survival and reproduction, is influenced by traits of the seed from which it originated. Seed size has been found to influence both fecundity (Kalisz 1989) and survival (Galen and Stanton 1991; Simons and Johnston 1999); the timing of germination also influences both fecundity (Baskin and Baskin 1972; Marks and Prince 1981; Kalisz 1986, Biere 1991b; Galen and Stanton 1991; Shitaka and Hirose 1993) and survival (Baskin and Baskin 1972; Marks and Prince 1981; Biere 1991b; Simons and Johnston 1999); and seed size influences 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 1999).

The two most commonly studied seed traits are seed size and the timing of germination. Among species, seed size varies by over ten orders of magnitude (Westoby et al. 1992). Seed trait variation observed among taxa attests to the effects of past selection, and may represent "coadapted syndromes" involving several seed traits (Venable and Brown 1988; Kalisz 1989; Rees 1997). 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), even in crop plants that have been subjected to artificial selection to control this variance (references in Silvertown 1984), although 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 has 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), but considerable variation in the timing of germination within a season also exists (e.g., Kalisz 1986; Biere 1991a; Simons and Johnston 1999). Because seed size and the timing of germination are closely associated with fitness, explaining variance in these traits is of broad evolutionary interest.

Possible sources of diversity in both seed size and germination timing include genetic variation, parental effects, the inbreeding coefficient of parents, and variation generated by positional (e.g., among fruits) or developmental timing differences within seed parents. In addition, variation in time to germination may be generated by adaptive plasticity in response to environmental differences among locations to which seeds have dispersed (Harper 1977), or by extreme sensitivity to internal or external environmental differences, which may be determined by developmental instability (Simons and Johnston 1997).

Fisher's "fundamental theorem" (1958) has been interpreted to imply that, within populations, genetic variation will erode through selection, particularly in fitness traits such as life-history traits. General trends in heritabilities ($\underline{h}^2$) measured for a variety of traits (Mousseau and Roff 1987) support this idea. Nevertheless, significant additive genetic variation for life history traits (average $\underline{h}^2 = 0.27$) is often found (Mousseau and Roff 1987). Seed traits are anomalous in that additive genetic variation is usually either not detected or is very low (Schaal 1980; Kalisz 1986; Mazer 1987; Schwaegerle and Levin 1990; Biere 1991a; Wolfe 1995; Mojonnier 1998), even if measured under homogenous conditions, which have been shown to inflate measures of heritability (Simons and Roff 1994).

An overriding proportion of the within-population variance in seed size and germination behaviour, when studied, 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 (Stanton 1984; Thompson 1984; Roach 1986; Wolfe 1992; 1995) and, in the case of germination variance, the effects of microhabitat (Galen and Stanton 1991; Shitaka and Hirose 1993; Baskin et al. 1994; Horvitz and Schemske 1994).

A yet untested hypothesis is that the variance in seed traits, rather than the mean trait values, has a genetic basis and is acted on directly by selection. Diversification bet-hedging strategies are expected to be 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). Diversification may also be selected for under spatial variability in seedling density (Geritz 1995) or under intense sibling competition (Nilsson et al. 1994). That the variable timing of germination, and thus bet hedging, is often under maternal control (Westoby 1981) does not imply that germination variance is predominantly an expression of maternal effects—maternal control may equally imply maternal genetic control: genotypes may differ in the level of variance expressed by their progeny. Maternal genetic control of variance would equally account for the ineffectual attempts to select for immediate or delayed germination: selected early or late germinators would merely be a random sample of a genotypically diversified germination schedule.

Although previous work on this species has shown a statistically significant influence of seed size on the timing of germination (Simons and Johnston 1999) through maternal and positional effects, much variance in the time to germination remains unexplained. In this experiment, I attempt to determine in detail the unexplained sources of variance in the timing of germination in Indian tobacco, Lobelia inflata (Campanulaceae). Using seeds from 84 genotypes collected from the wild, I perform offspring-on-parent regressions on germination data collected under highly controlled conditions to test for polygenic control of seed size as well as the timing of germination both within and among seasons. A genetic basis of diversification in the timing of germination is then analyzed by performing regressions using measures of variation in the timing of germination of offspring and parents. The design also allows for the examination of microenvironmental effects on germination. Associations between developmental instability (DI) and germination timing are measured to test the hypothesis (Simons and Johnston 1997) that DI is a possible mechanism whereby germination variance is generated.

Features of L. inflata lend themselves particularly well to this 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 is maximized here because interpretations of differences within plants are 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.

MATERIALS AND METHODS

The experiment consisted of two generations of seed germination in which seed size and the timing of germination were recorded for seeds within each of many genotypes (Fig. 3.1). This allowed for offspring-on-parent regressions of time to germination and seed size as well as of variance in seed traits. There were several differences between the first and second generations: In the first generation, the germination characteristics of a genotype were represented by 40 seeds of individuals from 100 genotypes. In the second generation, 30 seeds of individuals from 84 genotypes were used. Whereas seed size was measured on a subsample of the seeds used in the first generation, all seeds used in the second generation were measured. Only seeds produced in the first two fruits on the main

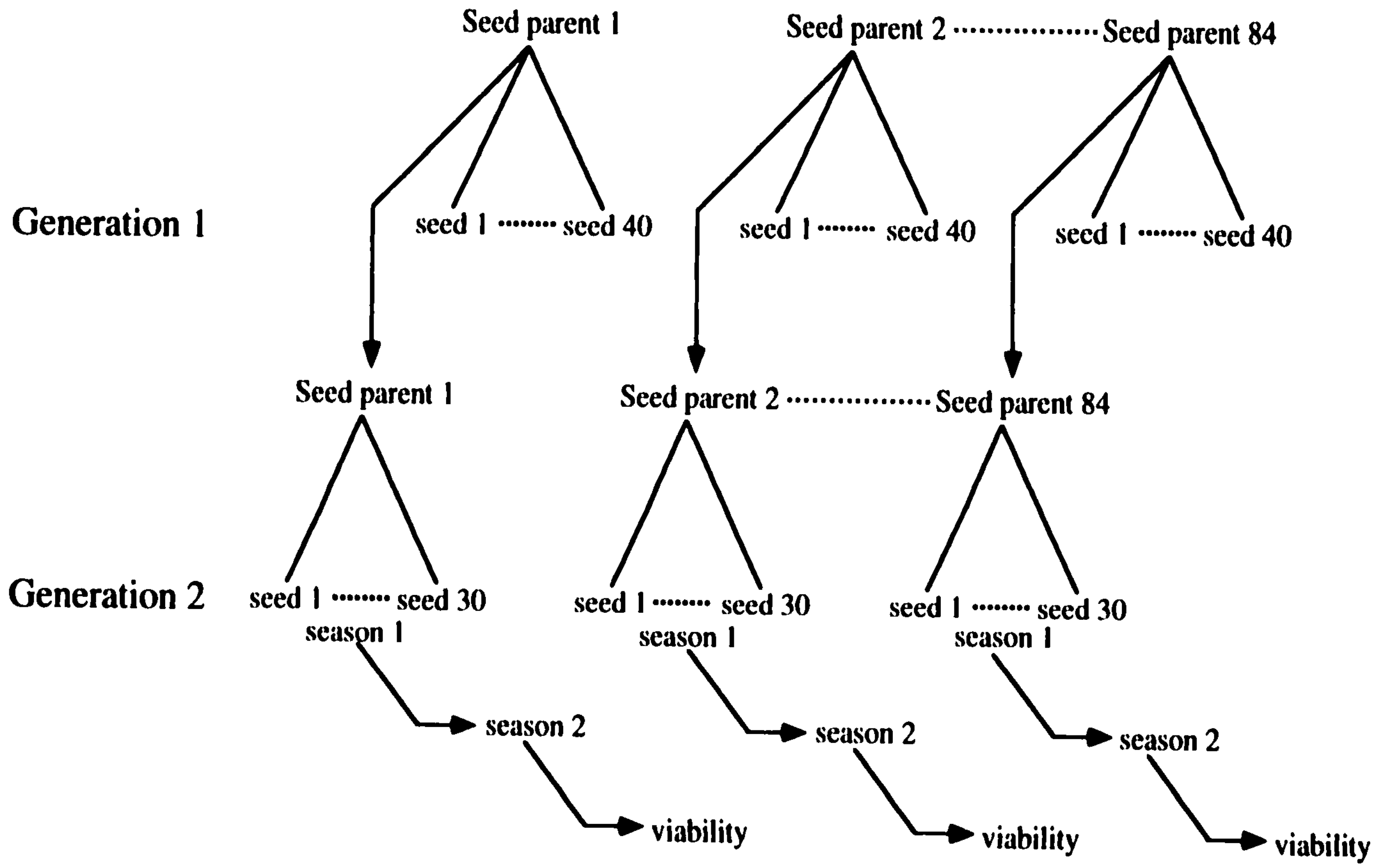

Figure 3.1. Experimental design. Seeds from the original seed parents were field-collected; all seeds were germinated in a growth chamber. In the first generation, seed size and cotyledon fluctuating asymmetry were measured for 10 seeds from each seed parent, and time to germination was recorded for all seeds. In the second generation, seed size and time to germination were measured for all seeds; seeds that did not germinate were restratified and allowed to germinate during a second season. This was followed by a third stratification and viability tests.

inflorescence were used for the second generation. Also, as a measure of developmental instability, the fluctuating asymmetry (FA) of the cotyledons of germinating seeds of the first generation was measured. Finally, for the second generation, the state of seeds that had not germinated by the end of the germination trial, referred to here as a season, was attributed to either dormancy or mortality by restratifying the seeds and following subsequent germination over two more seasons, followed by viability tests.

Seeds of 125 mature individuals were collected from Martock, Nova Scotia, Canada in autumn 1995, and were stored in dark and dry conditions until use. One hundred genotypes were used in the first generation of the growth chamber germination experiment, each represented by 40 seeds. Forty petri dishes were assembled, but in four batches of 10 plates staggered in time to avoid synchronous peak germination of the 4000 seeds. To avoid confounding genotypic and common environmental effects, only one seed of every genotype was represented on a petri dish. Because it is possible that positional effects within a petri dish exist, 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.

Ten seeds from each individual were randomly selected for each batch of 10 plates. The seeds were selected by emptying all seeds collected from an individual onto a glass petri plate that had been marked with a grid, shaking the seeds, and then removing all but the ten centremost seeds using an aspirator. Individual seeds were placed (dry) onto small hole-punched discs of filter paper grouped by genotype until the start of the experiment. This is the only time at which seeds were directly contacted. Damage or scarification of seeds was avoided, however, because transfer using forceps was effected using static electricity only; forceps were never closed on a seed.

Digital images of 10 dry seeds (plates 1-10) per genotype were made for later measurement. All seeds for a given batch were moistened simultaneously with distilled water before transfer to their correct randomized positions on the petri plates. Because light exposure prior to stratification affects germination behaviour (Simons and Johnston 1999), seeds spent five hours under florescent light, including the time taken to allocate seeds to their positions, before transfer to darkness at 5°C for 30 d. Seeds were then placed in the growth chamber under a 14h photoperiod and thermoperiod of 21°C/14°C for germination. In the growth chamber, ten plates were placed in each of four white doubled trays, covered by a dome lid. Trays were lined with paper towel, and contained standing water that had been previously boiled. The filter paper within each petri dish was kept moist by watering ad libitum with distilled water using an eye dropper. Each of the 4000 seeds was checked for germination every second day under a dissecting microscope. A seed was judged to have germinated upon protrusion of the radicle, whereupon the date of germination, position, and genotype were recorded.

Ten germinated seeds of each genotype were allowed to develop for a further 10-14 days to allow the emergence of the cotyledons for measurement. Digital images were made by removing the stem and placing the inverted leaves in water between a slide and coverslip. Both cotyledons were then measured using NIH Image (Wayne Rasband; http://rsb.info.nih.gov/nih-image/). Fluctuating asymmetry was measured as the absolute value of the difference between the length of the two cotyledons, divided by their mean length (Palmer and Strobeck 1986).

Sibling seeds of the seeds used in the first generation, rather than the seeds actually used in the first generation, were used to found the seed parents for the second generation for use in the offspring-on-parent analysis. Parental effects specific to individual seeds used in the first generation will thus not be present in offspring of their siblings. However, because siblings may be assumed to be nearly genetically identical, offspring of siblings are genetically identical to direct offspring. About twenty seeds of each genotype were placed in petri plates on moistened filter paper and placed in the refrigerator at 5°C. The plates were protected from desiccation by sealing with Parafilm. The seeds were transferred after 43 days for germination under 12h daylength conditions in a growth room. Eight germinated seeds of each genotype were transferred to cell packs containing ProMix at a density of two seeds per cell. Seedlings were then thinned to one per cell based on seedling vigour. For cases where genotypes did not produce enough germinated seedlings, additional seeds were added directly to the ProMix without stratification. Photoperiod was set to 13 hours light and gradually increased to 15h over 84 d and reduced again gradually to 14h over 21 d.

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. Plants that had not bolted by day 110 were transferred to the growth chamber (11h light; 16°C/10°C) in order to precondition the rosettes for cold treatment. By using seeds from both immediate bolters and those that bolted only following vernalization, selection on seed traits that might be correlated with flowering phenology was avoided. After a vernalization treatment of about 3 months, the rosettes were placed in the growth room. Many of the rosettes died during the cold treatment due to desiccation. Vigorous bolting was observed within one to two weeks of placement in the growth room; virtually every (106 of 107) living rosette bolted promptly. Seeds were collected from these plants as above. In the second generation, 84 genotypes from Martock were available for use in the offspring-on-parent analysis.

Seed plates were assembled for the second generation in the same manner as for the first generation, but the 84 genotypes were divided between two sets of plates; one set containing 49, and the other containing 35 genotypes. Each set consisted of 30 plates containing seeds from one individual from each genotype. A genotype's seed was randomly assigned to one of the 49 or 35 positions within a petri plate, and a different randomization was used for each plate. Thus, in the second generation, 2520 seeds from 84 genotypes were used, for a total of 6520 seeds over the two generations.

Seeds were removed from the growth chamber after germination on a plate had ceased, which extended from between 51 to 62 d, and were placed once again at 5°C for restratification for 129 d. Plates were again checked for germination immediately upon removal from the first restratification treatment (many germinated under complete darkness in the cold), and were checked for germination up until removal from the growth chamber after 30 d. A third stratification was then conducted at 5°C for 30 d, and seeds were examined for germination. Finally, to determine whether the remaining ungerminated seeds were viable, triphenyl tetrazolium chloride (TTC) viability tests were performed (Moore 1973).

Because no appropriate tetrazolium staining technique has been described for this 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 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 ungerminated seeds were left in their original petri dishes, and were tested for viability using the chipping/peeling treatment described above.

The time to germination is characterized by a skewed distribution, and was log-transformed prior to analysis; germination and viability fractions are proportions, and were transformed as arcsin(proportion$^{1/2}$). The means of each sibship's individual log-transformed germination times, seed widths, and arcsin-transformed germination fractions were thus used to calculate heritabilities of those traits. The coefficient of variation of the log-transformed time to germination was used as a variance measure for each sibship for the offspring-on-parent estimate of the heritability of diversification. All analyses were performed using SAS (1989).

RESULTS

The overall viability of seeds, as judged by the cumulative germination over three stratification treatments and the viability of the remaining seeds, was 91%. Time to germination showed high levels of variance within the first season of both generations: the CV of time to germination was 0.51 in the first generation and 0.46 in the second. The proportion of seeds germinating following one stratification (i.e. during the first season) was 0.71 in the first generation and 0.68 in the second. In the second generation, a further 0.59 of the seeds that had not germinated in the first season germinated during the second after restratification. Following a third stratification, 0.36 of the remaining seeds either germinated or were still viable during the third season. The viability of seeds not germinating during the experiment was 0.28.

Seed size was strongly related to both the time to germination within a season and the fraction delaying germination. A homogeneity-of-slopes analysis shows that large seeds in both generations of the experiment germinated earlier within a season ($\underline{df} = 1$; $\underline{F} = 86.4$; $\underline{P} < 0.001$), and that the time to germination did not differ between the two generations ($\underline{df} = 1$; $\underline{F} = 1.2$; $\underline{P} = 0.27$) although the slope describing the relationship between seed size and the timing of germination differed between generations (interaction $\underline{df} = 1$; $\underline{F} = 10.1$; $\underline{P} = 0.002$). Logistic regression shows that, for seeds that germinated in the first or second seasons, the probability of germinating during the first season was dependent on seed size ($\underline{N} = 2179$; Wald $\chi^2 = 17.8$; $\underline{P} < 0.001$). To analyze the relationship between seed size and among-season delays, seeds were assigned to one of four groups: those germinating during the first season, the second season, those germinating or viable during the third season, and those found to be inviable in the tetrazolium test. ANOVA reveals significant seed size variation among these four groups ($df = 3$; $F = 8.5$; $P < 0.001$). According to a Tukey test, which corrects for experimentwise type 1 error rate, the mean size of seeds germinating within the first season (0.301 mm) was larger than the mean size of seeds delaying germination until the subsequent season (0.297 mm), and was also larger than seeds that were deemed inviable following a tetrazolium test (0.296 mm). The mean size germinating or viable in the third season (0.297 mm), however, was not found to be significantly different from means of other groups.

Phenotypic variance in time to germination within a season is high for both generations (above), and no additive genetic variance for this trait was detected ($h^2 = 0.02$; $P = 0.84$). Delay in germination from the first season to the second also shows no significant genetic variance ($h^2 = -0.30$; $P = 0.20$), although the heritability value is high and negative. The only significant result implying a genetic effect for germination traits is a negative genetic correlation between germination fraction in the second season of the second generation and first season of the first generation ($N = 82$; $r = -0.27$; $P = 0.015$): genotypes that delay germination in one generation germinate more readily in the next generation. The h^2 of seed size is low and nonsignificant ($h^2 = -0.11$; $P = 0.45$). For calculations of the heritability of the diversification in time to germination and seed size, two measures of diversification were used: the variance (Var) of and the coefficient of variation (CV) of log-transformed time to germination. No significant quantitative genetic variance was detected for either measure (Var: $h^2 = -0.23$; $P = 0.19$; CV: $h^2 = -0.24$; $P = 0.11$). Similarly, the diversification of seed size shows no consistent relationship among parents and their offspring (Var: $h^2 = 0.13$; $P = 0.25$; CV: $h^2 = 0.18$; $P = 0.09$).

When additive genetic variance is zero, resemblance among progeny within individual parents may be attributed to parental effects. Parental effects within generations can be calculated for time to germination and for seed size but, because variance cannot be measured on individuals, diversification differences among parents could not be analyzed within generations. Although no significant heritability is found across generations (above), a significant effect of seed parent within generation on time to germination is found ($df = 181$; $F = 4.78$; $P < 0.001$) using a nested mixed-model ANOVA with parent nested within generation. The same model, but using seed size as the response variable shows a similar result for parent within generation ($df = 182$; $F = 16.00$; $P < 0.001$).

The germinable fraction of seeds in the first and second seasons differed by only 0.09 (above). Using the family mean transformed germination fractions for the second generation reveals that parents that produce seeds with a high germination fraction during the first season also produce seeds with a high germination fraction during the second season ($N = 82$; $r = 0.43$; $P < 0.001$). Furthermore, for the first generation, a significant correlation between time to germination within the first season and the fraction of seeds remaining dormant within that season ($N = 100$; $r = -0.52$; $P < 0.001$) implies a similar mechanistic basis for the two types of germination delay; however, no such correlation is observed during the second generation ($N = 83$; $r = 0.09$; $P = 0.43$).

The position of every seed randomized within petri plates, the position of petri plates within trays, and the position of trays within the growth chamber were recorded. To detect positional effects within a petri plate, a meaningful index of location had to be assigned to each seed: a seed was simply coded as occurring on the left- or right-hand side. Analysis for environmental effects on the timing of seed germination was performed by assigning numbers to trays, position within a tray, and position within a petri plate. A three-factor fixed effects ANOVA detected significance for all three main environmental effects (Table 3.1), and explained 31% of the variance in time to germination. Adding seed size as a covariate only increased the r-squared value of the model from 0.31 to 0.33 because, although seed size was found to explain a statistically significant portion of variation in time to germination, it explained only a small amount of the total variation.

Mean FA measurements were available for each genotype used in generation one. No significant correlation between mean FA and CV of log-transformed germination time

Table 3.1. The effects of microenvironment on time to germination in Lobelia inflata. The ANOVA was a three-way factorial fixed effects model. Each of 12 trays in the growth chamber contained five plates, and each plate contained a single seed from every genotype at a randomized position. The model is factorial because a particular location of a plate in a tray is assumed to correspond to the same location in another tray, as is a position (left or right half) within a plate.

Source of variation	df	SS	F	P
Tray (T)	11	55.32	25.74	< 0.001
Plate (P)	4	4.65	5.95	< 0.001
Position (S)	1	1.83	9.38	0.002
T x P	44	29.83	3.47	< 0.001
T x S	11	17.53	8.16	< 0.001
P x S	4	0.57	0.72	0.575
T x P x S	42	14.91	1.82	0.001
Error	1580	308.74		

was found ($N = 100$; $r = 0.04$; $P = 0.67$). Fluctuating asymmetry was found to be negatively associated with the time to germination, according to both a simple correlation and partial correlation that corrects for mean seed width (Table 3.2). High observed FA associated with larger seeds is driven by the association of FA and time to germination, and disappeared once time to germination was accounted for (Table 3.2).

DISCUSSION

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; Michaels et al. 1988; Venable and Brown 1988; Kalisz 1986; Westoby et al. 1992; Philippi 1993; Rees 1997; Simons and Johnston 1999). The present work is an attempt to understand the genetic and environmental roles of seed germination, both within and among growing seasons, by following the germination patterns of individual seeds, of known size and genotype of origin, over two generations and two seasons under highly controlled conditions.

The total phenotypic variation of seed traits observed in a population, unlike that for most traits studied, is rarely found to be composed of underlying genetic variance (Schaal 1980; Kalisz 1986; Mazer 1987; Schwaegerle and Levin 1990; Biere 1991a; Wolfe 1995; Mojonnier 1998). Because heritabilities may be inflated under conditions of reduced environmental variability (Bull et al. 1982; Simons and Roff 1994), the likelihood of detecting the presence of genetic variance in seed traits was increased by conducting germination experiments under growth-chamber conditions. Despite these circumstances, no genetic variation in seed size, time to germination, or germination fraction was found.

If the variance of these traits, and not the traits themselves, is under genetic control, then selection can modify the expression of trait diversification directly. The data

Table 3.2. Correlations between means of seed families measured in the first generation of the germination experiment. Results included only seeds that germinated, and FA represents the fluctuating asymmetry of cotyledons emerging from the germinating seeds. Pearson product-moment correlations are above the diagonal, partial correlations are below. P-values are given in parentheses.

	Mean FA	Mean log time to germination	Mean seed width
Mean FA	—	–0.354 (< 0.001)	0.292 (0.003)
Mean log time to germination	–0.225 (0.025)	—	–0.652 (< 0.001)
Mean seed width	0.087 (0.391)	–0.614 (< 0.001)	—

presented here offer no evidence that selection can operate directly on seed-trait variance; heritabilities of both seed size variance and of germination variance are not different from zero. It should be noted, however, that the detection of genetic differences in variance depend, first, on obtaining a biologically meaningful measure of variance and, second, on the power of the test performed. The qualitative results in this study, however, did not depend on the measure of variance used. With only one measurement of diversification per genotype per generation, the likelihood of detecting genetic effects is considerably reduced compared to that for simple trait values. The results do suggest that further attempts to measure h^2 of seed size variance should be made with more than 84 genotypes: the heritability of the coefficient of variance of seed size ($h^2 = 0.18$; $P = 0.086$) would be significant at a relaxed α–level of significance, and the relationship is stronger than that for seed size itself. Approximately 20 additional offspring-parent pairs would be required to show a significant heritability at $\alpha = 0.05$ with an offspring-parent relationship of this strength.

Although variation in seed size explains a significant amount of variation in time to germination within a season, the effect of a seed's position in a petri plate, the position of a petri plate within a tray, and the location of the tray within the growth chamber were far more important. Just to illustrate the importance of position within a petri plate, the mean time to germination in the left-hand side of all plates is 26.5 d, whereas it is 31.1 d on the right, a highly significant difference. The timing of germination of seeds in _L. inflata_, then, is extremely sensitive to small environmental differences found within the confines of a growth chamber, which is designed to maintain constant conditions. It is difficult to imagine that germination behaviour would not be substantially increased in the wild—innumerable additional environmental parameters such as light conditions prior to stratification or length of the stratification period (Simons and Johnston 1999), as well as their interactions, might influence germination. It could be argued that the growth-chamber conditions augmented germination variance in comparison to variance that would be expressed in nature, but this would be an extreme stand denying both conventional wisdom (e.g., Bull et al. 1982) and empirical findings (Simons and Roff 1994) related to the effects of environmental variance on trait expression.

Bradshaw (1965) pointed out that there is no clear delineation between plasticity and noise. I argue that the germination responses of some seeds might be regarded as an example of where plasticity and noise are indistinguishable. If the germination responses of seeds to environmental variance experienced in the present experiment are viewed as plasticity, it is inconceivable that the norms of reaction are adaptive, because plasticity occurs over an environmental gradient of no ecological relevance to the growth or survival of the seed. With adequate technology, though, norms of reaction could be established for any environmental parameter, internal or external to the organism, even if the variance that is generated appears random at an ecological scale relevant to the organism.

It was recently proposed (Simons and Johnston 1997) that diversification bet hedging might be produced by extreme sensitivity of germination to the internal or external environment through selection for developmental instability (DI). A seed would thus use its surroundings as a randomization mechanism to practice diversification akin to Kaplan and Cooper's "adaptive coin flipping" (1984). A common method to test for developmental instability is through the measurement of fluctuating asymmetry (FA) of paired characters. The reasoning is that asymmetry results from developmental errors. In this experiment, FA of cotyledons was measured for 100 genotypes in the first generation, and no correlation between developmental instability and diversification in the timing of germination was found. However, developmental instability and the timing of germination are related in that early-germinating seeds have higher levels of FA, even after removing the effects of seed size. Although FA "accounts" for a significant amount of variation in the timing of germination, no causal relationship between DI and germination behaviour may be implied.

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 under temporal environmental unpredictability. Hopper (1999) in a review of the insect bet hedging literature, states that, although bet-hedging theory is "voluminous," he was "unable to find any definitive tests with unequivocal results showing that risk-spreading has been a major factor in the evolution of insect behaviors or life histories" (Hopper 1999). 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 quantification of diversification strategies. The present work contributes toward the understanding of how diversification is attained, but the evolutionary significance of this diversification requires further empirical study.

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CHAPTER 4

The determinants and life-history consequences of reproductive behaviour in the monocarp, *Lobelia inflata*

Abstract—The timing of reproduction is an important life-history variable, especially for organisms, such as the monocarp Lobelia inflata, that die following a single reproductive episode. In this set of experiments I attribute variance in the timing of onset of reproduction in L. inflata to several sources, and examine the fitness consequences of variability in this life-history trait. Recent work on rosette-forming monocarps shows plant size to be a more important influence on the initiation of flowering (bolting) than is age. Here I find that the size requirement for bolting changes through time over a diminishing photoperiod regime. A novel conceptual model of the control of bolting, based on time-dependent size requirements, is presented. The magnitude of the heritability of bolting is found to depend on the photoperiod treatment under which it is measured, with higher heritabilities occurring later in the growing season. The extent of population differentiation with respect to bolting behaviour is also found to be dependent on the time of year in which it is measured, but among-population differences in bolting behaviour are more pronounced early in the season. No within-population heritability of the plasticity of bolting behaviour is detected. Differences among populations in the plasticity of bolting are found to be dependent on the environment or generation in which they are measured. An advantage to large rosette size in addition to the direct effects of size on fecundity is found: large rosettes require less time to flower once bolting has occurred.

INTRODUCTION

An organism's schedule of reproduction is a fundamental life-history trait; fitness is maximized only if this schedule is optimal with respect to an organism's particular pattern of growth, mortality and fecundity. A schedule of reproduction has two major components: the timing of reproduction, and the proportional allocation of energy to reproduction, or reproductive effort (Roff 1992). Organisms that die following a single bout of reproduction (semelparous), such as monocarpic plants, are ideally suited for the study of the life-history consequences of the timing of reproduction, because the issue of reproductive effort is not confounded by tradeoffs involving allocation among different reproductive episodes as it would be for iteroparous organisms.

For rosette-forming monocarpic plants, the reproductive mode is initiated at the time of stem elongation, or "bolting"; although the "decision" to flower is made at the time of bolting, actual flowering occurs later within the same season. Recent empirical work has advanced our understanding of the timing of reproduction in monocarps, but a considerable amount of work is still needed in what may be defined as three major areas: the mechanisms controlling the timing of reproduction, the genetic basis of the timing of reproductive behaviour, and the fitness consequences of timing "decisions." In this chapter I investigate each of these three areas using the monocarpic perennial, Lobelia inflata.

Previous work on monocarpic species suggests that the timing of the onset of reproduction may be influenced by both plant size (Werner 1975; Klinkhamer et al. 1987; Couvet et al. 1990; Klinkhamer et al. 1991) and age (Klinkhamer et al. 1987), although the effects of age are rarer (Lacey 1986a), as well as by interactions between plant size and conditions during vernalization (Klinkhamer et al. 1991; Karlsson et al. 1993). Size and age are often confounded, though, and bolting is consistently found to be influenced primarily by rosette size, and not directly by age in experiments that discern between these two variables (Werner 1975; Klinkhamer et al. 1991).

A large proportion of variation in the probability of bolting remains unexplained, however, and it is becoming evident that the basis of the flowering "decision" is more complex. The threshold size for reproduction, although it may be characterized for a given year, is not fixed. Even in cases where a strong relationship exists between rosette size and the probability of bolting, significant among-year variation in size requirements have been found (Wesselingh 1995).

Much is known, then, about the requirements for bolting for any particular season of growth. Because it has been demonstrated that these requirements can change dramatically through time within the same population, the ecological implications of this important life-history character cannot be well understood until progress is made toward determining the mechanisms underlying temporal variation in the requirements for initiation of reproduction. The observations of an effect of size on bolting behaviour within seasons combined with observations of changes through time in these size requirements strongly suggest that explicit tests for interactive effects of size and time would be an appropriate starting point.

For Cynoglossum officinale, a monocarpic plant that requires vernalization for stem elongation, de Jong et al. (1998) propose that the bolting "decision" for a given season is made during the previous season, perhaps in late autumn, and is dependent on environmental conditions at that time. I test a specific hypothesis based on this suggestion of de Jong et al. (1998); namely, that the probability of bolting depends on rosette size, photoperiod, and their interaction. "Environmental conditions" such as weather, then, are included in the interaction term if the size a rosette has attained by a given date has been influenced by growing conditions. If bolting depends on a changing size requirement through time, year-to-year variance in the fraction of the population bolting could be observed even if the mean rosette size at the end of the season is constant. To test the hypothesis of the existence of size requirements that change through time, an expedient method would be to use a plant with no vernalization requirement. This way, the interaction of size and time could be observed within a single season of growth provided that sufficient numbers bolt within one season, and provided that sufficient variation in rosette size at age is generated.

Response to selection, or evolution, of a trait is proportional to the heritable component of its phenotypic variation (Falconer 1989). The heritability (h^2) of a trait is thus a fundamental evolutionary parameter. Few studies have assessed the genetic basis of bolting, but the year of bolting in offspring has been shown to resemble that of its parents (Lacey 1986b) and, when measured, the heritability has been found to be substantial (Wesselingh and de Jong 1995). Wesselingh and de Jong (1995) selected for large and small threshold rosette size at bolting, and found $h^2=0.35$ and $h^2=0.32$ for the two lines. They proposed that the relatively high heritability for a life-history character (Mousseau and Roff 1987) results from a "flat fitness profile" near the optimal rosette size (Wesselingh and de Jong 1995). The present experiment differs from others in that the timing of bolting, rather than the threshold size at bolting (Wesselingh and de Jong 1995) is of interest, and it is measured within a single season of growth, rather than among seasons (Lacey 1986b). Both the genetics of bolting behaviour and the genetics of the plasticity of bolting behaviour under different environments are assessed in L. inflata. This is the first study to assess the quantitative genetics of the plasticity of a threshold character.

Plant fitness, as measured by final plant size or total fruit production, may be influenced by a great number of genetical and environmental factors. The timing of reproduction is of particular importance. For indeterminately growing semelparous organisms, for example, delayed reproduction is advantageous if a positive correlation between survival and size exists. This expectation has some empirical support in a monocarpic plant; Wesselingh et al. (1997) found a positive correlation between survival and threshold size for bolting in Cynoglossum officinale. Delayed reproduction is most often studied in terms of among-season delay. The timing of reproduction is important also within a season of finite length because, even for semelparous organisms, reproduction is not instantaneous. If reproduction is initiated during a period of favorable conditions late in the season, for example, there may not be sufficient time remaining for the successful development and maturation of seeds following this initiation. In higher northern and southern latitudes, diminishing photoperiod toward the end of the growing season acts as more than a simple timing cue: shorter daylengths must result in reduced photosynthetic assimilation and production.

The control of bolting, the quantitative genetics of bolting behaviour, and the fitness consequences of the timing of bolting in Lobelia inflata were studied in a single experiment. Rosette size and bolting behaviour were recorded under three continuously diminishing photoperiod treatments representing early, middle, and late summer (referred to as phototreatments A, B, and C), over two generations in the growth chamber. L. inflata has several favorable characteristics for such a study: First, individuals of this species are prevented from outcrossing through the formation of an "anther tube" that surrounds the stigma. Because heterozygosity decreases rapidly over generations of self-fertilization, seed parents and their offspring may be assumed to be genetically identical. Second, it reproduces only once in its lifetime; no alternative reproductive modes to seed production, such as adventitious rooting or spontaneous generation, exist. Furthermore, vernalization is not required for bolting (A.M. Simons, personal observation). Factors influencing reproduction within a single season may thus be examined in detail for L. inflata. It is hoped that conclusions reached here may at least provide a foundation for further work on other species.

MATERIALS AND METHODS

Seeds were collected from three populations: Martock (Sept. 20, 1995), Mt. St-Hilaire (Sept. 6, 1995), and Harvard Forest (autumn 1994, by P. Wilson). Seeds were stored in dark, dry conditions until use. Approximately 15 seeds from each of 12 genotypes from all three populations were placed, by genotype, on moistened filter paper in 6 cm petri plates. The seeds were exposed to cool white light for five hours before being placed at 5°C for 31 days. Three photoperiod treatments were established, each of which was designed to mimick the changing photoperiod of a different part of the growing season from early to late summer. On day one for phototreatment A, (early summer) the growth chamber was initially set at 21°C/12°C and a 15 h 22 min light photoperiod (Fig. 4.1). The thermoperiod was not adjusted along with the photoperiod; it remained at the original 15 h 22 min so that temperature effects would not confound those of photoperiod. One of three banks of lights was set for this photoperiod, the others were set for 15 minutes later in the morning, and 15 minutes earlier in the evening. So that seed germination within each phototreatment would be synchronized, only seeds germinating over the peak germination period of seven days were used. Upon germination, five seedlings from each population and each genotype were transplanted individually to Kord cellpacks filled with ProMix.

Seeds for phototreatments B and C were subject to the same procedures that were followed for phototreatment A, and germination in the growth chamber (and thus photoperiod) was timed to be offset by 35 d (Fig. 4.1). Photoperiod was no longer adjusted when it had diminished to 10h light (simulating the photoperiod of November 6). The 540 individuals were positioned in the growth chamber using a randomized block design: Phototreatment, population, and genotype were randomized within four blocks of 135 plants (Fig. 4.2a).

Rosette size was measured as the length of the longest leaf, which is strongly correlated with rosette dry weight in this species (Simons and Johnston, in press). Growth trajectories of each rosette were recorded until bolting, or until day 86 if the rosette did not bolt, and bolting date was recorded. Final size of nonbolters and final fruit set of bolters were recorded on day 126 of the experiment. Seeds of generation 1 were then harvested and all fruits of each individual were stored separately in glassine envelopes.

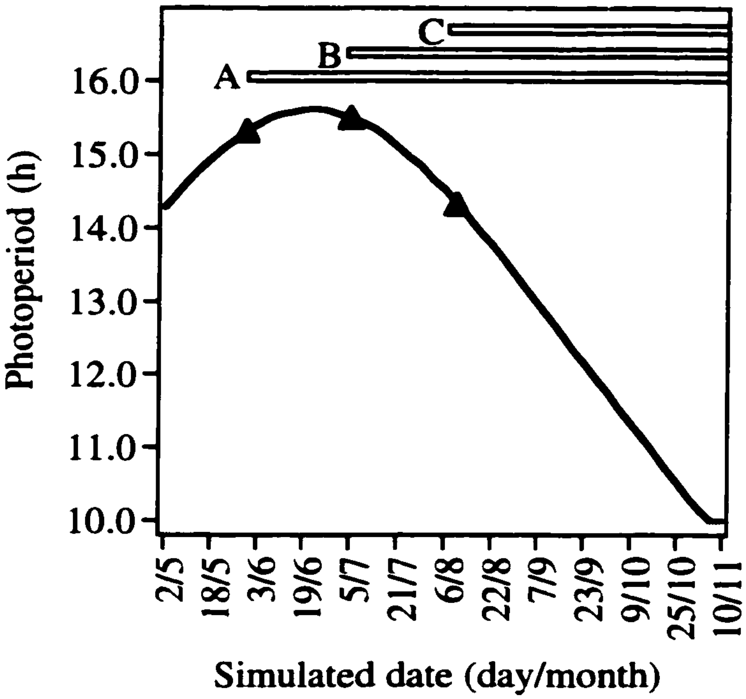

Fig. 4.1. Growth chamber photoperiod schedule. The 35-d separation of initiation of the three phototreatments, A, B, and C, is indicated.

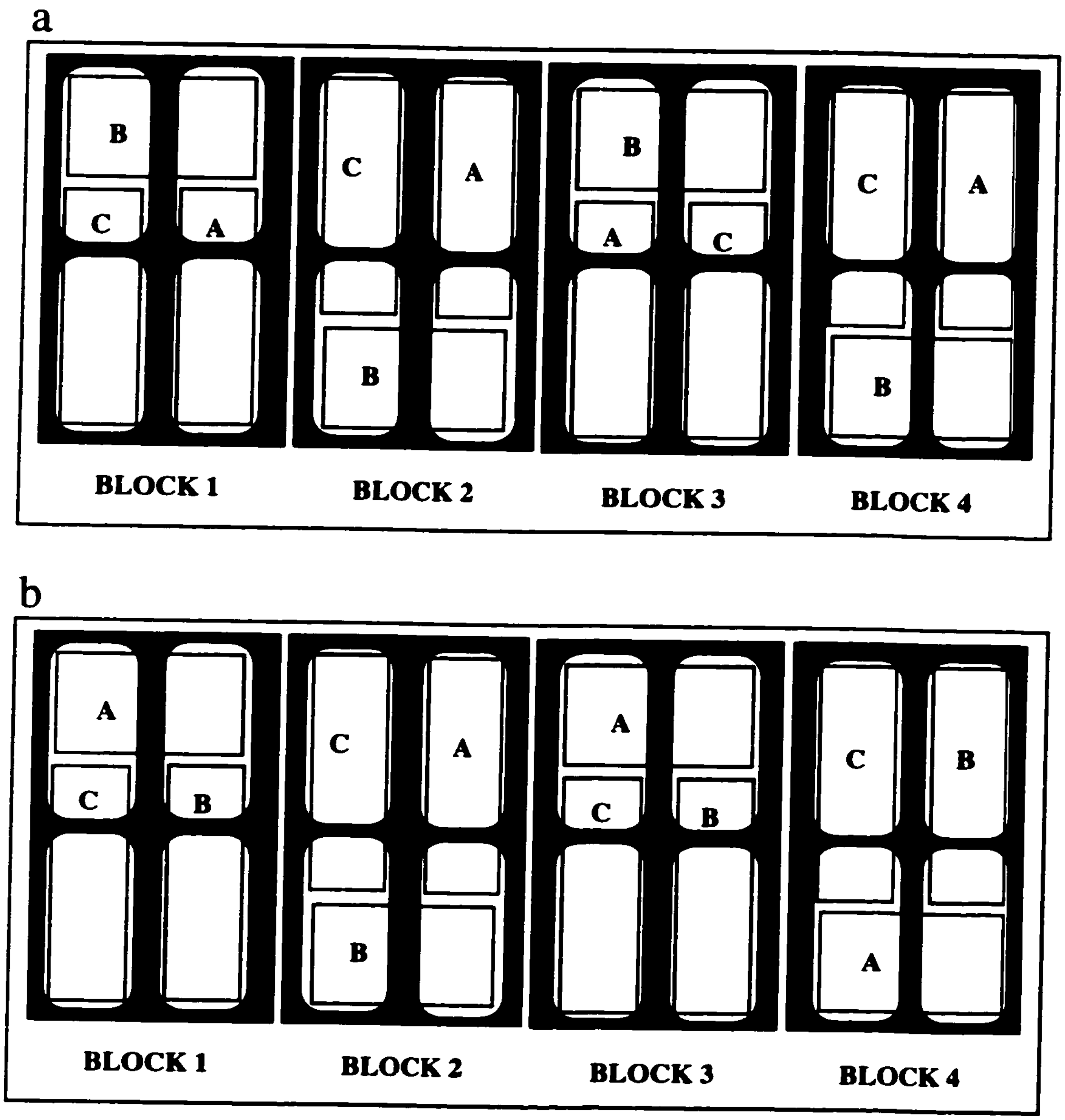

Fig. 4.2. Experimental design for generations 1 (a) and 2 (b). Each open oval represents a flat, and rectangles contain plants for a given photoperiod, as indicated by the letters A, B, and C. Each phototreatment is represented within each of four blocks.

Seeds for generation two were derived from bolting individuals from phototreatment A of generation one. Insufficient seed was available for two genotypes from Harvard Forest, and seed from phototreatment B was used in these two cases. In no case was seed from individuals of the short-day phototreatment (C) used. To further reduce possible maternal effects, seed from four individuals (from the different blocks) within each genotype were equally represented in founding generation two. Each genotype's seed dish contained twenty seeds, five from each parent whenever possible: of the 36 genotypes in generation two, 27 were represented by seed from four parents, 2 by 3 parents, 4 by 2 parents, and 3 by only one seed-producing parent. Furthermore, for this generation only seeds from the first two fruits on the main inflorescence were used.

The seeds were moistened and exposed to five hours of light before the petri plates were sealed and placed at 6°C for 31 days. The same temperature regime and photoperiod schedule (Fig. 4.1) was used in this generation as for generation one, but new randomized positions within the growth chamber were used (Fig. 4.2b).

Starting on day 27, rosette measurements (longest leaf) were taken once per week on all individuals, and were continued until day 139 for phototreatment A and until day 127 or until no growth in three successive weekly measurements was observed for phototreatments B and C. In addition to measurements made in generation one (final rosette size of nonbolters, rosette size at bolting, date of bolting, number of fruits produced), date of first open flower, weekly height measurements of bolters, and weekly counts of the number of fruits and open flowers were also recorded. Fruits were harvested upon ripening, and the flowering stalk (from 2 cm aboveground) was dried and weighed.

Requirements of bolting: Size and time

Bolting status and rosette size were recorded weekly for each individual of generation two over a period of 22 weeks. Data consisting of multiple measurements on each experimental subject may be analyzed either using a univariate repeated measures approach or using a multivariate approach (von Ende 1993). The univariate approach was taken here for several reasons. A high proportion of subjects (i.e. rosettes) contain missing values, and the multivariate approach discards subjects with missing values for any of the repeated measures. Furthermore, the univariate approach must be used when a covariate that changes with the repeated measures is included in the model. In the present design, rosette size is a covariate that changes through time. The univariate approach is generally more powerful than is the multivariate, but interpretation of its results should be made in light of the possible violation of its underlying "circularity" assumption about the variance-covariance matrix of the within-subject (repeated measures) factor (von Ende 1993).

The response variable, bolting behaviour, is dichotomous, whereas standard ANOVA assumes a continuous response. Repeated measures analyses specifically designed to address categorical responses (e.g. PROC CATMOD, SAS (1989)), however, are restricted to the multivariate approach. Therefore, results of the univariate repeated measures procedures were complemented by post-hoc logistic regression analyses to more closely examine the sources of any differences in bolting response at different, individual, dates. Logistic regression analyses, based on maximum-likelihood techniques, have previously been employed for the case of bolting as a function of rosette size observed at only one time (Klinkhamer et al. 1987). Because phototreatment A contained a relatively high proportion of bolting individuals, this phototreatment of generation two was chosen for these analyses.

Quantitative genetic basis of bolting behaviour

Traits that are influenced by a large number of loci (polygenic) may be phenotypically dichotomous if the phenotypic response depends on an underlying "liability" (Falconer 1989) exceeding a threshold value. Examples of such "threshold" traits include cricket wing dimorphisms (Roff and Simons 1997), and mating behaviour dimorphisms in thrips (Crespi 1986). Here I treat bolting behaviour as such a threshold trait and assume that the underlying liability of bolting is a product of a number of interacting physiological variables (Roff 1997). I analyze the variance in liability underlying bolting behaviour using the proportions of bolting observed among different genotypes. Genetic differences in bolting, then, should be interpreted as comprising all factors contributing to the determination of the bolting phenotype (including, for example, any genetic differences in the interactive effects of rosette size and photoperiod).

The data consist of both "affected" and "unaffected" parents and their offspring, and the offspring-on-parent regression could not be performed in the usual manner (Lynch and Walsh 1998), which uses only the affected parents and their progeny. Instead, liability values were calculated for each parental and offspring genotype. Thus, one value per genotype, based on several individuals, was obtained for each generation and for each phototreatment. Because this experiment included three phototreatments within two generations, liability could be calculated either by considering the phototreatments individually, or it could be calculated with respect to the entire generation. Liability was calculated using both methods, and is referred to as phototreatment-wide liability and generation-wide liability, respectively.

Phototreatment-wide liability was calculated as the deviation of the genotype's mean liability from the mean liability observed within a phototreatment. The distribution of liability within a phototreatment was assumed to be normal, with a mean of zero. The threshold for bolting within a phototreatment, $\underline{x}_p$, was obtained by converting the proportion bolting to the corresponding standard normal deviate, in units of standard deviations, using the PROBIT statement (SAS 1989). Similarly, the deviation of the threshold from the genotype's mean, $\underline{x}_g$, was then calculated for each genotype. Each genotype's mean liability was then obtained by subtracting $\underline{x}_g$ from $\underline{x}_p$. This procedure was repeated for each phototreatment, and for both generations. Generation-wide liability was calculated in the same manner, except that a genotype's value for a given phototreatment was calculated as the deviation of the genotype's mean from the mean liability observed within the generation.

The conversion of bolting on the scale of proportions within a genotype to underlying liability is not possible when the genotype contains either 100% or 0% bolting because the corresponding normal deviate is infinite. The standard solution to this problem is to simply drop those genotypes from the analyses (D.A. Roff, pers. comm.). Rather than dropping genotypes with no variance, a "pseudoliability" was calculated for these genotypes. The rationale behind this method is that an observed bolting frequency of 0 or 1 is based on a small sample of the true bolting frequency of the genotype which lies somewhere between 0 and 1. If a genotype contained zero bolters, one bolter was added to the genotype along with a number of nonbolters in a proportion consistent with the overall proportions observed in the phototreatment (or generation, in the case of generation-wide liability). Likewise, if a genotype contained only bolters, one nonbolter and an appropriate number of bolters were added. Liability values were then calculated as above from the proportions for each genotype. This method, although it is conservative in that it has the disadvantage of diminishing genotypic effects (and thus underestimating heritability), allows for the inclusion of genotypes with extreme values.

Heritability of bolting—Because data on individuals of the same genotypes within generations and over two generations were available, the heritability of liability of bolting could be calculated in two main ways: offspring on parent regressions, and analyses of variance. All analyses were performed using SAS (1989): offspring-on-parent regressions were performed using PROCs GLM and REG; analyses of variance were performed using PROC GLM, and the variance components used in the calculation of heritabilities were estimated using PROC VARCOMP. All *F*-tests were constructed based on Zar (1984, pp. 470-476).

L. inflata is completely self-fertilizing, and offspring are assumed to be genetically identical to parents. Therefore, the slope (not twice the slope) of the regression coefficient estimates the heritability. All regressions were corrected for the effect of population by including population as a covariate after testing for homogeneity of slopes. Heritabilities for each population were then calculated post hoc. An overall heritability estimate was first obtained through a regression based on the calculation of a single weighted average of the three phototreatment-wide liability values per generation for each genotype. This heritability thus corrects for differences in liability among phototreatments. Offspring-on-parent regressions were then performed for each of the three phototreatments. In addition, a heritability based on a single, generation-wide liability value for each genotype was performed. Thus, this heritability does not correct for differences in liability among phototreatments.

Although six estimates of each genotype's liability are available in the experiment, there is only one genotypic value per phototreatment per generation. Therefore, several ANOVA models, based on alternative ways of obtaining replicates within genotypes, were used to estimate the heritability of bolting: First, two genotypic liability values, one per generation for each phototreatment, were used as replicates. An overall estimate of heritability was obtained by modeling phototreatment-wide liability as dependent on phototreatment and population as random main effects, with genotype within population as a random nested effect. To obtain heritability estimates within each of the phototreatments, liability was modeled as the dependent variable with population as a random main effect, and genotype within population as a random nested effect. Second, a within-generation heritability was calculated for both generations separately by using the three liabilities, estimated for each phototreatment, as replicate values for each genotype within populations. This within-generation heritability was calculated using both the phototreatment-wide liabilities and the generation-wide liabilities.

With only one liability value per genotype available, a sibling analysis of the heritability of bolting within each phototreatment is not possible. Population differences with respect to bolting behaviour, however, were tested using a fully factorial mixed-model ANOVA including generation as fixed, and phototreatment and population as random main effects.

Heritability of plasticity of bolting—A plasticity value was calculated as the difference between two generation-wide liability values for that genotype. Because bolting was assessed with respect to three phototreatments, three plasticity measures were constructed: the plasticity of liability over phototreatments A to B (plastAB), that over B to C (plastBC), and that over A to C (plastAC). Similarly to the calculation of the heritability of bolting, the heritability of the plasticity of bolting was calculated using both offspring-on-parent regression and ANOVA.

An overall heritability of plasticity was first calculated by regressing mean offspring plasticity over phototreatments A to C on the mean parent plasticity over these phototreatments. Offspring-on-parent regressions were performed also for the plasticities over phototreatments A to B and B to C. After testing for homogeneity of slopes, all regressions were corrected for the effect of population by including population as a covariate. Post-hoc calculation of heritabilities of plasticity were then performed for each population.

A number of ANOVA models were used to address different questions. First, a nested random effects ANOVA tested the effect of population, and genotype within population on plasticity for each of the plasticity measures. These ANOVAs parallel the offspring-on-parent regressions in that the generations are used as the replicate observations within genotypes. To test for differences among populations in plasticity, the effect of population on plastAB, plastBC and plastAC were modeled in two-factor, mixed model ANOVAs including generation, population, and their interaction.

Fitness and the timing of reproduction

The direct and indirect influences of the timing of reproduction on fitness were studied through path analysis. In path analysis, a path diagram is constructed on the basis of a priori hypotheses about causal influences of independent variables on the response variable (Kingsolver and Schemske 1991). Here, fitness was measured as both fruit production and final plant size. The independent variables in the model include time of bolting, size at bolting, and the amount of time elapsed from bolting to first flowering. Not all variables were measured in both generations of the experiment (see above). All variables were standardized prior to analysis by subtracting each observation by the mean for the phototreatment, and dividing by the standard deviation (Lynch and Walsh 1998). Path coefficients, representing direct influence, were then determined by multiple regression, and indirect paths by simple correlations.

RESULTS

General results

The frequency of bolting over the two generations of the experiment was 0.31, and a smaller proportion bolted with diminishing photoperiod within both generations (Fig. 4.3a). One way ANOVAs show that the mean rosette size at bolting differed little among phototreatments (*F*=1.24; *P*=0.291), but plants bolted at a significantly smaller size in the second generation (*F* = 31.95; *P*<0.0001; Fig. 4.3b). The time from germination to bolting did not differ significantly among generations (*F*=3.02; *P*=0.083), but differed among phototreatments (F=4.31; *P*=0.014) consistently over the two generations (interaction *F*=00; 0.00; *P*=1.00; Fig. 4.3c). The final rosette sizes of the nonbolters differed between generations (*F*=86.25; *P*<0.0001) and decreased with diminishing photoperiods (*F*=22.43; *P*<0.0001) with the exception of large rosettes of the late-season

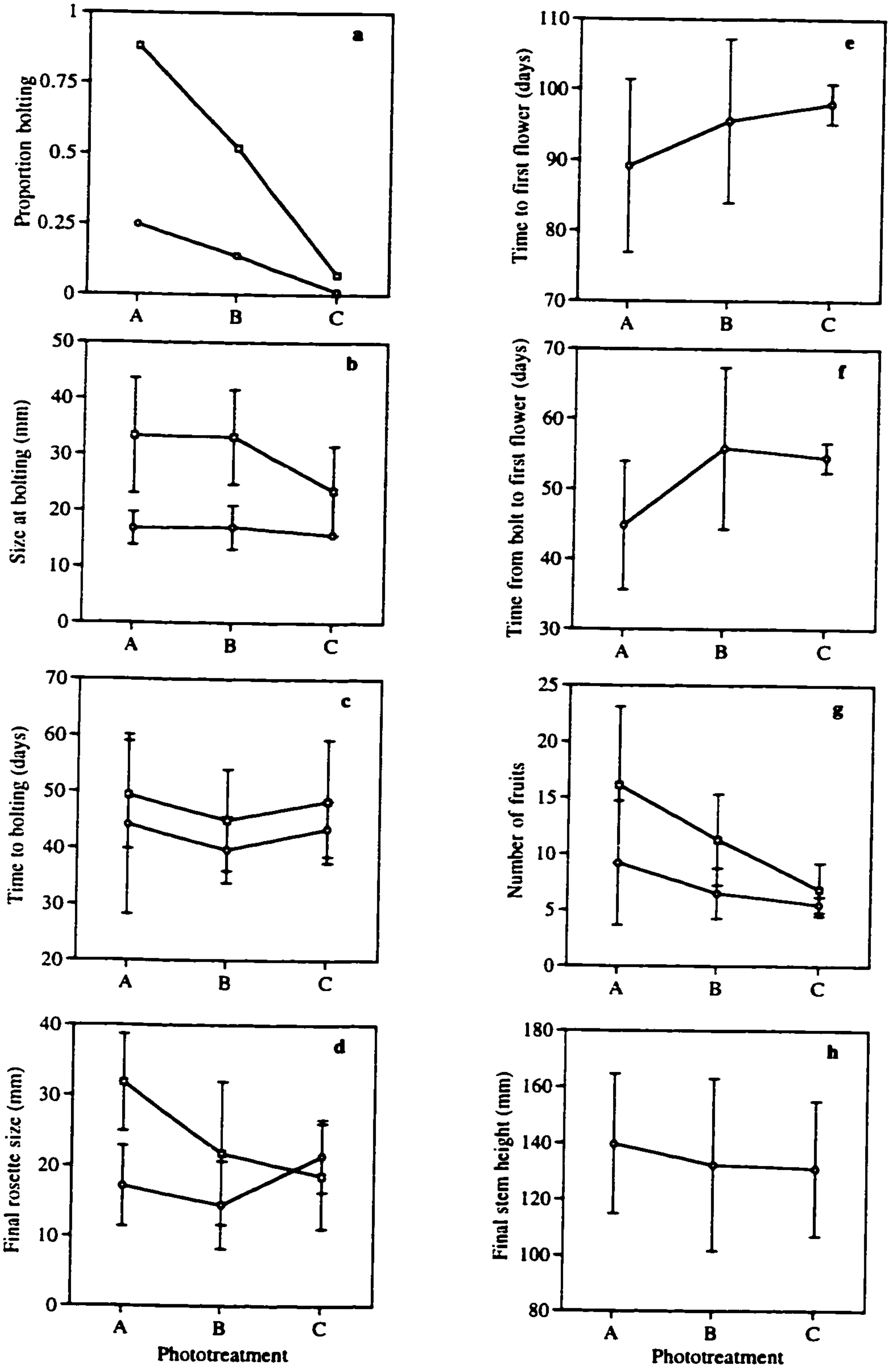

Fig. 4.3. Plasticity of various traits over the three phototreatments. Squares indicate measurements taken for generation 1, and circles indicate those taken for generation 2. Error bars indicate ± s.d.

phototreatment of generation two (F=61.72; P<0.0001; Fig. 4.3d). Whereas the time elapsed from germination to first flowering did not differ significantly among phototreatments (measured only in generation two) (F=2.57; P=0.084; Fig. 4.3e), less time was required to flower once bolting had occurred under longer daylengths (F=9.76; P=0.0002; Fig. 4.3f). The number of fruits produced by individuals changed across generations (F=7.26; P=0.008), but a consistent (interaction F=1.35; P=0.260) decreasing trend occurred with diminishing photoperiod (F=12.50; P<0.0001; Fig. 4.3g). No differences in final stem height (F=0.66; P=0.518) or dry weight (F=0.03; P=0.967), however, were detected (Fig. 4.3h).

Requirements of bolting: Size and time

The maximum rosette size attained during a season of growth is not a significant predictor of bolting behaviour (Fig. 4.4) except in phototreatment B of generation one (Bonferroni-adjusted P<0.0001). Rosettes continue to grow throughout the season and, even in phototreatments where no overall effect of maximum rosette size is observed, it is possible that rosette size is an important determinant of bolting for a given daylength.

Maximum rosette size attained in phototreatment A of generation two appears to have little influence on the probability of bolting (Fig. 4.4b), and is thus a suitable candidate for closer examination of the possible role of changing size requirements through time. Repeated measures ANCOVA including all rosette size measurements through time as the changing covariate shows no overall effect of rosette size on bolting behaviour, but the effect of time is significant (Table 4.1). However, the highly significant interaction term is evidence for the existence of size requirements for bolting that change through time. Almost 100% of bolting occurred within the first five weeks of measurements (d 55). If only the first five measurement dates are considered in the repeated measures ANCOVA, the effect of rosette size on bolting is significant, but the time and interaction terms remain highly significant as well (Table 4.2).

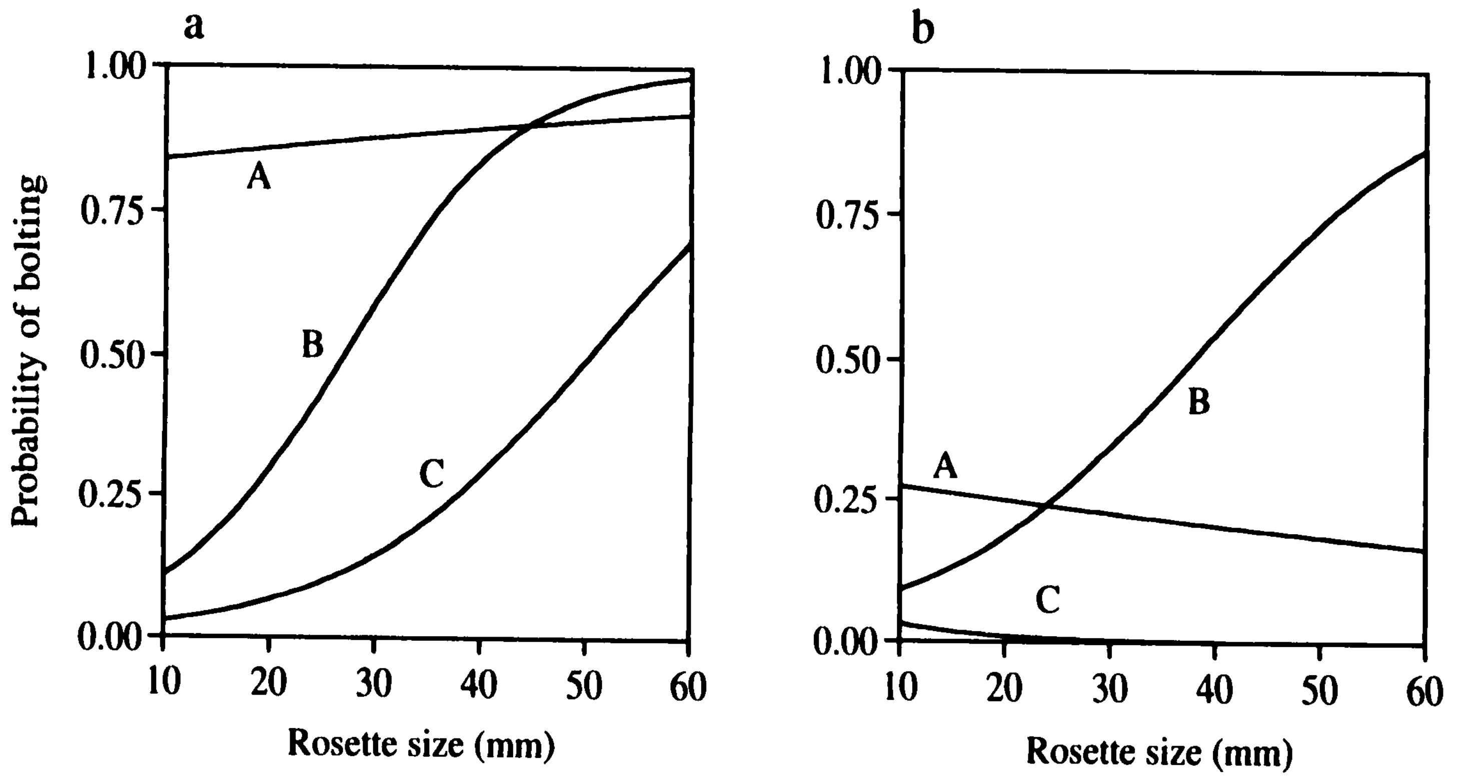

Fig. 4.4. The probability of bolting as a function of maximum rosette size attained by an individual under the three different phototreatments. Probability functions were derived from the log odds of bolting estimated by maximum likelihood (see text). Panels a and b represent results for the first and second generations, respectively.

Table 4.1. The probability of bolting based on repeated measures ANCOVA. Rosette size is a covariate that changes with the repeated response variable. The analysis includes all 22 measurement dates.

Source	df	SS	F-ratio	P-value
Rosette size	1	0.137	1.62	0.21
Individual	173	14.629	—	—
Time	21	0.433	2.18	0.002
Time x rosette size	21	2.947	14.80	<0.001
Time x individual	2593	24.590	—	—

Table 4.2. The probability of bolting based on repeated measures ANCOVA, using only the first five measurement dates. Rosette size is a covariate that changes with the repeated response variable.

Source	df	SS	*F*-ratio	*P*-value
Rosette size	1	1.013	12.18	<0.001
Individual	173	14.383	—	—
Time	4	0.501	3.61	0.006
Time x rosette size	4	3.151	22.67	<0.001
Time x individual	602	20.915	—	—

The changing size requirements for bolting over the first 55 d of growth were examined more closely through the use of post-hoc logistic regressions. Estimates of the rosette size and intercept parameters that maximize the likelihood of the observed bolting data are produced by PROC LOGISTIC (SAS 1989). The regression equation predicting the $\log_e$ odds of bolting may be easily converted to bolting probabilities (p):

$$p = \frac{e^{\mu + \alpha}}{1 + e^{\mu + \alpha}}$$

where μ and α are the intercept and rosette size parameters of the $\log_e$ odds of bolting estimated by maximum likelihood. This probability of bolting changes for a given rosette size over the first four measurement days (Fig. 4.5); there is a decreasing probability of bolting at a given rosette size through time, with no apparent rosette size requirement observed for bolting on d 41. Logistic regressions could not be performed for dates subsequent to d 55 because only four plants bolted after this date. However, 51 plants that did not bolt had attained a rosette size by the end of the season at which >50% would be expected to bolt if that size had been attained by d 34. All four plants that bolted after d 55 were at or below the rosette size at which the predicted probability of bolting is 0.05 for d 55. The most likely model for the control of bolting, given the observed bolting behaviour in the growth chamber, is one in which a rosette's "decision" to bolt is based on whether or not it attains a critical size for a given date (Fig. 4.6).

Heritability of bolting

The overall heritability of bolting on the underlying scale of liability was first calculated by offspring-on-parent regression for the entire dataset by lumping all phototreatment-wide liabilities. The homogeneity of slopes model indicates no significant interaction effect of population (P=0.127), and the heritability of liability of bolting is

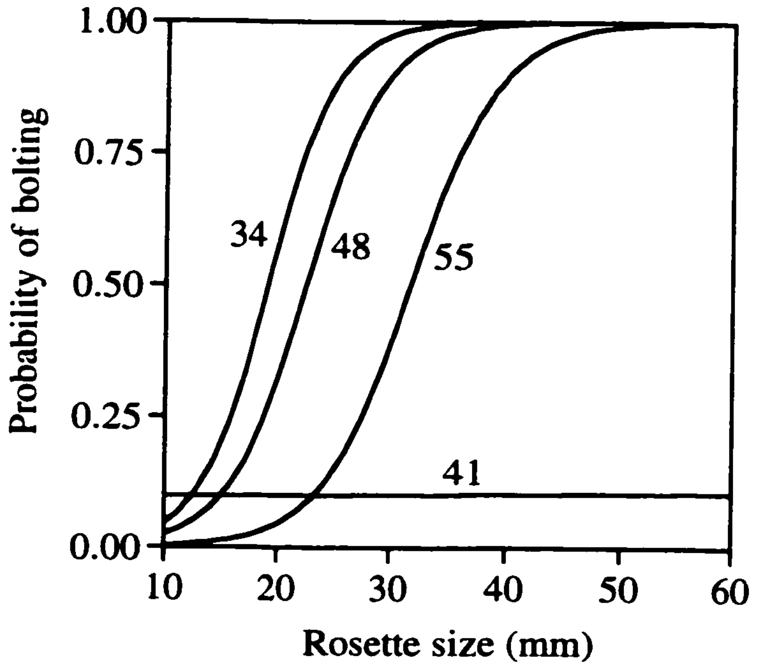

Fig. 4.5. The probability of bolting as a function of rosette size attained by an individual for phototreatment A of the second generation. Probability functions for each of four dates were derived from the log odds of bolting estimated by maximum likelihood (see text).

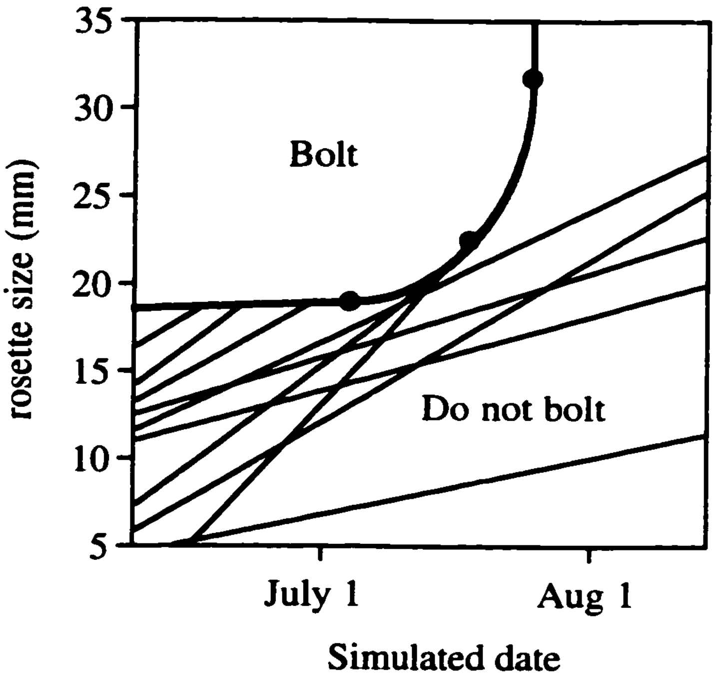

Fig. 4.6. A conceptual model for the "rules" controling bolting behaviour within a season. The heavy curve represents the minimum size requirement for bolting, which changes with time during a single growing season. The three plotted values (filled circles) are the size-date combinations at which the probability of bolting was observed to be 50% under the conditions of phototreatment A of the second generation of the growth chamber experiment.

highly significant (Table 4.3) with population entered as a covariate to correct for liability differences among populations. Genetic population differentiation (pop h^2), as estimated by the regression coefficient of the covariate, is also highly significant (Table 4.3). Heritability within each of the three phototreatments, calculated in the same manner, shows an increase as the season progresses (Table 4.3). In no case did the regression slope differ significantly among populations. In contrast to heritability, there is a decreasing trend in genetic population differentiation as the season progresses (Table 4.3). Ignoring the effect of phototreatment, the overall heritability based on a single liability value for each genotype per generation is lower than that for overall heritability based on phototreatment-wide liabilities, but remains significant ($h^2=0.30$; $P=0.02$).

Results of ANOVAs confirm the trends in heritability observed for the offspring-on-parent regressions. The estimate of heritability of liability that incorporates all phototreatments simultaneously is highly significant ($h^2=0.195$; $P<0.001$), genetic population differentiation, although weak, is detected (pop $h^2=0.073$; $P=0.034$), and this effect of population does not differ among phototreatments (interaction $P=0.78$). Separate ANOVAs for each phototreatment indicate that the heritability increases from 0.00 ($P=0.665$) when measured in the early phototreatment to 0.208 ($P=0.108$) in the intermediate, to 0.321 ($P=0.037$) in the late-season phototreatment.

Heritability of liability based on phenotypic resemblance among siblings cannot be computed for individual phototreatments, because only one liability value is available per genotype. However, three liability values, one from each phototreatment, are available per genotype per generation. The heritability of bolting, based on three phototreatment-wide liabilities, is 0.182 ($P=0.043$) in the first generation, and 0.152 ($P=0.066$) in the second generation. The effect of population explains no variance in the first generation (pop $h^2=0.00$, $P=0.421$), but explains a significant amount of variation in liability in the second generation (pop $h^2=0.204$, $P=0.001$). These heritabilities, because they are based on genotypic values measured in three environments, are reduced by the effects of genotype-

Table 4.3. Heritability of bolting (h^2), and population differentiation with respect to bolting (Pop h^2) on the underlying scale of liability, based on offspring-on-parent regressions. Probability values are given in parentheses.

	h^2	Pop h^2
Overall	0.42 (0.008)	0.25 (0.003)
Phototreatment A	0.15 (0.35)	0.55 (<0.001)
Phototreatment B	0.23 (0.17)	0.20 (0.11)
Phototreatment C	0.37 (0.001)	0.00 (0.96)

by-environment interactions. Using generation-wide liabilities in the same analysis results in a heritability of 0.00 in both the first (P=1.00) and second (P=1.00) generations. Through the use of generation-wide liabilities, the different phototreatments are completely ignored in this analysis. Thus, this heritability is further reduced by the environmental component of variance across the three phototreatments.

The availability of a single genotypic value of liability within phototreatments does not preclude population-level analyses within phototreatments. A fully-factorial mixed-model ANOVA reveals, besides a strong effect of phototreatment, a significant overall effect of population (P=0.035). The effect of population does not depend on phototreatment (interaction P=0.445) or generation (interaction P=0.481). Interestingly, the three-way interaction of generation x phototreatment x population is significant (P=0.012), indicating that the effect of the interaction between phototreatment and population on bolting behaviour differs between the two generations.

Heritability of plasticity of bolting

Bolting behaviour showed considerable plasticity over the three phototreatments in both generations (Fig. 4.3a), and an appraisal of the relative contribution of genetic variance to the total phenotypic variance in plasticity would be informative. Similarly to the overall heritability of bolting, the overall heritability of plasticity of bolting on the underlying scale of liability was calculated for the entire dataset using the plasticity of liability over phototreatments A to C for each genotype in an offspring-on-parent regression. No significant interaction effect of population is indicated by the homogeneity of slopes model (P=.704). The heritability of plasticity of bolting is low and nonsignificant (Table 4.4) when population is included as a covariate. There is evidence that the effect of population differs between generations, or of population-by-generation interaction: the regression coefficient of the covariate is strong and negative (pop $h^2=-0.461$, P=.003), and this is further explored using ANOVA (see below). Offspring-on-parent regressions

Table 4.4. Heritability of plasticity based on offspring-on-parent regressions. Probability values are given in parentheses.

	Overall Phototreatments A to C	Phototreatments A to B	Phototreatments B to C
phototreatment-wide	0.049 (0.74)	0.014 (0.94)	0.164 (0.33)
Martock	0.000	–0.146	0.001
Mt. St. Hilaire	0.062	–0.071	–0.057
Harvard Forest	0.085	0.053	0.295

performed on the two separate plasticity measures reveal no significant heritability for either plastAB or plastBC (Table 4.4), and post-hoc tests on populations separately show no heritabilities significantly different from zero (Table 4.4). Although nonsignificant, the population effects are negative for both of the separate plasticity measures (plastAB: pop $h^2=-0.313$, $P=0.077$; plastBC: pop $h^2=-0.172$, $P=0.212$).

The ANOVAs confirm the general results of the offspring-on-parent regressions: no significant within-population heritability of plasticity is found. Although two plasticity measures per genotype are available for each generation (plastAB and plastBC), these measures are not independent. Plasticity over phototreatments A to B is inversely related to the plasticity over phototreatments B to C in both the first ($N=32$, $r=-0.426$, $P=0.015$) and second ($N=36$, $r=-0.518$, $P=0.001$) generations. Therefore, the question of whether there are among-genotype differences in plasticity within generations could not be directly addressed. Analyses of variance were also performed specifically to test for differences in plasticity among populations. Two-factor, mixed model ANOVAs including generation, population and their interaction were performed for plastAB, plastBC and plastAC. These ANOVAs reveal no effect of population overall, due to a strong population-by-generation interaction (Table 4.5). However, the effect of population on overall plasticity (plastAC) is highly significant if included as a nested effect within generation ($P=0.006$). Whereas Martock exhibits the highest relative overall plasticity in the first generation, it shows the lowest in the second.

Fitness and the timing of reproduction

Correlation and path analyses indicate that the importance of the timing of reproduction to fitness depends on both the fitness measure and on the conditions under which the study is performed. In both generations, simple correlations (Table 4.6) show that later-bolting individuals are, in general, associated with lower fitness under phototreatment A, but this association is weak under the conditions of phototreatment B.

Table 4.5. Population and generation effects on the plasticity of bolting on the underlying scale of liability. The mixed-model ANOVA is for the overall plasticity (phototreatments A to C), and includes generation as fixed, and population and the interaction effect as random.

	ms	df	P
generation	10.44	1	0.232
population	0.17	2	0.707
generation x population	3.64	2	0.001
within population (error)	0.49	62	

Table 4.6. Simple Pearson correlations among traits of bolting plants. Results for generation 1 are above the diagonal, those for generation 2 are below. Results for phototreatments A and B are shown for each trait combination. Missing values are for traits that were not measured for generation 1.

Gen 1 / Gen 2		Time to bolt	Rosette size at bolt	Time to first flower	Bolt to flower time	Number of fruits	Stem height
Time to bolt	A		0.29***			-0.40***	
	B	—	-0.09	—	—	-0.13	—
Rosette size at bolt	A	0.34*				0.34***	
	B	-0.04	—	—	—	0.30**	—
Time to first flower	A	0.84***	0.20				
	B	0.26	-0.38†	—	—	—	—
Bolt to flower time	A	-0.67***	-0.37*	-0.16			
	B	-0.25	-0.36	0.87***	—	—	—
Number of fruits	A	-0.24	-0.33*	-0.28	0.11		
	B	0.30	0.28	-0.34	-0.50**	—	—
Stem height	A	-0.39**	-0.15	-0.62***	0.09	0.59***	
	B	0.02	0.51**	-0.37†	-0.38†	0.56**	—
Stem dry weight	A	-0.49***	-0.15	-0.43**	0.31*	0.69***	0.74***
	B	-0.24	0.57**	-0.46*	-0.35	0.43*	0.80***

†P ≤ 0.07; *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001

The correlation between the size at bolting and fitness (as measured as number of fruits) is negative under the conditions of phototreatment A of the second generation (Table 4.6); an unexpected result. This correlation, however, could be caused by a confounding relationship between the time and size at bolting, and this result points to the necessity of distinguishing the direct effect of a variable (i.e. controlling for all other variables) from simultaneous effects of independent variables through path analysis.

Path analysis provides evidence that, in phototreatment A of the first generation of the experiment, time to bolt had a strong direct effect on the number of fruits produced (Fig. 4.7a), but no such effect is evident under the conditions of phototreatment B Fig. 4.7b). In both phototreatments A and B, rosette size at bolting has a significant effect on fruit number after correcting for time to bolt. In generation two, no evidence exists for a direct effect of the timing of bolting on the number of fruits produced by a plant (Fig. 4.8). Later-bolting individuals, however, take less time to flower once bolting has occurred, and a marginally nonsignificant direct relationship indicates that the time taken to flower might be an important influence on the number of fruits produced under phototreatment B (Fig. 4.8b). Unfortunately, no meaningful analyses could be performed for phototreatment C, because only two plants bolted.

Separate analyses were performed using final stem height as a fitness measure. Interestingly, under phototreatment A of the second generation, both the timing of bolting and the time taken to flower once bolting has occurred have a significant and negative direct effect on final stem height (Fig. 4.9a), whereas under phototreatment B, only the size at bolting has a significant effect (Fig. 4.9b). Notice that, in phototreatment A, the direct effect of time to bolting on plant height (Fig. 4.9a) is much greater than is the simple correlation between these variables (Table 4.6), and that the effect of time from bolt to first flowering on plant height (Table 4.6) is significant only after the removal of the indirect effects of time of bolting and size at bolting (Fig. 4.9a).

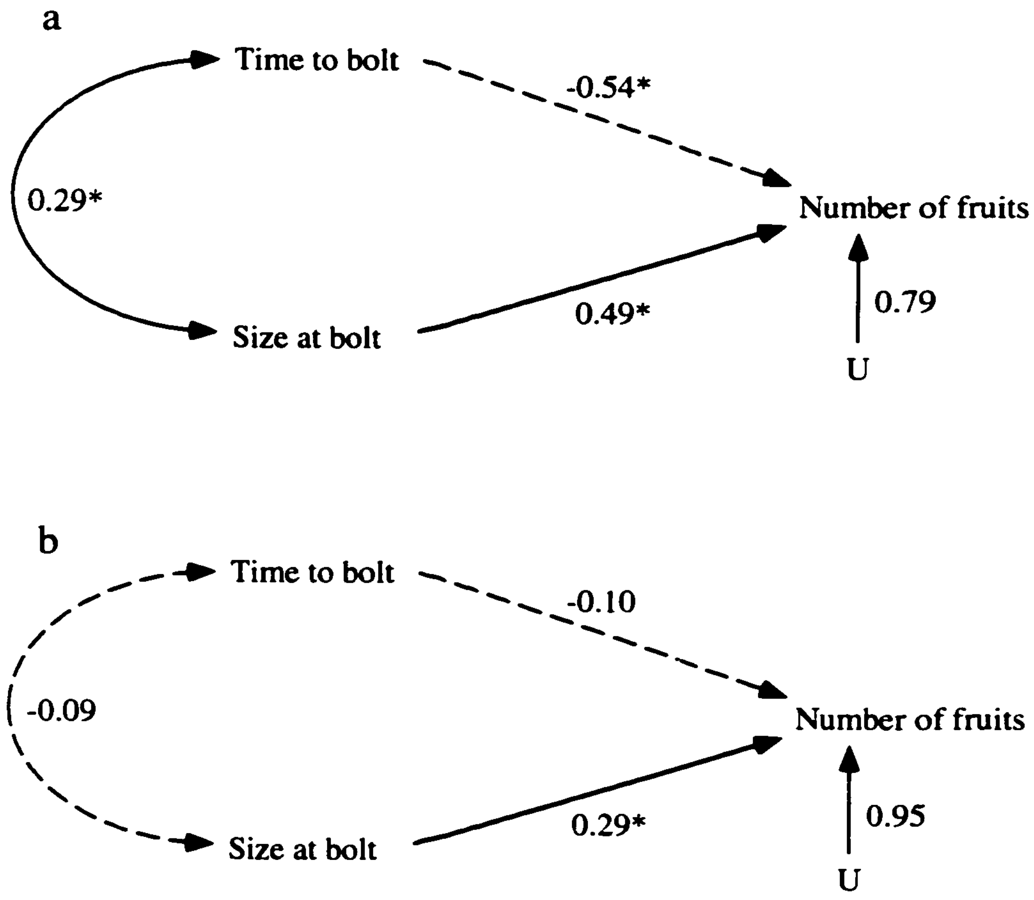

Fig. 4.7. Path diagrams (a; phototreatment A, b; phototreatment B) showing the direct effects of the time taken to bolt, the size at which a rosette bolted, and residual variables (U) on total fruit production for generation 1. Solid lines indicate positive effects, dashed lines indicate negative effects.

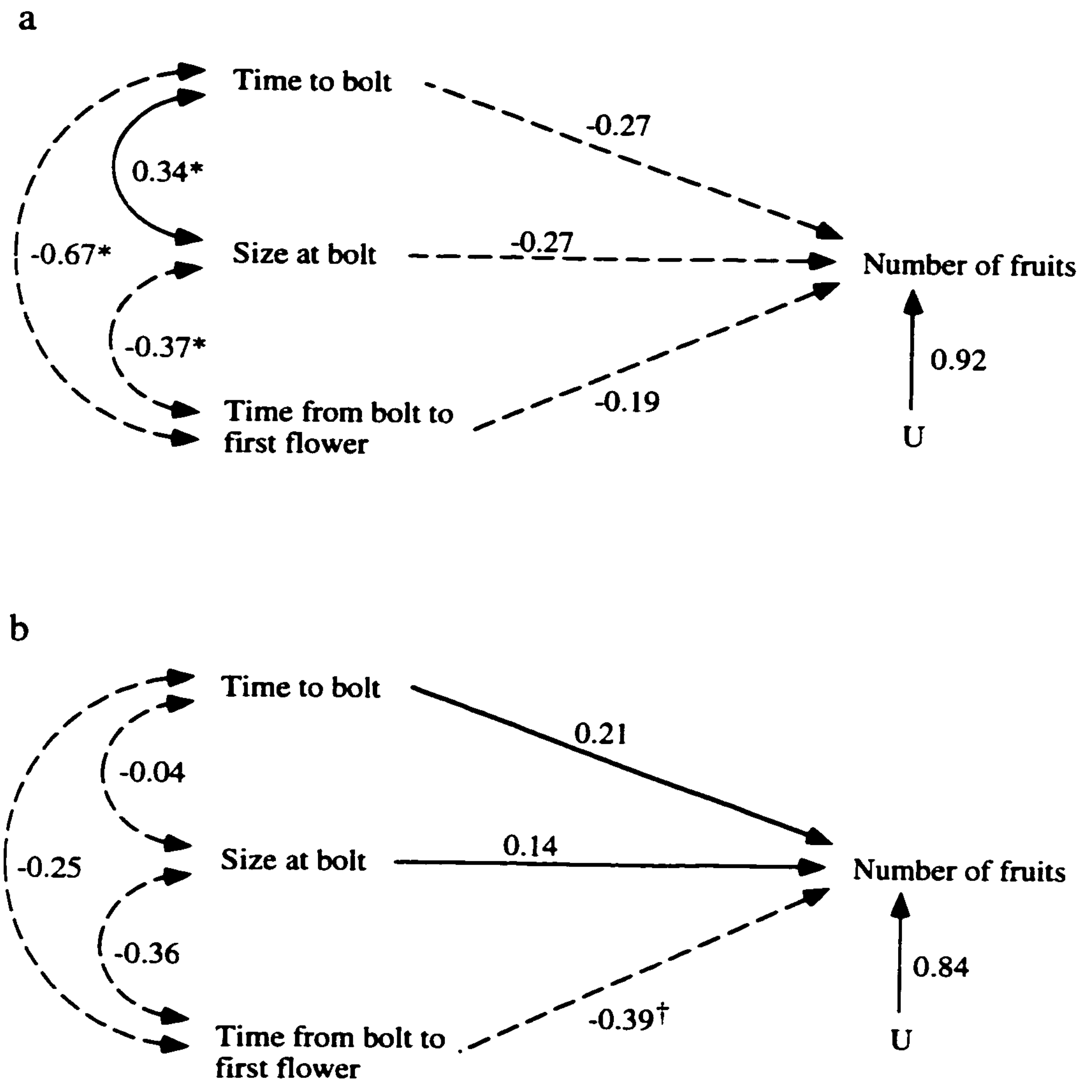

Fig. 4.8. Path diagrams (a; phototreatment A, b; phototreatment B) showing the direct effects of the time taken to bolt, the size at which a rosette bolted, the time elapsed from bolting to flower production, and residual variables (U) on total fruit production for generation 2. Solid lines indicate positive effects, dashed lines indicate negative effects.

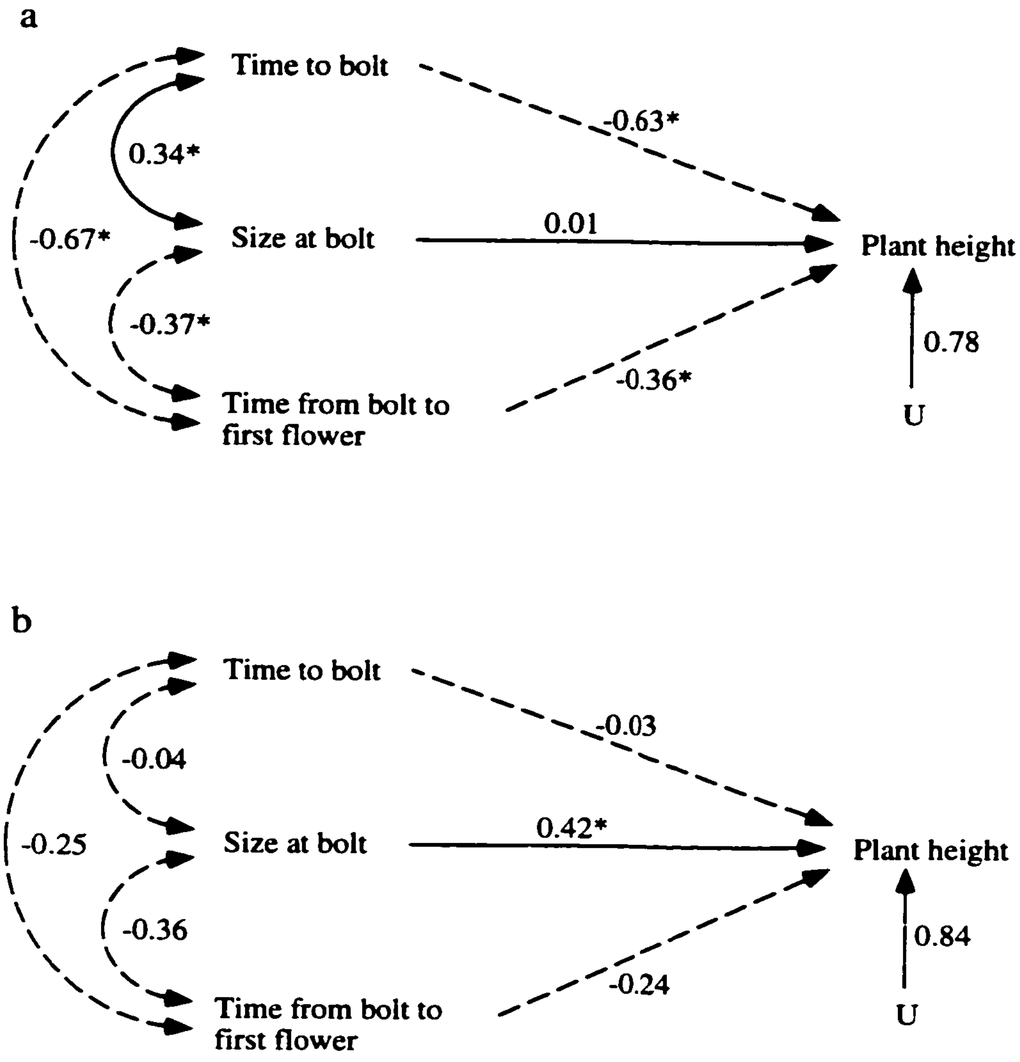

Fig. 4.9. Path diagrams (a; phototreatment A, b; phototreatment B) showing the direct effects of the time taken to bolt, the size at which a rosette bolted, the time elapsed from bolting to flower production, and residual variables (U) on final stem height for generation 2. Solid lines indicate positive effects, dashed lines indicate negative effects.

DISCUSSION

This experiment was designed to investigate the timing of reproduction in the monocarpic perennial, Lobelia inflata, from three main perspectives: its control, genetic basis, and fitness consequences. Bolting behaviour is highly plastic over the three photoperiod treatments (phototreatments) and two generations of the experiment in terms of the proportion bolting, rosette size at bolting, the time taken to flower, and other traits. This study partially confirms results of previous studies showing that rosette size (for a review, see Lacey 1986a) is important in determining whether an individual will initiate reproduction. The threshold size for bolting, however, changes depending on the time of the season: for a given rosette size, the probability of bolting decreases later in the season. A new model for the control of bolting behaviour, that takes the within-season time component into account, was presented in the results (Fig. 4.6). This is a conceptual model based on the present data and is meant as a hypothesis about the elements relevant to bolting decisions. However, it should be kept in mind that the critical dates and rosette sizes were observed under controlled condtions—critical rosette sizes and dates might differ under field conditions even if the principles of the conceptual model are valid.

With an overall heritability of 0.42, the response to selection on bolting might be expected to be rapid, given a nonzero selection differential. Estimates of h^2, though, are dependent both on population and environment (Falconer 1989). Based on previous work that directly compares heritabilities measured in homogeneous and heterogeneous environments (Simons and Roff 1994), the measured h^2 of 0.42 is probably an overestimate of the true h^2 in the field. Furthermore, the heritabilities differ substantially for the three phototreatments, and the overall heritability estimate might be misleading. The late-season phototreatment (C) is characterized by both the lowest frequency of bolting and the highest heritability; genetic variation for the timing of reproduction is expressed most strongly under comparatively severe environmental conditions. It is important to note that a high heritability does not imply elevated genetic variance, only an elevated level of genetic variance relative to total phenotypic variance. In this example, it is clear that the total phenotypic variance of bolting for phototreatment C is low in comparison to that of the other phototreatments.

The range of phenotypic expression of a character across different environments is known as phenotypic plasticity, and is commonly described by the character's "norm of reaction." A genotype capable of appropriate phenotypic expression in every possible environment would of course be at a selective advantage under changing environments. Given genetic variation for norms of reaction, adaptive norms of reaction should evolve as any other trait in response to selection. Although much resident variation in plasticity of bolting behaviour is found in the present experiment, the variation is as high within as among genotypes. At the among-population level, a strange phenomenon is observed: within generations, plasticity differs among populations, but a population exhibiting high plasticity in generation 1 exhibits low plasticity in generation 2. This highly significant generation-by-population interaction has no theoretical explanation at present, but should be investigated further because, if this phenomenon proves consistent, single measures of plasticity could not be taken as characteristic of that population.

A plant's fitness, as estimated by total fruit production and final stem height, is influenced in a complex manner by the time taken to bolt, the size at which it bolts, and by the time taken to flower once bolting has occurred. Whenever a significant direct effect of time to bolt on fitness is observed within a phototreatment, it is negative. This higher fitness of early bolters is especially apparent for the early-season phototreatment (A), after correcting for the larger rosette sizes attained by late bolters. Early bolters (with other variables held constant), then, benefit from a long season subsequent to bolting. Not surprisingly, larger rosettes result in plants of higher fitness. The relationship between rosette size and fitness highlights the added information gained through path analysis: in phototreatment A of generation 2, the negative correlation between rosette size and final plant size would lead to the erroneous conclusion that small rosettes produce larger bolters; this relationship disappears when only the direct effect is considered. Similarly, in phototreatment B of generation 2 the simple correlation between rosette size and final plant size is negative and high (although nonsignificant), but the direct effect of rosette size is positive and significant.

The effect on fitness of the timing of bolting, rosette size, and the time taken to flower once bolting had occurred were found to depend on what fitness measure (fruit number and final stem height) was used. Plant size is often used as a measure of fitness, both because of its relationship with fruit production and because of direct ecological benefits associated with size such as improved ability to compete for light. It is especially crucial to consider the importance of plant size when fitness is measured under artificial conditions where ecological effects of size are not necessarily evident. Inconsistencies between the two measures of fitness suggest that additional information would be gained by performing such experiments under more natural conditions.

Bolting marks an individual's transition from the vegetative mode to the reproductive mode. Bolting commits an individual to reproduction, but a substantial amount of time must be spent in the bolting mode prior to seed ripening, or successful reproduction. If a rosette bolts too late in the season, it may not successfully reproduce, even if the rosette is large. Therefore, a genotype's fitness depends not only on its rosette size and the time at which a rosette bolts, but also on the amount of time taken to reproduce once bolting has occurred. The direct effects of rosette size on fitness are clear, but an unexpected and consistent result of this experiment is that there is an additional advantage to large rosette size: larger rosettes require less time to flower. In summary, large rosettes that bolt early are, of course, at an advantage, but it takes more time to develop a large rosette. The time taken to flower once bolting has occurred is negatively associated with fitness, but large rosettes require less time to flower. Recall that bolting behaviour is found to be dependent on both rosette size and the interaction of size and time. The larger threshold rosette size for bolting as the season progresses is consistent with the observation that larger rosettes require less time to flower.

In the field, the date of first frost in the autumn and the effective length of the growing season are variable among years; the amount of time remaining in a particular season for growth cannot be predicted. A substantial amount of time is required to reproduce once bolting occurs, and the "rules" for bolting in Lobelia inflata would thus be expected to have been shaped by environmental unpredictability. Because only rosettes and seeds can survive the winter, an individual that bolts with insufficient time remaining in the season to develop mature fruits will have a fitness of zero. Under environmental unpredictability through time, selection is expected to maximize the geometric mean fitness (Gillespie 1974), and this measure is especially sensitive to low values. The "rules" governing bolting behaviour would be considered to be a conservative bet-hedging (Slatkin 1974; Seger and Brockmann 1987; Philippi and Seger 1989) strategey if a rosette "plays it safe" by not bolting even though its expected fitness, if it were to bolt at that size and date in an average season, would be higher.

The advantages of a growth chamber experiment are that it allows the description of the control of bolting through photoperiod manipulations, and indicates likely fitness effects of the timing of bolting and other reproductive traits. This experiment says nothing, however, about the appropriateness of the bolting "rules" discovered. It will be necessary to conduct experiments under field conditions to reveal whether the observed plasticity in bolting behaviour is adaptive; an attempt is made toward this end in the following chapter.

This study shows the importance of considering the interactive effects of rosette size and time of year within a season on bolting behaviour for a plant that requires no vernalization. Although these results pertain to within-season control of reproductive behaviour, they also provide a testable hypothesis for the observation of year-to-year variation in threshold size at bolting (Wesselingh 1995; de Jong et al. 1998): if the same mechanisms underlie the control of bolting in plants that do and do not require vernalization, this variability could be generated by yearly variation in growth trajectories of rosettes in relation to photoperiod.

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CHAPTER 5

The control of reproductive timing both within and among seasons: conservative bet hedging?

Abstract—A time-dependent size threshold for the onset of reproduction (bolting) in Lobelia inflata has been observed under controlled, growth-chamber conditions (Chapter 4). The observed threshold is suggestive of adaptive plasticity, but the adaptiveness of the "rules" of flowering cannot be known until their fitness consequences are measured under field conditions. The present work attempts to address whether observed bolting behaviour is appropriate under field conditions. To address this question, it was necessary to obtain measures of natural overwinter mortality, mortality within the growing season, size-specific growth rates, the relationship between rosette size and fecundity, and the fitness consequences of bolting at various times throughout the season. For plants naturally occurring in the field, overwinter mortality is found to be independent of rosette size but, if a rosette survives, both its probability of flowering and its fecundity are size dependent. Plants were observed to bolt only from the beginning of June until July 10 in the field, yet a manipulation experiment shows that plants induced artificially to bolt as late as August 29 may still produce mature fruits. In the manipulated plants, however, fecundity declines rapidly with later bolting dates. These empirical relationships are used in a model to investigate the optimality of bolting behaviour. It is concluded that observed bolting behaviour of plants growing naturally, although qualitatively similar to predictions of the optimality model, is suboptimal under the particular conditions of the season in which the observations were made. The observed bolting behaviour is better accommodated by the optimality model if unpredictability in season length is considered, and this indicates that bolting behaviour might be interpreted as a conservative bet-hedging strategy.

INTRODUCTION

The timing of reproduction affects fitness both through its direct effect on the Malthusian parameter, λ and, indirectly, through its correlated effects on, for example, plant size. Variance in the delay of reproduction observed both within and among species of monocarpic plants has led to an extensive literature on the size- vs. age-dependence of reproductive timing (for a review, see Lacey 1986). A conceptual model for the control of flowering, based on a growth chamber experiment under three different changing-photoperiod regimes, was presented in the previous chapter. The results show that reproductive behaviour in Lobelia inflata within a season is highly plastic, and is governed not only by rosette size attained, but also by the interaction of rosette size and date.

The control, or "rules," of bolting behaviour have presumably evolved through natural selection, and would thus be expected to maximize fitness, given various constraints. Growth chamber experiments are vital in studying the presence of decision-making "rules" and the genetic basis of alternative life-history strategies; progress in interpreting the fitness consequences of these alternative reproductive strategies would, ideally, be made through observations in nature. There are two goals of this chapter: first, to present empirical evaluations of fitness components measured under field conditions, and second, to ask whether L. inflata makes the right flowering "decision," or whether some other pattern of reproductive behaviour would confer higher fitness, based on the available data.

Information on Lobelia inflata is mostly derived from observation under controlled environments (Muenscher 1936; Baskin and Baskin 1992), and the behaviour of this species in nature is poorly understood. It is known that L. inflata has an absolute light requirement for germination (Muenscher, 1936; Baskin and Baskin 1992). Germination may occur following soil disturbance at any time during a growing season (Baskin and Baskin 1992), but a peak in germination occurs in May or June (Bowden 1959; personal observation), likely due to exposure following frost heave. Rosettes, even from the same year class, may thus be of any size at any time of year. L. inflata has no vernalization requirement for flowering. It may germinate and flower in the same growing year (Bowden 1959); it may act as a "biennial" or winter annual and bolt only after overwintering in the rosette stage (Bowden 1959); or it may delay reproduction further, acting as a monocarpic perennial. Observations of rosettes over three seasons, however, suggest that bolting in the first year is very rare; bolting occurs in year two, three, or even later. Nonetheless, the absence of a vernalization requirement in L. inflata means that analysis of flowering is not complicated by differences between threshold size for flowering and size at flowering (Wesselingh and De Jong 1995; De Jong et al. 1998).

Study of the timing of reproduction in monocarpic plants has centred on the fitness consequences of among-season delays (Lacey 1986; De Jong et al. 1987; Klinkhamer et al. 1991; Wesselingh et al. 1997). The bolting behaviour of an individual has traditionally been treated as dichotomous for any given season, and has been found to be influenced by rosette size (Werner 1975; Lacey 1986; Klinkhamer et al. 1987; Couvet et al. 1990; Klinkhamer et al. 1991) attained by the end of the previous growing season (Wesselingh et al. 1997). Because of this focus on reproductive delays among seasons, the possibility of a changing rosette size requirement for flowering within a season, as observed in the growth chamber (Chapter 4) has been overlooked. The time of bolting, both within and among seasons, may have strong fitness consequences: upon bolting, an individual may produce abundant viable seed if the rosette is large and, perhaps just as importantly, if sufficient time remains in the season for the development of mature fruits. Expected fitness of a rosette attaining a large size only later in the season, however, would be increased by overwintering in the rosette stage if the improvement in fecundity, gained both through the more abundant time in which to develop mature fruits and through additional rosette growth, compensates for the probability of overwinter mortality.

A thorough assessment of the fitness consequences of bolting behaviour requires a knowledge of several parameters. The influence of rosette size and time of year on total fruit production are important components of fitness, but fitness depends also on the probability of surviving to reproduce. The pattern of survival, which may be size dependent, will thus influence the evolution of a bolting threshold. Data on the survival of wild rosettes over three winters and over two growing seasons will be presented. Potential for rosette growth should also influence the evolution of bolting behaviour because a decision to bolt forgoes the additional fecundity which would be enjoyed by a larger rosette. In addition, inferences about the direct fitness consequences of the size at bolting and the timing of bolting under growth chamber conditions (previous chapter) will be verified against results using unmanipulated individuals followed over two summers in the field.

Results from the growth chamber (Chapter 4) indicate that, for a given rosette size, final fruit number declines with later date of bolting. Bolting, however, was confined to the first few weeks of the season. To assess the optimality of this bolting behaviour in L. inflata, I also ask what the fitness consequences would be if plants were to bolt later in the season than they do naturally. This is accomplished through a manipulation experiment in which newly bolted rosettes were transplanted into the field at intervals throughout the season. On the basis of these observations, comparisons could be made between what plants actually did, and what plants "should" have done, given the environmental conditions of the year of study.

The relationship between bolting behaviour and fitness depends on season length, which may vary unpredictably from year to year. If adaptive plasticity can evolve as an effective response to unpredictable environmental conditions, then this is always the tactic of highest fitness. Because the quality of the remainder of the season is unpredictable at the time the bolting "decision" is made, and bolting is irreversible, there is no single appropriate behaviour for a given set of immediate conditions. Under such conditions, plasticity is ineffectual, and bet-hedging strategies are expected to evolve. Bolting could be determined by a variety of cues, but the most likely is photoperiod. Maximization of the geometric mean fitness would be attained through bolting rules that may differ from rules that maximize the expected fitness for a growing season of average length. Notice that the argument here is that bolting rules, themselves norms of reaction, like any other trait, might be the subject of selection for bet hedging. Under low overwinter mortality, for example, an individual's fitness would be increased by following the advice, "a bird in the hand is worth two in the bush," by delaying bolting to the next season where reproduction is assured, rather than by bolting late in the season and thereby risking low or zero fitness if the season happens to be shorter than average. Such a tactic would be an example of a "conservative" bet hedging strategy (Seger and Brockmann 1987). The rules of bolting behaviour observed in these experiments are discussed in terms of the expectations of such a conservative bet-hedging strategy.

MATERIALS AND METHODS

For the analysis of overwinter survival, rosettes were located and marked with plastic swizzle sticks along the length of a gravel access road ascending Martock Mountain. Care was taken to locate all rosettes within specific areas, but it is possible that smaller rosettes were overlooked. Over three winters 412 rosettes were followed: 141 were marked in autumn 1995; 241 in 1996; and 58 in 1997. Twenty-eight rosettes survived more than one winter, yielding a total sample size of 440 overwintering rosettes. Because survival is a categorical response variable, and both continuous (autumn rosette size) and categorical (year) independent variables are of interest, PROC CATMOD (SAS 1989) was used for survival analyses. PROC CATMOD is an extension of the LOGISTIC procedure that uses maximum likelihood techniques for categorical data modeling.

The fate of rosettes that survived the winter was documented, and additional rosettes were marked and followed in both 1996 and 1997 for a total of 946 individuals. Unfortunately, an area containing 227 rosettes was destroyed by a large tree harvesting vehicle. Although disturbances of this magnitude are expected given enough time, and bet-hedging strategies appropriate over longer time scales should thus evolve (Chapter 7), mortality caused by this catastrophic event was deemed unnatural and these rosettes were excluded from analyses of survival during the growing season. Survival, bolting behaviour, rosette size at bolting, the date and stem height at first flowering, as well as total fruit production and final stem height were recorded for all 719 remaining rosettes based on weekly visits to Martock. Path analysis was performed using PROCs REG and CORR (SAS 1989) after standardizing all variables to zero mean and unit variance (Kingsolver and Schemske 1991; Lynch and Walsh 1998).

Martock was also used as the transplant site for the bolt time manipulation experiment. The production of bolting individuals at dates beyond the natural range for L. inflata was attempted in two ways: 1) by satisfying bolting requirements of rosettes under long days in the growth chamber and then transplanting newly bolted individuals to the field, and 2) by the application of Gibberellic acid (GA) to rosettes growing naturally in the field. Despite the success of GA application in preliminary trials in the growth chamber, none of the field rosettes could be kept in the bolting mode (perhaps because GA concentrations could not be consistently maintained). The experiment in which newly bolted individuals in the growth chamber were transplanted to the field was successful, and only the results from this manipulation will be presented.

To obtain sufficient numbers of rosettes for transplant throughout the summer, newly bolting rosettes were produced in three ways. Rosettes that had not bolted by the end of the second generation of the growth chamber experiment (Chapter 4) were used following vernalization in darkness at 6°. Seeds produced in the first generation of the growth chamber experiment were also used to produce rosettes which were vernalized in the same manner. Additional rosettes were produced using seeds collected from plants at Martock, and were subjected to the same vernalization treatment.

After vernalization, rosettes were transferred into long-day conditions suitable for bolting (15h 15min light, and a 19°/13° thermoperiod of 12h 30min). Date of bolt was recorded for each plant, and the length of the longest leaf was measured weekly so that it could be recorded just prior to bolting. To ensure that bolting had occurred, bolting individuals were not transplanted on the weekly transplant date directly following bolting, but were allowed a delay. Transplant occurred, on average, on the 12th day of bolting (mean = 11.93; s.d. = 3.29). Newly bolted plants (subject to their availability), were transferred to Martock at weekly intervals throughout the summer of 1997, where they were randomly assigned to one of two sites. In order to minimize transplant effects, plants were not removed from the individual cells of the Kord cellpacks. A total of 233 individuals were transplanted from June 30 to October 24, 1997. Date and stem height at transplant, date and stem height at first flowering, and final stem height and fruit production were recorded. Thirteen individuals were grazed, and are excluded from analyses.

RESULTS

The effects of rosette size under field conditions

Rosette size and survival—Overwinter rosette survival averaged 44% over the three winters (1995/96, 43%; 96/97, 47%; 97/98, 34%), and did not differ among winters (PROC CATMOD; d.f. = 2; χ^2 = 4.31, $\underline{P}$ = 0.1160). The present data offer no evidence that overwinter survival is influenced by rosette size in late autumn (PROC CATMOD; d.f. = 1; χ^2 = 0.50; $\underline{P}$ = 0.48) with year entered as a covariate in the model. Mortality is not restricted to the winter months, and was recorded throughout the 1997 growing season as well. Growing season mortality was assumed to occur at a constant rate, and is thus a function of the length of time spent as a rosette. Because rosettes were being added to the dataset continually through the season as they were located, survival of a rosette during the growing season was weighted by the proportion of the 162-d season (from May 22, the first assessment of overwintering rosettes, to October 31, the final measurement date in the fall) over which it was observed. All nonbolting rosettes, including 1996/97 overwinter survivors and newly-found rosettes in 1997, were included. The weighted survival for the 126 rosettes was 0.423.

Rosette size and bolting—Rosette size at the end of the previous season is a good predictor of bolting, given that a rosette survives the winter (PROC CATMOD; d.f. = 1; $\chi^2 = 17.42$; $\underline{P} < 0.001$). However, this probability differed between years (d.f. = 1; $\chi^2 = 27.02$; $\underline{P} < 0.001$), indicated by a significant interaction between rosette size and year (d.f. = 1; $\chi^2 = 32.31$; $\underline{P} < 0.001$). Logistic regressions, performed post hoc for the two seasons separately, indicate that there is a slightly negative nonsignificant relationship between rosette size and bolting for overwinter survivors for the first season (1995/96: $\underline{N} = 61$; d.f. = 1; Wald $\chi^2 = 2.65$; $\underline{P} = 0.10$), whereas this relationship is strong and positive for the second season (1996/97: $\underline{N} = 113$; d.f. = 1; Wald $\chi^2 = 30.95$; $\underline{P} < 0.001$).

Rosette size and fecundity—Rosette size the previous autumn is important to the success of bolters in terms of both the number of fruits produced and final stem height. Correlations including only those plants that both survived the winter and bolted during the subsequent summer show that larger rosettes at the end of 1995 produced plants with more fruits in 1996 ($\underline{N} = 24$; $\underline{r} = 0.48$; $\underline{P} = 0.02$). Similarly, in 1997, plants with higher total fruit production were derived from larger overwintering rosettes ($N = 39$; $r = 0.46$; $P = 0.003$), as were plants with higher final stem height ($N = 39$; $r = 0.49$; $P = 0.002$). Combining the two seasons, the regression equation relating autumn rosette size (Ros) and fruit number (FN) is

$$\mathrm{FN} = -7.74 + 0.69\ (\mathrm{Ros}). \tag{1}$$

Rosette size and growth rate—Because larger rosettes are characterized by higher fruit production upon bolting, the growth rate of rosettes influences the optimal size threshold for bolting. Size-specific rosette growth rate was calculated during the 1997 season based on a subsample of 69 nonbolting rosettes from May 22 to September 11. The effect of rosette size on growth rate is highly significant ($P < 0.001$), although much variation in growth rate remains unexplained ($r^2 = 0.26$). The relationship between growth rate (GR), in mm d^{-1} and initial rosette size (Ros), in mm, is given by

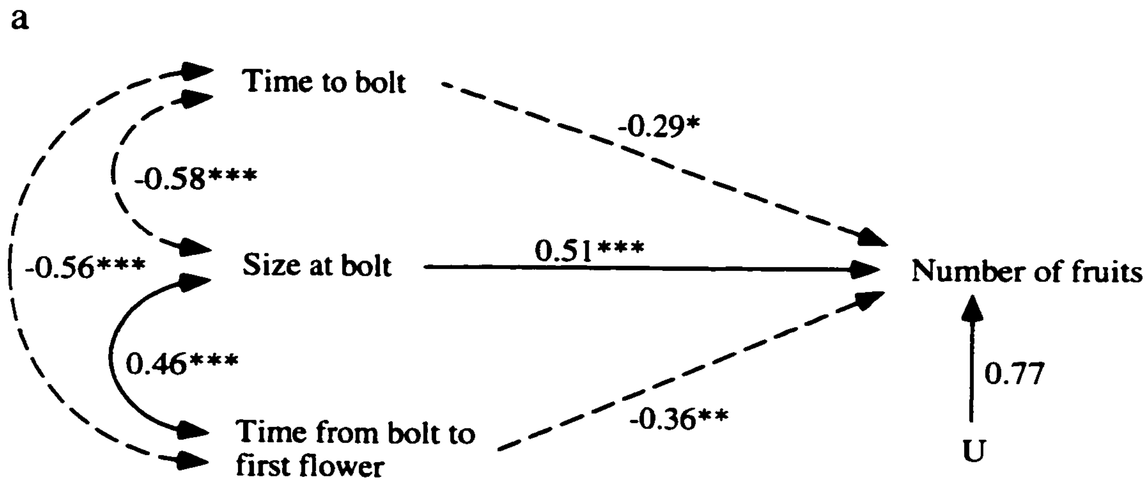
$$\mathrm{GR} = -0.020 + 0.011\ (\mathrm{Ros}). \tag{2}$$

The within-season timing of bolting

Field observations—The fitness effects of the timing of reproduction under field conditions agrees closely with results obtained under changing photoperiods under growth chamber conditions (Chapter 4). Simple correlations (Table 5.1) indicate that individuals that bolt later in the season are shorter and produce fewer fruits than those bolting earlier. There is a negative correlation between rosette size and time to bolting under field conditions, which probably results from large overwintering rosettes bolting almost immediately. Rosette size at bolting and fitness are strongly positively correlated before correcting for the time of bolting.

Results of path analysis for fitness as measured by fruit number (Fig. 5.1a) are similar to those for fitness as measured by final plant height (Fig. 5.1b). Time to bolt, after removing the effects of rosette size and the time taken to flower once bolting has occurred, has a strong and negative direct effect on both fruit production (Fig. 5.1a) and final plant height (Fig. 5.1b). Similarly, rosette size at bolting has a strong direct effect on both fruit number and final plant height, holding other variables constant. Individuals that bolt later in the season take less time to flower once bolting has occurred, and a negative direct relationship exists between the time taken to flower and fitness.

Manipulated transplants—Under natural field conditions, the median date of bolting was June 20, and no rosette was observed to have bolted after July 10. The transplant experiment, designed to determine the fitness consequences of later bolting, included individuals bolting from June 14 to November 13, 1997. Bolting up to August 29, well over a month after the last natural bolting event in the field, resulted in the successful production of fruit. However, a steady decline in the number of fruits produced (Fig. 5.2a) and final plant height (Fig. 5.2b) is observed with date of bolting. To restrict the correlations to the linear portion of the relationship between date of bolting and fruit production (Fig. 5.2; zero fruits would be produced by transplants through the winter), I decided on a cutoff date of bolting of September 23 because no individual that bolted after September 23 produced even a single flower. The simple correlations among measured variables (Table 5.2) show that the relationship between date of bolting and fitness (fruit number and stem height) is strong. Stem height at which a plant flowers depends neither on the date of bolting nor on the rosette size at the time of bolting; earlier-bolting plants, however, take less time to attain a height at which flowering occurs (Table 5.2). Because a variable amount of time elapsed between bolting and transplant (see Materials and



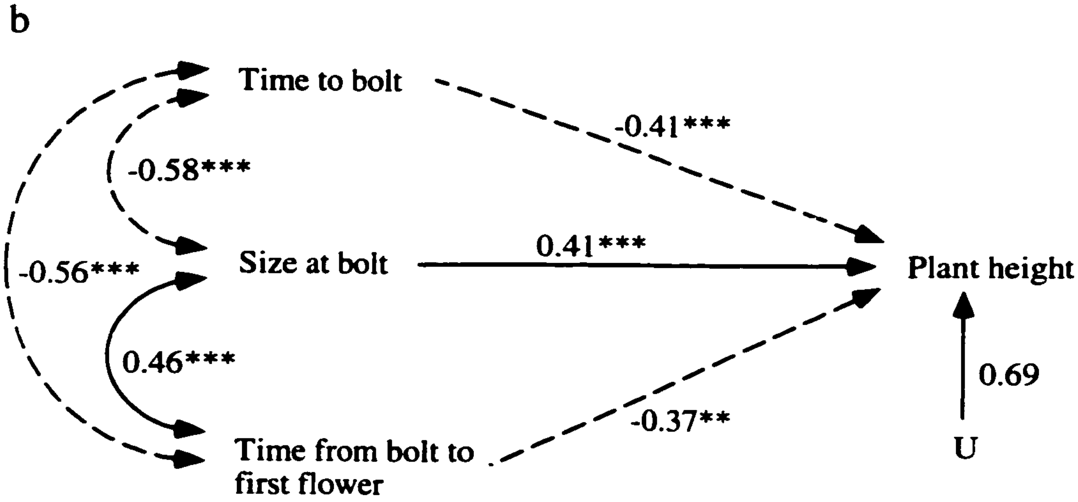


Fig. 5.1. Path diagrams showing the direct effects of bolting time, as measured by the number of days elapsed since June 1, the size at which a rosette bolted, the time elapsed from bolting to flower production, and residual variables (U) on a) total fruit production, and b) final plant height as measured in the field. Solid lines indicate positive effects, dashed lines indicate negative effects.

Table 5.1. Pearson correlations among eight traits of Lobelia inflata growing naturally in the field. Sample sizes are 58 for all correlations not involving flower characters, and 49 (all plants that flowered) for correlations involving flower characters. Correlations involving fruit number include all 58 individuals, even if zero fruits were produced. P values are given below each correlation coefficient.

	Bolt size	First flower day	First flower height	Bolt to flower time	Fruit number	Stem height
Bolt day	−0.58 <0.001	0.65 <0.001	−0.57 <0.001	−0.56 <0.001	−0.44 <0.001	−0.53 <0.001
Bolt size	—	−0.25 0.08	0.62 <0.001	0.46 <0.001	0.54 <0.001	0.52 <0.001
First flower day		—	−0.53 <0.001	0.27 0.06	−0.43 0.002	−0.55 <0.001
First flower height			—	0.14 0.34	0.90 <0.001	0.99 <0.001
Bolt to flower time				—	0.06 0.68	0.08 0.57
Fruit number					—	0.90 <0.001

Methods), the date of transplant is also included in the correlation table; no qualitative differences in results exist between correlations involving bolting date and transplant date.

In the same manner that path analysis was performed for the plants grown in the growth chamber (Chapter 4) and for plants growing naturally at Martock (above), path analysis was performed to partition the direct effects of time of bolting and size at bolting for the transplanted individuals (Fig. 5.3). Note that the time elapsed between bolting and flowering could not be meaningfully included as a direct effect in path analyses, because this value would be missing for all plants that did not flower; these plants, therefore, would have been unjustly excluded. This path analysis reveals that the effect of rosette size at bolting is unimportant in explaining variation in fruit production when bolting times span the entire summer (Fig. 5.3a). Although rosette size at bolting explains a significant portion of variation in final stem height (Fig. 5.3b), final height is not a good indication of reproductive success for plants that bolted late in the season.

The regression equation describing the dependence of fruit production on date of bolting was used to quantify the relative success of individuals bolting later in the season. The same cutoff date as used for the correlations (September 23) was used to restrict the regression to the linear portion of the relationship between date of bolting and fruit production. Fruit number (FN) as a function of time to bolt ($\underline{t}$) is given by

$$FN = 27.6 - 0.27(\underline{t});\ \underline{r}^2 = 0.65 \qquad (3)$$

where time to bolt is measured in days, beginning on June 1.

The fitness consequences of the observed "rules" of bolting—Several life history variables, including size-dependent fecundity, time-dependent fecundity, size-specific growth rates of rosettes, rates of overwinter mortality, and the "rules" of bolting have thus been measured. It is now possible to make at least a preliminary assessment of the

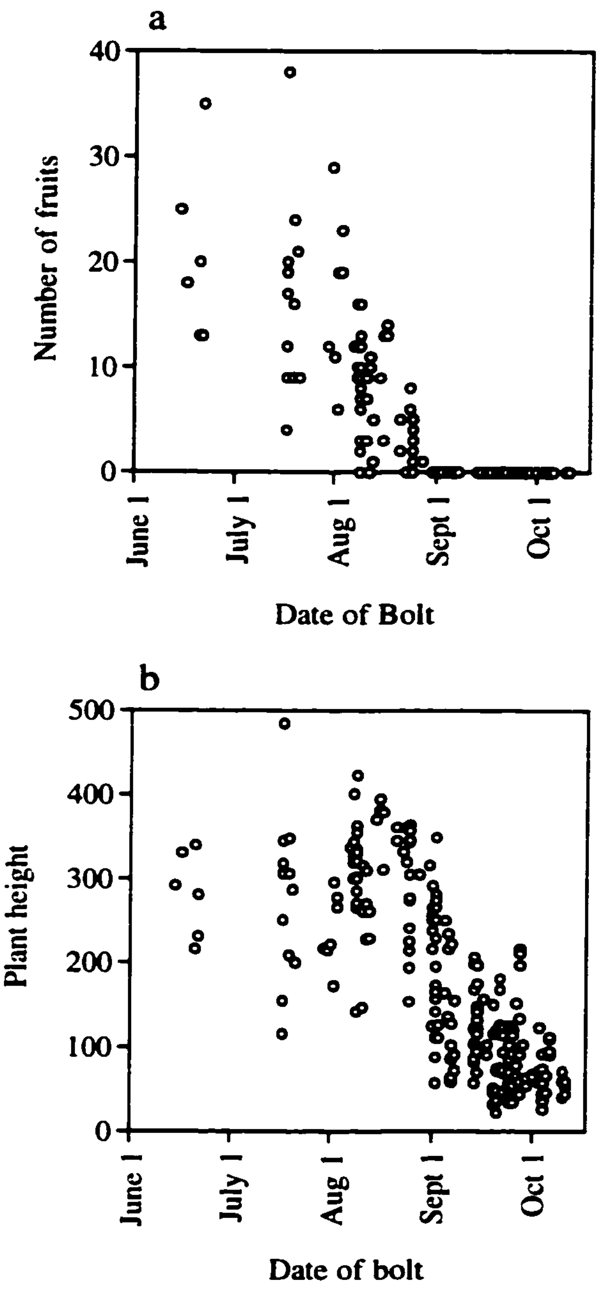


Fig. 5.2. Relationships between date of bolt and a) the number of fruits produced, and b) final plant height. These plots include only plants that were manipulated to bolt beyond dates of bolting naturally observed; rosettes were transplanted into the field shortly after bolting had occurred under growth chamber conditions.

Table 5.2. Pearson correlations among traits of *Lobelia inflata* transplanted to the field shortly after bolting had occurred. All individuals bolting up until September 23 (*N* = 156) are included, except for specific correlations involving flowering (*N* = 89). *P* values are given below each correlation coefficient.

	Trans-plant day	Bolt size	First flower day	First flower height	Bolt to flower time	Fruit number	Stem height
Bolt day	0.99 <0.01	–0.01 0.94	0.91 <0.01	0.08 0.48	0.31 <0.01	–0.81 <0.01	–0.61 <0.01
Trans-plant day	—	–0.01 0.94	0.90 <0.01	0.09 0.39	0.30 <0.01	–0.80 <0.01	–0.61 <0.01
Bolt size		—	0.14 0.19	0.14 0.19	0.06 0.58	–0.01 0.92	0.27 <0.01
First flower day			—	0.11 0.33	0.67 <0.01	–0.78 <0.01	–0.18 0.09
First flower height				—	0.11 0.32	0.07 0.49	0.88 <0.01
Bolt to flower time					—	–0.49 <0.01	–0.25 0.02
Fruit number						—	0.58 <0.01

appropriateness of bolting behaviour in *Lobelia inflata*.

Selection on the timing of bolting is influenced by the probability of surviving to reproduce, and on the relationship between rosette size and potential fecundity. The relationship between rosette size and fecundity, though, is further complicated by the fact that it is itself dependent on both the time of year and on size-specific rosette growth rate. I will first consider the fitness consequences of bolting within a season compared to a delay from one season to the next under the assumption of constant population size. The fitness, ω, associated with a probabilistic bolting behaviour, β_{yt}, governed by a particular rosette size (y) and time (t) relationship within a season is given by the fecundity function, m_1, associated with this bolting rule if the individual bolts, added to the probability of both delaying bolting and surviving to the subsequent season, considering the increased fecundity associated with rosette growth during the preceding season. This relationship may be described by the equation

$$\omega = \beta_{yt}\, m_1 + [l_r l_o\, (1 - \beta_{yt})\, m_2\,] \qquad (4)$$

where m_1 is the size- and time- dependent fecundity; m_2 is calculated from size-specific growth rates and the time remaining in the first season for rosette growth, and the relationship between rosette size and fecundity; l_r is the probability of surviving the remainder of the first season; and l_o is the probability of overwinter survival, all given by empirically-derived relationships.

The goal is to find the bolting "rule" or function that maximizes fitness. The plane containing every possible combination of time of year and rosette size can be divided into two areas: an area containing combinations for which immediate bolting would maximize fitness, and an area containing combinations for which a delay in reproduction would maximize fitness. The approach taken here is to find the function, dividing these two

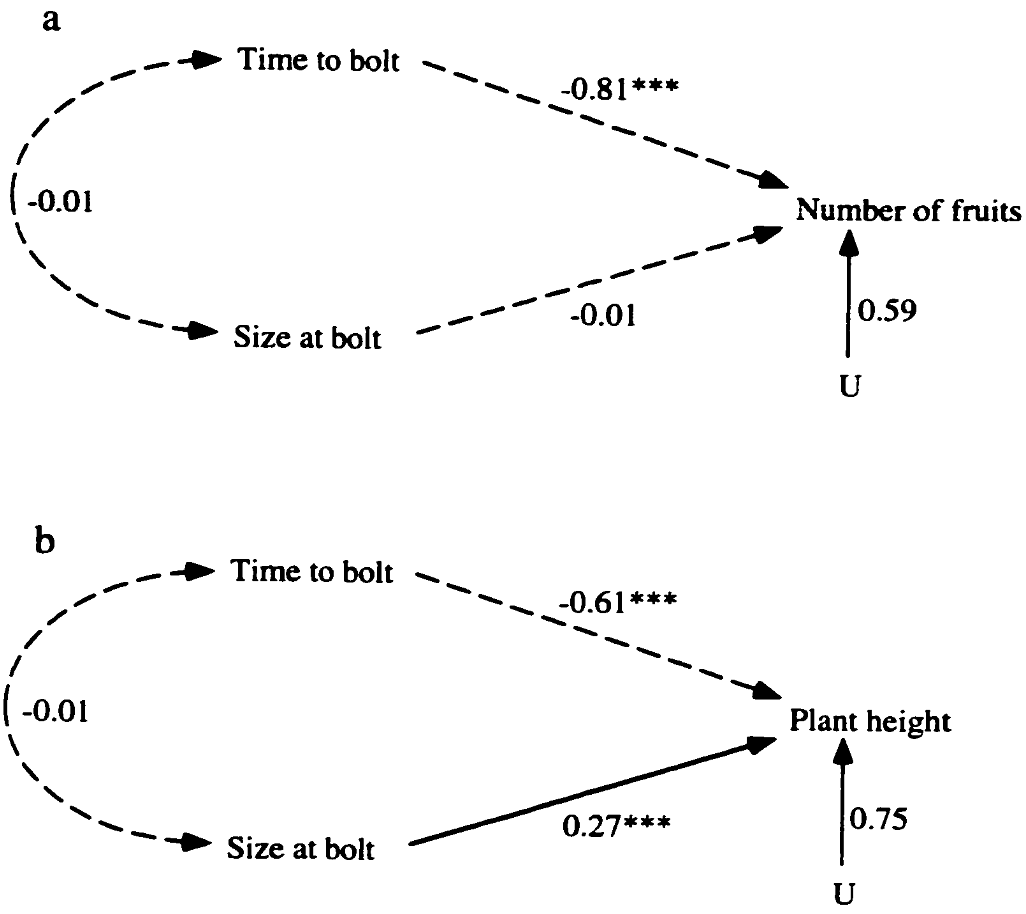


Fig. 5.3. Path diagrams showing the direct effects of the time taken to bolt, the size at which a rosette bolted, and residual variables (U) on a) total fruit production, and b) final plant height, when bolting was manipulated to occur throughout the season. Solid lines indicate positive effects, dashed lines indicate negative effects.

areas, that describes the bolting rule of equal fitness for bolting in the first season (ω_b), and delaying bolting until the subsequent season (ω_{nb}),

$$\omega_b = \omega_{nb}; \qquad (5)$$

where ω_b is a function of rosette size, y, (equation 1) multiplied by the proportional decline in fecundity associated with date of bolting, t, (equation 3) through time up to day 103.3 (the y-intercept, corresponding to the bolting date where fecundity is zero),

$$\omega_b = (-7.735 + 0.687y)\left(1 - \frac{t}{103.3}\right); \qquad (6)$$

and ω_{nb} is given by the time-dependent probability of surviving the remainder of the previous season, and by overwinter survival multiplied by a function of rosette size that takes into account additional growth after time t (equation 2) in the previous season:

$$\omega_{nb} = \left[0.423 + \left(1 - \frac{162 - t}{162}\right)0.577\right]0.44\left\{-7.735 + 0.687\left[y + (103.3 - t)(-0.02 + 0.011y)\right]\right\}. \qquad (7)$$

Setting $\omega_b = \omega_{nb}$ and simplifying yields the function describing the relationship between rosette size and time of year of equal fitness of immediate bolters and plants delaying bolting to the subsequent summer:

$$y = \frac{5.09 \times 10^5 - 7.32 \times 10^3 t + 1.82t^2}{3.49 \times 10^4 - 6.37 \times 10^2 t + t^2}. \qquad (8)$$

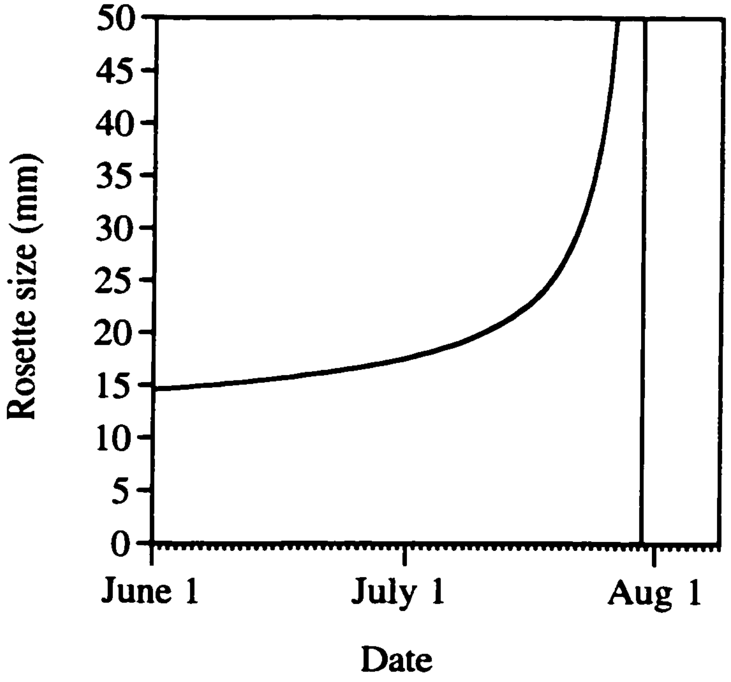


Fig. 5.4. The function describing the combinations of date and rosette size at which expected fitness of rosettes making a "decision" to bolt is equal to the expected fitness of those delaying bolting until the subsequent season. Individuals below the curve maximize fitness by delay of flowering, and those above the curve by immediate bolting. The function is derived from empirical information on the relationship between fecundity and rosette size, fecundity and bolting date, rosette growth rates, and rosette survival during both the growing season and over the winter.

The plotted function (Fig. 5.4) represents combinations of time of year and rosette size for which the expected fitness associated with an immediate decision to bolt is equal to the expected fitness associated with a reproductive delay until the next spring. The area at the upper left bounded by the curve contains values of rosette size and time of year at which fitness of immediate bolters would be higher than that of delayed bolters.

DISCUSSION

An array of reproductive behaviours exists among flowering plants; for example, species may be strictly annual, so-called biennial, monocarpic perennial, or perennial. Such variation has spawned much theoretical and empirical study of life-history evolution. Monocarpic species, in particular, provide an opportunity for empirical tests of theory, because it is rational to attempt to measure the important life-history parameters when reproductive effort cannot be spread among several reproductive episodes. For example, in a study of among-season reproductive delays in Cynoglossum officinale, Wesselingh et al. (1997) found the rank order of threshold size for bolting in three different habitat types to be consistent with life-history models including rates of growth and mortality. Lobelia inflata must geminate, produce a rosette, bolt, flower, and produce mature fruit within the confines imposed by seasonality. No single structure can be perfectly adapted to different extreme conditions, and survival through the different extremes imposed by seasonality has required the evolution of specialized structures.

Seeds and rosettes provide L. inflata with a high probability of survival through harsh conditions, but reproduction cannot occur until an individual makes the transition from a hardy rosette to the more vulnerable flowering stage. Fecundity has been treated simply as a function of rosette size in previous studies, but for a given rosette size, the manipulation experiment shows that fecundity decreases dramatically with time of year. In the manipulation experiment plants were induced to bolt, and it is possible that they exhibited lower fitness in the field if the bolting decision was physiologically "reversed" upon transplant to the field under short day conditions. The delay from bolting to transplant was seemingly sufficient to avert this effect: plants continued to grow and produce flowers, even if transplanted late in the season. Furthermore, the direct effect of date of bolting on both final plant height and fruit production observed in naturally growing individuals, especially considering that bolting is restricted to June and early July, is consistent with the transplant results. The success of an individual, then, depends critically on the timing of bolting; the within-season component of reproductive timing cannot be ignored.

Both final fruit number and final plant height were used as fitness measures in previous work conducted under growth chamber conditions (Chapter 4). It was noted in that chapter that it is important to consider some measure of plant size in addition to fruit production because ecological effects of size might not be evident when fitness is assessed under artificial conditions. The present results show that fruit production and plant height are more strongly correlated under field conditions (Table 5.1), and that the direct effects of the time to bolting, rosette size at bolting, and the time elapsed from bolting to flowering are much more consistent between the two measures of fitness as measured under field conditions than when measured under growth chamber conditions.

Although the general form of the conceptual model for bolting behaviour presented in the previous chapter is supported, the details cannot be exacted from the present data. In the growth chamber, an increasing threshold size for bolting was observed for dates later in the season. Under field conditions, a negative relationship between rosette size and bolting date was observed. However, the growth chamber study included only first-year rosettes, whereas the field study includes overwintering rosettes. If the conceptual model is expanded to include multiple seasons (Fig. 5.5), it can be seen that rosettes that have attained a large size during the previous season are expected to bolt immediately, and would produce a negative correlation between date of bolt and rosette size. The model as

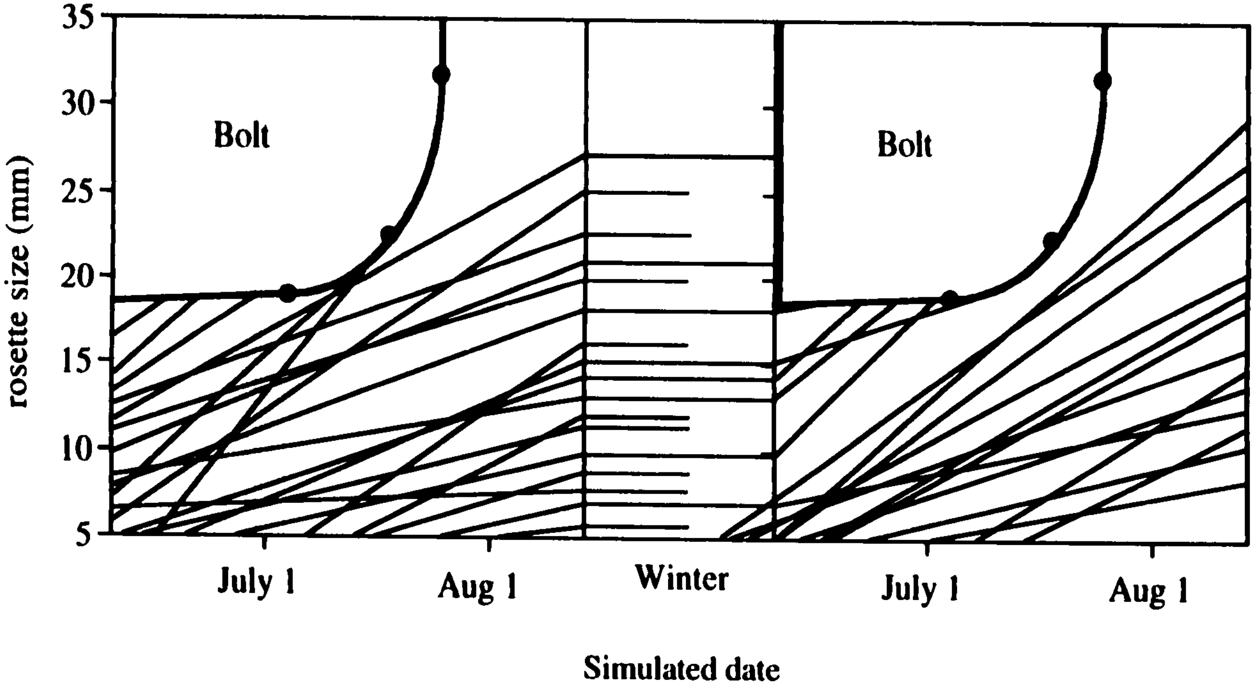


Fig. 5.5. A conceptual model for the within-season control of bolting over multiple seasons. The heavy curve represents the minimum size requirement for bolting, which changes with time during a single growing season. Rosettes that have attained a large size only toward the end of one growing season will not bolt during that season because they are below the threshold size for bolting for dates late in the season. However, these large rosettes, if they survive the winter, bolt immediately in the following season because they are well above the threshold size for bolting for early dates.

presented does not include possible differences in bolting thresholds for vernalized and unvernalized plants, but such effects may well exist even for plants such as Lobelia inflata that have no strict vernalization requirement. Given that rosettes of different ages are included, the data conform well to the conceptual model applied over multiple seasons.

The "rules" governing bolting that would maximize fitness under the observed conditions and assumptions (Fig. 5.4) are strikingly similar, at least in qualitative aspects such as general shape, to those that rosettes were observed to follow in the growth chamber (Chapter 4, Fig. 4.6). The function is contained within a realistic range of rosette sizes for bolting over the season, and implies that rosettes with a longest leaf greater than or equal to about 15 mm at the beginning of the season are expected to bolt immediately, and no rosette is expected to bolt after late July, no matter its size. Of the rosettes followed under field conditions, 58 were observed to bolt; one bolted on July 10, and all other bolting events took place between June 1 and July 4.

The similarity between the size- and time-dependent control of flowering discovered under artificial conditions and the fitness maximization model is encouraging, and the restriction of bolting to early in the season suggests that natural selection has been important in shaping the behaviour of bolting. A test of optimality, however, must be based on observations at the individual level (Orzack and Sober 1994). Because the ages of individuals in the field were unknown in the present study, a quantitative evaluation of bolting at combinations of size and date would be confounded by large overwintering rosettes that are well above the threshold size for bolting in the early summer. The asymptote of the function describing optimal bolting behaviour, though, is not confounded by this issue. That only one rosette bolted later than July 4 whereas the optimal bolting function predicts bolting until nearly the end of July suggests that bolting behaviour is either suboptimal, that not all relevant variables were taken into consideration in the construction of the model, or that an inappropriate fitness criterion is being used: rosette bolting behaviour might be a conservative bet-hedging strategy that has evolved to maximize the geometric mean fitness.

The possible effect of lifespan on the rate of increase associated with a bolting strategy was not taken into consideration in the model. In a population that increases without limit, rate of increase is sensitive to lifespan (Lewontin 1965; Harper 1977). For a monocarpic perennial under unconstrained population growth, an individual delaying reproduction until the subsequent season must produce seeds in the ratio of (n+1)/n to have the equivalent fitness of an immediate bolter (De Jong et al. 1987). The bolting rule of equal fitness (equation 5) for bolters in year $\underline{n}$ and $\underline{n}+1$ would thus be $\underline{\omega}_b = \underline{\omega}_{nb}^{(n+1)/n}$. However, it is unreasonable to assume indefinite population growth. De Jong et al. (1987) showed that, for monocarpic plants under assumptions of limits to population growth, lifespan is unimportant: delayed flowering is appropriate simply if increased fecundity outweighs the risk of mortality (De Jong et al. 1987). It is possible, though, that lifespan has some effect on fitness, and this might partially explain the restriction of bolting to early summer. An evaluation of the importance of lifespan in the evolution of delayed flowering would require a knowledge of both the direct effects of lifespan, if any, on fitness, and the ages of the rosettes.

Assuming, then, that all important influences on fitness are included in the model, the bet-hedging hypothesis can now be qualitatively assessed by comparing the behaviour of rosettes in the field to variations in the empirically-derived function corresponding to season-length variability. A more rigorous quantitative test of the predictions of the model, however, would be to observe bolting under field conditions over several generations, using rosettes of known age.

Lobelia inflata can be said to inhabit a temporally unpredictable environment. The date of last frost in the spring, first frost in the autumn, and accumulation of degree-days within a growing season are all characterized by year-to-year variability. Unpredictability has severe fitness consequences: once an individual has germinated, produced a rosette and bolted, it must use the remainder of the available season to flower and produce mature fruit. The time elapsed between the "decision" to bolt and fruit maturation is substantial and is shown to have direct fitness consequences: an individual becomes vulnerable to unpredictable risks such as herbivory (Simons and Johnston 1999) and season length. If, in one particular year, the remainder of the season following a bolting event happens to be too short to produce mature fruit, the fitness of the bolted individual is zero because only rosettes can survive the winter. If, on the other hand, the individual does not bolt and the season happens to be longer than average, the individual's relative fitness is low both because it has forgone its potential first year fecundity, and there is a risk of overwinter mortality.

The long-term fitness of a genotype inhabiting a temporally unpredictable environment is not simply the arithmetic mean of each of its yearly fitnesses; fitness is multiplicative and, hence, the appropriate measure of long term fitness is the geometric mean (Gillespie 1974) of its yearly fitnesses. Because low values have strong effects on the geometric mean, a genotype with a low variance in fitness has a high geometric mean fitness. Although this formulation of fitness is the more general definition, situations in which low fitness variance between years is gained at the expense of expected fitness within a generation is given the special name, "bet hedging" (Siatkin 1974; Seger and Brockmann 1987; Philippi and Seger 1989). Rosette bolting in L. inflata is a likely candidate for bet-hedging because time remaining within a season is unpredictable, and flowering with insufficient time remaining leads to extremely low or even zero fitness.

At present, variance in season length cannot be confidently included in a model because, even if accurate climate records were available, it is not known which of a potentially infinite set of combinations of environmental variables influence plant fitness. However, a variety of season lengths can be incorporated into the empirically-derived optimization model to ask how the general characteristics of the function change. From these characteristics, the expected behaviour of conservative bet-hedgers can be deduced.

The optimization equation, $\omega_b = \omega_{nb}$, based on the original empirical relationships can be altered to reflect the fitness effects of different season lengths. A shorter growing season is assumed to be characterized by a steeper negative slope for time-dependent fecundity, with a y-intercept occurring at the date of bolting that is expected to produce zero fruits. Expected fitness of delayed bolters is influenced by a change in season length through the proportion of the season remaining for rosette growth. Under the assumption that overwinter rosette survival and survival during the growing season are unaffected by season length, the bolting rule under season length ϕ is given by $\omega_b = \omega_{nb}$, where

$$\omega_b = (-7.735+0.687y)\left(1-\frac{t}{\phi}\right), \text{ and} \tag{9}$$

$$\omega_{nb} = \left[0.423+\left(1-\frac{162-t}{162}\right)0.577\right]0.44\left\{-7.735+0.687\left[y+(\phi-t)(-0.02+0.011y)\right]\right\}. \tag{10}$$

A plot of this function for values of ϕ = 80, 90, 103.3, 110 and 120 days (Fig. 5.6) reveals interesting characteristics expected of conservative bolting behaviour. Whereas one might intuitively expect a conservative strategy to consist of more stringent bolting requirements, the results show that a shorter season length should select for a smaller threshold size at bolting near the beginning of the season. Short seasons, however, are expected to select for a rapid increase in the threshold size requirement later in the bolting period. This results in the intersection of the curves late in the bolting season, and an earlier expected final date of bolting for shorter seasons, as depicted by the dates corresponding the asymptotes of the five curves (Fig. 5.6).

Bolting confined to the period between early June to early July is qualitatively consistent with conservative bet hedging. Although, in an average year, a rosette might

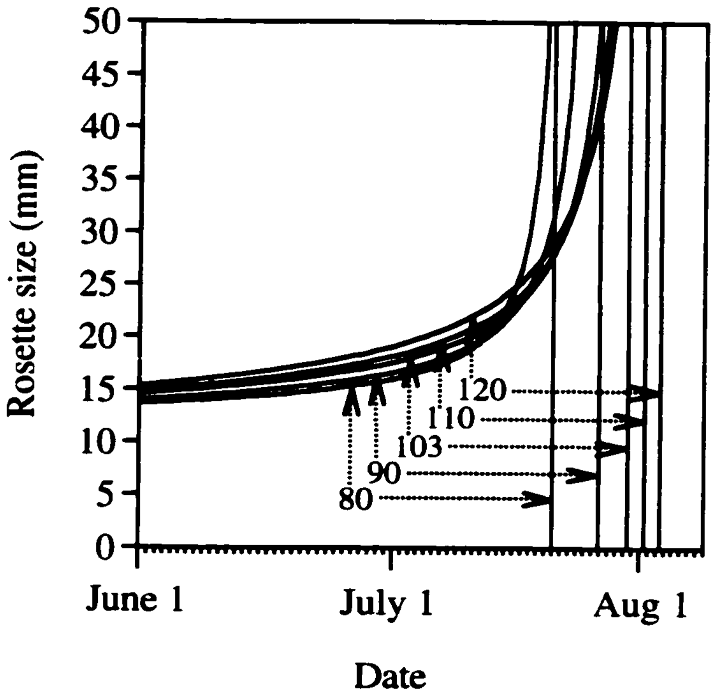


Fig. 5.6. Functions describing the combinations of date and rosette size at which expected fitness of bolting rosettes is equal to the expected fitness of delayed bolters, for the observed season length (103 d) and for four hypothetical season lengths (80, 90, 110, and 120 d).

maximize fitness by bolting, this rosette might maximize its longer-term fitness by remaining vegetative in case the season is shorter than average. The season length that would correspond to the function maximizing fitness for the latest observed date of bolting (July 10) is only 58 days, or 0.56 of the observed season length. The implication is that variation in season length is so pronounced that the bet-hedging behaviour that maximizes the geometric mean fitness is so conservative that it maximizes expected fitness only for seasons of 0.56 the length of the observed season. Other, unmeasured, parameters might also be important in explaining the confinement of bolting to the first few weeks of the season, or bolting behaviour may simply be suboptimal. Whatever the case, these results suggest that the evolution of bolting behaviour is shaped by seasonal differences; thus, a quantification of seasonal variability as it relates to fitness would be an important step toward assessing the importance of unpredictability in the evolution of the timing of reproduction.

The validity of any optimality model depends on the time scale over which natural selection can be effective. That bolting behaviour was not found to be optimal during the season in which the study was performed does not rule out optimality as an explanation for the observed control of flowering. Bet-hedging strategies are seldom assessed empirically because, by definition, a test of optimality for bet hedging requires allocation to research in proportion to the study's relative duration, but the benefit of performing such long-term research cannot be predicted at the outset. The present results, however, add to the growing sentiment that, if the significance of trait values in nature is to be better understood in terms of optimality, temporal fluctuations in selection will demand more rigorous empirical study.

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CHAPTER 6

The Cost of Compensation

INTRODUCTION

Much recent attention has focused on the apparent benefit accrued by a plant through its partial destruction by grazing (Owen 1980; Belsky 1986; McNaughton 1986; Crawley 1987; Paige and Whitham 1987; Vail 1992; Belsky et al. 1993; Mathews 1994; Tuomi et al. 1994; Järemo et al. 1996; Nilsson et al. 1996; Lennartsson et al. 1997). The subject has been characterized by heated debate on two related issues: the empirical question of whether fitness is, in fact, increased by the partial destruction of plant tissue (overcompensation) (Paige and Whitham 1987; Bergelson and Crawley 1992; Crawley 1993; Bergelson et al. 1996) and, if overcompensation exists, whether claims that grazing is thus beneficial to a plant are justified (Crawley 1987; Belsky et al. 1993; Mathews 1994; Vail 1993; Vail 1994).

Empirical studies have concentrated on testing a plant's capacity for compensation by comparing the performance of grazed plants to ungrazed controls (Paige and Whitham 1987; Maschinski and Whitham 1989; Bergelson and Crawley 1992; Paige 1992; Wegener and Odasz 1997) and the claim that plants benefit from herbivore damage has been made (Owen 1980; Paige and Whitham 1987; Paige 1992). As a result of differing interpretations of what constitutes a "benefit" (Crawley 1987), this claim is viewed by some as a paradox. A "benefit" of being partially destroyed might be observed in a population for two different reasons. First, grazing of aboveground tissue might alter the plant's architecture such that fitness is increased by the removal of constraints. If partial destruction is truly beneficial to the plant through the removal of such obstacles as architectural constraints, the plant-herbivore association might be regarded as mutualistic (mutualism hypothesis). Second, compensation could be the expression of an adaptive strategy which has evolved during a history of grazing (van der Meijden 1990; Lennartsson et al. 1997), or competition for light (Aarssen 1995; Järemo et al. 1996) in which case plants "cope" (Mathews 1994) with herbivores, but any benefit is only proximal (Belsky et al. 1993) because it is attained at an evolutionary cost (tradeoff hypothesis).

Bet-hedging models have been proposed that suggest that the capacity for compensation should evolve under size-selective herbivory (Vail 1992) and as a bet-hedging strategy under year-to-year variation in the risk of destruction (Nilsson et al. 1996). This chapter has three purposes: first, to present a model of compensation as a bet-hedging strategy that is simpler than, yet generalizes the formulation of Nilsson et al. (1996); second, to suggest that empirical studies might be more constructive if directed towards measuring the costs of compensation; and third, to present empirical evidence for the existence of a phenotypic tradeoff between reproductive success without destruction and reproductive success following destruction in Indian tobacco, Lobelia inflata, a monocarpic plant capable of compensatory regrowth.

A MODEL OF COMPENSATION AS BET HEDGING

Partial dedication of total resources to regrowth following destruction is expected to evolve as a bet-hedging strategy under among-season variation in the risk of herbivory (Nilsson et al. 1996). In the model of Nilsson et al. (1996), the intensity of herbivory is either low (good years) or high (bad years) and, in this respect, is reminiscent of Cohen's (1966) model of population-level optimal seed dormancy under environmental unpredictability. From the viewpoint of an individual plant, though, partial destruction is unpredictable even when the risk at the population level is predictable. Whereas Nilsson et al. (1996) restrict their results to year-to-year variation in herbivore pressure, the simple model developed below demonstrates that their results are in fact more general. In particular, the results apply also to individuals evolving in populations experiencing a constant risk of destruction.

Vail (1992), before showing that overcompensation should evolve when the probability of herbivore damage is size dependent, first models optimal allocation strategies under different constant levels of herbivory without size-selection. Because of its clarity, I use Vail's (1992) base model as a point of departure to examine the relative fitnesses of different compensation strategies under constant risk of destruction. If a is the probability of destruction of initial effort, and b is the fraction of total available resources dedicated to initial reproductive effort,

$$S = (1-a)b + a(1-b) \tag{1}$$

where S is expected fitness (Vail 1992). For monocarpic plants, expected fitness under this model is maximized either with complete dedication to initial reproduction if the probability of destruction is below 0.5, or complete dedication to secondary fruit production if the probability of destruction is greater than 0.5 (see Fig. 2 of Vail 1992). Selection, however, acts to maximize the geometric mean fitness (Seger and Brockmann 1987; Philippi and Seger 1989). The geometric mean principle, despite its perception as a "special case" is the more general fitness measure and applies also to environmental constancy (Roff 1992 pp. 41-42), although the geometric and arithmetic mean fitnesses are identical under the special case of no variation in the optimal trait value among generations. The optimal allocation to initial reproduction, b, then, under unpredictability of destruction, is given by the geometric mean fitness (S_{GM}) over n generations such that

$$S_{GM} = \left\{ \prod_{1,n} \left[(1-a')b + a'(1-b)\right] \right\}^{\frac{1}{n}} \tag{2}$$

where a’ represents destruction of initial reproductive effort, and is either 1, with probability a, or 0. Because a plant’s initial effort is either destroyed or is not destroyed each generation, the geometric mean fitness simplifies to

$$S_{GM} = \left\{ \left[b^{n(1-a)} \right] \left[(1-b)^{na} \right] \right\}^{\frac{1}{n}}. \tag{3}$$

This model reveals intermediate optimal values of initial reproductive effort (b) for different values of the probability of destruction of initial reproductive effort (a) (Fig. 6.1). The greater the probability of destruction, the greater the optimal allocation to potential regrowth: optimal initial reproductive effort (b) (corresponding to fitness maxima of Fig. 6.1) is simply equal to the probability of the initial reproductive effort not being destroyed (1−a). A constant risk of destruction, therefore, may select for any level of compensation, including an apparent “benefit” of destruction (overcompensation) when the risk of destruction is greater than 0.5.

COMPENSATION AS TRADEOFF

Previous models demonstrate that compensation is expected to evolve under size-selective herbivory (Vail 1992) and among-season variability in the risk of herbivory (Nilsson et al. 1996). An assumption of these models is that a proportion of the plant’s resources, measured as the total number of potential buds or meristems (Tuomi et al. 1994) is allocated to initial growth, and the remaining resources are available for compensatory regrowth in the event of partial destruction of aboveground tissue. If individuals dedicate reserves to post-destruction fruit production, and these reserves are unavailable for initial reproduction, a tradeoff is expected between initial and post-destruction reproductive potential. The “benefit” of partial destruction is thus achieved at a cost: if plants had

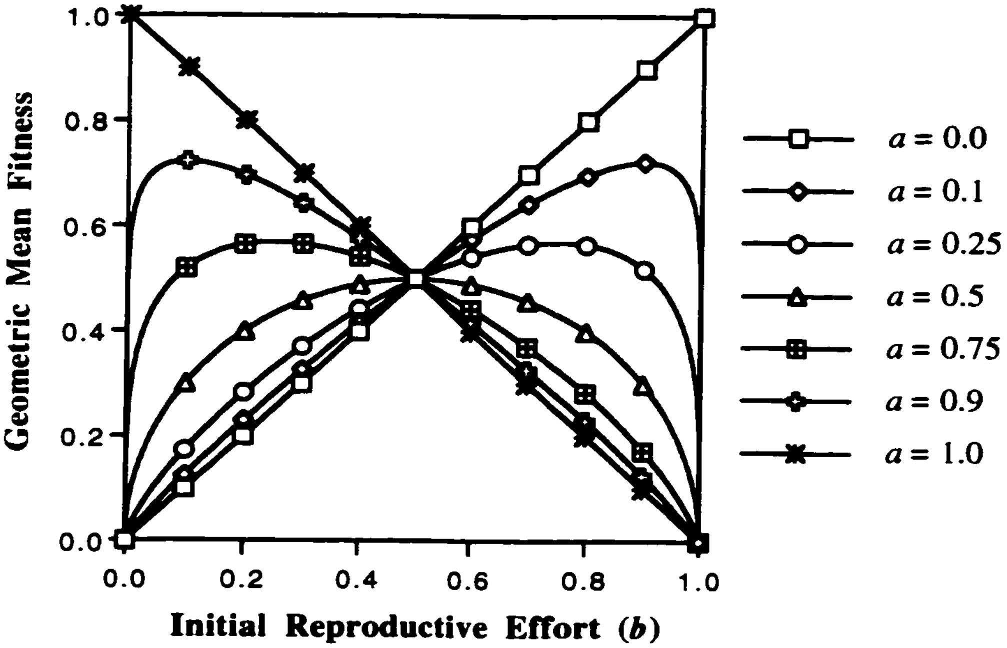

Figure 6.1: Geometric mean fitness as a function of initial reproductive effort, b, plotted for seven different constant probabilities of destruction, a. Maximum fitness values occur where the allocation to initial reproduction is 1 minus the probability of destruction.

evolved in the absence of destructive forces such as herbivory, all resources might be dedicated to initial fruit production, and total seed production would therefore be higher. The capacity for compensation is expected to evolve in proportion to the relative benefit of compensatory regrowth compared to primary growth, and should be viewed as occurring on a continuum of potential compensatory responses from undercompensation to overcompensation (Maschinski and Whitham 1989; Belsky et al. 1993; Nilsson et al. 1996).

The significance of overcompensation from the viewpoint of the tradeoff hypothesis is simply that it represents all values past the midpoint on the scale of zero to 100% dedication of resources to post-destruction fruit production. The very existence of overcompensation has been extensively debated (Belsky 1986; Bergelson et al. 1996) but, because overcompensation is predicted by both the mutualism and the tradeoff hypotheses, evidence of overcompensation cannot be used to distinguish between these hypotheses. Empirical evidence for a tradeoff between the dedication of reserves to primary and post-destruction fecundity, though, would support the hypothesis that destruction bears a cost, and would thus count against the mutualism hypothesis.

A PHENOTYPIC TRADEOFF IN LOBELIA INFLATA

Lobelia inflata (L.), an annual, biennial, or monocarpic perennial, typically inhabits disturbed areas (Baskin and Baskin 1992). This species exhibits compensatory regrowth following removal of aboveground tissue (pers. obs.) and because it reproduces only once in its lifetime and only by seed, it is ideally suited for studies on compensation. In plants capable of compensatory growth, resources are “wasted” if the plants are not damaged. The greater the allocation to regrowth, the smaller the proportional allotment to initial growth. If the proportion of unused resources allocated to regrowth could be measured for undamaged plants, then the tradeoff between initial and secondary growth could be quantified. In this experiment, I assess allocation to regrowth in undamaged plants by damaging the stems only after having allowed plants to completely mature without destruction. The tradeoff between initial and secondary growth is measured as the number of fruits produced initially compared to that produced secondarily but, because I am interested in allocation relative to total available resources, both initial and compensatory fruit numbers are corrected for rosette size immediately prior to bolting.

The expression of regrowth is known to be influenced by the timing of damage (Maschinski and Whitham 1989) or, more precisely, by the developmental stage at which damage occurs (Bergelson et al. 1996). The cutting treatment administered only following initial fruit maturation in the present experiment, however, does not represent herbivory late in the season. The plants were allowed ample time to regrow following partial destruction, and the late destruction is simply a means to reveal the tradeoff between allocation to initial vs. secondary fruit production.

Seeds of L. inflata were placed on moistened filter paper in petri plates, and allowed to germinate in a growth chamber. Seedlings were transplanted, one per cell, into Kord cell packs and were grown under a continuously diminishing photoperiod regime designed to simulate conditions through the summer. Rosette size was measured weekly, and size and date at bolting were noted. Plants bolted at various times throughout the "summer" and all plants were allowed to develop until no new flowers appeared, and all fruits had ripened. Individuals that remained vegetative (rosettes) were not used in the experiment, and total fruit production was recorded for plants that had bolted.

Stems were cut to 2 cm and the cell packs containing the remaining tissue were covered and placed at 5°C for 30 d before initiating regrowth. Plants that died up until this stage were discarded (see below). The 58 remaining plants were transferred into a growth room under long day conditions (15h L, 9h D; 21°C/14°C). Final secondary fruit number was recorded, and ranged from one to 69 with a mean of 10.5.

Because of the possibility that environmental correlations between initial and secondary fruit production might obscure a genetic tradeoff, fruit number was first corrected for rosette size, measured as the longest leaf, at the time of bolting. Rosette size at bolting is a significant predictor of total fruit number (A. M. Simons and M. O. Johnston, unpublished data), and I assume that it is an appropriate correlate of the total resources available to an individual. The tradeoff was thus measured as the correlation between the residuals from the relationship between log(initial fruit number) vs. rosette size at the time of bolting, and the residuals from the relationship between log(secondary fruit number) vs. rosette size at the time of bolting.

Empirical data support the existence of compensatory plant regrowth following partial destruction, but no previous study has directly asked whether the potential for secondary regrowth incurs a cost to initial flower production. In this simple study I find that, for a given rosette size, a phenotypic tradeoff exists between initial fruit production and secondary fruit production ($r = -0.262$; $P = 0.047$; $N = 58$) in *Lobelia inflata*.

Secondary fruit number (2°) is predicted by the multiple regression equation that includes rosette size (Ros) and initial fruit number (1°):

$$\log(2^\circ) = 2.16 + 0.041*(\mathrm{Ros}) - 0.815*\log(1^\circ).$$

Fruit number was log-transformed because the relationship between the absolute value of the residual secondary fruit number increases significantly with rosette size before (slope = 0.468; $P = 0.001$) but not after (slope = 0.013; $P = 0.175$) transformation (Zar 1984, p.288). Although this residual relationship for initial fruit number is nonsignificant (slope = 0.421; $P = 0.161$), logs were taken prior to analysis for consistency and because this transformation aided in detrending the data (slope = -0.002; $P = 0.699$). Furthermore, the use of untransformed initial fruit number and log-transformed secondary fruit number does not qualitatively alter the finding of a phenotypic tradeoff ($r = -0.282$; $P = 0.032$; $N = 58$).

The above results include only plants that were alive at the beginning of the regrowth period. Complete trays of plants desiccated during storage, and were excluded because it is most reasonable to attribute their demise to environmental factors unrelated to allocation to primary fruit production. It is possible, however, that the few (13) dead plants on the remaining trays might have been those with zero allocation to regrowth. The data were reanalyzed to include the individuals with zero secondary fruit production. The analysis was performed by first adding 1 to all fruit numbers, primary and secondary, before log transforming, but was otherwise identical to the above correlation analysis. The correlation between initial and secondary fruit production is stronger ($r = -0.317$; $P = 0.007$; $N = 71$) with the inclusion of the dead individuals: these individuals had, given their rosette sizes, produced disproportionately many fruits during initial flowering.

CONCLUSIONS

Theoretical analyses (Vail 1992; Tuomi et al. 1994; Nilsson et al. 1996) have assumed the existence of a tradeoff between initial and secondary growth. The tradeoff between initial and secondary fruit production observed in Lobelia inflata supports these analyses, and implies that there is a phenotypic cost associated with the potential for regrowth after partial destruction; individuals that dedicate a higher proportion of total resources to regrowth produce fewer fruits initially. Genetic correlations are a more appropriate measure of evolutionary costs (Reznick 1992) because positive phenotypic correlations may be driven by common environmental effects. In the present experiment, a negative phenotypic correlation is observed despite this possibility. Therefore, the idea that the increase in fitness following grazing has evolved as a mutualistic relationship between plants and herbivores (Owen 1980) is not supported.

Previous models suggest that compensation is expected under particular conditions (van der Meijden 1990; Vail 1992; Nilsson et al. 1996). Our model shows that compensation, and even overcompensation, may be expected under the more general conditions of a constant risk of destruction. As has been noted by Mathews (1994), evidence of overcompensation is not evidence that plants benefit from partial destruction: if they were immune to forces such as herbivory, drought, frost and trampling, plants dedicating resources to regrowth would be at a selective disadvantage relative to plants able to allocate all available resources to initial reproduction. Therefore, further empirical work to determine the genetic correlation between initial and secondary fruit production would greatly enhance our understanding of the evolutionary cost of compensation.

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CHAPTER 7

From Bet Hedging to Clade Persistence:
The Continuity of Microevolution and Macroevolution

Abstract—Disagreements between paleobiologists and population biologists are disturbing because it is assumed that these workers study the same phenomena from different perspectives. I argue that a mischaracterization of natural selection has led to the erroneous contention that micro- and macroevolution are decoupled. Environmental unpredictability selects for bet-hedging traits, traits that do not necessarily maximize expected fitness in an average environment. Selection for bet hedging has mistakenly been treated as an exception, wherein geometric mean fitness is maximized, to natural selection more generally, wherein expected fitness is maximized. That selection for the geometric mean fitness is the general case has been acknowledged, but the repercussions of accommodating this measure of fitness into evolutionary theory have neither been explored nor recognized. The application of bet-hedging theory to all time scales results in a self-consistent view of the way in which natural selection operates, and renders superfluous the numerous more elaborate explanations for individual extinction events as special cases of natural selection.

INTRODUCTION

The continuity of selective processes over microevolutionary and macroevolutionary time continues to be a source of disagreement in evolutionary biology, one that Maynard Smith (1989) describes as "unsatisfactory." This dispute is composed of two issues which are often confused. One, and the better-known, is whether the principles of population genetics can explain the observed irregular pattern in the rate of evolution over macroevolutionary time. Because, as has been sufficiently documented by Charlesworth et al. (1982), the rates of evolutionary events in the fossil record are readily explained by population genetics and the neo-Darwinian synthesis, this debate will be resolved only by an explanation of the observed episodic pattern (Maynard Smith 1989) and is reduced to a description of past selective environments (Charlesworth et al. 1982).

The second issue, and the one addressed in this paper, is whether the effects of selection operating over microevolutionary time, or at the population level, accumulate to produce the observed effects of selection over macroevolutionary time. That any particular evolutionary event can be explained by the neo-Darwinian synthetic theory does not imply that the evolutionary consequences of rare events such as mass and minor extinctions will not tend to oppose trends occurring between these events. This is a debate about the relevance of the process of natural selection operating at the "ecological moment" (Gould 1985) to the production of phenotypic trends over the long term and, according to Gould (1985), is the most important issue of the debate surrounding punctuated equilibria.

Resolution of the continuity issue is critical because if selection is discontinuous over different time scales, then paleontology and population genetics are not two approaches to the same evolutionary problems; they are only distantly related fields of study. The fact that the debate has not been extinguished means either that at least half of the experts are wrong, or that a single perspective of selection consistent with both micro- and macroevolution is still needed. I believe the truth to be the latter, and propose that such a perspective is attainable not through a new theory, but through the application of one with a previously underappreciated significance.

To understand the argument for the continuous nature of selection, it is first necessary to consider the rationale for the view of discontinuity. I therefore begin with an outline of the views of prominent scientists who argue that selection is fundamentally discontinuous over different time scales. The remainder of the paper presents the argument that the principles of bet-hedging theory, which are conventionally restricted to exceptional circumstances, should be integrated into evolution in general. Justification for the general applicability of bet-hedging ideas rests on the existence of parallel processes of environmental variance, selection, and heritable components of phenotypic variance at different temporal scales. Arguments for the existence of such processes are presented, and it is concluded that the integration of bet-hedging ideas into evolutionary theory results in a self-consistent view of the way in which natural selection operates.

VIEWS OF DISCONTINUITY

The existence of the terms "microevolution" and "macroevolution" reflects the controversy (Eldredge and Gould 1972; Stanley 1975; Charlesworth et al. 1982; Orzack 1981; Maynard Smith 1989; Gould and Eldredge 1993; Van Valen 1994; Bennett 1997) over the unity of the process of natural selection operating over different time scales. Gould (1985), for example, contends that the process of selection is of a different nature at different time scales. His argument is based on the observation that what is seemingly an adaptive trend over one time scale is not consistent with trends over another. For this reason, Gould sometimes believes that the synthetic theory, at least as portrayed by Mayr, is "effectively dead" (Gould 1980; but see Gould 1985); adaptation over a few generations is insignificant because major evolutionary trends are not established by processes circumscribed by the modern synthesis (Gould 1985). Nothing about the patterns observed in the fossil record is beyond explanation by population genetic theory (Charlesworth et al. 1982), but this argument has not satisfied many paleobiologists; it does not address the problem of why the fossil record should reveal trends contrary to those observed over microevolutionary time. Implicit in the disagreement is that the outcome of selection is qualitatively different when acting at different levels, but the relationship between these outcomes has remained indistinct.

Paleobiologists have developed a variety of explanations for trends on different scales, most treating the opposition of trends as reflecting a fundamental discontinuity of processes acting across these scales. A theory to account for evolution above the species level is presented by Stanley (1975). Stanley adapts the logistic growth equation for the description of species selection based on rate of speciation and extinction within taxa, but then proposes that higher level processes are necessary to explain evolutionary trends, and that natural selection provides "little more than the raw material and fine adjustment of large-scale evolution" (Stanley 1975).

For Jablonski (1986), there is a qualitative difference between processes operating during the mass extinctions and during background times. He proposes an alternation of macroevolutionary "regimes" to explain opposing trends in survival, stating that mass extinctions are not caused merely by an intensification of background selection. Survival over the longer term would depend on the "chance occurrence" of traits allowing survival under both regimes (Jablonski 1986).

Raup (1986) sees mass extinctions as one extreme on the continuum of environmental variance, but envisions a dichotomy between "destructive" and "constructive" forces operating over different time scales. He argues that, in order that it be constructive, an environmental event must occur often enough to be within the "'experience'" of the organism (Raup 1986). Extinction as a nonconstructive evolutionary force is proposed by Raup (1986) as "only hypothesis, just as the conventional Darwinian role is hypothesis."

Rather than an alternation between two processes, Gould (1985) proposes a three-tiered model of evolution to account for the "paradox" implied by the uncorrelated evolutionary trends across time scales. Evolution at the three tiers (ecological moments, normal geological time, and periodic mass extinctions) is governed by distinct processes with independent "rules and principles" (Gould 1985). Gould (1985) asserts "We will all, I trust, at least allow that creatures cannot prepare for catastrophes of such spacing and that their adaptive struggles at the first tier can therefore, at the very best, only produce exaptations for later debacles." Thus, for Gould (1985), trends over normal geological time are autonomous, and the accumulation of adaptations occurring at the first tier are "reversed, undone, and overridden."

Bennett (1997), with the intention of "linking evolution with ecology" which, in his view had been decoupled by Darwin, adds yet a fourth tier to Gould's three. He envisions different processes acting concurrently over four time scales to account for observed trends within each, and calls his view the "post-modern evolutionary synthesis" (Bennett 1997, p.184). Bennett outlines the relevance of individual lifespans to his arguments (Bennett 1997, p. 176): diurnal and seasonal changes, unlike fluctuations over longer time scales, are predictable and thus may form an organism's "environmental background" (Bennett 1997).

We could continue to accept the addition of discrete tiers as higher-resolution data permits, but a four-tiered process of evolution, while explaining more of the data than a three-tiered model (but perhaps not as much as would a five-tiered model), still falls short of reconciliation. The construction of the concept of a tier is merely a deferral of a description of the way in which natural selection operates which should be comprehensive enough to subsume any number of tiers. Jablonski (1986) concedes that "...a complete theory of evolution must incorporate the different sets of selective and random processes that characterize the background and mass extinction regimes."

BET HEDGING AND THE "SACRIFICE" FALLACY

Environments may vary unpredictably across generations, and a trait that contributes to a high relative fitness in one generation might be inappropriate in another. Under environmental uncertainty, and under circumstances in which adaptive plasticity is not a viable alternative, one might expect selection to produce phenotypes equipped to perform well in an "average" temporal environment: these forms would exhibit the highest average fitness over generations. Genotypes that perform best on average over generations, however, will not necessarily be represented by the highest number of descendants (Gillespie 1973; 1974) because fitness is determined by a multiplicative process. For this reason, the geometric mean (the nth root of the product of n values) is the appropriate measure of fitness under environmental unpredictability across generations (Gillespie 1977). Because the geometric mean is strongly influenced by low values, selection acts to reduce the variance in fitness over generations, even if this entails the "sacrifice" of expected fitness within any given generation. "Bet-hedging" (Slatkin 1974) traits are defined as those that reduce the expected within-generation fitness yet confer a higher geometric mean fitness (Seger and Brockmann 1987; Philippi and Seger 1989).

Bet-hedging theory has been given considerable attention during the decade following reviews of the field by Seger and Brockmann (1987), and Philippi and Seger (1989). Despite the attention paid to bet hedging, misunderstanding endures. This confusion appears to be over bet hedging itself but it is in fact over how selection for bet hedging can be reconciled with selection more generally.

The geometric mean principle is implicitly assumed to be a special case of the way in which natural selection works but, as has been acknowledged, is the general case and applies to all situations, even the implausible situation of complete constancy of environmental conditions through time (Roff 1992 pp. 41-42). In this latter situation, the arithmetic and geometric means just happen to be equivalent but, under temporally variable environments, calculating the simple arithmetic mean fitness over several generations might yield an inappropriate fitness estimate. What has not been acknowledged is that, notwithstanding its status as a defining concept of bet hedging, the notion of a "sacrifice" of expected fitness for geometric mean fitness is a deceptive one. There is no detrimental effect of maximizing the geometric mean fitness and, hence, no tradeoff between the mean and variance in fitness exists; the assumption that expected or within-generation fitness is maximized by natural selection is simply wrong. Therefore, the idea of bet hedging is a conceptual "spandrel"; it exists only because it has been defined.

Although the idea of a tradeoff between the mean and variance of fitness is faulty, the concept of bet hedging should not be discarded. Bet hedging theory says something general and true about the way natural selection operates but then, through the misguided tradeoff idea, specifies a smaller set of circumstances to which it should be applied. The idea of a tradeoff between the mean and variance of fitness has persisted because it reconciles the more accessible view of selection, wherein organisms are selected toward an optimal state for a given environment, with the more accurate view, wherein organisms are selected toward an optimal state given a series of different environments.

The evolution of a bet hedging strategy within a population occurs through the elimination of variants not exhibiting the strategy, even if these variants have the highest expected fitness for an average environment. As an example, consider the evolution of seed germination timing within a season in a hypothetical annual plant (with no seed bank) inhabiting a northern temperate region. Early-germinating seeds have the advantage of a relatively long season in which to grow and reproduce, but they run the risk of being killed by a late spring frost. Because the timing of the last spring killing frost is unpredictable, genotypes with reduced risk of complete failure through the production of seeds with high germination variance (diversification bet hedging) have higher fitness than those whose seeds germinate synchronously immediately following the most likely date of final frost (Simons and Johnston 1997). Nevertheless, as the bet-hedging strategy evolved, selection favored early germination during seasons in which no late killing frost occurred, and favored late germination during seasons of late frost. Furthermore, the evolution of early germination over the time scale of a few generations of early final frost is an adaptive trend over that time scale. If random variation in timing of spring frost continues over several generations, however, genotypes exhibiting the bet-hedging strategy would have the highest fitness. Selection for bet-hedging, then, is attained through the continual "reversal" of adaptive trends over some shorter time scale.

Selection for the geometric mean is the general case even if bet-hedging strategies, as they are defined, do not result. If we accept that the geometric mean principle is the general case over short time scales (and applies also to constant conditions), one might ask why reversals in adaptive trends over macroevolutionary time scales should be regarded as fundamentally different from the continual reversals of trends characterizing microevolution. The answer to this question will be explored along two lines: first, by addressing why bet-hedging principles have traditionally been restricted to short time scales and second, if there is no theoretical justification for the restriction of bet-hedging, by asking whether the components essential in the evolution of bet hedging might be present over all time scales. If all components are present, a rationale that supports discontinuity based on trend reversals observed across time scales is not valid.

GLOBAL BET HEDGING: LIMITS AND REQUIREMENTS

Traditional bet-hedging strategies are maintained through a history of fluctuating selection. Bet-hedging models reflect this variability and are constructed on the basis of a prescribed level of variance characterizing only recent environments. Although not made explicit, this is tacitly justified by a vague appreciation for the diminishing relevance of environments in the distant past; the "genetic memory" of an anomalous event in the distant past will no longer contribute to the maintenance of a contemporary bet-hedging strategy as new mutations and recombination occur and are acted upon by current selection pressures. No genetically based risk-averse strategy for a past anomalous event could persist at the population level indefinitely. By the same token, a current anomalous environmental event outside the range of recent historical variance might cause the extinction of the population because this event is beyond the scope of the bet-hedging strategy's "genetic memory."

Extinction at the population level does not imply, however, that "genetic memory" is no longer relevant and that longer-term bet hedging cannot evolve. Within any clade individuals tend to share certain characteristics, and a selection event will cause differential survival at some clade level. More severe events, because they are selective at deeper phylogenetic levels, act on traits that are more fundamental or embedded than would less severe events. Whereas environmental variation among generations is sufficient to maintain traditional bet-hedging strategies, major changes are rarer, and "genetic memory" for such events might be maintained only at deeper phylogenetic levels. Therefore, deeply-embedded bet-hedging strategies could be persistent even if no genetically-based risk-averse strategy persists at the population level.

During the period in which the validity of the arithmetic vs. geometric mean fitnesses was being debated, Lewontin and Cohen (1969) presented a formal argument showing the absurdity of the use of the arithmetic mean fitness under environmental variability. Their argument can be used to corroborate the above logical argument, and reveals the absurdity of considering environmental variability only over short time scales as being relevant to the evolution of bet-hedging strategies.

Lewontin and Cohen (1969) demonstrate that, even when expected fitness approaches infinity, the probability of extinction in a variable environment may rise to one (Lewontin and Cohen 1969). A bet-hedging strategy, then, has allowed survival through anomalous events even though it may be suboptimal (expected fitness is not maximized) under usual circumstances: individuals well adapted to usual circumstances have been eliminated. The geometric and arithmetic means are identical under environmental constancy, and a consecutive sequence of identical environments would thus select for what would effectively be the arithmetic mean fitness in these environments. Evolution during generations of "normal" variation is set somewhere in a longer sequence of environments, and the probability of encountering ever more deviant environments increases with time. When compared to the longer time scale, the "normal" environments may be considered approximately invariable. If environments are effectively constant over one time scale, however, the arithmetic mean is, in effect, selected for over this time scale. Therefore, from Lewontin and Cohen's (1969) argument, as one considers more generations in the series of environments, the inclusion of anomalous events leads to an increasing probability of extinction. Although such extinction events might be beyond the domain of population genetics, Lewontin and Cohen's (1969) argument holds: survivors necessarily consist of individuals with higher geometric mean fitness than nonsurvivors, even if these survivors might be found only among individuals of other populations, among members of related species, or even other genera. Thus, in principle, there is no reason to restrict the consideration of the evolution of bet-hedging strategies to traits relevant to environmental variance occurring over the short term.

Although the reason that bet hedging has been considered only over short time scales has not previously been addressed and cannot be known with certainty, it appears that such a rationale is not justified. In the absence of a viable argument for restricting bet-hedging strategies to the short term, a case for the existence of longer-term bet hedging can now be made. This will be done through an investigation of the components that would be necessary for the evolution of such strategies.

Analogously to the three components that are required for the traditional view of response to selection at all levels (Jablonski 1987; Vrba and Gould 1986; Williams 1992; Van Valen 1994), three components are required for response to selection for geometric mean fitness and thus produce bet-hedging strategies at all phylogenetic levels: a continuum of severity of environmental unpredictability, differential survival imposed by these unpredictable environments (for which I use the general term "selectivity"), and a heritable component or "phylogenetic nesting" of traits relevant to fitness.

The Continuum of Unpredictability

The description of environmental variability is a difficult undertaking, but it is generally agreed that the probability of an event is in some way inversely related to its severity. The severity of environmental conditions may be implied by the magnitude of extinction events. In the history of life on this planet, five mass extinctions are recognized along with a variable number of minor extinctions (see, for example, Raup 1986; Benton 1995). The minor extinctions, in turn, are set in a "background" of even more minor extinctions. Variation in the severity of the five mass extinctions (Raup 1986; Kitchell 1990; Benton 1995), and among the minor extinctions is pronounced (Benton 1995), which Stigler (1987) used to demonstrate that discontinuity of mass and background extinctions cannot be concluded. Raup (1986) states that "although names may imply that qualitatively different processes are operating, this may not be the case."

Extinction records of 2316 families of marine animals over Phanerozoic time (last 600 my), while apparently episodic, offer no evidence that variation of extinction intensity over the 79 geologic stages does not conform to a Poisson distribution (Fig. 1 in Raup 1986). Benton (1995) presents extinction data using four different metrics of event magnitude. The rank order of importance of events (Raup 1986; Benton 1995) is heavily dependent on the extinction metric used. For example, only two extinction events fall consistently in the top five across the four extinction metrics used by Benton (1995), and at least eight extinction events fall within the top five ranked considering all metrics. A continuum of event magnitudes, though, is apparent regardless of metric. Kitchell (1990) argues that background and mass extinctions cannot be distinguished, and Van Valen (1994) suspects that mass extinctions may be considered the extreme of a continuum of environmental variance; qualitative differences in effects, if they exist, may be a product of biotic thresholds of response (Van Valen 1994).

Some mass extinctions are not caused by a single factor; they illustrate the temporal clustering in geological time of several minor disturbances (Hoffman 1989). By allowing for both a range of single physical processes and for the cumulative effects of coincidental physical processes, the continuity of event intensities is made even more plausible. Temporal environmental change, then, may be viewed as a fundamental influence on evolution from common minor fluctuations within generations (Simons and Roff 1994; 1996), to less common but more severe perturbations among generations (e.g. Gibbs and Grant 1987), to extremely uncommon but catastrophic events (Benton 1995) or coincidences of events (Hoffman 1989).

Selectivity and Phylogenetic Nesting

Besides a continuum of intensities of environmental crises over Phanerozoic time, the bet-hedging perspective's validity depends on both the differential survival of clades and on the existence of heritable variation at higher phylogenetic levels. Selection may operate over a wide range of events (Gould 1989, p.306; Stanley 1984; Jablonski 1986; Raup 1986; Kitchell 1990; Van Valen 1994; McKinney 1997), although a consensus on the relative importance of nonrandom extinction to evolution will become more certain only as more studies which attribute fitness variance to its sources become available. Van Valen (1994) argues that conclusions about selectivity are influenced by the focal time scale of the observer, and that even extreme catastrophes are selective at some level. Raup (1986) affirms, "Most extinction episodes are biologically selective, and further analysis of the victims and survivors offers the greatest chance of deducing the proximal causes of extinction."

The term, "phylogenetically nested" (sensu McKinney 1997) will be used as an inclusive term implying the presence of heritable variation of a character, from the correlation among siblings within populations, to resemblance among members of a monophyletic lineage at any higher taxonomic level. Heritable variation may occur at any clade level (Williams 1992) regardless of the issue of trait emergence at that level (Van Valen 1994; but see Vrba and Gould 1986 for contrasting view).

McKinney (1997), in a comprehensive review of the empirical literature on selectivity, concludes that extinction vulnerability is "rarely random," and that there is considerable consistency of traits influencing differential survival across temporal scales. He compiles data on over 20 traits, 15 of which are individual-level traits which have been associated with patterns of selectivity over both ecological and geological time scales. Nonrandom extinction vulnerability is ascribed to phylogeny in a diversity of taxa including bird and plant families, echinoids, and fossil mollusks (McKinney 1997).

Anstey (1978), using 398 specimens of 123 Paleozoic genera of bryozoans, determined the relationship between extinction probability and character complexity based on 150 binomial character states. Complex taxa had higher overall relative extinction rates, but this extinction is attributable almost exclusively to the mass extinction events. Simple taxa, then, had relatively high survival during mass extinctions.

Three traits have been specified as conferring extinction-resistance on mollusks during late-Cretaceous background times: planktotrophic larval development, species richness, and species-level broad geographical range (Jablonski 1986). During the late-Cretaceous mass extinction, however, only broad geographic range is associated with diminished extinction proneness (Jablonski 1986), and selectivity could be demonstrated to occur only at deeper phylogenetic levels than it did during the preceding background extinctions (Jablonski 1986). Extinction is selective both during background and mass extinctions and, although Jablonski (1986) sees selectivity during mass extinctions as often "indifferent" to adaptations accumulated during background times, some continuity exists (Jablonski 1995). Jablonski (1995) cites two examples: the bivalve order Pholadomyoida suffered extensive extinction during the end-Permian event but was not vulnerable to the conditions of the end-Triassic; and mollusks showed "remarkably" low extinction during the Pleistocene climate changes which may have resulted from the previous elimination of vulnerable forms during the Pliocene (Jablonski 1995).

The survival of obligate autotrophic planktonic diatoms through the global darkness of the end-Cretaceous mass extinction was a well-known "enigma" until the work by Kitchell et al. (1986). Kitchell et al. (1986) were able to attribute survival of the autotrophs to a resting spore stage which had evolved in response to local environments of low light during background times. The resting spore stage is a phylogenetically nested trait restricted to the centric diatoms (Kitchell 1990). Similarly, terrestrial plant survival in the Northern Hemisphere during the Cretaceous-Tertiary event was dependent on the ability to enter a dormant stage through deciduousness, a perennating organ, or a seed bank (Spicer 1989).

Whereas phylogenetic nesting of clade-level variation is most readily studied for extinction events, it has also been argued that threatened status, and presumably extinction risk, of modern bird species is phylogenetically nested within families (Bennett and Owens 1997). The "filtering" effect of environmental disturbance in a biota's history may account for present day differences in vulnerability (Balmford 1996). Coope (1995), on the subject of insects' capacity to avoid climatic extremes, compares selectivity to a hurdle race; "any species that could leap the first gate could similarly leap the rest."

In summary, extinction events of any scale are neither entirely random nor selective. Differential survival, both over longer and shorter time scales, is attributable to individual-level traits (Ward and Signor 1983; Kitchell 1990; McKinney 1997) that tend to be phylogenetically nested (Ward and Signor 1983; McKinney 1995; 1997). A continuity of environmental variance, and selection on heritable traits at all phylogenetic levels means that the geometric mean principle should be applied to evolution occurring at all time scales. It is important to point out that the global bet hedging view is not a hypothesis about the existence of bet hedging traits; it is a perspective that readily incorporates bet hedging as a possible outcome. In addition, it should be noted that an exception to the geometric mean principle is the case of spatial and spatio-temporal variation with dispersal. Although spatio-temporal variation with dispersal must be considered, its importance is offset by the fact that more severe events also have more widespread effects. Surviving clades at any level may be bet hedgers in precisely the same way as are traditional bet hedgers; survivors may have "sacrificed" arithmetic mean for the geometric mean fitness.

DISCUSSION

Bet hedging at deep phylogenetic levels, rather than being an analogy, corresponds exactly to processes at the population level. At the population level, two kinds of bet hedging are recognized: diversification and conservative (Seger and Brockmann 1987; Philippi and Seger 1989). Diversification, such as polymorphism in seed germination behavior (Cohen 1966), is observable within a generation and is thus easier to recognize than is a potential conservative strategy. The mechanisms underlying the generation of traditional diversification strategies are poorly known (Cooper and Kaplan 1982; Simons and Johnston 1997), and it is unknown whether mechanisms exist at the clade level whereby diversification bet hedging could evolve. Some clades, however, seem to be characterized by consistently high rates of speciation and extinction, perhaps related to ecological amplitude or dispersal (Dennis et al. 1995), and such clades have been referred to as "boom and bust" (Anstey 1978; Jablonski 1986) or macroevolutionary *r*-strategists (Anstey 1978). Although this evidence is suggestive of clade-level diversification, the existence of long-term conservative strategies is more compelling.

A population will not go extinct if at least some members exhibit the appropriate phenotype for a given environment. An entire clade would be extinguished, though, upon encountering a particularly severe environment which none of its members are equipped to endure. Therefore, the deeper the phylogenetic nesting of a candidate conservative trait in an extant clade, the greater the probability that it is adequate under any circumstance even if it is not the most appropriate under any circumstance. For example, that the production of chlorophyll has evolved to depend on the availability of zinc does not imply that, for the present environment, chlorophyll production based on zinc is optimal.

Adaptation is constrained by various factors (Williams 1992 pp. 72-88) including phylogenetic history. The exact relationship between the ideas of optimality and phylogenetic constraint is elusive. Stearns (1982) describes organisms as "a mosaic of relatively new adaptations embedded in a framework of relatively old constraints" adding that the selective mechanisms underlying constraints deserves attention (Stearns 1982). If a fundamental characteristic of environments is that they are variable, the optimal trait, even locally, should be considered to be that which maximizes the geometric mean fitness. Global constraints, however, may have been produced by variable selection and should, by the same criteria, be considered potentially optimal: there is no correct time scale for assessing fitness. Thus, an implication of the bet-hedging perspective is that the evolution of an optimal trait at one time scale might be constrained by optimality over a greater time scale. This perspective exposes a close relationship and possible ambiguity between phylogenetic constraint and optimality.

The resting spore stage of diatoms provides a convenient illustration of the potential confusion between optimality and constraint. Although recently evolved families are not spore forming (instead forming a resting cell) (Simonsen 1979), older groups retain the primitive character, for which Kitchell (1990) provides two possible explanations, one genetic and one ecological: "spore formation may be genetically linked to other indispensable aspects of cell metabolism..." or "...unfavorable environmental conditions may be sufficiently frequent...that many phytoplankton have retained the primitive life history strategy." These explanations, though one is based on constraint and the other is adaptive, are identical: both explanations rest on the embedding of a trait as a result of selection over a particular time scale, but the trait is regarded as an adaptation from one perspective and as a constraint from another. In the field of animal behavior, suboptimal behavior under short-term environmental variability has been explained by "coarse behavior rules" that are optimal over "extended uncertainty" (Bookstaber and Langsam 1985). A potential example of a very deeply embedded conservative trait is the ubiquitous secondary structure of protein that is considered suboptimal, but was probably appropriate in the prebiotic environment (Mróz and Latajka 1997).

The global bet-hedging view of evolution should not be reduced to the expectation of generalist strategies, although long-term trends away from specialization are sometimes observed. Some traits conferring high relative extinction risk may be interpreted as enhancing specialization (McKinney 1997); traits such as temperature specialization (Stanley 1984), diet specialization, symbiosis, and complex morphology. Results of Anstey's (1978) analyses (see above) are particularly suggestive of short-term selection for specialization countered by long-term selection for generalist strategies. Character complexity can arguably be interpreted as degree of adaptive specialization in this group (Anstey 1978). Between mass extinctions simple forms were outperformed by complex genera, but their long-term fitness is greater once mass extinctions are accounted for.

It would be illogical to claim Anstey's (1978) findings as evidence that selection acts against specialization over the long term. The process of evolutionary change, from changes in allele frequency to extinction events, is a constant interplay between the inevitable adaptation to environmental parameters if they are constant at one temporal scale, and the elimination of those forms which have specialized to these constant environments if it turns out that the constancy is itself transitory, and the parameters prove to be unreliable on any larger scale. This interpretation implies that patterns of selection should exhibit self-similarity over different phylogenetic levels and time scales. Raup (1986) makes the intriguing conjecture that survivorship might have a fractal quality: self similarity of plots of survivorship are apparent when species survivorship on the scale of about 38 My is compared to survivorship on the family level over about 300 My.

True plant extinctions during the end-Cretaceous event seem to be the result of "the loss of specific environments to which a particular group or groups have become irreversibly specialized" (Spicer 1989). As Foote (1993) notes, no single pattern of evolution of generalist strategies exists among trilobites. Explanation of patterns is dependent on time scale and such patterns reflect a diversity of causes; the decline of a clade may or may not be accompanied by a concurrent reduction in morphological complexity (Ward and Signor 1983; Foote 1993). One exceptional trilobite lineage of the five studied shows little morphological variation through its history, suggesting that it is a "morphologically conservative group" (Foote 1993). This conserved clade is the sole survivor through the post-Devonian decline (Foote 1993). Conversely, clades might repeatedly evolve traits inappropriate for events over a longer time scale if genetic memory decays. The evolution of keeled from unkeeled planktonic foraminifera has occurred at least 16 times (Norris 1991), but the unkeeled species invariably survive the mass extinctions to once again found new species of keeled species (Norris 1991). These examples are not meant as evidence of bet hedging at deep phylogenetic levels, they are meant only to demonstrate how cases of apparent discontinuity are readily accepted under the bet-hedging perspective.

It has been assumed that if similar processes operate over all time scales, long- and short-term trends should not be in opposition. Many experts in the study of macroevolution, because of reversals in phenotypic trends observed in the fossil record, believe microevolution and macroevolution to be "decoupled" (Stanley 1975); that selection is characterized by alternating regimes (Jablonski 1986) and operates constructively or destructively (Raup 1986) depending on the time scale or "tier" (Gould 1985; Bennett 1997), and believe the opposite trends across tiers to be a "paradox" (Gould 1985) resolved only by accepting a hierarchical structure (Vrba 1980; 1983; Vrba and Eldredge 1984) of autonomous processes operating over these tiers (Gould 1985). However, a fundamental property of evolutionary trends is that they are continually being "reversed" from the viewpoint of any unit of selection. Extinction is a "fundamental cause" of evolution (Van Valen 1994) whether selection acts in similar or opposite directions during different events, just as intergenerational fluctuations of random direction select for bet hedging.

CONCLUSIONS

This paper is meant as a vehicle to expose the repercussions of a point that has previously been acknowledged yet has been ignored: that selection for a bet-hedging strategy is not a special case of the way in which natural selection operates. I have argued that ignoring this contention has led to a misconception now firmly embedded in bet hedging theory and may be responsible, at least in part, for the apparent confusion about the meaning of bet hedging. Furthermore, this tacit conviction, that the kind of selection that operates to produce bet-hedging strategies is exceptional, is a hindrance to recognizing the full importance of the axiomatic principles of bet-hedging theory as relevant not only to a broad range of microevolutionary studies, but also to macroevolution. The perspective of continuity through bet hedging has been prevented jointly by the failure of microevolutionary biologists to recognize the general applicability of the geometric mean principle and by the adherence of macroevolutionary biologists to a multi-tiered view of Earth history. The same selective forces that lead to bet-hedging strategies govern the evolution of traits that are not currently subsumed under the title of bet hedging, and the treatment of environmental variability as a continuous variable, spanning minor fluctuations to catastrophic events, in models of selection at all time scales leads to a more self-consistent view of evolution, one that should be acceptable to both paleontologists and population geneticists.

Gould (1980) predicts a unified theory that is "rooted in a hierarchical view of nature...It will possess a common body of causes and constraints, but will recognize that they work in characteristically different ways upon the material of different levels..." A new unified theory is neither forthcoming nor necessary. Mass extinctions, "so utterly beyond the power of organisms to anticipate" (Gould 1984) comfortably take the extreme position on the continuum of perturbations, none of which is anticipated: adaptation, although judged from the present, is always a relic of the past. A depiction of evolution based on environmental unpredictability coupled with genetic memory manifested concurrently at all levels of biotic organization offers a parsimonious perspective on selection operating over all time scales. Under this interpretation, the dichotomy between changes in allele frequency and extinction events vanishes. Although issues such as those over evolutionary rates remain unresolved, micro- and macroevolutionists are united by the proposed perspective on the issue which Gould (1985) describes as the central point of the debate over punctuated equilibria. Claims of qualitative differences in the process of natural selection depending on the severity of selection events become unnecessary and, therefore, should be bid a tierful goodbye.

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