

Delusions of grandeur: Seed count is not a good fitness proxy under individual variation in phenology

Lina Wen | Andrew M. Simons 

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

Correspondence

Andrew M. Simons, Department of Biology,
Carleton University, 1125 Colonel By Drive,
Ottawa, ON, K1S 5B6, Canada.
Email: andrew_simons@carleton.ca

Funding information

Natural Sciences and Engineering Research
Council of Canada, Grant/Award Number:
RGPIN-2015-03922

Abstract

The concept of fitness is central to evolutionary biology, yet it is difficult to define and to measure. In plant biology, fitness is often measured as seed count. However, under an array of circumstances, seed count may be a biased proxy of fitness, for example when individuals vary in allocation to sexual versus asexual reproduction. A more subtle example, but also likely to be important in natural populations, is when inter-individual variation in conditions during development results in variation in offspring quality among seed parents. In monocarpic (semelparous) plants, this is expected to result from variation in effective season length experienced among individuals that reach reproductive maturity at different times. Here, we manipulate growing season length to ask whether seed count is an accurate representation of parental fitness in the monocarpic herb *Lobelia inflata*. Simple seed count suggests a paradoxical fitness advantage under constrained-season length. However, we find that the apparent fitness advantage of a constrained-season length is overridden by low relative per-seed fitness. Furthermore, the fitness deficit in the constrained environment is associated primarily with an accelerating decrease in viability and seedling survival in seeds derived from fruits produced progressively later in the season. In this study, the overall fitness value of a seed under a constrained season is 0.774 of that observed under a long season.

KEYWORDS

environmental variation, fitness measures, inbreeding, offspring number, offspring quality, phenology, seed count

1 | INTRODUCTION

The concept of fitness is central to evolutionary biology, yet is notoriously difficult to define (c.f. Endler, 1986; de Jong, 1994; McGraw & Caswell, 1996). From an empirical perspective, fitness estimation is important to studies addressing key questions about life history

evolution (Endler, 1986; Roff, 2002; Stearns, 1992) and about adaptation in general (de Jong, 1994; Williams, 1966). Fitness, if understood to be the 'population growth rate of the individual' (McGraw & Caswell, 1996), can be difficult to measure, and empiricists often use fitness components such as survival, offspring number or mating success as proxies (Lande & Arnold, 1983; Roff, 1992). Assumptions

The peer review history for this article is available at <https://publons.com/publon/10.1111/jeb.13633>

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2020 The Authors. *Journal of Evolutionary Biology* published by John Wiley & Sons Ltd on behalf of European Society for Evolutionary Biology

underlying the association between fitness and proxies of fitness are often not tested, and their reliability as estimates of fitness is generally unknown (Lande & Arnold, 1983; McGraw & Caswell, 1996; Mousseau & Roff, 1987; Thoday, 1953).

The total number of seeds produced, or fecundity, is a commonly used fitness proxy in plant evolutionary ecological studies (Aarssen, 2014; Côté & Simons, 2020; Pan & Price, 2002; Wen, 2019). There are good reasons for this: seed number is generally easily obtained either through direct count or through estimation based on fruit count (Primack & Kang, 1989). Furthermore, seeds are a defining characteristic of gymnosperms and angiosperms, and are thus the most readily recognized structures that propagate genetic material across generations (Pan & Price, 2002).

However, the relationship between fitness and seed count may be weakened or dissociated under several natural scenarios including those detailed more fully in Wen (2019): some plant species can propagate both asexually—for example vegetative propagation—and sexually (Aarssen, 2014; Antonovics & Ellstrand, 1984), and seed count would thus provide a biased estimate of fitness when individuals invest differently in asexual and sexual reproduction; plant architecture may influence the relationship between fitness and seed count through factors such as dispersal or pollination success (O'Connell & Johnston, 1998); success as a male parent in outcrossing species is not reflected in an individual's seed count (Devlin, 1989); trade-offs between allocation to reproduction and defenses against herbivory may exist (Agrawal, Strauss, & Stout, 1999); individuals that allocate more energy to reproduction are expected to suffer lower residual reproductive value (Miller, Williams, Jongejans, Brys, & Jacquemyn, 2012); variation among individuals in the proportion of self-fertilized and outcrossed seeds may result in fitness variation due to inbreeding depression (Johnston & Schoen, 1996); individuals may express apparently low fitness as seed count as a result of a conservative bet-hedging strategy that maximizes fitness over the longer term (Simons, 2011); and finally, plastic adjustment of seed production observed to result from variation in flowering phenology among individuals (Hughes & Simons, 2014a) may bias the fitness–seed number relationship through variable seed quality (Galen & Stanton, 1991). Here, by manipulating seasonal constraints, we investigate the reliability of seed count as a fitness measure under this final scenario of variable phenology.

Phenology, or the seasonal timing of life history events and transitions, may have important fitness effects (Rafferty, CaraDonna, Burkle, Iler, & Bronstein, 2013; Weis, Turner, Petro, Austen, & Wadgymer, 2015; Zimmerman & Gross, 1984). For instance, in temperate insects, seasonal timing of egg hatch determines whether the larval stage coincides with optimal conditions for feeding, development and growth (Sniegula, Golab, & Johansson, 2016). Similarly, constraints on flowering phenology can impose fitness costs by causing asynchrony between flowering and pollinating insects (Forrest, 2015) or by influencing the allocation of resources to growth versus reproduction (Austen & Weis, 2015). Such variation in reproductive phenology may be caused by environmentally induced differences in germination and development. In the monocarpic

perennial, *Lobelia inflata* (Campanulaceae), the variable timing of seed germination within seasons results in variation in the phenology of reproductive phase or 'bolting' (Simons & Johnston, 2003). The phenology of this transition to reproduction is critical, because in semelparous plants, there should be strong selection for reproduction that completely exhausts resources prior to the end of the growing season (Hughes & Simons, 2014c; Simons & Johnston, 2003).

Previous work on this species demonstrates that individuals respond to variation in reproductive phenology using season length cues by adjusting post-bolting reproductive allocation patterns (Hughes & Simons, 2014b). Specifically, late-bolting individuals that experience a more constrained-season length respond plastically with a corresponding more 'extreme' semelparity (Hughes & Simons, 2014a): they express reduced time to first reproduction, smaller size at reproduction and multiplication of reproductive organs through increased branching (Hughes & Simons, 2014a). Remarkably, individuals experiencing a constrained-season seem to compensate and do not suffer the expected reduction in seed number (Hughes & Simons, 2014a). However, like in most studies of plant fitness, offspring quality was not assessed. A fuller understanding of the effect of variable phenology on the relationship between seed count and fitness requires an assessment of offspring quality because high seed production may not translate into high realized reproductive success.

The monocarpic herb, *Lobelia inflata*, makes an excellent model system for the study of effects of phenology on seed count and fitness for several reasons. First, as mentioned above, higher than expected seed production was observed in an earlier study when season length was experimentally shortened (Hughes & Simons, 2014a). Second, *L. inflata* is semelparous, and lifetime reproductive success can be observed during a single growing season in nature. Third, *L. inflata* has a simple acropetal flowering pattern, where fruits form sequentially along inflorescences, making it possible to track the packaging of reproductive effort through the growing season (Simons & Johnston, 2000a). Fourth, the populations under study are completely self-fertilizing (determined through microsatellite analysis; Hughes & Simons, 2015); therefore, the effect of mating system on genetic load found in some species can be ruled out as an explanation of fitness differences among siblings; and, critically, total reproductive success can be directly determined because *L. inflata* reproduces exclusively by seed.

In this study, we use *L. inflata* to test the hypothesis that inter-individual variation in phenology results in a disparity between seed count and fitness. Specifically, we hypothesize that a constrained-season length experienced by later bolting plants will result in a more extreme semelparous ('big bang') reproductive strategy (Hughes & Simons, 2014a) that trades off seed quality for seed number, thus biasing seed count as a measure of true fitness. In an observational study, later bolting could be correlated with other factors such as greater resource acquisition prior to bolting, which would confound the effect of phenology on realized fitness. We therefore manipulate phenology through post-bolting season lengths to preclude the possibility of confounding factors influencing timing of bolting. This

manipulation was performed by splitting newly bolted plants between a constrained and a long simulated season. Furthermore, the purpose was solely to elicit a response to season length; we do not ask to what extent temperature, photoperiod and their interaction affected response. We note that in the field, other variables may also provide cues of season length.

To determine individual parental fitness, we then assessed constrained and unconstrained plants as both a direct seed count and parental 'realized fitness' adjusted for offspring quality based on germination success and seedling survival under common conditions. We used slightly stressful, saline germination conditions to reveal underlying fitness variation that would otherwise be masked by benign laboratory environments and would inflate germination and survival rates. Offspring quality was measured both at the whole-plant level and as patterns of reproductive allocation to fitness among fruits within individuals to test whether any general disparity between seed count and fitness occurs uniformly or changes across sequential fruits, and whether this pattern differs between the constrained and full season. If the expected fitness return on a seed differs depending on interindividual variation in phenology, seed count should be used cautiously as a measure of fitness.

2 | MATERIALS AND METHODS

2.1 | *Lobelia inflata* and field collection

L. inflata is a monocarpic herbaceous perennial native to North America that grows well in disturbed and sandy areas. Seeds of *L. inflata* in the Ottawa area typically germinate in the spring and early summer and form basal rosettes (Bowden, 1959). *L. inflata*, once germinated, has two distinct phases of life: the vegetative rosette stage in which resources are accumulated, terminating with the transition to the reproductive stage and the formation of a flowering stalk ('bolting') in the first or second season (Bowden, 1959). Reproduction occurs in an acropetal pattern (i.e. from basal to distal positions) along the stalk, with most plants producing between 10 and 100 fruits, and many thousands of seeds, over the course of the reproductive season (Hughes & Simons, 2014b). Bolting is triggered by light intensity and photoperiod (day length), is irreversible and has to meet a threshold for rosette size that changes with time of year (Simons & Johnston, 2000b). The timing of bolting directly affects fitness; bolting late in the season decreases the time available for a plant to reproduce. Alternatively, the rosette may increase its effective season length by overwintering and bolting the following spring (Simons & Johnston, 2003). Upon fruit maturation (the browning of fruits), seeds are passively dispersed and the plant senesces.

Individuals appear to be exclusively self-fertilizing, resulting in complete homozygosity after a few generations (Hughes & Simons, 2015). A closed tube of fused anthers ensures self-fertilization and acts as a barrier to pollen release; pollen is released directly onto the stigma of the same flower. Recent research using nuclear microsatellite markers in *L. inflata* supports this assumption (Hughes

& Simons, 2015), with no observations of outcrossing or heterozygosity in the populations studied.

Our principal focus is on the comparison of patterns of fitness expression within individuals in contrasting environments rather than on genotypic or among-individual fitness expression. Thus, mature fruits of *L. inflata* were collected from ten field-collected individuals separated by an average distance of 6.6m and minimum distance of 2m in Gatineau Park, Quebec (Lat. 45°29'N, Long. 75°50'W). These ten seed 'lineages' were used to found the experimental plants.

2.2 | Season length manipulation

To produce parental plants for the study, 100 seeds from each field-collected lineage were placed in 10 replicate 60 × 15 mm Petri dishes lined with moistened 5.5-cm doubled filter paper and were cold-stratified for 14 days in darkness at 5°C. Seeds were then placed in Biochambers SG-30 seed germinators set to a 12-hr/12-hr day/night light regimen at 20°C with 80% humidity until germination.

Seedlings of each lineage germinating over a narrow time window were planted at randomized positions in cells (4 cm × 4 cm) of 32-celled trays with autoclaved Promix BX soilless growth medium. Trays were placed in a Biochambers/AC40 growth chamber set to a 16-hr/8-hr day/night photoperiod at 24°C to induce rosette growth. Trays were watered every 3–4 days as needed, and a 15-5-15 liquid fertilizer mixture was added every two weeks. Following 60 days of vegetative growth, rosettes were subjected to a 31-day vernalization treatment at 5°C in the dark to induce flowering. Only plants that bolted following vernalization were included in the study.

Effective season length variation among individuals in natural populations results from the initiation of bolting over an extended period beginning as early as late May through July, and flower and fruit production typically ends by October (Simons & Johnston, 2003). Manipulation of phenology (and thus season length) was accomplished by inducing bolting in experimental plants, and splitting these plants between a growth chamber environment that then provided cues of a constrained-season length (short photoperiod, low temperature), and one that simulated a long season (long photoperiod, high temperature). Previous work with this species—in which newly bolted rosettes transplanted from growth chambers into field plots in 4 different batches throughout the growing season—demonstrated that this method of season length manipulation is effective: individuals showed strong plasticity to time remaining in the season (Hughes & Simons, 2014). Thus, two contrasting experimental environments were set up, with a 'long' season growth chamber representing average early summer photoperiod and temperature conditions (16.5-hr/7.5-hr day/night photoperiod at 24°C) and a 'constrained' season chamber representing late-season photoperiod and temperature conditions (12-hr/12-hr day/night photoperiod at 20°C).

Thirty-six newly bolted rosettes were split equally into two groups for designation to the long and constrained experimental environments. Given that the goal was to study the effect of

season length on within-individual allocation patterns, the primary criterion for designation into the two groups was to ensure no initial difference in rosette size (constrained $\mu = 11.6 \pm 0.66$ mm; long $\mu = 12.3 \pm 0.63$ mm; $df = 1$, $F = 0.58$, $p = .452$) and, secondarily, to maximize replication of original genotypic lineages between the two environments (16 of 18). All bolted individuals were monitored until senescence or the browning of all fruits. Mature fruits were collected, including all fruits on both the main stem and branches, if branching occurred.

2.3 | Trait assays

To characterize trait expression in the two contrasting manipulated environments, traits at both the whole-plant (plant size, fruit number) and within-plant (fruit sizes, and fruit-specific seed size and number) levels were assessed for each individual. Plant size was measured as final plant stem height and as final stem diameter 2cm from the base of the stalk using digital vernier calipers (± 0.01 mm). Fruits on the main stem and branches were counted, and the diameter of each fruit was measured using digital vernier calipers (± 0.01 mm). The chronological order of fruit production (starting from the earliest fruit) is noted by position along the main raceme. Since it is difficult to determine the temporal order of fruits on branches from position, only the fruits on the main stem (1,096 of 2,673) were used in any analyses that included fruit position effects. Total fruit number per plant ranged from 11 to 150.

Seed number was sampled from a subset of fruit positions for each individual. Because total fruit number varies among individuals, fruit position was scaled to relative position (5th, 50th and 95th percentile). Seed number at the 5th, 50th and 95th percentile positions was determined by manual count under a light microscope. Manual seed counts were taken for 108 fruits over the full fruit size range. Because there is a consistent relationship between fruit size and seed number in the two environments (fruit size*environment interaction, $F = 0.027$; $p = .87$), a general relationship was obtained by regressing seed number on fruit size for all plants: seed number = $944.9 \times \text{Fruit size(mm)} - 231.1$ mm. This equation was then used to estimate the total number of seeds per plant, based on counts and measurements of every fruit for all individuals. Seed size was measured by (a) collecting thirty seeds from each fruit at the 5th, 50th and 95th percentile position along the raceme; (b) imbibing seeds on moistened filter paper-lined Petri dish for 24 hr; and (c) measuring seed width on stored images captured under a dissecting microscope using Image J 1.51.

Under laboratory conditions, seed fitness (e.g. germination success, seedling viability) is artificially inflated (St Juliana & Janzen, 2007), and true underlying variance in fitness will not be observed. Salinity is known to be a major abiotic stress, affecting plant growth and productivity (Hannachi & Van Labeke, 2018; Vu, Chang, Moriuchi, & Friesen, 2015) through factors including osmotic and oxidative stress. This stressor results in delayed germination and seedling mortality (Ibrahim, 2016; Papastilianou, Baokogianii,

Travlos, & Roussis, 2018) and is meant to represent a generalized stress rather than one that occurs in the field. Therefore, to unmask variation in seed fitness under laboratory conditions, salinity of 0.01 M NaCl was used as an abiotic stress, a concentration found to maximize survival variance in preliminary studies (data not shown).

To assess germination success, thirty cold-stratified seeds collected from each fruit position (5th, 50th and 95th percentile) of each of the 36 individuals were imbibed in water for 24h. Ten seeds were then placed in each of three replicate 60×15 mm Petri dishes on a double layer of filter paper, watered with a solution of 0.01 M NaCl and distilled water and allowed to germinate under a continuous 12-hr/12-hr day/night photoperiod at 20°C and 80% humidity in seed germinators. Germination was assessed daily and was defined as the protrusion of the radicle through the testa. Total germination percentage was recorded, and germination fraction was arcsine-square root transformed prior to analysis. Seedling success and survival were assessed as proportions (for each fruit and each individual); however, to determine the effect of seed size on fitness, seeds from all three positions of six of the 36 individual plants were individually tracked by placing seeds individually on labelled hole-punched 'islands' of filter paper. To distinguish viability from dormancy, nongerminating seeds were exposed to a no-salt germination trial after a second stratification.

To assess seedling survival, seeds that successfully germinated were transferred to 72-celled trays with autoclaved Promix and placed in a Biochambers/AC40 growth chamber set to a 16-hr/8-hr day/night photoperiod schedule at 24°C. Seedlings were initially treated with a solution of 0.01 M NaCl and distilled water and were then watered with only distilled water. Seedlings were observed daily for survival for 5 days and then every other day for an additional 20 days. Survival fraction was arcsine-square root transformed prior to statistical analysis.

2.4 | Statistical analyses

Analyses were performed in three stages: first, comparisons of the expression of fitness components (plant size, fruit number and size, seed number and size) at the individual level between two environments; second, patterns of allocation across fruits within and across environments; and finally, comparisons of realized fitness across the two environments that incorporate germination success and seedling survival. First, to assess differences in expression across environments, means for each trait were compared using the individual as the unit of replication. False discovery rate (FDR) was used to correct for multiple comparisons. To test for allocation patterns across the three main fruit positions in the two environments, a two-level factorial ANOVA included the effect of fruit position, environment and their interaction on each trait. Tukey's HSD tests were used to assess where differences among levels occur. Because we have individual fruit size data for all main-stem fruits, the proportion of reproductive allocation across fruits in the two environments was investigated further. A measure of an individual's proportional

reproductive allocation across fruits was obtained by standardizing to a mean fruit size of 1 for all main-stem fruits within each individual. An ANCOVA was then used to model the effect of relative fruit position, environment and their interaction on this standardized fruit size. Patterns of allocation in the two environments were further explored with linear and quadratic regression. Patterns of seed number and seed size across fruits were modelled in the same way as performed above for fruit size across the three main relative fruit positions.

To assess realized fitness, a two-factor ANOVA was used to model the effect of environment and fruit position (for the three positions) on germination fraction and seedling survival. Significant effects were followed by Tukey's HSD post hoc tests to establish where differences occurred among levels of both factors. To determine the extent to which the effect of fruit position on fitness can be accounted for by seed size within fruit, a mixed effects logistic regression was used, with seed size nested within fruit position, on germination and survival fractions. All statistical analyses were performed in R 3.4.2.

The effective fitness value of a single seed produced in the constrained environment relative to one produced in the long environment was calculated based on observed germination and survival

fractions across all of each individual's fruits weighted by number of observed seeds. Finally, the total expected fitness for individuals, without considering seedling success (i.e. purely counting seeds), was compared to realized fitness taking germination and survival into consideration. This was calculated using all fruits on the main raceme of all individuals in the two environments, as well as using all fruits of individuals under the assumption that the fruit size–seed survival relationship holds for fruits on branches.

3 | RESULTS

Overall, plants in the constrained and long growth chamber environments did not differ in height (constrained $\mu = 27.5 \pm 1.3$ mm; long $\mu = 29.6 \pm 1.7$ mm; $t_1 = 0.99$; $p = .33$) or stem diameter (constrained $\mu = 2.13 \pm 0.12$ mm; long $\mu = 2.41 \pm 0.16$ mm; $t_1 = 1.39$; $p = .175$). Several reproductive traits differed in the two environments (Figure 1): individuals in the constrained-season length environment produced fewer ($F_{1,35} = 14.23$, $p < .001$), but larger ($F_{1,107} = 90.3$, $p < .001$) fruit, that each contained more seeds ($F_{1,107} = 91.0$, $p < .001$). Total seed number per individual in all main-stem fruits—estimated using the observed relationship

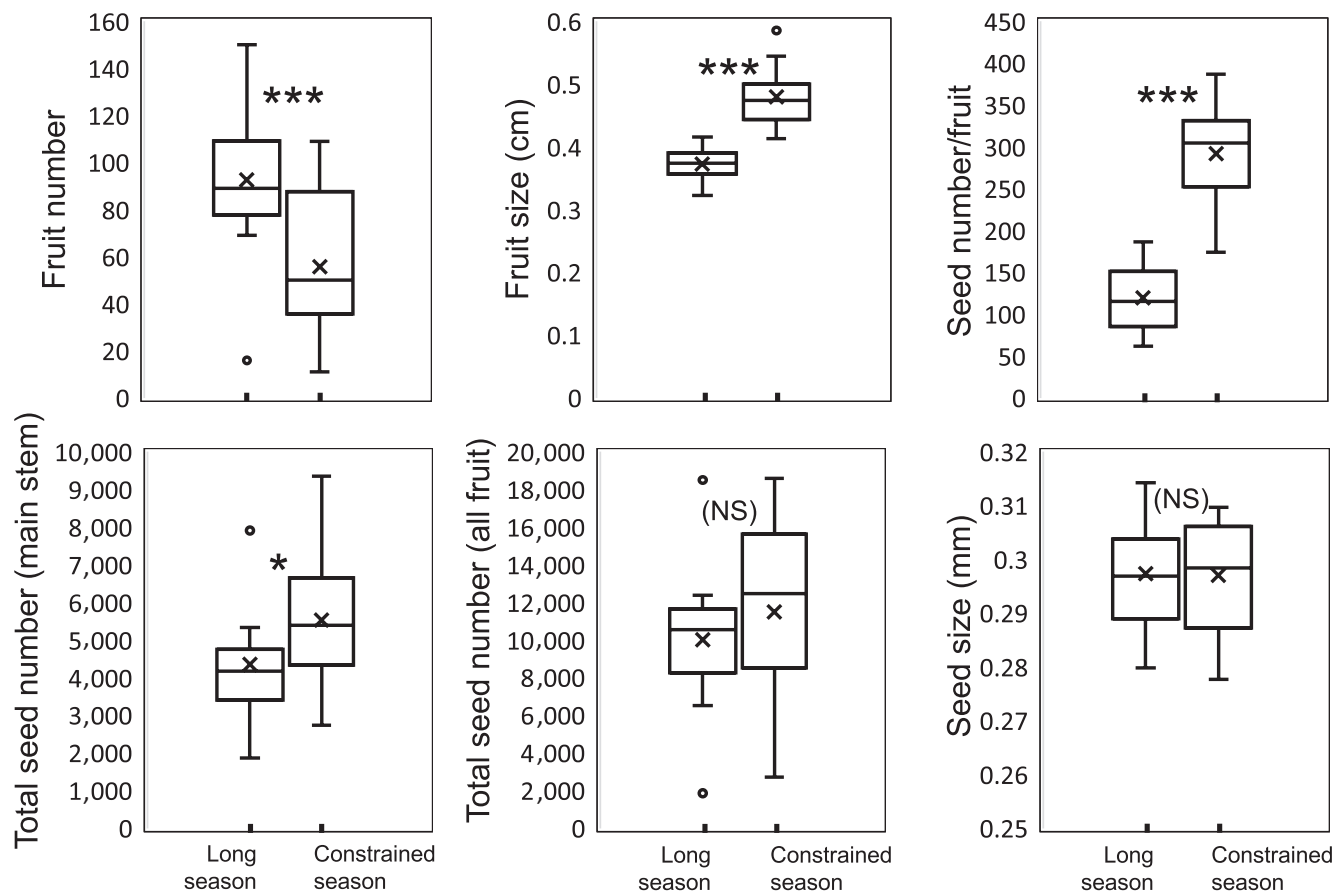


FIGURE 1 General comparison of reproductive trait expression measured at the whole-plant level in *Lobelia inflata* observed under manipulated long-season and constrained-season conditions. Boxplot midlines are medians, exes are means, and boxes contain 25th to 75th quartiles. Datapoints plotted outside whiskers (1.5 times interquartile range) are considered outliers. (***) $p < .001$; (**) $p < .01$; (*) $p < .05$; NS, $p > .05$)

between fruit size and seed number (Figure 1)—differed significantly between the constrained and long-season environments ($F_{1,35} = 4.70$, $p = .037$), but total seed number including branch fruits did not ($F_{1,35} = 1.18$, $p = .286$). No difference in seed size was found between the long-season and constrained-season environment at the whole-individual level ($F_{1,35} = 0.005$, $p = .946$; Figure 1). Fitness, however, is influenced by patterns of reproductive allocation through time (across fruits) and its effects on the relationship between seed quantity and quality.

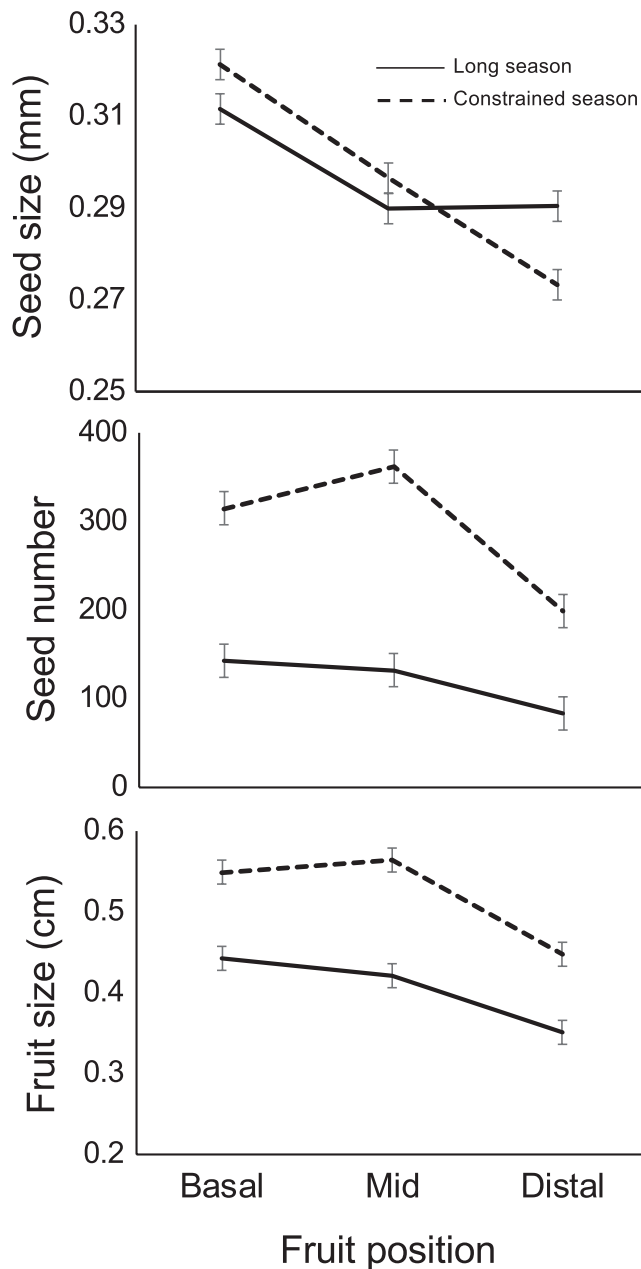


FIGURE 2 Effect of fruit position and environment on seed size, seed number and fruit size. Both fruit size and seed number—often used as fitness proxies without accounting for realized seed fitness—are greater in the constrained (dashed line) than in the long (solid line) environment. A strong environment \times fruit position interaction is observed for seed size. Error bars are $\pm 1SE$

3.1 | Patterns of allocation across fruit

A two-factor ANOVA using raw measurements confirms an overall fruit size difference between environments ($F_{1,102} = 90.9$, $p < .001$) reported in Figure 1, and also among the three relative positions (Figure 2; basal $\mu = 0.496 \pm 0.013$ mm, mid $\mu = 0.493 \pm 0.017$ mm, distal $\mu = 0.399 \pm 0.013$ mm; $F_{2,102} = 27.33$, $p < .001$), with no significant position–environment interaction effect ($F_{2,102} = 1.36$, $p = .262$). A post hoc Tukey's HSD test reveals that fruits at the most basal position were larger than fruits at the most distal position. However, the ANCOVA using individually standardized reproductive allocation across all main-stem fruits indicates, besides a significant effect of position ($F_{1,1092} = 31.1$, $p < .001$), a significant interaction between environment and fruit position ($F_{1,1092} = 6.57$, $p = .011$). Further examination of trends in standardized fruit size across positions in the two environments separately using quadratic regression confirms a negative linear decline in both environments and reveals a highly significant nonlinear (concave) decline in fruit size only for individuals under a constrained season (quadratic parameter estimate = -0.764 ± 0.109 , $t = 7.00$, $p < .001$) and not under a long season (quadratic parameter estimate = 0.143 ± 0.093 , $t = 1.54$, $p = .125$).

Seed number per fruit (Figure 2) differed between the two environments ($F_{1,102} = 127.0$, $p < .001$) and across positions ($F_{2,102} = 18.27$, $p < .001$), with a significant interaction effect ($F_{2,102} = 4.63$, $p = .012$). According to a post hoc Tukey's test for the main effect of fruit position, seed number is lower at the distal position (mean = 141.2 ± 13.27) than both the mid (mean = 247.1 ± 24.91) and basal (mean = 228.9 ± 19.94) positions; the interaction effect is attributable to a significant decrease in seed number in the distal compared to the other two positions in the constrained environment, with no difference among positions observed in the long chamber.

Analyses of seed size (Figure 2) confirmed no difference between environments ($F_{1,102} = 0.01$, $p = .920$), but revealed differences across fruit positions ($F_{2,102} = 56.7$, $p < .001$) and a significant interaction term ($F_{2,102} = 9.81$, $p < .001$). A follow-up Tukey's test suggests that seed size differed significantly between environments only at the distal position. Specifically, seeds were smaller at the distal position in a constrained-season environment (mean = 0.273 ± 0.003), compared to a long-season environment (mean = 0.290 ± 0.003), highlighted by a lower distal:basal position seed size ratio in the constrained (ratio = 0.851) compared to long (ratio = 0.932) season. Although these results are suggestive of differing patterns of allocation across fruits in the two environments, inferences about fitness through seed production require seed germination and seedling survival analyses.

3.2 | Fitness

Seed viability, measured as germination success under salt stress (Figure 3), differed significantly between the two environments ($F_{1,102} = 11.42$, $p < .001$) and among fruit positions ($F_{2,102} = 17.67$, $p < .001$), with a significant interaction term ($F_{2,102} = 5.07$,

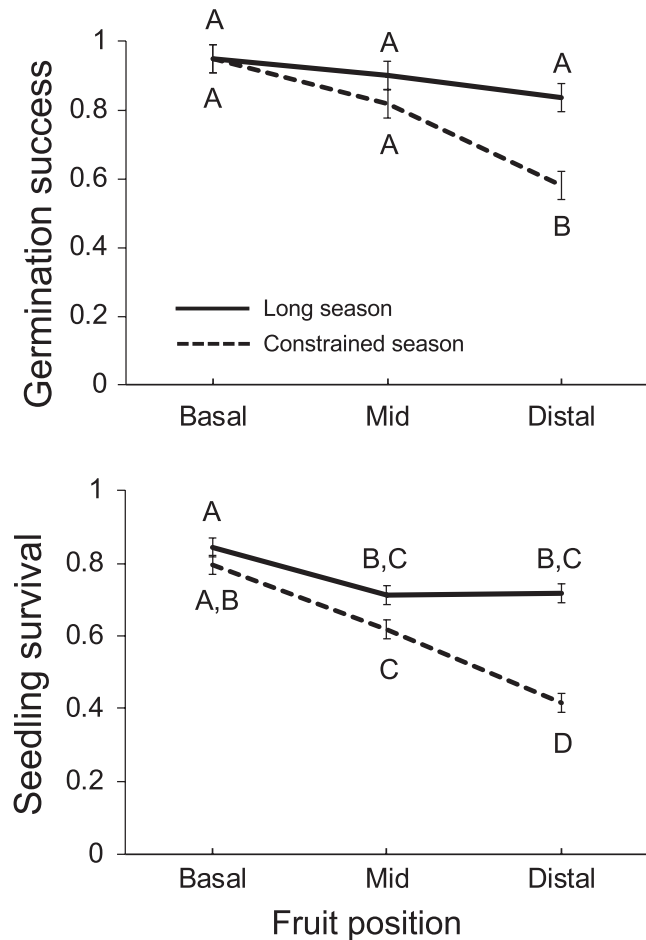


FIGURE 3 Effects of environment, fruit position and their interaction on germination success and survival. All effects are highly significant ($p < .01$) for both traits. Letters that are not shared among levels indicate significant differences in means according to post hoc Tukey's tests. Error bars are ± 1 SE

$p < .008$). Lower germination success was observed for seeds from the constrained-season environment, and the effect size was large: seeds of fruit at the distal position from a constrained-season environment showed substantially lower germinability (mean = 0.580 ± 0.058) than seeds from a long-season environment (mean = 0.835 ± 0.020). Germination trials in benign conditions following stratification of nongerminating seeds under salt stress indicate mortality rather than dormancy; only 14% of remaining seeds germinated overall, with a higher seed viability in the long-season environment (24.6%) than in the constrained-season environment (9.3%).

The seedling survival fraction (Figure 3)—including only those seeds that germinated—differed between the long-season (mean = 0.76 ± 0.01) and constrained-season (mean = 0.61 ± 0.03) environments ($F_{1,102} = 48.00$, $p < .001$) and differed across positions ($F_{2,102} = 47.76$, $p < .001$), and the environment-by-position interaction was also highly significant ($F_{2,102} = 13.34$, $p < .001$): seedlings from the distal fruit of constrained-season plants suffered a 30% reduction in survival. Post hoc Tukey's tests (Figure 3) find significantly lower seedling survival at this position than seedlings from the distal

position in the long-season environment, and lower survival than any other position in either environment (Figure 3). Furthermore, although seedling survival from the basal position in the long-season environment does not differ significantly from that position in the constrained environment, it is significantly higher than at any other position in either environment, as assayed under stressful salt conditions. Mixed effects logistic regressions, to ask to what extent seed size differences account for fruit positional effects on germination and survival fraction, found that the effect of seed size nested within position fully accounts for the effect of fruit position (Table 1).

At the level of the individual plant, realized fitness associated with a seed in a constrained-season environment is only 0.532 ± 0.03 of that given by seed count and is significantly less than ($F_1 = 10.6$, $p = .003$) realized fitness of a seed in a long-season environment (0.69 ± 0.03), yielding a relative per-seed fitness of 0.774 ± 0.034 under constrained conditions. This individual realized per-seed fitness was further broken down across the three fruit positions in the two environments (Figure 4), revealing that the overall reduction in realized fitness is accounted for to an increasing extent later in the reproductive season.

Using seed numbers—estimated using the empirical relationship between seed counts and main-stem fruit measurements—that is, purely counting seeds, the total expected fitness for individuals in a constrained-season environment is significantly higher than individuals in a long-season environment (Figure 5). However, the apparent fitness advantage in the constrained environment based only on seed count disappears when seedling success is included in fitness (Figure 5). Fitness (surviving seeds) including all fruits of individuals in the two environments may be calculated under the assumption that the fruit size–seed survival relationship observed for the main stem also holds for fruits on branches. Again, the greater fitness under constrained conditions based on seed count alone is reversed when seedling success is included; relative fitness in a constrained-season environment is 0.89 of that in a long-season environment.

TABLE 1 Sources of variation in germination and survival fraction as an effect of seed size nested within fruit position in the two environments. Results of a mixed effects logistic regression using the subset of seeds individually tracked for survival, germination and size

Trait	Source	df	Wald chi-square	p
Germination	ENV	1	43.77	<.001
	FP	2	0.245	.8846
	FP*ENV	2	45.80	<.001
	Seed size [FP]	3	198.46	<.001
Survival	ENV	1	29.66	<.001
	FP	2	3.50	.1737
	FP*ENV	2	6.32	.0425
	Seed Size [FP]	3	199.24	<.001

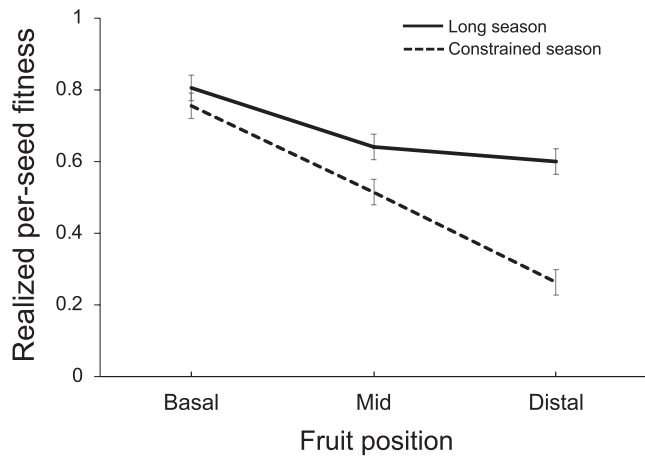


FIGURE 4 Realized per-seed fitness across fruit positions in the two experimental environments. Per-seed fitness in both the long (long-dashed line) and constrained (short-dashed line) season is measured as the product of realized seed and seedling survival assayed at each of the three fruit positions on a subsample of 30 seeds from every individual. Error bars are 1 SD

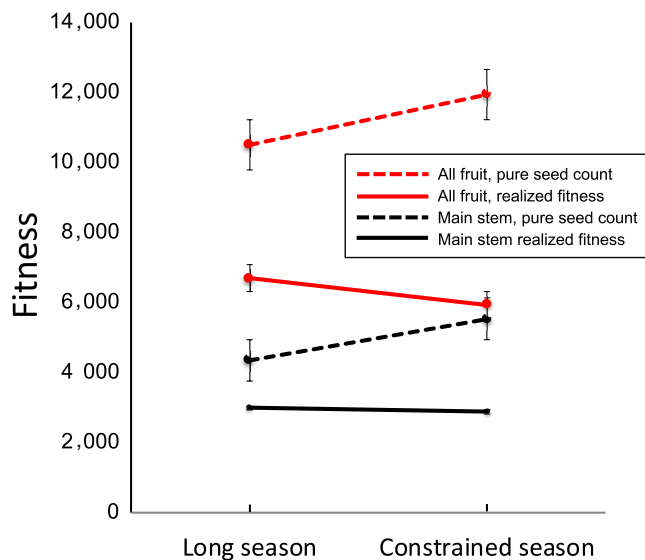


FIGURE 5 Total individual fitness based on pure seed count (dashed lines) and on realized fitness (solid lines), measured across the two environments. Realized fitness is total individual seed count weighted by germination success and seedling survival derived from fruit position-specific empirical relationships (see text; Figure 4). The upper pair of red lines are seeds from all fruits; the lower pair (black) are from the main-stem fruits only

4 | DISCUSSION

Seed number is often used as a fitness proxy in studies of plant evolutionary ecology and, perhaps even under most circumstances, may accurately represent relative reproductive success. However, seed count may be a biased estimate of fitness under some scenarios including when parents differ in phenology, which results in differences among individuals in effective season length. The use of seed

count in previous work on *L. inflata* implied that there was no fitness cost of developing under severely time-constrained environments (Hughes & Simons, 2014a); however, variation in offspring quality resulting from variation in temporal constraints among parents was not considered. Here, in a manipulation study, we find support for the hypothesis that variable constraints imposed by flowering phenology result in biased estimates of fitness based on seed count; specifically, seed count provides an inflated fitness estimate under constrained conditions. The counterintuitive finding that plants under constrained-season lengths produce fewer but larger fruit, and almost 1.3 times greater seed production, confirms earlier findings (Hughes & Simons, 2014a). As predicted, we find that individuals developing under a constrained season suffer reduced realized reproductive success through a substantial reduction (of about 23%) in per-seed fitness, gauged as germination success and seedling survival measured under slightly stressful laboratory conditions.

Our results demonstrate that the overall reduction in per-seed fitness is directly related to intraindividual patterns of allocation. Although a semelparous organism, *L. inflata* packages its reproductive effort over a distended period among many fruits. The role of within-individual variation across fruit positions in fruit size, seed number and seed size in the regulation of reproductive allocation is consistent with work on other species (Lu, Tan, Baskin, & Baskin, 2017; Nakamura, 1986; Stocklin & Favre, 1994). We further demonstrate that these allocation patterns of fitness decline in later-produced fruit are expressed more strongly under a constrained environment. These effects on realized fitness are largely accounted for by seed size differences among fruits, which is in agreement with the general finding that intraspecific variation in seed size is associated with germinability and survival (Metz et al., 2010; Moles & Westoby, 2004; Simons & Johnston, 2000a). The observed pattern of reproductive allocation also supports the semelparity–iteroparity continuum hypothesis (Hughes & Simons, 2014a); individuals that bolt later and experience a constrained growing season show a more extreme ‘big-bang’ semelparous reproductive strategy; they appear to ‘rush’ reproduction, resulting in high seed number. Under the manipulated conditions of this study, this does not result in high realized fitness because lower rates of germination and survival occurred.

Plants used in this study were reared from seeds that were field-collected, and, although seeds are small, maternal environmental effects may be expressed in the parental plants generated from them. However, fitness itself was measured on seeds that were produced under growth chamber conditions. Regardless, maternal effects, if present, do not confound differences observed in traits (including fitness) that were generated between growth chamber environments because these differences were observed in individuals randomly allocated to experimental treatments.

Also, we note that we allocated individuals of each genotype across the two environments where possible, but some genotypes were represented in only one environment (three in constrained, two in long). Although this is an improvement over randomization of individuals across treatments, we confirm that no anomalous genotype drives results by reanalysing (all analyses of effect of environment

and fruit position on fruit size, seed size, seed number, germination success, seedling survival), eliminating the unreplicated genotypes. Results are qualitatively identical to those reported for significance of all effects, as well as for differences among levels in Tukey's tests.

Our estimate of seed viability is unlikely to confound dormancy with mortality. The populations of *L. inflata* studied exhibit high germination fractions under conducive conditions following stratification, and the fraction of nongerminating seeds in the salt treatment were subjected to an additional no-salt germination test, a technique known to result in recurrent germination fractions in tests of viability (Simons & Johnston, 2006). Furthermore, viability of the nongerminating fraction produced in the constrained environment was lower than for that from the long environment, and analyses accounting for dormancy strengthen, but do not qualitatively alter results.

Inference from our manipulation results to the effects of natural variation in phenology in the field is moderated by several factors. First, although the apparent individual fitness advantage under constrained-season length disappears with the use of a measure of realized fitness, we cannot conclude that total fitness is significantly reduced under a constrained-season length. However, the magnitude of the difference between seed count and realized fitness depends on the severity of constraints imposed by the environment. Considering that in a stationary population, on average only 1 seed per parent survives to reproduce, natural conditions would be expected to be more stressful. Nonetheless, our salt stress treatment revealed otherwise-hidden fitness variation, in that per-seed fitness is significantly reduced and the decline in fitness is significantly steeper across fruit positions in the constrained environment as it was imposed.

Second, the two environments used in this study were meant to mimic short and long seasons and, although they might be interpreted as high contrast environments, the manipulated differences were in just two environmental parameters (temperature and photoperiod) of many that are likely to influence development. It is thus unknown how the variation generated relates to variation in phenology and realized fitness under field conditions. An alternative approach using observational field studies would provide insight into the potential range of phenotypic variation, but interpretation of underlying causal effects would be limited compared to that from manipulation studies for a number of reasons. Critically, in observational studies fitness variation is likely to be a driver of, in addition to a product of, variation in phenology. Also, especially for monocarpic perennials like *L. inflata*, reproductive delay across seasons would have to be accounted for in calculations of realized fitness. For example, an individual that delays bolting in year 1 will bolt early and at a large rosette size in year 2, thereby increasing its fitness through the production of high quality, plentiful seeds; however, the delay has also forfeited fitness through an increase in generation time.

Abundant individual variation in phenology exists in natural populations, resulting in the expression of plasticity in reproductive traits (Hughes & Simons, 2014a; Simons & Johnston, 2003; Weis et al., 2015); however, the effects of this phenological adjustment on fitness estimation have been incompletely studied. Our results

demonstrate that the resulting variation in patterns of reproductive allocation can disrupt the relationship between seed count and realized fitness. Therefore, when within-population variation in phenology exists, we conclude that fitness assessment using simple seed count should be interpreted with caution, and attempts to evaluate offspring viability among parents should be made. As outlined in the Introduction and detailed in Wen (2019), there are several additional scenarios in which seed count is expected to produce a biased estimate of relative fitness. Future research on phenological variation in the field, as well as on these additional scenarios, would increase our confidence in the estimation of fitness in natural populations.

ACKNOWLEDGEMENTS

We thank Jessica Forrest, Joe Bennett, Naomi Cappuccino and two reviewers for critical comments and R. Chlebak, W. Campeau, S. Raghu, R. Morris and H. Hitsman for constructive feedback. We also thank Isabelle Beaudoin-Roy for granting a Land Access Permit for Gatineau Park, QC. This work was supported by an NSERC Discovery Grant to AMS.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

AMS conceived of the study, LW and AMS designed the study and conducted field work, LW conducted all laboratory work, LW and AMS performed statistical analyses, and LW and AMS drafted, revised and approved the paper for submission.

DATA AVAILABILITY STATEMENT

Data deposited at Dryad: <https://doi.org/10.5061/dryad.0cfxpnvzc>.

ORCID

Andrew M. Simons  <https://orcid.org/0000-0002-0198-465X>

REFERENCES

- Aarssen, L. W. (2014). Estimating fitness from offspring counts in clonal seed plants. *Ideas in Ecology and Evolution*, 7(1), 77–83. <https://doi.org/10.4033/iee.2014.7.16.c>
- Agrawal, A., Strauss, S., & Stout, M. (1999). Costs of induced responses and tolerance to herbivory in male and female fitness components of wild radish. *Evolution*, 53(4), 1093–1104. <https://doi.org/10.2307/2640814>
- Antonovics, J., & Ellstrand, N. C. (1984). Experimental studies of the evolutionary significance of sexual reproduction. I. A test of the frequency-dependent selection hypothesis. *Evolution*, 38(1), 103–115. <https://doi.org/10.1111/j.1558-5646.1984.tb00263.x>
- Austen, E. J., & Weis, A. E. (2015). What drives selection on flowering time? An experimental manipulation of the inherent correlation between genotype and environment. *Evolution*, 69(8), 2018–2033. <https://doi.org/10.1111/evo.12709>
- Bowden, W. R. (1959). Phylogenetic relationships of twenty-one species of *Lobelia* L. Section *Lobelia*. *Bulletin of the Torrey Botanical Club*, 86, 94–108. <https://doi.org/10.2307/2482990>
- Côté, K., & Simons, A. M. (2020). Genotype-environment interaction and the maintenance of genetic variation: An empirical study of *Lobelia*

- inflata* (Campanulaceae). *Royal Society Open Science*, 7, 191720. <https://doi.org/10.1098/rsos.191720>
- De Jong, G. (1994). The fitness of fitness concepts and the description of natural selection. *The Quarterly Review of Biology*, 69(1), 3–29. <https://doi.org/10.1086/418431>
- Devlin, B. (1989). Components of seed and pollen yield of *Lobelia cardinalis*: Variation and correlations. *American Journal of Botany*, 76(2), 204–214. <https://doi.org/10.2307/2444662>
- Endler, J. A. (1986). *Natural selection in the wild*. Princeton University, NJ: Princeton University Press.
- Forrest, J. (2015). Plant-pollinator interactions and phenological change: What can we learn about climate impacts from experiments and observations? *Oikos*, 124(1), 4–13. <https://doi.org/10.1111/oik.01386>
- Galen, C., & Stanton, M. L. (1991). Consequences of emergence phenology for reproductive success in *Ranunculus adoneus* (Ranunculaceae). *American Journal of Botany*, 78(7), 978–988. <https://doi.org/10.2307/2445177>
- Hannachi, S., & Van Labeke, M. C. (2018). Salt stress affects germination, seedling growth and physiological responses differentially in eggplant cultivars (*Solanum melongena* L.). *Scientia Horticulturae*, 228, 56–65. <https://doi.org/10.1016/j.scienta.2017.10.002>
- Hughes, P. W., & Simons, A. M. (2014a). The continuum between semelparity and iteroparity: Plastic expression of parity in response to season length manipulation in *Lobelia inflata*. *BMC Evolutionary Biology*, 14(90), <https://doi.org/10.1186/1471-2148-14-90>
- Hughes, P. W., & Simons, A. M. (2014b). Changing reproductive effort within a semelparous reproductive episode. *American Journal of Botany*, 101(8), 1323–1331. <https://doi.org/10.3732/ajb.1400283>
- Hughes, P. W., & Simons, A. M. (2014c). Secondary reproduction in the herbaceous monocarp *Lobelia inflata*: Time-constrained primary reproduction does not result in increased deferral of reproductive effort. *BMC Ecology*, 14, 15. <https://doi.org/10.1186/1472-6785-14-15>
- Hughes, P. W., & Simons, A. M. (2015). Microsatellite evidence for obligate autogamy, but abundant genetic variation in the herbaceous monocarp *Lobelia inflata* (Campanulaceae). *Journal of Evolutionary Biology*, 28(11), 2068–2077. <https://doi.org/10.1111/jeb.12734>
- Ibrahim, E. A. (2016). Seed priming to alleviate salinity stress in germinating seeds. *Journal of Plant Physiology*, 192, 38–46. <https://doi.org/10.1016/j.jplph.2015.12.011>
- Johnston, M. O., & Schoen, D. J. (1996). Correlated evolution of self-fertilization and inbreeding depression: An experimental study of nine populations of *Amsinckia* (Boraginaceae). *Evolution*, 50(4), 1478–1491. <https://doi.org/10.2307/2410885>
- Lande, R., & Arnold, S. J. (1983). The measurement of selection on correlated characters. *Evolution*, 37(6), 1210–1226. <https://doi.org/10.2307/2408842>
- Lu, J., Tan, D. Y., Baskin, C. C., & Baskin, J. M. (2017). Effect of seed position on parental plant on proportion of seeds produced with non-deep and intermediate physiological dormancy. *Frontiers in Plant Science*, 8, 147. <https://doi.org/10.3389/fpls.2017.00147>
- McGraw, J. B., & Caswell, H. (1996). Estimation of individual fitness from life-history data. *The American Naturalist*, 147(1), 47–64. <https://doi.org/10.1086/285839>
- Metz, J., Liancourt, P., Kigel, J., Harel, D., Sternberg, M., & Tielborger, K. (2010). Plant survival in relation to seed size along environmental gradients: A long-term study from semi-arid and Mediterranean annual plant communities. *Journal of Ecology*, 98(3), 697–704. <https://doi.org/10.1111/j.1365-2745.2010.01652.x>
- Miller, T. E. X., Williams, J. L., Jongejans, E., Brys, R., & Jacquemyn, H. (2012). Evolutionary demography of iteroparous plants: Incorporating non-lethal costs of reproduction into integral projection models. *Proceedings of the Royal Society B*, 279(1739), 2831–2840. <https://doi.org/10.1098/rspb.2012.0326>
- Moles, A. T., & Westoby, M. (2004). Seedling survival and seed size: A synthesis of the literature. *Journal of Ecology*, 92(3), 372–383. <https://doi.org/10.1111/j.0022-0477.2004.00884.x>
- Mousseau, T. A., & Roff, D. A. (1987). Natural selection and the heritability of fitness components. *Heredity*, 59, 181–197. <https://doi.org/10.1038/hdy.1987.113>
- Nakamura, R. R. (1986). Maternal investment and fruit abortion in *Phaseolus vulgaris*. *American Journal of Botany*, 73(7), 1049–1057. <https://doi.org/10.2307/2444124>
- O'Connell, L. M., & Johnston, M. O. (1998). Male and female pollination success in a deceptive orchid, a selection study. *Ecology*, 79(4), 1246–1260. [https://doi.org/10.1890/0012-9658\(1998\)079\[1246:MAFPS\]2.0.CO;2](https://doi.org/10.1890/0012-9658(1998)079[1246:MAFPS]2.0.CO;2)
- Pan, J. J., & Price, J. S. (2002). Fitness and evolution in clonal plants: The impact of clonal growth. *Evolutionary Ecology*, 15, 583–600. <https://doi.org/10.1023/A:1016065705539>
- Papastylianou, P., Baokogianii, N.-N., Travlos, I., & Roussis, I. (2018). Sensitivity of seed germination to salt stress in black cumin (*Nigella sativa* L.). *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 46(1), 202–205. <https://doi.org/10.15835/nbha46110861>
- Primack, R. B., & Kang, H. (1989). Measuring fitness and natural selection in wild plant populations. *Annual Review of Ecology and Systematics*, 20, 367–396. <https://doi.org/10.1146/annurev.es.20.110189.002055>
- Rafferty, N. E., CaraDonna, P. J., Burkle, L. A., Iler, A. M., & Bronstein, J. L. (2013). Phenological overlap of interacting species in a changing climate: An assessment of available approaches. *Ecology and Evolution*, 3(9), 3183–3193. <https://doi.org/10.1002/ece3.668>
- Roff, D. A. (1992). *The evolution of life histories: Theory and analysis*. New York, NY: Chapman and Hall.
- Roff, D. A. (2002). *Life history evolution*. Sunderland, MA: Sinauer Associates Inc.
- Simons, A. M. (2011). Modes of response to environmental change and the elusive empirical evidence for bet hedging. *Proceedings of the Royal Society B*, 278(1712), 1601–1609. <https://doi.org/10.1098/rspb.2011.0176>
- Simons, A. M., & Johnston, M. O. (2000a). Variation in seed traits of *Lobelia inflata* (Campanulaceae): Sources and fitness consequences. *American Journal of Botany*, 87(1), 124–132. <https://doi.org/10.2307/2656690>
- Simons, A. M., & Johnston, M. O. (2000b). Plasticity and the genetics of reproductive behaviour in the monocarpic perennial, *Lobelia inflata* (Indian tobacco). *Heredity*, 85, 356–365. <https://doi.org/10.1046/j.1365-2540.2000.00760.x>
- Simons, A. M., & Johnston, M. O. (2003). Suboptimal timing of reproduction in *Lobelia inflata* may be a conservative bet-hedging strategy. *Journal of Evolutionary Biology*, 16(2), 233–243. <https://doi.org/10.1046/j.1420-9101.2003.00530.x>
- Simons, A. M., & Johnston, M. O. (2006). Environmental and genetic sources of diversification in the timing of seed germination: Implications for the evolution of bet hedging. *Evolution*, 60, 2280–2292. <https://doi.org/10.1554/05-396.1>
- Sniegula, S., Golab, M. J., & Johansson, F. (2016). Time constraint effects on phenology and life history synchrony in a damselfly along a latitudinal gradient. *Oikos*, 124, 414–423. <https://doi.org/10.1111/oik.02265>
- St Juliana, J. R., & Janzen, F. J. (2007). Can natural phenotypic variances be estimated reliably under homogeneous laboratory conditions? *Journal of Evolutionary Biology*, 20(4), 1406–1414. <https://doi.org/10.1111/j.1420-9101.2007.01343.x>
- Stearns, S. C. (1992). *The evolution of life histories*. Oxford, London: Oxford University Press.
- Stocklin, J., & Favre, P. (1994). Effects of plant size and morphological constraints on variation in reproductive components in two related species of *Epilobium*. *Journal of Ecology*, 82(4), 735–746. <https://doi.org/10.2307/2261439>

- Thoday, J. (1953). Components of fitness. *Symposium of the Society for the Study of Experimental Biology*, 7, 96–113.
- Vu, W. T., Chang, P. L., Moriuchi, K. S., & Friesen, M. L. (2015). Genetic variation of transgenerational plasticity of offspring germination in response to salinity stress and the seed transcriptome of *Medicago truncatula*. *BMC Evolutionary Biology*, 15, 59. <https://doi.org/10.1186/s12862-015-0322-4>
- Weis, A., Turner, K. M., Petro, B., Austen, E. J., & Wadgyman, S. M. (2015). Hard and soft selection on phenology through seasonal shifts in the general and social environments: A study on plant emergence time. *Evolution*, 69(6), 1361–1374. <https://doi.org/10.1111/evo.12677>
- Wen, L. (2019). *An evaluation of seed number as a measure of fitness: A review and experimental study (Unpublished master's thesis)*. Ottawa, ON: Carleton University. Retrieved from <https://curve.carleton.ca/9abef385-e747-4467-96dc-a85efedabc1f>
- Williams, G. C. (1966). *Adaptation and Natural Selection*. Princeton, NJ: Princeton University Press.
- Zimmerman, M., & Gross, R. S. (1984). The relationship between flowering phenology and seed set in an herbaceous perennial plant, *Polemonium foliosissimum* Gray. *The American Midland Naturalist*, 11(1), 185–191. <https://doi.org/10.2307/2425557>

How to cite this article: Wen L, Simons AM. Delusions of grandeur: Seed count is not a good fitness proxy under individual variation in phenology. *J Evol Biol*. 2020;33:1039–1049. <https://doi.org/10.1111/jeb.13633>