

**An evaluation of seed number as a measure of fitness: a
review and experimental study**

by

Lina Wen

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Abstract

Seed number is often used as a measure of fitness; however, situations exist where there is a discrepancy in the relationship between seed number and fitness. In Chapter 1, I identify eight main scenarios in nature where fitness is not well represented by seed count, and review existing empirical research that used seed count under these specific scenarios. Results suggest that the validity of seed number as a measure of fitness is largely under studied and should be supplemented with alternative metrics to appropriately quantify fitness. In Chapter 2, I use the monocarpic herbaceous plant *Lobelia inflata* to assess whether variable seasonal constraints can disrupt the relationship between seed count and fitness under controlled growth chamber conditions. Interestingly, the relative per-seed fitness is 0.774 ± 0.034 under constrained compared to long season conditions; higher total fitness is observed under a constrained season using simple seed count, but not after accounting for seedling viability.

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General Introduction

Fitness is a key concept in evolutionary biology and may be represented as the ability to survive and reproduce. Unfortunately, it is notoriously difficult to define and measure fitness (Dobzhansky, 1955; Kempthorne and Pollak, 1970; Wagner, 2009). Despite its central role, there is no universally accepted method to measure individual fitness. Endler (1986) famously discussed five classic definitions of fitness (Darwinian fitness, the natural selection rate coefficient, adaptedness, adaptability, and durability), each containing unique properties. Although there are differences in what these fitness concepts represent, all of them aim to quantify a rate of increase, and that is typically achieved by estimating the relative number of offspring produced per individual (Stearns, 1992; McGraw and Caswell, 1996; Godfrey-Smith, 2007).

In plant biology, a large number of studies have focused on seed production as a measure of fitness under the assumption that seed number is an indication of total recruitment (Pan & Price, 2002; Aarssen, 2014; Simons & Johnston, 2000a). Seed number is often used as a measure of fitness because it is a direct measure of reproductive success and is easily quantified (Primack and Kang, 1989). However, there are several scenarios in nature where there is a “disruption” between the relationship of seed number and fitness. Evolutionary theorists have recognized the pitfalls of solely using seed number as a measure of fitness (Primack and Kang, 1989; Eriksson and Jerling, 1990), and while this problem has been acknowledged in recent studies (Metsare *et al.*, 2015; Austen and Weis, 2016; Bigio *et al.*, 2017), no study to date has explicitly identified

situations where seed number may not be a reliable proxy for fitness, or recognized alternative measures that should be taken in those particular situations.

In this thesis, I focus on the question of whether seed number is a reliable estimate of plant fitness, by conducting two studies:

- 1) A literature study that identifies eight main scenarios in nature where fitness may not be well represented by seed number, and reviews existing empirical research for the use of seed number as a measure of fitness under these specific scenarios.
- 2) An empirical study to test one of these scenarios; specifically that variation in phenology under different quality growth conditions influences the relationship between seed count and fitness.

The first objective of the literature review is to describe the main scenarios in nature where seed count may not be a reliable measure of fitness. The second objective is to review existing empirical studies for the use of seed count as a proxy for fitness under the scenarios identified, have acknowledged that seed number may not be a reliable measure of fitness, have justified the use of seed count, or have used alternative or additional measures of fitness. The term “scenarios” will be used to describe circumstances or situations in which seed number is not a reliable measure of fitness, and the term “disruption” will be used to describe the disconnect between seed number and fitness under these scenarios.

For the empirical study, I use the monocarpic herbaceous plant *Lobelia inflata* (Campanulaceae) to assess whether variable constraints imposed by season length can disrupt the relationship between seed count and fitness. Although seed number might not be reduced under a constrained season length (Hughes and Simons 2014a), fitness costs may result from a reduction in seed quality. Plants that experience a long season will transition from vegetative to reproductive mode at a larger size, and might produce better quality seeds than a plant that has been forced to accelerate its development under a short season. Thus, together with evidence that late bolting plants show a progressive semelparous reproductive strategy (Hughes and Simons, 2014a), it seems reasonable to infer that a trade off in seed number and seed quality will occur.

The goals of the two chapters are to investigate situations in which seed number may be a biased estimate of fitness, and to provide solutions for when there are confounding effects on the relationship between seed number and fitness. This is important because investigators can be aware of the potential consequences of using seed number as a measure of fitness when conducting experimental or observational studies. This thesis addresses fundamental questions about the estimation of plant fitness, and provides insight on more suitable alternatives that can be used when feasible.

Chapter 1: A review of seed count as a measure of fitness

1.1 Introduction

Fitness is a fundamental concept in evolutionary theory and is meant to reflect the probability of survival and reproduction. One of the important reasons we measure fitness is to determine the strength of selection acting on life history traits, and how the differential expression of these traits result in evolutionary change and adaptation (Williams, 1966; Endler, 1968; Stearns, 1976; Mills and Beatty, 1979; Sober, 1984; Sober, 2001; Barker, 2009). Assessment of the pattern and strength of selection requires measuring the association of fitness to a particular property or trait of an individual (Lewontin, 1970; Lande and Arnold, 1983; Arnold and Wade 1984; Roff, 1992).

The fitness concept can vary among different fields of biology (i.e. in demography, quantitative genetics, population genetics developmental and physiological, systematics, comparative biology studies) (Brommer, 2000; Metz, 2008; Hansen, 2017), and the literature on the topic is vast. Reviews exist on the various ways in which the term is used across contexts (i.e. genes, genotypes, individuals, groups) (Hedrick and Murray, 1983, Endler, 1986). A widely used fitness metric is the intrinsic rate of increase, or “Malthusian parameter” (r), a measure of the per capita instantaneous rate of increase of a genotype or population with a stable age distribution (Stearns, 1992, Brommer, 2000). Net reproductive rate, R_0 , is the combination of age-specific survival and reproductive rates, and has been favoured by field biologists interested in variation in reproductive success (Endler, 1986; Brommer, 2000).

In contrast to r and R_0 , Rand *et al.* (1994) have based a fitness concept on the invasibility criterion in the context of evolutionarily stable strategies (ESS) (Metz *et al.*, 1992; Geritz *et al.*, 1998; Brommer, 2000). Furthermore, fitness may be expressed either as absolute or relative fitness. Absolute fitness measures the proportional growth (positive or negative) of a unit of selection (e.g. genotype), while relative fitness is success in comparison to either the mean fitness, or the fittest genotype in the population (Kimbrough, 1970; Orr, 2009). Individual fitness may also be measured across several generations. It may be quantified as the mean fitness; however, reproduction is a multiplicative process, and the long-term fitness of a genotype should be quantified as the geometric mean of individual fitness values (Roff, 2002; Wagner, 2010; Chevin, 2011; Starrfelt and Koko, 2012). Although each of these fitness concepts has different properties, the common goal is to quantify a rate of increase.

Regardless of the lack of a single theoretical fitness concept, evolutionary biologists must quantify fitness in empirical studies (Godfrey-Smith, 2009; Sober, 2001). Approaches to empirical measures of fitness are also diverse, and also differ among fields of biology. Studies in demography use age and size-specific variation in mortality and reproduction to infer fitness (Stearns, 1992; McGraw and Caswell, 1996; Engen and Sæther, 2014), whereas quantitative genetics studies focus on selection differentials and trait correlations with fitness (Lande, 1982). In population genetics, fitness differences are quantified at the genetic level and are expressed in terms of a change in allele frequency at individual loci (de Jong, 1994; Barker 2009).

Evaluating fitness is straightforward in some organisms, and especially so in some plants. For evolutionary studies, it is helpful to work with organisms that reproduce quickly, so fitness can be assessed across an entire, or several lifetimes. For example, in clonal populations of microbes, one commonly used method of quantifying fitness is to calculate the maximum growth rate (V_{max}) of a culture by measuring the optical density of the culture over time (Andersson and Hughes, 2010, Wisser and Lenski, 2013). There are several advantages of these methods; for example, a spectrophotometer can measure many samples quickly and efficiently, and systems can be programmed to take measurements of a full growth cycle of a culture (Wisser and Lenski, 2013).

It is more difficult to measure long-term reproductive success in longer-lived organisms, and researchers will often use a proxy for fitness. For example, insects have several traits that can be used as fitness proxies: size, longevity, fecundity, mating ability and vagility. All these proxies are positively correlated with the lifetime fitness of an individual (Doyon and Boivin, 2005). In more complex organisms such as mammals, body condition indices are frequently used as surrogate of fitness and are generally positively related to fitness (Green, 2001; Milenkaya *et al.*, 2015).

Fitness assessment in plants is facilitated by several factors. First, plants are sessile, facilitating studies of growth, survival and reproduction (Primack and Kang, 1989). Second, they can be grown in controlled conditions. Moreover, genetically identical replicates can often be obtained by cloning, which can be beneficial in

determining environmental effects on the trait(s) under investigation. Last, plants have a diversity of sexual systems that can be used to study the genetic and morphological mechanisms on differential fitness patterns (Bawa and Beach, 1981). In experimental studies, several traits can readily be used as proxies for fitness (i.e. biomass, size, or performance), but fitness is most commonly represented as lifetime seed production (Strauss, 1997, Geber and Griffen 2003, Younginger, 2017).

Seed number is often used as a measure of fitness for many reasons; most notably, it can be readily counted (Pan and Price, 2002). However, there is a strong underlying assumption that seed count accurately represents (relative) fitness. In current literature, there is seldom a justification for the use of seed number as a reliable method for estimating fitness, and there are reasons to suspect that seed number might provide a biased estimate of fitness under a variety of circumstances.

There are several scenarios in nature where there is a ‘disruption’ in the relationship between seed number and fitness. For example, for individuals that can reproduce both sexually and asexually, seed number may not be an appropriate measure of fitness as it only represents the sexual component of fitness (Antonovics and Ellstrand, 1984, Aarssen, 2014). Thus, the validity of the assumption that seed count is an accurate measure of fitness is not universal.

This review will aim to:

1. Outline the main scenarios in nature in which seed count may not be an appropriate measure of fitness.
2. Provide evidence from published studies for the use of seed count as a measure of fitness, and quantify the number of studies that have acknowledged that seed count may be a biased estimate of fitness, justified the use of seed count as a proxy for fitness, provided solutions to problems with using seed count as a measure of fitness, or have offered alternative metrics.
3. Establish appropriate experimental criteria to evaluate which specific metrics most accurately reflect fitness and consider the appropriate methodological and experimental requirements to measure fitness in plant populations.

1.1.1 The “Scenarios”

Here, I identify eight main scenarios in nature in which the relationship between seed number and fitness may be disrupted. It is worth noting that these eight scenarios do not represent the entirety of possible scenarios, but are the most prominent in current literature; nor are they mutually exclusive. They can fall into broader categories of trade-offs, environmental heterogeneity, and differential reproductive modes.

Trade-offs

i. Plant architecture

Plant form may be variable within species (e.g. height, degree of branching). Architecture may influence fitness, through factors such as dispersal, independent of seed number. For instance, height would confer a fitness advantage for several reasons: by attracting greater pollinator visitation, thus allowing larger pollen loads and producing more vigorous offspring (Donnelly *et al.*, 1998); through more effective pollen and seed dispersal (Lortie and Aarssen, 1999); through variation in susceptibility to herbivore damage (Cipollini and Bergelson, 2002); or through competition for light (Aarssen, 2008). Trade-offs in resource allocation between reproductive versus vegetative function may also affect fitness (Charnov, 1982; Reekie and Bazzaz, 1987; Taylor *et al.*, 1990). A plant that invests more into vegetative growth might suffer in reproductive output in terms of number and quality. For instance, plants that invest in interference strategies (i.e.

allocating more into stem and leaf growth to shade neighbours) might produce fewer seeds than a plant that allocates primarily to reproduction (Falster and Westoby 2003; de Jong and Klinkhamer, 2005). Thus, because of differences in plant architecture among individuals, counting seeds may not be an accurate representation of fitness, and should be used with another fitness measure such as plant biomass.

ii. Trade-offs between defense against herbivory and seed number

Interactions of herbivores with plant reproductive tissues can cause negative effects on plant fitness (Feeny, 1976; Bazzaz *et al.*, 1987, McArt *et al.*, 2013). If variation in allocation to defense against herbivory exists among individuals, seed count of defended individuals would underrepresent their relative fitness. For example, plants can allocate more energy to chemical defenses to avoid predators, and in turn produce fewer seeds that are more protected and fitter (Lehndal and Agren, 2015). Results from a meta-analysis demonstrate that the production of antiherbivore defenses imposes a fitness cost on plants through negative correlations between defense and fitness measures (Koricheva, 2002). This analysis also suggests that fitness costs may arise not only due to trade-offs in the allocation of common limited resources between defense, growth and reproduction within an individual plant, but also through interactions between plants and external factors in their environment. For instance, plant responses to herbivory may cause changes in floral attraction, which can then affect seed production indirectly through attractiveness to pollinators (Strauss, 1997). Thus, these results suggest that seed

number may not be an appropriate measure of fitness due differences in allocation, and should be supplemented with metrics reflecting growth and defense.

iii. Bet-hedging

Bet-hedging strategies are expected to evolve that maximize geometric-mean fitness rather than maximizing fitness under average conditions (Seger and Brockmann 1987; Philippi and Seger 1989; Simons 2002). Plants with relatively low apparent fitness in particular generations might produce more offspring in the long run if they express traits that reduce inter-generational variance in fitness. A classic example is delayed germination in desert plants, where spreading germination over multiple seasons can reduce the risk of complete seedling mortality from unfavourable conditions in a particular year (Cohen 1966; Venable 2007). If all seeds germinate in a single year, 100% mortality may result if environmental conditions are particularly harsh in that year (e.g. a drought). In fact, in many temperate and tropical species, seeds do not germinate despite conducive conditions but remain dormant as a risk-reducing strategy in unpredictable environments (Liu, 2014, Simons and Johnston, 2006). Therefore, seed count may not be a reliable measure of fitness when plants use bet-hedging strategies. An appropriate measure of fitness for plants expressing bet-hedging strategies would include measuring fitness across several generations.

iv. Variation in degree of iteroparity

An iteroparous species may divide its resources between reproduction and somatic maintenance; individuals that allocate more energy to reproduction might suffer lower residual reproductive value. Thus, a trade-off can occur between reproduction and survival when the degree of somatic maintenance affects the reproductive success of the parent (Young, 1981). For example, in colder months, plants that overwinter can allocate more energy to storage reserves (somatic maintenance) rather than seed production as a trade-off between vegetative and reproductive strategies (Lu *et al.*, 2015). Therefore, assessing fitness as seed production may be inaccurate for iteroparous species, as current reproduction is only one component of the overall fitness of an individual. In order to examine how effects on different components of fitness are translated into effects on overall fitness, it is necessary to assess the entire life cycle of an individual and to include lifetime seed production (Garcia and Ehrlén, 2002).

Differential modes of reproduction

v. Selfing vs. outcrossed progeny

Plants with mixed mating systems produce variable ratios of self-fertilized and outcrossed seeds, which may differ in fitness. Self-fertilization may be favoured in the short term and often results in a larger number of offspring than outcrossing; however,

there are fitness consequences associated with self-fertilization including inbreeding depression, which is the reduced fitness of inbred progeny relative to outcrossed progeny (Charlesworth and Willis, 2009). A meta-analysis of inbreeding depression in various fitness components showed differential inbreeding depression between self-compatible species and self-incompatible species (Angeloni *et al.*, 2011). Thus, seed count may not represent fitness under mixed mating, because individuals will vary in their degree of inbreeding and inbreeding depression. The relationship between outcrossing and fitness can be complex: a study by Ghazoul (2014) suggests that, according to the sacrificial sibling hypothesis, nonviable seed set enhances plant fitness. Inbred seeds can be retained and used as a sacrifice during seed predator attacks for the benefit of outcrossed seeds, therefore increasing individual fitness. Therefore an assessment of survivability or seedling establishment of offspring should be used with seed number for plants with mixed mating systems.

vi. Sexual vs. asexual reproduction

Many plants exhibit dual reproductive strategies, where they can reproduce sexually, producing seeds, and asexually, through vegetative reproduction or apomixis (Askers and Jerling, 1992). Vegetative reproduction exploits a variety of plant structures to initiate new generations (e.g. budding from roots, rhizomes, stems, bulbils, or storage organs) (Eckert, 1999). Thus, seed number may not be reflective of overall fitness because it only represents one component of fitness. A complete measure of fitness

would include other measures such as plant biomass, or parentage analyses to assess the relative success of sexually vs. asexually reproducing individuals (Silvertown, 2008).

vii. Male and female success

Since all sexually reproducing plants have a maternal and paternal parent, it is important to account for the male component of reproduction. Female fitness can be readily estimated by the number of seeds that an individual produces (Burd *et al.*, 2009). However, male fitness, or the numbers of seeds sired by pollen, accounts for half of the genes contributed to the next generation (Sutherland and Delph, 1984). Thus, the exclusive use of seed production is a female-biased view because success as a male parent is not reflected in an individual's seed count when plants outcross. Determining male fitness can be difficult in the field. However, there are several methods available to obtain measures of male fitness: through an evaluation of the number, viability, and growth of pollen (Delph *et al.*, 1998); and through molecular methods such as the combination of genetic markers with paternity analysis (Meagher 1986; McGraw & Caswell, 1996). With the use of these methods and seed count, fitness of sexually reproducing plants can be obtained.

Environmental heterogeneity

viii. Variation in phenology

The seasonal timing of growth and reproduction can cause differences in fitness among individuals in many ways (Galen and Stanton, 1991, Forrest and Miller-Rushing, 2010). Delays in phenology can result in diminishing time available for reproduction, leading to variable seed production and seed quality. For example, a plant may produce many seeds in a constrained season, but those seeds may be of lower quality because of constrained time for allocation to reproduction (see Chapter 2, p. 34). Thus, seed number may not be a reliable measure of fitness when there is variation in phenology because seed quality may be reduced. A measure of seedling survival may provide a more accurate depiction of overall fitness.

1.1.2 Methodology

In this review paper, I attempt to assess the prevalence of the use of seed number as a measure of fitness in published research. I performed this review to clarify which metrics are frequently used to estimate plant fitness and also to determine if the use of seed number as a measure of fitness correlates with alternative metrics in the estimation of plant fitness. I am not claiming that the use of seed number is more or less valuable than other metrics of plant fitness, nor is this meant to be an exhaustive review of the papers published that have examined plant fitness; rather, it is meant to be a representative sample. In order to retrieve relevant papers, I attempted to filter for studies that used seed number or other metrics to examine plant fitness using Web of Science and Google Scholar databases. I used the strict combination of the following search terms for

keyword and abstract searching: ‘plant fitness’ and ‘offspring quality’ and also screened reference lists of collected articles to obtain additional relevant papers. I only included peer-reviewed research papers from established journals and excluded any review or opinion papers, or papers that did not measure plant fitness; some papers examined herbivore fitness or were outside of biological sciences; these were not included. No priority was given to specific journals, authors, or year of publication, which ranged from 1975- 2018.

The final data set consisted of information from 147 articles that were assessed at the full-text level and sorted into eight scenarios based on two criteria of relevance to each scenario: i) assessing the goal of measuring fitness as explained by the author, and ii) identifying the natural history of the plant system used in the study. The author’s reasoning behind measuring plant fitness was the main determinant of the relevance of the study to the scenario it was sorted into. I provide a critical analysis of the experimental methodologies that have been used to assess fitness in plants, justified seed count as a measure of fitness, and provided solutions to measuring total plant fitness. Alternative measures of fitness were noted and classified into seven trait categories: plant biomass, total seed biomass, seed size, germinability, survival, male success, and seedling establishment. Fruit number, fruit size and flower number are surrogates for seed number, and thus were treated as estimates of seed production. All of the studies used in this review and the categories of their fitness estimations are included in Appendix A.

1.2 The Literature Review

The literature review reveals that 83.7% of studies (123/147) have used seed number (or a surrogate of seed number) as a measure of fitness (Figure 1.1). Although the review is not exhaustive, this suggests that seed number is the most frequently used measure of fitness in plant evolutionary studies, likely because it is assumed to be closely related to reproductive success (Primack and Kang, 1986; Burd *et al.*, 2009; Campbell, 2000), and can be easily measured (Pan and Price, 2002).

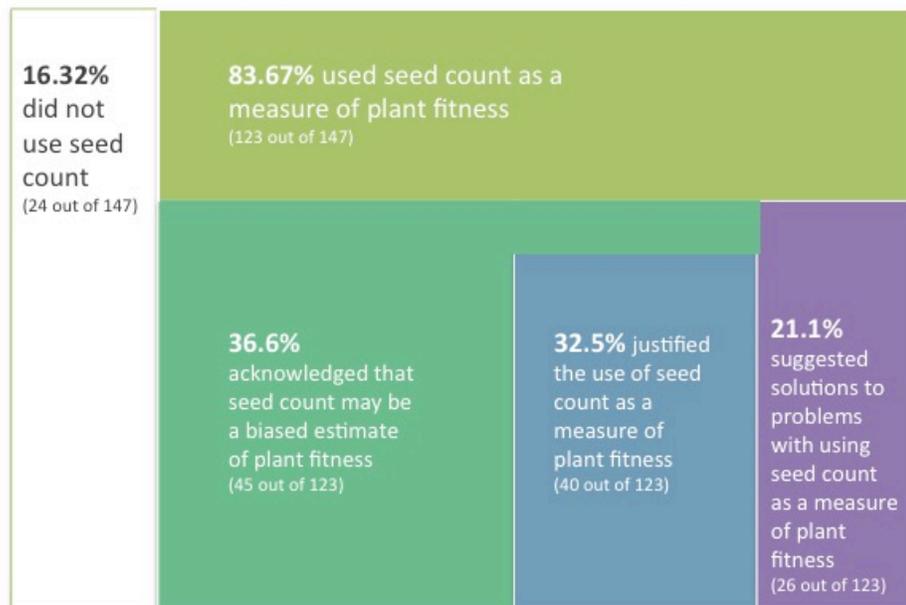


Figure 1.1 An area graph showing the number of studies that have used seed number as a measure of plant fitness, the number of studies that have acknowledged that seed number may be a biased estimate of plant fitness, the number of studies that have justified seed number as a measure of

fitness, and the number of studies that have suggested solutions to problems with using seed count as a measure of plant fitness. The categories “acknowledged seed count as a biased estimate of fitness”, “justified the use of seed count as a measure of fitness”, and “suggested solutions to problems with using seed count as a measure of fitness” are nested within the fraction of studies that have used seed count as a measure of fitness.

All studies were first categorized in terms of their recognition of potential issues in relating seed count to fitness (Appendix A). Among the studies that used seed number as a measure of fitness, 45 studies acknowledged that seed count may be a biased estimate of fitness, 40 studies justified its use as a measure of fitness, and only 26 studies have suggested practical solutions or alternatives to using seed number as a measure of fitness (Figure 1.1). It is important to note that 47% of studies do include an alternative measure of fitness alongside seed count, which suggests that the problem is recognized, even if not explicitly mentioned in the paper (Figure 1.4). Nonetheless, seed count is by far the single most used metric, and papers that acknowledge that seed number may be a biased estimate of fitness are in the minority in the literature.

These recognition categories are not completely hierarchical; some studies that have justified the use of seed count as an appropriate measure of fitness have not acknowledged that seed number may be a biased estimate of fitness. I emphasize that papers falling into the category “acknowledged seed number as a biased measure of fitness” have discussed that seed number may not be an appropriate measure, but have not explicitly tested this. I will provide an overview of the basic distribution of all studies

among the eight scenarios before going on to examine where these studies fall among these categories of level of recognition of the seed count issue.

1.2.1 Distribution of studies among scenarios

I found that 16.3% of studies could be classified under more than one scenario. In particular, I found that the scenarios of male and female fitness, variation in phenology and trade-offs between allocation to reproduction and defense against herbivory overlapped with other scenarios (Figure 1.2). For example, one study investigated pollinator-mediated selection on flowering phenology and floral traits through female function, and also examined how herbivores affected the relationship between floral traits and female fitness (Chen *et al.*, 2017). Because herbivory can affect female fitness through direct effects on floral traits, this study may fall under two scenarios: variation in phenology, and trade-offs between allocation to reproduction and defense against herbivory. Moreover, Ehrlen (2015) provides a path analysis showing positive and negative associations between flowering date (variation in phenology), pollination activity (male and female success), and herbivory. This path analysis shows that variation in flowering time can affect interactions of pollinators and herbivores with fitness; pollinators can have a positive effect on seed production, while herbivores have negative effects. A trade-off may also occur between seed production and the probability of flowering. Thus, direct effects of flowering time and indirect effects of pollinators and herbivores can influence fitness as measured through seed count.

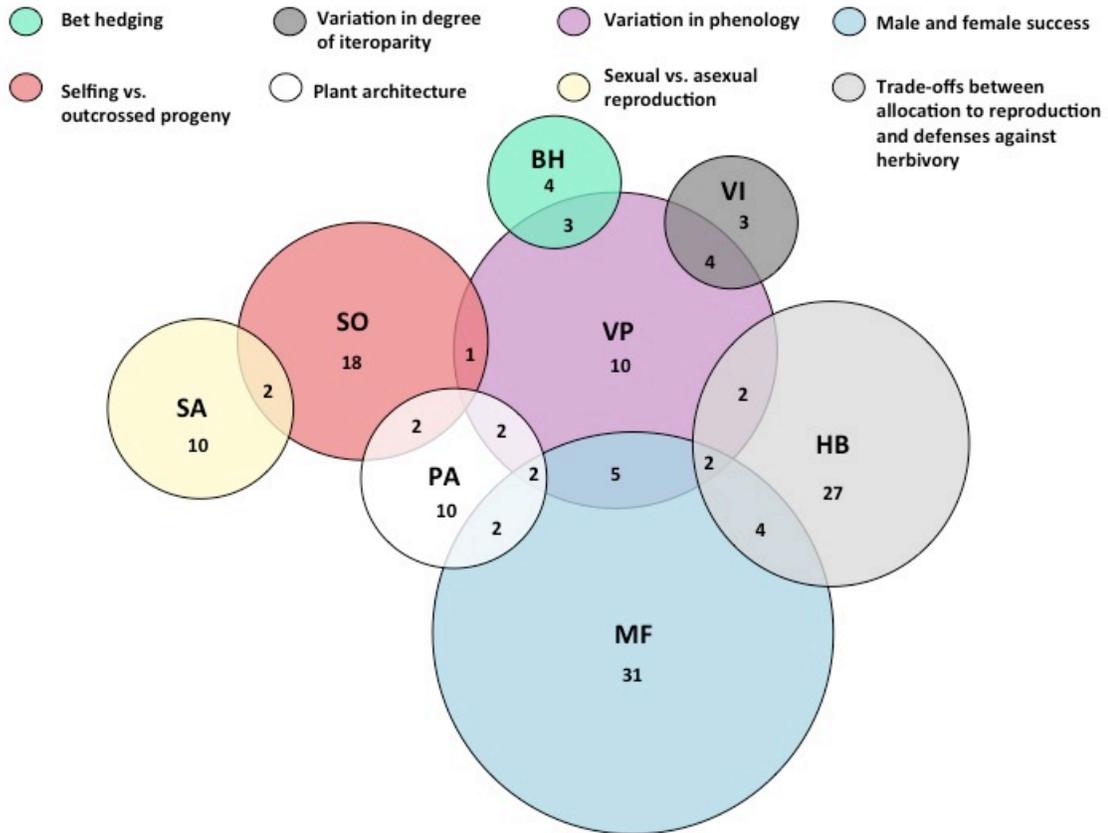


Figure 1. 2 A Venn diagram showing the overlap between the different “scenarios” (see text). The size of each circle represents the number of studies in each scenario. Scenarios: PA= plant architecture, HB= trade-offs between defense against herbivory and seed number, MF= male and female fitness, SO= selfed vs. outcrossed progeny, SA= sexual vs. asexual reproduction, VP= variation in phenology, VI= variation in degree of iteroparity, BH= bet hedging.

Of the 147 total studies reviewed, the highest proportion of studies that have used seed count as a measure of fitness fall under the scenarios of male and female success, variation in phenology, and the trade-off between allocation to reproduction and defense against herbivory (Figure 1.3). In contrast, relatively few studies that used seed number to measure plant fitness fall under the scenarios of sexual vs. asexual reproduction, variation in degree of iteroparity, and bet hedging (Figure 1.3). It should be noted that a subset of

studies examined was classified under scenarios that were not in the eight main scenarios identified. The ‘other’ category included density-dependent effects, hybridization, maternal effects, and symbiosis. Density-dependent interactions can differentially influence individual fitness due to competition among siblings (Campbell *et al.*, 2017), introgressive hybridization can lead to trait combinations that enhance fecundity, and/or pest resistance (Campbell and Snow, 2007); maternal environmental effects can cause variable offspring fitness due to variation in nutrient availability across generations (Jacobs and Lesmeister, 2012); and the effect of mycorrhizal symbiosis can mediate variable effects on plant reproductive success by altering hormone levels in parental plants (Nuortila *et al.*, 2004).

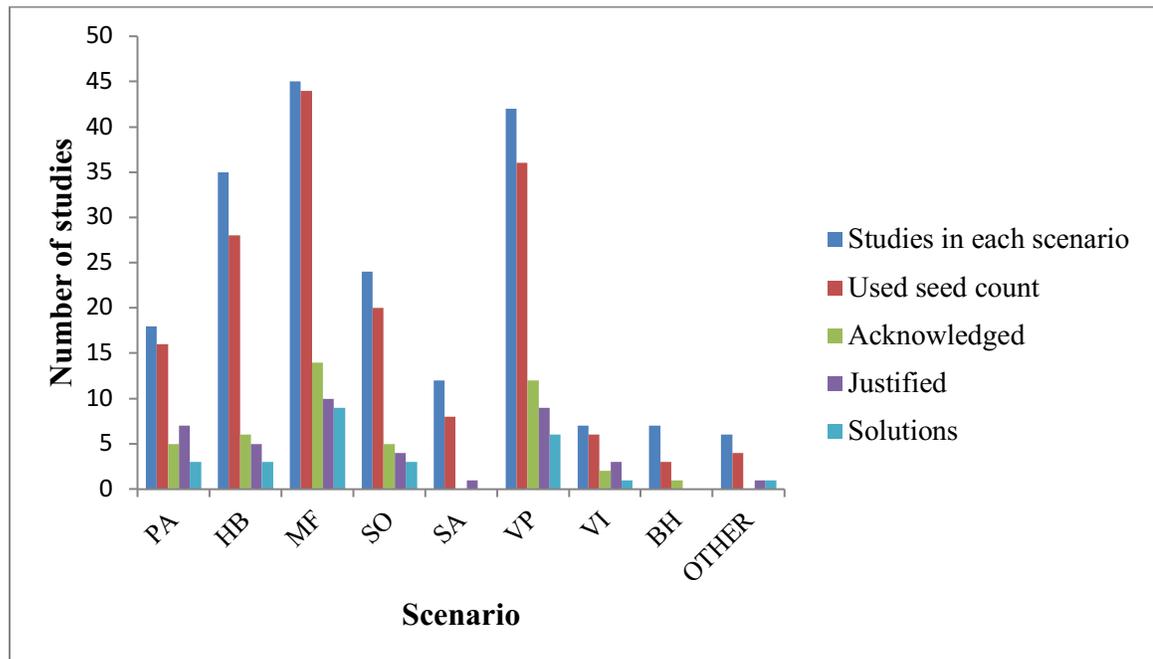


Figure 1.3. Breakdown of reviewed studies into categories of recognition of problems associated with using seed count as a proxy for fitness under each “scenario”. The total number of studies that have investigated plant fitness are represented by the dark blue bar, studies that have acknowledged seed count as a biased measure of fitness in red, studies that have justified seed number as a measure of

fitness in green, studies that have suggested solutions in purple, and studies that have suggested solutions in light blue. Some of the 147 studies examined fall under multiple scenarios, and each occurrence is included in this analysis; therefore, the total number of studies exceeds 147. See Figure 1.2 for scenario abbreviations.

From the fraction of studies that use seed number as a measure of fitness, the largest number of studies that acknowledged that seed number may be a biased estimate of fitness were found under the scenarios of male and female fitness, variation in phenology, and a trade-off between defense against herbivory and seed number (Figure 1.3). The lowest number of studies that have acknowledged seed number as a biased estimate of fitness was found under the scenarios of variation in degree of iteroparity, and bet-hedging. Notably, no studies under the scenario sexual vs. asexual reproduction explicitly acknowledged that seed number might be a biased estimate of fitness (Figure 1.3).

The highest proportion of studies that justified the use of seed number as an estimate of fitness were found under the scenarios of male and female fitness, variation in phenology, and plant architecture. In contrast, few studies that justified the use of seed number as an estimate of fitness were found under the scenarios of variation in degree of iteroparity, sexual and asexual reproduction, and bet-hedging (Figure 1.3). Similarly, most studies that have suggested solutions to problems of using seed count as a measure of fitness were under the scenarios of male and female fitness, and variation in phenology. No studies under the scenarios of sexual vs. asexual reproduction and bet-hedging suggested solutions.

1.2.2 Alternative metrics used

Of the 147 experimental studies reviewed, 53% of studies exclusively used seed number as a measure of fitness, and 47% of all studies used an alternative metric of fitness with or instead of seed number (Figure 1.4).

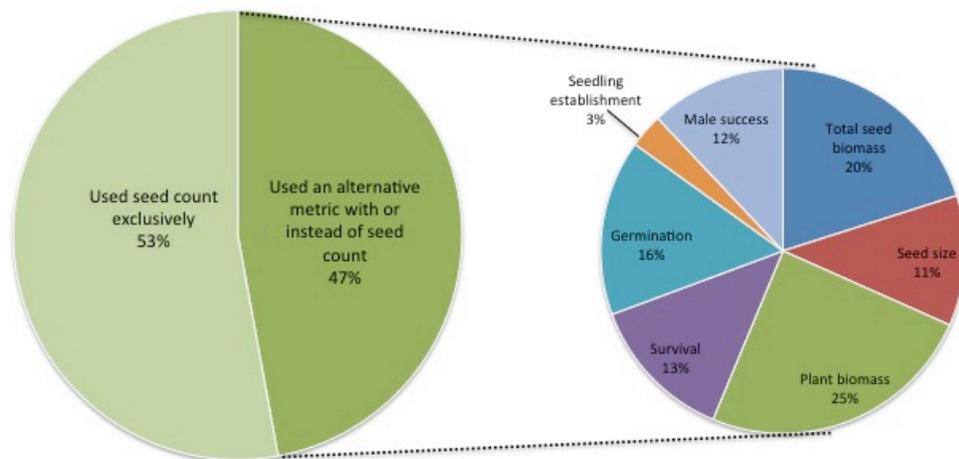


Figure 1.4 Pie charts showing the percentage of all studies that have exclusively used seed number as a measure of fitness and the percentage of studies that have used an alternative measure of fitness with or instead of seed count. The second pie chart represents the percentage of various alternative fitness metrics used in studies that have used an alternative metric with or instead of seed count.

Out of the seven alternative metrics used in studies investigating plant fitness, most studies used growth-related metrics (plant biomass), and seed-related metrics (seed size, seed biomass). Particular alternative metrics were preferentially used under the different scenarios. Plant biomass was the most-used metric under the scenarios of plant architecture, herbivory, sexual vs. asexual reproduction, variation in phenology, and variation in degree of iteroparity; total seed biomass was the most-used metric under the scenarios of male and female success and selfing vs. outcrossed progeny; and germination success was the most-used metric under the scenario of bet hedging in comparison to other metrics (Figure 1.5). Not surprisingly, measures of male success were also strongly represented in the scenario male and female fitness.

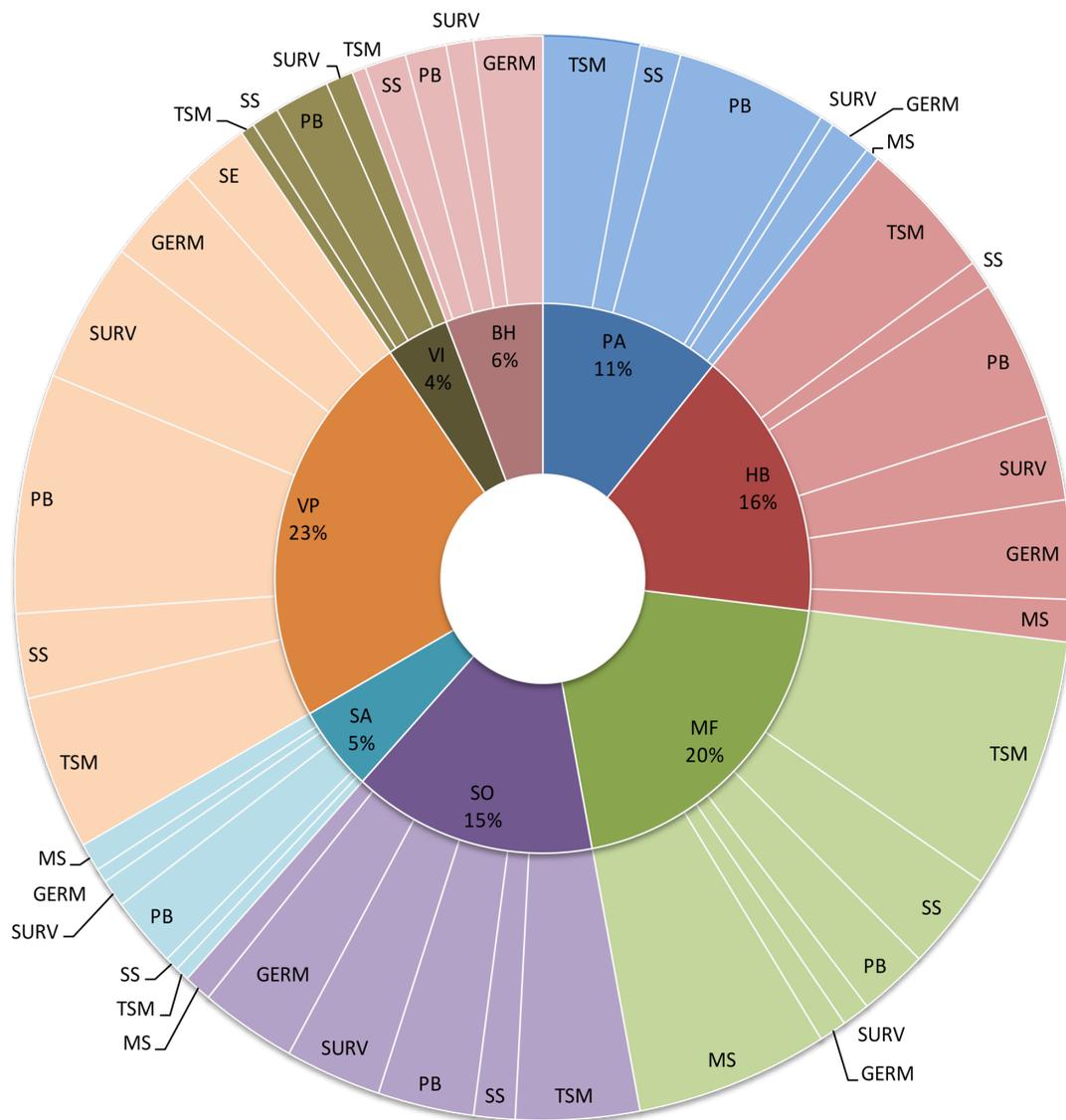


Figure 1.5 Sunburst chart showing the proportions of alternative fitness metrics to seed count used under each scenario (see Fig. 1.2 for scenario abbreviations). The abbreviations for the alternative fitness metrics are: PB= plant biomass, SS= seed size, TSM= total seed biomass, GERM= germination success, SURV= survival, SE= seedling establishment, and MS= male success.

Seed size (diameter and mass) is a key life-history trait and is closely linked to fitness; larger seeds have a higher chance of success (Smith and Fretwell, 1974; Giles, 1990).

However there are situations where there is a resource trade-off between seed size and

seed number; larger seeds are often more successful than smaller seeds, but smaller seeds are more numerous and are therefore likely to have higher dispersal rates (Eriksson, 1999; Smith and Fretwell, 1974). There is supporting evidence that seed sizes are negatively correlated with fecundity, and in some situations smaller seeds may be favoured over larger ones (Simons, 2007). In order to appropriately quantify overall individual fitness, seed size should be considered in conjunction with seed number.

Measurements of plant size or biomass are commonly used as surrogates for fitness and often display positive correlations to fitness as seed count: larger plants generally have more reproductive output, leading to a greater likelihood of leaving viable offspring (Aarssen and Taylor; 1992; Lortie and Aarssen, 1999; Aarssen and Jordan, 2001). Allocation to below-ground biomass can serve as a conservative bet-hedging strategy under unpredictable environmental conditions (e.g. drought, herbivory) (Childs *et al.*, 2010), and is the basis for considering iteroparity itself to be a bet-hedging trait. Additionally, for perennials that may live long and reproduce clonally, plant growth may represent an appropriate measure of fitness if used alongside seed production, especially when there are trade-offs between allocation to growth and reproduction. Thus, the use of growth or biomass as fitness proxies may be appropriate for predominantly clonal species (Hereford, 2009), but seed number should also be considered for plants with mainly sexual strategies. It is currently not known whether the existence of trade offs between asexual and sexual reproduction is general, or whether positive phenotypic correlations generally exist. Further research on this topic is necessary to address whether seed count is an adequate fitness proxy.

The use of male fitness was well represented in the scenario “male and female success”; however, it was under-represented in other scenarios. This result was expected, as it is notoriously difficult to conduct paternity analyses in the field (Bernasconi, 2003). Regardless, the male contribution may be essential to the accurate estimate of individual total fitness, and this is a problem because studies do not tend to evaluate male and female fitness.

I found that seedling establishment and germination success are among the least frequently used metrics across the eight investigated scenarios (Figure 1.5). This result is surprising in that offspring number adjusted for viability is the most direct route to estimating realized fitness through sexual reproduction, but not surprising in that it requires the effort to produce at least a partial additional generation to complete a study. Seedling establishment and successful germination have previously been recognized as critical to fitness estimation (Ackerman *et al.*, 1996). An example of a scenario under which this might be particularly important is in mixed mating systems where selfing may result in a large number of offspring, but these offspring may be of reduced viability relative to outcrossed offspring depending on the severity of inbreeding depression (Baker *et al.*, 1994; Berg; 1999; Angeloni *et al.*, 2011). Thus, seedling establishment and germination success should be considered along with seed number when measuring plant fitness.

Selection acting on traits can vary in intensity and direction, and the use of any single measure of fitness can lead to a misleading interpretation of overall fitness (McGraw and Caswell, 1996; Rausher, 1992). Of the studies that used an alternative fitness metric along with seed number, three in particular revealed a disparity between these fitness measures (Metsare *et al.*, 2015; Bigio *et al.*, 2017; Campbell *et al.*, 2017). These three studies fall under the scenarios of density-dependent interactions, variation in phenology, and selfing vs. outcrossed progeny, respectively. Because there were not sufficient data to do a formal quantitative analysis of disparity among fitness measures, I give an overview of the studies and discuss the differences in fitness found when using seed number versus an alternative metric. Thus, these three studies serve as circumstantial evidence that seed number does not align with alternative metrics, and that alternatives are needed to accurately quantify plant fitness.

1.2.3 Case studies

Case study #1: Do different measures of maternal fitness affect estimation of natural selection on floral traits? A lesson from *Linum pubescens* (Linaceae) (Bigio *et al.*, 2017)

Selection on floral traits is largely influenced by pollinator behaviour, leading to differential fitness (Chen *et al.*, 2017). Investigating pollinator-mediated selection on floral traits with just one fitness measure may not be appropriate because different pollinator behaviour may have different effects among various fitness measures. For

instance, variation in floral colour or size can affect pollinator visitation rate, which can create fitness differences among individuals. Bigio *et al.* (2017) compared the relative roles of multiple fitness measures (fruit set, number of fruits, number of seeds, and seed mass) for estimating pollinator-mediated selection on floral colour and flower diameter in *Linum pubescens*. Their goal was to identify the most appropriate measure of fitness to accurately assess selection on floral traits.

They found that using different maternal fitness measures produced inconsistent results in determining selection on the two floral traits of *L. pubescens*. First, seed mass was a positive indicator of selection for the colour combination of floral tube throat and stamen colour; however, floral diameter was under positive directional selection when using seed number or fruit number as a measure of fitness. Their results suggest that using multiple fitness measures can accurately estimate selection on floral traits, but the exclusive use of seed number or fruit number would misrepresent selection on flower colour. Thus, using several fitness measures should be taken when investigating mechanisms underlying pollinator-mediated selection on floral traits.

Case study #2: Four seed-quality measures in orchids with different pollination systems (Metsare *et al.*, 2015)

In this study, Metsare *et al.* (2015) used four metrics (seed number, seed abortion, seed viability by tetrazolium test and germination in vitro) to assess seed viability in one or more species simultaneously. The focus of their study was to evaluate differences in seed quality metrics across two different orchid species (one nectar rewarding, one

deceptive) with two pollination systems (cross-pollination vs. self-pollination). Evidence has shown that there are differences in fruit set and inbreeding depression between rewarding (flowers that contain nectar) and deceptive species (flowers that do not contain nectar); outcrossing individuals produce better quality seeds compared to geitonogamous individuals (Johnsson and Nilsson 1999). Here, they use three treatments to assess how different quality measures reveal actual seed quality. The three treatments performed were: (1) cross-pollination, (2) geitonogamous self-pollination and, (3) natural pollination as a control.

Interestingly, they found that in the rewarding species *Platanthera clorantha*, seed number, viability, and germination tests suggests higher fitness for outcrossing fruits (higher seed number, viability, and germination), but this trend was only true for the deceptive *Orchis militaris* when exclusively using seed number as a measure of fitness. Although naturally pollinated *O. militaris* seeds had a relatively higher seed count, their seeds had the lowest germination out of all three treatments. Moreover, seed number did not correlate with seed viability or germination in either species, suggesting that more than one uncorrelated fitness measure should be used when investigating fitness in plants with mixed mating systems. Thus, their results show that only using seed production as a fitness measure can be misleading because important fitness-affecting factors may be ignored without germination or viability tests.

Case study #3: Is plant fitness proportional to seed set? An experiment and spatial model (Campbell *et al.*, 2017)

Most empirical studies have used a component of individual fitness such as fecundity to estimate overall fitness. However, using fecundity may be biased if density-dependent interactions between siblings affect survival and reproduction of offspring from high- and low-fecundity parents differently. Moreover, there are mechanisms that can differentially influence individual fitness during transitions between life cycles. Campbell *et al.* (2017) proposed that a high production of seeds does not translate into high seedling survival to reproduction. Specifically, they focused on how spatial distribution of offspring and effects of sibling interactions can differentially influence individual fitness during the gap between seed input and seedling emergence. Using seeds of a wildflower *Ipomopsis aggregata*, they mimicked natural seed dispersal, with areas of overlapping seed shadows and used genetic markers to track offspring in order to test for differences in offspring success. Spatial models revealed that relative fitness is modified by sibling interactions taking place between seed dispersal and juvenile recruitment.

Their results revealed that post-dispersal density dependent interactions caused differences in plant fecundity among individuals. As well, individual differences in fecundity arose from sibling competition, which depended on seed dispersal. It was found that high-fecundity parents that produced twice as many seeds as low-fecundity parents produced fewer than twice the number of successful seedlings. Therefore, a trade-off between high seed production and offspring survival suggests that competition among siblings due to density-dependent interactions can result in lower survivability of seeds.

In this situation, measuring relative fitness based solely on seed number would not be accurate and should be supplemented with survivability of seeds.

1.3 Conclusion

In conclusion, seed count is widely used as a measure of fitness despite a lack of information on its reliability. While a small percentage of studies have recognized the problems that may arise with using seed number as a measure of fitness, a majority of studies have not addressed the consequences associated with the exclusive use of seed number as a measure of fitness. We acknowledge that there are situations in which seed number may be the most appropriate measure of fitness, but the notion that seed number is a reliable estimate of fitness is unclear for many scenarios in nature.

This literature review aimed first to help recognize or identify the specific situations in which there are confounding effects in the relationship between seed number and fitness. It is of course important that investigators be aware of the consequences of using seed number as a measure of fitness when conducting experimental or field studies, and appropriate fitness metrics are expected to vary among scenarios and study species. We note that there may be other scenarios that have not been mentioned in this review, but the discussions outlined here are meant to provide fruitful avenues for future research. While this review is not an exhaustive study, results suggest particular situations under which the exclusive use of seed number may either under- or over-estimate fitness, and that it is important to obtain alternative measures of fitness, especially when large

discrepancies are expected. We therefore emphasize a need for data reporting on more than one fitness measure within individual studies; this would allow a meta-analysis suggesting appropriate fitness measures for different scenarios.

Ideally, then, plant fitness measurement would include a combination of seed number with an alternative metric. This could include measuring fitness components over multiple stages of the individual's life cycle (i.e. vegetative, reproduction, and survival). It is hoped that this review cautions researchers to be mindful of the potential bias in the exclusive use of seed count as a measure of fitness, and to encourage the use of multiple measures of fitness when feasible.

Chapter 2: Variable constraints disrupt the relationship between seed count and fitness

(Sections 2.1-2.5 is in manuscript format and is being prepared for publication as: Wen L. and Simons A.M ‘Delusions of grandeur: seed count is not fitness under variable environments’)

2.1 Introduction

The concept of fitness is central to evolutionary biology--yet difficult to define--and often described in terms of the number of descendants produced. For example, fitness can be conceptualized as the realized values of an individual’s fitness components (i.e. survival of offspring) (de Jong, 1994; McGraw and Caswell, 1996). Our ability to measure fitness is thus important for addressing key questions about life history evolution (Endler, 1986; Stearns, 1992; Roff, 2002), and about adaptation in general (Dobzhansky, 1968; Williams, 1966; Sober, 1984; de Jong, 1994). However, direct measures of fitness can be difficult to obtain. Therefore, empiricists often use fitness proxies that are assumed to be associated with total fitness (Roff, 1992). Assumptions underlying the association between fitness and proxies of fitness are often not tested, and their reliability as estimates of fitness is generally unknown (Thoday, 1953; Lande and Arnold, 1983; Mousseau and Roff, 1987; Charnov, 1997).

The majority of plant evolutionary ecological studies use seed number as a measure of fitness (see Chapter 1, p. 4). There are good reasons for this: seed number is

generally easily obtained either through direct count, or through estimation based on fruit count. Furthermore, seeds are often the most recognizable structure that transfers genetic material across generations, and disperse from the parent plant (Pan and Price, 2002). However, the relationship between true fitness and seed count may be weakened or “disrupted” under several natural scenarios: For example, some plant species can propagate both asexually—e.g. vegetative propagation, budding—and sexually (Pan and Price, 2002), thus, seed count would provide a biased estimate of fitness when individuals invest differently in asexual and sexual reproduction; plant architecture may influence fitness through factors such as dispersal or pollination success (O’Connell and Johnston, 1998); success as a male parent is not reflected in an individual’s seed count when plants outcross (Devlin, 1989); trade-offs between allocation to reproduction and defenses against herbivory may exist (Agrawal et al., 1999); individuals that allocate more energy to reproduction might suffer lower residual reproductive value (Miller *et al.*, 2012). Moreover, the use of seed count assumes a strong association between the number of seeds produced by an individual and number of successful offspring, yet variation among individuals in self-fertilized and outcrossed seeds may result in fitness variation due to inbreeding depression (Berg and Torstensson, 1999); individuals may have relatively low fitness in some years as a result of a bet-hedging strategy that increases the geometric mean fitness under fluctuating conditions (Simons, 2011); finally, plastic adjustment of seed production observed to result from variation in flowering phenology among individuals may affect seed quality (Galen and Stanton, 1991). Here, we investigate the reliability of seed count as a fitness measure under this final scenario of variable phenology by manipulating seasonal constraints.

Phenology, or the seasonal timing of life history events and transitions, may have important fitness effects (Zimmerman and Gross, 1984; Rafferty *et al.*, 2013, Weis *et al.*, 2014), especially when timing influences species interactions. For instance, in temperate insects, precise seasonal timing of egg hatch ensures that the larval stage coincides with optimal conditions for feeding, development and growth (Sniegula, 2016), and adjustment of the transition to the adult stage ensures optimal conditions for feeding, reproduction and dispersal (Danks, 2008). Similarly in plants, constraints on flowering phenology can impose fitness costs by causing asynchrony between flowering and pollinating insects (Forrest, 2016), or by influencing the allocation of resources to growth versus reproduction (Austen and Weis, 2015). For example, yearly variation in temperature and precipitation during a flowering season may affect pollinator abundance and activity and lead to variation in plant reproductive success (Asikainen, 2005).

Variation in phenology can also affect fitness independently of species interactions. Environmentally induced differences in germination and development can result in variance in reproductive phenology: in monocarpic plants, the transition from the vegetative to reproductive phase is often marked by the emergence of a reproductive stalk from a basal rosette, termed bolting. The phenology of this transition to reproduction is critical, because semelparous plants must reproduce successfully before the season ends, and selection should act against individuals with incompletely exhausted resources (Simons and Johnston, 2003; Hughes and Simons, 2014c). Previous work on the monocarpic herb, *Lobelia inflata*, has shown that a semelparous species' pattern of

reproductive allocation is phenotypically plastic to season length cues (Hughes and Simons, 2014b). Although semelparous organisms can be classified as those that show a single, “big bang” reproductive episode, typical reproduction in semelparous organisms occurs over a prolonged reproductive episode. Thus, parity may be considered a continuum between pure semelparity, and pure iteroparity (Hughes and Simons, 2014a). Specifically, in *L.inflata*, individuals that bolt later experience a more constrained season length, and show even more extreme semelparity (Hughes and Simons, 2014a): they express reduced time to first reproduction, smaller size at reproduction, and multiplication of reproductive organs through increased branching, (Hughes and Simons, 2014a). Remarkably, individuals experiencing a constrained season seem to compensate and do not suffer the expected reduction in seed number (Hughes and Simons, 2014a). However, in this work, offspring quality was not assessed. A fuller understanding of the effect of phenology on the relationship between seed count and fitness requires an assessment of offspring quality because high seed production may not translate into high reproductive success.

2.1.1 Model system: *Lobelia inflata*

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 when season length was experimentally shortened (Hughes and 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 and Johnston, 2000a). Fourth, the populations under study are completely self-fertilizing (Hughes *et al.* 2014); 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 (Hughes and Simons, 2015). Finally, total reproductive success can be directly determined because *L. inflata* reproduces exclusively by seed.

2.1.2 Goals of study

In this study, we test the hypothesis that interindividual variation in phenology results in a disruption in the relationship between seed count and fitness, using the monocarpic plant, *Lobelia inflata*. Specifically, we predict that a constrained season length after bolting results in the production of numerous seeds but of lower quality. Later bolting plants showing a more extreme semelparous (“big bang”) reproductive strategy (Hughes and Simons, 2014a) will trade 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 greater resource acquisition prior to bolting, which would affect seed production. However, the manipulation of season lengths only following bolting precludes this possibility in the present study. Manipulated phenology and effective season length was accomplished by inducing bolting in experimental plants, and splitting these plants between a growth-chamber environment that provided cues of a constrained season length (shorter photoperiod, lower temperature), and one that mimicked a long

season (longer photoperiod, higher temperature). Seed number and quality were then assessed for constrained and unconstrained plants by direct seed and fruit counts, and then by observing germination and seedling success under common conditions. Germination and seedling survival were measured both at the whole-plant level and also as patterns of reproductive allocation and fitness among fruits within individuals to test whether seed quality declines within individuals or is expressed uniformly 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 effective season length, seed count should be used cautiously if variation in reproductive phenology exists.

2.2 Materials and methods

2.2.1 *Lobelia inflata* and field collection

L. inflata (Campanulaceae) is an herbaceous, monocarpic perennial native to North America that grows well in disturbed and sandy areas. Seeds of *L. inflata* typically germinate in the spring and early summer and form basal rosettes. *L. inflata* has two distinct phases of life: the vegetative rosette stage in which resources are accumulated, which terminates with the transition to the reproductive stage (expending resources on the production of offspring) upon “bolting”, or the formation of a flowering stalk in the first or second season (Hughes and Simons, 2014c). Reproduction occurs in an acropetal pattern (i.e. from basal to distal positions) along the stalk, with most plants producing between 10-100 fruits, and many thousands of seeds, over the course of the reproductive

season (Hughes and Simons, 2014b). Bolting is triggered by light intensity and photoperiod (daylength), is irreversible, and has to meet a threshold for rosette size that changes with time of year (Simons and Johnston, 2003). Late bolting decreases the time available for a plant to reproduce, leading to potential fitness consequences. If the plant does not enter the reproductive stage during the first year, the rosette is capable of overwintering (Simons and Johnston, 2000b). Upon fruit maturation (the browning of fruits), seeds are passively dispersed and the plant senesces.

Individuals are exclusively self-fertilizing, resulting in complete homozygosity after a few generations (Hughes & Simons, 2015). A closed tube of fused anthers ensures self-fertilization; pollen is released directly onto the stigma of the same flower. Aside from enforcing self-fertilization, the anther tube also prevents outcrossing by acting as a mechanical barrier to pollen release. Offspring have been assumed to be genetically identical to the parent, with populations consisting of independent genetic lineages (Simons and Johnston, 2000b). Recent research using nuclear microsatellite markers developed for genotyping reproductive lineages of *L. inflata*, supports this assumption (Hughes *et al.* 2015; Hughes & Simons, 2015), with no observations of outcrossing or heterozygosity in the populations studied.

Mature fruits of *L. inflata* were collected during the autumn of 2016 from natural populations in Gatineau Park, Gatineau, Quebec (Lat. 45°29'N, Long. 75°50'W). Seeds from ten field-collected individuals separated by an average distance of 6.6m and minimum distance of 2m were used to found experimental populations. Here we are

principally interested in the expression of fitness in contrasting environments, and thus focus on within- rather than among-individual fitness expression patterns.

2.2.2 Season length manipulation

To produce offspring plants, 100 seeds from each lineage were placed in 10 replicate petri dishes (60 x 15mm Fisherbrand) lined with moistened 5.5cm doubled filter paper for up to 14 days. Seeds were cold stratified for 14 days in darkness at 5°C. Seeds were then placed in Biochambers SG-30 seed germinators set to a 12h/12h day/night light regimen at 20°C with 80% humidity until germination.

Upon germination, seedlings of different lineages were planted at randomized positions in cells (4cm x 4cm) of 32-celled trays with autoclaved soil (Promix 860B). Trays were then transferred to a Biochambers AC-40 growth chamber set to a 16h/8h day/night photoperiod schedule at 24°C to induce growth and bolting. Trays were watered every 3-4 days, and a 15-5-15 liquid fertilizer mixture was added once every two weeks. Plants that did not bolt within 60 days (± 1 day) were subjected to a 31 day vernalization treatment (induction of flowering by exposure to prolonged cold) at 5°C in the dark. All plants that bolted were included in the experiment.

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). To study the effect of season length, upon bolting, plants were split equally between two experimental environments: a “long” season representing average summer-like photoperiod and temperature conditions (16.5h/7.5 day/night photoperiod at 24°C) and a “constrained” season representing late-season photoperiod and temperature conditions (12h/12h day/night photoperiod at 20°C). The two contrasting conditions remained constant through time. Thirty-six bolted individuals were monitored until senescence, which was the termination of flowering, and the observation of browning of all fruits. Full duration of senescence of a plant ranged from 90-100 days. Mature fruits were collected, including all fruits on main stem and branches, if branching occurred.

2.2.3 Trait assays

With the aim of characterizing trait expression in the two contrasting manipulated environments, traits at both the whole-plant and within-plant level were assessed for each individual. These included measures of plant size, fruit number and individual fruit sizes, and fruit-specific seed size and number.

Final plant height was measured as the distance from the base of the stalk to tip of the latest fruit for all plants. The final stem diameter was measured 2cm from the base of the stalk using a Vernier caliper (± 0.01 mm).

Fruits were counted, and fruit position on main stem was recorded for all plants at senescence. The total number of fruit produced by a plant included all fruits at all

positions, including on the main stem and branches. The width of each fruit was measured as the diameter using a Vernier caliper (± 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 (1096 out of 2673) were used in any analyses that included fruit position effects. Total fruit number ranged from 11 to 150. Because total fruit number varied among individuals, position was scaled to relative position (5th, 50th, and 95th percentile) for each plant for analyses of reproductive allocation.

Seed number per fruit at the 5th, 50th, and 95th percentile position was determined by manual count under a light microscope. Seed size was measured by: i) collecting thirty seeds from three fruits at the 5th, 50th, and 95th percentile position along the raceme; ii) imbibing seeds on a moistened filter paper-lined petri dish for 24 hours; and iii) measuring seed width using Image J 1.51. Seed counts were taken for a total of 108 fruits over the full fruit size range; thus, a general relationship between seed number and fruit size was obtained by fitting a second-degree polynomial. This equation was then used to estimate the total reproductive output (the number of seeds) per plant, based on measurements of every fruit of all individuals.

Under laboratory conditions, fitness (e.g. germination success, seedling viability) is artificially inflated (St Juliana and Janzen, 2007), and true underlying variance in fitness will not be observed. To introduce variation in seed fitness under laboratory conditions, salinity was used as an abiotic stress (For details, see Appendix B). Salinity is

known to be one of the major abiotic stresses that affect plant growth. Seed germination and seedling growth are the stages most sensitive to salinity, where germination or survival of seeds is often delayed or prevented (Papastylianou *et al.*, 2017). Thirty cold-stratified seeds collected from each fruit position (95th, 50th, and 5th percentile) of the 36 individuals were imbibed in water for 24h. Seeds were then placed on 6 x 5 cm petri dishes on a double layer of filter paper, and watered with a solution of 0.01M NaCl and distilled water, and allowed to germinate under a continuous 12h/12h day/night photoperiod at 20°C and 80% humidity in seed germinators. Seeds of a subsample of six of the 36 individual plants were individually ‘tracked’ (recorded seed size) at each of the three fruit positions for germination and survival in order to determine the effect of seed size on fitness. These seeds were placed individually on labelled hole-punched ‘islands’ (made from Fisherbrand filter paper). Germination was assessed as the protrusion of the radicle through the testa, and counts were made daily. Time (days) to germination and total germination percentage were recorded. Germination fraction was transformed by taking the arcsine of its square root prior to analysis.

To assess survival, seedlings were transferred to 72-celled trays with autoclaved soil (Promix 860B), and placed in a Biochambers AC-40 growth chamber set to a 16h/8h day/night photoperiod schedule at 24°C. Seedlings were initially treated with a solution of 0.01M NaCl and distilled water, and were then watered with only distilled water. To assess vigour, seedlings were observed daily for survival, and measurements of developing rosettes were taken daily for 5 days, and then every other day for 20 days with a Vernier caliper (± 0.01 mm). To determine viability, non-germinating seeds were

subjected to a 14 d cold stratification and a second germination trial. Survival fraction was transformed by taking the arcsine of its square root prior to any statistical analyses.

2.2.4 Statistical analyses

Analyses were performed on three levels: general comparisons of trait expression between two environments, patterns of allocation across fruits, and fitness across the two environments. Seven main reproductive traits were investigated: fruit number, fruit size, seed number, seed size, germination fraction, mean germination time, and seedling survival.

Overall trait means in the two environments were first compared using t-tests, simply using environment as the unit of replication. A two-factor ANOVA was then used to model the effect of fruit position and environment on each trait. Tests showing a significant effect of fruit position were followed by Tukey HSD tests to assess which groups differed. Proportional allocation across fruits was obtained by standardizing to a mean fruit size of 1 within each individual. For analyses of proportional allocation across fruits, an ANCOVA was used to model the effect of environment and relative fruit position on standardized fruit size (all fruit sizes standardized within individuals). Patterns of allocation in the two environments were further explored with linear and quadratic regression.

A two-factor ANOVA was used to model the effect of environment and fruit position on germination fraction, mean germination time, and seedling survival. Tests showing a significant effect of fruit position were followed by Tukey HSD post hoc tests to determine which groups differed.

A mixed effects logistic regression was used with seed size as a nested effect within fruit position to examine fruit positional effects (accounting for seed sizes differences) on germination and survival fractions. All statistical analyses were performed in R 3.4.2.

To estimate the effective fitness of one seed produced in the short environment relative to one produced in the long environment, the mean seed survival was calculated for each individual based on observed germination and survival fractions across fruits weighted by number of observed seeds.

Finally, the total expected fitness for individuals, without considering seedling success (i.e. purely counting seeds), was determined for both environments using the total seed numbers calculated from seed counts and their relationship with fruit measurements. The realized fitness, i.e. taking survival into consideration, was calculated for fruits on the main raceme of individuals in the two environments using the estimated seed numbers from fruit measurements and germination and survival fractions. This measure will be referred to as “realized” or “true fitness” for simplicity. Realized fitness was also

calculated including all fruits of individuals in the two environments, under the assumption that the fruit size-seed survival relationship holds for fruits on branches.

2.3 Results

Overall, plants in the two growth-chamber environments did not differ in height ($P=0.33$) or stem diameter ($P=0.175$), but those in the constrained season length environment produced fewer fruit, and larger fruits that each contained more seeds (Table 2.1). Total seed number in main stem fruits per individual—estimated using the observed relationship between fruit size and seed number—differed between the constrained and long-season environments, but total seed number including branch fruits did not differ significantly (Table 2.1). Overall seed sizes did not differ between the long season and constrained season environment (Table 2.1). Fitness, however, is influenced by patterns of reproductive allocation through time (across fruits) and its effects on the relationship between seed quantity and quality.

2.3.1 Patterns of allocation across fruit

A two-factor ANOVA shows that fruit size differed between environments and among positions, with no significant interaction effect (Table 2.2). A post-hoc Tukey HSD test reveals that fruits at the most basal position were larger than fruits at the most distal position.

Because we have individual fruit size data for all main stem fruits, the proportion of reproductive allocation across fruits in the two environments could be investigated further. ANCOVA of standardized fruit size indicates, besides a significant effect of position ($p < 0.001$), a highly significant interaction ($p = 0.01$) between environment and fruit position. Further examination of trends in standardized fruit size across positions in the two environments separately using quadratic regression reveals a highly significant nonlinear decline in fruit size for individuals under a constrained season only (Table 2.3).

Seed number per fruit differed between the two environments and across positions, with a significant interaction effect (Table 2.2). According to a post-hoc Tukey test, seed number is lowest at the distal position (mean=141.2) and highest at the basal position (mean=247.1) with pooled environments as expected; however, the interaction effect is attributable to a significant difference in seed number between the top and other two positions that is expressed in the short chamber only.

Analyses on seed size confirmed no difference between environments as observed in the simple t-test (Table 2.1), but revealed differences across fruit positions and a significant interaction term (Table 2.2). Results using the subsample of individuals in which seeds were individually tracked yielded qualitatively identical results. A follow-up Tukey 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), compared to a long-season environment (mean=0.290). 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.

2.3.2 Fitness

Germination fraction differed significantly between the two environments and among fruit positions, with a significant interaction term (Table 2.4). Lower germination fractions were observed in the constrained-season environment, and seeds of fruit at the distal position from a constrained-season environment showed substantially lower germinability (mean=0.580) than seeds from a long-season environment (mean=0.835). Germination trials following stratification of nongerminating seeds indicate mortality rather than dormancy; 42 of 171 non-germinating seeds germinated following stratification from a long-season environment (24.6%), whereas only 33 of 355 germinated from the constrained-season environment (9.3%).

Mean time to germination did not differ between environments, but differed among fruit positions (Table 2.4). Seeds from the distal position in both environments took longer to germinate than seeds from the intermediate and basal positions. Although seeds in the distal position from the constrained season took on average two days longer to germinate than those from the long environment, the interaction term is marginally non-significant.

The survival fraction—of those seeds that germinated—differed between environments (long-season environment mean = 0.76; constrained-season environment mean = 0.61) and positions, and the environment-by-position interaction was also highly significant (Table 2.4). Post-hoc Tukey tests (Table 2.5) find that seedling survival from the basal position in the long-season environment does not differ significantly from that in the constrained environment, but is higher than at any other position in either environment. Seedlings produced from the distal position fruit in the constrained-season environment have lower survival than any other position in either environment, and 30% lower survival than seedlings from the distal position in the long-season environment (Table 2.5).

Mixed effects logistic regressions, to ask to what extent seed size differences account for fruit positional effects on germination and survival fraction, found no independent effect of fruit position beyond the effect of seed size nested within position (Table 2.6).

The true or realized fitness associated with a seed in a constrained-season environment (0.532 ± 0.03) is significantly less than ($F=10.6$, $df=1$, $p=0.003$) 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.

Using seed numbers—estimated using the empirical relationship between seed counts and main-stem fruit measurements—i.e. 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 2.1). However, the apparent fitness advantage in the constrained environment based only on seed count disappears when seedling success is included in fitness (Figure 2.1). Total 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 2.1 General comparison of reproductive trait expression between two manipulated environments observed in *Lobelia inflata*.

Trait	<i>df</i>	<i>F</i> <i>Ratio</i>	P	Mean value for environment	
				Long	Short
Fruit number (all)	1	14.23	0.0006	92.72	55.78
Fruit size (mm; main stem)	1	60.42	<0.001	0.405	0.520
Fruit size (mm; all)	1	450.10	<0.001	0.359	0.462
Seed number/fruit (3 positions)	1	91.04	<0.001	119.59	291.93
Total seed number/ individual (main stem)	1	4.70	0.0374	4349.00	5526.33
Total seed number/individual (all)	1	1.18	0.2863	10025.00	11493.83
Seed size (mm)	1	0.0045	0.9464	0.2973	0.2970

Table 2.2 Sources of variation in reproductive traits (fruit size, seed number, seed size) across three fruit positions (FP) and two environments (ENV).

Trait	Source	<i>df</i>	Sum of squares	<i>F</i>	<i>P</i>
Fruit size	ENV	1	0.360	90.86	<0.001

	FP	2	0.217	27.34	<0.001
	FP*ENV	2	0.011	1.36	0.2618
Seed number	ENV	1	801867.00	126.96	<0.001
	FP	2	230852.07	18.27	<0.001
	FP*ENV	2	58482.67	4.63	0.0119
Seed size	ENV	1	0.00000199	0.01	0.9464
	FP	2	0.022	56.66	<0.001
	FP*ENV	2	0.004	9.81	0.0001

Table 2.3 Exploratory quadratic regressions of fruit size allocation (where fruit size is standardized to a mean of 1 for each individual) on relative fruit position for all fruits produced on the main stem.

Environment	Term	Estimate	Std. Error	<i>t Ratio</i>	<i>P</i>
Long season	Intercept	1.068	0.016	66.01	<0.001
	Relative FP	-0.155	0.024	-6.43	<0.001
	Quadratic term	0.143	0.093	1.54	0.1253
Constrained season	Intercept	1.093	0.019	57.52	<0.001
	Relative FP	-0.057	0.028	-2.04	0.0415
	Quadratic term	-0.764	0.109	-7.00	<0.001

Table 2.4 ANOVA results for sources of variation in germination fraction, mean germination time, and survival fraction across positions in the two contrasting growth-chamber environments.

Trait	Source	<i>df</i>	Sum of squares	<i>F</i>	<i>P</i>
Germination fraction	ENV	1	0.345	11.42	0.001
	FP	2	1.066	17.67	<0.001
	FP*ENV	2	0.306	5.07	0.008
Mean germination time	ENV	1	3.786	0.498	0.482
	FP	2	345.39	22.73	<0.001
	FP*ENV	2	43.89	2.89	0.060
Survival	ENV	1	0.586	48.00	<0.001
	FP	2	1.15	47.76	<0.001
	FP*ENV	2	0.325	13.34	<0.001

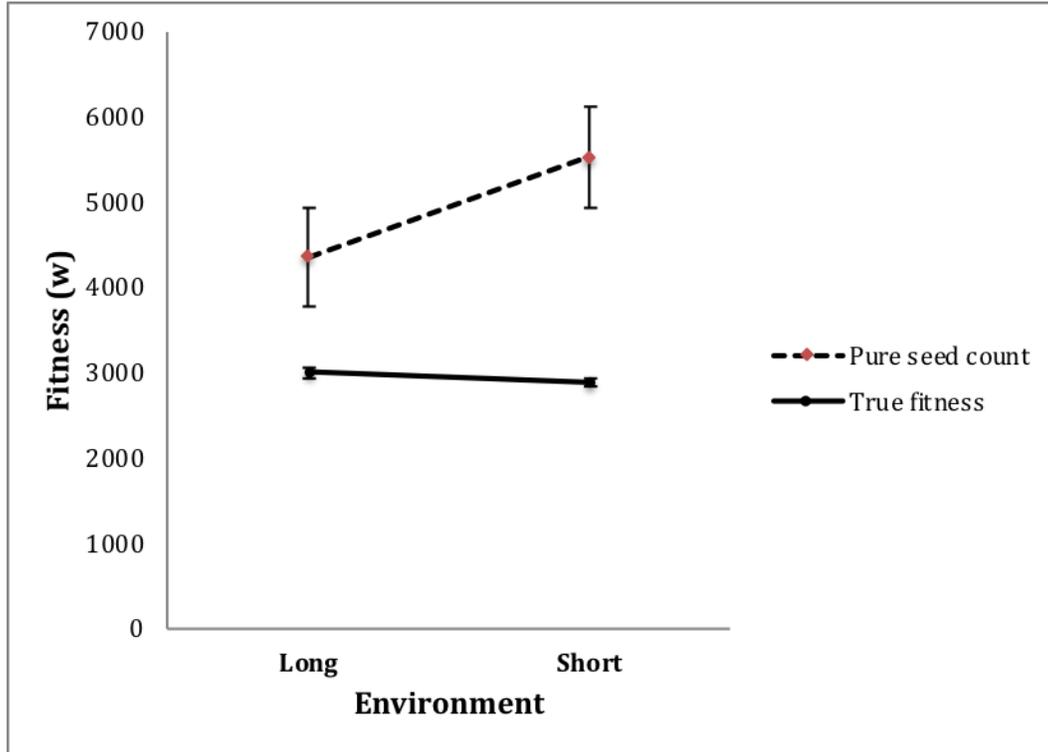
Table 2.5 Post-hoc Tukey test results for differences in means in survival between groups and across positions in the two growth-chamber environments. Letters that are not shared indicate significant different mean values.

Level (ENV:FP)	Letters showing homogenous subsets		Least sq. Mean
Long: Bottom	A		0.843
Short: Bottom	A	B	0.795
Long: Top	B	C	0.717
Long: Middle	B	C	0.712
Short: Middle		C	0.618
Short: Top		D	0.416

Table 2.6 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	<i>df</i>	Wald chi-square	<i>P</i>
Germination	ENV	1	43.77	<0.001
	FP	2	0.245	0.8846
	FP*ENV	2	45.80	<0.001
	Seed size [FP]	3	198.46	<0.001
Survival	ENV	1	29.66	<0.001
	FP	2	3.50	0.1737
	FP*ENV	2	6.32	0.0425
	Seed Size [FP]	3	199.24	<0.001

A.



B.

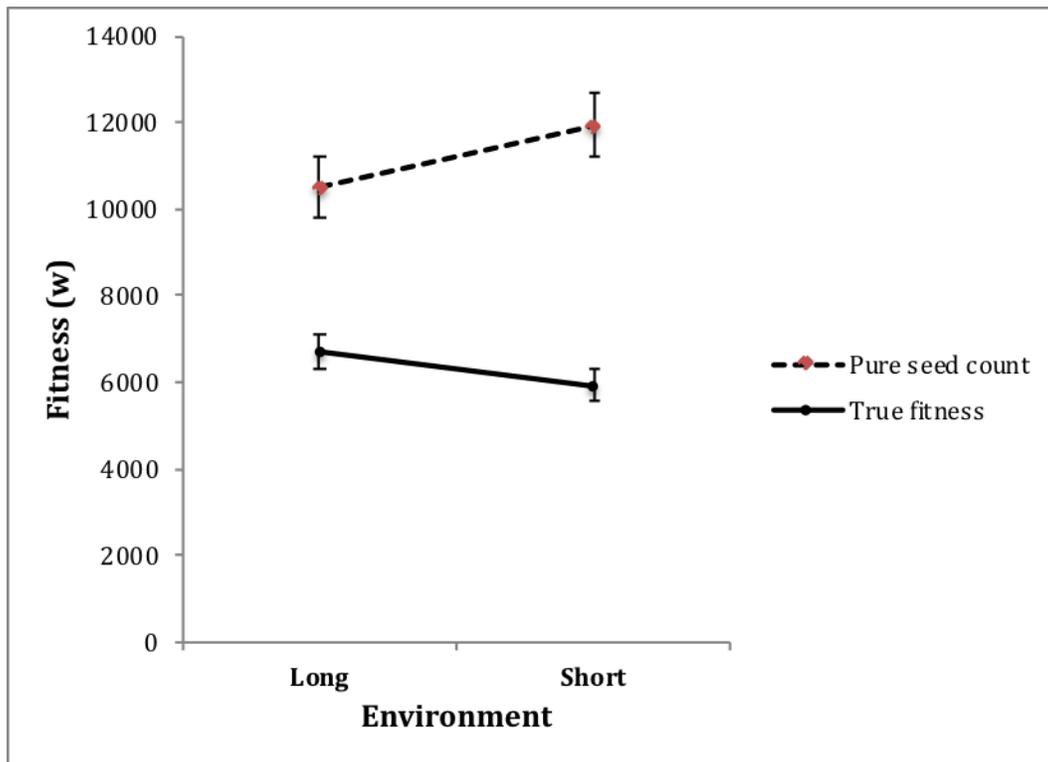


Figure 2.1 Average fitness estimates based on pure seed count and "true fitness" that accounts for successful seed germination and survival, measured across the two manipulated environments. A) Seeds from the main stem fruits, and B) Seeds from all fruits, including fruits from branched stems. Solid lines=true fitness, dashed lines= pure seed count. Error bars represent standard error.

2.4 Discussion

Seed number is often used as a measure of fitness. However, it may not accurately represent reproductive success, and may be a biased estimate of fitness under some situations. Previous work found that *L. inflata* showed plastic responses to variable season lengths in total seed production, implying little to no cost of constrained environments, by exhibiting a more extreme semelparous reproductive strategy (Hughes and Simons, 2014a). However, this study assessed fitness as seed count, and did not test for offspring seedling quality. In the present study, we ask if variable constraints on season length disrupt the relationship between seed number and fitness. Specifically, we test the hypothesis that a constrained season length results in the production of numerous but low quality seeds, thus biasing seed number as a measure of fitness.

The finding that plants under constrained season lengths produce fewer but larger fruit that contained more seeds in total is consistent with Hughes and Simons (2014a) study. However, we found that, although under a constrained season length *L. inflata* plants produced numerous seeds, they were of lower quality, Total fitness, measured as seed number adjusted for success (germinability and survival), was associated with fruit positional effects and seed size, and importantly, was lower for constrained-season plants. Thus, our findings support our hypothesis, that variable effective season lengths

experienced by individuals result in deviations in the relationship between seed number and realized fitness. Because we are interested in within-individual fitness expressions, patterns of reproductive allocation among fruits within individuals were further explored.

Patterns of allocation across fruit

Within-individual variation across fruit positions in fruit size, seed number and seed size suggests that fruit position effects play an important role in the regulation of fecundity and reproductive allocation within individuals, which is consistent with work on other species (Nakamura, 1986; Stocklin and Favre, 1994; Lu *et al.*, 2017). For example, seeds produced later in the season, positioned at the distal end, were smaller. Importantly, the effect of fruit position on reproductive traits was environment-dependent, with an exponential decline in fruit size observed only under constrained conditions, and a significant decline in seed size in distal fruits in the constrained season; however, this decline was not reflected in seed number alone. The observed pattern supports the continuum hypothesis; individuals that bolted later (constrained-season plants) showed a more extreme “big-bang” semelparous reproductive strategy. Because plants in a constrained-season environment have less time to allocate energy to reproduction, plants may “rush” reproduction at the end of the season, resulting in high reproductive effort (seed number) that may not result in high realized fitness.

Fitness- germinability and survival across fruits

Fitness in a constrained vs. in a long-season environment was interpreted in three ways: as the total estimated fitness (purely counting seeds), as total realized fitness (fraction of surviving seedlings, given germination), and as relative per seed fitness. Under the assumption that simple seed count on the main stem represents success, plants in the constrained environment have 1.27 times the fitness of plants in a long season. However, realized parental fitness, estimated as the number of germinating seeds that survived, was no longer significantly higher for constrained season plants: individuals in a constrained-season environment produced many seeds, but both germination success and seedling viability were significantly reduced. This realized fitness reduction under constrained season was due to reduced survival at distal fruit positions.

Seed size differences produced significant effects on germination and survival within fruits; larger seeds had higher germinability and survival than smaller seeds. This result is supported by previous research, where seed size is positively correlated with higher survival and faster early growth under environmental stress (Simons and Johnston, 2000a; Moles, 2004; Metz *et al.*, 2010).

Based only on seed count, apparent fitness was higher (i.e. larger fruits, higher number of seeds) for individuals experiencing a constrained season length. The true or realized fitness in the two environments can be compared as the average relative fitness value of a single seed produced in the constrained season compared to that in a long-

season environment, which was observed to be 0.774 ± 0.034 . This shows intriguing evidence in support of the residual reproductive value-dependent hypothesis; in a constrained season length environment, current reproductive value is increased (i.e. by producing numerous seeds) at the expense of decreased residual reproductive value (Hughes and Simons, 2014b).

The apparent fitness advantage under the constrained season disappears after adjusting seed numbers for seedling fitness, suggesting that using seed number alone as a measure of fitness under these circumstances does not reflect true individual fitness. Although an individual under a constrained-season environment produces an average of 11,493 total seeds (or 5,526.33 seeds on its main stem), only 5,930 (or 2,887 on the main stem) contribute to individual reproductive success.

The results are consistent across a random sample of individuals grown in two environments; however, several limitations on inferences from our findings should be noted. First, plants used in this study were reared from seeds that were field-collected, and it is thus possible that the expression of reproductive traits in growth chambers was influenced by maternal environmental effects. However, this is unlikely to generate differences between growth chamber environments, and the observed among- and within-individual fitness differences were consistent among individuals reared in each environment; furthermore, fitness was measured on seeds that were produced under growth-chamber conditions. Second, because we have only mean seed size in the main dataset and observed variation in germination and survival, we could not distinguish

between effects of individual seed size, environment, and fruit position. However, our analyses using seeds of a subsample of individual plants that were individually ‘tracked’ showed that seed size nested within fruit position had a significant effect on germination and survival. Thus, this subsample of individuals with tracked seed sizes corroborates analyses of the individuals with ‘untracked’ seed sizes, showing that fitness is associated with seed size, which differs across fruit positions. Third, germination and plant growth of *L. inflata* occurred under homogeneous growth chamber conditions that may generate lower variances in reproductive traits compared to under field conditions. However, this would imply that our results are conservative, in that the disparity between seed count and “true” fitness might be higher under field conditions. Finally, the two contrasting environments used in this study meant to mimic short and long seasons are artificial, and it is unknown how the variation generated under these conditions relates to variation in phenology under field conditions. Results of observational field studies would provide insight into the range of phenotypic variation, but interpretation of cause is limited compared to manipulation studies.

2.5 Conclusion

In conclusion, our results demonstrate that variation in effective season length results in variation in patterns of reproductive allocation, and this variation disrupts the relationship between seed count and realized fitness using seedling viability. Other studies have shown evidence of plasticity in reproductive traits under varying phenology (Simons and Johnston, 2003; Hughes and Simons, 2014a; Weis *et al.*, 2015), but the fitness effects of this phenological adjustment have been incompletely studied. When

within-population variation in phenology exists, we conclude that fitness assessment using simple seed count should be interpreted with caution. Future research on phenological variation in the field would increase our understanding of the relationship between seed count and fitness.

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Appendices

Appendix A Complete table of research articles used for the literature review

A total of 147 articles obtained from Web of Science, Google Scholar and screened references lists for literature review. Articles included are the results of the search terms 'plant fitness', 'seed number', and 'offspring quality'. The column heading "Scenario" refers to the specific scenario the article is classified under ("PA"= plant architecture, "HB"= trade-offs between defense against herbivory and seed number, "BH"=bet-hedging, "VI"= variation in degree of iteroparity, "MF"= male and female fitness, "SA"= sexual vs. asexual reproduction, "SO"= selfing vs. outcrossed progeny, "OTHER"= density dependent effects, hybridization, maternal effects, pollination limitation, and symbiosis). Studies that have used seed count as a measure of fitness, or used an alternative metric to measure fitness were noted with a "Yes/No" under each column. The column heading "Metric" corresponds to the alternative metrics to measure plant fitness ("SS"= seed size, "TSM"= total seed biomass, "PB"=plant biomass, "SURV"= survival, "GERM"= germination, "SE"= seedling establishment, "MS"= male success). Studies that have acknowledged seed number as a biased estimate of fitness, justified its use, or provided solutions were noted with a "Yes/No" under each column.

Article name	Authors	Year	Journal	Scenario 1	Used seed count?	Used an alternative metric?	Metric	Acknowledged?	Justified?	Solutions?
Between-species patterns of covariation in plant size, seed size and fecundity in monocarpic herbs	Aarssen, L.	2001	Ecoscience	PA	Yes	Yes	PB, SS, TSM	No	No	No
Fecundity allocation in herbaceous plants	Aarssen, L.	1992	Oikos	PA	Yes	Yes	PB, TSM	Yes	No	No
Genotypic variation in fecundity allocation in <i>Arabidopsis thaliana</i>	Aarssen, L.	1992	Journal of Ecology	PA	Yes	Yes	PB, GERM	No	Yes	No
Seedling establishment in an epiphytic orchid : An experimental study of seed limitation	Ackerman et al.	1996	Oecologia	OTHER	No	Yes	SE, SURV	Yes	No	No
Effects of apical meristem mining on plant fitness architecture, and flowering phenology in <i>Cirsium Altissum</i> (Asteraceae)	Adhikari & Russell	2014	American Journal of Botany	HB	No	Yes	GERM, PB	No	No	No
Induced responses to herbivory and increased plant performance	Agrawal, A.	1998	Science	HB	Yes	No		No	No	No
Transgenerational consequences of plant responses to herbivory: An adaptive maternal effect?	Agrawal, A.	2001	The American Naturalist	HB	No	Yes	SS, SURV	No	No	No
Costs of induced responses and tolerance to herbivory in male and female fitness components of wild radish	Agrawal, A.	1999	Evolution	HB,MF	Yes	Yes	SS, MS	Yes	Yes	Yes
Reciprocal transplants demonstrate strong adaptive differentiation of	Agren & Schemske	2012	New Phytologist	VP	Yes	Yes	SURV, SE	Yes	No	No

the model organism Arabidopsis thaliana in its native range											
Pollination Success across an Elevation and Sex Ratio Gradient in Gynodioecious Daphne laureola	Alonso, C.	2005	American Journal of Botany	MF	Yes	No		Yes	No	No	
Plant patch structure influences plant fitness via antagonistic and mutualistic interactions but in different directions	Andersson et al.	2016	Oecologia	HB	Yes	Yes	GERM	No	No	No	
Effects of mating system on adaptive potential for leaf morphology in Crepis tectorum (Asteraceae)	Andersson & Ofori	2013	Annals of Botany	SO, PA	No	Yes	SURV	No	No	No	
Experimental studies of evolutionary significance of sexual reproduction	Antonovic s & Ellstrand & Asikainen	1985	Evolution	SA	Yes	No		No	Yes	No	
Pollen and Resource Limitation in a Gynodioecious Species	Mutikainen	2005	American Journal of Botany	MF	Yes	Yes	TSM	No	No	No	
Within-plant variation in reproductive investment: consequences for selection on flowering time	Austen et al.	2015	Journal of Evolutionar y Biology	MF	Yes	Yes	MS	No	No	No	
The causes of selection on flowering time through male fitness in a hermaphroditic annual plant	Austen EJ, Weis AE	2016	Evolution	MF	Yes	No		No	Yes	Yes	
Estimating selection through male fitness: three complementary methods illuminate the nature and	Austen EJ, Weis AE	2016	Proceedings of The Royal Society B	MF	No	Yes	TSM, MS	Yes	Yes	Yes	

causes of selection on flowering time											
What drives selection on flowering time? An experimental manipulation of the inherent correlation between genotype and environment	Austen EJ, Weis AE	2015	Evolution	VP, PA, HB	No	Yes		PB, TSM	No	No	No
Fitness constraints on flower number, seed number and seed size in the dimorphic species <i>Primula farinosa</i> and <i>America maritima</i>	Baker & Richards	1994	New Phytologist	MF	Yes	Yes		SS, GERM	No	No	No
Effects of above- and belowground herbivory on growth, pollination, and reproduction in cucumber	Barber et al	2011	Oecologia	HB	Yes	Yes		MS	No	No	No
Seed production in <i>gentiana newberri</i> (Gentianaceae)	Barnes & Rust	1994	The Great Basin Naturalist	MF	Yes	No			No	No	No
Pollen limitation and its influence on natural selection through seed set	Bartkowska and Johnston	2015	Journal of Evolutionary Biology	VP	Yes	No			No	No	No
The balance between sexual and asexual reproduction in plants living in variable environments	Bengtsson & Ceplitis	2000	Journal of Evolutionary Biology	SO	Yes	Yes		MS	Yes	No	No
Offspring performance in three cleistogamous <i>Viola</i> species	Berg et al.	1999	Plant Ecology	SO	Yes	Yes		SS, GERM, SURV, PB	No	No	No
Do different measures of maternal fitness affect estimation of natural	Bigio et al.	2017	Journal of Plant Ecology	MF	Yes	Yes		TSM, MS	Yes	Yes	Yes

selection on floral traits? A lesson from <i>Linum pubescens</i> (Linaceae)											
Climate adaptation is not enough: warming does not facilitate success of southern tundra plant populations in the high Arctic	Bjorkman et al.	2017	Global Change Biology	VP	No	Yes	PB, SE	No	No	No	
Seed production and pre-dispersal reproductive losses in the narrow endemic <i>Euphorbia pedroi</i> (Euphorbiaceae)	Boieiro & Rego	2012	Plant Ecology	HB	Yes	No		No	No	No	
Are mismatches the norm? Timing of flowering, fruiting, dispersal and germination and their fitness effects in <i>Frangula alnus</i> (Rhamnaceae)	Bolmgren & Eriksson	2015	Oikos	VP	Yes	Yes	TSM, GERM, SS	No	No	No	
Adaptive value of phenological traits in stressful environments: Predictions based on seed production and laboratory natural selection	Brachi et al	2012	PLoS One	VP	Yes	Yes	PB, GERM, SE	Yes	Yes	No	
Effects of experimental manipulation of inflorescence on pollination and pre-dispersal seed predation in the hummingbird-pollinated plant <i>Ipomopsis aggregata</i>	Brody & Mitchell	1997	Oecologia	VP	Yes	Yes	SS	No	No	No	
Male Reproductive Success and Variation in Fruit and Seed Set in	Brunet, J	1996	Ecology	MF	Yes	Yes	MS, TSM	Yes	No	Yes	

Aquilegia Caerulea											
Clonal Growth and Ramet Performance in the Woodland Herb, Asarum Canadense	Cain and Damman	1997	Journal of Ecology	SA	Yes	Yes	SURV, PB	No	No	No	
Effects of Floral Traits on Sequential Components of Fitness in Ipomopsis aggregata	Campbell, Diane	1991	The American Naturalist	MF	Yes	Yes	MS	Yes	Yes	No	
Selection for a floral trait is not mediated by pollen receipt even though seed set in the population is pollen-limited	Campbell, Diane	2013	Functional Ecology	MF	Yes	No		No	No	No	
Is Plant Fitness Proportional to Seed Set? An Experiment and a Spatial Model	Campbell, Diane	2017	The American Naturalist	PA, OTHER	Yes	No		No	No	Yes	
Competition alters life history and increases the relative fecundity of crop-wild radish hybrids (Raphanus spp.)	Campbell & Snow	2007	New Phytologist	OTHER, HB	Yes	Yes	PB	No	No	No	
Isolated and Community Contexts Produce Distinct Responses by Host Plants to the Presence of Ant-Aphid Interaction: Plant Productivity and Seed Viability	Canedo-Junior et al	2017	PLoS One	HB	Yes	Yes	TSM, GERM	Yes	No	No	
Direct and indirect effects of pollinators and seed predators to selection on plant and floral traits	Cariveau et al	2004	Oikos	MF	Yes	Yes	PB	Yes	No	No	
Effects of florivory on plant-pollinator	Carper et al	2016	American Journal of	HB, MF	Yes	Yes	MS	No	No	No	

interactions: Implications for male and female components of plant reproduction			Botany							
Selection on floral and carbon uptake traits of <i>Lobelia siphilitica</i> is similar in females and hermaphrodites	Caruso & Yakobowski	2008	Journal of Evolutionary Biology	MF	Yes	No		Yes	No	No
Seed production timing influences seedling fitness in the tropical live oak <i>Quercus oleoides</i> of Costa Rican dry forests	Center et al.	2016	American Journal of Botany	VP	Yes	Yes	SE	No	Yes	No
Offspring for the next generation: most are produced by small plants within herbaceous populations	Chambers, Jesse; Aarssen, Lonnie W.	2009	Evolutionary Ecology	PA	Yes	Yes	PB	No	Yes	No
Spatial variation in pollinator-mediated selection on phenology, floral display and spur length in the orchid <i>Gymnadenia conopsea</i>	Chapurlat et al.	2015	New Phytologist	VP	Yes	No		No	No	No
Pollinator-mediated selection on flowering phenology and floral display in a distylous herb <i>Primula alpicola</i>	Chen et al.	2017	Scientific Reports	VP, MF, HB	Yes	No		No	No	No
Variation in sex ratio, morph-specific reproductive ecology and an experimental test of frequency-dependence in the gynodioecious	Cuevas et al.	2008	Journal of Evolutionary Biology	MF	Yes	Yes	TSM	No	No	No

Kallstroemia grandiflora (Zygophyllaceae)											
Responses of phenology and seed production of annual <i>Koenigia islandica</i> to warming in a desertified alpine meadow	Cui et al.	2017	Agricultural and Forest Meteorology	MF	Yes	Yes	SS, TSM	No	No	No	
Components of Seed and Pollen Yield of <i>Lobelia cardinalis</i> : Variation and Correlations	Devlin, B.	1989	American Journal of Botany	MF	Yes	Yes	MS, TSM	Yes	Yes	Yes	
Effect of pollen load size and source (self, outcross) on seed and fruit production in highbush blueberry cv. 'Bluecrop' (<i>Vaccinium corymbosum</i> ; Ericaceae)	Dogterom et al	2000	American Journal of Botany	SO	Yes	Yes	GERM	No	No	No	
Pollination in <i>Verbascum Thapsus</i> (Scrophulariaceae): The Advantage of Being Tall	Donnelly et al.	1998	American Journal of Botany	PA, MF, SO	Yes	Yes	PB	No	No	No	
The Evolution and Maintenance of Monoecy and Dioecy in <i>Sagittaria Latifolia</i> (Alismataceae)	Dorken et al	2002	Evolution	SA, SO	No	No		No	No	No	
Sex allocation in clonal plants: Might clonal expansion enhance fitness gains through male function?	Dorken and van Drunen	2010	Evolutionary Ecology	SA	No	No		No	No	No	
Proximate Limits to Seed Production in a Herbaceous Perennial Legume, <i>Lathyrus Vernus</i>	Ehrlen, Johan	1992	Ecology	VP, HB, MF	Yes	Yes	TSM	No	No	No	
Flowering schedule in a	Ehrlen et	2015	Ecology	VP, VI	Yes	No		Yes	Yes	Yes	

perennial plant; life-history trade-offs, seed predation, and total offspring fitness	al.									
Timing of Flowering: Opposed Selection on Different Fitness Components and Trait Covariation	Ehrlen, Johan	2009	The American Naturalist	VP, HB	Yes	No		No	No	Yes
Population size and relatedness affect fitness of a self-incompatible invasive plant	Elam et al	2007	Proceedings of the National Academy of Sciences of the United States of America	SO	Yes	No		No	No	No
Experimental Studies of the Evolutionary Significance of Sexual Reproduction Ii. a Test of the Density-Dependent Selection Hypothesis	Ellstrand, Norman	1985	Evolution	SA	No	Yes	PB	No	No	No
Phenotypic selection varies with pollination intensity across populations of <i>Sabatia angularis</i>	Emel et al.	2017	New Phytologist	VP, MF	Yes	Yes	PB	Yes	No	Yes
Climate change alters reproductive isolation and potential gene flow in an annual plant	Franks and Weis	2009	Evolutionary Applications	VP	No	Yes	TSM	No	No	No
Consequences of Emergence Phenology for Reproductive Success in <i>Ranunculus adoneus</i>	Galen & Stanton	1991	American Journal of Botany	VP	Yes	Yes	SS, SE	No	No	No
Reproductive effort and herbivory timing in a	Garcia, MB;	2002	American Journal of	HB, VP, VI	Yes	Yes	SURV, PB	Yes	No	No

perennial herb: Fitness components at the individual and population levels	Ehrlen, J		Botany								
Nonviable seed set enhances plant fitness: the sacrificial sibling hypothesis	Ghazoul & Satake	2009	Ecology	SO	Yes	No		No	Yes	No	
Timing is everything: does early and late germination favor invasions by herbaceous alien plants?	Gioria et al.	2018	Journal of Plant Ecology	VI, VP	No	No		No	No	No	
Population Sex Structure and Reproductive Fitness in Gynodioecious <i>Sidalcea malviflora</i>	Graff, Alison	1999	Evolution	MF	Yes	Yes	TSM	No	No	No	
Bet hedging in desert winter annual plants: Optimal germination strategies in a variable environment	Gremer and Venable	2014	Ecology Letters	BH	No	Yes	GERM, SURV, PB	No	No	No	
Are tetraploids more successful? Floral signals, reproductive success and floral isolation in mixed-ploidy populations of a terrestrial orchid	Gross & Schiestl	2015	Annals of Botany	MF	Yes	No		No	Yes	No	
Effects of Pollen and Resources on Seed Number and Other Fitness Components in <i>Amelanchier arborea</i> (Rosaceae : Maloideae)	Gorchov	1988	American Journal of Botany	MF	Yes	Yes	TSM	No	No	No	
Sources and Consequences of Seed Size Variation in <i>Lupinus Perennis</i>	Halpern, Stacey	2005	American Journal of Botany	PA	Yes	Yes	SS, TSM, GERM,	Yes	No	No	

(Fabaceae): Adaptive and non-adaptive hypotheses								PB			
Induced Floral and Extrafloral Nectar Production Affect Ant-pollinator Interactions and Plant Fitness	Hernandez Cumplido et al.	2016	BioTropica	HB	Yes	No		No	No	No	
Environmental Unpredictability and Plastic Energy Allocation Strategies in Polygonum Cascadense (Polygonaceae)	Hickman	1975	Journal of Ecology	VI	Yes	Yes	PB, TSM	No	Yes	No	
Herbivore-induced changes in flower scent and morphology affect the structure of flower–visitor networks but not plant reproduction	Hoffmeister et al.	2016	Oikos	HB	Yes	Yes	TSM, GERM	No	No	No	
Seed set variation in wild Clarkia populations: teasing apart the effects of seasonal resource depletion, pollen quality, and pollen quantity	Hove et al.	2016	Ecology and Evolution	VP, SO	Yes	No		No	No	No	
Predicted global warming scenarios impact on the mother plant to alter seed dormancy and germination behaviour in Arabidopsis	Huang et al.	2018	Plant, Cell & Environment	BH, VP	Yes	Yes	SS, TSM, GERM	Yes	No	No	
The continuum between semelparity and iteroparity: plastic expression of parity in response to season length manipulation in Lobelia inflata	Hughes & Simons	2014	BMC Evolutionary Biology	VI, VP	Yes	Yes	SS, PB	No	No	No	

Changing reproductive effort within a semelparous reproductive episode	Hughes & Simons	2014	American Journal of Botany	VI	Yes	Yes	SS	No	Yes	No
Secondary reproduction in the herbaceous monocarp <i>Lobelia inflata</i> : time-constrained primary reproduction does not result in increased deferral of reproductive effort	Hughes & Simons	2014	BMC Ecology	VI, VP	Yes	Yes	SURV, PB	No	No	No
Maternal environmental effects on fitness, fruit morphology and ballistic seed dispersal distance in an annual forb	Jacobs & Lesmeister	2012	Functional Ecology	PA, OTHER	Yes	No		No	Yes	No
The effects of quantitative fecundity in the haploid stage on reproductive success and diploid fitness in the aquatic peat moss <i>Sphagnum macrophyllum</i>	Johnson & Shaw	2016	Heredity	SA	No	Yes	SS, PB	No	No	No
Pollen Limitation of Female Reproduction in <i>Lobelia Cardinalis</i> and <i>L. Siphilitica</i>	Johnston, Mark	1991	Ecology	MF	Yes	Yes	TSM	No	No	No
Effects of Cross and Self-Fertilization on Progeny Fitness in <i>Lobelia cardinalis</i> and <i>L.</i>	Johnston, Mark	1992	Evolution	SO	Yes	Yes	SS, TSM	Yes	Yes	No
Context-dependent autonomous self-fertilization yields reproductive assurance and mixed mating	Kalisz et al.	2004	Nature	SO	Yes	Yes	TSM, SURV, PB	No	No	No
Temporal Variation of Flower and Fruit Size in	Kang & Primack	1991	American Journal of	VP, PA	Yes	Yes	PB, TSM	Yes	Yes	Yes

Relation to Seed Yield in Celandine Poppy	Botany										
Effects of Herbivores on Growth and Reproduction of their Perennial Host , Erigeron Glaucus	Karban & Strauss	1993	Ecology	HB	Yes	Yes	TSM, PB, SURV	Yes	No	No	
Growth, phenology, and seed viability between glyphosate-resistant and glyphosate-susceptible hairy fleabane	Kaspary et al.	2017	Bragantia	HB	Yes	Yes	SURV	No	No	No	
Reduced fecundity and o spring performance in small populations of the declining grassland plants Primula veris and Gentiana lutea	Kery et al.	2000	Journal of Ecology	SO, PA	Yes	Yes	SS, TSM	No	No	No	
Maternal effects and heterosis influence the fitness of plant hybrids	Kirk et al.	2005	New Phytologist	OTHER	No	Yes	PB	No	No	No	
Optimal defense theory explains deviations from latitudinal herbivory defense hypothesis	Kooyers et al.	2017	Ecology	HB	No	Yes	GERM	No	No	No	
The Effect of Floral Herbivory on Male and Female Reproductive Success in Isomeris arborea	Krupnick & Weis	1999	Ecology	HB, MF	Yes	Yes	SURV	No	No	No	
The Consequences of Floral Herbivory for Pollinator Service to Isomeris arborea	Krupnick et al	1999	Ecology	HB, MF	Yes	No		No	Yes	No	
Effects of pollination intensity on offspring number and quality in a	Labouche et al.	2017	Journal of Ecology	MF	Yes	Yes	SS, TSM, SURV,	Yes	Yes	No	

wind-pollinated herb								MS			
Herbivory Differentially Affects Plant Fitness in Three Populations of the Perennial Herb <i>Lythrum salicaria</i> along a Latitudinal Gradient	Lehndal & Agren	2015	PLoS One	HB	Yes	Yes		PB	No	No	No
Responses of plant phenology, growth, defense, and reproduction to interactive effects of warming and insect herbivory	Lemoine et al.	2017	Ecology	HB, VP	Yes	Yes		TSM	Yes	Yes	No
Effects of Sowing Date on Phenotypic Plasticity of Fitness-Related Traits in Two Annual Weeds on the Songnen Plain of China	Li et al.	2015	PLoS One	VP	Yes	Yes		PB, TSM	Yes	No	No
Life history trait differentiation and local adaptation in invasive populations of <i>Ambrosia artemisiifolia</i> in China	Li et al.	2015	Oecologia	MF, VP, PA	Yes	Yes		PB, TSM	No	Yes	No
Experimental inbreeding reduces seed production and germination independent of fragmentation of populations of <i>Swertia perennis</i>	Lienert & Fischer	2004	Basic and Applied Ecology	SO	Yes	Yes		GERM, TSM	No	No	No
Lack of early inbreeding depression and distribution of selfing rates in the neotropical emergent tree <i>Ceiba Pentandra</i> : Assessment from several	Lobo et al.	2015	American Journal of Botany	SO	Yes	Yes		TSM, GERM, SURV	No	No	No

reproductive events										
The advantage of being tall: Higher flowers receive more pollen in <i>Verbascum thapsus</i> L. (Scrophulariaceae) ¹	Lortie & Aarssen	1999	Ecoscience	PA	Yes	Yes	PB	No	No	No
Effects of germination season on life history traits and on transgenerational plasticity in seed dormancy in a cold desert annual	Lu et al.	2016	Scientific Reports	VP	Yes	Yes	GERM, PB	No	No	No
Enforced Clonality Confers a Fitness Advantage	Martínková and Klimešová	2016	Frontiers in Plant Science	SA	Yes	Yes	GERM	No	No	No
Effects of herbivore-induced nutrient stress on correlates of fitness and on nutrient resorption in scrub oak (<i>Quercus ilicifolia</i>)	May and Killingbeck	1995	Canadian Journal of Forest Research	HB	Yes	Yes	PB	No	No	No
Fitness consequences of occasional outcrossing in a functionally asexual plant (<i>Oenothera biennis</i>)	Maron et al.	2018	Ecology	SO, HB	Yes	No		No	No	No
Leaf herbivory increases plant fitness via induced resistance to seed predators	McArt et al.	2013	Ecology	HB	Yes	No		No	Yes	No
Avoiding the crowds: the evolution of plastic responses to seasonal cues in a density-dependent world	Metcalfe et al.	2015	Journal of Ecology	BH	No	Yes	GERM	No	Yes	No
Four seed-quality measures in orchids with different pollination systems	Metsare et al.	2015	Botany Letters	SO	Yes	Yes	GERM, SURV	Yes	Yes	Yes
Parental environmental	Metz et al.	2015	Journal of	VP	Yes	Yes	SURV,	No	Yes	No

effects due to contrasting watering adapt competitive ability, but not drought tolerance, in offspring of a semi-arid annual Brassicaceae			Ecology					PB			
Relative performance of selfed and outcrossed progeny in <i>Impatiens capensis</i>	Mitchell-Odds, Waller	1985	Evolution	SO	No	Yes		TSM, SS	No	No	No
Inbreeding Depression and Maternal Effects in <i>Aquilegia Caerulea</i> , a Partially Selfing Plant	Montalvo, Arlee	1994	Ecology	SO	Yes	Yes		TSM	Yes	Yes	Yes
Effects of climate on reproductive investment in a masting species: assessment of climatic predictors and underlying mechanisms	Moreira et al.	2015	Journal of Ecology	VP	Yes	No			No	No	No
Clonal growth and its effects on male and female reproductive success in <i>Prunus siori</i> (Rosaceae)	Mori et al.	2009	Population Ecology	MF,SA	Yes	Yes		MS	No	No	No
Fitness Impacts of Herbivory through Indirect Effects on Plant-Pollinator Interactions in <i>Oenothera macrocarpa</i>	Mothershead Marquis	2000	Ecology	HB	Yes	Yes		TSM	No	No	No
Contrasting effects of ploidy level on seed production in a diploid–tetraploid system	Munzbergova et al.	2017	AoB Plants	VP, MF	Yes	No			Yes	Yes	Yes
Inbreeding Depression in Gynodioecious <i>Lobelia siphilitica</i> : Among-Family	Mutikainen	1998	Evolution	SO, HB	No	Yes		GERM, PB	No	No	No

Differences Override Between-Morph Differences										
Sex expression and inbreeding depression in progeny derived from an extraordinary hermaphrodite of <i>Salix subfragilis</i>	Nagamitsu and Futamara	2014	Botanical Studies	SO	Yes	Yes	PB, SURV	No	No	No
Mycorrhizal symbiosis has contrasting effects on fitness components in <i>Campanula rotundifolia</i>	Nuortila et al.	2004	New Phytologist	OTHER	Yes	Yes	GERM, SURV, PB, TSM	No	No	No
Pollinators exert natural selection on flower size and floral display in <i>Penstemon digitalis</i>	Parachnowitsch et al.	2010	New Phytologist	HB	Yes	No		Yes	Yes	Yes
Macroevolutionary constraints to tolerance: trade-offs with drought tolerance and phenology, but not resistance	Pearse et al.	2017	Ecology	HB	Yes	Yes	PB	No	No	No
Adaptation of flowering phenology and fitness-related traits across environmental gradients in the widespread <i>Campanula rotundifolia</i>	Preite et al.	2015	Evolutionary Ecology	VP	Yes	No		Yes	No	No
Male and Female Pollination Success in a Deceptive Orchid, a Selection Study	O'Connell and Johnston	1998	Ecology	VP, MF, PA	Yes	Yes	MS	No	No	No
The shape of the female fitness curve for <i>Cynoglossum officinale</i> : Quantifying seed dispersal	Rademaker and de Jong	1999	Plant Biology	PA	Yes	Yes	GERM, PB	Yes	Yes	No

and seedling survival in the field

Fitness-consequences of geitonogamous selfing in a clonal marine angiosperm (<i>Zostera marina</i>)	Reusch, T	2001	Journal of Evolutionary Biology	SO, SA	Yes	Yes	GERM, SURV	No	No	No
Frequency dependent fitness in gynodecious <i>Lobelia siphilitica</i>	Rivkin et al.	2015	Evolution	MF, PA	Yes	No		No	No	No
Reproductive consequences of variation in flowering phenology in the dry forest tree <i>Enterolobium cyclocarpum</i> in Guanacaste, Costa Rica	Rocha et al	2018	American Journal of Botany	VP	Yes	Yes	GERM, TSM, SURV	No	No	No
The effects of plant size and light availability on male and female reproductive success and functional gender in a hermaphrodite tree species, <i>Magnolia stellata</i>	Setsuko and Tomaru	2011	Botany	PA, MF	Yes	Yes	MS	Yes	Yes	Yes
Selection for increased allocation to offspring number under environmental unpredictability	Simons, Andrew	2007	Journal of Evolutionary Biology	BH	No	No		Yes	No	No
Suboptimal timing of reproduction in <i>Lobelia inflata</i> may be a conservative bet-hedging strategy	Simons & Johnston	2003	Journal of Evolutionary Biology	BH, VP	Yes	Yes	PB	No	No	No
Environmental and Genetic Sources of Diversification in the Timing of Seed	Simons and Johnston	2006	Evolution	BH	Yes	Yes	SS, GERM	No	No	No

Germination: Implications for the Evolution of Bet Hedging										
Variation in seed traits of <i>Lobelia inflata</i> (Campanulaceae): Sources and Fitness Consequences	Simons & Johnston	2000	American Journal of Botany	BH, VP	No	Yes	SURV, SS, GERM, PB	No	No	No
Consequences of Floral Variation for Male and Female Reproduction in Experimental Populations of Wild Radish, <i>Raphanus sativus</i> L.	Stanton et al.	1991	Evolution	MF	Yes	Yes	MS, SS, GERM	No	No	No
Ecological Consequences and Phenotypic Correlates of Petal Size Variation in Wild Radish, <i>Raphanus sativus</i> (Brassicaceae)	Stanton & Preson	1988	American Journal of Botany	MF	Yes	Yes	TSM	No	No	No
Reproductive performance of <i>Helianthemum caput-felis</i> along its fragmented distribution in the Mediterranean coasts	Sulis et al.	2017	Flora	VP, MF	Yes	No		No	No	No
On the Importance of Male Fitness in Plants: Patterns of Fruit-Set	Sutherland & Delph	1984	Ecology	MF	Yes	No		Yes	Yes	No
Interacting effects of genetic variation for seed dormancy and flowering time on phenology, life history, and fitness of experimental <i>Arabidopsis thaliana</i> populations over multiple generations in the field	Taylor et al.	2017	New Phytologist	VP	No	Yes	PB	Yes	Yes	No
Antiherbivore defenses	Thompson	2016	Evolution	HB	No	Yes	TSM	No	No	No

alter natural selection on plant reproductive traits	& Johnson										
Trade-offs between clonal and sexual reproduction in sagittaria latifolia (alismataceae) scale up to affect the fitness of entire clones	Van-drunen & Dorken	2012	New Phytologist	SA, MF	Yes	Yes	PB	No	No	No	
Consequences of clonality for sexual fitness: Clonal expansion enhances fitness under spatially restricted dispersal	Van-Drunen et al	2015	Proceedings of the National Academy of Sciences of the United States of America	SA	Yes	Yes	MS	No	No	No	
Parasitoid-plant mutualism: parasitoid attack of herbivore increases plant reproduction	van-Loon et al.	2000	Entomologia Experimentalis et Applicata	HB	Yes	Yes	GERM, SURV, TSM, SS	No	No	No	
Floral Plasticity in an Iteroparous Plant : The Interactive Effects of Genotype , Environment , and Ontogeny in Campanula rapunculoides (Campanulaceae)	Vogler et al.	1999	American Journal of Botany	MF	Yes	Yes	MS	No	No	No	
Inbreeding depression in Campanula rapunculoides L. I. A comparison of inbreeding depression in plants derived from strong and weak self-incompatibility phenotypes	Vogler et al.	1999	Journal of Evolutionary Biology	SO	Yes	Yes	GERM, SURV, PB	Yes	No	Yes	
Correlated patterns of	Volis,	2007	Evolutionary Biology	VP	Yes	Yes	SS,	No	No	No	

variation in phenology and seed production in populations of two annual grasses along an aridity gradient	2007		Ecology					GERM, SURV			
Seed Set and Seed Mass in <i>Ipomopsis aggregata</i> : Variance Partitioning and Inferences about Postpollination Selection	Waser et al.	1995	Evolution	MF, SO	Yes	Yes		TSM	No	No	No
The shape of selection: using alternative fitness functions to test predictions for selection on flowering time	Weis et al.	2014	Evolutionary Ecology	VP	Yes	No			No	No	No
Hard and soft selection on phenology through seasonal shifts in the general and social environments: A study on plant emergence time	Weis et al.	2015	Evolution	VP	Yes	Yes		SURV, PB	No	No	No
The snow and the willows: earlier spring snowmelt reduces performance in the low-lying alpine shrub <i>Salix herbacea</i>	Wheeler et al.	2016	Journal of Ecology	VP, SA	Yes	Yes		PB	No	No	No
Floral Dimorphism, Pollination, and Self-Fertilization in Gynodioecious <i>Geranium richardsonii</i> (Geraniaceae)	Williams et al.	2000	American Journal of Botany	SO, MF	Yes	Yes		MS	No	No	No
Parasitoid wasps indirectly suppress seed production by stimulating consumption rates of their seed-feeding hosts	Xi et al.	2015	Journal of Animal Ecology	HB	Yes	No			No	No	No

Influences of Floral Variation on Pollen Removal and Seed Production in Wild Radish	Young et al	1990	Ecology	MF	Yes	Yes	MS	No	No	No
The relationship between flowering phenology and seed set in an herbaceous perennial plant, polemonium foliosissimum gray	Zimmerman and Gross	1984	The American Midland Naturalist	VP	Yes	No		Yes	Yes	Yes
Growth-defense tradeoffs for two major anti-herbivore traits of the common milkweed <i>Asclepias syriaca</i>	Zuest, Tobias; Rasmann, Sergio; Agrawal, Anurag A.	2015	Oikos	HB	No	Yes	PB	Yes	No	Yes

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Appendix B Supporting data for the effects of salinity on germination and seed yield of *Lobelia inflata*

Analysis of fitness variation under laboratory conditions is notoriously problematic because even individuals that would have low fitness in the field perform well under benign laboratory conditions (St Juliana and Janzen, 2007). Seed number is often used as a surrogate for fitness, but under lab conditions, variance in seed fitness- measured as variance in seed germination and viability- may be much reduced. In order to introduce variation in seed fitness under laboratory conditions, an abiotic stress such as salinity can be used (Vu et al., 2015; Hannachi and Van Labeke 2018). Salinity is known to be one of the major abiotic stresses that affect plant growth. Seed germination and seedling growth are the stages most sensitive to salinity, where germination or survival of seeds are often delayed or prevented (Papastylianou et al., 2017). The goal of this study was to investigate laboratory conditions under which seed germination and viability better reflect variable seed fitness, by using varying levels of salinity. *Lobelia inflata* seeds and new seedlings will be subjected to salinity conditions that are harsh enough to reveal fitness differences, but not so harsh that mortality is 100%.

Two fitness assays were performed: 1) seed viability (germination assay) and, 2) seedling survival. Forty-eight randomly selected seeds from eight genotypes were used per treatment. To account for true fitness differences, four genotypes that were “fit” and four genotypes that were “less fit” were used. In order to estimate fitness for these genotypes, 30 seeds were randomly selected from each genotype and measured for seed

size. Because seed size known to affect the fitness of the offspring (i.e. larger seeds often have higher fitnesses) (Giles, 1990; Mandal, 2008; Souza, 2014), the mean seed size for the thirty seeds were used to categorize “fit” and “unfit” genotypes; the larger the mean seed size, the more “fit” the genotype.

For the germination assay, seeds were cold stratified for 14 days and then subjected to varying concentrations of a NaCl solution. Four treatments were used, each with different concentrations of NaCl:

Salinity treatments:

- 1) Control (no NaCl)
- 2) Treatment 1: seeds treated with 0.01M NaCl (292.2mg/500mL)
- 3) Treatment 2: seeds treated with 0.05M NaCl (1.461g/500mL)
- 4) Treatment 3: seeds treated with 0.1M NaCl (2.922g/500mL)
- 5) Treatment 4: seeds treated with 0.15M NaCl (4.383g/500mL)

Seeds were initially watered with NaCl solutions, and then watered with distilled water henceforth. Germinated seeds were counted daily for 31 days. Germination was considered successful when a 2mm radicle is visible. Seeds that did not germinate after 31 days were considered unviable. The final germination percentage and mean germination time were concluded for analyses. Non-germinating seeds were subjected to a cold stratification process for two weeks to test for dormancy, and then re-tested for germination under no NaCl treatment.

For the second part of the fitness assay, seedlings were planted in cell-packs (4cm x 4cm) in 32-celled trays with autoclaved soil (Promix 860B) in a randomized fashion. Trays were then transferred to Biochambers AC-40 set on a 16h/8h day/night photoperiod schedule at 24°C to induce growth. The four salinity treatments were applied at the beginning of seedling transplant. Survival of seedlings (either alive or dead) was observed for 20 days and rosette size measurements were taken every 3 days.

The first stage of the fitness assay revealed that germination did not significantly differ between treatments ($F(5,42)=0.5733$, $P=0.597$); mean germination percentage ranged from 97.5% to 100% for all treatments, regardless of seed size. All non-germinating seeds successfully germinated after a second trial.

The second stage of the fitness assay showed that survival differed between treatments; seedling survival decreased with higher salinity concentrations control (seedling survival %= 93.5), treatment 1 (seedling survival %= 88.125), treatment 2 (seedling survival %=81.5), treatment 3 (seedling survival %= 71.875), treatment 4 (seedling survival %= 55.25). Treatment 3 in particular produced the most variable results out of all the treatments among all genotypes, where survivability ranged from 45% to 81%. Thus, treatment 3 (0.1M NaCl) was determined to be the most effective in determining true fitness differences and was used in the main experiment.