Empirical evidence for the continuity
of semelparity and iteroparity

by

P. William Hughes

B.Sc., Pg.C.

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Lobeliaceae.

Lobelia inflata L.
Aufgeblasene Lobelie.~

“*Lobelia inflata* L.” Colour chromolithograph by Artus Kirchner (1848)

Abstract

Semelparity—the life history featuring a single, massive bout of reproduction followed by death—has been traditionally considered as a discrete trait, and mathematical models that compare the intrinsic rates of increase between annual semelparity and perennial iteroparity are often used to explain why organisms have semelparous or iteroparous reproductive strategies. However, some authors have proposed that semelparity and iteroparity may represent different extremes along a continuum of possible modes of parity, rather than discrete alternatives.

In this thesis, I provide experimental evidence for this “continuum” hypothesis by assessing the degree of variation in the simultaneity and uniformity of semelparous reproduction in the semelparous herb *Lobelia inflata* (Campanulaceae). I report four points of interest: (1) that reproductive characters can strategically vary among offspring within a semelparous reproductive episode; (2) that phenotypically plastic responses to constrained reproduction cause variation in the “semelparousness” of a semelparous reproductive episode; (3) that *L. inflata* is capable of deferring reproductive effort to another season, and that this deferral is also phenotypically plastic; and (4) that the degree of genetic variation in a field population of *L. inflata* suggests non-random survival of ecotypes related to differences in parity. I conclude that the continuum hypothesis more accurately characterizes life history variation among semelparous organisms.

Acknowledgements

First and foremost, I would like to thank Dr Andrew Simons for giving me the ideal combination of structure and freedom while in graduate school. He has been an outstanding teacher and mentor, and I am very grateful to have had the chance to learn from him.

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Statement of Coauthorship

This thesis contains one review chapter and five data chapters—four of these (Chapters 2-5) have been published, and the other two (Chapters 1 and 6) are currently in preparation or under review. Chapter 1 is presented here as a general overview of the literature, and is authored by me alone. Chapters 2-4 and 6 are the product of collaboration between Professor Andrew Simons and myself: we conceived of each of these projects together, and I conducted the experiments and collected the data. Professor Simons and I performed the project described in Chapter 5, the development of microsatellite markers for *Lobelia inflata*, in collaboration with Allison Jaworski and Professor Susan Aitken (at Carleton University), as well as Corey Davis (at the University of Alberta). For this chapter, Professors Simons, Aitken, and I planned this study together, and I performed the experiments with Ally and Corey.

Thesis Overview

Chapter 1: General Introduction – The continuity of semelparity and iteroparity

- submitted as: Hughes PW. The continuity of semelparity and iteroparity.

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Chapter 2: Changing reproductive effort within a semelparous reproductive episode

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Chapter 3: Semelparity and iteroparity: phenotypically plastic modes of parity in *Lobelia inflata*

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Chapter 4: Facultative secondary reproduction in *Lobelia inflata*

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Chapter 5: Development of microsatellite markers for *Lobelia inflata*

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Chapter 6: Evidence for fluctuating selection on genetic lineages of an obligately self-fertilizing plant

- submitted as: Hughes PW, Simons AM. Obligate autogamy in *Lobelia inflata* (Campanulaceae): genetic variation is partitioned by genetic lineage. Manuscript in review as of December 2014.

“One of the most significant of the possible classifications of life histories rests on the distinction between species which reproduce only once in a lifetime and those in which the individuals reproduce repeatedly. This being the case, it is very surprising that there seem to be no general terms to describe these two conditions. The writer proposes to employ the term semelparity to describe the condition of multiplying only once in a lifetime, whether such multiplication involves fission, sporulation, or the production of eggs, seeds, or live young. Thus nearly all annual plants and animals, as well as many protozoa, bacteria, insects, and some perennial forms such as century plants and the Pacific salmon, are semelparous species. The contrasting condition will be referred to as iteroparity.”

Lamont Cole (1954)

The population consequences of life history phenomena.
Quarterly Review of Biology 29:103-137

“The octopus as a rule does not live the year out. It has a natural tendency to run off into liquid; for, if beaten and squeezed, it keeps losing substance and at last disappears. The female after parturition is peculiarly subject to this colliquescence; it becomes stupid; if tossed about by waves, it submits impassively; a man, if he dived, could catch it with the hand; it gets covered over with slime, and makes no effort to catch its wonted prey. The male becomes leathery and clammy. As a proof that they do not live into a second year there is the fact that, after the birth of the little octopuses in the late summer or beginning of autumn, it is seldom that a large-sized octopus is visible, whereas a little before this time of year the creature is at its largest. After the eggs are laid, they say that both the male and the female grow so old and feeble that they are preyed upon by little fish, and with ease dragged from their holes; and that this could not have been done previously; they say also that this is not the case with the small and young octopus, but that the young creature is much stronger than the grown-up one. Neither does the sepia live into a second year.”

Aristotle, (~350 BCE)

History of Animals BkIX, Sec. 37

(translated by D’Arcy Wentworth Thompson).

The oldest known reference to a semelparous life history.

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Chapter 1: The continuity of semelparity and iteroparity

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“If we consider our unit of time to be a single year, annuals can be termed semelparous and perennials iteroparous. A further division is possible within annuals, for some reproduce once and are, therefore, semelparous within any time scale, while others flower repeatedly throughout the summer and, hence, are iteroparous with respect to annuals that flower only once, but semelparous with respect to perennials.” (Derek Roff, *The Evolution of Life Histories*, p. 248)

1.1 Overview

The number of times an organism reproduces—its mode of parity—is one of its most fundamental life-history characters. In this thesis I will argue that the traditional consideration of semelparity and iteroparity as discrete life history habits is not an accurate empirical description of the continuity of reproductive behaviours, and that semelparity and iteroparity are best characterized as endpoints along a continuum of possible modes of parity. I will further argue that the importance of accurately describing the relationship between semelparous and iteroparous habits is especially important for life history theory, which attempts to provide evolutionary and ecological explanations

for the expression of these behaviours. To this end, in the following general introduction and literature review, I will: (1.2) explain the development of the discrete model of parity, as well as the development of the problem of the evolution of semelparity and iteroparity according to the discrete model; (1.3) consider the empirical support for the discrete model; (1.4) introduce the continuous model of parity; and (1.5) consider the empirical support for the continuous model. I will then end with (1.6) a short conclusion—where I explain how this empirical work shows that the continuous model is a more general conception of parity than the discrete model—and (1.7) an explanation of the importance of the experimental treatment of parity in the data chapters of this thesis.

1.2 The problem of semelparous life histories and the development of the discrete model of parity

Identifying the evolutionary reasons why semelparity, the life history defined by a single, sizable bout of reproduction, and iteroparity, the life history defined by repeated (i.e. “iterative”) bouts of reproduction throughout life, are adopted by different organisms is one of life history theory’s oldest problems. Although numerous exceptions have been identified, the received view is that semelparity (which is synonymous with the botanical term “monocarpy”) and iteroparity (“polycarpy”) are discrete and discontinuous life-history strategies—I refer to this as the “discrete model” of parity. The alternative view, which I argue for in this chapter, is that semelparity and iteroparity are endpoints along a continuum of possible life histories, with many species displaying intermediate strategies (i.e. the “continuum model”).

According to the discrete model, for any given species, a semelparous or iteroparous strategy evolves in response to strong directional selection for values of specific reproductive characters—e.g. inflorescence size, offspring number—that maximize the annual rate of intrinsic increase. Although Linnaeus (1744) was the first to construct a mathematical model of the intrinsic rate of increase of annual plants, Cole (1954) was the first to categorize life histories into dichotomous “semelparous” and “iteroparous” groups: a semelparous organism is one that “dies upon producing seed” and therefore “potential population growth may be considered on the assumption that generations do not overlap” (p. 109), while iteroparous organisms include a variety of cases, from those where “only two or three litters of young are produced in a lifetime” as well as “various trees and tapeworms, where a single individual may produce thousands of litters” (p. 118). Thus, Cole created, and contemporary theorists have inherited, a conception of parity as a discrete variable: an organism either reproduces more than once or it doesn’t.

Cole also identified what would come to be called “the paradox of semelparity”, saying:

“for an annual species, the absolute gain in intrinsic population growth which could be achieved by changing to the perennial reproductive habit would be exactly equivalent to adding one individual to the average litter size” (Cole 1954, p. 118).

This answer was surprising because it implies that iteroparity, not semelparity, should be rare, while in nature, iteroparous life histories are generally more common than semelparous ones.

The paradox was resolved by including age-specific rates of mortality, which would affect semelparous and iteroparous habits differently. Charnov and Schaffer (1973) noted that mortality rates were not global; when rates varied between age classes, the expected fitness value of individuals at juvenile (i.e. prereproductive) and adult (i.e. reproductively mature) developmental stages were different. They argued that when the survival of adults was more assured than the survival of juveniles, the iteroparous habit would have a distinct advantage over the semelparous one. Young (1981) extended this insight into a new general model of intrinsic rates of increase, which incorporated not only differences in age-specific survivorship, but also differences in prereproductive development time and time between reproductive episodes. Young's model was therefore able to provide three major reasons why semelparity might be favoured by natural selection. First, high adult mortality or the early onset of reproductive senescence might prevent iteroparous species from accruing fitness gains from established parents over long timescales. Second, a high population growth rate would favour semelparity outright. Finally, when the marginal cost of additional offspring is inversely proportional to the number of offspring produced, fecundity is maximized by investing all reproductive effort into a single episode, i.e. adopting a semelparous life history—see also Schaffer (1974) and Schaffer and Gadgil (1975).

1.3 Empirical evidence supporting the discrete model of parity

The discrete model accurately characterizes key differences in demographic parameters between semelparous and iteroparous congeners with very different reproductive strategies. In a comparison of Mount Kenya *Lobelia* species, Young (1984)

found that in the semelparous *Lobelia telekii* both juvenile and adult mortality were higher than in the closely related iteroparous species *Lobelia keniensis*. Young concluded that the difference in age-specific rates of mortality would strongly influence the expected value of future reproduction for each species, leading to iteroparity in one species and semelparity in the other (Young 1990).

Empirical support for the discrete model of parity is strongest when iteroparous and semelparous congeners have starkly different life histories. Many studies focus on reproductive effort, since a declining marginal cost of offspring in terms of reproductive effort should select for a semelparous life history over an iteroparous one; this is the cited cause of the evolution of semelparity in *Digitalis purpurea* (Sletvold 2002), and in *Antechinus agilis* (Smith and Charnov 2001; Fisher and Blomberg 2011). Growth rate is also important; in two subspecies of *Yucca whipplei*, the semelparous variety showed higher viability and faster time to germination than the iteroparous variety did (Huxman and Loik 1997). Other studies highlight the mortality differences between juveniles and adults, which explains the evolution of semelparity in a variety of long-lived semelparous plants (Young and Augspurger 1991), as well as in salmon (Fleming 1998; Crespi and Teo 2002; Hendry et al. 2004; Sloat et al. 2014). Models have been developed that use demographic parameters to predict age and size at first flowering for semelparous (monocarpic) plants; however, these models are more appropriate for long-lived than short-lived species (Metcalf et al 2003, Rees and Rose 2002).

However, the discrete model of parity fails to account for life history variation in many semelparous species, or between iteroparous and semelparous species that have similar life histories. Mathematical models based on the discrete model predict threshold

values—in mortality rate, size at initiation of reproduction, or expected growth rate—that do not agree with empirical observation (Omielan 1991; Lessells 2005; Piñol and Banzon 2011; Su and Peterman 2012; Trumbo 2013; Vaupel et al. 2013). In addition, there are empirical cases that explicitly do not conform to the predictions of the discrete model. An analysis of 12 winter-establishing primrose species (Oenothera: Onagraceae) found no significant differences in mortality estimates or in environmental determinants of fitness for semelparous and iteroparous species (Evans et al. 2005). Many salmon species do not fit neatly into semelparous and iteroparous classifications (Unwin et al. 1999). Other research has suggested that deterministic models of investment may better fit long-lived than short-lived semelparous species, given that many annual semelparous species (usually plants) show substantial phenotypic plasticity in phenology (e.g. size at first flowering), offspring quality and overall fecundity (Burd et al. 2006).

1.4 The continuous model of parity

The fact that semelparous reproduction rarely occurs “once”—i.e. in exactly one place, at exactly one time—has led to a new treatment of parity as continuously varying between extremes of “pure” semelparous and iteroparous reproduction. This approach has gained traction because there is considerable ambiguity in “breeding once”, and “annuality” and “perenniality”—terms that refer to the number of years in which organisms reproduce—cannot be used interchangeably with “semelparity” and “iteroparity”, which refer to the number of reproductive episodes organisms have (Fritz et al. 1982; Kirkendall and Stenseth 1985). In *The Evolution of Life Histories* (1992), Derek Roff noted,

“If we consider our unit of time to be a single year, annuals can be termed semelparous and perennials iteroparous. A further division is possible within annuals, for some reproduce once and are, therefore, semelparous within any time scale, while others flower repeatedly throughout the summer and, hence, are iteroparous with respect to annuals that flower only once, but semelparous with respect to perennials.” (p. 248)

That is, it is the simultaneity (i.e. the property of being non-iterative) of the reproductive episode that defines perfect semelparity. Therefore, the continuous model considers “extreme” semelparity to be a single, complete, and exhaustive reproductive episode where all reproductive effort is invested at once. Canonical examples of this strategy—which Kirkendall and Stenseth (1985) termed “uniparity”—would include mayflies (Edmunds et al. 1976; Corkum et al. 1997), and Adactylid mites (where offspring devour the mother from the inside out, making them obligately semelparous (Elbadry and Tawfik 1966; Goldrazena et al. 1997)).

1.5 Empirical evidence supporting the continuous model of parity

The theoretical critique of the discrete model of parity—as well as the proposal of the continuous model—occurred in the 1980s and 1990s; what is new at the present time is the abundance of evidence that now counts in favour the continuous model. We now know that many species cannot be neatly classified as “classically” semelparous or iteroparous as per the discrete model, but instead have intermediate life histories. Here I will consider the empirical evidence for such “intermediate” strategies, including: (1) facultative iteroparity; (2) facultative semelparity; and (3) phenotypically plastic

semelparity. More than one intermediate strategy may exist within a single species—for instance, arctic cod (*Boreogadus saida*) are semelparous in nature, but can reproduce in two consecutive years in captivity (Hop et al. 1995; Hop and Gjosaeter 2013), making them facultatively iteroparous. However, males and females seem to have different life histories – males begin to reproduce at an earlier age, and can allocate extreme amounts of reproductive effort to a single instance of reproductive activity; semelparity in this species is also phenotypically plastic (Nahrgang et al. 2014). I will cite empirical evidence supporting each of the intermediate strategies below.

1.5.1 Facultative Iteroparity

Many semelparous species have shown the ability to facultatively reproduce a second time—this is termed “facultative iteroparity”. Facultative iteroparity can be adaptive when it either: (1) provides an opportunity to realize fitness gains from an unexpected abundance of resources, or (2) shifts reproductive effort from inopportune to opportune times. However, facultative iteroparity can also be non-adaptive, since an incomplete transition from an ancestral iteroparous strategy to a present semelparous one may result in vestigial secondary reproductive episodes.

The first type of adaptive facultative iteroparity occurs when additional bouts of reproduction increase fitness by permitting unexpected “bonus” resources to be invested in new offspring. For example, mothers of the semelparous crab spider *Misumena vatia* (Araneae, Thomsidae) typically lay and provision a single brood of eggs (Gertsch 1939; Morse 1979), however in response to high food availability and/or usually warm environmental conditions, they are capable of laying and caring for a second brood if

sperm supplies are not depleted (Morse, 1994). A similar facultative double-broodedness in response to unusually favourable environment has been observed in the green lynx spider *Peucetia viridans* (Fink 1986), and A small proportion of Chinook salmon (*Onchorhynchus tshawytscha*), which typically reproduce only once, can survive and reproduce in two or three additional seasons (Unwin et al. 1999). Tallamy and Brown have shown that large, well-provisioned female burying beetles in multiple species in the genus *Nicrophorus* can reproduce more than once, despite the fact that small females can typically breed only once (Tallamy and Brown 1999).

The second form of adaptive facultative iteroparity occurs when deferral of reproductive effort—from a primary reproductive episode to a secondary one—allows an organism to reproduce at a more opportune time. Reproduction is deferred to seek the highest marginal fitness return on invested reproductive effort. For example, when high organic pollution levels disrupt primary reproduction in the freshwater leech *Erpobdella octoculata*, reproduction ceases and remaining reproductive effort is deferred to a second reproductive bout produced the next year (Maltby and Calow 1986). Similar behaviour has been seen in another Erpobdellid leech, *Erpobdella obscura* (Peterson 1983; Davies and Dratnal 1996) as well as in many cephalopods (Rocha et al. 2001). Adaptive deferral of reproductive effort is common in crab spiders. In *Lysiteles coronatus*, artificial brood reductions resulted in the production of a second brood, and the degree of deferral was proportional to the degree of the original reduction (Futami and Akimoto 2005). This was also observed in the field in Eresid spiders of the genera *Anelosimus* and *Stegodyphus*, both of which facultatively produce a second brood in response to nest predation (Schneider and Lubin 1997; Schneider et al. 2003; Grinsted et al. 2014).

Nonadaptive facultative iteroparity is the vestigial expression of a second bout of reproduction in response to particular environmental or developmental stimuli. In this case investing reproductive effort in multiple bouts may not enhance fitness, and may merely reflect the life history of an iteroparous ancestor. For example, in the semelparous herb *Lobelia inflata* (Campanulaceae), photoperiod cues cause some individuals to defer reproductive effort from the primary reproductive episode to a second episode. However, the timing of this second episode precludes fitness gains, since it occurs in midwinter (Hughes and Simons 2014a). A similar expression of vestigial iteroparity was seen in the semelparous polychaete worm *Nereis diversicolor*, which can produce nonadaptive gametogenesis in response to high levels of endocrine activity (Golding and Yuwono 1994).

1.5.2 Facultative Semelparity

Facultative semelparity occurs when species that are normally iteroparous—in the sense of having multiple, discontinuous reproductive episodes, often in multiple years—are capable of expressing only a single reproductive bout. For example, in the short-lived mustard *Boechera fecunda* (also known as *Arabis fecunda*; Brassicaceae), plants are capable of a continuum of reproductive strategies, from near-simultaneous semelparity to multi-year iteroparity. This is because *B. fecunda* can produce many small axillary inflorescences in any given year, and their production does not preclude flowering by the same rosette in the subsequent year. However, plants can also produce large “terminal inflorescences” that exhaust remaining resources and lead to senescence and death. Although some plants produce axillary inflorescences for several years before a terminal

inflorescence, others produce a terminal inflorescence in their first year (Lesica and Shelly 1995; Lesica and Young 2005). A similar system is seen in common foxglove, *Digitalis purpurea* (Scrophulariaceae), which is predominantly biennial or perennial, but can be facultatively semelparous if resource availability in the first year is high (Sletvold 2002). Facultative semelparity has also been observed in capelin (Christiansen et al. 2008; Loïc et al. 2012), soil microarthropods (Siepel 1994), dasyurid marsupials (Kraaijeveld et al. 2003; Martins et al. 2006), and in the flowering plant *Ipomopsis aggregata* (Silvertown and Gordon 1989). Some facultatively semelparous species show a continuous range of types of reproductive episode, rather than discretely fatal or non-fatal ones. *Erysimum capitatum* (Brassicaceae) produces multiple reproductive episodes in environments where water is plentiful; where water is scarce it expresses a semelparous strategy (Kim and Donohue 2011).

1.5.3 Phenotypically Plastic Semelparity

Phenotypically plastic semelparity occurs when different forms of semelparity are expressed in response to variation in the environment. Different forms of semelparity can occur both: (1) among individuals; or (2) within the reproductive episode of a single individual. Phenotypically plastic semelparity is common source of intraspecific variation in life history characters; this is a major source of confusion for mathematical models that predict a single optimal value for all semelparous habits, leading one paper to note that,

“A glaring inconsistency between the models and the data is that all models predict a specific threshold flowering condition...but data from natural population show a graded response” (Metcalf et al. 2003, p. 479).

That is, models predict that semelparous strategies should not vary, since phenotypic plasticity cannot happen when reproduction occurs “once”; in contrast, observations from natural systems can show a continuum of strategies expressed among individuals of the same species.

In plants, empirical evidence of phenotypically plastic semelparity is found in sea beets (*Beta spp.*), which display reproductive strategies along “a gradient from pronounced iteroparity to pronounced semelparity” (Hautekèete et al. 2001, p. 796). As per the discrete model of parity, the authors predicted that: (1) in semelparous species, reproductive effort per reproductive episode would be invariant—since it should be expected to be fixed at the maximum value possible, given physiological constraints; and (2) in iteroparous species were predicted to display environment-dependent phenotypic plasticity in allocation of reproductive effort. However, both semelparous and iteroparous species alike responded to environmental stressors by trading off future fecundity for increased immediate reproductive effort (Hautekèete et al. 2001, 2009). Similar trade-offs were observed among individuals of *Yucca whipplei* (Huxman and Loik 1997), *Chusquea ramosissima* (Montti et al. 2011), and *Onopordum illyricum* (Rees et al. 1999). *Lobelia inflata* is capable of producing different semelparous strategies, from nearly complete semelparity, where plants produce many similar flowers quickly and simultaneously, to (nonadaptive) facultative iteroparity, where as much as half of all reproductive effort is invested in a second reproductive episode; the most important predictor of life history strategy in *L. inflata* is the time of initiation of reproduction (Hughes and Simons 2014a,c).

Many semelparous insect species are also capable of displaying a range of forms of semelparity (Trumbo 2013). In the assassin bug (*Atopozelus pallens*), females deposit eggs in small clutches, approximately every two days; the number of clutches—and hence how prolonged this reproductive episode is—can vary substantially (Tallamy et al. 2004). European earwigs (*Forficula auricularia*) show a bivariate form of semelparity that is condition-dependent; females either deposit all eggs into a single clutch or lay two clutches (Meunier et al. 2012). Other invertebrates, including pitcher-plant mosquitoes (Bradshaw 1986), and ascidarians (Grosberg 1988), as well as some semelparous mammals (Wolfe et al. 2004; Mills et al. 2012), also adopt more or less extreme versions of semelparity depending on environmental cues.

Phenotypic plasticity within the reproductive episode of a single individual is notable when a semelparous organism displays a changing reproductive strategy—varying along the continuum of parity—that cannot be attributed to developmental, environmental, or architectural constraints (see: Diggle 1995, 1997). This pattern is more difficult to detect than phenotypically plastic strategies that differ between individuals, but in many systems observable differences exist between the “packaging” of reproductive effort, resulting in adaptive variation in phenology or offspring quality through time. In the semelparous plant *Lobelia inflata*, late fruits show accelerated phenology and higher offspring quality than early fruits. This pattern, which indicates that more reproductive effort is invested in later fruit, shows that *L. inflata* does not “reproduce once” but dynamically allocates reproductive effort throughout a sequence of repeated fruiting events (Hughes and Simons 2014b). In populations of the semelparous plant *Centaurea corymbosa*, plants showed highly variable life cycles—dynamically

varying the proportion of reproductive effort allocated to sequential flowers—depending on environmental conditions and crowding (Acker et al. 2014).

1.6 General Conclusions

Recent empirical work has provided substantial support for the continuous model of parity. Although some systems are described well by the discrete model of parity—in particular those where there is a direct comparison between an annual semelparous species and a perennial iteroparous one (e.g. Kitajima and Augspurger 1989; Young and Augspurger 1991; Metcalf et al. 2003; Burd et al. 2006; Davies and Gan 2012; Pausas and Keeley 2014)—these should be understood as special cases (i.e. extremes) of the continuum of possible strategies between semelparity and iteroparity. Future research may include new mathematical models that accurately predict the relationship between continuous parity and life history characters, especially phenological variables that are strong determinants of fitness. Currently, only a few such models exist (Zeineddine and Jansen 2009; Bonnet 2011; Su and Peterman 2012).

1.7 An introduction to the data chapters of this thesis

To date, the theoretical prediction of continuity between semelparous and iteroparous reproductive strategies has not been substantiated through controlled experimentation. In this dissertation I explore the degree to which a single organism—the semelparous herb *Lobelia inflata* (Campanulaceae)—expresses variation in parity along the semelparity-iteroparity continuum. The discrete model of parity predicts that semelparous reproduction is: (1) instantaneous, i.e. all offspring are produced as quickly

and simultaneously as possible, and are not subject to time-specific adaptive variation in reproductive characters; and (2) complete, in the sense that semelparity as “breeding once” requires that all reproductive effort is realized within a single reproductive bout. I will test these assumptions in the following data chapters.

In Chapter 2, I explore the degree of variation in reproductive strategy within a single semelparous reproductive episode in *L. inflata*. As Roff (1992) and Kirkendall and Stenseth (1985) have noted, reproductive effort realized during semelparous reproduction is rarely “packaged” into a single clutch of offspring; most semelparous organisms realize reproductive effort by producing multiple litters or fruit at different points in time. The existence of developmental, architectural or environmental constraints (see: Diggle, 1995) makes it likely that these “packages” will vary in predictable ways – offspring developing at apical flower positions along an inflorescence often receive fewer resources than those developing at basal positions, resulting in slower development and smaller size (e.g. Galloway, 2002; Ollerton & Lack, 1998; Wyatt, 1982). This is what would be expected if the discrete model of parity were true for *L. inflata*. Alternatively, if the continuum conception of parity were true, one would expect *L. inflata* to show putatively adaptive variation—that is, fitness-enhancing and not explainable as a consequence of constraints—in offspring traits within a reproductive bout (e.g. faster development or larger seed at an apical flower position). Chapter 2 reports data showing that *L. inflata* does indeed show adaptive variation throughout its reproductive episode, and thus provides empirical support for the continuum conception of parity.

In Chapter 3, I report the results of a manipulation experiment that examines whether *L. inflata* expresses dynamic reaction norms governing reproductive phenology

and allocation in response to variation in timing of the initiation of reproduction. Although *L. inflata* naturally flowers between May and mid-July in the field (Simons and Johnston 2003), it is possible to use a growth chamber with an astronomic clock to manipulate when plants initiate flowering. Replicate groups (RGs) of plants, composed of groups of genetically identical individuals grown under common conditions, were released into a field and lab site on four dates in the late summer; the response to different flowering times was then observed. This test allowed me to determine whether, once *L. inflata* initiates reproduction, it produces a single reproductive phenotype, regardless of the timing of initiation of reproduction (as would be predicted by the discrete model of parity), or whether adaptive reaction norms facilitate faster reproduction when less time is available to reproduce (as would be predicted by the continuous model of parity). The continuous model of parity allows semelparous reproduction initiated at different points in time to be modelled as a series of incrementally different reproductive strategies, from a “prolonged” iteroparous-like semelparity, where variation in reproductive phenotype (e.g. offspring size) slowly changes through the season, to an “extreme” semelparity characterized by an instantaneous reproductive bout. Our results confirmed the prediction of the continuous model of parity. Both lab and field components were incorporated into the experimental design—a field site was used to emulate growing conditions in the wild, while lab plants, grown under controlled conditions, permitted the observation of underlying reproductive behaviours, unconstrained by adverse environmental conditions. Early-bolting RGs showed a “prolonged” semelparity, with gradual changes in offspring size and flowering phenology occurring over time. In contrast, late-bolting RGs showed an “extreme”

semelparity where reproduction took place quickly and completely, as plants accelerated fruiting phenology and produced larger offspring. These results were consistent in both lab and field environments, among all 21 genotypic lineages included in the RGs, and across each of the three consecutive years that this study was replicated. Taken as a whole, I argue that this experiment provides conclusive empirical support for the idea that semelparity is a phenotypically plastic trait—at least in *L. inflata*—and that “prolonged” semelparity is merely a quasi-iteroparous form of annual semelparity.

In Chapter 4, I address a novel behaviour in *Lobelia inflata*—facultative secondary reproduction—and report results of a manipulation experiment linking expression of this behaviour to timing of initiation of reproduction. Secondary reproduction (in semelparous organisms) refers to an uncommon second bout of reproduction after the primary reproductive episode has been completed. The main explanation of a secondary reproductive episode (SRE) has been that it is an adaptive mechanism facilitating the deferral of reproductive potential when primary reproduction is constrained (Futami and Akimoto 2005). For example, the Eresid crab spider *Stegodyphus lineatus*, which typically dies after laying a single clutch of eggs, can respond to offspring predation (leading to the loss of the primary brood) by producing a smaller second clutch (Schneider and Lubin 1997; Schneider et al. 2003). Secondary reproduction is generally explained as a strategy facilitating the adaptive deferral of reproductive effort from a time that is unlikely to result in realized fitness gains to another, more congenial time in the future. Chapter 4 reports the results of an experiment designed to assess whether plants subjected to time constraints during reproduction were more likely to defer reproductive effort to a second episode than plants not subjected to

time constraints. Although *Lobelia inflata* does not typically exhibit an SRE in the field (at least at Petawawa), when protected from extrinsic mortality due to snow cover and subzero temperatures, some plants produce flowers a second time. To assess the importance of secondary reproduction as a mechanism of deferring reproductive effort, I used an experimental framework—similar to the one described in Chapter 3—where the timing of initiation of reproduction was manipulated in order to present plants with more or less time to complete their (primary) semelparous reproductive episode. I observed both whether or not plants subjected to strong time constraints were more likely than unconstrained plants to express an SRE, as well as, among plants that did express an SRE, whether or not constrained plants allocated a greater proportion of their total reproductive effort to the second bout. The results of this experiment showed that, contrary to the adaptive deferral hypothesis, those plants subjected to strong reproductive constraints were less likely to express an SRE than unconstrained plants, and invested less reproductive effort if they did. This finding is consistent with the results of Chapter 3; if the continuous model of parity is correct, and if—at least in *L. inflata*—parity is a phenotypically plastic trait, then the fact that late-bolting plants are more likely to forego a second bout of reproduction is further evidence that late-bolting plants favour an “extreme” semelparous strategy instead of a “prolonged” semelparous strategy (as do early-bolting plants that do not display an SRE) or even facultative iteroparity (as do early-bolting plants that display an SRE). For this reason, I include the plasticity in SRE display as further empirical support for the continuous model of parity.

Chapters 5 and 6 are closely related. In Chapter 5, I describe the isolation and quantification of 28 unique microsatellite loci in *L. inflata*. These markers were

developed using a microsatellite-enriched library, and were tested for polymorphism using: (1) samples of *L. inflata* from three sites in Eastern North America (Petawawa ON, Martock NS, and Petersham, MA); and (2) samples of two congeners, *Lobelia cardinalis* and *Lobelia siphilitica*, both sourced from populations in Algonquin Park. Of these loci, 24 showed polymorphism in *L. inflata*, and although tests of 58 individuals revealed eight distinct lineages, all individuals genotyped were homozygous for all alleles at all microsatellite loci.

In Chapter 6, I report findings of a more comprehensive exploration of the genetic structure of the Petawawa population of *L. inflata* using these microsatellite markers. I genotyped 105 plants at 22 microsatellite loci in order to assess: (1) whether *L. inflata* is obligately self-fertilizing; (2) whether genetic variation in *L. inflata* is structured between genetic lineages; and (3) whether these lineages are associated with significant differences in reproductive phenotype. Among the 2,310 loci genotyped, I found no heterozygotes, and conclude that *L. inflata* is entirely or very nearly entirely selfing. The 21 source plants collected from Petawawa were resolved into 8 microsatellite lineages, which were associated with significant phenotypic variation in flower colour and phenology. These findings corroborate the hypothesis that the tight-closed flower of *L. inflata* is constructed in such a way that the anther tube prevents pollen release (Simons and Johnston 2000a). Because genetic variation is higher than would be expected given zero outcrossing, I speculate that temporal fluctuating selection on genetic lineages preserves genetic variation in *L. inflata*. Although Chapters 5 and 6 do not specifically address the continuum between semelparity and iteroparity in *L. inflata*, genetic lineages are found to explain a substantial proportion of the variation in life history traits related to

parity. It is therefore possible that fluctuating selection of life history characters is de facto fluctuating selection on the degree of semelparity or iteroparity itself.

Chapter 2: Changing Reproductive Effort Within a Semelparous Reproductive Episode

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2.1 Abstract

Life-history theory predicts a tradeoff between current and future reproduction for iteroparous organisms—as individuals age, the expected value of future reproduction declines, and thus reproductive effort is expected to be higher in later than in earlier clutches. In contrast, models explaining the evolution of semelparity treat semelparous reproduction as instantaneous, with no scope for intra-individual variation. However, semelparous reproduction is also extended, but over shorter time scales; whether there are similar age- or stage-specific changes in reproductive effort within a semelparous episode is unclear. In this study, we assessed whether semelparous individuals increase reproductive effort as residual reproductive value declines by comparing the reproductive phenotype of flowers at five different floral positions along a main inflorescence.

Using the semelparous herb *Lobelia inflata*, we conducted a longitudinal study of 409 individuals including both lab and field populations over three seasons. We recorded six reproductive traits—including the length of three phenological intervals as well as

fruit size, seed size and seed number—for all plants across floral positions produced throughout the reproductive episode.

We found that while the rate of flower initiation did not change, flowers at distal (late) floral positions developed more quickly and contained larger seed than flowers at basal (early) floral positions did. Our results were consistent with the hypothesis that, like iteroparous organisms, *L. inflata* increases reproductive effort in response to low residual reproductive value.

2.2 Introduction

2.2.1 Overview

Semelparity – the life history characterized by a single, exhaustive reproductive episode – is usually considered to be categorically different from life histories involving more than one reproductive episode, e.g. iteroparity (Cole 1954; Charnov and Schaffer 1973; Young 1981). Although semelparity can be counted as a discrete adaptation, whether or not semelparous reproduction is a single event (i.e. “big bang” reproduction) is unclear; in this study, we ask whether or not the pattern of variation in the sequential “packaging” of reproductive effort in a semelparous plant represents altered reproductive allocation in the same way that iteroparous plants express strategic changes in reproductive allocation throughout their lifetimes.

2.2.2 Residual Reproductive Value

In iteroparous organisms, life-history changes often occur in response to changes in expected future reproduction. This is because reproductive value, the expected

reproductive success of an individual, is not constant; it is both age- and context-dependent (Fisher 1930). Williams (1966) identified two components to reproductive value: (1) current reproductive value; and (2) residual reproductive value (RRV). The former term expresses reproductive value that can be realized immediately, whereas the latter expresses the future expectation of reproduction. Natural selection should favor life histories that maximize rate of increase integrating across all ages (Pianka and Parker 1975), but, in iteroparous organisms, residual reproductive value gradually declines as individuals age (Roach 2003; Roach et al. 2009). Thus, older individuals are expected to exhaust accumulated resources in large bouts of reproduction rather than defer reproductive effort to future bouts (Clutton-Brock 1984; Isaac and Johnson 2005; Bercovitch et al. 2009; Javoš 2013).

Most studies of RRV-specific reproductive effort are of long-lived iteroparous animals, particularly birds (Part et al. 1992; Gustafsson et al. 1994; Forslund and Part 1995; Ots and Horak 1996; Hanssen 2006; Velando et al. 2006), but more recent studies have also explored the applicability of this hypothesis to many short-lived iteroparous organisms, including pipefish, rotifers and multivoltine insects (Langley and Clutton-Brock 1998; Stelzer 2001; Sadd et al. 2006; Billing et al. 2007; Creighton et al. 2009; Kivleniece et al. 2010; Heinze and Schrempf 2012; Gonzalez-Tokman et al. 2013). There is empirical evidence that some iteroparous plants express greater reproductive effort in late flowering events than in earlier ones, which includes the production of larger offspring, proportionately high investment in reproductive structures, and a faster rate of reproduction (Herrera 1992; Mazer and Wolfe 1992; Guitián and Navarro 1996; Wolfe and Burns 2001; Kliber and Eckert 2004; Zeng et al. 2009).

2.2.3 Semelparity and Reproductive Phenotype

Relative to iteroparity, where longevity is required to reproduce more than once, semelparity is an adaptation to low adult-stage survival (Cole 1954; Charnov and Schaffer 1973; Young 1981; Orzack and Tuljapurkar 1989). Models explaining the adaptive value of semelparous reproduction generally compare the cumulative fitness of an annual semelparous strategy with a perennial iteroparous strategy. For annual semelparous organisms, where survival to the next year (or other reproductive “cycle”) is low, completing growth and reproduction within a single year is expected to maximize reproductive success (Clutton-Brock 1984; Charlesworth 1994; Kaitala et al. 2002; Zeineddine and Jansen 2009). Thus, extant models predict instantaneous, “big bang” semelparous reproduction—such as that of mayflies (Edmunds et al. 1976; Elliot and Humpesch 1980)—and thereby implicitly consider the production of a single offspring phenotype (Cole 1954; Young 1981, 2010; Kaitala et al. 2002).

However, it is rare for individual semelparous organisms to display only a single reproductive phenotype during their reproductive episode. For example, variation has been observed in clutch and/or offspring number (Omielan 1991; Siepel 1994; Unwin et al. 1999; Schneider et al. 2003; Futami and Akimoto 2005; Christiansen et al. 2008; Meunier et al. 2012; Hughes and Simons 2014a), in size and type of reproductive structures (Biere 1995; Simons and Johnston 1999; Tallamy and Brown 1999; Gallardo et al. 2006; Kim and Donohue 2012; Hughes and Simons 2014c), and in phenology (Grosberg 1988; Amir and Cohen 1990; Simons and Johnston 2003, 2006; Imaizumi and Kay 2006; Välimäki et al. 2008; Hughes and Simons 2014c). Semelparous reproduction is also rarely instantaneous, and in many species is realized through packaging

reproductive effort in many separate events (i.e. fruits, seeds, etc.) produced successively over an extended period of time. Changes in flower or seed phenotype over the reproductively active phase of a semelparous organism's lifespan may be analogous to the changes among successive broods produced by iteroparous organisms; the main difference being that since semelparous organisms cannot defer reproduction to later reproductive bouts, tradeoffs between current and future reproduction would take place entirely within a single reproductive season. Thus, semelparous organisms may alter reproductive behavior according to changing reproductive value, and exhibit higher reproductive effort later in life. No studies of RRV-specific tradeoffs of reproductive function in semelparous organisms have been performed, although there is evidence that facultative semelparity is associated with higher reproductive output in marsupial mammals (Fisher and Blomberg 2011). In this paper, we distinguish between two hypotheses: the "RRV-dependent" hypothesis that semelparous organisms show age-specific increases in reproductive effort over sequential bouts of reproduction as residual reproductive value decreases, and the "RRV-independent" null hypothesis that semelparous reproduction is a single event, and no age- or RRV-specific changes are expressed.

2.2.4 Constraints

The existence of constraints makes it unlikely that any plant is capable of maintaining uniform allocation of resources across all floral positions of an inflorescence (Diggle 1995). Therefore, actual reproductive investment among floral positions may decline whether or not a plant increases reproductive effort late in its life (Zeng et al.

2009). Common constraints include architectural effects, both direct and indirect (reviewed by Stephenson 1981 and Diggle 1995), competitive disadvantage at distal inflorescence positions (Casper 1984; Solomon 1988; Herrera 1991a, 2009; Guitián and Navarro 1996), and environmental constraints including the effects of environmental heterogeneity associated with season length, such as changes in photoperiod or ambient temperature (Flood and Halloran 1982; Mazer and Wolfe 1992; Wolfe 1992; Searle and Coupland 2004; van Kleunen and Fischer 2005; Imaizumi and Kay 2006). It is notoriously difficult to identify the degree to which variation in phenotype is attributable to one type of constraint or another (Herrera 2009), but—despite a few notable exceptions—most constraints act more severely on distal floral positions more than on basal floral positions when the inflorescence is tapered.

Therefore, several common trends regarding the effect of constraints on trait values among floral positions on an inflorescence can be identified: (1) that seeds of flowers at distal positions are generally smaller than seeds of flowers at basal positions (Cavers and Steele 1984; Levy 1988; Winn 1991; Ashman 1992; Wolfe 1995, 1992; Obeso 1993; Brunet and Charlesworth 1995; Brunet 1996; Vaughton and Ramsey 1997; Herrera 2009); (2) that flowers at distal positions are generally smaller than those at basal positions (Herrera 1988; Macnair and Cumbes 1990; Kang and Primack 1991; Brunet 1996; Navarro 1996; Wolfe and Denton 2001; Moravcová et al. 2005); and (3) that flowers at distal positions will have longer intervals between successive stages of floral development and/or develop more slowly than those at basal positions (Wyatt 1982; Herrera 1992; Wolfe 1992; Wolfe and Burns 2001).

Therefore, the null “RRV-independent” hypothesis that we use in this study—that no differences in reproductive allocation due to declining RRV are apparent—does not necessarily imply that there will be no variation in trait values (e.g. seed size) between floral positions (since uniform allocation to all floral positions is unlikely if floral positions along an inflorescence experience direct or indirect architectural effects (reviewed in Diggle 1995). Rather, our null hypothesis predicts that any differences in trait value (e.g. seed size) between floral positions will display common patterns of lower investment at distal relative to basal floral positions. This null hypothesis follows other tests of reproductive effort in iteroparous organisms (e.g. Kliber and Eckert 2004; Zeng et al. 2009), including a congener of the model system used in this study (Caruso 2006), and is statistical rather than concerned with the proportion of variation in traits that is attributable to specific constraints (architectural or otherwise) in our model system. We note that because severe constraints may mask a real underlying increase in reproductive effort, no increase in apparent reproduction cannot be taken as evidence of no underlying increase in reproductive effort, and that, for offspring produced late in life, investment may be greater even if structures are smaller (Clutton-Brock 1984). Therefore, prediction of an increase in reproduction may be considered a conservative test of the RRV-dependent hypothesis.

2.2.5 Predictions

In this study, we test this hypothesis using the semelparous herb *Lobelia inflata* (Campanulaceae), and evaluate whether or not *L. inflata* gradually increases reproductive effort per flower as it “packages” reproductive effort in successive flowering events

within a semelparous reproductive episode, or whether it expresses a single flowering strategy for all offspring. Because *L. inflata* reproduces by producing flowers in an acropetal pattern (i.e. in sequence from basal to distal floral positions along an inflorescence), the chronological order of flower formation can be readily tracked. Although current and residual reproductive value cannot be directly measured for the plant as each flower is produced, the relative reproductive effort expended on early (basal) and late (distal) flowers can be compared. If *L. inflata* adheres to the predictions made by the RRV-dependent hypothesis, we would expect to observe higher reproductive investment in distal (late) floral positions relative to basal (early) floral positions. Alternatively, if *Lobelia inflata* expresses an underlying reproductive allocation pattern that is not sensitive to residual reproductive value (i.e. according to the RRV-independent hypothesis), we would expect either: (1) no difference in trait values among floral positions; or (2) higher investment in basal floral positions relative to distal floral positions, if reproductive investment at distal positions is strongly constrained.

2.3 Materials and Methods

2.3.1 Study Species

The semelparous plant *Lobelia inflata* (Campanulaceae) is ideally suited for this study for several reasons. Because its anthers form a sealed tube, *L. inflata* is obligately autogamous (i.e. does not outcross), and genetic variation within populations exists only between monotypic genetic lineages (Hughes et al. 2014). This feature allows experimental replication of genotypes and facilitates partitioning of sources of phenotypic variation. In addition, reproductive success is dependent on plant phenology and

environmental conditions such as resource availability, and its assessment is thus not confounded by availability of pollinators. Furthermore, the temporal pattern of reproductive allocation is easily reconstructed because fruits are produced in a simple acropetal series as the inflorescence develops. Since *L. inflata* is semelparous, tradeoffs in energy allocation among growing seasons do not confound measures of reproductive success.

Previous studies have shown that reproduction in *L. inflata* is responsive to environmental cues (Simons and Johnston 2000b, 2003, 2006). Finally, studies on *Lobelia siphilitica*, an iteroparous congener of *L. inflata*, have shown that architectural constraints have a powerful influence on reproductive characters (Caruso et al. 2003; Caruso 2006).

2.3.2 Protocol

L. inflata seeds were collected from 21 plants (representing distinct genetic lineages) separated by at least 50m from a natural population in the Petawawa Research Forest (Petawawa, Canada, 45°99'N, 77°30'W). Field-collected seeds were placed on moistened filter paper for up to 14 days in a Biochambers (Canada) SG-30 seed germinator on a 12h/12h and 20°C/20°C day/night schedule at 85% humidity. Seedlings were planted in autoclaved soil in cellpacks (7.6 cm X 7.6 cm) and transferred to a Biochambers AC-40 growth chamber on a 16h/8h and 24°C/18°C day/night schedule. All plants were grown in this common growth-chamber environment until the initiation of reproduction (“bolting”), which was assessed by the early formation of an inflorescence.

Plants that bolted within a four-day window (June 15th +/- 2d) were used, and split between a field site (at Ottawa, Canada: 45°23'N, 75°41'W), and a second AC-40 chamber programmed to mimic the light conditions and photoperiod of the field site (and on a 24°C/18°C day/night temperature schedule). Plants were left to grow and reproduce under observation until they senesced, which typically occurred approximately 120 days later, during early October. Measurements of inflorescence height, number of flowers, stage of flower formation, flower maturation and seed formation were made once every 4-6 days for all flowers on all plants. Following senescence, plants were harvested and seeds collected. This experiment was repeated in three consecutive field seasons.

Plants varied in total number of fruit; thus, comparisons among plants were made by indexing percentile floral position (1st fruit=0%; Last=100%). We included five floral positions in our analysis, closest to the 0th, 25th, 50th, 75th and 100th percentile for each plant (e.g. for a plant with 50 flowers, this would be the 1st, 13th, 25th, 38th and 50th flower, respectively). These floral positions were used in a previous study of *L. inflata* that suggested seed traits varied across floral positions of the main inflorescence (Simons and Johnston 2000b). We assessed all phenotypic traits under discussion at each floral position.

2.3.3 Response Variables

We used six measures to assess reproductive phenotype: days from bud formation (preflowering) to anthesis, days from anthesis to fruit inflation, days from fruit inflation to fruit maturation, fruit diameter, mean seed diameter and seed number. Fruit diameter was recorded after maturation and harvest using a Vernier caliper ($\pm$ 0.01 mm). Mean

seed diameter—also recorded after harvest—was measured by: (1) taking a 20-seed sample from each floral position; (2) imbibing seeds on moistened filter paper (inside a Petri dish) for three days; and (3) measuring seed diameter under a light microscope using digital photographs and NIHImage 1.62b7. Measurements of imbibed seeds were used in order to facilitate the recording of maximum seed size, both to reduce experimental error and to replicate seed size recording protocols used in other studies (e.g. Simons and Johnston 2000a). Seed number was determined by manual count.

These phenotypic variables were chosen as surrogate measures of reproductive effort because they permitted testing of the RRV-dependent hypothesis against the RRV-independent null hypothesis (i.e. fruit and seed size and number in early vs. late fruits). The three phenological stages were chosen because they each act as different measures of the time taken to complete floral development. In a late-season semelparous plant such as *L. inflata*, selection on completing reproduction before frosts arrive is presumably strong. We chose fruit and seed diameters as measures of offspring “packaging” because seed size represents parental provisioning, is correlated with dormancy, a key fitness trait in this organism (Simons and Johnston 2003), and previous results from a small sample of 12 plants suggested that differences in fruit and seed diameter occur among floral positions (Simons and Johnston 2000b). Although we do not measure reproductive effort directly, the relative size, number, and rate of production of reproductive structures are known to be subject to tradeoffs between current and future reproduction, and thus direct comparisons between the values of these traits at different times are a useful indirect measure (see Herrera 1991b; Shine and Schwartzkopf 1992)

2.3.4 Statistical Methods

Because the duration of the three phenological stages of flowers may be correlated, the three phenological traits we measured were analyzed using a factorial between-subjects MANOVA. The dependent variables we included were (1) time from first flower to anthesis; (2) time from anthesis to fruit inflation; and (3) time from fruit inflation to fruit maturation, all measured in days. The three independent variables included as fixed effects were: (1) floral position (0th, 25th, 50th, 75th or 100th percentile flower produced); (2) year; and (3) environment (lab or field site). We assessed significance of floral position as a multivariate effect using Pillai's Trace, due to its robustness and power (Olejnik and Algina, 2000). Where the MANOVA indicated a significant main effect of floral position, the pairwise comparisons between different floral positions were investigated using factorial ANOVAs and post-hoc Tukey HSD tests. Effect sizes are reported using partial η^2 .

The statistical model for fruit and offspring traits was similar. Because fruit diameter, seed diameter and seed number at any floral position may also be correlated, we used a second between-subjects MANOVA to analyze this data. The dependent variables included were: (1) fruit diameter; (2) seed diameter; and (3) seed number. The independent variables included were: (1) floral position; (2) year; and (3) environment. Again, we assessed the multivariate significance using Pillai's Trace and followed the MANOVA with factorial ANOVAs for each dependent variable and post-hoc Tukey HSD tests on floral position. Effect size was reported using partial η^2 .

2.4 Results

Mean trait values for all six traits by floral position are shown in Table 2.1. Although there was considerable variation among plants in the rate of flower initiation between the first and second halves of the reproductive episode, there was no evidence that the rate of flower initiation changed over time ($t = 0.241$, $df = 408$, $p = 0.81$; Figure 2.1). According to the multivariate response of the MANOVA model, all effects were significant predictors of the time spent in flowering phases (Table 2.2). Floral position had the largest multivariate effect size, while floral position*environment had the lowest. Floral position was a significant predictor of all three flowering phases we measured, according to follow-up ANOVAs for each response variable (Table 2.3). Late flowers completed all phenological stages more rapidly than early flowers did, but the fruit inflation to maturation phase showed the greatest change in duration (Figure 2.2).

Likewise, all effects were significant predictors of the multivariate MANOVA model for the three measures of reproductive investment (Table 2.4). Floral position was the predictor with the largest effect size, while year had the lowest effect size. Follow-up ANOVAs on floral position revealed that floral position was a significant predictor for all measures of reproductive investment that we considered (Table 2.5). Basal floral positions expressed significantly larger fruits than distal floral positions, but late floral positions also produced significantly larger seeds (Figure 2.3). The effect of floral position on seed number was less straightforward, as fruits at the 0 and 100 floral positions showed significantly higher seed counts than intermediate floral positions did.

2.5 Discussion

In general, our results show that higher reproductive effort was observed at later floral positions relative to earlier floral positions, although the strength of this pattern varied among traits measured (Table 2.1). Overall, this pattern of increasing reproductive effort as flowers are produced late in life was consistent with the RRV-dependent hypothesis (i.e. that, late in life, when current reproductive value is high and residual reproductive value is low, *L. inflata* channels reproductive effort that could potentially be deferred into current reproduction). Although there was considerable variability between plants, *L. inflata* expressed the phenotype requiring the highest reproductive effort (i.e. the fastest phenology, the largest offspring) at the most distal floral position for five of six traits, for example, seed diameter was consistently the largest at the most distal fruit position. Another trait—seed number—showed the highest mean number of seeds at the most distal floral position, although this was statistically the same value as for the most basal floral positions and significantly differed only from intermediate floral positions. Another trait—fruit diameter—showed a pattern of expression that was inconsistent with increasing reproductive effort; however, although fruits produced toward the end of season were not larger, they were produced more quickly and contained more seeds. Nevertheless, the overall pattern was largely consistent with the RRV-dependent hypothesis.

Each of the three phenological intervals we measured was consistently completed more rapidly as the reproductive episode progressed. Despite shortening photoperiod, flowers at distal floral positions developed more quickly than those at basal floral positions did. The “acceleration” of reproduction via faster flower and fruit development

requires greater resource input per unit time—especially given that the rate of flower initiation did not vary (Figure 2.1)—and is therefore an expression of proportionately higher reproductive effort (Williams 1966; Javoš 2013); thus, we conclude that this pattern is consistent with the RRV-dependent hypothesis. Several interaction effects were significant, and showed that field fruits generally matured slightly faster than lab plants. There was significant variation among years, possibly due to aging effects associated with the seed used to found the experimental populations of plants. In addition, the fact that there was no significant environment $\times$ year effect, but year $\times$ environment $\times$ year was highly significant (with later fruits, particularly in the field, showing fast maturation and small size), which suggested that environmental heterogeneity among years affected fruit positions differently.

Of the three stages of flower formation and maturation, the greatest proportional reduction in time across floral positions occurred during the time spent in the fruit inflation to maturation phase. This is meaningful given the life history of *L. inflata*. Because it is obligately autogamous, extended floral display does not affect pollen receipt or dispersal and thus does not increase fitness; indeed, the time spent in these pre-seeding flowering phases is short and facilitates a quick transition to seed production, which occurs after fruit inflation (Simons and Johnston 2000a, 2003). If seed production is accelerated in late fruits, reducing time spent in the lengthy post-inflation stage will save the most time. Unlike pre-seeding stages, which have no adaptive benefit in early or late fruits, long post-inflation periods in early fruits may accrue fitness gains from prolonging the total amount of time spent seeding by producing a few extra seeds.

In general, late-produced fruits were smaller than early fruits. This pattern was not consistent with the RRV-dependent hypothesis. Although this result is what might be expected when a single strategy is expressed under progressively more severe architectural and environmental constraints—since acropetally-flowering plants constrained by architecture typically express smaller reproductive structures at distal positions (Brunet 1996; Navarro 1996; Vaughton and Ramsey 1997; Vallius 2000; Moravcová et al. 2005)—given the pattern of increasing reproductive effort in other reproductive traits, we cannot conclude that architectural effects (and not some other factor or tradeoff) prevent the formation of large fruits at distal fruit positions.

The effect of floral position on seed diameter was strong: each of our five measured floral positions produced seeds that were significantly larger than those produced at the floral position that immediately preceded it. Other studies have shown that seed mass is positively correlated with higher survival and faster early growth, particularly when seedlings confront stress early in life (Bonfil 1998; Lloret et al. 1999; Moles and Westoby 2004). This finding suggests that the fitness contributions of individual seeds produced at late floral positions are as important as seeds produced at early floral positions. Moreover, the finding that the number of seeds produced at the most distal floral positions was significantly higher than for the 25th, 50th, and 75th percentile floral positions (although non-significantly higher than the most basal floral position), strongly suggests that per-fruit reproductive effort expressed late in the reproductive episode is higher than at any other point in the episode, especially when the fact that seeds produced at distal positions are significantly larger than those produced at basal positions.

There are important limitations to our findings. First, we did not test germination and dormancy fractions across different floral positions; instead we used a surrogate measure of offspring viability (seed diameter), which is correlated with dormancy and early germination in *L. inflata* (Simons and Johnston 2003). Second, we did not directly evaluate fitness of seeds produced by early and late fruits. However, larger seeds produced at distal (late) floral positions are likely to have high fitness, since seed mass is often associated with high survivability and above average stress tolerance (Moles and Westoby 2004).

2.6 Conclusion

In conclusion, our results indicate that *L. inflata*, despite being semelparous, behaves like an iteroparous organism in that it adopts a changing reproductive strategy as it gradually increases per-fruit reproductive effort. These findings highlight the incompleteness of existing models of semelparity, which do not consider that although offspring are produced in a single “big bang” reproductive episode, changes in reproductive value or variation in offspring phenotype may occur within this single episode. The fact that reproductive phenotypes change within the reproductive episode of single individuals corroborates the hypothesis of Kirkendall and Stenseth (1985), who noted that formally defining semelparity as “breeding once”, while mathematically and logically simple, creates problems when applied to actual biological systems. As a corollary, our results also support the idea that semelparity and iteroparity are not discrete life-history strategies, but instead represent endpoints along a continuum of possible modes of reproduction (Orzack and Tuljapurkar 1989; Roff 1992, 2001; Stearns 1992;

Hughes and Simons 2014c). Thus we conclude that *L. inflata*'s reproductive allocation pattern is consistent with an age-specific, plastic strategy in which reproductive value decreases with age, favoring higher reproductive effort late in their short life. This behavior, which is common among iteroparous species, may also be common for semelparous species whose reproduction is prolonged.

2.7 Tables

Table 2.1 - Summary data by floral position

Summary statistics for trait values for six reproductive traits of Lobelia inflata by floral position.

Trait	Mean Value for Floral Position (SE)				
	0	25	50	75	100
Flower Initiation to Anthesis (d)	3.93 (0.10)	3.61 (0.12)	3.20 (0.10)	3.09 (0.08)	2.93 (0.08)
Anthesis to Fruit Inflation (d)	10.26 (0.30)	8.93 (0.24)	9.149 (0.25)	8.84 (0.23)	8.44 (0.21)
Fruit Inflation to Maturation (d)	27.60 (0.82)	21.66 (0.78)	18.31 (0.62)	13.02 (0.47)	10.53 (0.25)
Fruit Diameter (mm)	5.77 (0.06)	5.16 (0.07)	4.97 (0.07)	4.24 (0.06)	3.87 (0.07)
Seed Diameter (mm)	0.288 (0.03)	0.296 (0.04)	0.308 (0.04)	0.318 (0.04)	0.348 (0.04)
Seed Number	265.28 (3.47)	246.70 (3.26)	235.77 (3.37)	240.84 (3.45)	275.94 (3.57)

Table 2.2 - MANOVA results: phenological traits

Test results for flowering phases using a multivariate model. MANOVA model: $Time_{F-A} + Time_{A-I} + Time_{I-M} = \text{Floral Position} + \text{Year} + \text{Environment}$.

Effect	<i>Pillai's Trace</i>	<i>F</i>	<i>df</i>	<i>p</i>	<i>partial η^2</i>	power
Floral Position	0.21	38.27	12, 6045	< 0.001	0.07	1.00
Year	0.01	4.53	6, 4028	< 0.001	0.01	0.99
Environment	0.01	9.37	3, 2013	< 0.001	0.01	0.99
Floral Position*Year	0.03	2.45	24, 6045	< 0.001	0.01	1.00
Floral Position*Environment	0.01	1.84	12, 6045	< 0.001	0.00	0.90
Year*Environment	0.02	5.45	6, 4028	0.037	0.01	1.00
Floral Position*Year*Environment	0.03	2.52	24, 6045	< 0.001	0.01	1.00

Table 2.3 - ANOVA results: phenological traits

Univariate test results for significance of floral position on flowering phases. ANOVA Model: Timephase = Floral Position + Year + Environment

Response	<i>F</i>	<i>df</i>	<i>p</i>	<i>partial η^2</i>
Time from Flower-Anthesis (Days)	16.20	4, 2015	< 0.001	0.03
Time from Anthesis-Inflation (Days)	10.01	4, 2015	< 0.001	0.02
Time from Inflation-Maturation (Days)	104.39	4, 2015	< 0.001	0.17

Table 2.4 - MANOVA results: fruit traits

Test results for fruit and seed diameter using a MANOVA model. Model: Seed Diameter + Fruit Diameter = Floral Position + Year + Environment.

Effect	<i>Pillai's Trace</i>	<i>F</i>	<i>df</i>	<i>p</i>	<i>partial η^2</i>	power
Floral Position	0.40	77.47	12, 6045	< 0.001	0.13	1.00
Year	0.19	69.42	6, 4028	< 0.001	0.09	1.00
Environment	0.18	146.52	3, 2013	< 0.001	0.18	1.00
Floral Position*Year	0.18	16.07	24, 6045	< 0.001	0.06	1.00
Floral Position*Environment	0.14	25.45	12, 6045	< 0.001	0.05	1.00
Year*Environment	0.06	20.16	6, 4028	< 0.001	0.03	1.00
Floral Position*Year*Environment	0.07	5.84	24, 6045	< 0.001	0.02	1.00

Table 2.5 - ANOVA results: fruit traits

Univariate tests for significance of floral position on fruit and seed diameter. ANOVA Model: $Time_{phase} = \text{Floral Position} + \text{Year} + \text{Environment}$.

Response	<i>F</i>	<i>df</i>	<i>p</i>	<i>partial η^2</i>
Fruit diameter	253.06	4, 2015	< 0.001	0.334
Seed diameter	26.04	4, 2015	< 0.001	0.049
Seed number	23.58	4, 2015	< 0.001	0.045

2.8 Figures

Figure 2.1 - Flower initiation rate per day

Box plot of flower initiation rate per day for intervals before and after the 50th percentile flower. Stems indicate quartiles and outliers are labelled. Categorical labels specify: (1) the first 50% of the fruit set, measured as the interval (in days) between the initiation of the first flower and the initiation of the 50th percentile flower; and (2) the second 50% of the fruit set, measured as the interval (in days) between the initiation of the 50th percentile flower and the initiation of the last flower. The mean flower initiation rate of the first phase ($M = 0.85$, $SD = 0.58$) does not significantly differ from the mean flower initiation rate of the second phase ($M = 0.84$, $SD = 0.66$; $t = 0.241$, $df = 408$, $p = 0.81$).

Figure 2.2 - Phenological traits by floral position

Boxplot of phenological intervals by indexed floral position, by environment. Traits shown are time (in days) elapsed for three flowering intervals: from flower formation to anthesis (a), from anthesis to fruit inflation (b), and from fruit inflation to fruit maturation (c). Differences between environments were not statistically significant. Letters above x-axis show homogenous subsets found using Tukey HSD tests; letters not shared between two floral positions have significantly different mean values ($\alpha = 0.05$). Error bars show standard error.

Figure 2.3 - Fruit traits by floral position

Boxplot of size and number of reproductive structures by indexed floral position by environment. Traits shown are fruit diameter in millimeters (a), seed diameter in millimeters, and seed number per fruit by floral position. Differences between environments were not statistically significant. Letters above x-axis show homogenous subsets found using Tukey HSD tests; letters not shared between two floral positions have significantly different mean values ($\alpha = 0.05$). Error bars show standard error.

Chapter 3: Phenotypically plastic expression of parity in *Lobelia inflata* in response to season length manipulation

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3.1 Abstract

Semelparity and iteroparity are considered to be distinct and alternative life-history strategies, where semelparity is characterized by a single, fatal reproductive episode, and iteroparity by repeated reproduction throughout life. However, semelparous organisms do not reproduce instantaneously; typically reproduction occurs over an extended time period. If variation in reproductive allocation exists within such a prolonged reproductive episode, semelparity may be considered iteroparity over a shorter time scale.

This continuity hypothesis predicts that “semelparous” organisms with relatively low probability of survival after age at first reproduction will exhibit more extreme semelparity than those with high probability of adult survival. This contrasts with the conception of semelparity as a distinct reproductive strategy expressing a discrete, single, bout of reproduction, where reproductive phenotype is expected to be relatively invariant.

Here, we manipulate expected season length—and thus expected adult survival—to ask whether *Lobelia inflata*, a classic “semelparous” plant, exhibits plasticity along a semelparous-iteroparous continuum.

Groups of replicated genotypes were manipulated to initiate reproduction at different points in the growing season in each of three years. In lab and field populations alike, the norm of reaction in parity across a season was as predicted by the continuity hypothesis: as individuals bolted later, they showed shorter time to, and smaller size at first reproduction, and multiplied their reproductive organs through branching, thus producing offspring more simultaneously.

This work demonstrates that reproductive effort occurs along a semelparous-iteroparous continuum within a “semelparous” organism, and that variation in parity occurs within populations as a result of phenotypic plasticity.

3.2 Introduction

3.2.1 The idea of the continuity of semelparity and iteroparity

The evolution of the age schedule of reproduction is of central concern to life-history theory. Organisms may be categorized according to their reproductive schedules: semelparous organisms (e.g. octopus, Pacific salmon) have a single, “big-bang” fatal reproductive episode, whereas iteroparous organisms (e.g. humans, Atlantic salmon) are capable of multiple reproductive episodes per lifetime (Roff 1992, 2001; Stearns 1992; Meunier et al. 2012). Cole (1954) presented a formal comparison of the fitness consequences of semelparity and iteroparity. He famously identified the persistence of iteroparity as a paradox: why is it so common, given that: (1) survival from one

reproductive episode to the next is costly; and (2) to be fitness-equivalent to an iteroparous strategy, a semelparous strategy need only produce one additional offspring (to replace the parent)? This problem was first resolved by pointing out that low juvenile establishment or survival lent the iteroparous strategy a fitness advantage (Charnov and Schaffer 1973). This model, which focused on age-specific mortality, has since been refined (Young 1981; Bulmer 1985), but remains a general framework to explain the evolution of semelparity and iteroparity as discrete strategies.

As has been noted by others (e.g. Kirkendall and Stenseth 1985), defining semelparity as instantaneous and fatal reproduction is ambiguous. It is unclear whether “fatal” precludes prolonged senescence, and whether “instantaneous” reproduction precludes production of more than a single offspring. Unless only a single offspring is produced, total reproductive effort is by necessity packaged in multiple offspring that are rarely produced simultaneously. Many examples of within-individual variation in the timing of semelparous reproduction in nature have been recognized. Although some semelparous species reproduce relatively rapidly, especially long-lived semelparous plants (Young and Augspurger 1991), others are reproductively active for an extended period of time (Golding and Yuwono 1994; Morse 1994; Christiansen et al. 2008; Montti et al. 2011; Meunier et al. 2012). For example, semelparous species such as capelin and crab spiders are capable of facultative iteroparity (Futami and Akimoto 2005; Christiansen et al. 2008) and many cephalopods, although considered semelparous, exhibit lengthy postreproductive senescence, with some capable of a second bout of reproduction (Rocha et al. 2001). Although each of these life histories is semelparous in the sense that there is normally a terminal reproductive episode, they do not reproduce in

a “single, massive, fatal reproductive episode” (Young 2010), but distribute their total reproductive effort in multiple offspring over time.

Attempts to model the fitness effects of reproductive strategies have often compared intrinsic rates of increase of annuals and perennials, where annuals are considered to be semelparous and perennials to be iteroparous. However, many semelparous organisms, such as bamboo, cicadas, *Yucca* spp., are not annual (Young and Augspurger 1991). Multivoltine insects lay more than one brood per year, and their classification as semelparous or iteroparous may depend on the time scale of reference (Fritz et al. 1982). In seasonal climates, the digitizing effect of the cost of adult survival through an especially harsh event (winter, dry season) results not only in dramatic integer changes in voltinism (Roff 1980) but also in annual and perennial life histories and the illusion of a strict dichotomy between semelparity and iteroparity. Thus, categorization of life histories into semelparous and iteroparous is useful, but does not fully reflect an underlying biological reality.

3.2.2 Predictions

We ask whether prolonged reproduction in species considered to be semelparous, although within a short lifespan, may be treated as iteroparous strategic packaging of reproductive effort in multiple bouts throughout life. Together with evidence that semelparity is evolutionarily labile, with closely related species exhibiting both semelparous and iteroparous life histories (Crespi and Teo 2002), it seems reasonable to consider parity as a continuum of intermediate strategies between endpoints of pure semelparity (“uniparity”, sensu Kirkendall and Stenseth (1985) and pure iteroparity, a

large number of small clutches, produced in discrete reproductive bouts. Following this logic, prolonged semelparity refers to a strategy where reproduction is expressed over a longer period of time than under pure semelparity.

Although numerous examples of prolonged semelparity exist, no study has attempted to address the life-history question of whether this phenomenon is indicative of phenotypic continuity between semelparity and iteroparity (Bradshaw 1965). Two alternative explanations for prolonged semelparity exist: it may be a single reproductive episode that simply cannot be expressed instantaneously, in which case there would be no reason to consider the phenomenon to be iteroparity. In contrast, we hypothesize that prolonged semelparity is iteroparity on a short time scale, in which case reproductive allocation within a lifetime will vary depending on expected adult survival. Differences in parity among semelparous species may exist, but it is not obvious how the continuum hypothesis could be tested in a species comparison. However, within a species, variation in reproduction along the semelparity-iteroparity continuum (i.e. more or less instantaneous semelparity) may be expressed as phenotypic plasticity, i.e. the capacity for one genotype to express multiple environment-dependent phenotypes (Schlichting 1986; Stearns 1989; Hendry et al. 2004). If a species' reproductive allocation pattern is phenotypically plastic, manipulating season length cues should change the instantaneousness of the reproductive episode. This hypothesis has not yet been explicitly tested in either plants or animals, but anecdotal evidence in animals exists. For example, age at first reproduction influenced the amount of reproductive effort invested in offspring production and defense in Sockeye salmon, *Oncorhynchus nerka* (Cohen 1971; Unwin et al. 1999; Morbey and Abrams 2004). Previous work has modeled the optimal

timing of initiation of the single, irreversible transition to reproduction in annual plants (King and Roughgarden 1982, 1983; Amir and Cohen 1990; Thomson et al. 2011). However, because reproduction in these organisms is treated as a single event, no study has addressed the question of how reproductive effort is expressed or packaged following the initiation of reproduction.

In this study, we test the parity continuum hypothesis by manipulating effective season length—and thus, expected reproductive lifespan—available to replicated genetic lineages of the semelparous plant *Lobelia inflata*. This species provides an appropriate model for five main reasons: (1) it is classically semelparous; (2) reproductive effort is realized over an extended period of time during its single growing season in nature; (3) it is obligately autogamous, and therefore genotypically-invariant lineages are readily obtained; (4) total reproductive effort can be directly assessed because reproduction is exclusively by seed, and both male and female fitness contributions are obtained in one plant; and (5) *L. inflata* has a simple acropetal flowering pattern, where fruits form sequentially along inflorescences, making it possible to track the packaging of reproductive effort.

Manipulation of effective season length was accomplished by inducing bolting in groups of experimental plants at different times (June, July, August, September) during the growing season. Progressively later bolting over the period June through September results in diminishing time available for reproduction before the onset of frosts in mid-October. If the semelparity-iteroparity continuum hypothesis is correct, late bolting should elicit a progressively more semelparous reproductive strategy; that is, late-bolting plants should exhibit a reproductive episode that trades off other aspects of reproductive

success for relatively prompt and simultaneous reproduction. Specifically, we predict that, for *L. inflata*, more extreme semelparity in response to late bolting will be expressed as short time to first flowering and small size at first flowering, more synchronous flowering (through rapid development of raceme and parallel development of fruits via branching), and the production of smaller and/or fewer seeds. In contrast, if prolonged semelparity is the expression of a single strategy (the single strategy hypothesis), late bolting will result either in no change or decelerating reproduction as a direct (nonadaptive) plastic response to declining resources and deteriorating conditions toward the end of the growing season.

3.3 Materials and Methods

3.3.1 Study Species

Lobelia inflata (Campanulaceae) is a semelparous plant native to Eastern North America. It has multiple flowering schedules in the wild (both annual and biennial patterns have been observed), but reproduction is always semelparous in that the plant senesces after completion of flowering. Upon germination, *L. inflata* seeds form rosettes capable of overwintering. Reproduction is initiated as a reproductive stalk forms from a mature rosette; this event is termed “bolting” and occurs predictably if size, photoperiod and light quality thresholds are exceeded (Simons and Johnston 2000a). *L. inflata* has perfect flowers, reproduces sexually, and is obligately self-fertilizing. Outcrossing is prevented by a stamen tube, a structure which permits the release of pollen directly onto the stigma, but does not permit the release of pollen into the air, since it is sealed (Simons

and Johnston 2003). Analyses of polymorphic microsatellite loci (Hughes et al. 2014) have revealed no evidence that outcrossing occurs in nature.

Bolting, which marks the beginning of a transition from a vegetative to a reproductive phase, is irreversible for *L. inflata*, and thus the timing of this “decision” has important fitness consequences. Inflorescences show an acropetal flowering pattern, where flowers are produced in series from the base to the tip of the stalk (and along each branch). Each flower progresses through easily observable stages: from bud, to flower formation, to anthesis, to “inflation” (where fruits resemble small balloons – hence the name *Lobelia “inflata”*) and finally to fruit maturation. Reproduction occurs as seeds are formed inside inflated ovules; the number of seeds in a fruit has been observed to depend on environmental unpredictability and reproductive timing (Simons and Johnston 2000b; Simons 2007). During reproduction, one or more shoots may branch off from the main stalk. Seeds disperse passively upon fruit maturation; once all fruits have reached this stage, a plant senesces.

3.3.2 Experimental Design

Of central importance to our design was the ecological significance of the timing of bolting, marking the (irreversible and terminal) initiation of reproduction. By manipulating the date of initiation of reproduction, we were able to control the length of time that plants had to reproduce. Because reproduction is terminated relatively consistently among individuals each year (around October 15th) with the onset of hard frosts – a phenotypically plastic reproductive response to a range of manipulated bolting dates could be effected.

We collected seeds from dead plants at the Petawawa Research Forest (Lat. 45°99'N, Long. 77°30'W) in eastern Ontario, Canada in October 2007. To maximize the potential inclusion of a variety of genetic lineages (and preclude the possible influence of atypical genotypes), we collected seeds from 21 parent plants in the field (each at least 50m from each other). Each seed sample was used to found an experimental population of genotypically identical replicate plants, yielding 21 (potentially distinct) genetic lineages. To obtain offspring plants from each lineage we first placed groups of 100-200 seeds on moistened filter paper in petri dishes, then germinated seeds for 10-14 days in a BioChambers SG-30 seed germinator using a 12h /12h day/night light regimen (at 20°C with 85% humidity).

Seedlings were then moved to individual cells (dimensions: 7.6 X 7.6 cm) within trays of autoclaved soil, and were then transferred to a Biochambers AC-40 growth chamber for prebolting rosette growth under a 16h/8h day/night schedule (at 24°C/18°C day/night). Trays were watered twice weekly, and a 15-5-15 liquid fertilizer mixture (200ppm N) was added once every two weeks. Seedlings were grown for approximately 8-9 weeks, forming small rosettes. Rosettes grew undisturbed until bolting; the emergence of a reproductive stalk upon bolting may be reliably detected (Simons and Johnston 2003); a stalk taller than 4cm is diagnostic of the onset of bolting. Seed germination and seedling translocation was planned so that plants would initiate bolting at four regular intervals, targeting the 15th of each month ($\pm$ 2 days) from June through September. Plants that bolted before the 13th or after the 17th of the month were excluded. The distribution of plants included in the study is shown in Table 3.1.

Bolted rosettes within each group were randomly assigned to one of two environments: a field site (at 45°23'N, 75°41'W) or to a growth chamber, which was programmed to mimic the photoperiod and light intensity of the field site. Lab plants were simply moved into the new chamber in their planting trays, and to minimize the difference in soil conditions between lab and field environments, field rosettes were planted along with the soil from their planting cell. Translocated plants were left to grow, reproduce and eventually senesce. Reproducing plants were monitored every two days until death.

Measurements of longest living leaf — a surrogate for rosette biomass (Simons and Johnston 2000a), stalk height, stage of flower formation (visible bud, anthesis, mature flower, etc...), fruit maturation and branch initiation were performed on growing plants once every 4-6 days for all plants. Once they had senesced, plants were taken to the lab, measured, and harvested. At harvest, fruits were measured and removed to storage vials.

3.3.3 Traits Measured

Seven traits were assessed in this manipulation: three phenological traits, two fruiting and two seeding traits. The three phenological traits were days to first flower, size at first flowering and flowering duration. 'Days to first flower' is simply the number of days between bolting and the formation of the first flower. 'Size at first flowering' is the distance between the base of the stalk and the pedicel of the emerging terminal flower bud (measured by Vernier caliper - error: +/- 0.01mm); this is the plant's height as the

first flower forms. Flowering duration is the number of days between the formation of the first flower and the maturation of the last flower (as it becomes a fruit).

The two fruiting traits included branches per plant and the total number of fruits produced. “Branches per plant” was the simple count of rami protruding from the main stalk. Upon death of a plant, fruits were counted and fruit location (on branch or reproductive stalk) recorded. The “total number of fruit” produced by a plant included all fruits at all positions, both on branches and the main reproductive stalk.

The two seed traits included seed size and the total number of seeds produced. To obtain a sufficient sample size while ensuring adequate replication, seed traits were obtained from a subsample of individuals from the June and September (early and late) bolting groups. Seed size was measured by: i) sampling the ten fruits at the 100th/90th/80th/... percentile position along the raceme – all fruits were used if there were fewer than 10 in total; ii) imbibing seeds on a moistened filter paper-lined petri dish for 72 hours; and iii) measuring seed dimensions using NIHimage 1.62b7. Seed number per fruit was determined by manual count under a light microscope. Estimates of total reproductive output (the number of seeds per plant) were calculated as the product of the mean number of seeds per fruit and the number of fruits per plant.

3.3.4 Statistical Analyses

Our aim was to assess the effect of bolting month as a predictor in a multivariate response; however, we first tested whether differences in any reproductive traits were explained by the genotype random effect. We used likelihood ratio tests to compare the proportion of total variability in response accounted for by two models (Pinheiro and

Bates 2000). The first model was a generalized linear model (GLM) that included only fixed effects and their interaction terms; and the second was a generalized linear mixed model (GLMM) that included the fixed effects and interaction effects from the first model, as well as genotypic lineage — which we considered a random effect — along with two interaction terms that included genotype. We then compared the restricted log likelihood values for each of these models to assess whether the inclusion of genotype significantly increases the predictive power of the model. This process was repeated for each of the seven reproductive traits measured. Where the GLMM did not significantly differ from the GLM in terms of the proportion of total variation explained by the model, we dropped the random effects, opting instead for the more parsimonious model. Although excluding the random effect—and reducing the GLMM to a GLM—reduces the unit of replication to the individual, we chose to prioritize model parsimony over realism. Below (Model 1 and 2) are the specifications for the two models we used.

For each of the seven reproductive traits, we constructed a GLM that included six predictors, and used a Poisson distribution and a log link. We included four fixed effects: year (categorical), bolting month (categorical), environment (i.e. lab or field - categorical), plant size (continuous, based on prebolting rosette leaf length), and two crossed effects: bolting month X year and environment X year. Effect sizes were measured using partial η^2 . Our full GLM model is below (Model 3.1).

Model 3.1. GLM

REPRODUCTIVE TRAIT =

BOLTING MONTH + SIZE + ENVIRONMENT + YEAR + BOLTING
MONTH*YEAR + ENVIRONMENT*YEAR

We also constructed a GLMM for each variable, which included: (1) all six factors from the GLM; as well as (2) “genotypic lineage”, a random effect; and (3) “genotypic lineage X environment”, “genotypic lineage X bolting month”, and “bolting month X year and environment X year”, three interaction effects. Our full GLMM model is specified below (Model 3.2).

Model 3.2. GLMM

REPRODUCTIVE TRAIT =

BOLTING MONTH + GENOTYPE + SIZE + ENVIRONMENT + YEAR +
 GENOTYPE*ENVIRONMENT + GENOTYPE*BOLTING MONTH + BOLTING
 MONTH*YEAR + ENVIRONMENT*YEAR

This analysis contains biologically relevant random crossed effects (i.e. GENOTYPE*BOLTING MONTH), and we constructed the GLMM so that parameters were fitted using REML-based estimation, to avoid underestimation of the standard deviation of random effects (Venables and Dichmont 2004; Bolker et al. 2009).

3.4 Results

Over the course of this experiment, 1,509 plants from 21 genotypic lineages were tracked (Table 3.1). These plants represent only those that germinated on time, rooted successfully, initiated bolting during the five-day window for each bolting group, and reproduced undisturbed (field plants were subject to herbivorous attacks from grasshoppers, which were particularly severe in 2008) until the onset of senescence.

Likelihood ratio tests showed that a GLMM explained significantly more variation in the response for three traits (size at first flower, number of fruit and number of branches), whereas a GLMM and GLM explained similar proportions of the total variation in the response variable for the other four traits (days from bolting to first flower, flowering duration, seed size and seed number; Table 3.2). Using these models, we found significant differences among bolting months for all seven reproductive traits included in this manipulation experiment (Table 3.3). In general, early-bolting plants reproduced more slowly and produced fewer, larger seeds than late-bolting plants.

Plasticity in the expression of all three phenological traits—time from bolting to first flower, height at first flower, and total flowering duration—was observed across manipulated bolting dates (Table 3.3). A GLM showed a significant effect of bolting month on days from bolting to first flower (Table 3.4); as plants bolted later in the season, they initiated flowering earlier (Figure 3.1A,B). All other predictors except year*environment were also significant for this trait. A GLMM showed a significant effect of bolting month on height at first flower (Table 3.5). As plants bolted later in the season, they initiated flowering at a smaller size (Figure 3.1C,D). Other significant predictors of size at first flowering included year*environment, bolting month*environment and year*bolting month (Table 3.5). A GLM showed a significant effect of bolting month on flowering duration (Table 3.6). Late-bolting plants flowered sooner after bolting than early-bolting plants, and the total length of time spent reproducing was significantly shorter (Figure 3.1E,F).

Plasticity was observed across manipulated bolting dates in the expression of all fruiting traits considered in this study (Table 3.3). A GLMM for mean number of

branches showed a highly significant effect of bolting month on number of branches (Table 3.7). Late-bolting plants produced a greater number of branches (Figure 3.2A,B). Other significant predictors of number of branches included environment, year*environment, and year*bolting month. A GLMM for total number of fruit showed a highly significant effect of bolting month on number of fruit (Table 3.8). As plants bolted later in the season, they produced more fruit (Figure 3.2C,D). All interaction terms included in the GLMM were also significant predictors of number of fruit.

Both seed traits analyzed in this study showed phenotypic plasticity across bolting dates (Table 3.3). A GLM showed a significant effect of bolting month on seed size (Table 3.9); late-bolting plants produced smaller seeds than early-bolting plants (Figure 3.3A,B). No other predictors were significant. A GLM showed a significant effect of bolting month on seed number (Table 3.10). Late-bolting plants produced significantly more seeds than early-bolting plants (Figure 3.3C,D). Other significant predictors of seed number included: year, environment, year*environment and year*bolting month.

3.5 Discussion

Reproduction in *L. inflata* showed significant phenotypic plasticity of key reproductive traits diagnostic of semelparity in response to time constraints imposed by the manipulation of bolting date. Variation in the expression of the degree of semelparity was consistent with the continuum hypothesis and inconsistent with the single strategy hypothesis: in lab and field environments alike, reproductive traits varied continuously as plants bolted later on in the season. June-bolting plants expressed pro- longed semelparity (where reproductive effort was realized slowly), while September-bolting plants

expressed a more instantaneous or extreme semelparity (where reproductive effort was realized quickly). Although there was substantial variation among individual plants that bolted at different times throughout the season, there were no consistent differences among the 21 genetic lineages. Analyses of differences among environments revealed that field plants were slightly larger and matured slightly faster than lab plants; the highly significant interaction between environment and bolting month revealed that this effect was stronger late in the season than earlier in the season. Year was an important predictor of several reproductive traits, likely due to the difference in seed age at the founding of each year's experimental population.

Our results support the continuum hypothesis for each of our main predictions with respect to flowering traits. Perhaps most importantly, late-bolters initiated reproduction sooner after bolting and at a smaller size than did early-bolters. Flowering soon after bolting and flowering at a small size allows a plant to reproduce sooner, but may cause plants to forego fitness gains associated with production of a larger stalk, which can hold more fruit and disperses seeds farther (Thomson et al. 2011). That late-bolting plants would trade off such gains for the ability to initiate reproduction sooner and at a smaller size is consistent with the general prediction of the continuum hypothesis that late-bolting plants respond to a constrained reproductive season by adopting a more extreme semelparous reproductive strategy. Late-bolters also flowered more synchronously, by fruiting in parallel more frequently and producing many more fruit than early bolters. Although producing many flowers simultaneously may increase maximum fecundity, competition for resources between them may eventually lead to diminishing fitness gains for additional flowers (Diggle 1995; Ollerton and Lack 1998).

Branching and fruiting patterns were phenotypically plastic across bolting groups. Late-bolting plants produced significantly more fruit and more branches than early-bolting plants. This pattern was not necessarily predicted by the continuum hypothesis, but it makes sense in view of the morphology of our study species: producing fruit on multiple branches allowed late-bolting plants to overcome constraints on fruit production related to the growth of the meristem; late-bolters were able to produce flowering in parallel rather than serially along the main stalk. Greater numbers of fruit also helped late-bolters produce a greater number of seeds—although early-bolters produced larger seeds, the total fecundity of late-bolters was significantly higher than that of early-bolters. Presumably, there is a context-dependent fitness cost associated with branching in *L. inflata*; otherwise, early-bolting individuals should also express branching architecture. We speculate that advantages of main stem dominance in early bolters may include better dispersal in taller plants, and higher diversification in timing of seed production, and thus in timing of germination (Simons 2009). For time-constrained plants late in the season, however, branching provides an outlet for reproductive potential that would otherwise be wasted.

Early-bolters produced fewer, larger seeds than late-bolters, a pattern that is consistent with the prediction that late-bolters would express “pure” semelparity, while early-bolters might express a more iteroparous-like semelparity (Bolmgren and D. Cowan 2008). Larger seeds show reduced dormancy (Simons and Johnston 2003), and are more likely than smaller seeds to germinate and establish rosettes within the same season. In contrast, late-bolters produced seeds in many fruits simultaneously, realizing higher fecundity at smaller seed size, although small seeds produced late in the season will be

required to overwinter before forming rosettes (Simons and Johnston 2000b, 2006; Larsen and Andreasen 2004). In *L. inflata*, differences in offspring traits among fruits produced at different times suggest that a transition from a high-quality to high fecundity strategy occurs as the prospect of offspring establishment diminishes through the season (i.e. from early fruit to late fruit) (Simons and Johnston 2000b); in our study, plants bolting at different times exhibit a similar pattern. This is likely due to the fact that seeds produced early in life are more likely to survive to reach reproductive maturity (Cole 1954; Stanton 1985; Roff 2001; Larsen and Andreasen 2004; Bolmgren and Cowan 2008; Zeineddine and Jansen 2009; Young 2010).

Bolting month was consistently the best predictor of reproductive traits. This signifies that reproductive allocation is phenotypically plastic with respect to time, and that environmental factors related to bolting month (i.e. photoperiod and/or light intensity) act as cues to trigger different allocation strategies. There was considerable consistency in trait values between lab and field bolting month groups, despite the fact that lab plants were sheltered from various stressors (e.g. wind, rain and temperature variation) experienced by their field counterparts; this suggests that photoperiod and light intensity may be potent environmental cues governing allocation strategy. Because all bolting month groups were composed of the same 21 genotypic lineages, and genotype was included as an effect in our mixed model design, genotypic differences were excluded as an explanation for differences among bolting groups. Rosette size was included as an effect in our model, but did not consistently predict phenotypic differences between bolting groups, as we would have expected if plant size, or direct effects associated with plant size, largely determined reproductive allocation patterns.

Other fixed effects included in our model (environment and year), as well as interaction effects (bolting month*year, bolting month*environment, and year*environment) were significant predictors of for one or more reproductive traits, although the effect sizes (calculated for GLMs) were typically small (Tables 3.4-3.10). Differences between plant growth environments showed a consistent pattern: relative to lab-grown plants, field-grown plants generally initiated flowering at a larger size, and produced more branches and seeds, but also showed greater variability in reproductive characters. Year was not a significant predictor of most of our reproductive traits, but where it was, this was presumably due to maternal effects related to seed age (e.g. seeds germinating in 2009 were a year older than those that germinated in 2008), or, in the case of significant year*bolting month interaction effects, maternal effects that affected bolting date-specific reaction norms (i.e. size at first flowering). Easier to interpret were year*environment interaction effects, which predicted a significant amount of the variation in reproductive traits, mostly because differences in seasonal weather among years affected field plants and not lab plants. For instance, in 2008 eastern Canada experienced a warm, abnormally rainy summer, resulting in all field plants flowering at a smaller size, and producing more branches, fruit and seed. Significant bolting month*environment interaction effects showed that fluctuating weather affected plant growth at some bolting months more than others; for instance, in 2008, the pattern of increased branching became more pronounced in later bolting months, with field plants bolting in September 2008 showing the greatest number of branches produced by any block in the study. Despite the importance of these additional effects, bolting month was the only effect that significantly predicted all reproductive traits in our study.

3.6 Conclusion

In conclusion, our data demonstrate that a classically semelparous plant exhibits variation in parity expression that is consistent with adaptive phenotypic plasticity. Other studies (Hendry et al. 2004; Christiansen et al. 2008) have shown intriguing evidence of plasticity in reproductive life histories, and here we explicitly test whether plasticity in reproductive behaviour can be explained as plasticity in the expression of parity along a continuum. That reproductive traits vary predictably with bolting date implies that, in *L. inflata*, degree of parity responds in a plastic manner to environmental cues and its expression is continuous. This substantiates the notion that there is a meaningful continuum of reproductive traits from a pure semelparous strategy to a prolonged semelparous strategy of iteroparous-like reproductive packaging over a substantial proportion of its lifespan.

Conceptual and mathematical models identify the conditions under which annual semelparity has a selective advantage over perennial iteroparity, where semelparous and iteroparous life histories are discrete alternatives. Our data suggest that these models, because they implicitly consider invariant extreme semelparity and iteroparity, describe the special cases of endpoints of a continuum. Our results suggest that parity may be treated as phenotypically plastic and continuous over shorter time scales, as variation in key reproductive traits yields a life history that falls between the absolute extremes of pure iteroparity and semelparity. Inferences about the generality of these conclusions will require study of reproductive allocation in other classically semelparous organisms, or in iteroparous organisms in which reliable cues for residual reproductive value may be perceived by individuals.

3.7 Tables

Table 3.1 - Number of plants summary data

Summary data table for bolting groups by year.

Year	Number of Plants								
	June		July		August		September		Total
	Lab	Field	Lab	Field	Lab	Field	Lab	Field	
2008	54	29	48	29	54	23	52	12	301
2009	98	66	80	70	88	66	97	33	598
2010	98	64	107	68	103	51	90	29	610
Total									1509

Table 3.2 - Likelihood ratio tests for reproductive traits (GLM vs GLMM)

Likelihood ratio test results for all seven dependent variables.

Dependent Variable	Parameters		Restricted Log Likelihood		χ^2	Critical Value	df	sig p>0.05	Model Used
	GLMM	GLM	GLMM	GLM					
Days from bolting to first flower	22	19	9792.829	9793.545	0.716	7.82	3	ns	GLM
Size at First Flower	22	19	16200.799	16215.686	14.887	7.82	3	*	GLMM
Flowering Duration	22	19	11144.377	11145.496	1.119	7.82	3	ns	GLM
Branch Number	22	19	5008.693	5026.915	18.222	7.82	3	*	GLMM
Fruit Number	22	19	12626.805	12645.419	18.614	7.82	3	*	GLMM
Seed Size	14	11	-2666.507	-2666.473	0.034	7.82	3	ns	GLM
Seed Number	14	11	13478.815	13480.608	1.793	7.82	3	ns	GLM

Table 3.3 - Summary data for reproductive traits

Means (+/- standard error) and pairwise comparisons by bolting month for all reproductive traits assessed. Homogenous subsets indicate pairwise differences assessed using Tukey tests at $\alpha = 0.05$; levels that do not share a letter are significantly different.

	Mean (by bolting month)				Homogenous Subsets ($p < 0.05$)			
	Jun	Jul	Aug	Sept	Jun	Jul	Aug	Sept
Time from Bolting to First Flower (days)	37.58 (+/- 0.33)	28.91 (+/- 0.34)	24.37 (+/- 0.35)	16.48 (+/- 0.44)	A	B	C	D
Height at First Flower (mm)	126.88 (+/- 3.66)	134.62 (+/- 3.69)	120.59 (+/- 3.79)	119.83 (+/- 4.41)	AB	A	B	B
Flowering Duration (days)	42.79 (+/- 0.53)	30.10 (+/- 0.53)	25.38 (+/- 0.56)	29.11 (+/- 0.69)	A	B	C	B
Number of Branches	1.13 (+/- 0.08)	1.54 (+/- 0.08)	2.14 (+/- 0.08)	2.90 (+/- 0.10)	A	B	C	D
Number of Fruit	30.58 (+/- 1.09)	38.59 (+/- 1.10)	35.23 (+/- 1.13)	43.89 (+/- 1.32)	A	B	C	D
Seed Size (mm)	.318 (+/- .002)	N/A	N/A	.276 (+/- .003)	A	N/A	N/A	B
Seed Number	7920.51 (+/- 162.7)	N/A	N/A	9221.42 (+/- 213.9)	A	N/A	N/A	B

Table 3.4 - GLM results for days to first flower

*Test of fixed effects from a GLM used to predict days from bolting to first flower. * $p < 0.05$, ** $p < 0.001$*

Source	df	F	p	partial η^2
Intercept	1, 1491	20616.23	< 0.0005**	0.933
Year	2, 1491	6.50	0.002*	0.009
Bolting Month	3, 1491	540.74	< 0.0005**	0.521
Environment	1, 1491	11.49	0.001*	0.008
Year * Environment	2, 1491	0.53	0.589	0.001
Bolting Month * Environment	3, 1491	23.20	< 0.0005**	0.045
Year * Bolting Month	6, 1491	4.46	< 0.0005**	0.008

Table 3.5 - GLMM results for size at first flower

*Test of fixed effects from a GLMM (fitted using REML-based approximation) used to predict size (stem height) at first flower. * $p < 0.05$, ** $p < 0.001$*

Source	df	F	p
Intercept	1, 56.58	3214.48	< 0.0005**
Year	2, 1445.08	0.003	0.997
Bolting Month	3, 65.77	3.52	0.020*
Environment	1, 43.46	0.70	0.406
Year * Environment	2, 1350.08	69.27	< 0.0005**
Bolting Month * Environment	3, 1481.45	107.54	< 0.0005**
Year * Bolting Month	6, 1416.41	18.08	< 0.0005**

Table 3.6 - GLM results for flowering duration

*Test of fixed effects from a GLM used to predict flowering duration. * $p < 0.05$, ** $p < 0.001$*

Source	df	F	p	partial η^2
Intercept	1, 1491	11726.30	< 0.0005**	0.887
Year	2, 1491	5.98	0.003**	0.008
Bolting Month	3, 1491	196.88	< 0.0005**	0.284
Environment	1, 1491	3.88	0.049*	0.003
Year * Environment	2, 1491	11.70	< 0.0005**	0.015
Bolting Month * Environment	3, 1491	14.83	< 0.0005**	0.029
Year * Bolting Month	6, 1491	0.98	0.437	0.004

Table 3.7 - GLMM results for number of branches

*Test of fixed effects from a GLMM (fitted using REML-based approximation) used to predict number of branches. * $p < 0.05$, ** $p < 0.001$*

Source	df	F	p
Intercept	1, 26.81	1237.98	< 0.0005**
Year	2, 1392.70	0.90	0.406
Bolting Month	3, 1475.18	98.38	< 0.0005**
Environment	1, 19.39	92.46	< 0.0005**
Year * Environment	2, 1223.24	11.30	< 0.0005**
Bolting Month * Environment	3, 1479.68	1.22	0.300
Year * Bolting Month	6, 1477.21	3.94	0.001**

Table 3.8 - GLMM results for number of fruit

*Test of fixed effects from a GLMM (fitted using REML-based approximation) used to predict number of fruit. * $p < 0.05$, ** $p < 0.001$*

Source	df	F	p
Intercept	1, 53.87	2867.38	< 0.0005**
Year	2, 1447.87	0.68	0.507
Bolting Month	3, 77.41	25.49	< 0.0005**
Environment	1, 44.03	0.58	0.452
Year * Environment	2, 1390.47	46.93	< 0.0005**
Bolting Month * Environment	3, 1479.94	8.11	< 0.0005**
Year * Bolting Month	6, 1410.42	16.54	< 0.0005**

Table 3.9 - GLM results for seed size

*Test of fixed effects from a GLM used to predict seed size. * $p < 0.05$, ** $p < 0.001$*

Source	df	F	p	partial η^2
Intercept	1, 712	33930.27	< 0.0005**	0.979
Year	2, 712	0.29	0.747	0.001
Bolting Month	1, 712	178.86	< 0.0005**	0.201
Environment	1, 712	0.09	0.771	0.001
Year * Environment	2, 712	2.01	0.135	0.006
Bolting Month * Environment	1, 712	0.71	0.401	0.001
Year * Bolting Month	2, 712	1.12	0.327	0.001

Table 3.10 - GLM results for seed number

*Test of fixed effects from a GLM used to predict seed number. * $p < 0.05$, ** $p < 0.001$*

Source	df	F	p	partial η^2
Intercept	1, 712	3999.55	< 0.0005**	0.849
Year	2, 712	25.43	< 0.0005**	0.067
Bolting Month	1, 712	23.84	< 0.0005**	0.032
Environment	1, 712	34.33	< 0.0005**	0.046
Year * Environment	2, 712	20.94	< 0.0005**	0.056
Bolting Month * Environment	1, 712	0.33	0.566	0.001
Year * Bolting Month	2, 712	3.87	0.021*	0.011

3.8 Figures

Figure 3.1 - Phenological traits by bolting date

Phenological traits by bolting date over three years. Days to first flower (charts A and B) indicates the time elapsed between bolting and the formation of the first flower. Size at first flower (charts C and D) denotes the length of the main flowering stalk on the day the first flower is produced. Flowering duration (charts E and F) indicates the time elapsed between the formation of the first flower and the maturation of the last flower. Charts on the left (A, C, E) show lab results and those on the right (B, D, F) show field results.

Figure 3.2 - Fruiting traits by bolting date

Fruiting traits by bolting date over three years. Number of branches (charts A and B) represents the mean number of branches per plant by bolting month. Total fruit number (charts C and D) represents the mean number of fruits per plant by bolting month. Charts on the left (A and C) show lab results and those on the right (B and D) show field results.

Figure 3.3 - Seed traits by bolting date

Offspring (seed) traits by bolting date over three years. Seed size (charts A and B) indicate mean seed length by bolting month (data available only for June and September bolting months). Total seed output (charts C and D) indicates mean number of seeds produced per plant by bolting month. Charts on the left (A and C) show lab results and those on the right (B and D) show field results.

Chapter 4: Secondary reproduction and semelparous reproduction in

Lobelia inflata

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4.1 Abstract

Although semelparity is a life history characterized by a single reproductive episode within a single reproductive season, some semelparous organisms facultatively express a second bout of reproduction, either in a subsequent season (“facultative iteroparity”) or later within the same season as the primary bout (“secondary reproduction”). Secondary reproduction has been explained as the adaptive deferral of reproductive potential under circumstances in which some fraction of reproductive success would otherwise have been lost (due, for example, to inopportune timing). This deferral hypothesis predicts a positive relationship between constraints on primary reproduction and expression of secondary reproduction. The semelparous herb *Lobelia inflata* has been observed occasionally to express a secondary reproductive episode in the field. However, it is unknown whether secondary reproduction is an example of adaptive

reproductive deferral, or is more parsimoniously explained as the vestigial expression of iteroparity after a recent transition to semelparity. Here, we experimentally manipulate effective season length in each of three years to test whether secondary reproduction is a form of adaptive plasticity consistent with the deferral hypothesis.

Our results were found to be inconsistent with the adaptive deferral explanation: first, plants whose primary reproduction was time-constrained exhibited decreased (not increased) allocation to subsequent secondary reproduction, a result that was consistent across all three years; second, secondary offspring—although viable in the laboratory—would not have the opportunity for expression under field conditions, and would thus not contribute to reproductive success.

Although alternative adaptive explanations for secondary reproduction cannot be precluded, we conclude that the characteristics of secondary reproduction found in *L. inflata* are consistent with predictions of incomplete or transitional evolution to annual semelparity.

4.2 Introduction

4.2.1 Overview

Semelparity (in plants, “monocarpy”) has generally been studied from a demographic perspective that directly contrasts semelparity and iteroparity. Cole (1954) found that the fitness advantage gained by an iteroparous over a semelparous strategy was very slight; a semelparous organism had to produce only a single additional offspring to offset the advantage of surviving to reproduce again. This finding was initially puzzling because it implied that semelparity should be common. Later authors noted that

differential juvenile-to-adult mortality, age-specific schedules of reproduction and other life-history traits affect the fitness of the semelparous habit (Charnov and Schaffer 1973; Young 1981, 1984, 1990; Fritz et al. 1982; Young and Augspurger 1991; Tallamy and Brown 1999; Stegmann and Eduard Linsenmair 2002). More recently, iteroparity and semelparity have also been thought to be endpoints of a continuum of life histories (Kirkendall and Stenseth 1985; Maltby and Calow 1986; Orzack and Tuljapurkar 1989; Unwin et al. 1999; Ranta et al. 2001; Crespi and Teo 2002; Kraaijeveld et al. 2003; Futami and Akimoto 2005; Martins et al. 2006; Meunier et al. 2012; Su and Peterman 2012).

There is mounting empirical support for this continuum hypothesis; semelparous species display significant reproductive variation, and many reproductive modes are not “pure” or “classical” semelparous or iteroparous habits. Some semelparous organisms facultatively reproduce a second time (i.e. in a discrete bout that is discontinuous from the first). For organisms subject to seasonal variation in living conditions, this second bout can occur either: (1) within the same season as the first (primary) bout, where it has been loosely termed “secondary reproduction” or “partial semelparity” (Morse 1994; Schneider and Lubin 1997; Unwin et al. 1999; Futami and Akimoto 2005; Martins et al. 2006; Christiansen et al. 2008); or (2) in a subsequent season, where it has been termed “facultative iteroparity”, or “facultative polycarpy” in plants (Morse 1994; Lesica and Shelly 1995; Tallamy and Brown 1999; Verkaar and Schenkeveld 2006). To avoid confusion between these terms, in this paper we refer exclusively to “secondary reproduction”, operationally defined as a second, non-continuous reproductive episode in an organism that is considered semelparous. This is meant to encompass both within-

season “secondary reproduction” and true “facultative iteroparity”. We also refer to an occurrence of secondary reproduction or facultative iteroparity as a “secondary reproductive episode” or SRE. Here, we investigate the life-history significance of SREs in *Lobelia inflata*.

4.2.2 Secondary reproduction

That a semelparous organism may be facultatively capable of reproducing more than once is surprising because the general benefit of semelparity is theorized to be the gain of a demographic advantage through a simultaneous, exhaustive reproductive episode (Charnov and Schaffer 1973; Young 1981; Orzack and Tuljapurkar 1989; Young and Augspurger 1991; Roff 1992; Murphy and Rodhouse 1999; Crespi and Teo 2002; Lesica and Young 2005). Identifying the conditions under which secondary reproduction or facultative iteroparity may occur is correspondingly important.

The most common explanation for SREs is that they are an adaptively plastic response to environmental stressors that constrain primary reproduction. Under conditions where normal semelparous reproduction may result in lost reproductive effort, deferral of reproductive effort to a second reproductive bout can realize fitness gains (Paige and Whitham 1987; Morse 1994; Simons and Johnston 1999; Schneider et al. 2003; Futami and Akimoto 2005; Christiansen et al. 2008). We term this the “adaptive deferral hypothesis”. According to this explanation, since the SRE is elicited as an adaptively plastic response to constrained reproduction, semelparous organisms capable of SREs should display “pure” semelparity only under ideal (unconstrained) conditions; constraints on primary reproduction should result in deferral of reproductive effort to a

second bout. An important corollary to this explanation is that the likelihood that reproductive effort will be deferred to a SRE (as well as the degree to which total reproductive effort is deferred) is proportional to the severity of the restriction of primary reproduction. Therefore, according to the adaptive deferral hypothesis, SREs should: (1) be expressed at more opportune times than primary reproductive episodes, since deferral of reproductive effort to an equally or more greatly constrained time would not increase fitness; and (2) show a proportional relationship between the degree to which reproductive effort is deferred to an SRE and the degree of constraint on the primary reproductive episode.

Several studies have supported the ability of the adaptive deferral explanation to account for SRE display. For example, when primary reproduction was artificially restricted in the crab spider *Lysiteles coronatus*, mothers compensated by reducing reproductive effort invested in the primary reproductive episode, and deferred reproductive effort to a second (Futami and Akimoto 2005). There is also evidence for a positive relationship between the degree of reproductive constraint and the expression of secondary reproduction - in a study of the semelparous Eresid spider *Stegodyphus lineatus*, Schneider *et al.* also artificially restricted primary reproduction (Schneider *et al.* 2003), but did so at different times – either at two or ten days after hatching. In these studies, both of which were performed in the lab, the authors found that the likelihood of observing a SRE was proportional to the severity of restriction of primary reproduction. In the field, the latent capacity to facultatively express an SRE has been observed in the erpobdellid leeches *Erpobdella octoculata* (Young and Ironmonger 1982) and *Nephelopsis obscura* (Baird *et al.* 1986; Davies and Dratnal 1996).

The most common alternative explanation for secondary reproduction is that semelparous organisms with a recent evolutionary transition from iteroparity to semelparity, or with a highly plastic expression of parity, may display reproduction a second time when the fitness cost of doing so is not high. We term this alternative explanation the “transitional hypothesis”, since it emphasizes the continuity between iteroparity and semelparity. According to this view, SREs may occur as part of a presumably adaptive (but potentially vestigial or maladaptive) opportunistic reaction norm: if selection for semelparity is not strong, an SRE can realize additional fitness. This hypothesis implies that, for seasonal semelparous organisms, the expected value of an SRE should correlate with the likelihood of being able to successfully complete that SRE. As semelparity is considered to be an adaptation to low adult survival (Young 1981, 2010; Orzack and Tuljapurkar 1989), where conditions are not conducive to prolonged adult survival (i.e. late reproduction may risk loss of reproductive potential) selection for more complete or extreme semelparity will be favoured over life-histories where adults survive to exhibit SREs. For semelparous organisms in seasonal environments, the timing of reproduction is usually critical, and while plants reproducing early may prolong the reproductive bout in time without losing fitness, the importance of semelparity increases as reproduction is initiated later in the season, since these plants will then under stronger selection for rapid, simultaneous and exhaustive reproduction (because they will have no future opportunity to realize fitness). The transitional hypothesis therefore predicts: (1) that SREs may occur at any time, including inopportune times; and (2) that increased SRE display will be associated with earlier primary reproduction.

4.2.3 Testing factors related to the expression of SREs in *Lobelia inflata*

Our focus for this study is the semelparous herb *Lobelia inflata* (Campanulaceae), which, according to several lines of evidence, evolved semelparity comparatively recently. Semelparity is considered a derived trait elsewhere in the genus *Lobelia*: of 21 species of *Lobelia* L. sect. *Lobelia*, 20 are iteroparous, with only *L. inflata* being consistently reported as semelparous. *L. appendiculata* has also been variably described as semelparous (Lammers 2011); even so, this character is clearly derived from a iteroparous ancestor (Bowden 1959). Furthermore, *Lobelia cardinalis*, the species most closely related to *L. inflata*, is iteroparous (Caruso et al. 2003), and previous studies have demonstrated the lability of parity in the genus *Lobelia* (Young 1990).

Fitness in semelparous herbs like *L. inflata* depends on a schedule of reproduction that exhausts energy reserves just as the season ends; selection on reaction norms governing reproductive strategies is strong and reproducing at the right time is critical (Simons and Johnston 2003). For many semelparous organisms, the developmental transition to reproduction is initiated in response to and depends on the plants' evolved response to seasonal cues (Stearns 1976; Flood and Halloran 1982; Stearns and Koella 1986; Roff 1992, 2001; Johnson and Shite 1997; Karban et al. 1999; Hautekèete et al. 2001; Wolfe and Mazer 2005; Bäumle and Dean 2006; Simons and Johnston 2006; Karban 2008; Ratcliff et al. 2009). For *L. inflata*, transition from a prereproductive vegetative phase to a reproductive phase—marked by the formation of a reproductive stalk and termed “bolting”—occurs in response to seasonal changes in day length and light quality (Lane et al. 1965; Harris 1968; Roff 1992; Preston 1998; Simons and Johnston 2000b; Bernier and Périlleux 2005; Bäumle and Dean 2006; Imaizumi and Kay

2006). The timing of initiation of reproduction is critical, since plants need to exhaust accumulated resources before the season ends; plant phenology and offspring phenotype are especially sensitive to the timing of initiation of reproduction (Simons and Johnston 2006). Due to this sensitivity, an effective experimental method to restrict the ability for plants to complete primary reproduction without damaging plant tissue is to manipulate photoperiod to manipulate the cue for initiation of reproduction. We note that restricting primary reproduction might also have been accomplished via flower or inflorescence destruction, but plants generally do not provide maternal care to seeds, and many plants exhibit a compensatory growth response to herbivory or flower destruction that would confound the response to the restriction of reproduction itself, an issue that is avoided by using time restriction to constrain primary reproduction (Juenger and Bergelson 1997; Simons and Johnston 1999; Pilson and Decker 2002; Freeman et al. 2003).

Laboratory experimental studies have an advantage over observational (or correlational) approaches when it is important to attribute response to a particular agent, or when a question demands extending the observed natural phenotypic range of expression (Schmitt et al. 1999). For *L. inflata*, an observational study *in situ* cannot be used to assess the extent to which reproductive effort is deferred, because adverse conditions in the field suppress the expression of SREs. We therefore used a lab-based phenotypic manipulation experiment to study the underlying potential (unexpressed in the field) for plants to express an SRE, and thus whether, and to what extent, plants exhausted their reproductive potential before then onset of winter. For this experiment, we subjected replicate groups of plants in a growth chamber to increasingly severe time

constraints on primary reproduction by manipulating the photoperiod and light intensity to mimic the natural changing conditions of the site of collection (Petawawa, ON).

4.2.4 Predictions

An increase in the expression of SREs in response to more severe time constraints during the primary reproductive episode would be consistent with the “adaptive deferral hypothesis” comparable to SREs elicited by restricted primary reproduction in other taxa (Schneider and Lubin 1997; Schneider et al. 2003). This hypothesis specifically predicts that SRE expression should be proportional to restriction severity; i.e. the less time a plant has to complete reproduction, the more likely it is to display a SRE, and the greater investment it will make in the SRE. Alternatively, if SREs in *L. inflata* are a vestigial expression of the iteroparous history of the genus *Lobelia* or an adaptation to an ancestral environment, time-restricting primary reproduction should be negatively correlated with SRE expression if SREs waste reproductive potential (especially under temporal constraints that select for more extreme semelparity), or should be uncorrelated if SREs have little or no fitness cost. It should be noted that, even if results show that SREs are not effective as a deferral of reproductive effort, alternative explanations to the vestigial hypothesis exist. For example we cannot discount the possibility that SREs may have been adaptive in ancestral environments (or in areas with winters even milder than the southern margins of its current range), or that SREs are adaptive for unknown reasons.

4.3 Materials and Methods

4.3.1 Test Species – *Lobelia inflata* (Campanulaceae)

L. inflata is an herbaceous plant in the family Campanulaceae, and is found throughout Eastern North America. It prefers sandy soils and thrives on the margins of roadways or in disturbed areas. Like many semelparous plants, *L. inflata* has two discrete phases of life: vegetative (accumulating resources as it is a growing rosette) and reproductive (expending resources on the production of offspring). Once a plant forms a stalk ('bolts'), vegetative growth ceases and leaves senesce. Reproduction occurs acropetally (i.e. in series from basal to apical positions) as the stalk grows, with most plants producing between 10-100 fruit, followed by senescence (Hughes and Simons 2014c). To trigger bolting, thresholds for rosette size, light intensity and photoperiod (day length) must be met (Simons and Johnston 2003). The "decision" to bolt is irreversible; however, if rosettes do not bolt in a given year, they are capable of overwintering (Simons and Johnston 2000a,b; Simons et al. 2010). At Petawawa, *L. inflata* rosettes typically bolt any time from late May to mid July during their second season post germination.

Reproduction occurs along the stalk (or small branches) as flowers are produced acropetally over the course of the reproductive season. Because the timing of the decision to bolt indicates an irreversible transition to the terminal reproductive phase of a plant's life history, and late bolting limits the time available to a plant to survive and reproduce (Simons and Johnston 2003), bolting is a critical fitness trait. After primary reproduction finishes, plants normally senesce and become dry, brittle and brown. In the field, secondary reproduction, which is very rare, is observed most often in warm spells in late

autumn. Although the main stalk is apparently “dead” or fully senesced, SREs occur through the production of new shoots from leaf axils. Typically, shoots produced during a SRE do not grow taller or longer than 10cm, remain unbranched, and flower acropetally until resources are exhausted.

L. inflata is an obligately self-fertilizing hermaphrodite, and produces offspring that are genetically identical to their parent. 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. No examples of outcrossing have been recorded, and heterozygosity in the Petawawa population appears to be zero (Hughes et al. 2014).

4.3.2 Seed Collection and Rosette Growth

L. inflata seeds were collected in October 2007 from the Petawawa Research Forest in Ontario, Canada (Lat. 45°99’N, Long. 77°30’W). Because it is self-fertilizing, *L. inflata* persists in isolated genotypic lineages; thus, to reduce the likelihood of studying an atypical genotype, seeds from spatially separated parental plants (a minimum of 50m apart) were used to found an experimental population of 21 (potential) genetic lineages. Eight of these 21 lineages were used in the 2008 experiment, and all 21 were used in the 2009 and 2010 experiments. Note that although we include genotype as a factor in analyses, a study aimed at assessing genetic variation for expression of SRE would comprise more than 8-21 genotypes; our intent here was to include a representative random sample of existing genetic variation.

4.3.3 Manipulation of Effective Reproductive Season Length

We manipulated available time to reproduce by controlling the timing of rosette bolting (initiation of reproduction) in a two-stage design implemented yearly from 2008-2010. In the vegetative, or first stage, we grew plants from seed to bolting rosette under ideal conditions: 400-800 seeds of each genotype were germinated on moist filter paper in a Biochambers SG-30 seed germinator under a regimen of 12h /12h (day/night) at 20°C with constant 85% humidity for 10-14 days. Seedlings were then planted in sterilized soil in 32-well cell soil trays and transferred to a Biochambers AC-40 growth chamber (on a 24°C/18°C 16h/8h day/night regimen) for approximately 40 days of growth. After this growth period, bolted plants were transferred to the reproductive chamber for the second, reproductive, stage. In each year, this process was repeated four times; once each to produce bolted rosettes by June 15th, July 15th, August 15th and September 15th. This created four bolting groups – one per month from June to September. Plants that did not bolt on the 15th (+/- 1 day) were not included in the bolting group. Each bolting group (BG) was translocated (on the 15th of the month) into another AC-40 growth chamber designed to simulate the outdoor environment at Petawawa (following outdoor photoperiod and light intensity via astronomic clock; temperature 20°C/16°C day/night). We followed the BGs as they expressed primary reproduction, senesced, and expressed SREs (or not). Plants were monitored until no further flowering occurred.

We observed each plant and recorded: (1) initial (prebolting rosette) size; (2) whether or not plants expressed a SRE; (3) how many fruits were produced during both primary reproduction and SRE; and (4) what proportion of fruits was allocated to the

SRE. Initial plant size was assessed as rosette size, measured as the length of longest living leaf (LLL), the best available surrogate measure of biomass in *L. inflata* (Simons et al. 2010; Hughes and Simons 2014c). Two traits were followed to assess secondary reproduction: we noted whether a plant produced a SRE, defined as any flowering occurring after initial senescence of the main reproductive stalk. Second, we counted the number of fruits produced in both the primary and secondary reproductive episodes. The proportion of all fruits expressed in the SRE was used as the estimate of the reproductive effort invested in the SRE. After harvest we measured the stalk height of all plants and subjected a subsample of seeds (from only the June and September BGs) to test viability.

4.3.4 Seed Viability

We examined whether SREs produced viable seeds to determine if reproductive effort realized during a SRE resulted in potential fitness gains. To assess viability, we measured seed length and germination fraction of subsamples of fruits from both the primary and secondary reproductive episodes from all plants in the June and September BGs in all three years. Seed measurements were calculated from digital photos (after allowing 72 hours for water absorption) and a stage micrometer. Germination fraction was assessed by placing seeds on moistened filter paper on Petri plates in an SG-30 seed germinator running on a 24°C/14°C 16h/8h 85% humidity schedule for 45 days, examining seeds every other day under an Olympus B061 light microscope.

4.3.5 Statistical Analyses

Three statistical approaches were used to evaluate plant investment in secondary reproduction, and for two of these a GLMM (mixed model) was run to assess the importance of random effects as well as fixed effects. First, to examine the likelihood of SRE expression, we used binomial logistic regression to model the equation governing SRE likelihood and to compare the relative weighting of bolting month, year, genotype, and prebolting rosette size on the response. A binomial logistical regression was chosen since the response was binary (i.e. “SRE expressed/No SRE expressed”), and can be understood as a series of Bernoulli trials with the log of the odds ratio as the linking function. Second, to examine the count of SRE fruits as a proportion of total fruits produced, we again used binomial logistic regression (where we modeled the total number of fruits as the set of trials and the number of fruit produced during the SRE as the number of “events” or “successes” in that set) to model the equation governing SRE investment and compare the relative weighting of different predictors on the response. Finally, to predict total (primary and secondary) fruit produced, we used a factorial ANCOVA. This analysis was run to examine whether or not SRE expression was associated with a direct cost (i.e. having fewer total fruit). Before running this ANCOVA, we used a Shapiro-Wilk test to assess the distribution of all predictor and response variables: none had a distribution that significantly differed from normality. For the ANCOVA analysis, we treated bolting group and year as fixed effects and included prebolting rosette size as a covariate.

To examine whether genotypic lineage was a significant predictor of SRE expression, we ran all three analyses as mixed-effect models (i.e. GLMMs), including

genotype as a random effect. To estimate total variance attributable to genotype, we used REML-based estimation (Harville 1977; Piepho et al. 2003). We then compared the fixed-effect-only models to the mixed-effect models using a likelihood ratio test; where the model including the random effect did not explain significantly greater proportion of total variation in the response, we discarded it and used a fixed-effect binary logistic regression or ANCOVA instead. Tests showing a significant effect of bolting group were followed by Tukey HSD post-hoc tests to assess which bolting groups differed. Seed viability proportions were compared by F-test. All statistical analyses were performed in SPSS 21.0 (IBM Corp).

4.4 Results

4.4.1 Likelihood of SRE occurring

According to our binomial logistic regression model, the timing of bolting was a strong predictor of likelihood of SRE expression ($X^2_6 = 274.52$, $p < 0.005$; Table 1). The likelihood of SRE expression was higher in early-bolting, less time-constrained plants, and lower in late-bolting, more time-constrained plants (Figure 4.1). Likelihood of SRE display did not depend on year or prebolting rosette size (Table 4.1). A second binomial regression—run as a mixed-model analysis with genotype included as an additional random effect—did not explain significantly more variation in the response ($X^2_1 = 1.87$, $p = 0.171$), and thus genotypic lineage did not predict likelihood of SRE expression.

4.4.2 Proportion of total fruits allocated to SRE

According to a second binomial logistic regression model, the timing of bolting was a strong predictor of the proportion of total fruit expressed during the SRE ($X^2_{32} = 2200.57$, $p < 0.005$; Table 2). This analysis showed that early-bolting, less time-constrained plants expressed a greater proportion of their total fruits in the SRE than did late-bolting, more time-constrained plants (Figure 4.2). Year and prebolting rosette size were not significant predictors of the proportion of total fruits produced during the SRE (Table 4.2). This binomial logistic regression analysis was also repeated with genotype included as an additional random effect, but again the resulting mixed model did not explain significantly more variation in the response ($X^2_1 = 2.34$, $p = 0.126$), and therefore genotypic lineage did not predict the proportion of total fruit expressed in the SRE.

4.4.3 Fruit number

A factorial ANCOVA model including bolting month, prebolting rosette size and year, significantly predicted the mean total number of fruits produced per plant ($F_{12,956} = 36.53$, $p < 0.01$). Tukey HSD tests on bolting group revealed two homogenous subsets; September-bolting plants produced the most fruit (mean= 46.93, SE = 2.9), which was significantly more than any other group, with July-bolting plants (mean= 36.0, SE = 1.1), August-bolting plants (mean= 33.4, SE = 1.0), and June-bolting plants (mean= 31.2, SE = 2.1) all producing significantly fewer fruit. This model indicated that bolting month, year, bolting month*year and prebolting rosette size (the covariate) were all significant predictors of the response.

4.4.4 Timing of SRE

SRE expression occurred from January to early February the following year. A large proportion of plants (431 of 969, or 41.7%) expressed a SRE, all between 95 and 127 days after the termination of primary reproduction and apparent senescence (Figure 4.3). The overall mean time between the cessation of primary reproduction and the initiation of the SRE was 108.3 days (SE = 16.6 days). Because early- and late-bolting plants ended primary reproduction at approximately the same time (~October 15), SREs for all BGs took place at approximately the same time: late January to mid February of the year following primary reproduction.

4.4.5 Seed germination

Our analysis revealed no phenotypic differences among offspring resulting from primary reproduction and SREs with respect to germination fraction ($F= 0.069$, $df= 1,101$, $p = 0.794$) or days to germination ($F= 1.63$, $df= 1,101$, $p = 0.204$).

4.5 Discussion

4.5.1 Overview

The adaptive deferral hypothesis, which predicted that plants under the severest time constraints would be the most likely to express SREs, was not supported by our findings. Each of the four measures we assessed in this manipulation study—likelihood of SRE expression, proportional reproductive investment in SRE, total fruits produced, and timing of reproduction—showed a trend opposite to what would be expected given

that the deferral hypothesis were true, and instead were consistent with the transitional hypothesis.

First, we observed that late-bolting, heavily time-constrained plants were less likely than early-bolting, less time-constrained plants to express a SRE (Figure 4.1), and produced proportionately fewer SRE fruits if they did (Figure 4.2). This pattern is inconsistent with the deferral hypothesis, which predicts that time-restriction of primary reproduction should be positively correlated with both SRE expression and investment.

Second, although SREs were given the opportunity for expression in the lab, they have no opportunity for expression under field conditions in a temperate climate: in our growth-chamber study, reproduction generally ended in October, followed by an inter-reproductive period of over 100 days. Also, plants transplanted from our field site to a glasshouse in October 2008 (just before the first snow) exhibited SREs the following January (P.W. Hughes pers. obs.). It is therefore clear that deferral of reproductive effort to an SRE does not contribute to reproductive success, contrary to predictions of the adaptive deferral hypothesis. In examples where SREs are explained by adaptive deferral of reproductive effort, the SRE generally takes place either: (1) shortly after primary reproduction (i.e. within 25 days for *S. lineatus*; Figure 4.3); or (2) in the next season, as for the pitcher plant *Wyeomyia smithii* (Bradshaw 1986). In either case, SREs increase fitness because SRE occur under conditions that are as or more favourable than the conditions under which primary reproduction took place (Schneider and Lubin 1997; Schneider et al. 2003). Although in principle it is possible that the inter-reproductive period for *L. inflata* would be longer under field conditions than it was in the lab, and an SRE could occur the following spring, to the best of our knowledge this has not yet been

observed. For these reasons, we conclude that SRE expression in *L. inflata* cannot be accounted for by the adaptive deferral hypothesis.

4.5.2 SREs do not represent a deferral of reproductive effort in *L. inflata*

Our results did support the transitional hypothesis, both because SREs were produced at an inopportune time, and because the underlying allocation pattern showed that heavily time-constrained plants were less likely to defer offspring than were less time-constrained plants. If these underlying trends are also expressed in the field, these trends suggest that: (1) early-bolting plants that opt to defer reproduction may die with significant unspent reproductive potential, representing a substantial fitness cost; and (2) late-bolting plants compensate for time-constraints by producing flowers more quickly as the season progresses. Furthermore, the observation that no differences in seed viability were detected between primary and secondary reproductive episodes indicates that SREs cannot be discounted as simply pseudo reproduction that incurs no energetic costs; this further suggests that any reproductive effort allocated to SREs in the field is wasted. For these reasons, our main conclusion is that SREs do not represent an effective avenue for the adaptive deferral of reproductive effort in *L. inflata*, which is the primary hypothesis explaining why SREs exist in semelparous organisms; that deferral results in wasted reproductive effort may have contributed to the evolution of semelparity in *L. inflata*.

4.5.3 Limitations

It is important to recognize the limits on the interpretation of data collected under growth-chamber conditions, but also that we are not making assumptions about how SRE

would be expressed in the field. It is because we expect the expression of SRE to be suppressed under field conditions that we used phenotypic manipulation to expose the trait of interest across a particular array of laboratory conditions. Here, we use photoperiod, a cue known to be ecologically relevant and that elicits a strong reproductive response in *L. inflata* (Hughes and Simons 2014c) to reveal an otherwise unobservable expression of SRE. We thus make an assumption common to all phenotypic manipulations: that the expression of a trait (SRE) will not respond in an opposite manner to a manipulation (photoperiod) in the lab compared to its potential expression in the field. We focused on photoperiod cues in *L. inflata*; other cues, correlated with an unknown factor germane to expectation of realized reproductive effort, may influence the developmental decision to defer resources for a SRE. Finally, we note that our study was designed to test the adaptive deferral hypothesis, and rejection of this hypothesis does not conclusively establish that SREs are maladaptive or nonadaptive in *L. inflata*. Further study of SRE expression in *L. inflata* elsewhere in its range would allow us to better assess the fitness consequences for plants expressing SREs.

4.6 Conclusion

In conclusion, we found that while *L. inflata* plants had the ability to produce viable seeds in SRE fruits, the pattern in which they did so was inconsistent with the adaptively plastic deferral of reproductive effort seen in animal systems: here, time-constraining primary reproduction resulted in semelparity with reduced secondary reproduction. This norm of reaction is consistent with the expression of vestigial iteroparity that decreases as time constraints increase. Where seasonality is strong and

winter is long (i.e. throughout most of *L. inflata*'s current range), SREs likely represent a loss of reproductive effort. Thus, secondary reproductive episodes in *L. inflata* appear to be a vestige of their iteroparous evolutionary past.

4.7 Tables

Table 4.1 - Logistic regression results for SRE likelihood

Results of binomial logistic regression analysis of factors affecting likelihood of SRE expression. The four bolting groups were experimentally produced using a photoperiod manipulation, and this manipulation was repeated for three years from 2008-10. Prebolting plant size was measured as the length (in mm) of the longest living leaf of the unbolted rosette. The regression model significantly predicts likelihood of SRE expression ($X^2 = 274.52$, $df=6$, $p < 0.005$). Odds ratios express relative likelihood of SRE expression.

Term	Parameter Estimate ($\pm$ SE)	Wald chi-square statistic	df	p	Estimated Odds Ratio
Constant	-2.34 (0.35)	45.25	1	0.23	-
Year=2008	-0.30 (0.20)	2.27	1	0.74	1.07
Year=2009	0.07 (0.17)	0.16	1	1.07	0.74
Year=2010	0	-	-	-	1.00
Bolting month=June	2.93 (0.24)	149.82	1	< 0.001*	18.79
Bolting month=July	2.45 (0.23)	108.93	1	<0.001*	11.61
Bolting month=August	0.81 (0.24)	11.49	1	<0.001*	2.26
Bolting month=September	0	-	-	-	1.00
Size	0.02 (0.01)	3.33	1	0.07	1.02

Table 4.2 - Logistic regression results for proportion of fruit in SRE

Results of binomial logistic regression analysis of factors affecting proportion of total fruits allocated to SRE. The four bolting groups were experimentally produced using a photoperiod manipulation, and this manipulation was repeated for three years from 2008-10. Prebolting plant size was measured as the length (in mm) of the longest living leaf of the unbolted rosette. The regression model is a significant predictor of the response ($X^2 = 2200.57$, $df=32$, $p < 0.005$). Odds ratios express relative likelihood of fruit being allocated to the SRE.

Term	Parameter Estimate ($\pm$ SE)	Wald chi-square statistic	df	p	Estimated Odds Ratio
Constant	-4.88 (0.20)	589.81	1	<0.001*	-
Year=2008	-0.40 (0.32)	1.51	1	0.22	0.67
Year=2009	0.18 (0.23)	0.61	1	0.44	1.19
Year=2010	0	-	-	-	1.00
Bolting month=June	3.35 (0.17)	389.09	1	<0.001*	28.50
Bolting month=July	2.96 (0.17)	300.49	1	<0.001*	19.30
Bolting month=August	1.71 (0.18)	87.45	1	<0.001*	5.52
Bolting month=September	0	-	-	-	1.00
Size	0.02 (0.00)	0.37	1	0.54	1.01

4.8 Figures

Figure 4.1 - Proportion of plants expressing an SRE

Proportion of plants expressing a secondary reproductive episode (SRE) by bolting group (BG). The four bolting groups were experimentally produced using a photoperiod manipulation. Levels not sharing letters indicate significantly different means by Tukey HSD test following a factorial ANCOVA performed for illustrative purposes.

Figure 4.2 - Proportion of fruit allocated to SRE

Proportion of fruit allocated to SRE across the four experimental bolting groups. Boxes indicate 2nd/3rd data quartiles, horizontal lines show median value and whiskers show range of data dispersal. Levels not sharing letters indicate significantly different means by Tukey HSD test following a factorial ANCOVA performed for illustrative purposes.

Figure 4.3 - Reproductive phase diagram

Reproductive phase diagram comparing Lobelia inflata bolting groups (means from 2008-10) and Stegodyphus lineatus (data from Schneider and Lubin 1997). Boxes indicate successive stages of growth and whiskers indicate standard error (some data unavailable for S. lineatus).

Chapter 5: Development of microsatellite markers for *L. inflata*

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5.1 Abstract

Nuclear microsatellite markers were developed for *Lobelia inflata* (Campanulaceae), an obligately self-fertilizing plant species, for use in the study of temporal fluctuation in allele frequency and of the genetic structure within and among populations.

We developed 28 primer pairs for *L. inflata*, all of which amplify CT dinucleotide repeats. We evaluated amplification of these loci in 53 *L. inflata* individuals at three sites in Eastern North America, and found that 24 loci showed microsatellite polymorphism. We also found that 16 loci amplified successfully in *Lobelia cardinalis*, and 11 amplified successfully in *Lobelia siphilitica*.

These primers will be useful for assessing allelic diversity within and among populations of *Lobelia inflata*, and show potential for use in congeneric species.

5.2 Introduction

Lobelia inflata (Campanulaceae) is an herbaceous plant native to North America in the cosmopolitan genus *Lobelia*, which contains over 400 species (Lammers 2011). *L. inflata* is semelparous and is capable of expressing either an annual or biennial life history; in the latter case, the plant overwinters as a frost-hardy rosette. Systematists have placed *Lobelia inflata* in *Lobelia* sect. *Lobelia*, along with 20 other (primarily) North American species (Murata 1992). Of these species, *Lobelia cardinalis*, *L. siphilitica*, *L. kalmii*, *L. nuttallii*, *L. spicata* and *L. dortmanna* all coexist sympatrically with *L. inflata* in the northeastern United States and Canada. The phylogenetic relationships between these species are not well understood, but morphological analysis has suggested that *L. inflata* is most closely related to *L. cardinalis*, *L. siphilitica* and *L. dortmanna* (Lammers 2011).

Populations of *L. inflata* consist of myriad distinct genetic lineages, which are expected to be reproductively isolated from one another. This is because *L. inflata* is assumed to be obligately autogamous—i.e. it is incapable of outcrossing—since it possesses a closed anther tube, which permits pollen release only onto the stigma of the same flower. As such, plants will produce offspring that are genetically identical to the parent, and therefore heterozygosity is expected to be zero in natural populations (Simons and Johnston 2006). Although quantitative genetic variation is present for some traits, the extent of genetic variation among *L. inflata* ecotypes is unknown (Simons and Johnston 2000a). Highly variable molecular labels (i.e. polymorphic microsatellite loci) would permit the genotyping of reproductive lineages, and the tracking of gene flow among populations. In this study, we characterized 28 microsatellite loci for *L. inflata*, tested

these markers in 58 individuals from three eastern North American populations, and assessed cross-amplification success in two congeneric species within *Lobelia* sect. *Lobelia*: *Lobelia cardinalis* and *Lobelia siphilitica*.

5.3 Methods

Genomic DNA was extracted from a single *L. inflata* specimen collected from the Petawawa Research Forest (individual DAO-887897 – see Section 5.6). To obtain high-quality DNA for enrichment, total genomic DNA was extracted using the CTAB extraction method of Murray and Thompson (1980). We prepared a CT microsatellite-enriched DNA library using the method of Hamilton et al. (1999) and linker sequences of Glenn and Schable (2005). Ninety-six clones from this library were sequenced using the BigDye 3.1 kit on an ABI 3730 DNA analyzer (Applied Biosystems, Carlsbad, USA). Twenty-eight clones contained dinucleotide motifs with at least 7 repeat units and were used to design primer pairs using the Primer3Plus software package (Untergasser et al. 2007). Selection of the final 28 primer sets was based on: (1) reliable and repeatable amplification by PCR (using the PCR parameters listed below); and (2) distinct banding pattern visualization on 3% agarose gel. All 28 primer pairs met these inclusion criteria and were used to assess genetic diversity.

To assess amplification, we performed PCR in 20µL reaction volumes, using the Phire II Direct PCR Kit (Thermo Fisher Scientific, Waltham, USA). Unlike two-step protocols that require separate DNA isolation and PCR steps, direct PCR allows amplification directly from plant material (Bellstedt et al., 2010). Here, the genomic DNA template for the direct PCR reaction was taken from a circular punch of dried leaf

or fruit capsule material using a 0.35mm Harris Uni-Core micropunch (Thermo Fisher Scientific). Each 20µL reaction contained 0.5µM primers and 1.5mM MgCl₂. A standard three-step PCR protocol was used, including an initial denaturation at 98°C for 5 min, followed by 30 cycles of denaturation at 98°C for 5s, annealing at 50-58°C for 5s, extension at 72°C for 20s, and a final extension step of 72°C for 10 min. The annealing temperatures for each primer set used in this study are given in Table 5.1. The PCR reactions were performed using a T-3000 ThermoCycler (Biometra, Goettingen, Germany). We initially screened primer sets for polymorphism using 58 individuals from three *L. inflata* populations: Petawawa, ON (*n*=43); Martock, NS (*n*=8); and Petersham, MA (*n*=7). We then tested cross amplification of these primer sets in *L. cardinalis* (*n*=12) and *L. siphilitica* (*n*=3) (see Section 5.6 for collection location and voucher deposition information). Amplicon size was determined using a Generuler 100bp DNA Ladder (Thermo Fisher Scientific). Gel images for all samples were compared via Sequentix GelQuest (Sequentix Digital DNA Processing, Klein Raden, Germany) from 3% agarose gel electrophoresis (run at 60V for 90min).

5.4 Results

Genetic diversity parameters for the three populations are presented in Table 1.2. Among *L. inflata* individuals, 24 loci were polymorphic, with 2-4 alleles per locus. Four of the loci (1, Linflata6, Linflata11, and Linflata24) were monomorphic in all *L. inflata* individuals tested. Notably, some loci showed substantial differences in allele size; for example, two alleles were found for Linflata5 in the Petawawa population – one was 215 bp and the other 331 bp. Although the variability of SSR repeats undermines their

usefulness as precise molecular clocks, in the absence of outcrossing, differences in SSR allele size may be proportional to lineage divergence time (Neff 2004). We used GenePop version 4.2 to test linkage disequilibrium and Hardy-Weinberg equilibrium (Rousset 2008). Observed heterozygosity was zero across all loci in *Lobelia inflata*, a finding that supports the hypothesis that outcrossing is rare or nonexistent in field populations of *L. inflata*.

Cross-amplification success of these 28 loci in *L. cardinalis* and *L. siphilitica* is also presented in Table 2. There was successful amplification at 16 loci for *L. cardinalis*, with 2-3 alleles per locus, and at 11 loci for *L. siphilitica*, with 1-2 alleles per locus. For *L. cardinalis*, we found 3 loci (Linflata2, Linflata12, and Linflata26) to be polymorphic. We found no polymorphism for any loci in *L. siphilitica*.

5.5 Discussion and Conclusion

These microsatellite markers are the first to be developed for *L. inflata*, and offer a new opportunity to investigate allelic diversity and gene flow within and among populations of *L. inflata*. Observed homozygote excess is likely due to lack of gene flow between *L. inflata* reproductive lineages. Successful cross-amplification of these loci in both *L. cardinalis* and *L. siphilitica* suggests that these loci may also be used to assess genetic diversity in congeneric species.

5.6 Supplementary Voucher Information

The data below describe voucher specimens used to characterize microsatellite markers in *Lobelia inflata*. All specimens were collected in Canada and are deposited at

the Canadian National Collection of Vascular Plants (DAO), in Ottawa, Canada. Information for each sample is listed below, including: taxon, voucher specimen, collection site and geographic coordinates.

Lobelia inflata sect. *Lobelia* (Campanulaceae); Voucher Accession Number DAO-887897; Cultivated at Carleton University (45.3854°N, 75.6922°W)

Lobelia inflata sect. *Lobelia* (Campanulaceae); Voucher Accession Number DAO-887899; Collected from Petawawa Research Forest, Ontario (45.9902°N, 77.4413°W)

Lobelia inflata sect. *Lobelia* (Campanulaceae); Voucher Accession Number DAO-887901; Collected from Martock, Nova Scotia (44.9560°N, 64.1060°W)

Lobelia inflata sect. *Lobelia* (Campanulaceae); Voucher Accession Number DAO-887902; Collected from Harvard Forest, Petersham, Massachusetts (42.5314°N, 72.1899°W).

Lobelia siphilitica sect. *Lobelia* (Campanulaceae); Voucher Accession Number DAO-887903; Cultivated at Carleton University (45.3854°N, 75.6922°W)

Lobelia cardinalis sect. *Lobelia* (Campanulaceae); Voucher Accession Number DAO-887904; Cultivated at Carleton University (45.3854°N, 75.6922°W)

5.7 Tables

Table 5.1 - Characteristics of 28 microsatellite markers for *L. inflata*

Characteristics of microsatellite markers developed for Lobelia inflata

Locus	Primer	Repeat Motif	Allele size range (bp)	Ta	GenBank Accession No.
Linflata1	F: TTCCAGAGACATGCTTCACG R: TTTCTCAAACTGGCACATGA	(CT) ₃₈	181	59.3	KF855960
Linflata2	F: CTCTCCCCCTTCGTTTCTC R: GCAACAACACGAACACGATT	(CT) ₁₈	165-201	59.6	KF855961
Linflata3	F: GGCCATTGGGTTGCTTTTAT R: GTTTCGGCAACCAACAGAAT	(CT) ₂₄	145-163	59.9	KF855962
Linflata4	F: GCCTATGCTGGGGTTACTTG R: ACACGGTAGCGGAAAAATGT	(GA) ₇	158-166	59.5	KF855963
Linflata5	F: TTTCAACCTTCATGAGGAAATATG R: TTGCTTAGTGCTTATGGTTGAAG	(GA) ₃₇	215-331	58.6	KF855964
Linflata6	F: TGGGAATTGTATTCCCTCAA R: AGAGGCTATTGCGCATCTTG	(GA) ₂₃	189	59.2	KF855965
Linflata7	F: TCGATCTAATTGTGCAAACTGC R: ATTGGGCTCATGCATCTCT	(CT) ₂₈	144-154	59.2	KF855966
Linflata8	F: TGTCTTTAATCAGGTCAACCA R: GGTCAACTTTGCAAGCCATT	(CT) ₃₂	207-225	59.4	KF855967
Linflata9	F: AAATTGACCAACATACCCCTAGTC R: CGCTGCTCCTCCCTTACTC	(CT) ₇	207-311	59.5	KF855968
Linflata10	F: AAGAAAGTTTGTAGGCCATCC R: AACCAAGGGGACTGAAGCAT	(GA) ₉ TA(GA) ₁₁	113-231	59.5	KF855969
Linflata11	F: ATGAGTGCTGGTCCGCTTT R: CTAATCCCATCCCCCATTTT	(GA) ₁₅	162	59.8	KF855970
Linflata12	F: AACAAGGAGACGGGGATTTT R: TGCCTTTCTACGGCTACTTTG	(CT) ₁₈	126-184	59.5	KF855971
Linflata13	F: ACAAAACATCATCAGTCACCCATA R: GGGGGTTTCCTCTCTCCATA	(GA) ₂₂	102-118	59.2	KF855972
Linflata14	F: GGCAACTGCATTTAGGGATG R: TCAAAGGCCCTTCTCTCTGA	(CT) ₃₅	185-207	60.0	KF855973
Linflata15	F: TTTCAGGCTGTCTTTTGACC R: TTCAAATACTTGTATTTCGACGTTT	(GT) ₉ (GA) ₈	168-180	59.8	KF855974
Linflata16	F: TGTTCGAGAAACAAACACCTT R: AGCCACCTGGTATTCAAGA	(GA) ₁₂	178-256	59.5	KF855975
Linflata17	F: CTGGTGAACAATTGCCAAAA R: GGTGTTTGTCTTCTGCAACATT	(GA) ₁₅	175-191	59.0	KF855975
Linflata18	F: CCGTATCCTATCCATCTCCTACA R: AGGGGAGTGAGGAAGACGTT	(CT) ₂₅	176-184	59.3	KF855976
Linflata19	F: GACCCTCCAAATAACCAAGTGA R: CACACTGCCATAATCCTCA	(CT) ₁₇ (CA) ₈	164-168	59.5	KF855977
Linflata20	F: AAGGGTGAGGAAGTCCGATT R: ACCTTCAAAACCACAGCAC	(GA) ₁₆	129-189	59.9	KF855978
Linflata21	F: ACTGCTATCGCTCTCGATCTC R: TTTCCCAACAAACCTCCATC	(GA) ₁₉	180-214	58.8	KF855979
Linflata22	F: CTTCATTTGAGCCTCGTAACC R: ACCTATATAAAAGACAATGTCAGCAA	(GT) ₁₀ (GA) ₁₈	93-119	57.6	KF855980
Linflata23	F: CTCTCCCCCTTCGTTTCTC R: GCAACAACACGAACACGATT	(CT) ₁₈	165-244	59.6	KF855961
Linflata24	F: TCGTCAATTTTGTGGTTGTCA R: GCGGCAGTGAAGGAGAAATA	(CT) ₂₁ (CA) ₂₀	208	60.0	KF855981
Linflata25	F: TTCAGGACATAAGCCTTGAAGTC R: AAAAGAAATTTGCGCCACAC	(CT) ₁₃ (CA) ₁₇	155-177	59.7	KF855982
Linflata26	F: GGATCATGCCTTGAATCTGC R: AACGGAGAAGCAACCCTTTT	(CT) ₁₄	104-143	60.1	KF855983
Linflata27	F: CCACCTCTCAGCAAGAAGAC R: TCCCTCCCATATTTGTGTGT	(GA) ₂₀	175-181	59.8	KF855984
Linflata28	F: CAAACACATGCAAAACACACAA R: AGCAGTGCCCGTTTAGATTG	(GA) ₉	183-197	59.0	KF855984

Table 5.2 - Allele diversity for 28 microsatellite loci.

*Allele numbers and cross amplification for L. inflata microsatellite loci. Data for L. inflata includes 58 individuals from 3 populations. *Note: zero alleles detected for cross-amplification indicates failed amplification*

Locus	<i>L. inflata</i> (N=58)									Number of alleles detected for cross-amplification*	
	Petawawa, ON (<i>n</i> =43)			Martock, NS (<i>n</i> =8)			Petersham, MA (<i>n</i> =7)				
	Allele No.	H _o	H _e	Allele No.	H _o	H _e	Allele No.	H _o	H _e	<i>L. cardinalis</i> (<i>n</i> =12)	<i>L. siphilitica</i> (<i>n</i> =3)
Linflata1	1	0.000	0.000	1	0.000	0.000	1	0.000	0.000	1	0
Linflata2	3	0.000	0.651	2	0.000	0.469	1	0.000	0.000	2	1
Linflata3	2	0.000	0.214	1	0.000	0.000	2	0.000	0.490	0	0
Linflata4	2	0.000	0.467	1	0.000	0.000	1	0.000	0.000	1	0
Linflata5	2	0.000	0.089	2	0.000	0.219	2	0.000	0.245	0	0
Linflata6	1	0.000	0.000	1	0.000	0.000	1	0.000	0.000	1	1
Linflata7	3	0.000	0.662	3	0.000	0.594	3	0.000	0.653	1	1
Linflata8	3	0.000	0.467	1	0.000	0.000	2	0.000	0.408	0	0
Linflata9	2	0.000	0.500	2	0.000	0.469	1	0.000	0.000	0	0
Linflata10	2	0.000	0.130	1	0.000	0.000	2	0.000	0.245	0	0
Linflata11	1	0.000	0.000	1	0.000	0.000	1	0.000	0.000	1	0
Linflata12	4	0.000	0.586	2	0.000	0.375	2	0.000	0.408	2	0
Linflata13	2	0.000	0.478	1	0.000	0.000	2	0.000	0.490	0	0
Linflata14	2	0.000	0.439	1	0.000	0.000	1	0.000	0.000	1	1
Linflata15	1	0.000	0.000	1	0.000	0.000	2	0.000	0.490	0	0
Linflata16	2	0.000	0.273	2	0.000	0.219	1	0.000	0.000	1	1
Linflata17	2	0.000	0.130	2	0.000	0.469	2	0.000	0.490	1	0
Linflata18	2	0.000	0.576	1	0.000	0.000	1	0.000	0.000	0	0
Linflata19	2	0.000	0.169	1	0.000	0.000	1	0.000	0.000	0	1
Linflata20	2	0.000	0.283	2	0.000	0.469	1	0.000	0.000	0	0
Linflata21	3	0.000	0.580	2	0.000	0.469	3	0.000	0.388	1	1
Linflata22	2	0.000	0.423	1	0.000	0.000	1	0.000	0.000	0	1
Linflata23	2	0.000	0.357	1	0.000	0.000	1	0.000	0.000	0	0
Linflata24	1	0.000	0.000	1	0.000	0.000	1	0.000	0.000	1	0
Linflata25	1	0.000	0.000	2	0.000	0.375	1	0.000	0.000	1	1
Linflata26	3	0.000	0.597	2	0.000	0.500	2	0.000	0.408	2	1
Linflata27	2	0.000	0.498	1	0.000	0.000	2	0.000	0.490	1	1
Linflata28	2	0.000	0.498	1	0.000	0.000	2	0.000	0.245	1	0

Chapter 6: Obligate autogamy, but abundant genetic variation in the herb *Lobelia inflata* (Campanulaceae)

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6.1 Abstract

Although high levels of selfing (>85%) are not uncommon in nature, organisms reproducing entirely through selfing are extremely rare. Organisms reproducing predominantly through self-fertilization are expected to have low genetic diversity because genetic variation is distributed among rather than within lineages, and is readily lost through genetic drift. We examined genetic diversity at 22 microsatellite loci in the semelparous herb *Lobelia inflata*, and found (1) no evidence of outcrossing, and (2) high rates of genetic polymorphism occurring among lineages: we genotyped 2,310 loci—105 plants at 22 microsatellite loci—and found no heterozygous loci despite high levels of polymorphism (2-4 alleles per locus). Furthermore, this genetic variation among lineages was significantly associated with phenotypic traits (e.g. flower colour, size at first flower). The extent of genetic variation observed here cannot be explained by mutation-selection balance and therefore must be explained by another mechanism. Patterns suggest that temporal genotype-by-environment interaction might maintain genetic

variation and, because genetic variation occurs only among lineages, this simple system offers a unique opportunity for a test of this mechanism.

6.2 Introduction

The long-term viability of self-fertilization (selfing) as a reproductive strategy has been the subject of considerable attention (Lande and Schemske 1985; Schemske and Lande 1985; Barrett and Eckert 1990; Johnston 1998; Herlihy and Eckert 2005; Hereford 2010; Pérez et al. 2012). Self-fertilization is favoured over outcrossing when fitness gains due to reproductive assurance, transmission advantage, isolation from maladaptive genes, or density-dependent interactions outweigh negative effects associated with inbreeding depression or gamete discounting (Fisher 1941; Jain 1976; Lande and Schemske 1985; Schemske and Lande 1985; Barrett and Eckert 1990; Cheptou and Dieckmann 2002; Goodwillie et al. 2005; Grossenbacher and Whittall 2011). Inbreeding depression may have slight (Willis 1993, 1999; Husband and Schemske 1996) to severe fitness consequences (Eckert and Barrett 1994; Sletvold et al. 2012), and may vary in intensity (Hauser and Loeschcke 1995, 1996; Willis 1999; Hayes et al. 2005; Chang 2007). The strength of inbreeding depression is negatively associated with the proportion of self-fertilization present in a species with a mixed mating system (Willis 1993; Dudash et al. 1997; Crnokrak and Barrett 2002; Kokko and Ots 2006). Although 10-20% of species are predominantly (>50%) selfing (Barrett 2002), only a small minority is highly (>95%) selfing (Wright et al. 2013). In these species, reproductive assurance is cited as the main evolutionary mechanism favouring extreme selfing over a mixed mating system (Takebayashi and Morrell 2001; Zhang et al. 2014). However, Wright et al. (2013) note,

“marker-based estimates of self-fertilization rates suggest that very few if any plant species are completely selfing”—that is, obligate selfing is extremely rare or unknown in nature.

Extant mating-system models generally assume that—in the absence of gene flow among individuals within a population—genetic diversity will gradually erode for several reasons. Because the effective population size of selfers is half that of outcrossers (Lande and Schemske 1985; Schemske and Lande 1985; Jarne and Charlesworth 1993; Charlesworth and Charlesworth 1995), the effects of genetic drift may be strong. Alternative alleles are “captured” within lineages and cannot be recombined; thus, both drift and selective purging of deleterious alleles will result in low genetic diversity (Husband and Schemske 1996; Dudash et al. 1997; Crnokrak and Barrett 2002; Glémin et al. 2006; Michalski and Durka 2007). This leads to the speculation that selfing may be irreversible and is therefore an “evolutionary dead end” (Darwin 1877; Marshall and Brown 1983; Schoen and Brown 1991; Barrett and Harder 1996; Takebayashi and Morrell 2001; Igic and Busch 2013). Thus, despite the obvious benefits of selfing—reproductive assurance and transmission advantage—the danger of committing to a “dead end” explains the rarity of very high selfing in nature.

In a comparison of predominantly inbreeding and outbreeding plant species (Schoen and Brown 1991), allelic diversity of inbreeders was found to be less than half that of outcrossers, although the variance in allelic diversity among selfing populations was higher. There are also numerous examples of organisms showing lower allelic diversity in selfing than outcrossing populations or congeners (Jarne and Charlesworth 1993; Dudash et al. 1997; Igic 2001; Wendt et al. 2002). For example, lower genetic

diversity has been observed in highly selfing populations of *Arabidopsis thaliana* than in populations with a higher degree of outcrossing (Abbot and Gomes 1989; Todokoro et al. 1995). However, high levels of within-population allelic diversity have also been found within predominantly selfing populations of groups including fungi (Winton et al. 2006), legumes (Bonnin et al. 1996; Siol et al. 2007), proteaceous trees (Ayre et al. 1994), ginger (Zhang and Li 2008), brittle stars (Boissin et al. 2008), snails (Jarne et al. 1994; Viard et al. 1996; Trouvé et al. 2003), and killifish (Tatarenkov et al. 2007).

There is thus no simple relationship between mating system and intrapopulation genetic diversity, and little is known about how genetic diversity is maintained in highly selfing species, despite the fact that the partitioning of genetic variation in species with mixed-mating systems is an important and well-studied question in evolutionary ecology (Stebbins 1974; Barrett and Eckert 1990; Holsinger 1991; Stephenson et al. 2000; Kalisz et al. 2004; Goodwillie et al. 2005; Igic and Kohn 2006).

In this study, we determine the selfing rate and assess the degree of genetic diversity in the semelparous herb *Lobelia inflata* (Campanulaceae). *L. inflata* has been assumed to be obligately self-fertilizing in previous studies (Simons and Johnston 2000b, 2003; Hughes and Simons 2014a,c), presumably via autogamy in cleistogamous flowers. However, genetic variation in *Lobelia inflata* appears high (Hughes et al. 2014), despite the fact that studies of outcrossing congeners show limited allelic diversity at genotyped loci (Antonelli 2008; Geleta and Bryngelsson 2012). One suggestion is that self-fertilization can create multigenerational genetic lineages, as each mother produces genotypically identical offspring; in these cases genetic variation could be maintained by fluctuating selection on lineages with distinct phenotypes (Winton et al. 2006;

Tatarenkov et al. 2007; Leys et al. 2014). Moreover, models of genetic variation in predominantly selfing species have predicted that relatively high levels of genetic variation can be maintained by fluctuating selection (Ellner and Sasaki 1996; Barrière and Félix 2005; Brys et al. 2011). Therefore, if—through autogamous self-fertilization—*L. inflata* forms genetically identical lineages, temporal fluctuations in fitness among reproductive phenotypes may preserve the diversity of genetic lineages, and therefore of alleles, through time. For *L. inflata*, fitness differences may be related to flowering phenology and reproductive effort (Hughes and Simons 2014a,b).

To examine these questions, we performed three main tests in this study: first, we determined the degree of autogamous selfing, assessed using microsatellite marker diversity found in *L. inflata* specimens sampled from a field site. Second, we genotyped plants to identify genetic lineages. Finally, we evaluated the degree of variation in phenotype—particularly with respect to reproductive traits known to affect the variable expression of semelparous reproduction in *L. inflata*—that was associated with variation among genetic lineages.

6.3 Materials and Methods

Lobelia inflata L. (Campanulaceae) is a rosette-forming semelparous biennial distributed throughout Eastern North America. The genus *Lobelia* contains more than 400 species, distributed globally, and is structured by sections and subgenera confined to particular geographic regions (Murata 1992; Lammers 2006). *L. inflata* has been placed in *Lobelia* sect. *Lobelia*, along with most other Eastern North American *Lobelia* species, e.g. *L. cardinalis*, *L. siphilitica*, *L. dortmanna* (Lammers 2011). Although *L. cardinalis*

and *L. siphilitica* are iteroparous and produce (self-compatible) chasmogamous flowers, *Lobelia inflata* is the only species in this section that is semelparous and produces only cleistogamous flowers (Simons and Johnston 2000a; Caruso 2006; Bartowska and Johnston 2014; Hughes and Simons 2014c). *L. inflata* has been presumed to be obligately or nearly obligately autogamous (Simons and Johnston 2003; Hughes and Simons 2014a,b), since anthesis is extremely brief, and the onset of fruit development can occur very shortly after the initiation of flower formation. Moreover, in each flower the short stigma is completely enclosed by a tube of anthers, and pollen does not appear to disperse outside flowers.

The plants used in this study were sourced from a wild population in the Petawawa Research Forest (Petawawa, Canada 45°99'N, 77°30'W). Wild-grown plants ($n=28$) represented the parental generation (which we term “P₁”; here, we use parental/filial labels to indicate generations, not hybrids); seed samples were collected in situ in October 2007. Offspring plants ($n=811$) representing the first filial generation (F₁) were grown under lab and field conditions in 2008, 2009 and 2010. We produced the F₁ generation under lab conditions in order to adequately replicate all lineages and to control for maternal effects that might differ among field-harvested plants. To produce the F₁ generation, groups of 100-200 seeds collected from P₁ mothers were placed on 70mm circles of moistened Whatman #5 filter paper (Thermo Fisher Scientific, Waltham MA, USA) inside 4” petri plates, and germinated in a Biochambers (Winnipeg, MB, Canada) SG-30 germination chamber for 10-14 days under a day/night light cycle of 12h/12h, an ambient temperature of 20°C, and an ambient humidity level of 85%.

Upon germination, seedlings were planted in 4 X 4cm cellpacks of autoclaved topsoil, in trays of 32, and moved to a Biochambers AC-40 growth chamber where they were left to grow under a 16h at 24°C/12h at 18°C day/night light and temperature cycle, with an ambient humidity of approximately 30%. Each tray was watered with 1 L of water twice per week, and 15 mL of a solution of 5% by weight liquid fertilizer (15-5-15) was added once every two weeks. Seedlings were left to grow for 55-65 days until they had formed small rosettes, and were undisturbed until they initiated reproduction (i.e. “bolted”), which we assessed as the formation of an inflorescence taller than 400mm. Once plants had begun bolting, plants were randomly allocated for translocated to one of two sites: (1) a field site at Carleton University (Ottawa, Canada 45°23’N, 75°41’W); or (2) another AC-40 growth chamber, designed to emulate the prevailing photoperiod, light intensity, and day/night temperature fluctuation at the field site (see Hughes and Simons 2014c). Field plants were planted in the ground and lab plants were transferred to the new chamber in their rosette planting trays, and both groups of plants were left to grow until their semelparous reproductive episode was completed and all individuals had senesced. Two groups of plants were produced in each replicate season: one with an approximate bolting and translocation date of June 15, and another with an approximate bolting and translocation date of July 15. This experiment was replicated in 2008, 2009, and 2010. Demographic data for the plants included in the study are shown in Tables 6.1-6.2 in the supplementary material.

We genotyped P_1 and F_1 individuals at 22 microsatellite loci found to be polymorphic in the source population (Hughes et al. 2014), in order to: (1) evaluate the distribution of alleles at microsatellite loci in (F_1) daughters for evidence of outcrossing

in the field-raised (P_1) generation; and (2) assess whether P_1 mothers were genetically identical to F_1 daughters, and thus were members of identifiable genetic lineages. To ensure replicability of our comparison of F_1 and P_1 genotypes, five daughters from each of the P_1 individuals were screened at all 22 microsatellite loci.

Amplification of all microsatellite alleles was performed using a Phire II Direct PCR Kit (Thermo Fisher Scientific), according to the method described in Hughes et al. (2014). We used a direct PCR protocol in which DNA extraction and PCR are combined into a single step. A microsample of unprocessed plant tissue was used as the genomic DNA template for amplification, acquired by using a 0.35mm Harris Unicore Micro Punch (Thermo Fisher Scientific) to obtain tissue from a dried leaf or fruit. We performed PCR according to the kit's recommended protocol for 20 μ L reaction volumes: each tube contained a fresh punch of plant tissue, 0.5 μ M of each primer, 1.5 mM $MgCl_2$, 1 X Phire Plant PCR Buffer, and 1 μ L of Phire Hot Start II DNA Polymerase per reaction. The PCR protocol, which was performed in a T-3000 thermocycler (Biometra, Goettingen, Germany) included: (1) an initial denaturation step of 5 minutes at 98°C; (2) 30 cycles of PCR with a denaturation step of 5 seconds at 98°C, an annealing step of 5 seconds at 50-58°C (T_m values and other details concerning SSR loci is in Hughes et al. 2014), and an extension step of 20 seconds at 72°C; and (3) a final extension step of 10 minutes at 72°C, after which the sample was held at 4°C.

Immediately following amplification, tubes were removed from the thermocycler and PCR products were prepared for high resolution melt (HRM) analysis. 2.0 μ L of a 1/4000 dilution of SYBR Green I (Life Technologies, Carlsbad, CA, USA) was added to each tube; SYBR Green was not included in the original PCR mixture because it may

affect the efficiency of the PCR reaction. Tubes were then placed in a Rotor-Gene 6000 thermocycler (QIAGEN Inc., Valencia, CA, USA) for HRM analysis as per Arthofer et al. (2011). The HRM protocol included: (1) an initial denaturation step of 5 minutes at 95°C; (2) a cooling period of 5 minutes at 72°C; and (3) a melting period, where the temperature ranged from 75°C to 95°C, rising by 0.1°C every 5 seconds. The HRM curve analysis was performed using Rotor-Gene ScreenClust HRM Software (QIAGEN Inc.), which provided peak melting temperatures and fluorescence difference plots for all samples, which were used to provide data on which alleles were present at the test locus for the given sample. We confirmed differences in allele size using agarose gel electrophoresis to separate amplicons; gels were run at 60V for 75 minutes according to the method of Wang et al. (2009).

We assessed genetic diversity by calculating estimates of F_{IS} (inbreeding coefficient), H_O / H_E using version 4.2 of the GENEPOP software package (Raymond and Rousset 1995; Rousset 2008) to analyze allele frequencies. F_{IS} estimates were computed according to the estimation procedure found in Weir and Cockerham (1984). Because we found no heterozygotes, it was not possible to use common methods for estimating outcrossing rate (e.g. David 2000; David et al. 2007; Koelling et al. 2012). With an observed outcrossing rate of zero, we instead established a conservative theoretical maximum outcrossing rate consistent with our data, given our sample size. Given that we found n SSR loci to be homozygous, we calculated what the outcrossing rate would have been, had the next locus sampled—i.e. the $(n + 1)^{th}$ —had been found to be heterozygous. So from this sample of plants ($n = 2,311$), we estimated maximum outcrossing rates as though we had obtained 2,310 negative results (i.e. 2,310 homozygous loci), and one

positive result (i.e. the hypothetical heterozygote at locus $n + 1$). This resulted in a theoretical observed heterozygosity rate of 4.33×10^{-4} , which we compared to various theoretical outcrossing rates ranging from 0.1 to 0.0001 using a chi-square test.

We examined patterns of allelic variation to determine whether *L. inflata* individuals are members of one of a number of non-outcrossing, genetically identical lineages, as well as how many genetic lineages were represented by our sample of 21 P_1 individuals from the Petawawa Research Forest. Lineages were identified by sorting individuals into groups based on the detected alleles present at each of the 22 loci.

In order to assess whether phenotypic variation exists among microsatellite lineages we examined the association between genetic lineage and four reproductive traits: (1) size at formation of the first flower; (2) size at formation of the 50th percentile flower; (3) size at formation of the last flower; and (4) flower colour. Plants were monitored every 2-4 days from bolting until natural senescence to track size (stalk height) at flower formation. Upon the emergence of each flower, plant stalk height (± 0.1 mm) was recorded; only the stalk heights at the formation of the first flower (i.e. Flower 1), the last flower (i.e. Flower x), and the 50th percentile flower (i.e. Flower x/2) are reported here. The total number of flowers produced per plant was determined after the plant had senesced. Flower colour was determined by visual inspection, and was classified as pink, purple, or white. The assignment of colour was performed blind; i.e. without regard to plant label.

To ask whether genetic lineage was a significant predictor of the three phenological traits, we used a hierarchical analysis that included models both with and without genetic lineage (as a random effect), and used likelihood ratio tests to assess the

effect the inclusion of lineage. The first model in each pair was a generalized linear model (GLM) that included only fixed effects, including year (2008, 2009, or 2010), environment (lab or field), and year $\times$ environment as fixed effects, as well as prebolting rosette size (in mm) as a covariate. The second model included these predictors, but added lineage (A-H), lineage $\times$ year, lineage $\times$ environment, and lineage $\times$ year $\times$ environment as random effects. We used residual maximum likelihood (REML) based estimation of variance components for each of the random effects, as it results in a more accurate estimator of variance and covariance than maximum likelihood estimation (Venables and Dichmont 2004; Bolker et al. 2009). The GLM/GLMM used a Poisson distribution and a logarithmic canonical link function. A likelihood ratio test was used to determine whether the GLMM explained a significantly greater proportion of variation in the value of the reproductive trait than did the GLM (i.e. whether the inclusion of genetic lineage and its associated interaction effects resulted in a model with greater explanatory power). Post hoc tests were performed on genetic lineage and related interaction effects when the inclusion of genetic lineage was deemed significant.

We used chi-square goodness-of-fit tests to determine whether observed proportions of flower colour are significantly different from the proportions of flower colour that would be expected by chance, given the proportion in the population as a whole (i.e. white=0.424, pink=0.274, purple=0.302). One goodness-of-fit test was performed for each genetic lineage.

6.4 Results

6.4.1 Selfing Rate and Genetic Diversity

An analysis of microsatellite marker data of 2,310 loci—105 plants at 22 marker loci—revealed significant polymorphism, but no heterozygous loci, nor any mother-daughter (i.e. P_1 - F_1) pairs with different alleles at any SSR locus. Population genetics parameters calculated by GENEPOP v4.2 (Raymond and Rousset 1995; Rousset 2008) are shown in Table 6.1. Notably, observed heterozygosity was much lower than expected heterozygosity given observed allele frequencies in a panmictic population. Although our analysis found no evidence in favour of outcrossing in the Petawawa population of *L. inflata*, we calculated the consistency of our data with several theoretical selfing rates; these results are shown in Table 6.2.

A small sample ($n = 16$) of F_2 individuals—offspring of the F_1 generation reared in 2008—including plants descended from all P_1 individuals, was also genotyped (data not shown). Allelic genotypes of F_2 individuals were identical to those of their respective F_1 parents and P_1 grandparents. All F_2 plants genotyped were obtained from F_1 plants raised in the field sample, since—given that there is no putative pollination mechanism for *L. inflata*—it was desirable to avoid the confounding fact that F_1 lab plants were raised in an environment without access to insect pollinators.

6.4.2 Covariation between Lineage and Phenotype

After genotyping P_1 and F_1 plants, we identified eight distinct *L. inflata* genetic lineages, each of which had a unique pattern of alleles at our 22 SSR loci (Table 6.8).

Using a hierarchical modeling approach to assess the significance of genetic lineage as a predictor of three reproductive traits, we found that GLMM predictive models explained significantly more variation for size at first flower and size at 50th percentile flower. However, the GLMM model did not explain significantly more variance for size at last flower than the GLM (Figure 6.1; Table 6.3). The individual F-tests for all random effects in all GLMMs—including those that did not show better fit than the GLM alternative—are shown in Table 6.4.

Genetic lineage, genetic lineage $\times$ environment, and genetic lineage $\times$ year were not significant predictors of any phenological traits, but the interaction between genetic lineage, environment, and year was a highly significant predictor of variation in size at first flower ($F_{12,766} = 2.13$, $p < .01$), size at 50th percentile flower ($F_{12,766} = 2.04$, $p = .02$), and size at last flower ($F_{12,766} = 1.97$, $p = .02$).

Chi-square goodness-of-fit tests revealed that lineages were strongly associated with flower colours (Table 6.5), and that all lineages were invariant for colour.

6.5 Discussion

6.5.1 Selfing Rate

Complete selfing is extremely rare in nature. Despite the high degree of polymorphism present in the Petawawa population of *L. inflata*, we found no heterozygotes—and hence no evidence of outcrossing—at any loci in any plants we genotyped. The F_{IS} for this sample was 1.00, indicating purely inbred lineages. If we assumed a hypothetical heterozygote at the next locus genotyped, the maximum outcrossing rate that would be consistent with our data at $p = 0.05$ would be

approximately 0.0025% (Table 6.2), which is much lower than outcrossing rates typically found in mixed mating systems (Wright 1921; Kalisz et al. 2013). An outcrossing rate this low supports the hypothesis that *L. inflata* is in the extreme high selfing end of the mating system continuum, and is likely obligately autogamous (Lande and Schemske 1985; Schemske and Lande 1985). Furthermore—again despite the ubiquity of polymorphism—we found that all P₁ and F₁ plants had identical allelic genotypes at all microsatellite loci, and this finding supports the hypothesis that, via autogamy, *L. inflata* forms genetically distinct lineages.

The reason why *L. inflata* has an obligately self-fertilizing strategy—despite the fact that many highly selfing plant species retain the ability to outcross—is unknown. We suggest that the importance of reproductive assurance cannot be considered independently of life history; thus, in a semelparous species like *L. inflata*, extreme or obligate selfing may be favoured because reproductive assurance becomes relatively more important when the possibility of reproductive failure due to low pollen availability, or mistimed coordination represents a substantial risk (Lloyd and Schoen 1992; Agren and Schemske 1993).

6.5.2 Genetic Diversity in *L. inflata*

The degree of allelic polymorphism present at the 22 microsatellite loci—with a mean of 2.50 alleles per microsatellite locus—is also remarkable given that *L. inflata* is obligately selfing. Because new haplotypes cannot be generated through recombination in populations of highly inbred selfers (Narain 1966), the erosion of allelic diversity is expected over time (Nordborg et al. 2014). Many species with mixed mating systems

show lower allelic diversity than *L. inflata*: an analysis of microsatellite loci in the angiosperm *Leavenworthia uniflora* (Brassicaceae), with a selfing rate of approximately 90%, showed a mean allelic diversity of 1.42 alleles per locus (Busch and Werner 2012), and a similar analysis in the freshwater snail *Lymnaea trunculata*, with a selfing rate of ~80%, showed a mean allelic diversity of 2.36 alleles per locus (Trouvé et al. 2003). The level of allelic diversity found in the Petawawa population of *L. inflata* was significantly higher than might be expected given an entirely selfing mating system, and is greater than might be suggested by even the most liberal estimates of ambient mutation rates at microsatellite loci (i.e. at Chernobyl - see Kovalchuk et al. 2000).

Selection on genetic lineages may be the mechanism that is responsible for preserving genetic diversity. Our analyses revealed eight distinct genetic lineages in our sample of *L. inflata*, all of which showed allelic differences at three loci or more. We also found significant variation associated with genetic lineage in four traits (Figure 6.1): size at first flower, size at 50th percentile flower, size at last flower and flower colour. Thus, we conclude that the microsatellite loci we use in this study are not mere genetic labels, but are associated with real phenotypic differences such as flower colour.

Importantly, rather than genetic lineage alone, it was the interaction between genetic lineage, environment, and year that was the most important predictor of the three phenological traits. This makes sense given that significant variation in key environmental parameters—i.e. temperature, rainfall, and wind—existed between experimental environments as well as between years (see Figures 6.2-6.4). This, coupled with the fact that *L. inflata*'s reproductive phenotype is highly sensitive to environmental variation (see Simons and Johnston 2003; Hughes and Simons 2014b,c), makes it

unsurprising that interactions including genetic lineage, rather than genetic lineage alone, predict variation in reproductive traits.

We speculate that genetic variation among lineages is maintained by genotype-by-environment interaction through time: fluctuating selection favours different phenotypes (associated with the genetic lineages) at different times. This hypothesis has also been proposed as the cause of relatively high allelic diversity in other highly selfing species, although in these cases the association between microsatellite lineage and phenotype has not been established (e.g. Sponer and Roy 2002; Winton et al. 2006). Although we acknowledge that a rigorous test of this hypothesis—which would involve measuring lineage fitness in the field over time—is beyond the scope of this study, we suggest that our data are more consistent with temporal fluctuating selection than with alternative explanations. Further research on genetic lineage fitness *in situ* should be performed in order to better substantiate this hypothesis.

There are limitations associated with this work. First, because we did not initially know if *L. inflata* was entirely autogamous, whether genetic variation existed among lineages, or how many lineages were present among source plants, the sample of genetic lineages included here is small, and the proportion of field plants that each lineage accounts for is unknown. Future studies aiming to understand the mechanisms maintaining genetic variation in field populations of *L. inflata* should use a broader sample of genetic lineages. Second, although we did not find any heterozygotes at any of the SSR loci we tested, we cannot conclude that absence of proof is proof of absence. Therefore we cannot rule out that outcrossing is possible, if extremely rare, in *L. inflata*.

6.5.3 Conclusion

In conclusion, we have shown that the population of *Lobelia inflata* studied is obligately or near-obligately self-fertilizing. Although there is no genetic variation among offspring, substantial genetic variation occurs among lineages. Furthermore, variation among lineages accounted for significant differences in reproductive traits. We speculate that genetic variation among lineages may be maintained by fluctuating selection; specifically, by temporal genotype-by-environment interaction in which different lineages are favored at different times.

6.6 Tables

Table 6.1 - Genetic diversity summary data

Genetic diversity summary data by locus for L. inflata sampled from the Petawawa Research Forest. Data was collected from 105 plants for each locus (5 replicate plants for each of the 21 maternal lines collected). Inbreeding coefficient (F_{IS}) was computed according to the method of Weir and Cockerham (1984); note that no heterozygotes were found at any locus.

<i>Locus</i>	<i>Alleles</i>	<i>Heterozygosity</i>		<i>Homozygosity</i>		F_{IS}
		<i>Expected (H_e)</i>	<i>Observed (H_o)</i>	<i>Expected</i>	<i>Observed</i>	
<i>Linflata2</i>	4	0.62	0.00	0.38	1.00	1.00
<i>Linflata3</i>	2	0.42	0.00	0.58	1.00	1.00
<i>Linflata4</i>	2	0.42	0.00	0.58	1.00	1.00
<i>Linflata5</i>	2	0.32	0.00	0.68	1.00	1.00
<i>Linflata7</i>	4	0.67	0.00	0.33	1.00	1.00
<i>Linflata8</i>	3	0.57	0.00	0.43	1.00	1.00
<i>Linflata9</i>	2	0.18	0.00	0.82	1.00	1.00
<i>Linflata10</i>	2	0.09	0.00	0.91	1.00	1.00
<i>Linflata12</i>	4	0.62	0.00	0.38	1.00	1.00
<i>Linflata13</i>	3	0.40	0.00	0.60	1.00	1.00
<i>Linflata14</i>	2	0.46	0.00	0.54	1.00	1.00
<i>Linflata16</i>	2	0.50	0.00	0.50	1.00	1.00
<i>Linflata17</i>	2	0.18	0.00	0.82	1.00	1.00
<i>Linflata18</i>	2	0.18	0.00	0.82	1.00	1.00
<i>Linflata19</i>	2	0.37	0.00	0.63	1.00	1.00
<i>Linflata20</i>	2	0.37	0.00	0.63	1.00	1.00
<i>Linflata21</i>	3	0.39	0.00	0.61	1.00	1.00
<i>Linflata22</i>	2	0.25	0.00	0.75	1.00	1.00
<i>Linflata23</i>	2	0.51	0.00	0.49	1.00	1.00
<i>Linflata26</i>	3	0.59	0.00	0.41	1.00	1.00
<i>Linflata27</i>	3	0.59	0.00	0.41	1.00	1.00
<i>Linflata28</i>	2	0.51	0.00	0.49	1.00	1.00

Table 6.2 - Chi-square tests for theoretical outcrossing rates

Chi-square tests for outcrossing rates based on frequencies of heterozygotes and homozygotes, including a single hypothetical heterozygous locus.

Outcrossing Rate		n	Heterozygotes		Homozygotes		χ^2	P
Theoretical	Observed		Expected	Detected*	Expected	Detected		
0.1	4.33×10^{-4}	2311	231.10	1	2080.00	2310	254.44	2.80×10^{-57}
0.01	4.33×10^{-4}	2311	23.11	1	2287.90	2310	21.36	3.81×10^{-6}
0.0025	4.33×10^{-4}	2311	5.78	1	2305.22	2310	3.96	0.05
0.001	4.33×10^{-4}	2311	2.31	1	2308.69	2310	0.74	0.39
0.0001	4.33×10^{-4}	2311	0.23	1	2310.77	2310	2.56	0.11

**Note: the single detected heterozygote is hypothetical, and is included for the purposes of estimating the range of outcrossing rates compatible with the other 2,310 homozygous loci. Chi-square tests are therefore based on 2,311 loci.*

Table 6.3 - Likelihood ratio test results (GLM vs GLMM)

Likelihood Ratio Test Results Comparing GLM and GLMM Predictive Validity for Six Reproductive Traits in Lobelia inflata

Dependent Variable	Model	-2RLL	AIC	Parameters	Chi Square	df	p	GLMM>GLM
Size at First Flower	GLM	8625.1	8627.1	13	20.65	8	8.01 E-03	Y
	GLM M	8604.4	8622.4	21				
Size at 50 th Percentile Flower	GLM	8768.4	8770.4	13	46.83	8	1.65 E-07	Y
	GLM M	8721.6	8739.6	21				
Size at Last Flower	GLM	8974.4	8976.4	13	12.46	8	0.13	N
	GLM M	8961.9	8979.9	21				

Table 6.4 - F-tests of random effects in GLMMs

F-tests evaluating significance of random effects included in GLMMs predicting three phenological reproductive traits. F-tests conducted using REML-based estimation of variance parameters.

Dependent Variable		Random Effect			
		Genetic lineage	Genetic lineage × Environment	Genetic lineage × Year	Genetic lineage × Environment × Year
Size at First Flower	F	3.00	1.03	1.01	2.13
	df	7, 3.52	7, 13.15	12, 12.01	12, 766
	p	0.17	0.46	0.49	<0.01**
Size at 50 th Percentile Flower	F	4.25	0.88	1.31	2.04
	df	7, 4.33	7, 13.21	12, 12.01	12, 766
	p	0.08	0.55	0.33	0.02*
Size at Last Flower	F	2.65	0.79	1.17	1.97
	df	7, 3.41	7, 13.25	12, 12.01	12, 766
	p	0.21	0.61	0.39	0.02*

Table 6.5 - Chi-square tests on flower colour

Chi-square goodness-of-test results for flower colour by lineage. Expected frequency of each colour was calculated based on overall population frequencies: white=0.424, pink=0.274, purple=0.302.

Lineage	n	Observed Flower Colour			Expected Frequency of Colours	Chi Square	df	p
		White	Pink	Purple				
A	219	219	0	0	73.00	297.51	2	<0.01
B	62	62	0	0	20.67	84.23	2	<0.01
C	190	0	0	190	63.33	258.11	2	<0.01
D	123	0	123	0	41.00	167.09	2	<0.01
E	55	0	0	55	18.33	74.72	2	<0.01
F	24	0	24	0	8.00	32.60	2	<0.01
G	75	0	75	0	25.00	101.89	2	<0.01
H	63	63	0	0	21.00	85.58	2	<0.01

Table 6.6 - Summary demographic data

Summary demographic data for F_1 plants used in this study by year and environment. Six variables are shown: (1) size at first flower; (2) size at 50% flower; and (3) size at final flower. Mean values shown ($\pm$ SE).

<i>Year</i>	Environment	<i>n</i>	Size at First Flower (mm)	Size at 50 th Percentile Flower (mm)	Size at Final Flower (mm)
2008	Field	102	108.67 (6.53)	149.47 (7.53)	203.17 (7.42)
	Lab	58	141.88 (5.09)	181.92 (5.72)	234.18 (6.43)
2009	Field	178	91.07 (4.79)	129.53 (4.95)	183.94 (5.49)
	Lab	136	188.26 (4.62)	231.57 (5.18)	292.12 (6.21)
2010	Field	205	117.59 (5.41)	154.34 (5.60)	211.44 (6.54)
	Lab	132	127.7 (8.50)	166.27 (3.74)	219.11 (4.22)

Table 6.7 - Lineage frequencies of F_1 plants

Lineage frequencies of F_1 plants used in this study by year and environment.

Year	Lab	Field	Genetic lineage								
			A	B	C	D	E	F	G	H	TTL
2008	102	58	41	20	21	38	24	0	0	16	160
2009	178	136	79	24	76	47	13	12	36	27	314
2010	205	132	99	18	93	38	18	12	39	20	337
TTL	485	326	219	62	190	123	55	24	75	63	811

Table 6.8 - Allele genotypes for *L. inflata*

Observed alleles at 22 microsatellite loci and flower colour phenotype for 21 P₁ individuals. Observed alleles and flower colour phenotype for F₁ and F₂ individuals followed the same pattern.

SSR Locus	P ₁ Individual																				
	138	140	143	144	148	149	150	151	152	153	156	157	158	159	160	165	166	171	186	189	190
Linflata2	0101	0101	0101	0101	0101	0101	0404	0202	0303	0101	0404	0404	0303	0101	0101	0202	0101	0101	0101	0303	0303
Linflata3	0101	0101	0101	0101	0101	0101	0101	0202	0202	0101	0101	0101	0202	0101	0101	0202	0101	0101	0101	0202	0202
Linflata4	0101	0101	0101	0101	0101	0101	0101	0202	0202	0101	0101	0101	0202	0101	0101	0202	0101	0101	0101	0202	0202
Linflata5	0101	0101	0202	0101	0202	0101	0101	0101	0101	0101	0101	0101	0101	0101	0101	0101	0202	0101	0202	0101	0101
Linflata7	0404	0404	0303	0404	0303	0101	0101	0202	0101	0101	0101	0101	0101	0101	0404	0101	0303	0404	0303	0101	0101
Linflata8	0101	0101	0303	0101	0303	0101	0101	0101	0202	0101	0101	0101	0202	0404	0101	0303	0303	0101	0303	0303	0303
Linflata9	0101	0101	0101	0101	0101	0101	0101	0101	0202	0101	0101	0101	0202	0101	0101	0101	0101	0101	0101	0101	0101
Linflata10	0101	0101	0101	0101	0101	0101	0101	0202	0101	0101	0101	0101	0101	0101	0101	0101	0101	0101	0101	0101	0101
Linflata12	0404	0404	0101	0404	0101	0101	0101	0202	0303	0101	0101	0101	0303	0101	0404	0404	0101	0404	0101	0101	0101
Linflata13	0101	0101	0101	0101	0101	0101	0101	0101	0202	0101	0101	0101	0202	0404	0101	0202	0101	0101	0101	0303	0303
Linflata14	0101	0101	0202	0101	0202	0101	0101	0202	0202	0101	0101	0101	0202	0101	0101	0101	0202	0101	0202	0101	0101
Linflata16	0101	0101	0202	0101	0202	0202	0202	0202	0101	0202	0202	0202	0101	0101	0101	0101	0202	0101	0202	0202	0202
Linflata17	0101	0101	0101	0101	0101	0101	0101	0101	0202	0101	0101	0101	0202	0101	0101	0101	0101	0101	0101	0101	0101
Linflata18	0101	0101	0101	0101	0101	0101	0101	0202	0101	0101	0101	0101	0101	0101	0101	0202	0101	0101	0101	0101	0101
Linflata19	0101	0101	0101	0101	0101	0101	0101	0101	0202	0101	0101	0101	0202	0101	0101	0202	0101	0101	0101	0202	0202
Linflata20	0101	0101	0101	0101	0101	0101	0101	0101	0202	0101	0101	0101	0202	0101	0101	0202	0101	0101	0101	0202	0202
Linflata21	0101	0101	0101	0101	0101	0101	0101	0101	0202	0101	0101	0101	0202	0101	0101	0303	0101	0101	0101	0202	0202
Linflata22	0101	0101	0101	0101	0101	0101	0101	0101	0202	0101	0101	0101	0202	0101	0101	0202	0101	0101	0101	0101	0101
Linflata23	0202	0202	0101	0202	0101	0202	0101	0101	0202	0202	0101	0101	0202	0202	0202	0202	0101	0202	0101	0101	0101
Linflata26	0202	0202	0101	0202	0101	0202	0202	0202	0303	0202	0202	0202	0303	0202	0202	0303	0101	0202	0101	0101	0101
Linflata27	0303	0303	0101	0303	0101	0101	0101	0101	0202	0101	0101	0101	0202	0303	0303	0202	0101	0303	0101	0101	0101
Linflata28	0202	0202	0101	0202	0101	0202	0101	0101	0202	0202	0101	0101	0202	0202	0202	0202	0101	0202	0101	0101	0101
Flower Colour	pu	pu	w	pu	w	pi	pi	w	w	pi	pi	pi	w	pu	pu	pu	w	pu	w	pi	pi
Genetic lineage	C	C	A	C	A	F	G	H	B	F	G	G	B	C	C	E	A	C	A	D	D

Notes: (1) “0101” indicates two copies of allele 01; (2) Flower Colour: pu = purple, pi = pink, w = white

6.7 Figures

Figure 6.1 - Reproductive traits by lineage

Box plots of reproductive traits by genetic lineage, environment, and year. Panels A-C show: (A) size at first flower (mm); (B) size at 50th percentile flower (mm); and (C) size at last flower (mm) for lab-grown plants. Panels D-F show: (D) size at first flower (mm); (E) size at 50th percentile flower (mm); and (F) size at last flower (mm) for field-grown plants. Box plots show quartiles and median value. Points represent outlier values.

Figure 6.2 - Mean temperature at Petawawa (2008-10)

Mean monthly temperature (°C) at Petawawa by year from 2008-10. Readings taken by an Environment Canada weather station at 45.97 N -77.25 N at 137.2m elevation.

Figure 6.3 - Minimum-maximum temperature at Petawawa (2008-10)

Minimum (dashed line) and maximum (solid line) monthly temperature (°C) at Petawawa by year from 2008-10. Readings taken by an Environment Canada weather station at 45.97 N -77.25 N at 137.2m elevation.

Figure 6.4 - Mean precipitation at Petawawa (2008-10)

Mean monthly precipitation (in mm) at Petawawa by year from 2008-10. Readings taken by an Environment Canada weather station at 45.97 N -77.25 N at 137.2m elevation.

Chapter 7: General Conclusion

I believe that this thesis offers persuasive evidence that parity is continuous, and therefore that semelparity and iteroparity are not discrete strategies, but endpoints along a continuum of possible modes of parity. Although other studies have shown limited life history plasticity with respect to parity, none have comprehensively explored continuous variation in parity within a single species. In doing so I hope to have conclusively established that conceiving of parity as continuous is not merely a useful heuristic; it is the most accurate representation of real reproductive behaviour.

I also hope to have shown the ways in which parity can vary along this continuum. In Chapter 2, I showed that *Lobelia inflata* expresses continuous variation in phenology throughout its reproductive episode, and becomes more semelparous over time. In Chapter 3, I presented data showing that semelparity is phenotypically plastic—plants that initiate reproduction late in the season are more semelparous than plants that initiate reproduction earlier. In Chapter 4, I showed that secondary reproduction is also phenotypically plastic—some plants express true multi-season iteroparity, albeit nonadaptive, unless they bolt late in the season. Finally, in Chapters 5 and 6 I showed that even though *Lobelia inflata* is obligately self-fertilizing—itsself a nature history phenomenon that is extremely rare—there exists substantial variation in reproductive phenotype that is linked to genotype.

There are important new avenues of research that can extend this work. First, new mathematical models are needed, specifically ones that can address the tradeoff between investment of reproductive effort and senescence along the continuum from ‘extreme’ to ‘iterative’ semelparous reproduction. Second, the correlation between genetic lineage and

phenology (which is related to parity) in *L. inflata* is suggestive of a genetic basis for different forms of semelparity; that is, under controlled conditions, different genetic lineages of *L. inflata* may express different modes of parity, despite phenotypic plasticity. Finally, the relative importance of different reproductive traits as part of the “semelparousness” of an individual plant’s reproductive episode should be made explicit.

I believe that the data chapters of this thesis provide a unique and rigorous empirical justification for the continuous model of parity. Although this model was proposed some time ago (see Fritz et al. 1982), remarkably few papers have been willing to test the predictions that it makes—specifically, that semelparous reproduction could adaptively vary through time, and that parity could be phenotypically plastic. In providing evidence that parity can vary within a species in a predictable way, I hope to provide resolution to this debate.

Appendices

Appendix A Availability of Supporting Data

A.1 For Chapter 3

The data set supporting the results of Chapter 3 is available in the Data Dryad repository.

Full citation:

Hughes PW, Simons AM (2014) *Reproductive Traits of Lobelia inflata*. Dryad Digital Repository. <http://datadryad.org/resource/doi:10.5061/dryad.2d218>

A.2 For Chapter 4

The data set supporting the results of Chapter 4 is available in the Data Dryad repository.

Full citation:

Hughes PW, Simons AM (2014) *Data describing secondary reproduction in Lobelia inflata*. Dryad Digital Repository. <http://datadryad.org/resource/doi:10.5061/dryad.m02bt>

Appendix B Other Publications

The following publications were also completed from 2009-2014, but are not included in this thesis.

- **Hughes P.W.** (2015) An introduction to population genetics: Theory and applications. *Plant Science Bulletin* (in press – BR)
- Imbeault E. and **Hughes P.W.** (2015) Phenomenal consciousness, affectivity, and conation: where extended cognition has never gone before. *Constructivist Foundations*. 10(3) (in press)
- **Hughes P.W.** (2015) Paradoxes in scientific inference. *Journal of Applied Statistics*. 42(2):459-460 (BR)
- **Hughes P.W.** (2014) Global Collaboration: NextGen VOICES. *Science*. 346(6205):48 (Commentary)
- **Hughes P.W.** (2014) Teaching scientific inquiry: inquiry-based training for biology graduate teaching assistants improves undergraduate learning outcomes. Toronto: *Higher Education Quality Council of Ontario*
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