The role of fluctuating selection in the maintenance of genetic variation in *Lobelia inflata*

By

Kristen Côté

A thesis submitted to the Faculty of Graduate and Postdoctoral Affairs in partial fulfillment of the requirements for the degree of

Master's of Science

in

Biology

Carleton University
Ottawa, Ontario

ABSTRACT

Understanding the mechanisms that are responsible for maintaining genetic variation continues to be the focus of much research in evolutionary ecology. It has been suggested that the abundant genetic variation found in *Lobelia inflata* is maintained by fluctuating selection coupled with temporal genotype-environment interaction. I begin by asking whether microsatellite genotypes exhibit variation in key life-history traits including timing of germination, bolting, flowering and maturation. I used a common garden experiment to show that phenotypic variation exists, that this variation occurs in life-history traits, and that this variation has a genetic basis. Next, I looked at how the microsatellite genotypes that differed in life-history traits expressed differential fitness across environments in a "space-for-time" experiment: I grew multiple lineages under varying conditions to simulate differing natural conditions. Results offer tentative support for the hypothesis that fluctuating selection is responsible for maintaining variation in this system.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank Dr. Andrew Simons for giving me the opportunity to work with him. He has been so patient and understanding while guiding me through my Master's Thesis, and I am very grateful for everything that he has taught me. I also want to thank Dr. P. William Hughes, a mentor and friend, who has helped me tremendously with various aspects of my project; for his words of encouragement through it all and his support with molecular work. Thanks to Dr. Susan Aitken for allowing me to work in her lab and for her help with troubleshooting when I thought things weren't going according to plan. I'd like to thank my lab mates (Ryan Cleback and Hebah Mejbel) for their help and advice. I want to thank my family and friends. Amy Glover for her friendship and never wavering support and encouragement. And most of all, I need to thank my parents for their unconditional love and support. I am so grateful for their willingness to take this journey with me by always lending an ear, speaking words of encouragement and seeing me through it all.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

GENERAL INTRODUCTION

Natural environments are variable over time and space, and organisms may respond to this change in many ways. Some may be well adapted to a variety of environments, while others simply cannot survive when a change occurs (Skelly et al., 2007). Populations can react to changing conditions through "strategies" that include phenotypic plasticity (Bradshaw, 1965; Schlichting, 1986; Via et al., 1995) or bet hedging (Cohen, 1966; Gillespie, 1974; Slatkin, 1974) or, if enough genetic variation exists, they may adapt directly to environments as they change (Bell & Gonzalez, 2009; Anderson et al., 2012). However, genetic variation is expected to decline in populations both through random genetic drift (Wright, 1931) and natural selection (Wright, 1932; Fisher & Ford, 1947; Fisher, 1930; Lande, 1976) that increase mean fitness of a population by eliminating genotypes that are less fit (Mousseau & Roff, 1987; Roff & Mousseau, 1987; Haasl & Payseur, 2013). But declines in genetic variation due to drift and selection do not always occur; many traits in natural and experimental populations are found with high levels of genetic variation (Mousseau & Roff, 1987; Roff & Mousseau 1987). Therefore, understanding how genetic variation is maintained in natural populations despite the effects of selection and drift remains an important unresolved problem in evolutionary ecology.

Researchers have studied the extensive genetic variation since the surprising discovery back in the late 1950's (Markert & Moller, 1959). Since the early discovery of abundant genetic variation from enzymes (Markert & Moller, 1959) and proteins (Zuckerlandl & Pauling, 1965), in the early 1980's it was also found through DNA analyses (Kimura, 1983). Because of this, research turned towards an evolutionary

quantitative genetics approach to study the underlying variation in genes. Since then, abundant genetic variation has been described in nature (e.g. Zhang & Hill, 2005). In many mating system models, the assumption is that in the absence of gene flow, genetic diversity will gradually be eliminated. Outcrossing species are generally more genetically diverse with new alleles less likely to be lost due to drift or population extinction (Hamrick & Godt, 1996). However in predominantly selfing species, where there is less gene flow, there is a greater chance for new alleles to be lost because drift is acting on an effective population size that is half that of self-incompatible species, which leads to less overall genetic diversity (Charlesworth & Charlesworth, 1995; Hamrick & Godt, 1996; Hughes & Simons, 2015). Yet, high levels of within-population allelic diversity have been found in selfing populations (e.g. in fungi (Winton et al., 2006) and shrubs (Ayre et al., 1994)) and have shown to be maintained through time (Hughes & Simons, 2015). Therefore, the main question of what mechanism(s) can account for this variation in different traits in natural populations, particularly in complete selfing species, continues to lack consensus.

Researchers have proposed that the abundant variation noted in natural populations could be maintained by mechanisms such as mutation-selection balance (Lande, 1976; Turelli, 1984; Bulmer, 1989), overdominance (Bulmer, 1973; Falconer, 1989; Gillespie, 1984), frequency-dependent selection (Ayala & Campbell, 1974), antagonistic pleiotropy (Rose, 1982) and variable selection in space and/or time (Fisher & Ford, 1947). New mutation maintains genetic variation at equilibrium frequencies determined by a balance between mutation rate and loss due to drift and selection (Zhang & Hill, 2005). Overdominance, also known as heterozygote advantage,

maintains allelic diversity because heterozygotes are fitter than either homozygote causing a variety of alleles to be maintained in a population (Old et al., 1993). Frequency-dependent selection, where the fitness of a phenotype is dependent on its frequency relative to the other phenotypes in a population, is also a possible mechanism for the maintenance of genetic variation (Ayala & Campbell, 1974). Antagonistic pleiotropy, where one gene (or set of genes) controls more than one trait and the traits have opposing fitness effects, can also result in the maintenance of genetic variation (Keightley & Hill, 1990; Santiago & Sanjuan, 2003; Russell et al., 2010). Finally, variable selection occurs when natural selection fluctuates in time, whereby the relative fitness of different phenotypes changes in time or space resulting in the maintenance of a variety of genotypes in a population (Ellner & Hairston, 1994). Although several mechanisms have been proposed to maintain variation, there remains a lack of empirical evidence from natural populations, and thus a lack of agreement on which mechanism(s) have the greatest effect.

Determining which mechanism(s) can account for the "excess" variation observed in any species can be a complex task. For example, a natural population of *Mimulus guttatus,* both antagonistic pleiotropy (where alleles increase flower size and fecundity at a cost of reduced viability) and variable selection on a yearly and spatial scale maintained variation (Mojica et al., 2012). However, in *Collinsia verna*, variable selection was the mechanism responsible for maintaining the variation observed in germination time (Kalisz, 1986). Many species exhibit substantial genetic variation, and determining which of the proposed mechanisms can account for the observed variation in natural populations remains the central focus of much research in evolutionary ecology.

Lobelia inflata L. (Campanulaceae), or Indian tobacco, provides a unique opportunity to address questions on the maintenance of genetic variation because of its particular features. This species displays abundant genetic variation despite being completely self-fertilizing: in a sample of 21 field-collected individuals, 8 genetically distinct lineages were found (Hughes & Simons, 2015). In addition, it was shown that this plant exhibits 100% homozygosity and that all genetic variation occurs among lineages (Hughes & Simons, 2015). Because lineages cannot recombine, alternative alleles are retained within lineages and should therefore be eliminated due to selection and drift (Hughes & Simons, 2015). However, microsatellite analysis shows a mean of 2.50 alleles per locus (Hughes & Simons, 2015), which is an unexpectedly high degree of polymorphism for a selfing species. *Lobelia inflata's* characteristics are thus conducive to the study of the maintenance of genetic variation.

Lobelia inflata's distinctive qualities allow for the elimination of several mechanisms that have been shown to preserve variation in other species. For example, because heterozygosity has not been observed in this species, overdominance is not responsible (Simons & Johnston, 2006; Hughes et al., 2014; Hughes & Simons, 2015). It is possible that frequency-dependent selection maintains genetic variation but is unlikely because it is generally the result of competition between species or genotypes with distinct differences in phenotype (Ronsheim, 1996). For example, because *L. inflata* is completely self-fertilizing, flowering phenology is not subject to selection by pollinators (Robertson, 1895). Mutation-selection balance cannot account for the mean of 2.50 alleles per microsatellite locus observed in this inbred species (Keightley & Halligan, 2009; Hughes & Simons, 2015). Antagonistic pleiotropy is not

responsible for maintaining polymorphisms in inbred species with offspring demonstrating complete homozygosity (Hedrick, 1998). The observation of a high degree of polymorphism—with few viable explanations for its maintenance—provides a unique opportunity to test the hypothesis that fluctuating selection maintains genetic variation.

Because *L. inflata* colonizes disturbed habitats characterized by unpredictable change, suggests that fluctuating selection coupled with temporal genotype-environment interaction is maintaining genetic variation. This mechanism is independent of mating system and heterozygosity and allows for subsets of genotypic lineages to be selectively favoured in some years, while others favoured in other years. Sometimes referred to as "zig-zag selection" (Stanley & Yang, 1987; Bell, 2010), this mechanism allows for a variety of genotypes to be maintained in the population (Roff, 1997) and causes the rank order of fitness to change through time. This mechanism has garnered great interest over the last few decades (Haldane & Jayakar, 1963; Mueller et al., 1985; Bull, 1987; Hairston & Walton, 1986; Lynch, 1987; Seger & Brockman, 1987; Hairston & Dillon, 1990; Hedrick, 1998; Mousseau et al., 2000) with multiples studies being conducted in the last 10 years (O'Hara, 2005; Johnson, 2006; Huerta-Sanchez et al., 2007; Bell, 2010; Gossmann et al., 2014). Studies on freshwater copepods highlight the role of this mechanism, and fluctuating selection was found to maintain variation in the timing of diapause (Hairston & Walton, 1986; Hairston & Dillon, 1990). Variable selection was found to maintain wing polymorphism in the scarlet tiger moth (Fisher & Ford, 1947) and, in a population of *Daphnia*, fluctuating selection played a role in generating differences in gene frequency of the magnitude often observed between adjacent populations (Lynch, 1987). In a recent review article, it was shown that shifts in the

magnitude and/or direction of selection occur in many other species (Bell, 2010). Some examples include flowering time in *Erythroxylum, Carlina,* and *Digitalis* (Bell, 2010). Here, I propose that this mechanism is acting to maintain genetic variation in *Lobelia inflata*.

The maintenance of genetic variance in *L. inflata* through fluctuating selection coupled with temporal genotype-environment interaction requires that phenotypic variation exists, that this variation occurs in life-history traits (traits that influence a life-table and have important implications for growth and reproduction), and that this variation has a genetic basis. Therefore, the following chapters aim to address these components. Chapter 1 reports on the phenotypic variation in life-history traits among replicated field-collected individuals and asks whether this phenotypic variation corresponds to microsatellite variation. Here I look at the phenotypic variation that was observed in an experimental garden study and how microsatellite genotypes exhibit this variation in key life-history traits including timing of germination, bolting, flowering and maturation. Chapter 2 explores how microsatellite genotypes exhibit fitness variance through time that is associated with life-history phenotypes, whereby selection results in a change in rank order of fitness across years. In this chapter we address how the genotypes that differed in key life-history traits from chapter 1, also express fitness differences and have differential success in different environments. To address this, I conducted a "space-for-time" study in which I grew multiple replicate lineages under varying environmental conditions to assess relative success under simulated potential environmental variation among seasons, or "year types". In order to supplement the "space-for-time" study, two years of fieldwork was conducted in order to include natural seasonal variation. However, a

decade or more of fieldwork would potentially be necessary to provide a complete answer on how important fluctuating selection is in maintaining genetic variation. The goal is to provide concrete evidence for or against the plausibility of variable selection coupled with genotype-by-environment interaction maintaining variation in *Lobelia inflata*. If many genotypes exist within populations, and these genotypes are associated with phenotypes that are shown to affect fitness differently across environments, this could help explain the maintenance of genetic variation—a longstanding problem in evolutionary ecology.

Chapter 1: Phenotypic variation in life-history traits associated with microsatellite genotypes in two populations of *Lobelia inflata*

INTRODUCTION

Understanding the mechanisms that are responsible for maintaining genetic variation continues to be the focus of much theoretical and empirical research in evolutionary ecology (Svardal et al., 2014; Gulisija & Kim 2015; Culumber & Tobler 2016). Genetic variation in fitness traits is expected to decline in populations both through drift and natural selection, yet high levels of genetic variation are often found (Fisher & Ford, 1947 Mousseau & Roff, 1987; Roff & Mousseau, 1987; Roff, 1992; Hendrick, 1998; Kliman et al., 2008; Haasl & Payseur, 2013). Findings of higher than expected genetic variation have led to the development of numerous explanations for its maintenance.

Population genetic models have shown that natural selection can preserve genetic variation through various mechanisms including mutation-selection balance (Lande, 1976; Bulmer, 1989; Zhang & Hill, 2005; Lynch, 2010), overdominance (Gillespie, 1984; Old et al., 1993; Falconer & MacKay, 1996), frequency-dependent selection (Ayala & Campbell, 1974), antagonistic pleiotropy (Rose, 1982; Santiago & Sanjuan, 2003) and variable selection in space and/or time (Fisher & Ford, 1947; Lynch, 1987; Hairston & Dillon, 1990; Gillespie, 1991; Ellner & Hairston, 1994; Hedrick, 1998; O'Hara, 2005; Huerta-Sanchez et al., 2007; Bell, 2010; Gossmann et al., 2014).

The mechanism(s) are context dependent, and determining which mechanism can account for "excess" variation observed in any specific case is a challenging task. However, empirical examples of the maintenance of genetic variance through the various mechanisms exist, and are presented earlier (see General Introduction), including one in which genetic variation is maintained by temporally fluctuating selection on the timing of seed germination in the annual, *Collinsia verna* (Kalisz, 1986).

Because of particular features, *Lobelia inflata*, a monocarpic perennial native to eastern North America, provides a unique opportunity to address longstanding questions on the maintenance of genetic variation. This plant has shown substantial genetic variation, which is unexpected. Hughes and Simons (2015) recently showed that in a sample of 21 field-collected individuals there were 8 genetically distinct lineages, all of which demonstrated allelic differences at three or more loci (Hughes & Simons, 2015). But because populations of *L. inflata* studied have been assumed to be self-fertilizing, having a closed anther tube that surrounds the stigma, this degree of genetic variation at the 22 microsatellite loci tested is unanticipated. Evidence of complete self-fertilization was recently provided by microsatellite analysis showing 100% homozygosity (Hughes et al, 2014). Since recombination cannot create new haplotypes with even a short history of complete selfing (Nordbord et al., 2014; Hughes & Simons, 2015), genetic variation occurs among lineages and, whether it is associated with fitness variance or is neutral, should be rapidly eliminated either through lineage selection or genetic drift. Therefore, *L. inflata*'s characteristics make it an appropriate model system for questions on variation-maintaining mechanisms.

Lobelia inflata's unique suite of features both helps narrow down the scope of possible mechanisms acting to maintain variation, and makes a test feasible. Overdominance is not responsible since heterozygosity has not been observed (Hughes & Simons, 2015). It is possible that frequency-dependent selection maintains genetic variation, however is unlikely because it is generally the result of, for example, predation on, or competition between discrete forms (Ronsheim, 1996), and no obvious discontinuous forms have been observed. For example, because *L. inflata* is completely self-fertilizing, flowering phenology is not subject to selection by pollinators (Robertson, 1895) therefore unlikely to maintain the variation in this species. Mutation-selection balance cannot account for a mean of 2.50 alleles per microsatellite locus observed in this completely self-fertilizing species (Hughes & Simons, 2015). Antagonistic pleiotropy occurs when one gene (or set of genes) influences more than one trait, and the traits have opposing fitness effects (Santiago & Sanjuan, 2003). However, it is unlikely that this mechanism can maintain polymorphism in inbred species characterized by high levels of homozygosity (Curtsinger, 1994; Hedrick, 1998). One mechanism that has the potential to account for the abundant genetic variation observed in this species is variable selection. *L. inflata* colonizes temporally variable, disturbed habitats, and temporal genotype-environment interaction under fluctuating selection may maintain genetic variation (Hughes & Simons, 2015). This mechanism is independent of mating system and heterozygosity and, in the case of *L. inflata*, predicts that different subsets of genotypic lineages are selectively favoured across years; i.e. the rank order of lineage fitness differs through time. Furthermore, a test of this mechanism is simplified

in this self-fertilizing species because microsatellites may be used as consistent genotypic "labels."

In recent years, the concept of variable selection has become increasingly prevalent in research on the maintenance of genetic variation (Hairston & Dillon, 1990; Hedrick, 1998; Mousseau et al., 2000; O'Hara, 2005; Huerta-Sanchez et al., 2007; Bell, 2010; Gossmann et al., 2014). Variation in selection may favor different genotypes at different times, resulting in the maintenance of a variety of genotypes in a population (Roff, 1997). For example, the strength and direction of selection on the timing of germination in *Collinsia verna* varied both within and between years for the overall population (Kalisz, 1986). Fluctuating selection has been shown to maintain genetic variation in timing of diapause in freshwater copepods (Hairston & Walton, 1986; Hairston & Dillon, 1990), wing polymorphism in the scarlet tiger moth (Fisher & Ford, 1947) and in the flowering time of *Carlina* and *Digitalis* (Rees et al., 2004; Sletvold & Grindeland, 2007).

A direct test of fluctuating selection would require documenting the fate of several lineages over multiple seasons and is beyond the scope of this study. However, the plausibility of this mechanism depends strongly on whether observed genotypic variation is related to variation in fitness traits. Although microsatellite loci themselves are not expected to be associated with fitness variance, in this species microsatellite polymorphisms distinguish consistently among genetic lineages that may differ in phenotype. Thus, microsatellite variation that corresponds to phenotypic variation in life-history traits in *L. inflata* would be the first step towards supporting the hypothesis that temporal fluctuating selection maintains lineage variation. Here we assess variation of

life-history traits (fitness-related traits) in replicated offspring of *L. inflata* to determine whether phenotypic variation exists among microsatellite lineages. Life-history traits were measured in a common garden experiment so that any consistent phenotypic differences among replicated lineages can be attributed to mostly genotypic differences. Microsatellite genotyping using PCR followed by High-resolution melt (HRM) analyses with SYBR Green (Hughes et al. 2014), was conducted to determine whether multiple genotypes exist in the populations, and their association with life-history traits.

MATERIALS AND METHODS

Model System

Lobelia inflata (Campanulaceae), or Indian tobacco, is a herbaceous monocarpic perennial native to North America that prospers in sandy or disturbed soils. Its closed anther tube has been assumed to enforce complete self-fertilization. After several generations, this would result in complete homozygosity, and offspring genetically identical to the parent, with populations consisting of independent genetic lineages (Loveless & Hamrick, 1984). Recent genetic work on *L. inflata* supports this assumption (Hughes et al., 2014; Hughes & Simons, 2015); no examples of outcrossing have been observed in the populations studied, and heterozygosity was found to be zero.

This plant typically germinates in the spring and early summer and forms rosettes that accumulate resources. If it does not enter the reproductive stage during the first year, the rosette is capable of overwintering. Bolting, or the initiation of reproduction, is influenced by a combination of photoperiod and rosette size (Simons & Johnston, 2003),

and is characterized by the production of a central stalk, which may have side branches. Flowers occur in leaf or bract axils and will remain open for approximately 3-10 days. The ovaries inflate, eventually turn brown, and two valves on the top of the fruit open allowing the hundreds of seeds to be dispersed by wind or other disturbance (Kelly, 1992).

Growth Conditions

To produce replicated individuals, I obtained *L. inflata* seeds from 20 individuals from two field locations: Gatineau Park (Gatineau, Quebec: 45°31'00N, 75°47'00W) and Petawawa Research Forest (Petawawa, Ontario: 45°57'00N, 77°19'00W). Seeds of each individual were grown in replicate 60mm petri dishes lined with moist filter paper and placed in a BioChambers AC-40 growth chamber set at 12 hour day/ 12 hour night photoperiod at 20° C until germination. Upon germination, seedlings were randomly assigned to cells (4cm x 4cm) in a 32-celled tray with autoclaved soil. The trays were then placed in a growth chamber set at 15 hour day/9 hour night photoperiod at 24°C/20°C to induce growth and bolting. Individuals were transferred to the experimental garden (Carleton University, Ottawa, Ontario: 45°57'00N, 77°19'00W) prepared with freshly tilled soil. A mean of 19.4 individuals of each genotype were randomly allocated to a position within each of four blocks. The plants were transferred from their 4x4cm cells into the ground on the first day of summer where they were exposed to natural conditions and provided with partial shade using strips of 30% shade cloth, with 50% coverage, suspended above the plots. All plants were measured and checked for life-history events every 2 days.

Phenotyping

I examined the association between microsatellite lineage and phenotypic variation in several life-history traits in replicated offspring of *L. inflata*. These traits included: time to germination, longest leaf before bolting, time to bolting, time from bolting to first flower, flowering time and time from first flower to first fruit maturation. Because a rosette-size threshold for initiation of bolting has been found in many species (Kachi & Hirose, 1983; Klinkhamer et. al., 1991; Simons, 1999), length of longest leaf prior to bolting was measured as a fitness-related trait (leaf length prior to bolting is a good predictor of number of flowers and therefore number of seeds produced). The timing of several early life-history events, such as time to germination and time to bolting, were also measured because the timing of the initiation of semelparous reproduction in *L. inflata* has been shown to display strong phenotypic plasticity (Hughes & Simons, 2015). In addition, timing of flowering events including time from bolting to first flower and time from first flower to fruit maturation, were measured because they represent key developmental stages in the plant's life cycle. Flower colour was documented because it is useful in determining whether microsatellite genotypes are associated with variation in phenotype (Hughes & Simons, 2015).

Microsatellite Genotyping

Microsatellite genotyping was performed to identify multiple genotypes in the 20 field-collected individuals. The use of microsatellites for genotyping has been common practice since their discovery in the early 1980s (for a review, see Vieira et al., 2016). They are informative multi-allelic, codominant genetic markers that are experimentally

reproducible and can be used to address a diversity of questions in evolutionary studies (Mason, 2015; Vieira et al., 2016). I carried out microsatellite genotyping using the protocol outlined in Hughes *et al*. (2014). In this earlier work, twenty-two loci were found to be polymorphic in the Petawawa population (Hughes et al., 2014). In addition to using a direct PCR protocol where DNA extraction and PCR are combined into a single step, I extracted DNA by clean prep methods using DNeasy Plant Mini Kit (QIAGEN Inc.) followed by the PCR protocol. Amplification was conducted using a Phire II Direct PCR Kit (Thermo Fisher Scientific) and the PCR was preformed in a T-3000 thermocycler (Biometra, Goettingen, Germany). PCR products then underwent HRM (high resolution melt) analysis using SYBR Green protocols in a Rotor-Gene 6000 thermocycler (QIAGEN Inc.) with curve analysis being performed using Rotor-Gene ScreenClust HRM Software (QIAGEN Inc.). HRM analysis was used to identify single nucleotide polymorphisms and allele frequencies (Erali & Wittwer, 2010; Wittwer et al., 2003).

Statistical Analyses

One-way ANOVAs were used to determine whether there were significant differences in the means of important life-history traits among lineages or genotypes. Calculations were carried out using JMP version 12.1 for each life-history trait where genotype was treated as a fixed effect. Although in most studies the effect of genotype is considered random, here 20 lineages were chosen that correspond to 16 genotypes which I considered as fixed effects because I was interested in particular (suites of) life-history traits rather than testing the greater hypothesis that genotypes in general differ.

When multiple ANOVAs are performed, there is an increased probability of incorrectly rejecting the null hypothesis (Zaykin et al., 2002). To account for the number of inappropriately rejected null hypotheses, false discovery rate (FDR) (Benjamini & Hochberg, 1995) was used. To examine relationships among life-history traits, Pearson correlations were also conducted.

Furthermore, to examine multivariate relationships among traits, discriminant analyses on all life-history characters were conducted. Canonical discriminant analysis (Hair et al., 1987) is a multivariate technique that describes the relationship between two or more variables through linear combinations that are maximally correlated (Tabachnick & Fidell, 2001). Dominant gradients of variation among groups are analyzed (Sherry & Henson, 2005). This analysis is often used in palaeontology where species sex can be identified based on the visual assessments of traits (Walker, 2008) and has been successful to demonstrate genetic variation within and between species (Goodwin et al., 1993; Jordana et al., 1993). The canonical plots show the two canonical variables that best separate genotypes and how life-history traits contribute towards genotype determination. Standardized coefficients show the contribution of the life-history traits to the discrimination between genotypes; the larger the coefficient, the greater the contribution.

RESULTS

Significant differences in important life-history traits among field-collected lineages were found (Table 1.1). Of the initial 20 field-collected individuals with a mean of 19.4 replicates each, 14 lineages bolted with a mean of 3.6 replicates each.

Microsatellite genotyping detected polymorphisms confirming all individuals within field-collected lineages are identical with respect to microsatellite alleles, but not all lineages (i.e. original field-collected individuals) are necessarily distinct genotypes. Of the 20 lineages, 16 unique genotypes were found (Table 1.2). A mean of 3.1 replicates grown in the garden for each of the 13 bolted genotypes allowed life-history traits to be examined for those genotypes (Table 1.3; Figure 1.1; Figure 1.2). The highest genotype mean time to germination was 9.0 (± 0) days compared to the lowest genotype mean time of 5.0 (± 0) days. The highest genotype mean time to bolting was 157.0 (±9.54) days compared to the lowest mean of 58.0 (± 0) days. Genotype means for time to first flower from bolting was highest at a mean of 33.6 (± 7.75) days and the lowest at 11.0 (± 0) days. The longest genotype mean flowering time was 89.3 (±8.07) days compared to the shortest of 52.0 (±17.33) days. No significant differences among microsatellite genotypes were found for longest leaf length prior to bolting, time from bolting to first flower or time from first flower to fruit maturation. Flower colour was useful in determining whether microsatellite genotypes were associated with variation in phenotype (Table 1.2).

In addition, phenotypic correlations among traits were conducted, with the greatest partial positive correlation occurring between longest leaf length prior to bolting and time from bolting to first flower (Table 1.4; Figure 1.1; Figure 1.2). Because a few

traits showed moderate correlations (Evans, 1996), I used canonical discriminant analysis to separate genotypes on the basis of a linear combination of six life-history response variables. A canonical correlation was conducted using 6 life-history traits as predictors of 4 genotypes common to all environments. The full model was not statistically significant (Table 1.5). Standardized discriminant coefficients are presented in Table 1.6. Discriminant functions were extracted; with significance only found with the Roy's Max root test (Table 1.6), genotypes do not display suites of characters that they can be identified.

Table 1.1 One-way ANOVA to test for differences in life-history traits among 20 field-collected individuals with a mean of 19.4 replicates each where 13 lineages bolted with a mean of 3.6 replicates each. Asterisks indicate significance at $\alpha<0.05$ after correcting for false discovery rate.

Life-history trait	Sum of squares	F-ratio	*P*-value
Time to germination	554.81	184.94	<0.0001*
Longest leaf length before bolting	4126.11	1.17	0.34
Time to bolting	19871.42	5.16	<0.0001*
Time from bolting to 1st flower	821.05	2.16	0.0364*
Duration of flowering	12912.67	3.96	0.0005*
Time from 1st flower to fruit maturation	1309.93	1.44	0.20

Table 1.2 Microsatellite genotyping results and flower colouration for 20 field-collected lineages. Genotype identification based on 4 primer sets. P indicates lineages collected in 2014 from Petawawa, Ontario (45°57'00N, 77°19'00W) and G indicates lineages collected in 2014 from Gatineau, Quebec (45°31'00N, 75°47'00W) in 2014. PI indicates lineages with pink flowers, PU purple flowers and PB purple-blue flowers. Superscript highlights lineages with genotypes that are the same at the microsatellite loci tested.

Field-collected lineage	Population	Genotype	Flower colour
8	P	CAEA	PI
17	P	CCEA	PB
22	P	AEAA	
28	P	CAAA	PI
35	P	CAAD	PI
38[1]	P	CCAA	PU
44	P	ACBA	PU
64	P	DAEA	PI
70	P	DCBA	PB
71[1]	P	CCAA	PI
84	G	AABD	PU-PB
85	G	DAAD	PI
87	G	CCAD	PU
92	G	CADA	PB
104	G	ACED	PI
117[1]	G	CCAA	PU
120	G	CEDA	PB
121[1]	G	CCAA	PB
125[1]	G	CCAA	PB
130	G	ACAA	PI

Table 1.3 One-way ANOVA to test for differences in life-history traits among 16 unique genotypes. Asterisks indicate significance at $\alpha < 0.05$ after correcting for false discovery rate.

Life-history trait	Sum of squares	F-Ratio	*P*-value
Time to germination	501.46	115.52	<0.0001*
Longest leaf length before bolting	3407.21	1.11	0.38
Time to bolting	19699.20	6.23	<0.0001*
Time from bolting to 1st flower	694.87	2.03	0.05
Duration of flowering	12063.70	4.22	0.0004*
Time from 1st flower to fruit maturation	1309.93	1.44	0.21

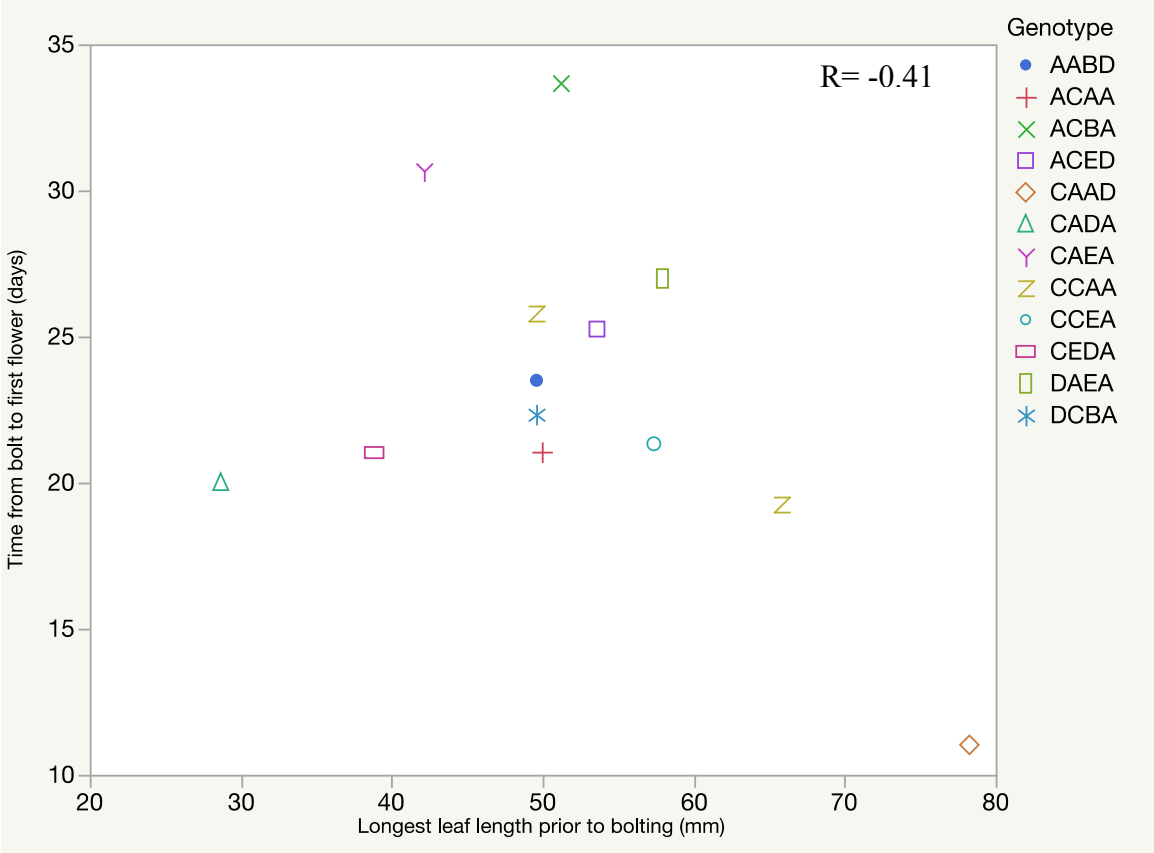

Figure 1.1 Trait correlation of the mean longest leaf length prior to bolting and mean time from bolting to first flower for all bolted replicates of the 13 genotypes present in the garden environment. A Pearson correlation of -0.41 is observed between the two life-history traits.

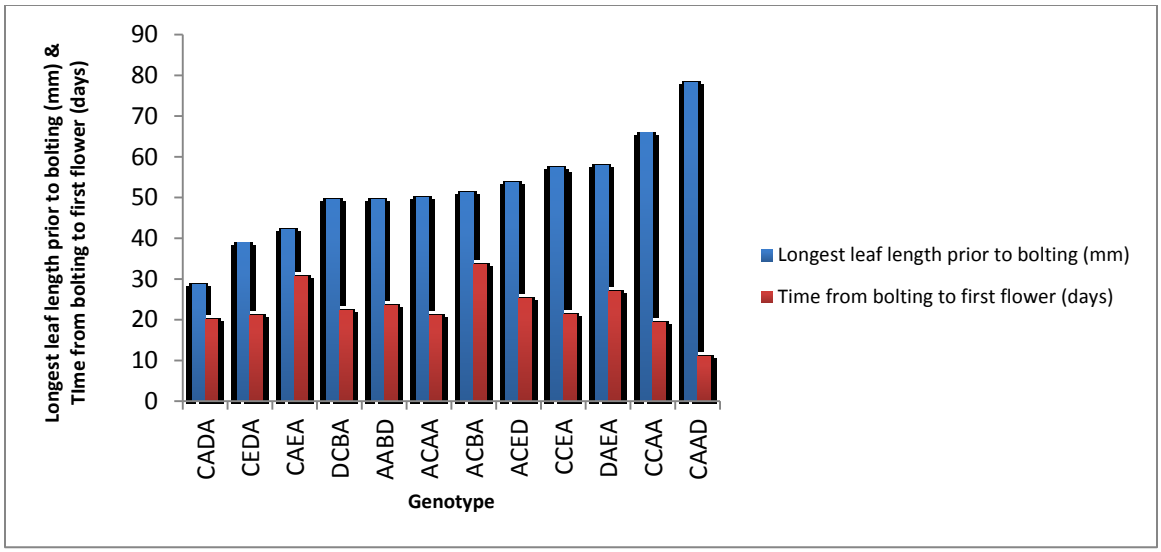

Figure 1.2 Mean longest leaf length prior to bolting and mean time from bolting to first flower for all bolted replicates of the 13 genotypes present in the garden environment.

Table 1.4 Phenotypic correlations among life-history traits. Pearson correlations are shown above diagonal, partial correlations below.

	Time to germination	Longest leaf length before bolting	Time from bolting to first flower	Time from first flower to first fruit maturation	Duration of flowering	Time to bolt
Time to germination	-	0.06	0.09	-0.17	0.14	0.16
Longest leaf length before bolting	0.16	-	-0.41	0.13	-0.19	0.62
Time bolt to first flower	0.15	0.45	-	-0.12	0.13	-0.55
Time first flower to fruit maturation	0.16	0.10	-0.06	-	0.06	0.04
Duration of flowering	0.18	0.29	-0.26	-0.03	-	-0.45
Time to bolt	-0.15	-0.15	-0.11	-0.11	-0.11	-

Table 1.5 Summary of canonical discriminant functions. Full discriminant analysis model is not significant. Although Roy's Max Root test (*P*=0.0024) was significant, Wilks's Lambda (*P*=0.057), Pillai's Trace (*P*=0.062) and Hotelling-Lawley (*P*=0.07) were all nonsignificant.

Function	Eigen value	Cumulative Variance	Canonical Correlation	Squared canonical correlation	Likelihood ratio	Approx F	*P*-value
1	1.52	68.25	0.78	0.61	0.22	1.79	0.06
2	0.53	92.18	0.59	0.35	0.56	1.16	0.35
3	0.17	100	0.38	0.144	0.85	0.78	0.55

Table 1.6 Standardized canonical coefficients for life-history traits across canonical functions.

Variable	Canon1	Canon2	Canon3
Time to germination	0.00	0.00	0.00
Longest leaf length prior to bolting	0.26	1.57	0.94
Time to bolting	0.89	-0.90	-0.35
Time from bolting to first flower	0.60	1.04	0.68
Time from first flower to fruit maturation	-0.36	-0.36	0.60
Flowering time	0.45	0.23	-0.54

DISCUSSION

High levels of genetic polymorphism in populations of *Lobelia inflata*—despite obligate self-fertilization—have previously been reported. Here, I study the plausibility of temporal genotype-by-environment interaction as a variance maintaining mechanism by documenting the variation. The maintenance of genetic variation in *L. inflata* through this mechanism under fluctuating selection requires that phenotypic variation exists in life-history traits (i.e. fitness components) and that this variation has a genetic basis. My results show that there are differences in life-history traits among replicated lineages derived from individuals collected from two field sites, and that these phenotypic differences correspond to microsatellite genotypes.

Consistent with earlier findings (Hughes & Simons, 2015) the level of genetic diversity found is greater than expected for a self-fertilizing plant population. There is no genetic exchange among selfing lineages; thus we would expect *L. inflata* to display low levels of variation due to the effects of both genetic drift and natural selection (Mousseau & Roff, 1987; Roff & Mousseau, 1987; Haasl & Payseur, 2013). Microsatellite genotyping detected polymorphisms demonstrating that genetic differences occur among field-collected lineages, and that all individuals within lineages are identical with respect to microsatellite alleles. Of the 20 lineages propagated from field individuals, 16 turned out to be distinct microsatellite genotypes. This is unexpected given that other plants in the Campanulaceae family with the ability to outcross show limited allelic diversity at genotyped loci (Antonelli, 2008; Geleta & Bryngelsson, 2012).

Therefore, an explanation for the maintenance of genetic diversity in this species is needed.

Although microsatellite variation is selectively neutral, markers here serve as genetic labels for whole genomes; thus, we can ask whether this genetic variation is associated with variation in traits related to fitness. Results show abundant genetic diversity inferred from these molecular markers, and analyses also show that this genetic variation corresponds to observed phenotypic variation measured on life- history traits. Different genotypes display differing times to germination, times to bolting and flowering time, which may be good predictors for the number of fruits and seeds produced (Ehrlen & Münzbergová, 2009) and can have important fitness effects in plants. Past research has found a rosette-size threshold for initiation of bolting that is dependent on external factors such as photoperiod (Simons & Johnston, 2000), and much variation in size at bolting was found in this study. This may be due to leaf herbivory from insects, which has been shown to reduce plant size (Baskin & Baskin, 1998) and therefore interfere with the initiation of bolting. Because most traits are genetically and phenotypically correlated with suites of other traits (Fischer et al., 2016), I examined whether there were any genotypes that displayed a suite of characters.

Life-history traits were overall found to display weak correlations. For example, there is a very weak correlation for time from first flower to fruit maturation and longest leaf length prior to bolting. However, there are life-history traits that did show stronger partial correlation (Table 1.4). For example, longest leaf length prior to bolting and time from bolt to first flower showed a partial correlation of 0.45 and correlations between the traits can be observed by genotype in Figures 1.1 and 1.2. Because of this, discriminant

analyses were used to see if there are suites of life-history characters that are associated with genotypes. Although some genotypes showed the possibility of possession of suites of characters for example ACED consistently had longer leaf lengths prior to bolting and had longer time from bolt to first flower, there was no suite of characters that defined genotypes (Table 1.5). Significance for life-history traits determining genotypes was found under Roy's Max test (Table 1.6), but is not accepted since it is the square of the largest canonical correlation (the extreme eigenvalues) that is based on maximums, and considered not significant when all other tests are nonsignificant (Johnstone & Nadler, 2013). Suites of fitness-related traits are thought to have evolved so that the timing of key life-history events maximizes reproduction and fecundity, for example the time to initiation of reproduction has a strong influence on the number and survival of progeny (Roff, 1992; Sinervo & Doughty, 1996). However, due to restricted parameters (i.e. a genotype sample size of 13 with a mean of 3.1 replicates, and life-history traits observed in only 1 environment), it is possible that suites of life-history characters exist, but are just not being displayed by these genotypes or in this environment.

An earlier finding (Hughes & Simons, 2015) showed that flower colour could be useful in determining whether microsatellite genotypes are associated with variation in phenotype. Flower colour may have important fitness effects, for example, it may influence herbivory with different colours being produced as a result of differing chemical composition (Irwin et al., 2003). For example, ACED genotypes possessed pink flowers while CADA genotypes were purple-blue (Table 1.2). It was observed that some genotypes could display flowers on a spectrum from purple to purple-blue while others

tended to look similar in hue. A chi-square test of independence was performed to examine the relation between genotype and flower colour and was found to be significant, $X^2(14, N = 44) = 46.02$, $p < 0.0001$*. Due to low sample size, it should be noted that the chi-square test for this data set is suspect, although the association between genotype and colour is highly significant in Chapter 2. Genetically based relationships among the various life-history traits observed in this study were found and can be primary determinants of the potential for adaptive phenotypic evolution (Stearns, 1989; Lynch & Walsh, 1998; Roff & Fairbairn, 2007).

Adaptive evolution results from differential reproduction of genotypes in a given environment (Kinnison & Hairston, 2007) and may consist of strategies such as phenotypic plasticity, where genotypes express differing phenotypes across environments (Via et al., 1995). *L. inflata* displays strong phenotypic plasticity in some life-history traits (Hughes & Simons, 2015), yet the genotypes used in this study were grown under a single environmental setting. Therefore, it is possible that the phenotypic expression observed among genotypes is the result of phenotypic plasticity, where the genotypes in this study with high fitness are better adapted to this season's environment, but may show lower relative fitness in a different year. In order to test the greater hypothesis that fluctuating selection coupled with temporal genotype-environment interaction maintains genetic diversity, the next step will require testing the fitness of various genotypes across multiple seasons, or environments. This will allow us to ask whether life-history variation observed here results in changes in the rank order of fitness across generations.

CONCLUSION

The topic of "excess" genetic variation in populations has long been a problem for evolutionary ecologists, and the mechanisms by which such variability is generated and maintained remains an important open question. *Lobelia inflata* offers an ideal system in which to test temporal genotype-environment interaction as a variance-maintaining mechanism because several explanations can be eliminated, and because genotypes can be readily identified using microsatellite markers. The present findings, that differences in life-history characters are associated with microsatellite genotypes (Table 1.3), suggests that substantial non-neutral genetic variation is being maintained. The results of this study and the fact that *L. inflata* colonizes disturbed habitats characterized by unpredictable change implies that fluctuating selection, coupled with temporal genotype-environment interaction, is a plausible mechanism maintaining variation. I can now perform a more direct test of this mechanism, which requires asking whether microsatellite genotypes, associated with life-history phenotypes, exhibit variance in fitness rank order of relative fitness through time.

Chapter 2: Variance in relative fitness of lineages of *Lobelia inflata* across years suggests fluctuating selection may be responsible for maintaining genetic variation

INTRODUCTION

Evolutionary biologists have long been puzzled by the abundant genetic variation that exists in populations and what is responsible for maintaining it. Drift can result in the loss of variation due to chance events, and natural selection erodes genetic variation through the elimination of suboptimal phenotypes (Roff, 1992; Stearns, 1997). Yet, despite the effects of drift and selection, high levels of genetic variation have been observed in natural populations (Fisher et al., 1958; Mousseau and Roff, 1987; Roff and Mousseau, 1987; Roff, 1992; Hendrick, 1998; Kliman et al., 2008; Haasl and Payseur, 2013). Because of this, much research attention has focused on mechanisms that could account for the observed genetic variation in natural populations.

Researchers have proposed that mutation-selection balance (Lande, 1976), overdominance (Falconer, 1989), frequency-dependent selection (Ayala & Campbell, 1974), antagonistic pleiotropy (Rose, 1982) and variable selection in space and/or time (Fisher & Ford, 1947) are all possible mechanisms maintaining genetic variation. However, it is often challenging to determine which mechanism is responsible in a given population. For example, in a 3-year study of selection on intrapopulation quantitative trait loci (QTL) of flower size in *Mimulus guttatus* (Mojica et al., 2012), researchers found that the QTL demonstrate antagonistic pleiotropy, where alleles increase flower size and fecundity at a cost of reduced viability, while also demonstrating that the

magnitude and direction of selection fluctuates yearly and on a spatial scale (Mojica et al., 2012). Therefore, trying to determine which mechanism is in effect can be a complex task that is often species or population-dependent and requires the disentanglement of multiple mechanisms in effect at once.

Because of particular features that allow the elimination of particular mechanisms, *Lobelia inflata* (Indian Tobacco) provides a unique opportunity to address questions on the maintenance of genetic variation. In a sample of 21 field-collected individuals, Hughes and Simons (2015) showed that populations of this plant contain considerable genetic variation, with 8 of the 20 lineages being genetically distinct. At the 22 microsatellite loci tested, there was a mean allelic difference of 2.5 between lineages and this high degree of genetic variation is unexpected in *L. inflata* because the populations studied are completely selfing (Hughes & Simons, 2015). This is supported by microsatellite analysis showing 100% homozygosity and zero outcrossing events observed (Hughes et al, 2014). Under these circumstances, several mechanisms that have been accepted as maintaining variation in other species would not be applicable to *L. inflata*.

Of the mechanisms proposed in other species, several can be eliminated as possibilities in *L. inflata*. Overdominance can be ruled out because heterozygosity has not been observed (Hughes & Simons, 2015). It is possible that frequency-dependent selection is maintaining genetic variation, but unlikely in this species because it is generally the result of predation on or competition between discrete forms (Ronsheim, 1996). With no obvious discontinuous forms having been observed frequency-dependent selection is therefore improbable. Fruiting or flowering at different times could affect

susceptibility to herbivory or florivory in a density- dependent way but is unlikely to account for the abundant variation in this species. Since it has recently been shown that this plant is completely inbred with variation occurring among lineages (Hughes et al., 2014), antagonistic pleiotropy is also unlikely. It has been shown that this mechanism is unlikely to take place in species characterized by high levels of homozygosity (Curtsinger, 1994; Hedrick, 1998). Therefore, variable selection is a likely alternative explanation.

Independent of mating system and heterozygosity, fluctuating selection is a potential variance-maintaining mechanism since *L. inflata* colonizes temporally variable habitats characterized by unpredictable change (Hughes & Simons, 2014). In the case of *L. inflata*, it also predicts that different subsets of genotypic lineages are selectively favored across years, which can feasibly be tested in this self-fertilizing species through the use of microsatellite genetic markers. Therefore, we will examine whether temporal genotype-environment interaction through time coupled with fluctuating selection is responsible for the maintenance of genetic variation in this species. This would result in variation in the rank order of lineage fitness through time and for a variety of genotypes to be maintained in a population (Roff, 1997; Hughes et al., 2015).

Fluctuating selection has been of interest to researchers since the early 1960s (Haldane & Jayakar, 1963; Mueller et al., 1985; Bull, 1986; Hairston & Walton, 1986; Lynch, 1987; Seger & Brockman, 1987; Hairston & Dillon, 1990; Hedrick, 1998; Mousseau et al., 2000; O'Hara, 2005; Huerta-Sanchez et al., 2007; Bell, 2010; Gossmann et al., 2014). This mechanism is responsible for maintaining variation in timing of diapause in freshwater copepods where, in the field, the mean timing of

diapause shifts between years as a result of fluctuating selection (Hairston & Walton, 1986; Hairston and Dillon, 1990). Wing polymorphism in the scarlet tiger moth (Fisher & Ford, 1947), flowering time of *Carlina* (Rees et al., 2004) and *Digitalis* (Sletvold & Grindeland, 2007) and seed germination time of *Collinsia verna* (Kalisz, 1986) also showed similar responses to shifts in the strength and magnitude of selection. The overall result of fluctuating selection is that different genotypes are favoured at different times in natural populations, and diversity to be maintained (Roff, 1997). Therefore tests of fluctuating selection require documenting the fate of several lineages over multiple seasons.

Although a direct test of fluctuating selection in *L. inflata* would require a multi-generational approach, the plausibility of this mechanism can be tested in a 2-year timeframe by incorporating both temporal variation and additional environments in a "space for time" replacement approach. The effectiveness of this mechanism depends on whether phenotypic variation exists, whether this variation occurs in life-history traits, and whether there is a genetic basis for this variation. In Chapter 1, I found that phenotypic variation in life-history traits among replicated field-collected individuals corresponds to multiple microsatellite genotypes in populations of *L. inflata.* This chapter will explore how microsatellite genotypes exhibit fitness variance through time that is associated with life-history phenotypes. In order to document the fate of several lineages over multiple seasons, a "space for time" approach is used in addition to two field seasons. This allows for multiple summer seasons to be simulated in a short amount of time using several growth chambers set with different environmental parameters (i.e. shorter/colder and longer/hotter summer seasons). Fitness measures in the form of

number of fruit and branches, plant height and stem diameter, were measured under simulated and natural conditions.

MATERIALS AND METHODS

Model System

Lobelia inflata (Campanulaceae) is a monocarpic perennial native to eastern North America and is often found along roads and trails where the soil is sandy and disturbed. Its anthers form a closed tube around the stigma, which has been assumed to enforce complete self-fertilization. Germination occurs in the summer when plants form rosettes to accumulate resources, and these rosettes are capable of overwintering if they do not enter the reproductive stage in their first year of growth. Under particular conditions (e.g. photoperiod and rosette size) the plant will initiate its reproductive phase by bolting (Simons & Johnson, 2003). Bolting is characterized by the production of a central flowering stalk. Individual flowers will remain in bloom for a mean of 7 days, and may have a white, pink, purple or purple-blue colouration (Hughes & Simons, 2015). It has an acropetal flowering pattern, where fruits form sequentially along inflorescences (Hughes & Simons, 2014). When the flowers die, the ovaries will turn brown and inflate, causing two valves on the top of the fruit to open, which allows the hundreds of seeds to be dispersed (Kelly, 1992).

I used two field locations, Gatineau Park (Gatineau, Quebec: 45°31’00N, 75°47’00W) and Petawawa Research Forest (Petawawa, Ontario: 45°57’00N, 77°19’00W), to obtain *L. inflata* seeds. In October 2014 and October 2015, seeds were

collected from both locations and final fitness measures documented.

Growth Conditions

Using *L. inflata* seeds obtained from the 2014 collection, seeds of each field-collected lineage were grown in replicate 60mm petri dishes lined with moist filter paper and placed in a BioChambers AC-40 growth chamber set at 12 hour day/ 12 hour night photoperiod at 20° C until germination. Seedlings were then randomly assigned to 4cm x 4cm cells in a 32-celled tray with autoclaved soil, and placed in a growth chamber set at 15 hour day/9 hour night photoperiod 24° C/20°C to induce growth and bolting. Prior to bolting and the first day of summer, individuals (hereafter called Group 1) were transferred to an experimental garden (Carleton University: 45°23'00N, 75°43'00W) that was divided into 4 blocks. Each block contained a mean of 19.4 individuals of each genotype and was provided with 30% shade cloth to ensure partial shade. Final fitness measures were obtained in November 2015.

To test the possibility of temporal fluctuating selection acting to maintain genetic variation and to document the fate of several lineages over multiple seasons, I used a "space for time" approach to supplement the field seasons. Another set of individuals (hereafter called Group 2) were grown under the same growth chamber conditions set for the seedling stage of Group 1; however, Group 2 remained in the chamber for their entire life cycle. Final fitness measures were obtained when the plants' fruits turned brown. This group was exposed to a set of conditions that remained constant through time, chosen as typical of average summer temperature and photoperiod conditions in the Ottawa area.

Using the progeny seeds obtained from Group 2 samples, two more groups of individuals (Group 3 and 4) were grown. The use of Group 2 progeny seeds further minimizes maternal parental effects from the field. The same methods outlined for Group 1 individuals were used to prepare Group 3 and 4 seeds. Upon germination, seedlings were placed in a growth chamber set at 16 hour day/8 hour night photoperiod 24°C/18°C. Upon bolting, half of the plants (Group 3) were transferred to a growth chamber set at a 15 hour day/9 hour night photoperiod 22° C/16° C, as a discrete environment that may represent a short-cold summer season. The other half of the plants (Group 4) remained under the 16 hour day/8 hour night photoperiod 24° C/18° C, as a discrete environment meant to represent a long-warm summer season. Each tray was watered (1L) 2-3 times a week, and 15ml of a solution of liquid fertilizer (15-5-15) was added once every two weeks. Final fitness measures were obtained once all the plants' fruits had matured.

Phenotyping

Results from Chapter 1 show that microsatellite genotypes are associated with life-history traits. Because Chapter 2 focuses on fitness in multiple environments, data on life-history traits are available to corroborate these earlier findings. Therefore, several life-history traits: including time to germination, time to bolting, time from bolting to first flower, flowering time and time from first flower to fruit maturation were examined as outlined in Chapter 1.

For assessment of temporal genotype-environment interaction in fitness, measures of fitness included final number of fruits and branches, final plant height and final stem diameter. Plant fitness measurements often focus on the number of offspring produced

through analyses of seed production (Primack & Kang, 1989) and here we use the number of fruits as a proxy measure of fecundity. Plant height may represent fitness in that it is speculated that taller plants will produce more fruit, and may also have a greater ability to disperse seeds (Hughes & Simons, 2014). Stem diameter is a measure of overall plant size independent of architecture. Branching allows the plants to overcome constraints on fruit production related to growth of the meristem and to maximize reproductive success under short growing season (Hughes & Simons, 2014).

Microsatellite Genotyping

I carried out microsatellite genotyping for 2014 field-collected lineages (83) and a subsample from the 2015 season (26) at 4 microsatellite loci using methods outlined in Chapter 1.

Statistical Analyses

ANOVA was used to determine whether there were differences in life-history traits among genotypes. Calculations were carried out as outlined in Chapter 1. Fitness traits were analyzed using factorial fixed-effect ANOVAs, where the interaction effect (genotype x environment) is of greatest interest. Genotype sample size is unknown at the time of collection, limited to the number of unique genotypes found among lineages, and genotypes collected in 2014 are not necessarily present in samples collected at the same sites in 2016. Furthermore, subsets of field-collected lineages were used in the growth chambers. The number of genotypes common to environments thus constrains analyses that include genotype-by-environment interaction. The main analysis includes all

environments. However, only four genotypes could be used for the full model with all environments, so two supplementary ANOVAs are performed with fewer environments to increase the number of genotypes included; one with the two temporal field environments (2014 and 2015), and one that includes only the growth chamber "space-for-time" environments. With a low number of genotypes common to all environments and an interest in the particular life-history traits that characterize the genotypes (Chapter 1), I treated genotype as a fixed effect (in most studies it is random). With six environments, including manipulated "good/long" and "bad/short" year types, environment was also treated here as a fixed effect although, ideally, the study would have been run for several years where environment would have been a random effect. A drawback of this approach is that conclusions are limited to genotypes and environments used in the study. ANOVAs were performed for each fitness trait listed above.

As in Chapter 1, to control for the number of inappropriately rejected null hypotheses, false discovery rate (FDR) (Benjamini & Hochberg, 1995) was used to correct for multiple comparisons; Pearson correlations were used to examine relationships between life-history traits; discriminant analyses were conducted on the life-history traits for which we had genotype data to determine suites of traits associated with genotypes.

RESULTS

Using additional data from multiple environments, I begin by confirming the results of Chapter 1; there are significant differences in life-history traits among genotypes. In a

sample of 109 individuals, there were 45 unique genotypes and a mean of 5.3 alleles per microsatellite locus tested (Table 2.1). Significant differences in life-history traits among genotypes were found using an ANOVA with all genotypes included regardless of environment (Table 2.2). Microsatellite genotyping detected polymorphisms confirming all individuals within field-collected lineages are identical with respect to microsatellite alleles (Table 2.1), and that these genotypes correspond to significant differences in life-history phenotypes (Table 2.2).

The 20 genotypes where life-history characters were examined across 4 environments had a mean of 16.1 bolted replicates, and showed differences in all the key life-history traits tested. The highest genotype mean time to germination was 12 (± 0.5) days compared to the lowest genotype mean time of 5.0 (± 0) days. The largest genotype mean leaf length prior to bolting was 55.7 (± 18.95) mm and the smallest mean was 24.6mm (± 1.95). The highest genotype mean time to bolting was 129.0 (± 0) days compared to the lowest mean of 27.0 (± 0) days. Genotype means for time to first flower from bolting was highest at a mean of 30.7 (± 2.0) days and the lowest at 13.7 (± 7.1) days. The longest genotype mean flowering time was 119.3 (± 4.8) days compared to the shortest of 74.0 (± 0) days. Genotype means for time from first flower to first fruit maturation was longest at a mean of 60.6 (± 5.7) days and shortest time at 37.7 (± 3.5) days. Flower colour was useful in determining whether microsatellite genotypes were associated with variation in phenotype using a chi-square test of independence, which showed a significant association, $X^2(15, N = 272) = 205.21$, $p < 0.0001$*.

Analysis of variance indicates that fitness differences exist among genotypes and among environments. Of greater importance here is whether the rank order of fitness

differs across environments. In the main analysis that includes all environments, a significant genotype-by-environment (GxE) interaction exists for all fitness traits except final stem diameter (Table 2.3). This analysis requires the elimination of genotypes that were not present in all environments (see Materials and Methods) and thus included 4 genotypes. The change in rank order in fitness (using fruit number as an example) of the 4 genotypes across all environments is shown in Table 2.4 and Figure 2.1 demonstrates the mean number of fruits produced by each genotype for which the ranking was based. In order to maximize the number of genotypes used in analyses, ANOVAs were also run using fewer environments. A test using Groups 2, 3 and 4 (growth chamber environments) included 14 genotypes, and showed significant GxE interaction for final fruit number, final stem diameter and final branch number (Table 2.5). An example of how rank order of the 14 genotypes changes across all environments is shown in Table 2.6. However, analysis of variance for fitness traits that includes only the two field collections includes 11 genotypes, and finds no significant genotype-by-environment interaction for any trait (Table 2.7).

Phenotypic correlations among fitness traits were found, with a positive partial correlation between final fruit number and final stem diameter (0.64) and a negative partial correlation between final height and final fruit number (-0.27) (Table 2.7).

A canonical correlation was conducted using life-history traits as predictors of 14 genotypes. The full model was statistically significant (Table 2.9). Because several traits were highly correlated, we used canonical discriminant analysis to separate the genotypes on the basis of a linear combination of 6 life-history traits as response variables. The dimension reduction analysis tests the hierarchical arrangement of functions for statistical

significance (Sherry & Henson, 2005). The first five discriminant functions were found to be significant (Table 2.9). Canonical function 6, which was the only function that was tested in isolation, did not explain a significant amount of shared variance between variable sets. The standardized canonical coefficient and total variance within functions shows that the first two canonical functions account for 40% and 18% of shared variance, respectively. For Canonical 1 structure coefficients (Table 2.11), the relevant variable was time to germination. The other side of the equation involves the predictor set (Table 2.12), and shows that Canonical 1 discriminates several genotypes including ACEA, CAAA, CCAD and DCAD. Relevant variables possess larger coefficients and will therefore contribute more towards predicting genotypes (Tabachnick & Fidell, 2001). Positive coefficients indicate a negative association and negative coefficients indicate a positive association (Sherry & Henson, 2005). Therefore, ACEA, CAAA and CCAD were positively related to time to germination, and DCAD was negatively related. For Canonical 2 structure coefficients (Table 2.11), the relevant variables were longest leaf prior to bolting, time to germination, time to bolting and flowering time. The predictor set (Table 2.12) shows that Canonical 2 discriminates genotype ACED, CAAA, CCBD and DCAD. Genotype ACED was positively related, while CAAA, CCBD and DCAD were negatively related to the suite of traits.

Table 2.1 Microsatellite genotyping results for field-collected lineages. Genotype identification based on 4 primer sets. P indicates lineages collected from Petawawa, Ontario (45°57'00N, 77°19'00W) in 2014 and G indicates lineages collected from Gatineau, Quebec (45°31'00N, 75°47'00W) in 2014. Superscript indicates lineages with genotypes that are the same at the microsatellite loci tested. Sample size of 109 lineages and 45 genotypes.

Field-collected lineage	Genotype	Collection year	Population
1^{18}	CCBD	2014	P
2^{1}	CCAA	2014	P
3^{18}	CCBD	2014	P
4	CADD	2014	P
5	BCBF	2014	P
6	CABA	2014	P
7^{7}	ACBA	2014	P
8^{15}	CAEA	2014	P
9^{4}	AAEA	2014	P
10	AADD	2014	P
12^{15}	CAEA	2014	P
13	BECC	2014	P
16	ACDA	2014	P
17^{19}	CCEA	2014	P
18^{15}	CAEA	2014	P
20	BECA	2014	P
22^{12}	AEAA	2014	P
28^{13}	CAAA	2014	P
32^{18}	CCBD	2014	P
33^{8}	ACBC	2014	P
34^{3}	AABA	2014	P

Table 2.1 (cont'd)

Field-collected lineage	Genotype	Collection Year	Population
35^{14}	CAAD	2014	P
37^{9}	ACBD	2014	P
38^{1}	CCAA	2014	P
40^{7}	ACBA	2014	P
43^{10}	ACEA	2014	P
44^{7}	ACBA	2014	P
46	AAAD	2014	P
47^{6}	ACAA	2014	P
48^{1}	CCAA	2014	P
49^{2}	AAAA	2014	P
51^{6}	ACAA	2014	P
57^{7}	ACBA	2014	P
58^{2}	AAAA	2014	P
60^{5}	AAED	2014	P
61	DADA	2014	P
63	ACCA	2014	P
64^{22}	DAEA	2014	P
65^{10}	ACEA	2014	P
66^{20}	CEDA	2014	P
67^{24}	DEEA	2014	P
68^{17}	CCBA	2014	P
69	DAAA	2014	P
70^{23}	DCBA	2014	P
71^{1}	CCAA	2014	P
72	DCAD	2014	P
73^{16}	CCAD	2014	P
74	PCBA	2014	P
75^{9}	ACBD	2014	P

Table 2.1 (Cont'd)

Field-collected lineage	Genotype	Collection year	Population
76[1]	CCAA	2014	G
78[2]	AAAA	2014	G
79[7]	ACBA	2014	G
80	AEAD	2014	G
83	DABA	2014	G
84	AABD	2014	G
85[21]	DAAD	2014	G
86[6]	ACAA	2014	G
87[16]	CCAD	2014	G
89[8]	ACBC	2014	G
90	DCAA	2014	G
91[23]	DCBA	2014	G
92	CADA	2014	G
93	CCDA	2014	G
94[3]	AABA	2014	G
95	ACEC	2014	G
96[7]	ACBA	2014	G
97[7]	ACBA	2014	G
98[19]	CCEA	2014	G
100[23]	DCBA	2014	G

Table 2.1 (Cont'd)

Field-collected lineage	Genotype	Collection year	Population
101[17]	CCBA	2014	G
102[7]	ACBA	2014	G
104	ACED	2014	G
107[11]	ACED	2014	G
108	PAXD	2014	G
114[7]	ACBA	2014	G
115[17]	CCBA	2014	G
116[13]	CAAA	2014	G
117[1]	CCAA	2014	G
120[20]	CEDA	2014	G
121[1]	CCAA	2014	G
122[7]	ACBA	2014	G
125[1]	CCAA	2014	G
130[6]	ACAA	2014	G

Table 2.1 (cont'd)

Field-collected lineage	Genotype	Collection year	Population
1	CEBA	2015	P
2^{25}	ECED	2015	G
2^{24}	DEEA	2015	P
3^{14}	CAAD	2015	P
4^{21}	DAAD	2015	P
6	EAAA	2015	P
9^{22}	DAEA	2015	P
12	DAED	2015	G
19^{5}	AAED	2015	G
20	ABFD	2015	P
23	DCEA	2015	P
24^{13}	CAAA	2015	P
28^{2}	AAAA	2015	P
30	FAED	2015	P
32^{19}	CCEA	2015	P
35^{15}	CAEA	2015	P
36^{24}	DEEA	2015	P
37^{4}	AAEA	2015	P
38	DAEE	2015	P
40	EAED	2015	P
47^{25}	ECED	2015	G
49^{24}	DEEA	2015	P
54	DEDA	2015	P
60	PCEA	2015	G
61^{11}	ACED	2015	G
106^{12}	AEAA	2015	G

Table 2.2 One way ANOVA to test for differences in life-history traits among genotypes. Asterisks indicate significance at $\alpha < 0.05$ after correcting for false discovery rate. To maximize genotype sample size, all 45 genotypes in all 6 environments were used.

Life-history trait	Sum of squares	F-Ratio	*P*-value
Time to germination	1289.87	9.68	<0.0001*
Longest leaf length before bolting	12447.77	2.94	<0.0001*
Time to bolting	179125.51	10.66	<0.0001*
Time from bolting to 1st flower	1964.74	2.07	0.0051*
Duration of flowering	61621.13	5.07	<0.0001*
Time from 1st flower to fruit maturation	12465.40	3.60	<0.0001*

Table 2.3 Two-way factorial ANOVA to test for differences in fitness traits among genotypes for all environments (Groups and field collections). Asterisks indicate significance at $\alpha < 0.05$ after correcting for false discovery rate. With few genotypes in common across all 6 environments, sample size was low at 4 genotypes.

Fitness Trait	Source	Sum of squares	F-Ratio	*P*-value
Final fruit number	Genotype	63636.20	2.46	0.07
	Environment	2012283.00	46.74	<0.0001*
	Genotype x Environment	420965.10	3.26	0.0004*
Final height	Genotype	205.88	0.99	0.40
	Environment	4835.64	13.97	<0.0001*
	Genotype x Environment	5217.28	5.02	<0.0001*
Final stem diameter	Genotype	1.09	0.46	0.71
	Environment	178.26	45.00	<0.0001*
	Genotype x Environment	24.43	2.06	0.02
Final branch number	Genotype	52.28	1.38	0.26
	Environment	371.07	5.88	0.0001*
	Genotype x Environment	622.88	3.29	0.0004*

Table 2.4 Rank order of genotypes ACED, CCEA, DAEA and DCBA according to final fruit number, a fitness measure, across all environments. The lower the rank number (e.g. 1), the higher the fruit production.

Genotype	Group 1 (Experimental Garden)	Group 2 (Average season)	Group 3 (Short-cold)	Group 4 (Long-warm)	Collection 2014	Collection 2015
ACED	2	2	2	4	4	4
CCEA	1	1	1	3	2	3
DAEA	4	4	3	1	1	2
DCBA	3	3	4	2	3	1

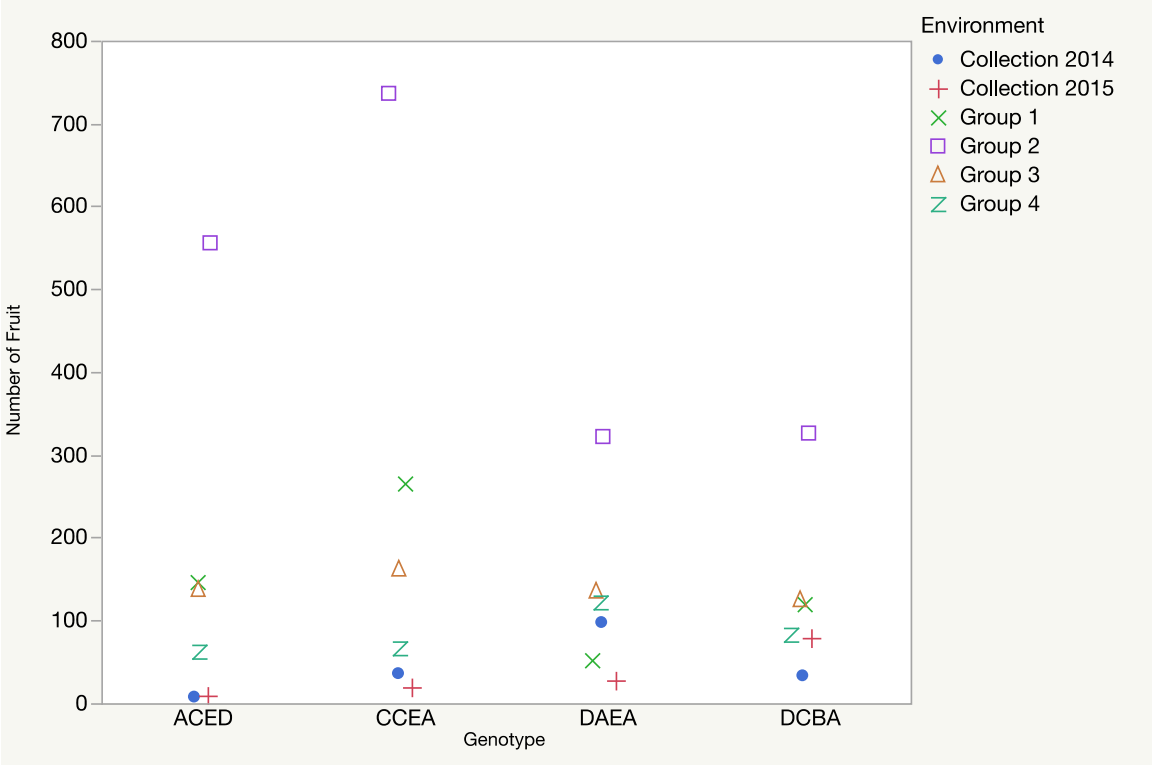

Figure 2.1 The mean number of fruit produced by each genotype across all environments.

Table 2.5 Two-way factorial ANOVA to test for differences in fitness among genotypes for Groups 2, 3 and 4 (growth chamber environments). Asterisks indicate significance at $\alpha < 0.05$ after correcting for false discovery rate. All 14 genotypes that were common to the 3 "space-for-time" environments were used.

Fitness Trait	Source	Sum of squares	F-Ratio	*P*-value
Final fruit number	Genotype	200351.64	8.04	<0.0001*
	Environment	219426.54	57.55	<0.0001*
	Genotype x Environment	244528.92	4.93	<0.0001*
Final height	Genotype	1528.71	4.60	<0.0001*
	Environment	956.11	18.68	<0.0001*
	Genotype x Environment	793.82	1.19	0.25
Final stem diameter	Genotype	24.17	6.65	<0.0001*
	Environment	25.12	44.94	<0.0001*
	Genotype x Environment	29.12	4.01	<0.0001*
Final branch number	Genotype	179.28	2.37	0.0055*
	Environment	250.18	21.52	<0.0001*
	Genotype x Environment	304.84	2.02	0.0037*

Table 2.6 Rank order of fitness (final fruit number) of 14 genotypes across the "space-for-time" environments. The lower the rank number (e.g. 1), the higher the fruit production.

Genotype	Group 2 (Average season)	Group 3 (Short-cold)	Group 4 (Long-warm)
AABD	1	7	7
ACBA	3	10	10
ACEA	8	9	9
ACED	9	3	3
CAAA	11	14	14
CCAA	12	8	11
CCAD	2	13	13
CCBD	4	1	1
CCEA	10	6	6
CEDA	5	7	8
DABA	7	2	2
DAEA	6	12	12
DCAD	12	11	4
DCBA	14	14	5

Table 2.7 Two-way factorial ANOVA to test for differences in fitness traits among genotypes for the two field collections. All 11 genotypes that were common to the two field collection years were used.

Fitness Trait	Source	Sum of squares	F-Ratio	*P*-value
Final fruit number	Genotype	5307.63	0.72	0.69
	Environment	44.34	0.06	0.81
	Genotype x Environment	4670.69	0.64	0.76
Final height	Genotype	2453.56	1.55	0.26
	Environment	98.63	0.62	0.45
	Genotype x Environment	2958.74	1.86	0.18
Final stem diameter	Genotype	7.05	0.94	0.54
	Environment	0.19	0.26	0.62
	Genotype x Environment	3.12	0.42	0.91
Final branch number	Genotype	271.27	1.32	0.34
	Environment	0.49	0.02	0.88
	Genotype x Environment	161.46	0.79	0.65

Table 2.8 Phenotypic correlations among fitness traits. Pearson correlations are shown above diagonal, partial correlations below.

	Final height	Final fruit number	Final stem diameter	Final branch number
Final height	-	0.36	0.59	0.52
Final fruit number	-0.27	-	0.77	0.65
Final stem diameter	0.46	0.64	-	0.65
Final branch number	0.31	0.37	0.11	-

Table 2.9 Summary of canonical discriminant functions. Tests of Wilks's Lambda (P<0.001*), Pillai's Trace (P<0.001*), Hotelling-Lawley (P<0.001*), and Roy's Max Root test (P<0.001*), were all found to be significant.

Function	Eigen value	Cum Variance	Canonical Correlation	Likelihood ratio	Approx F.	*P*-value
1	0.97	60.20	0.70	0.28	3.69	<0.001*
2	0.22	73.82	0.42	0.55	2.08	<0.001*
3	0.14	82.52	0.35	0.67	1.83	<0.001*
4	0.13	90.71	0.34	0.76	1.73	0.0018*
5	0.09	96.45	0.29	0.87	1.48	0.0496*
6	0.57	100.00	0.23	0.95	1.22	0.2609

Table 2.10 Standardized canonical coefficient for life-history traits across canonical functions.

Variable	Canon1	Canon2	Canon3	Canon4	Canon5	Canon6
Time to germination	1.11	0.03	0.10	-0.00	-0.04	0.11
Longest leaf length prior to bolting	0.35	-1.01	0.08	0.20	-0.28	0.76
Time to bolting	-0.50	1.03	-0.15	-0.16	0.55	0.46
Time from bolting to first flower	0.11	-0.21	0.45	0.71	0.6	0.26
Time from first flower to fruit maturation	-0.25	-0.12	0.85	-0.50	0.10	-0.58
Flowering time	0.41	0.55	0.18	0.49	0.01	0.43

Table 2.11 Structure coefficient of life-history traits across canonical functions.

Variable	Canon1	Canon2	Canon3	Canon4	Canon5	Canon6
Time to germination	0.9	0.36	0.2	-0.09	-0.09	0.1
Longest leaf length prior to bolting	-0.068	-0.46	-0.23	-0.25	-0.04	0.82
Time to bolting	-0.05	0.32	-0.26	-0.38	0.44	0.7
Time from bolting to first flower	0	-0.08	0.36	0.74	0.55	-0.12
Time from first flower to fruit maturation	-0.12	-0.01	0.85	-0.51	-0.05	0.06
Flowering time	-0.11	0.4	0.37	0.44	-0.69	0.14

Table 2.12 Class means on genotype across canonical functions.

Variable	Canon1	Canon2	Canon3	Canon4	Canon5	Canon6
AABD	-0.61	-0.44	-0.47	0.05	0.43	-0.19
ACBA	0.84	-0.02	-0.16	0.47	-0.34	0.53
ACEA	-1.18	-0.05	-0.20	-0.19	0.24	-0.16
ACED	0.35	-0.58	0.18	-0.09	0.12	0.18
CAAA	-1.93	0.48	0.35	0.38	-0.34	0.07
CCAA	-0.61	-0.18	0.12	-0.15	0.12	0.17
CCAD	-1.25	-0.18	-0.50	0.37	-0.55	-0.20
CCEA	0.65	-0.31	-0.54	0.57	0.09	0.12
CEDA	0.82	-0.39	0.60	0.29	0.38	-0.16
DABA	0.52	0.33	0.48	0.26	-0.08	-0.11
DAEA	0.65	-0.41	-0.09	-0.19	0.085	0.16
DCAD	1.23	0.60	0.00	-0.13	0.017	-0.15
DCBA	-0.05	-0.24	0.08	-0.60	-0.40	-0.12

DISCUSSION

Despite obligate self-fertilization, high levels of genetic polymorphism in populations of *Lobelia inflata* exist, and understanding how such variation is being maintained remains an open question. Here, I examine the possible maintenance of genetic variation in *L. inflata* through fluctuating selection coupled with temporal genotype-environment interaction. This requires that microsatellite genotypes exhibit fitness variance through time that is associated with life-history phenotypes, whereby selection results in a change in rank order of fitness across years. My results show significant differences in life-history phenotypes among genotypes, significant genotype-by-environment interaction in fitness traits and changes in the rank order of fitness across environments.

In Chapter 1, I examined the phenotypic variation in key life-history traits among replicated 20 field-collected individuals to show how the variation corresponded to microsatellite genotypes. Microsatellite variation is predominantly selectively neutral (Selkoe & Toonen, 2006) with markers being used here as genetic labels for whole genomes. In this study, I grew additional lineages (109) in replicates under a variety of environmental conditions, and found 45 unique genotypes at the microsatellite loci tested (Table 2.1). In addition, flower colour is useful in determining whether microsatellite genotypes are associated with variation in phenotype. A chi-square test of independence was performed using 16 genotypes grown in chamber environments (Group 3, 4, and 5) to examine the relation between genotype and flower colour and was found to be significant, $X^2(15, N=272) = 205.21$, $p<0.0001$*. There is therefore evidence that

genotypes were associated with particular flower colours, which is consistent with earlier findings (Hughes & Simons, 2015). The abundant genetic variation found is much greater than expected for a plant that is completely self-fertilizing and therefore is being maintained.

The possibility of variable selection maintaining variation requires life-history phenotypes to vary significantly among genotypes. Through the incorporation of all genotypes regardless of environment in the analysis, genotypic variation in life-history characters found in Chapter 1 is supported here (Table 2.1). If environment is included in factorial ANOVAs, sample size is limited by the fact that only 4 genotypes were in all environments, and only 1 life-history trait remains significant. Rosette size at bolting was not found to differ among genotypes in Chapter 1, but is found to differ here. Rosette-size threshold for initiation of bolting is often a good predictor of reproductive success (Kachi & Hirose, 1983; Klinkhamer et. al., 1991; Simons & Johnston, 2000); for example, smaller rosettes often lead to lower seed production (Springate & Kover, 2014).

Life-history characters are directly related to survival and reproduction, and their fitness effects are highly contingent on the environment (Stearns, 1992). In order to test the possibility of fluctuating selection maintaining genetic variation in *Lobelia inflata,* there must therefore be overall differences in environments. Results showing a strong main effect of environment on fitness traits (Table 2.3) suggest that the six environments (including "space-for-time" chambers) successfully generated variation and presented the genotypes with a range of environments to which fitness is sensitive. However, environmental fluctuation alone does not maintain genetic variation (Johnson, 2006); it requires relative performance to differ across environments.

Differential genotypic response to environmental variation is known as genotype-by-environment (GxE) interaction (Bowman, 1972) and this can maintain variation if the rank order of genotypes changes across environments (Mitchell-Olds, 1992). In order to analyze GxE interaction, genotypes common to all environments must be used. With only 4 in common in the full model including all 6 environments, significant GxE interaction for fitness measures was found (Table 2.3). Ranking of genotypes is based on final fruit number (Figure 2.1), one of the most commonly used measures of fitness (e.g. Russell, 2010), and is found to change across environments (Table 2.4). Genotype DAEA ranked best in the long-warm summer season (Group 4), but worst in the experimental garden environment (Group 1), while genotype DCBA has the same relative success in either environment. The increase in fruit production (and therefore fitness) of genotype DAEA would suggest that the long-warm environment is a more conducive environment for that genotype. Genotype DCBA is relatively well adapted to both environments and therefore performs equivalently in both. However, the use of only 4 genotypes in 6 environments resulted in the rank order being determined by a limited dataset. In addition, the use of field-collected seeds for Group 2 individuals in the analyses incorporates some maternal environmental effects, which could contribute to the fitness effects of genotypes in the Group 2 environment.

Further exploratory analyses were therefore conducted to ask whether GxE interaction would also be significant for fitness traits if genotype sample size increased. To accomplish this, environments were eliminated from analyses, which increased the possibility that common genotypes may be found. For 14 replicate genotypes grown under the "space-for-time" component (Groups 2, 3 and 4), significant GxE interactions

for final fruit number, branch number, and stem diameter (Table 2.5) were found. The use of Group 2 progeny seeds for Group 3 and 4 helped minimize maternal parental effects from the field, which could influence genotypes performance in different environments. It was found that genotypes performed differently based on the environment they were in and that the relative rank order of fitness changes (Table 2.6). For example, genotype DAEA ranked better than genotype DCBA in final fruit number for two of the three environments, consistent with what was just shown when all environments were being analyzed. However, further examination revealed that 5 and 13 genotypes are better ranked than genotype DAEA and DCBA respectively. Therefore, DAEA and DCBA are not as well suited to those environments as implied by the analysis restricted to four genotypes, where fitness is compared to only three other genotypes. Once again, these results are considered in light of the maternal environmental effects that may have been introduced by the use of field-collected seeds for the Group 2 environment.

Variation in genotype fitness across environments implies that particular suites of traits might be well suited for particular environment types. This could be thought of as specialization for particular temporal conditions. An increase or decrease in the expression of one of these traits may have a positive or negative influence on the suite of characters (Olinjnyk & Nelson, 2013). For example, a faster time to bolting decreases maturation time, possibly resulting in a shorter generation time and therefore higher fitness (Roff, 2000). However, there are often environmentally induced limits between traits affecting survival and reproduction resulting in negative associations (Reznick, 1985; Stearns, 1989). For example, a negative partial correlation is observed between final fruit number and final plant height (Table 2.6). Therefore, discriminant analyses

were conducted to see if there are suites of fitness-related characters that are associated with genotypes that may make them well suited for particular environments (Table 2.9).

Canonical discriminant functions show that there is a significant relationship between life-history traits and genotype grouping. The first two functions explain a reasonable amount of variance (i.e. 40% and 18% of variance respectively within their functions), and are used in interpretations. All other functions explain less than 15% of variance and are not explored further for risk of finding an effect that may be statistically significant but not replicable in future studies (Sherry & Henson, 2005). Standardized coefficients (Table 2.10) show the contribution of the life-history traits to the discrimination between genotypes. Time to germination accounts for 40% of the variation within Canonical 1 and a suite of life-history characters found (longest leaf prior to bolting, time to germination, time to bolting and flowering time) accounts for 18% of the variation within its function. Canonical 2 was able to discriminate genotype ACED and CAAA. Genotype ACED is shown to have a positive association to the suit of characters (Table 2.12) and ranks relatively high (3^{rd}) in fitness (final fruit number) for both the long-warm environment (Group 4) and the short-cold environment (Group 3), but relatively lower (9^{th}) for the average season (Group 2) compared to 14 other genotypes (Table 2.6). Genotype CAAA is shown to have a negative association to the suite of characters (Table 2.12) and ranks relatively low (14^{th}) in fitness (final fruit number) for both the long-warm environment (Group 4) and the short-cold environment (Group 3), but relatively higher (11^{th}) for the average season (Group 2) (Table 2.6). Therefore, this shows suites of life-history characters are associated with genotypes and may contribute to their variable success across environments. Suites of fitness-related traits are thought

to have evolved because timing of key life-history events maximizes reproduction and fecundity (Roff, 1992). For example the time to initiation of reproduction has a strong influence on the number and survival of progeny (Roff, 1992; Sinervo & Doughty, 1996). Therefore, particular suites of life-history characters may result in genotypes being better suited to certain environment types.

If across environments there is genetic variation associated with fitness-related traits, and covariation between fitness and life-history phenotypes, then traits can evolve in response to natural selection (Stearns, 1989; Falconer & Mackay, 1996; Roff, 1997; Fischer et al., 2016). Fluctuating selection coupled with temporal GxE interaction may be maintaining genetic variation in *L. inflata*. Only two observations of final fitness could be made under true natural conditions (Collection 2014 and 2015). An analysis of fluctuating selection on this limited dataset finds no significant GxE interaction. However, in the field there is no control over environmental conditions, how many genotypes are found, or are in common year after year. I was able to find 11 microsatellite genotypes common to these two temporal environments. But because of *L. inflata's* monocarpic life cycle and ability to overwinter in a rosette form, it is possible that cohorts are at least partially non-overlapping, thus explaining why there are few genotypes in common from one year to the next. A limited field-study could not accurately take into account the possibility of non-overlapping cohorts. Also, the natural environmental conditions encountered during the two field-seasons does not necessarily represent environmental variation *L. inflata* may encounter over generations in the field. Although GxE interaction for fitness was strong when all environments were included, indicating the possibility that fluctuating selection coupled with temporal GxE interaction

is maintaining genetic variation, a fuller test of the importance of this mechanism would require several more field seasons. For example, in a study on *Oenothera biennis,* GxE interaction was found to impose variable selection on flowering strategy, with the use of 3 more field environments (Johnson, 2007) than was used in this study.

In this study, I looked into the fitness variance of different genotypes under simulated environmental conditions in the "space-for-time" approach, and supplemented this approach with two years of fieldwork, to show how the genotypes express fitness variance across environments. Although this work does not assume that the environmental parameters of each simulated environment corresponds exactly to a different environment that could occur in nature, it does assume that the response to these environments represents potential natural responses. Therefore, the results should be interpreted cautiously as it is a preliminary look at the plausibility of fluctuating selection and temporal genotype-environment interactions' role in maintaining genetic variation in *L. inflata*.

CONCLUSION

Although a test of fluctuating selection coupled with temporal genotype-by-environment interaction would require a multi-generational approach, I provide evidence of its plausibility in *L. inflata* in a short 2-year timeframe. This mechanism's role in maintaining genetic variation depends strongly on whether phenotypic variation exists, whether this variation occurs in traits that influence fitness and whether there is a genetic basis for this variation. I showed that the phenotypic variation in life-history traits among

replicated field-collected individuals results from the presence of multiple microsatellite genotypes in the population. It was also determined that microsatellite genotypes exhibit variance in relative fitness through time that is associated with life-history phenotypes. The next step towards providing evidence for the importance of fluctuating selection in this system will be to study additional field seasons to determine the extent of variance in relative fitness through multiple generations.

REFERENCES

Anderson, J. T., Panetta, A. M., & Mitchell-Olds, T. (2012). Evolutionary and ecological responses to anthropogenic climate change. *Plant Physiology, 160* (4): 1728-1740.

Antonelli, A. (2008). Higher level phylogeny and evolutionary trends in campanulaceae subfamily Lobelioideae. *Molecular Phylogenetics and Evolution, 46 (1)*: 1-18.

Ayala, F. J., & Campbell, C. A. (1974). Frequency- dependent selection. *Annual Review of Ecology and Systematics, 5* (1): 115-138.

Ayre, D., Whelan, R., & Reid, A. (1994). Unexpectedly high levels of selfing in the Australian shrub *Grevillea barklyana* (Proteaceae). *Heredity, 72 (2)*: 168-174.

Baskin, C. C., & Baskin, J. M. (1998). *Seeds- ecology, biogeography, and evolution of dormancy and germination.* San Diego, CA, USA: Academic Press.

Bell, G. (2010). Fluctuating selection: the perpetual renewal of adaptation in variable environments. *Philosophical Transactions of the Royal Society B., 365* (1537): 87-97.

Bell, G., & Gonzalez, A. (2009). Evolutionary rescue can prevent extinction following environmental change. *Ecology Letters, 12* (9): 942-948.

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society, 57 (1)*: 289-300.

Bowman, J.C. (1972). Genotype x environment interactions. *Annales de Génétique et de Selection Animale.* 4 (1): 117-123.

Bradshaw, A. (1965). Evolutionary significance of phenotypic plasticity in plants. *Advances in Genetics, 13 (21)*: 115-155.

Bull, J. J. (1987). Evolution of phenotypic variance. *Evolution, 41* (2): 303-315.

Bulmer, M. G. (1973). Maintenance of genetic variability of polygenic characters by heterozygous advantage. *Genetics Research Cambridge, 22 (1)*: 9-12.

Bulmer, M. G. (1989). Maintenance of genetic variability by mutation-selection balance: a child's guide through the jungle. *Genome, 31* (2): 761-767.

Cohen, D. (1966). Optimizing reproduction in a randomly varying environment. *Journal of Theoretical Biology, 12 (1)*: 119-129.

Culumber, Z., & Tobler, M. (2015). Spatiotemporal environmental heterogeneity and the maintenance of the tailspot polymorphism in the variable platyfish (*Xiphophorus variatus*). *Evolution, 70* (2): 408-419.

Curtsinger, J. W., Service, P. W., & Prout, T. (1994). Antagonistic pleiotropy, reversal of dominance, and genetic polymorphism. *American Naturalist, 144 (2)*: 210-228.

Ehrlén, J., & Műnzbergovà, Z. (2009). Timing of flowering: opposed selection on different fitness components and trait covariation. *American Naturalist, 173* (6): 819-830.

Ellner, S., & Hairston, N. G. (1994). Role of overlapping generations in maintaining genetic variation in a fluctuating environment. *American Naturalist, 143* (3): 403-417.

Erali, M., & Wittwer, C. T. (2010). High resolution melting analysis for gene scanning. *Methods, 50 (4)*: 250-261.

Falconer, D. S. (1989). *Introduction to quantitative genetics* (4th ed.). Essex: Longman.

Falconer, D.S. & MacKay, T. F. C. (1996). *Introduction to quantitative genetics*. Longman, London.

Fischer, E. K., Ghalambor, C. K., & Hoke, K. L. (2016). Plasticity and evolution in correlated suites of traits. *Journal of Evolutionary Biology, 29*: 991-1002.

Fisher R. A. (1930) *The genetical theory of natural selection*. 2d ed. Oxford Univ. Press, Oxford, U.K

Fisher, R. A., & Ford, E. B. (1947). The spread of a gene in natural conditions in a colony of the moth *Panaxia dominula* L. *Heredity, 1* (2): 143-174.

Geleta, M., & Bryngelsson, T. (2012). Population genetic analysis of *Lobelia rhynchopetalum* Hemsl. (Campanulaceae) using DNA sequences from ITS and eight chloroplast DNA regions. *The Scientific World Journal, 26471*: 1-10.

Gillespie, J. H. (1974). Natural selection for within-generation variance in offspring number. *Genetics, 76 (3)*: 601-606.

Gillespie, J. H. (1984). Pleiotropic overdominance and the maintenance of genetic variation in polygenic characters. *Genetics, 107* (2): 321-330.

Gillespie, J. H. (1991). *The causes of molecular evolution.* New York: Oxford University Press.

Goodwin, S. B., Maroof, M.A.S. & Allard, R. W. (1993). Isozyme variation within and among populations of Rhynchosporium secalis in Europe, Australia and United States. *Mycological Research*, 97 (1): 49-58.

Gossmann, T. I., Waxman, D., & Eyre-Walker, A. (2014). Fluctuating selection models and McDonald-Kreitman type analyses. *PloS one, 9* (1): e84540.

Gulisija, D., & Kim, Y. (2015). Emergence of long-term balanced polymorphism under cyclic selection of spatially variable magnitude. *Evolution, 69 (4)*: 979-992.

Haasl, R. J., & Payseur, B. A. (2013). Microsatellites as targets of natural selection. *Molecular Biology and Evolution, 30* (2): 285-298.

Hair, J. F., Anderson, R. E., & Tatham, R. L. (1987). *Multivariate data analysis.* New York, New York, USA: Macmillan Publishing Company.

Hairston, N. G., & Dillon, T. A. (1990). Fluctuating selection and response in a population of freshwater copepods. *Evolution, 44* (7): 1796-1805.

Hairston, N., & Walton, W. E. (1986). Rapid evolution of a life history trait. *Proceedings of the National Academy of Sciences USA, 83* (13): 4831-4833.

Haldane, J. B., & Jayakar, S. D. (1963). Polymorphism due to selection of varying direction. *Journal of Genetics, 58* (2): 237-242.

Hedrick, P. W. (1999). Antagonistic pleiotropy and genetic polymorphism: a perspective. *Heredity, 82* (2): 126-133.

Huerta-Sanchez, E., Durrett, R., & Bustamante, C. D. (2008). Population genetics of polymorphism and divergence under fluctuating selection. *Genetics, 178* (1): 325-337.

Hughes, P. W., Jaworski, A. F., Davis, C. S., Aitken, S. M., & Simons, A. M. (2014). Development of polymorphic microsatellite markers for indian tobacco, *lobelia inflata* (campanulaceae). *Applications in Plant Sciences, 2* (4): 1300096.

Hughes, P.W., & Simons, A.M. (2014a). Secondary reproduction in the herbaceous monocarp *Lobelia inflata*: time-constrained primary reproduction does not result in increased deferral of reproductive effort. *BMC Evolutionary Biology, 14* (15): 1-10.

Hughes, P.W., & Simons, A.M. (2014b). The continuum between semelparity and iteroparity: plastic expression of parity in response to season length manipulation in *Lobelia inflata*. *BMC Evolutionary Biology, 14* (90): 1-17.

Hughes, P.W., & Simons, A. M. (2015). Microsatellite evidence for obligate autogamy, but abundant genetic variation in the herbaceous monocarp *Lobelia inflata* (Campanulaceae). *Journal of Evolutionary Biology, 28* (11): 2068-2077.

Irwin, R.E., Strauss, S.Y., Storz, S., Emerson, A., & Guibert, G. (2003). The role of herbivores in the maintenance of a flower color polymorphism in wild radish. *Ecology, 84* (7): 1733-1743.

Johnson, M. T. (2006). Genotype-by-environment interaction leads to variable selection on life-history strategy in Common Evening Primrose (*Oenothera biennis*). *Journal of Evolutionary Biology, 20 (1)*: 190-200.

Johnstone, I. M., & Nadler, B. (2013). Roy's largest root test under rank-one alternatives. arXiv.1310.6581 .

Jordana, J., Ribo, O., & Pelegrin, M. (1993). Analysis of genetic relationships from morphological characters in Spanish goat breeds. *Small Ruminant Research, 12 (3)*: 301-314.

Kachi, N., & Hirose, T. (1983). Bolting induction in *Oenothera erythrosepala* Borbás in relation to rosette size, vernalization and photoperiod. *Oecologia, 60 (1)*: 6-9.

Kalisz, S. (1986). Variable selection on the timing of germination in *Collinsia verna* (scrophulariaceae). *Evolution, 40* (3): 479-491.

Keightley, P. D., & Halligan, D. L. (2009). Analysis and implications of mutational variation. *Genetica, 136* (2): 359-69.

Keightley, P. D., & Hill, W. G. (1990). Variation maintained in quantitative traits with mutation-selection balance: pleiotropic side-effects on fitness traits. *Proceedings of the Royal Society B., 242 (1304)*: 95-100.

Kelly, C. (1992). Reproductive phenologies in Lobelia inflata (Lobeliaceae) and their environmental control. *American Journal of Botony, 79* (10): 1126-1133.

Kinnison, M. T., & Hairston, N. G. (2007). Eco-evolutionary conservation biology: contemporary evolution and the dynamics of persistence. *Functional Ecology, 21 (3)*: 444-454.

Kliman, R., Sheehy, B., & Schultz, J. (2008). Genetic drift and effective population size. *Nature Education, 1* (3): 3.

Klinkhamer, P., de Jong, T., & Meelis, E. (1991). The control of flowering in the monocarpic perennial *Carlina vulgaris*. *Oikos, 61* (1): 88-95.

Lande, R. (1976). Natural selection and random genetic drift in phenotypic evolution. *Evolution, 30* (2): 314-334.

Loveless, M., & Hamrick, J. (1984). Ecological determinants of genetic structure in plant populations. *Annual Review of Ecology, Evolution, and Systematics, 15*: 65-95.

Lynch, M. (1987). The consequences of fluctuating selection for isozyme polymorphisms in daphnia. *Genetics, 115* (4): 657-669.

Lynch, M. (2010). Evolution of the mutation rate. *Trends in Genetics, 26 (8)*: 345-352.

Lynch, M., & Walsh, B. (1998). *Genetics and analysis of quantitative traits.* Sunderland, MA: Sinauer Associates.

Markert, C. L., & Moller, F. (1959). Multiple forms of enzymes: tissue, entogenetic, and species-specific patterns. *Proceedings of the National Academy of Sciences USA, 45 (5)*: 753-763.

Mason, A. S. (2015). SSR genotyping. In I. B. (ed), *Plant Genotyping* (pp. 77-89). Springer, New York, New York.

Mitchell-Olds, T. (1992). Does environmental variation maintain genetic variation? A question of scale. *Trends in Ecology and Evolution, 7 (12)*: 397-398.

Mojica, J. P., Lee, Y. W., Willis, J. H., & Kelly, J. K. (2012). Spatially and temporally varying selection on intrapopulation quantitative trait loci for a life history trade-off in mimulus guttatus. *Molecular Ecology, 21* (5): 3718-3728.

Mousseau, T. A., & Roff, D. A. (1987). Natural selection and the heritability of fitness components. *Heredity, 59* (2): 181-197.

Mousseau, T., Endler, J. A., & Sinervo, B. (2000). *Adaptive genetic variation in the wild.* New York: Oxford University Press.

Mueller, L. D., Barr, L. G., & Ayala, F. J. (1985). Natural selection vs. random drift: evidence from temporal variation in allele frequencies in nature. *Genetics, 111* (3): 517-554.

Nordborg, M., Charlesworth, B., & Charlesworth, D. (1996). Increased levels of polymorphism surrounding selectively maintained sites in highly selfing species. *Proceedings of the Royal Society B., 263* (1373): 1033-1039.

O'Hara, R. B. (2005). Comparing the effects of genetic drift and fluctuating selection on genotype frequency changes in the scarlet tiger moth. *Proceedings of the Royal Society B., 272* (1559): 211-217.

Old, R., Sewell, R., Norris, M., & Joyce, C. (1993). Heterozygote advantage: why are some deleterious genes so common. *The Lancet, 341* (8839): 214.

Olijynk, A. M. & Nelson, W. A. (2012). Positive phenotypic correlations among life-history traits remain in the absence of differential resource ingestion. *Functional Ecology,* 27 (1): 165-172.

Primack, R., & Kang, H. (1989). Measuring fitness and natural selection in wild plant populations. *Annual Review of Ecology and Systematics, 20*: 367-396.

Rees, M., Childs, D. Z., Rose, K. E., & Grubb, P. J. (2004). Evolution of size-dependent flowering in a variable environment: partitioning the effects of fluctuating selection. *Proceedings of the Royal Society B., 271* (1538): 471-475.

Reznick, D. (1985). Costs of reproduction: an evaluation of empirical evidence. *Oikos*, 44 (2): 257-267

Robertson, C. (1895). The philosophy of flower seasons, and the phaenological relations of the entomophilous flora and the anthophilous insect fauna. *American Naturalist, 29 (338):* 97-117.

Roff, D. A. (1992). *The evolution of life histories.* New York: Chapman & Hall.

Roff, D. A. (1997). *Evolutionary quantitative genetics.* New York: Chapman & Hall.

Roff, D. A. (2000). Trade-offs between growth and reproduction: an analysis of the quantitative genetic evidence. *Journal of Evolutionary Biology,* 13 (3): 434-445.

Roff, D.A, & Fairbairn, D. (2007). The evolution of trade-offs: where are we? *Journal of Evolutionary Biology, 20* (2): 433-447.

Roff, D., & Mousseau, T. (1987). Quantitative genetics and fitness: lessons from drosophila. *Heredity, 58* (1): 103-118.

Ronsheim, M. L. (1996). Evidence against a frequency- dependent advantage for sexual reproduction inallium vineale. *American Naturalist, 147* (5): 718-734.

Rose, M. R. (1982). Antagonistic pleiotropy, dominance and genetic variation. *Heredity, 48* (1): 63-78.

Russell, P. J., Wolfe, S., Hertz, P., Starr, C., Fenton, M., Addy, H., et al. (2010). *Biology* (1st canadian ed.). Toronto: Nelson Education.

Santiago, E., & Sanjuán, R. (2003). Evolution: climb every mountain. *Science, 302* (5653): 2074-2075.

Schlichting, C. D. (1986). The evolution of phenotypic plasticity in plants. *Annual Review of Ecology and Systematics, 17:* 667-693.

Segar, J., & Brockmann, H. J. (1987). What is Bet-hedging? *Oxford Surveys in Evolutionary Biology, 4:* 182-211.

Selkoe, K. A., & Toonen, R. J. (2006). Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. *Ecology Letters, 9 (5):* 615-629.

Sherry, A., & Henson, R. K. (2005). Conducting and interpreting canonical correlation analysis in personality research: a user-friendly primer. *Statistical developments and applications, 84* (1): 37-48.

Simons, A. M. (2009). Fluctuating natural selection accounts for the evolution of diversification bet hedging. *Proceeding of the Royal Society B., 276 (1664):* 1987-1992.

Simons , A. M. (2011). Modes of response to environmental change and the elusive empirical evidence for bet hedging. *Proceedings of the Royal Society B., 278* (1712): 1601-1609.

Simons, A. M., & Johnston, M. O. (2000). Plasticity and the genetics of reproductive behaviour in the monocarpic perennial, *Lobelia inflata* (Indian tobacco). *Heredity, 85* (4): 356-365.

Sinervo, B., & Doughty, P. (1996). Interactive effects of offspring size and timing of reproduction on offspring reproduction: experimental, maternal and quantitative genetic aspects. *Evolution, 50:* 1314-1327.

Skelly, D. K., Joseph, L. N., Possingham, H. P., Freidenburg, L. K., Farrugia, T. J., Kinnison, M. T., et al. (2007). Evolutionary responses to climate change. *Conservation Biology, 21* (5): 1353-1355.

Slatkin, M. (1974). Hedging ones evolutionary bets. *Nature, 250*: 704-705.

Sletvold, N., & Grindeland, J. M. (2007). Fluctuating selection on reproductive timing in *Digitalis purpurea*. *Oikos, 116 (3):* 473-481.

Springate, D. A., & Kover, P. X. (2014). Plant responses to elevated temperatures: a field study on phenological sensitivity and fitness responses to simulated climate warming. *Global Change Biology, 20 (2):* 456-465.

Stanley, S. M., & Yang, X. (1987). Approximate evolutionary stasis for bivalve morphology over millions of years; a multivariate, multilineage study. *Paleobiology, 13* (2): 113-139.

Stearns, S. (1989). Trade-offs in life-history evolution. *Functional Ecology, 3* (3): 259-268.

Stearns, S. (1992). *The evolution of life histories.* Oxford: Oxford University Press.

Stearns, S. (1977). The evolution of life history traits: a critique of the theory and a review of the data. *Annual Review of Ecology and Systematics, 8:* 145-171.

Svardal, H., Rueffler, C., & Hermisson, J. (2015). A general condition for adaptive genetic polymorphism in temporally and spatially heterogeneous environments. *Theoretical Population Biology, 99:* 76-97.

Tabachnick, B. G., & Fidell, L. S. (2001). *Using multivariate statistics.* (Fifth, Ed.) Boston: Pearson.

Turelli, M. (1984). Heritable genetic variation via mutation selection balance- Lerch's zeta meets the abdominal bristle. *Theoretical Population Biology, 25 (2)*: 138-193.

Via, S., & Lande, R. (1987). Evolution of genetic variability in a spatially heterogeneous environment. *Genetics Research, 49 (2):* 147-156.

Via, S., Gomulkiewicz, R., De Jong, G., Scheiner, S. M., & Schlichting, C. D. (1995). Adaptive phenotypic plasticity: consensus and controversy. *Trends in Ecology and Evolution, 10 (5):* 212-217.

Vieira, M. L., Santini, L., Diniz, A. L., & Munhoz, C. d. (2016). Microsatellite markers: what they mean and why they are so useful. *Genetics and Molecular Biology, [online]*.

Walker, P. L. (2008). Sexing skulls using discriminant function analysis of visually assessed traits. *American Journal of Physical Anthropology, 136* (1): 39-50.

Winton, L., Hansen, E., & Stone, J. (2006). Population structure suggests reproductively isolated lineages of *Phaeocryptopus gaeumannii. Mycologia, 98 (5):* 781-791

Wittwer, C., Reed, G., Gundry, N., Vandersteen, J., & Pryor, R. (2003). High resolution genotyping by amplicon melting analysis using LC Green. *Clinical Chemistry, 49* (6): 853-860.

Wright, S. J. (1931). Evolution in Mendelian populations. *Genetics,* 16 (2): 97-159.

Wright, S. J. (1932). The roles of mutation, inbreeding, crossbreeding and selection in evolution. *Proceedings of the VI International Congress of Genetics, 1*: 356-366.

Zaykin, D. V., Zhivotovsky, L. A., Westfall, P. H., & Weir, B. S. (2002). Truncated product method for combining p-values. *Genetic Epidemiology, 22 (2):* 170-185.

Zhang, X., & Hill, W. (2005). Genetic variability under mutation selection balance. *Trends in Ecology and Evolution, 20* (9): 468-470.

Zuckerlandl, E., & Pauling, L. (1965). *Evolutionary divergence and convergence in proteins. In: Evolving Genes and Proteins.* (V. B. Vogel, Ed.) New York: Academic Press.