Florivory and Clonal Reproduction of *Clintonia borealis*: A Study Using Modeling and a Natural Experiment

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THESIS

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JMP

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LIST OF ABBREVIATIONS

AIC	Akaike Information Criterion
AINL	Apostle Islands National Lakshore
DNA	Deoxyribonucleic Acid
DAPC	Discriminant Analysis of Principal Components
FNA	Flora of North America
MLG	Multilocus Genotype
PCoA	Principal Coordinate Analysis of Variance
SBDNL	Sleeping Bear Dunes National Lakeshore
SHLT	Shirley Heinze Land Trust

SUMMARY

The balance of sexual and clonal reproduction has important implications for plant populations. While all plants reproduce sexually via flowers, most plants also have some form of clonal reproduction. Environmental factors such as florivory or geographic isolation can shift the reproductive balance of a population. Highly clonal populations may be difficult to differentiate from more clonally diverse populations since genets are not always morphologically distinct. My work aimed at quantifying the effect florivory and isolation can have on the clonal diversity of *Clintonia borealis*, bluebead lily, populations.

In Chapter 1, I review and compare sexual and clonal reproduction in plants. The primary difference between the two modes of reproduction is that sexual reproduction produces genetically unique offspring while clonal reproduction produces offspring that are genetically identical to the parent. This distinction is fundamental to studying plant populations since it may be impossible to differentiate reproductive units without molecular analysis. In plant studies, a single plant, usually identified as one stem protruding from the ground, is referred to as a ramet. The term ramet is in contrast to the term genet, which is defined by all the ramets that share a unique genotype. Hence, in plant populations that do not exhibit clonal growth, each ramet is an individual genet. However, the number of ramets belonging to a genet can vary widely in populations of plants which do exhibit clonal growth. Chapter 1 also contains a description of the study species, *Clintonia borealis*, including it's reproductive biology, ecology, and phylogenetic relationships.

In Chapter 2, I describe an agent-based model written in order to investigate the influence florivory can have on the ramet demography and clonal diversity of bluebead lily populations.

Agent-based modeling was chosen because of its ability to track the fate of genets as well as ramets. The model was parameterized largely with published data from excavation studies. While florivory had no significant effect on ramet density, florivory kept genet density an order of magnitude lower than in runs without florivory. The model suggested that florivory can have a substantial impact on the balance between sexual and clonal reproduction.

One interesting result of the model was the influence of altering the clonal reproduction rate on the survival rate of populations. While altering the seedling recruitment rate did not change the survival rate of populations, lowering the clonal reproduction rate dramatically decreased the survival rate of populations. This supports the theory that for certain plant species clonality provides reproductive assurance in situations such as colonizing and remnant populations. The reproductive assurance allows the populations to persist even when sexual reproduction is absent due to various ecological factors such as florivory, lack of compatible mates, or lack of pollinators.

In Chapter 3, a natural experiment was used to evaluate the impact of florivory on bluebead lily populations. Bluebead lily populations exist on most Great Lakes islands while *Odocoileus virginianus*, white-tailed deer, have historically populated only some of the islands. Patches were sampled from six islands at Apostle Islands National Lakeshore (AINL) and Sleeping Bear Dunes National Lakeshore (SBDNL), and the mainland at AINL. Patches were sampled regularly in a 1 m^2 square.

Microsatellite markers were developed in order to assign leaf samples to genets and characterize the clonal diversity of the patches. Microsatellite markers are useful because of their codominance, putative neutrality, and ease of genotyping. A single genome-wide shotgun

sequencing run was performed on an Illumina Mi-Seq. Analysis of the paired-end reads provided 196,416 potential loci. After optimization and testing for polymorphism, ten loci were characterized, of which three were di-, one was tri-, one was tetra-, one was penta-, and four were hexa-nucleotide motifs. The number of alleles for the 10 loci varied from five to 15, with a mean of 7.60. Observed heterozygosities ranged from 0.283 to 0.944, and expected heterozygosities ranged from 0.475 to 0.788.

The microsatellite markers were used to genotype the 350 samples from AINL and SBDNL. The samples were assigned to 118 multilocus genotypes (MLG's). The genet-to-ramet ratio (G:N) averaged 0.104 genets per ramet on islands with deer, and 0.458 genets per ramet on islands without deer. A linear mixed effects model was used to determine significance of the difference (ΔAICc = 3.94, Akaike weight = 0.878). The difference in clonal structure associated with the presence of deer illustrates the impact florivory can have on the reproductive mode of clonal plant species.

In Chapter 4, a remnant bluebead lily population in northwest Indiana was analyzed in order to determine what conservation practices might best protect the population. Concerns about the population size and geographic distance from conspecific populations generated interest among conservation practitioners in translocating genotypes into the population. Translocation is the incorporation of individuals from distinct populations into a population that is thought to be vulnerable to reduced genetic diversity. However, the Indiana bluebead lily population is at the southern extent of the species range and is unlikely to persist in the face of climate change. While the population may have adaptations that have allowed it to survive in a slightly warmer climate than its conspecifics, the isolation of the population makes it unlikely that those

adaptations will be transmitted to the rest of the species. The assisted migration of offspring northward may preserve the evolutionary potential of the population better than reinforcing the genetic diversity of the population via translocation. Assisted migration is generally seen as the movement of leading edge populations into habitats that have become or will soon be within the bioclimatic envelope of the species, but here I consider the assisted migration of a lagging edge, remnant population into the core range of the species.

The Indiana bluebead lily population consisted of between 41 and 86 ramets from 1999-2012 according to volunteer collected data. However, due to the clonal nature of bluebead lily, the number of ramets is not an accurate measure of the number of genets, and there was concern that the population consisted of only two or three genets. Leaf samples were taken from every ramet in the population, and extracted DNA was genotyped with 10 microsatellite loci. All fruit were also collected and dissected to count viable seed. The data were compared with random samples taken from AINL and SBDNL.

Results showed that the Indiana population was comprised of 12 genets, which, from fruit and seed set, seemed to be reproducing sexually at rates consistent with previous work on bluebead lily. Analysis of seed set by genet found two genets, E3 and E6, to have significantly higher seed set than other genets, which implies that their offspring may be important to future sexual reproduction in the population. Genetic comparisons of the populations showed SBDNL and Indiana to be highly divergent from AINL. Further analyses showed the Indiana population to have reduced allelic richness, which may indicate loss of alleles due to genetic drift or local adaptation. Translocation of new genotypes into the population would increase the population's genetic diversity, but maladapted genotypes may not be able to survive at the southern edge of

the species range long enough to have a significant effect. Alternatively, the evolutionary potential of the Indiana population could be preserved by the assisted migration of offspring northward, into the current species range. If the Indiana population does have adaptations for slightly warmer conditions, it could aid the species in the face of climate change.

CHAPTER 1: *CLINTONIA BOREALIS* AS A STUDY SPECIES FOR INVESTIGATING SHIFTS IN SEXUAL AND CLONAL REPRODUCTION

1.1 Introduction

Many plant species balance resource allocation between sexual and asexual reproduction. Ecological factors that alter this balance can have important effects on plant populations. By eliminating flowers, floral herbivory (florivory) reduces sexual reproduction and consequently shifts reproductive allocation towards asexual reproduction. Over time, the clonal structure of the population may change. Similarly, long term isolation of small populations can lead to the loss of genetic diversity through genetic drift or reduced reproductive output due to inbreeding depression.

Here, I review sexual and clonal reproduction in plants with respect to evolutionary advantages and disadvantages of each, and how sexual and clonal reproduction can influence each other. I describe the utility of *Clintonia borealis*, bluebead lily, as a model of the balance between sexual and clonal reproduction, especially since bluebead lily flowers are a preferred food of *Odocoileus virginianus*, white-tailed deer. Since mate availability is an important factor in floral reproduction, I review studies that have investigated self-sterility in bluebead lily. I conclude by briefly describing the history of the taxonomic placement of *Clintonia* and recent phylogenetic findings that place the genus firmly within Liliaceae.

1.2 Sexual and Clonal Reproduction in Plants

While flowering plants reproduce sexually, most species also exhibit some form of clonal growth (Klimeš et al. 1997). Sexual and clonal reproduction each provide different ecological and evolutionary advantages (Vallejo-Marín et al. 2010). Sexual reproduction involves the

formation and combination of gametes which produces genetically unique offspring. Clonal reproduction involves the multiplication of vegetative parts which produces offspring genetically identical to the parent. Plants have evolved multiple forms of clonal reproduction in many different lineages (Fischer and van Kleunen 2002). I am limiting my focus in this chapter to vegetative reproduction, such as produced by rhizomes or stolons. Both sexual and clonal reproduction produce new individual plants, or ramets, but only sexual reproduction produces new genotypes. Ramets sharing the same genotype are collectively known as a genet. Evaluation of plant population dynamics requires discerning ramets into genets because, at one extreme, a group of ramets belonging to a single genet is not a population but an individual.

The evolutionary advantages of sexual reproduction have been the subject of a long and ongoing debate (Barton and Charlesworth 1998). Sexual reproduction maintains genetic diversity and allows for the formation of new genetic combinations which may provide adaptability to novel or changing environments. For most plants, a significant advantage of sexual reproduction is the relatively long distance dispersal of seed (Howe and Smallwood 1982). Seed dispersal can distribute offspring much farther from the parent ramet than most forms of vegetative reproduction. However, as sessile organisms, plants are largely dependent upon stochastic processes for sexual reproduction. In most cases, flowers require an agent (e.g. wind or animals) in order to transfer pollen and successfully combine gametes. The patterns of the pollination depend upon the behavior of the pollinator, the distribution of conspecifics, and the genetic relationship between mating flowers. In highly isolated populations, such as in the case of colonizing or remnant populations, floral function may suffer from a lack of compatible mates due to self-incompatibility or inbreeding depression.

On the other hand, clonal growth provides plants several advantages for local population growth. Clonal growth can provide a foraging mechanism to explore the local conditions and move the genet toward more favorable environmental conditions or protect nutrient rich patches from interspecific competition (Hutchings 1988). Connections between ramets can persist for multiple years (Pitelka et al. 1985), and the resultant networks may act as conduits for resource allocation and division of labor among clonal sections (Stueffer et al. 1996). Clonal growth also protects the genet from risks associated with demographic stochasticity by spreading the risk of death among many ramets (Klimeš et al. 1997). Clonal growth also provides reproductive assurance when sexual reproduction is reduced or inhibited by extrinsic factors (Silvertown 2008).

The architecture of clonal growth in plants exists on a continuum between two distinct growth forms: phalanx and guerilla (Lovett Doust 1981). Phalanx type clonal architecture is characterized by tightly clustered ramets descendent from a single genet. The tight clustering of the phalanx architecture provides for the protection and consumption of limited resources in a patchy environment (Klimeš et al. 1997). That is, a seedling that germinates in a favorable habitat spreads clonally in a relatively tight spatial scale such that the genet monopolizes the resource which made the habitat favorable. Guerilla type clonal architecture is characterized by loosely clustered ramets descendent from a single genet. The loose aggregation of ramets in guerilla clonal architecture is thought to allow for greater spread of a genet through a homogeneous environment (Klimeš et al. 1997). Genets are not impeded from introgressing into each other, so clonal diversity should be higher in populations of species with guerilla clonal architecture than in phalanx type populations.

Clonal growth can influence pollen availability by effectively decreasing the number of compatible mates in an area (Eckert 2002). In self-compatible species, since low clonal diversity implies a high density of ramets of the same genet in an area, low clonal diversity increases the rate of geitonogamous mating, pollination by a flower belonging to the same genet. Self-pollination can lead to genetic erosion and inbreeding depression. Inbreeding depression can result in lower seed set in the population and further diminish the importance of sexual reproduction in the population (Barrett 2015). Self-incompatible species have mechanisms that prevent seed production from self-fertilization events. By avoiding self-fertilization, these species can avoid the negative consequences of genetic erosion and inbreeding. However, successful fertilization requires a compatible genotype with which to mate, and a lack of compatible mates can lead to pollen limitation. Further, if the population has low clonal diversity, self-pollen can outnumber outcross pollen and result in a swamping effect, effectively lowering seed set (Vallejo-Marín and Uyenoyama 2004). Colonizing and remnant populations may consist of one or a few closely related genets and thus lack compatible mates in the case of self-incompatible species. Sexual reproduction can be reduced in colonizing and remnant populations by inbreeding depression in the case of self-compatible species.

The relative importance of sexual versus clonal reproduction varies significantly by species (Silvertown 2008). For instance, in models compiled by Silvertown et al. (1993), longevity and ramet growth were highly important to forest herbs whereas the influence of fecundity (sexual reproduction) was negligible. Longevity, growth, and fecundity were more or less important in species from different habitats and with different life histories (Klimeš et al. 1997). Within a species, the reproductive balance of a ramet can be affected by resource allocation factors. Experimentally reduced floral function was shown to increase clonal

reproduction, especially when female flower function was removed (Van Drunen and Dorken 2012). Resources not used in fruit and seed production were instead used to produce more clonal ramets. Flower function in natural populations can be reduced by limited pollen availability or by direct removal of the flower by another organism, such as in florivory.

Florivory has been shown to have a significant influence on clonal growth (Tobler et al. 2006; Van Drunen and Dorken 2012). Florivory can affect clonal diversity in two ways. First, the consumption of flowers reduces the potential for sexual reproduction. Lowering reproductive potential leads to a reduction in fruit and seed production. Fewer seeds diminishes seedling recruitment and results in a lowered recruitment rate of new genets. Without new genets, clonal diversity cannot increase. Second, as demonstrated by experiments, the resources required for fruit and seed production are available for vegetative growth. In clonal species, the shift in available resources increases clonal growth which results in more ramets belonging to the same genet.

1.3 *Clintonia borealis* as a model organism for clonal plant species

The woodland herb species, bluebead lily, presents a good model for long-lived clonal species and their interactions with the environment. Bluebead lily is a long-lived perennial native to boreal forest and woodland habitats of eastern North America. The core range of bluebead lily extends from Minnesota, USA, to the Atlantic Ocean, and from Pennsylvania, USA, to Quebec, Canada. In the eastern portion of its range, bluebead lily extends southward through Appalachia at higher elevations. In the western portion of its species range, it is often a generalist species inhabiting many different forest communities. However, at the southern extent of its range,

bluebead lily is generally restricted to fen and bog type habitats (Swink, F, Wilhelm 1994; Reznicek, AA, Voss, EG, Walters 2011).

Ramets of bluebead lily produce 1-4 basal leaves. Sexual ramets also produce an inflorescence on a peduncle which extends vertically from the center of the ramet. The inflorescence extends 50 cm above the basal leaves and bears 3-5 flowers (Galen et al. 1985). Each flower contains 10-14 ovules (Dorken and Husband 1999). The flowers are pale yellow and are pollinated by bumblebees (Pitelka et al. 1985). The fruit characteristics are consistent with bird seed dispersal (Eriksson 1997), but no studies have identified any species interacting with the mature fruit. It is likely that bluebead lily does not constitute a primary component of any species' diet, as mature fruit have been observed on peduncles as late as early December (personal observation).

Underground rhizomes produce clonal ramets in bluebead lily. The frequency and architecture of clonal reproduction (producing secondary ramets) were measured by two excavation studies (Pitelka et al. 1985; Angevine and Handel 1986). Pitelka et al. (1985) documented ramet demography of 1498 ramets in 16 patches in Maine, USA. Excavations of the patches were performed to investigate frequencies of clonal growth in relation to size and production of flowers. Frequency of the production of both flowers and secondary rhizomes were found to be positively correlated (both, $r = 0.57$, $p < 0.05$) with nutrient availability as measured by mean soil resistance (Pitelka et al. 1985). These correlations suggest that nutrient availability has a significant effect on the growth of bluebead lily but it should not favor one form of growth over the other. Angevine and Handel (1986) excavated 194 clonal fragments in New Hampshire, USA in order to describe the clonal architecture of bluebead lily in great detail. They confirmed previous studies of bluebead lily's guerilla style of clonal architecture.

Flowers of bluebead lily are a primary component of the spring diet of white-tailed deer. While the diet of white-tailed deer shifts during the year, the spring diet of white-tailed deer consists primarily of wildflowers, particularly members of Liliaceae (McCaffery et al. 1974; Miller et al. 1992; Augustine 1997). Bluebead lily, a member of Liliaceae (see below), has been used as an indicator species in studies investigating the impact of deer overabundance on northern woodland plant communities. One study compared vegetation communities after 50 years of deer overabundance and found dramatic differences in species composition (Wiegmann and Waller 2006). Bluebead lily was a slight loser in the study based on presence/absence data, but it is likely that the strongly clonal nature of bluebead lily and its longevity hid the true impacts on bluebead lily populations. Balgooyen and Waller (1995) showed a threshold effect of peduncle height based on historic deer densities. However, their study did not consider the impact of reduced floral function on clonal diversity nor the impact of clonality on the longevity of the bluebead lily populations.

1.4 Self-sterility in *Clintonia borealis*

Self-incompatibility is an important factor in plant breeding systems and the maintenance of genetic diversity in many plant species. Self-incompatibility can play a role in the avoidance of geitonogamous selfing (Honnay and Jacquemyn 2008). While mechanisms of self-incompatibility for bluebead lily have been suggested, studies of the bluebead lily mating system have shown mixed results.

Four studies have investigated self-sterility in bluebead lily, with all of them exhibiting low seed set from self-pollination compared to out- or cross-pollination. Barrett and Helenurm (1987) studied the reproductive biology of 12 boreal forest herbs in central New Brunswick,

Canada. In bluebead lily, they found that self-pollination produced 57% fewer seeds per fruit than cross-pollination. Galen et al. (1985) studied the reproductive biology of bluebead lily patches in eastern Ontario, Canada, where self-pollination resulted in 32% seed set and cross-pollination resulted in 91% seed set. However, they found high variance in seed set between patches within treatments. Galen and Weger (1986) re-evaluated the results of Galen et al. (1985) for trends in seed set and seed weight with respect to assumed clonal identity. Clonal identity was determined by geographic distance such that ramets at least 10 m apart from one another were defined as different genets. Average seed set was lower in selfed than outcrossed flowers in their study, but only three of the fifteen clones showed statistical significance in pairwise contrasts between selfed and outcrossed flower seed set.

Dorken and Husband (1999) found highly significant differences in seed set among pollination treatments and only marginally significant differences among patches. They suggested that differences in selfing seed set among patches was probably influenced by resource availability. As opposed to previous studies, they coarsely defined genotypes with electrophoretic phenotypes of the enzyme phosphoglucose isomerase and were able to cross-pollinate with pollen from genotypes that they knew to be different from the flower genotype. Comparisons between fertilization frequency and seed set proportions suggest a prezygotic mechanism of self-sterility. However, differences in the rate of endosperm division and a difference in seed size between selfed and outcrossed seeds indicate that postzygotic mechanisms are also reducing seed set upon selfed bluebead lily flowers.

1.5 Phylogeny of *Clintonia borealis*

The genus *Clintonia* was first described by Constantine Samuel Rafinesque in 1832 who named the genus after his friend Dewitt Clinton (Mosquin 2012). Most of the 30 species he described in the genus were later reduced to synonyms of 5 currently accepted species. Taxonomic relationships underwent many changes during the 1980's and 1990's, with *Clintonia* frequently being placed in different orders, families, subfamilies, and tribes (*reviewed in* Hayashi et al. 2001).

Attempts to resolve taxonomic differences in Liliaceae by analyzing the *rbcL* and *matK* genes in select species showed *Medola* and *Clintonia* to be closely related, sister to core Liliaceae genera (Hayashi and Kawano 2000; Hayashi et al. 2001). Hayashi et al. (2001) made comparisons within the genus based on molecular data along with morphological and cytological traits. They concluded that *C. udensis*, the only Asian species, is basal to the four North American species. All five species displayed 2n = 28 chromosome numbers, although 2n = 14 was found in some individuals of *C. udensis* and some aneuploids (2n = 32) were found in *C. borealis* (Utech 1975; Li et al. 1996). Hayashi et al. (2001) suggested that 2n = 14 is the ancestral karyotype which became 2n = 28 through an ancient allopolyploid event. Flow cytometry shows *C. borealis* has a very large genome, pg/2C = 37.7, or roughly 36.9 Bbp (Bharathan et al. 1994).

Further analyses of the *rbcL* and *ndhF* genes generated more support for a *Clintonia* and *Medola* subfamily, Medeolioideae, and their relationship within Liliaceae (Givnish et al. 2006), shown in Figure 3. Analyses of sequenced plastid genomes and fossil evidence further supported the phylogeny of Medeolioideae described by Hayashi and Givnish (Givnish et al. 2016). From the plastid genomes, researchers deduced that Liliales diverged from other monocots some 124

Mya in Australia (Givnish et al. 2016). Givnish et al. (2016) suggested that Liliaceae diverged from Liliales in western North America 67 Mya. Ancestors of Medoloidae appeared in eastern North America by 50 Mya then migrated westward, with *Clintonia* migrating to East Asia through Beringia some 30 Mya.

1.6 Conclusion

The distinction between population growth resulting from sexual reproduction or clonal growth is important. Clonal growth increases population size in terms of the number of ramets in a population, but it is a fundamentally different type of population growth from sexual reproduction. Due to the identical genetic constitution of the offspring ramets, clonal growth promotes offspring with the exact same adaptive potential as the parent. While clonal growth spreads the risk of demographic death among its ramets, all of the ramets are susceptible to the same environmental stochasticities (diseases, climate changes, etc.) which may befall the population. Further, extensive clonal growth or prolonged isolation may lead to genetic erosion due to forced self-mating or mating between closely related individuals. Sexual reproduction produces novel genotypic combinations which maintain genetic diversity in a population. Genetic diversity in a population may provide adaptability to environmental stochasticity.

Factors that influence clonal structure can have long term effects on the population dynamics of clonal species. Florivory and isolation can both have strong influences on clonal structure. By removing floral function and increasing clonal reproduction, florivory lowers the clonal diversity of a population. Prolonged florivory combined with reduced clonal diversity can have a cascading effect that shifts the population into a largely asexual reproductive mode. Populations dominated by a few genets can lack the adaptive potential to withstand the effects of

environmental stochasticity. The isolation of a population reduces the availability of mates and makes the population vulnerable to loss of genetic diversity due to genetic drift. Loss of genetic diversity can lead to the expression of deleterious alleles which would produce maladapted offspring. Translocation of new genotypes into the population can introduce genetic diversity into an isolated population. Translocation, however, carries the risk of introducing genotypes that are maladapted for local environmental conditions.

In Chapter 2, I describe an agent-based model designed to determine the effects of florivory on the population dynamics of bluebead lily. Specifically, I focus on how florivory influences ramet and genet growth and the implications thereof. In Chapter 3, I test the results of the model by sampling bluebead lily patches on islands in the Great Lakes with and without white-tailed deer. Samples were genotyped with DNA microsatellite markers developed for the study. Genotyped samples were aggregated into genets in order to determine the effects of white-tailed deer on clonal diversity. In Chapter 4, I census a highly isolated, remnant population of bluebead lily in northwest Indiana, USA. Genetic data was analyzed to address conservation concerns regarding the population. Both translocation and assisted migration were considered as conservation strategies. Translocation would introduce new genotypes into the population and perhaps increase its adaptive potential and long-term persistence. Assisted migration would move offspring from the isolated population northwards into more contiguous populations and reconnect the isolated lineage with the species on a whole.

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CHAPTER 2: THE IMPACT OF FLORIVORY ON THE CLONAL STRUCTURE OF BLUEBEAD LILY SHOWN WITH AN AGENT-BASED MODEL

2.1 Introduction

Ecologists use mathematical models to direct their field research and discoveries from field research to refine their models. Since the early 1980's, mathematical models have informed a substantial amount of research in plant clonality and the ecological and evolutionary consequences of plants shifting between sexual and asexual reproduction (Oborny et al. 2012). While each species' potential to shift resource allocation toward sexual or asexual reproduction will depend upon its reproductive biology, most plant species are capable of some form of vegetative reproduction (Klimeš et al. 1997; Vallejo-Marín et al. 2010). In these species, ecological factors will determine current resource allocation and reproductive success (Silvertown 2008). For instance, loss of floral function, such as occurs with florivory, can increase asexual reproduction while reducing or eliminating sexual reproduction. While these shifts have been demonstrated empirically for individual plants (Dorken and van Drunen 2010; Chen et al. 2015), studies of how the effect of florivory scales to the population level are largely lacking (Tobler et al. 2006).

Clonality provides plants with reproductive assurance even when environmental conditions inhibit sexual reproduction. Sexual reproduction can be limited when a seedling pioneers a new habitat, flowers lack pollinators, or florivory reduces or eliminates flowers (Honnay and Bossuyt 2005). While inhibition of sexual reproduction can be offset by clonal reproduction, clonal offspring inherit the identical genetic composition of the parent ramet. An increase in the clonality of a population effectively decreases the genetic diversity of the population because a greater proportion of ramets in the population belong to identical

genotypes. Mating between ramets of identical genotypes, geitonogamous mating, can lead to a reduction in seed set or inbreeding depression (Charpentier 2002). Diminished seed set further reduces the number of offspring formed by meiotic recombination by reducing the number of seed available to germinate and produce new genets. Genetic erosion may increase the expression of deleterious alleles in a population which would lead to lower fitness. Ecological interactions such as florivory are known to shift resource allocation at the individual level, but to what extent can florivory shift an entire population towards clonality? In such an extreme case, a single genet may dominate an entire patch, and an entire population may eventually be comprised of one or a very few genets. The monoclonal patch, however, may appear indistinguishable from a multiclonal patch.

I developed a model based on empirical data from excavation studies of bluebead lily, *Clintonia borealis,* to investigate the relationship between florivory and clonal structure of populations. The relationship will depend on the relative rates of sexual and clonal reproduction. Since factors such as variability among individuals and local interactions influence these rates, I used an agent-based model to simulate the system (Grimm et al. 2006). Agent-based models can incorporate any number of parameters and these parameters can be adjusted to consider the overall influence each parameter has on the modeled system (Grimm 1999). In the case where the system involves multiple forms of reproduction, the agent-based model can be designed to track the mode of reproduction that produces an offspring. Subsequent analyses can determine the degree to which specific parameters, including florivory rates, influence the relative contributions of sexual and clonal reproduction to the composition of the population.

Bluebead lily presents an excellent species for investigating how florivory affects the balance of reproductive modes at the population level. First, bluebead lily reproduces both

sexually, via perfect flowers, and asexually, via rhizomes. All plant species reproduce sexually and most have some form of vegetative reproduction (Klimeš et al. 1997). Therefore, the results should be broadly applicable to many plant species. Second, excavation studies of bluebead lily provide empirical data on which to base many of the requisite parameters (Pitelka et al. 1985; Angevine and Handel 1986). With solid rationale for establishing key parameters, missing parameters can be approximated or tested over a range of values to check the robustness of the model. Last, deer preferentially consume bluebead lily flowers during the spring (McCaffery et al. 1974; Miller et al. 1992; Augustine 1997). Bluebead lily was one of 66 species incorporated in Lefkovitch model analyses of clonal plant species (Silvertown et al. 1993). While the model described the relative importance of seed and seedling recruitment versus clonal reproduction to overall population growth, the model did not differentiate the products of sexual reproduction from the products of vegetative reproduction. Ramet population growth is an important life history factor, but the underlying genotypic diversity and clonal structure can have important implications for the evolutionary trajectory of the population (Barrett 2015).

I addressed two main questions for this agent-based model of the interaction between bluebead lily and white-tailed deer florivory. First, how does deer florivory impact ramet density? While sexual reproduction is reduced via florivory, non-flowering ramets may produce compensatory secondary ramets through clonal reproduction such that population sizes, as counted by ramet number, may appear the same in populations with and without florivory. Second, how does florivory impact the clonal structure of populations? If florivory increases asexual reproduction and reduces the rate of genet formation, how does this impact clonal structure, genotypic diversity, and the potential for geitonogamous mating at the population level?

2.2 Methods

2.2.1 *Study Species*

Bluebead lily is a common perennial herb of the boreal forest understory of eastern North America. Clonal reproduction occurs through rhizomes with a classic guerrilla type clonal architecture, where clones are loosely aggregated (Angevine and Handel 1986). Flowering ramets typically have 3-5 flowers with 10-14 ovules per flower (Dorken and Husband 1999). The terminal inflorescence is raised about 50 cm above the basal leaves, so florivory can occur without disturbing the rest of the plant (Galen et al. 1985). The flowers are pollinated by bumble-bees (Pitelka et al. 1985). While the seeds are thought to be dispersed by birds, reports regarding the species involved in seed dispersal are lacking.

White-tailed deer is a common large herbivore of eastern North America. Despite having once been on the verge of extinction, deer overpopulation is currently considered a concern to the native biodiversity of North American woodlands (Rooney and Waller 2003). Wildflowers comprise a significant portion of the spring diet of white-tailed deer (McCaffery et al. 1974; Miller et al. 1992; Augustine 1997).

2.2.2 *Determining Parameter Values*

To model the growth of bluebead lily patches under florivory, demographic parameters of ramets were based on excavation studies (Pitelka et al. 1985; Angevine and Handel 1986). Pitelka et al. (1985) recorded the ramet demography of over 1500 ramets in 16 patches for two years in Maine, USA. After two years, they excavated the patches and recorded the rhizomatous connections. Angevine and Handel (1986) excavated 194 clonal fragments of bluebead lily in New Hampshire, USA. They measured the rhizomatous connections between ramets in order to

describe the clonal geometry of bluebead lily. For ramet demographic parameters, the model employed the three ramet size classes representing the basal leaf area of the ramet in cm^2 used by Pitelka et al. (1985). Since accurate aging of clonal species is difficult (De Witte and Stöcklin 2010), no age dependent demographic parameters (such as age-dependent mortality or fertility) could be developed. Table I, developed from Pitelka et al. (1985), presents the cumulative probability for a ramet of a particular size class of transitioning into a size class for the coming year. To minimize assumptions, one of the four sub-tables (a-d) was randomly chosen each year for each living ramet. The probability of a ramet flowering based on its size class is given in Table II. Since the flowering state of a ramet influences the likelihood that ramet producing a secondary rhizome (vegetatively reproducing), Table III divides the probability of producing a secondary rhizome by size class and flowering state. The size class of the secondary rhizome was determined by Table IV, which shows the cumulative probability of the determination of the size class of the secondary rhizome based on the size class of the primary rhizome. Note that while the probability of a <200 cm^2 primary rhizome appears to capable of producing larger secondary rhizomes, the certainty that the secondary rhizome will also be <200 cm^2 negates the possibility of secondary rhizomes of the larger size classes.

Table I – Size dependent annual ramet growth adapted from Pitelka et al. (1985). Each cell represents the cumulative probability that a ramet of a given size class in year, t, will become a ramet of a particular size class in the following year, t + 1. The four tables (a,b,c, and d) present data gathered from 4 plots excavated over the span of 3 years.

		t + 1			
(a)	1979-1980	Dead	< 200	200-400	>400
	<200	0.06	0.90	0.99	1.00
t	200-400	0.01	0.23	0.82	1.00
	>400	0.00	0.02	0.35	1.00

(b)	1980-1981	Dead	< 200	200-400	>400
	<200	0.06	0.80	0.99	1.00
t	200-400	0.02	0.21	0.81	1.00
	>400	0.00	0.00	0.37	1.00

(c)	1980-1981 b	Dead	< 200	200-400	>400
	<200	0.08	0.78	0.99	1.00
t	200-400	0.04	0.19	0.80	1.00
	>400	0.00	0.00	0.24	1.00

(d)	1981-1982	Dead	< 200	200-400	>400
	<200	0.05	0.76	0.97	1.00
t	200-400	0.03	0.10	0.63	1.00
	>400	0.00	0.00	0.17	1.00

Table II - Probability matrix for the flowering of a ramet of a given size class.

	Size Class		
	<200	200-400	>400
p_{flower}	0.02	0.16	0.40

Table III – Probability matrix for the production of a secondary rhizome based on flowering state and size class of the ramet.

		Size Class		
		<200	200-400	>400
p_{clone}	No Flower	0.05	0.11	0.14
	Flower	0.01	0.04	0.11

Table IV - Probability matrix of the cumulative likelihood of the size of the secondary ramet based on the size class of the primary ramet.

		Size Class of 2° Clone		
		<200	200-400	>400
Size Class of 1° Clone	<200	1.00	1.00	1.00
	200-400	0.55	0.98	1.00
	>400	0.22	0.98	1.00

Directionality was based on a 16-point system, representing approximately 3 cm length of rhizomes. In the 16-point system, each direction varies from its neighbor by 22.5°, which matches well with empirical data for bluebead lily rhizome growth patterns (Angevine and Handel 1986). Further, excavations showed that secondary rhizomes of bluebead lily deviated at an average of 48.1°, which can be approximated by two increments of the 16-point system for a 45° deviation.

Because of the limitations of the empirical data, some parameters required for the model had to be estimated. I set the seedling recruitment rate – the probability that an inflorescence produced a seed that germinated into a distinct ramet – to 2.5% per inflorescence. No conclusive data exists on recruitment rate for bluebead lily, although recruitment rate is thought to be low in the species (Pitelka et al. 1985). The rate I used was within the wide range reported for species with similar seed dispersal attributes and seedling recruitment versus clonal growth trade-offs according to Eriksson (1997). Due to the nature of excavation studies, age-dependent rhizome mortality could not be accurately parameterized. Since the oldest rhizomes from patches in the Pitelka et al. (1985) study ranged from 6 to 13 years, rhizomes had 100% chance of persisting for 6 years, with a progressively increased chance of decay until a 0% chance of persisting in its 15^{th} year.

2.2.3 *Model Runs*

The agent-based model was written and run in Python 3.3.3 using the jupyter Notebook environment (Python Software Foundation, www.python.org; Kluyver et al. 2016). The model tracked the fates of ramets and rhizomes in matrices constructed using Pandas (McKinney 2010). Uniform random values were generated by the random.randint() function in NumPy (Van Der Walt et al. 2011). A flow chart of the model is presented in Figure 2.1.

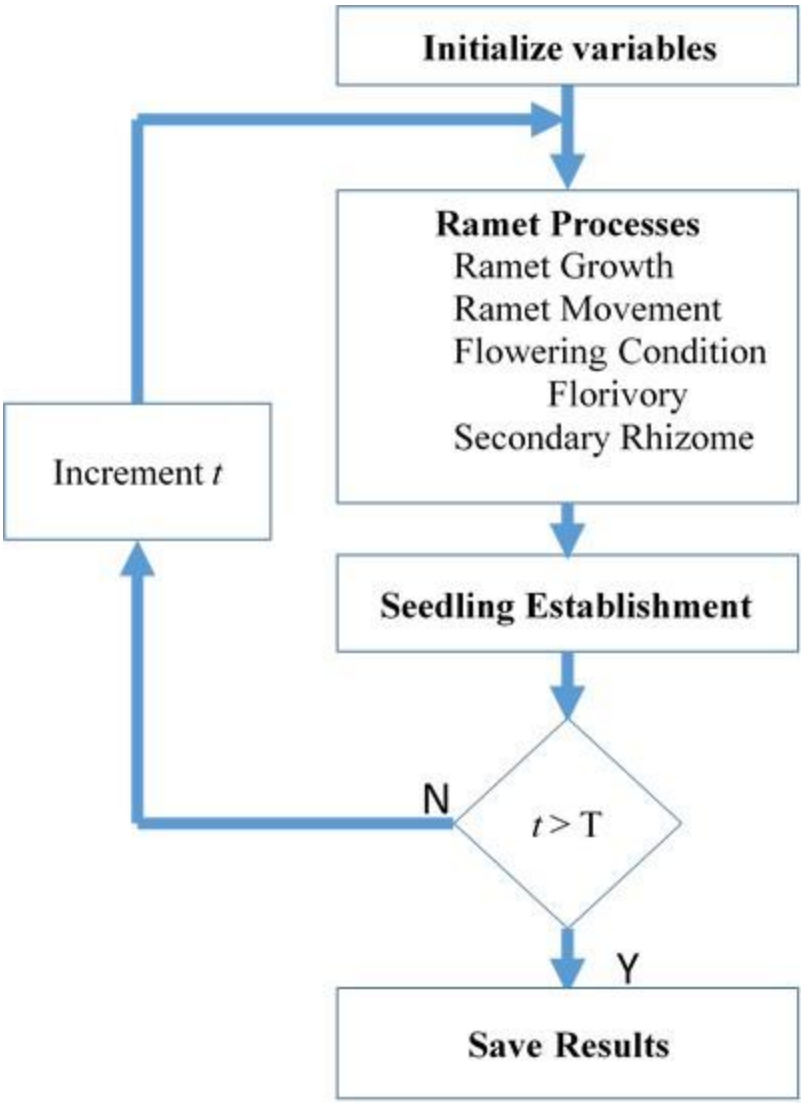

Figure 2.1 - Organization of one iteration of the agent-based model. Each iteration begins with the initialization of variables and tables used to store information. State changes are decided by comparing uniformly drawn random numbers against prepared look up tables. Time in years (t) is incremented until it exceeds the maximum time for each run (T). At the end of each iteration, the results were saved to disk and the next iteration was begun.

Each iteration of the model began with the placement of a single ramet with random directionality. Initial ramet size class was set at 200-400 cm^2. After the first year, ramet size class was determined by comparing random values against Table I. Ramet movement was based on

frequency and angle of deviation as determined by Angevine and Handel (1986). No two ramets were allowed to occupy the same position; ramets were given a new direction if such a conflict occurred. The flowering state of each ramet was determined by comparing randomly drawn values against Table II. In the runs with florivory, another random draw determined whether or not the ramet reverted to a non-flowering state due to florivory. Another random draw was compared against Table III, based on the ramet size class and flowering state, to check for the growth of a secondary rhizome. If a secondary rhizome was produced, a random draw against Table IV determined the size class of the new, clonal ramet. After the fates of all ramets had been decided for the year, each of the flowers was considered for seed recruitment. A successful recruitment placed a new ramet on the landscape. Ramets generated by seed recruitment were given new genet identifiers. The number of living ramets and genets were collected at the end of each year and output to a file. Populations were allowed to grow for 200 time increments with each time increment corresponding to one year.

For the main comparison, deer florivory was set at either 0% (control) or 95% (florivory). Each of the main runs was comprised of 1000 iterations. For parameter checking, each of the runs was comprised of 200 iterations. The model was run eight times to determine the influence different rates of florivory had on the model. Florivory during these runs was set between 15% and 85% at 10% intervals. The model was run with and without florivory to determine the influence seedling recruitment rates had on the model. Seedling recruitment rates varied from 0.25% to 12.5%. In order to investigate the relative influence of rates of clonal reproduction, clonal reproduction was manipulated by altering the probability of producing a secondary rhizome (Table III) by factors of 25% to 200%.

Graphs were produced in R (3.3.2) using normal plot functions and ggplot2 (R Core Team 2016). Runs that resulted in no living ramets at the end of 200 years were removed from most analyses. R was used to conduct Welch two sample t-tests to compare ramets and genets between control and florivory runs. Linear models were evaluated with the lm() function in the base R package. The genet-to-ramet ratio (G:N) was adjusted by subtracting one from both the number of genets and the number of ramets such that runs resulting in ramets from a single genet would be represented by zero (Dorken and Eckert 2001). I defined the survivorship rate for a run as the number of iterations that had one or more ramets at 200 years divided by the total number of iterations.

2.3 Results

2.3.1 *Main Results*

For my main agent-based model, population growth and reproductive mode were tracked for 200 years for populations subject to 95% florivory or control populations without florivory. Mean ramets at 200 years were not significantly different ($t = 1.290$, p-value = 0.199) in the florivory run (mean = 436.3 ramets, SE = 9.247) than in the control run (mean = 420.1 ramets, SE = 8.477). Ramet growth over time for the two main runs was very similar (Figure 2.2). Survivorship rates were also similar, 59.5% for the control run and 57.5% for the florivory run. The average number of genets at 200 years was significantly greater in the control run (mean = 44.80 genets, SE = 1.177) than the florivory run (mean = 3.174 genets, SE = 0.1442) ($t = 35.10$, $p < 2.2e\text{-}16$). Although ramet densities were similar for both runs, genet numbers quickly diverged with the control run accumulating genets at a much faster rate over time (Figure 2.3). The adjusted G:N at 200 years was significantly greater in the control run (mean = 0.1039, SE =

0.001677) than the florivory run (mean = 0.004914, SE = 0.0003174) (t = 58.04, p-value < 2.2e-16). Figure 2.4 shows that the confidence intervals for adjusted G:N over time for both the control and florivory runs never overlap. Note that Figure 2.4 begins at year 13 in order to avoid complications caused by the 95% confidence interval of the florivory run overlapping with zero on a logarithmic scale.

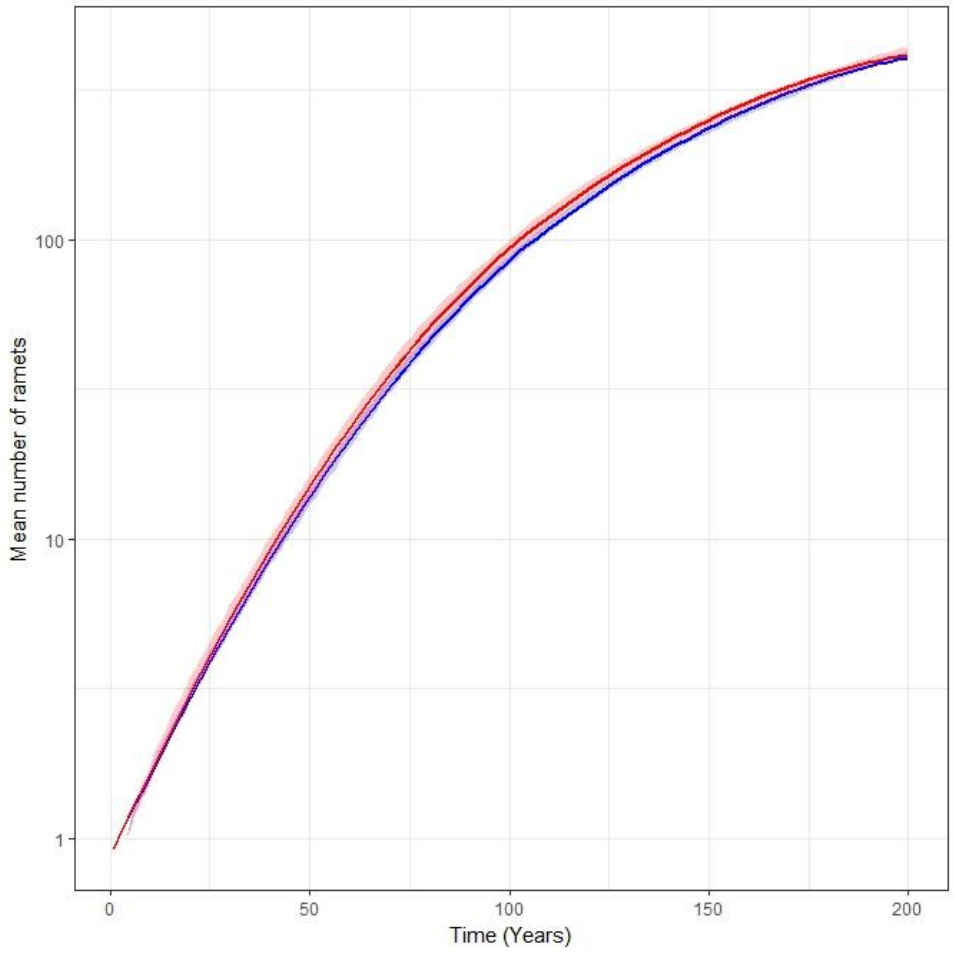

Figure 2.2 – Semi-log graph of mean number of ramets for runs with (red) florivory and without (blue) florivory. Shaded areas represent 95% confidence intervals.

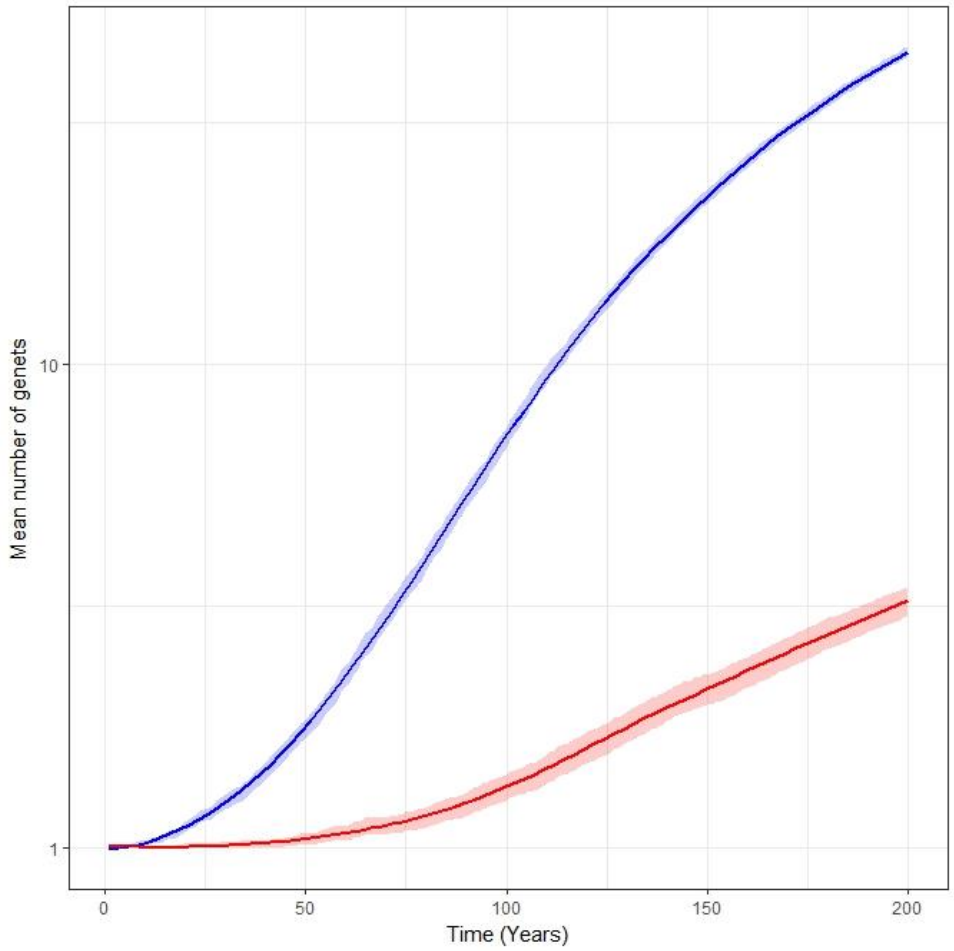

Figure 2.3 – Semi-log graph of mean number of genets for runs with (red) florivory and without (blue) florivory. Shaded areas represent 95% confidence intervals.

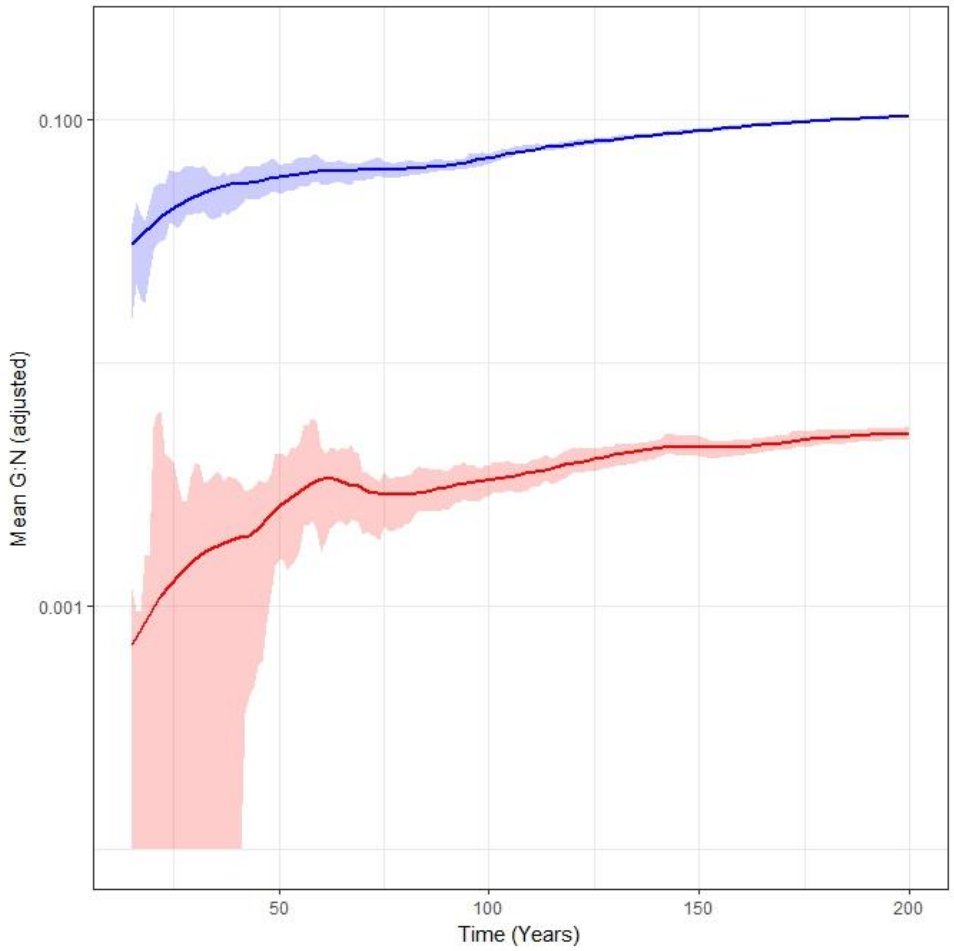

Figure 2.4 – Semi-log graph of mean G:N (adjusted) for runs with (red) and without (blue) florivory. Shaded areas represent 95% confidence intervals. Time begins at 13 years since confidence intervals that cross zero are problematic for semi-log graphs.

2.3.2 *Parameter Checking*

Florivory had a strong, negative, linear relationship on adjusted G:N (slope = -0.001036, p-value = 2.78e-13, adjusted R^2 = 0.9989) as shown in Figure 2.5. For florivory and control runs, changes in seedling recruitment rate resulted in proportional changes in G:N. Increases in seedling recruitment rate above the 2.5% value used for the main runs increased G:N, whereas decreases in seedling recruitment rate below 2.5% decreased G:N (Figure 2.6). Differences

between mean adjusted G:N for control and florivory runs were significant even at 0.25% recruitment rate ($t = 17.43$, p-value < 2.2e-16). Testing of clonal rates showed a slight decrease in G:N over all ranges of clonality for runs both with and without florivory (Figures 2.7 and 2.8). Mean adjusted G:N stabilized over time (Figure 2.9). Runs with florivory appeared to have more variability than control runs.

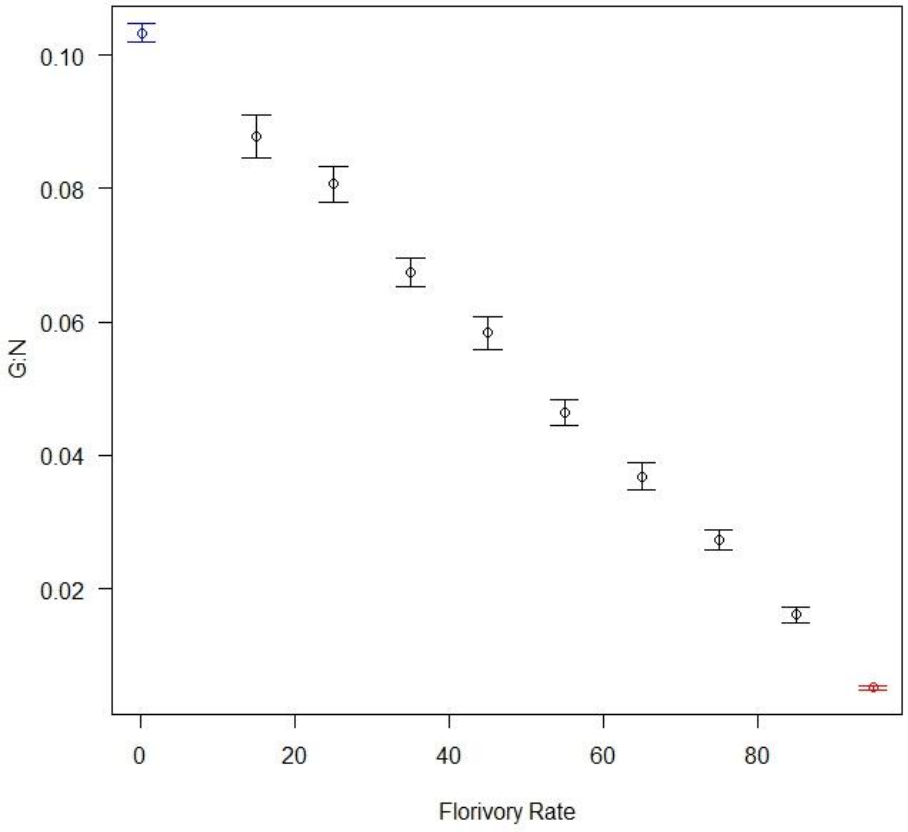

Figure 2.5 - Graph of adjusted G:N for a range of florivory rates representing the percentage chance of an inflorescence being consumed. The mean G:N for the model runs fit a linear model with a slope of -0.001036 (p-value = 2.778e-13, adjusted $R^2 = 0.9989$).

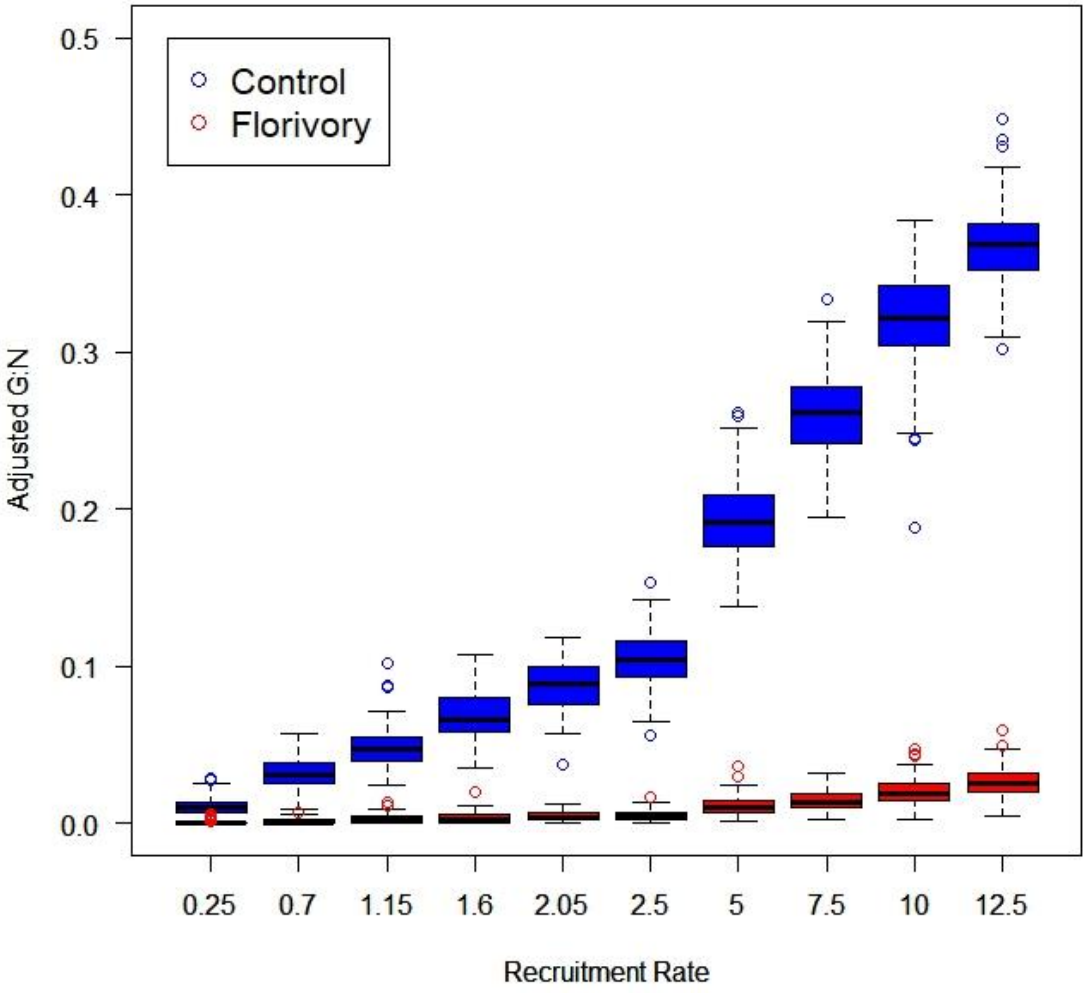

Figure 2.6 - Boxplots of adjusted G:N for model runs with different seedling recruitment rates, under control and florivory conditions. The seedling recruitment rate was 2.5% for the main runs. Note that the horizontal axis is not equally spaced.

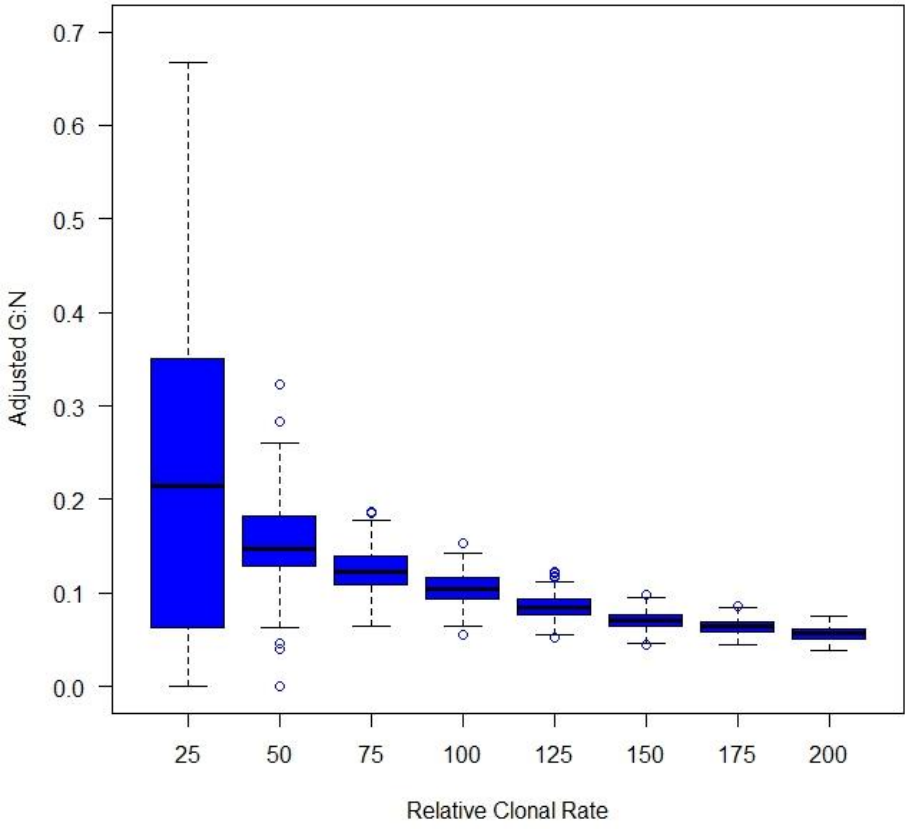

Figure 2.7– Boxplots showing the effect of relative rate of clonal reproduction on the adjusted G:N of runs without florivory. Relative clonal rate is a percentage adjustment of standard clonal reproduction rates (Table I), such that 100 corresponds to the main control run.

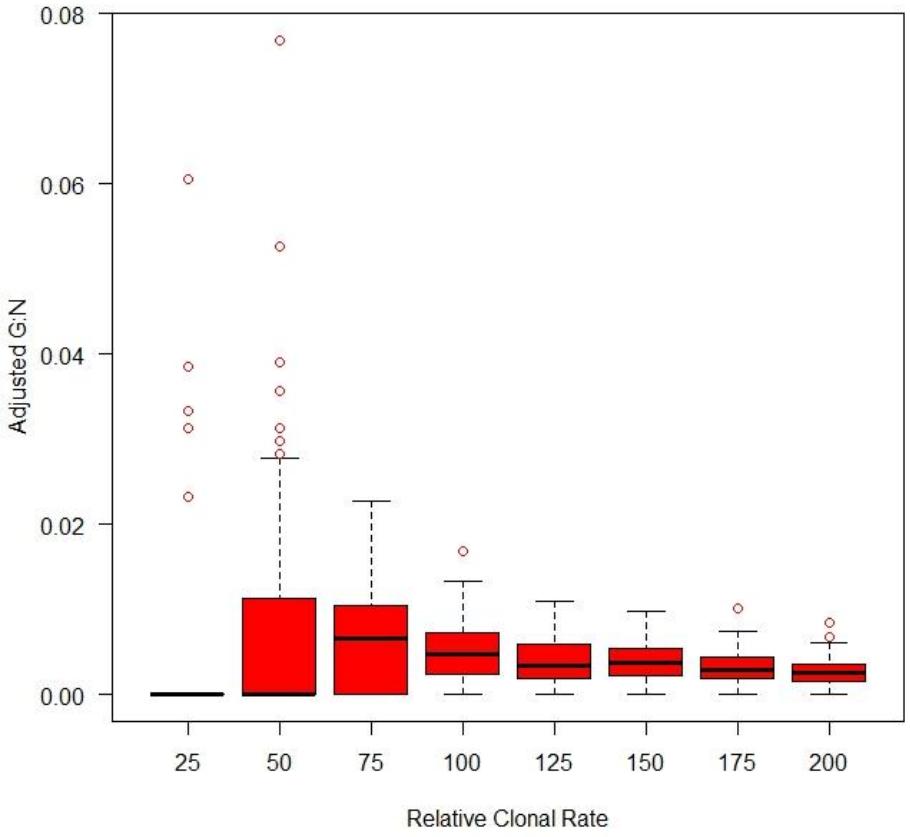

Figure 2.8 - Boxplots showing the effect of relative rate of clonal reproduction on the adjusted G:N of runs with florivory. Relative clonal rate is a percentage adjustment of standard clonal reproduction rates (Table I), such that 100 corresponds to the main florivory run.

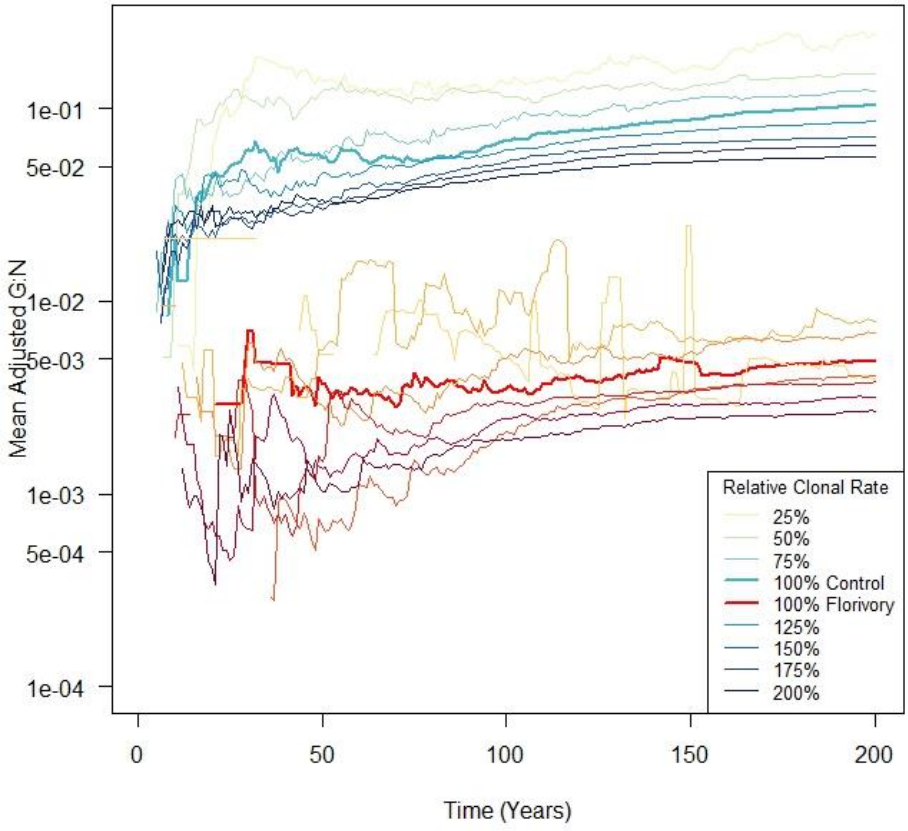

Figure 2.9 – The influence of clonal rates on adjusted G:N with (red hues) and without (blue hues) florivory. Clonal rates are measured as a fraction of reported clonal rates (Table 3), such that 100% represents the main runs. Relative clonal rates lower than 100% represent a reduction in clonal rate while numbers greater than 100% represent an increase in clonal rate. In general, increasing clonal rate reduces G:N.

Survivorship, the proportion of iterations within a run that had one or more ramets at the end of the iteration, ranged from 0.235 to 0.735. Both the large and small survivorship outlier values came from runs where the clonal reproductive rate was manipulated. Figure 2.10 shows the distribution of survivorship across a gradient of relative clonality rates. The points fit an $x + log(x)$ model ($F_{2,13} = 120.4$, p-value = 4.086e-9). A linear model fitted to survivorship as a

function of seedling recruitment rate (Figure 2.11) showed no significant relationship ($F_{1,18}$ = 0.2488, p-value = 0.624).

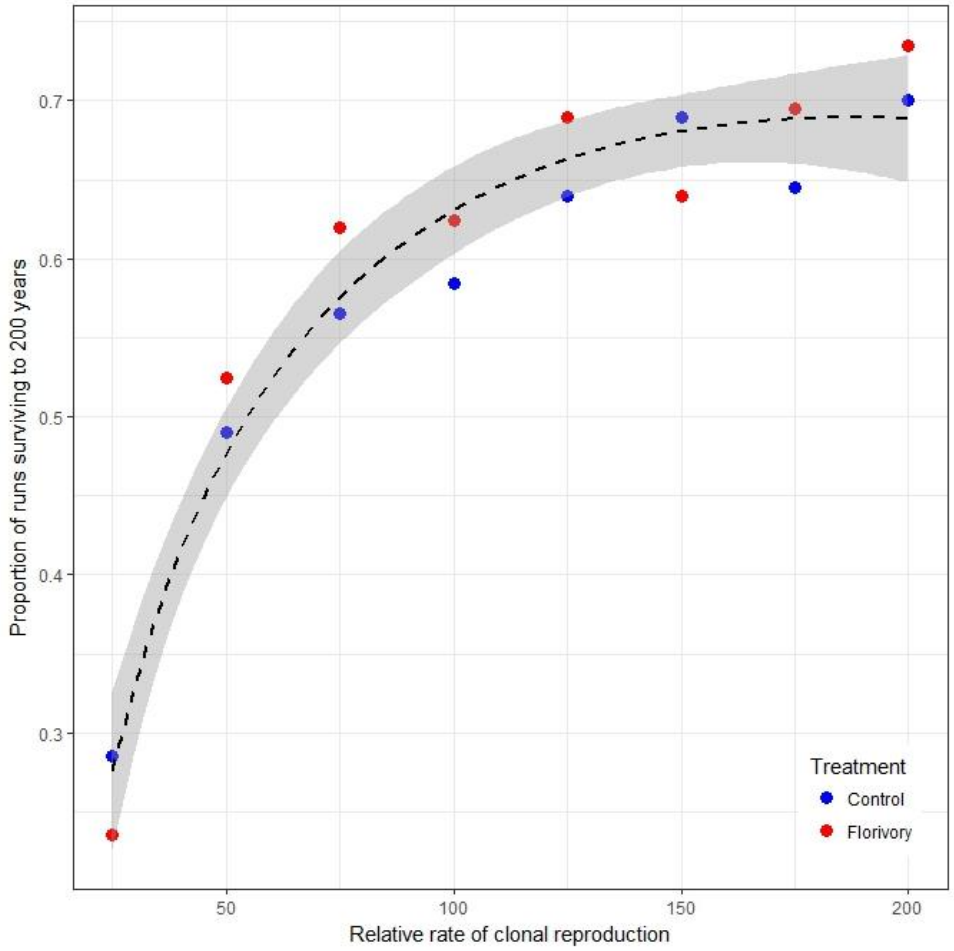

Figure 2.10 – The relationship between survivorship and rate of clonal reproduction. Clonal reproduction is shown as a percentage of reproduction rates used in the main runs (Table III) such that 100 correlates with the main runs. Each point represents the proportion of runs within an iteration which had 1 or more ramets at the end of the run. An x + log(x) linear model of the curve results in an adjusted R^2 of 0.9409 with a p-value of 4.086e-9.

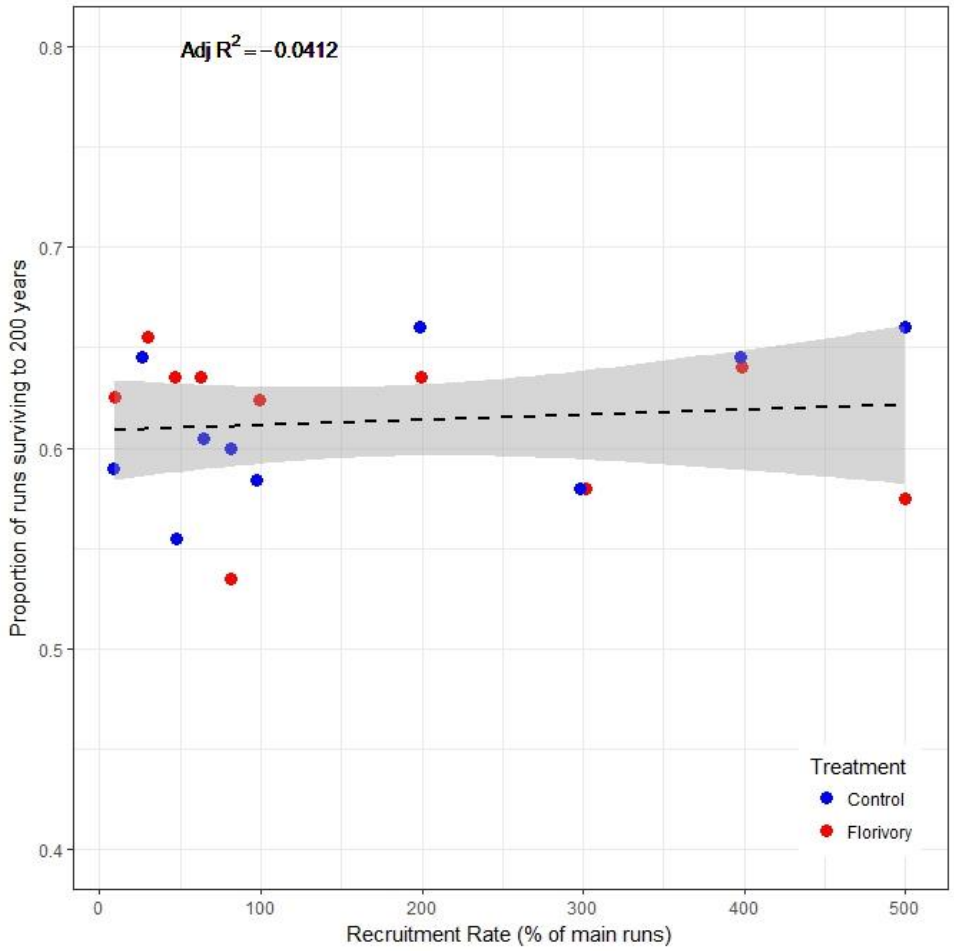

Figure 2.11 – The relationship between survivorship and seedling recruitment rate. Seedling recruitment rate is shown as a percentage of recruitment rate used in the main runs (2.5%) such that 100 correlates with the main runs. Each point represents the proportion of runs within an iteration which had 1 or more ramets at the end of the run. The linear model, represented by the dashed line, results in an adjusted R^2 of -0.0412, indicative that a horizontal line is a better fit of the data than the model.

2.4 Discussion

An agent-based model was programmed to describe the effects of florivory on the reproductive balance of bluebead lily populations. The model was parameterized with data from excavations studies which had described the demographic trends as well as flowering and cloning rates for bluebead lily. The two main runs consisted of control (no florivory) and high florivory (95%) conditions. Ramet fates were computed and recorded over a span of 200 years,

with seedling recruitment generating ramets belonging to new genets and clonal reproduction generating ramets belonging to the same genet as the parent ramet. Surprisingly, populations with and without florivory had similar ramet densities and followed comparable growth patterns over the course of 200 years (Figure 2.2). In terms of ramet population growth, the compensatory clonal growth of populations experiencing florivory essentially matched the ramet growth resulting from seedling recruitment. The number of genets in the main runs (Figure 2.3) diverged within just a few years and resulted in an order of magnitude difference by 200 years. The divergence of genet production strongly influenced the divergence between the two treatments of clonal structure (Figure 2.4). Despite a dramatic difference in clonal structure due to florivory, populations in the natural world would be indistinguishable based on ramet count. With survivorship of both control and florivory runs being similar (59.5% for control and 57.5% for florivory) and the long-lived nature of bluebead lily genets the negative effects on the population dynamics of high levels of florivory might not be detectable by demographic studies for decades.

As florivory rates were reduced in the model, clonal structure decreased linearly (Figure 2.5). Confidence intervals did not overlap with the control run even as low as 15% florivory, so florivory can have a strong influence on clonal structure even when florivory rates are relatively low. Clonal structure was also influenced by variation in recruitment rate. Lowering the recruitment rate from 2.5% narrowed the difference between control and florivory treatments (Figure 2.6). The differences between mean adjusted G:N were significant even at the lowest seedling recruitment rate tested, 0.25% chance that an inflorescence produced a seed that germinated within the vicinity. For higher recruitment rates, the difference in clonal structure increased. Hence, while decreasing the recruitment rate by an order of magnitude still yielded a

significant difference in clonal structure, increasing recruitment rate increased the effect of florivory.

Adjusting the relative clonal rate had a somewhat predictable effect on runs with and without florivory (Figures 2.7 and 2.8). As clonal rate increased, the adjusted G:N decreased for both treatments. This trend is easily explained by the increased production of clonal offspring increasing the denominator, N. Notably, however, variance of the results increased as the clonal rate decreased. While the increased variance is evident in both Figures 2.7 and 2.8, it is most pronounced in the time series data shown in Figure 2.9, which shows the mean clonal structure of runs with and without florivory over the span of 200 years with different clonal rates. While most of the means begin to normalize after 100 years, runs with low clonal rates maintain high levels of variation. The high levels of variation are attributable to reduced sample sizes caused by population mortality. Of the parameters tested, survivorship of populations was most heavily influenced by clonal rate. Runs with low clonal rates showed substantially lower survivorship than other runs (Figure 2.10). In contrast, adjusting recruitment rate did not vary survivorship (Figure 2.11). The finding that survivorship of patches is most influenced by variations in clonal reproduction rate supports the idea that clonal reproduction provides a safeguard against demographic stochasticity (Honnay and Bossuyt 2005; Silvertown 2008). Colonizing ramets, unable to sexually reproduce due to a lack of available mates or pollinators, can still produce clones of themselves. Clones increase the ability to forage resources from the environment while also spreading risks of death among ramets. However, clonality also increases the chances of self or geitonogamous mating. While this model does not consider the impact of geitonogamy or inbreeding, the influence of florivory on clonal structure described by this model can impact the reproductive success and the genetic diversity of the population.

Reproductive success, parameterized as recruitment rate in the model, lumps many different ecological factors. Deconstructing recruitment rate into sub-parameters – such as pollinator visitation behavior, mate availability, habitat quality, etc. – may provide details into the maintenance of genetic diversity of the system. Such considerations were outside the scope of the model, but future versions of the model could include pollinator behavior, variation in traits of particular genotypes, or more complex florivore behavior. For instance, simulations could incorporate different models of florivore foraging (i.e. random walk or density-dependent foraging) and factors such as predation (i.e. the effect of wolves on deer foraging (Callan et al. 2013)). These factors may influence the spatio-temporal distribution of genets which may in turn influence floral reproductive success.

My model shows that even when florivory results in very low sexual recruitment, clonal reproduction can produce patches of similar ramet density as those populations not experiencing florivory. Hence, patches of bluebead lily in the field may appear similar regardless of their clonal diversity. Assessing clonal structure in the field is impossible unless distinct genotypes are distinguishable in morphology. Even when there are quantifiable morphological differences between genotypes, genetic identity is assumed rather than known. Molecular genetic approaches provide the only unambiguous identification of the genetic identity of ramets. Use of multiple, hypervariable markers can distinguish even closely related genets with a high degree of confidence. The model presented in this paper can be further refined by field studies which quantify the clonal structure of patches of bluebead lily with and without deer florivory.

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CHAPTER 3: DEER FLORIVORY IS ASSOCIATED WITH CHANGES IN CLONAL STRUCTURE OF THE WOODLAND PLANT BLUEBEAD LILY

3.1 Introduction

Many plant species exhibit some form of clonal growth but require sexual reproduction for long-term maintenance of genetic diversity and adaptive evolution. An estimated 80% of angiosperms have some form of vegetative reproduction (Klimeš et al. 1997). In most species both sexual and asexual reproduction occur simultaneously, which can lead to trade-offs and antagonism between reproductive modes (Vallejo-Marin et al. 2010). Clonal reproduction allows populations to persist in habitats where sexual reproduction is restricted and allows adaptive genotypes to replicate rapidly by avoiding costs associated with sexual reproduction (Barret 2015). Mortality risk is spread among ramets, reducing the likelihood of death for each clone (Klimeš et al. 1997). However, clonality may limit resources allocated to flower and seed production (Van Drunen and Dorken 2012) and often has a disruptive effect on mating and fertility (Barrett 2015). Large clones may reduce the number of compatible mates for self-incompatible species. In self-compatible species, there may be an increase in geitonogamous mating (mating between two flowers of the same clone), resulting in fitness costs associated with inbreeding depression and pollen discounting.

In plants exhibiting both reproductive modes, a distinction must be made between the number of *genets* in a population, that is, individuals that arose by the union of gametes through sex, and the number of *ramets*, individuals that arose through clonal propagation. This distinction has important implications because the genet count will impact the genetic diversity of the population, the effective population size (N_e), the number of potential mates, and the spatial genetic structure of the population, while the ramet count is largely irrelevant to these parameters. Most studies that involve estimates of plant abundances and densities, however, are

based on ramet counts, and will not capture important population dynamics that require identification of genetic individuals.

Resource allocation between sexual and asexual reproduction may be strongly influenced by environmental conditions, including light intensity, nutrient availability, temperature and soil moisture (Zhang and Zhang 2007; Yang and Kim 2016). For example, high nutrient conditions have been shown to promote clonal growth in some plant species, presumably to take advantage of microhabitat resource abundance (Bai et al. 2009; Nicholls 2011). Ecological interactions with other species in the community, such as pollinators, seed dispersers and herbivores, also influence the relative rates of vegetative and sexual reproduction. Changes in species composition and/or their abundances may cause a clonal species to shift its reproduction towards more sexual or more asexual reproduction. A lack of pollinators can limit sexual reproduction. For example, the absence of sphingid moth pollinators causes pollen limitation in the clonal cactus *Stenocereus eruca* (Clark-Tapia and Molina-Freaner 2004), and the resulting reduction in sexual reproduction leads to lower clonal diversity and greater clone size in the population (Ricardo et al. 2006). Herbivory limits resources available to either mode of reproduction and reduces overall fitness. One particular form of herbivory, floral herbivory or florivory, is notable because it specifically impacts sexual reproduction. However, it may have little effect on asexual reproduction or may even promote vegetative growth if the plant can reallocate resources that would have been used for fruit or seed production (Kerley et al. 1993; Westley 1993).

White-tailed deer, *Odocoileus virginianus*, and their increased abundance in recent decades, have been shown to have large effects on plant communities (Côté et al. 2004; Russell, Zippin, and Fowler, 2001; Rooney and Waller 2003). Increased deer herbivory was a key driver in shifting plant communities in the Upper Great Lakes, with understory forbs showing the

greatest declines (Wiegmann and Waller 2006). The spring diet of white-tailed deer consists primarily of flowers, so herbaceous species that flower in the spring are particularly susceptible to the effects of deer (McCaffery et al. 1974; Miller et al. 1992; Augustine 1997). For instance, deer herbivory was shown to drive a population of *Trillium grandiforum* to such low flower densities that the population became pollen limited (Knight 2004). Despite the plethora of studies demonstrating deer impact on plant community dynamics, to date few studies have examined deer effects on reproductive mode. Increases in clonal growth in the wetland plant *Iris hexagona* have been shown when deer florivory limits floral reproduction in high salinity conditions (Geddes, N, Mopper 2006; Tobler et al. 2006). The high abundance of white-tailed deer in many woodland ecosystems combined with their preference for flowers predicts that they should have a considerable negative impact on sexual reproduction of herbaceous plants.

Bluebead lily (*Clintonia borealis*) is a long-living (>100 years), rhizomatous plant that can serve as a model organism for deer impact studies on reproductive mode. In bluebead lilies, the inflorescence is borne upon a relatively long peduncle that shoots well above the low-lying leaves. This allows white-tailed deer to browse the inflorescence without disrupting the rest of the plant. Bluebead lilies produce a single inflorescence per flowering season. Hence, the damage to the plant is limited to the loss of one inflorescence, but its capacity for sexual reproduction of that ramet is eliminated for that year. The inhibition of sexual reproduction by deer florivory should skew the reproductive effort of bluebead lilies to vegetative growth and, over time, alter the local clonal structure.

I tested this prediction by examining populations on islands in the Great Lakes which are located within the species distributions of both white-tailed deer and bluebead lily. Many of the Great Lakes islands have populations of bluebead lily, but the presence of deer depends on the

isolation and history of each island. Specifically, islands at Apostle Islands National Lakeshore (AINL) in Lake Superior and Sleeping Bear Dunes National Lakeshore (SBDNL) in Lake Michigan have bluebead lilies, but some of these islands have deer and some do not. I used this natural experiment to examine the effect of white-tailed deer herbivory on the clonal structure of bluebead lily at the patch scale. I selected six islands in the Great Lakes, three with a historic record of deer populations and three without a history of deer. Patches of bluebead lily were sampled and genotyped using newly developed DNA microsatellites to characterize the clonal structure of each patch. I analyzed the data to compare the clonal structure of bluebead lily patches and to determine if the presence of white-tailed deer was associated with differences in clonal structure and diversity.

3.2 Methods

3.2.1 *Study Species*

Bluebead lily (*Clintonia borealis*) is a widespread perennial herb of the northern woodlands of North America. It is a strongly clonal species which uses rhizomes to spread locally (Pitelka et al. 1985; Angevine and Handel 1986). Individual ramets have two to five basal, oblong leaves. The inflorescence consists of three to five yellow-green, perfect flowers that are borne on a peduncle approximately 50 cm tall (FNA). The flowers rely on bumblebees and solitary bees for pollination (Barrett and Helenurm 1987). Partial self-sterility has been reported in bluebead lily (Galen and Weger 1986; Barrett and Helenurm 1987, Dorken and Husband 1999; Galen, Plowright, and Thomson 1985), with substantial variation in levels of self-sterility among studies, with reported seed set upon selfing ranging from 0.09 (Dorken and Hustand 1999) to 0.38 (Barrett and Helenurm 1987).

White-tailed deer is a medium-sized deer native to North America and widely distributed east of the Rocky Mountains. Anthropogenic land use changes and extirpation of natural predators and competitors over the past 150 years have favored white-tailed deer, and current densities greatly exceed pre-settlement densities over much of the eastern United States (reviewed in Côté et al. 2004).

3.2.2 *Sampling*

Sampling occurred on six islands in the Great Lakes and on the northern coastline of Wisconsin. Three islands have never had deer: Raspberry (RI) and Devil's (DV) islands of Apostle Island National Lakeshore (AINL), and South Manitou (SM) island of Sleeping Bear Dunes National Lakeshore (SBDNL). Three islands have historically had deer: Oak (OI), Basswood (BW), and Stockton (SI) islands of AINL. Based on hunting records and aerial surveys, OI and BW have had deer since the 1950's. Hunting records show large numbers of deer on SI prior to 1964, when records stopped being kept for individual islands (United States Department of the Interior National Park Service 2013). Aerial surveys in the early 2000's do not report on the status of deer on SI, but deer were present in 2014 (USDOI National Park Service 2013; personal comm.). White-tailed deer occur on the mainland, but due to differences in population dynamics (i.e., exposure to hunting, roads, the presence of wolves, and other anthropogenic influences), I consider it a different treatment than the islands with white-tailed deer populations.

A custom made, 1 m^2 PVC quadrat (Figure 3.1) was used to quantify ramet density of patches. Patches of 60 ramets m^{-2} were targeted, but due to natural variations in plant densities, samples were taken from patches that had ramet densities between 40 and 80 ramets m^{-2}. Two

patches on each island and two patches from the Wisconsin mainland were sampled. Sampled patches were at least 200 m apart.

Figure 3.1 – A custom-made 1m x 1m PVC used to quantify ramet density and to select ramets sampled for genotyping.

Patch sampling was conducted by taking leaf samples from individual ramets closest to each of the 25 vertices of the PVC quadrat and rooted within the quadrat. Each sample came from a distinct ramet. Samples were individually packaged in labelled coin envelopes and dried

with Drierite crystals ($CaSO_4$) as quickly after sampling as possible. Dried samples were kept at -20°C until DNA extraction.

3.2.3 *Microsatellite Development*

Total genomic DNA was extracted from leaf samples using DNeasy plant kits and following recommended protocol (Qiagen, Valencia, California, USA). Whole genomic DNA from one individual, E1 (from an Indiana population), was used to prepare a sequencing library using the KAPA DNA Library Preparation Kit for Illumina Mi-Seq whole genome shotgun sequencing (Kapa Biosystems, Wilmington, Massachusetts, USA) following the manufacturer's instructions. The KAPA Library Quantification Kit for Illumina was used to quantify the final library. The DNA library was sequenced by an Illumina MiSeq Benchtop Sequencer using a 2x 300 bp paired end MiSeq Reagent Kit v3 (Illumina, San Diego, California, USA) at the Pritzker Laboratory in the Field Museum of Natural History (Chicago, IL, USA).

Paired-end reads were joined using *Fastq-Join* in iPlant (now CyVerse) with default options for percent maximum difference (0.20) and minimum overlap (6) (Merchant et al. 2016). The resultant file was divided into 14 smaller files with custom Python script using iPython Notebook running Python 3.4.2 (Python Software Foundation, https://www.python.org/; Perez and Granger, 2007). MSATCOMMANDER-0.8.2 was used to scan each file for regions of at least 6 repeats for di-nucleotide motifs and 4 repeats of tri- through hexa-nucleotide motifs (Faircloth 2008). The output files were concatenated and the potential loci were separated by motif and sorted by number of repeats. Potential loci with repeat motifs beginning more than 50 base pairs from the start of the read were exported to Primer3Plus with default settings

(Untergasser et al. 2007). Reads with discontinuous repeat regions or multiple motifs were removed from consideration. A total of 66 primer pairs were selected for optimization.

Of the 66 loci tested, 40 amplified successfully and were assessed for polymorphisms in 16 samples taken from three geographically distinct populations. Forward primers were tagged with an M13 primer tail (TGTAAAACGACGGCCAGT) and labeled as described in Schuelke (2000) and Abraham et al. (2011). All PCR reactions were performed in a total volume of 10 µL, with 10 ng of template DNA, 0.6 µM of reverse primer, 0.15 µM of forward primer, 0.25 µM each dNTP, 0.6 µL bovine serum albumin (BSA; 10% w/v), 1 µL 10x reaction buffer with 15 mM $MgCl_2$, and 0.2 µM florescent M13(-21) universal primer. The thermocycling profile consisted of an initial denaturation at 94°C for 5 min; then 35 cycles of 94°C for 30 s, T_a for 30 s (Table 2), 72°C for 30s; and a final extension at 72°C for 7 min. The PCR amplicons were run on an ABI 3730 DNA analyzer with ALEXA custom size standard (Maddox and Feldheim 2014). Genotypes were scored using GeneMapper version 3.7 (Applied Biosystems, Foster City, CA, USA).

3.2.4 *Microsatellite Genotyping*

A total of 350 ramets sampled from the study patches were genotyped. Total genomic DNA was extracted from leaf samples as described above. DNA concentration was quantified using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA) and samples were diluted to 10 ng/µL concentrations. Seven polymorphic microsatellite loci were chosen for genotyping and these were amplified and scored with the same protocol described above. Samples were amplified at locus specific annealing temperature.

3.2.5 *Genetic Analyses*

Multilocus genotypes (MLGs) were identified using the ALLELEMATCH package in R (Galpern et al., 2012). The function amUniqueProfile determined the appropriate value of the alleleMismatch parameter, $\hat{m}$, as 2, meaning that individuals can differ by up to 2 alleles and still be placed in the same MLG. The P_{sib} value is the probability that two individuals share the same MLG due to sibling relationship rather than being clones. For further analyses, individual ramets belonging to an MLG were collapsed down to a single genet. Heterozygosities, deviation from HWE, and the probability of identity, P_{ID}, were computed for all samples using GenAlEx (Peakall and Smouse 2006, 2012). The program FStat 2.9.3.2 was used to determine allelic richness (Goudet 2002).

3.2.6 *Statistical Analyses*

Diversity indices were computed for each of the islands. I used $\frac{G-1}{N-1}$ to determine genet to ramet ratio, G:N, such that a monoclonal stand would have a G:N of 0 (Dorken and Eckert, 2001). Effect size of deer on G:N was calculated using Cohen's *d* (Cohen 1988). For each plot, the complement of Simpson's index corrected for finite size, *D*, was computed as a measure of clonal diversity (Simpson 1949; Pielou 1969), and Pielou's evenness index, *J'*, was calculated for clonal evenness (Pielou 1975; Arnaud-Haond et al. 2007). The role of deer presence was tested with linear mixed-effects models (Bates et al. 2015). When analyzing genets, I log transformed the response variable. When analyzing *D* and *J'*, I logit transformed the response variable because they were values bounded by 0 and 1. Transformations were performed to meet assumptions about residual distributions and homoscedasticity. I chose the best model using AICc (Burnham and Anderson 2002).

3.3 Results

The Illumina Mi-Seq genome wide shotgun sequencing resulted in a total of 196,416 potential loci which represented 3.128% of all paired reads, summarized in Table V. No reads containing repeat regions were found to be duplications probably due to the large genome size of bluebead lily, approximately 44.8 Bbp (Bai et al. 2012). Of the 40 loci that successfully amplified, 12 were polymorphic in the samples tested, but two were discarded as they consistently had more than two alleles in certain individuals, perhaps due to gene duplication. The 10 polymorphic loci are characterized in Table VI. Three of these 10 loci were omitted from this study because of inconsistent amplification. Further optimization may have resolved these inconsistencies, but the seven remaining loci provided high confidence for clonal identification.

Table V – DNA microsatellite loci discovered by one Illumina Mi-Seq paired-end genome-wide shotgun sequencing run of a Clintonia borealis sample. The loci are grouped by motif. The raw number of loci of each motif and percentages of each motif out of total loci and total reads are reported.

Motif	Number of Loci	% of loci with motif	% of reads with motif
Dinucleotide	103425	52.66	1.647
Trinucleotide	82726	42.12	1.318
Tetranucleotide	7233	3.68	0.115
Pentanucleotide	1425	0.73	0.023
Hexanucleotide	1607	0.82	0.026
All (6,278,793 paired-end reads)	196416		3.128

Table VI – Characteristics of microsatellite primers developed for Clintonia borealis. Repeat motif shows the locus motif and the number of repeats observed in the sequenced sample. Number of alleles (A), allele size range, annealing temperature (T_a), number of amplified samples (N), observed and expected heterozygosities (H_o, H_e), and inbreeding coefficient (F) are based on 16 random samples taken from three populations.

Locus	Primer sequences (5' - 3')	Repeat motif	A	Allele size range (bp)	T_a (°C)	N	H_o	H_e	F
CLBO21	F: AATCTCATCGCCCATCAATC R: ACCCTCTTCGCAATCCATAA	$(ATGGCT)_6$	7	232-280	58.5	16	0.361	0.485	0.174
CLBO30	F: TCTAGGAAGTGAGAGACCCATGT R: TCATTCCTTCATGGCTGAGA	$(GATAT)_9$	6	185-285	59.3	16	0.556	0.546	0.083
CLBO46	F: TCCATATCGGTTTGGTATCG R: CGGGATTTCTCCTTCCATTT	$(GTTTTT)_{10}$	7	200-245	55.3	16	0.528	0.594	0.139
CLBO48	F: CAAAATGGGGATGGAAATTG R: CTTCATCTGCTTCGGTGACA	$(CT)_{22}$	15	204-248	54.1	16	0.583	0.788	0.287
CLBO49	F: GTGAGCCCTGAATTTTTCCA R: TGCGGTTTCTCTCTCTCTAAAGT	$(AG)_{22}$	7	224-240	60.3	14	0.417	0.629	0.348
CLBO57	F: CGGCGAAGGAGAATATGAAG R: TTTCCCGAGTTCGATGGTTA	$(AAG)_{27}$	5	142-199	60.3	15	0.233	0.502	0.634
CLBO62	F: TGGCAGCTCACATAAACCTG R: ACCCTGTCTTCCACCACAAG	$(AAAT)_{12}$	6	198-237	61.5	15	0.878	0.772	-0.134
CLBO63	F: TGAAGCTCGAACTCGAAAGT R: ACTCCCTCAGGACTCGCATA	$(AG)_{21}$	10	219-242	60.3	14	0.722	0.787	0.075
CLBO73	F: TGGAGCATGGTTTCCCTATT R: TCCTTCTGCAGCTTCATTGTT	$(AAGATG)_{14}$	6	230-258	54.1	14	0.283	0.475	0.394
CLBO78	F: GGGCACTTGTTTCTGGATGT R: ACAATTGGCGAAAGCAAAAC	$(CTTTTT)_9$	7	186-219	52.8	15	0.944	0.697	-0.357

After scoring all 350 sampled ramets at seven loci, AlleleMatch identified a total of 118 multilocus genotypes (MLGs). The value of P_{sib} was less than 0.048 for all MLGs, indicating that the probability that individuals with matching MLGs were siblings rather than members of the same clone was less than 5%. After removing duplicate MLGs, descriptive statistics of genetic variation were calculated (Table VII). All loci were highly variable, with 7-14 alleles per locus and gene diversity ranging from 0.545 to 0.873.

Table VII – Descriptive statistics for the loci used to identify MLG's in island and mainland samples. Number of amplified loci (N), observed and expected heterozygosities (H_o, H_e), inbreeding coefficient (F), allelic richness (A_r), and probability of identification (P_{ID}) were computed for 118 MLGs discovered from the 350 genotyped samples. P_{ID} presents the cumulative probability of identifying unique genotypes with the addition of each locus.

Locus	N	H_o	H_e	F	A_r	P_{ID}
CB21	117	0.615	0.668	0.079	7.97	1.8E-01
CB30	118	0.678	0.545	-0.245	6.97	4.5E-02
CB46	118	0.644	0.830	0.224	10.98	2.1E-03
CB62	118	0.780	0.750	-0.039	6.98	2.2E-04
CB63	118	0.890	0.873	-0.019	13.95	6.5E-06
CB73	116	0.440	0.729	0.397	10.00	6.9E-07
CB78	118	0.737	0.762	0.032	8.98	6.2E-08

All patches were multiclonal. A summary of the results by patch is presented in Table VIII. Islands without deer averaged 12.0 genets per patch with a range from 8 to 21 genets. Islands with deer averaged 3.5 genets per patch with a range from 2 to 4 genets. Genet-to-ramet ratios (G:N) from patches on islands without deer averaged 0.458 genets per ramet, SE = 0.025. Patches on islands with deer had an average G:N of 0.104 genets per ramet, SE = 0.014. The effect size of deer presence on G:N was 1.995, which is considered extremely large (Sawilowsky 2009). The two mainland patches were found to have 11 and 14 genets, with an average G:N of

0.480 genets per ramet, SE = 0.063. Differences between mean G:N for islands with deer and islands without deer were significant (ΔAICc = 3.94, Akaike weight = 0.878). A two-tailed t-test also showed a significant difference between islands based on deer presence (t = -6.0687, df = 3.2657, p = 0.007).

Table VIII – Summary of genet structure of 14 plots sampled on 6 islands (Basswood, BW; Oak, OI; Stockton, SI; Devil's, DV; Raspberry, RI; and South Manitou, SM) and the Apostle Islands mainland (AM). Microsatellite genotyping identified the number of genets discovered in 25 samples of each 1 m^2 plot. The genet-to-ramet ratio, G:N, was computed as (G-1)/(N-1) such that monoclonal stands would have a value of 0. Simpson's complement, *D*, and Pielou's evenness, *J'*, were computed for each plot.

Treatment	Patch	Genets	G:N	*D*	*J'*
Deer	BWP1	3	0.083	0.227	0.404
Deer	BWP2	4	0.125	0.590	0.695
Deer	OIP1	4	0.125	0.297	0.437
Deer	OIP2	4	0.125	0.410	0.548
Deer	SIP1	2	0.042	0.080	0.242
Deer	SIP2	4	0.125	0.467	0.621
No Deer	DVP1	21	0.833	0.987	0.984
No Deer	DVP2	9	0.333	0.837	0.859
No Deer	RIP1	10	0.375	0.913	0.951
No Deer	RIP2	16	0.625	0.943	0.930
No Deer	SMP1	8	0.292	0.903	0.951
No Deer	SMP2	8	0.292	0.833	0.881
Mainland	AMP1	14	0.542	0.950	0.957
Mainland	AMP2	11	0.417	0.857	0.864

Clonal diversity, *D*, and clonal evenness, *J'*, both showed the same general trends as G:N; islands without deer and the mainland are higher in diversity and evenness than islands with deer. Clonal diversity on islands with deer ranged from 0.080 to 0.590, with a mean of 0.345, SE = 0.0743. Clonal diversity on islands without deer ranged from 0.833 to 0.987, with a mean of

0.903, SE = 0.0245. Differences between *D* for islands with deer and islands without deer were significant (ΔAICc = 9.66, Akaike weight = 0.992). Clonal evenness on islands with deer ranged from 0.242 to 0.695, with a mean of 0.491, SE = 0.0669. Clonal evenness on islands without deer ranged from 0.859 to 0.984, with a mean of 0.926, SE = 0.0193. Differences between *J'* for islands with deer and islands without deer were significant (ΔAICc = 11.41, Akaike weight = 0.997).

3.4 Discussion

Natural populations of plants can be difficult to accurately census and monitor because the relative rates of sexual and vegetative reproduction are often unknown, and it is often impossible to distinguish ramets belonging to the same or different genets in the field (Wilk et al. 2009; Kim et al. 2015). Sampling and genotyping these populations with highly variable genetic markers is often the only method to accurately census the number of genets and to describe the population's clonal structure. Bluebead lilies have been reported to have extensive rhizomatous growth but have not previously been characterized using genetic markers. An excavation study by Pitelka et al. (1985) reported that patches of bluebead lily at study sites in Maine were dominated by one or a few genets, but excavation studies can only identify clones with intact rhizomatous connections. Thorough sampling and genotyping with several highly variable microsatellite loci developed for this study revealed a wide range in the number of genets comprising each patch (Table VIII), but I found no monoclonal patches and only two patches comprised of three or fewer genets. Thus, either the patches I sampled had higher clonal diversity than those sampled by Pitelka et al. (1985), or the excavation method used by these authors greatly underestimated clonal diversity.

By characterizing clonal structure of bluebead lily on Great Lakes islands with and without deer, I tested my hypothesis that deer florivory impacts the reproductive mode and clonal structure of patches. Our findings strongly suggest that the consumption of flowers by white-tailed deer has a significant influence on the clonal structure of bluebead lily. On islands without deer, genet richness in patches was much higher, with an average G:N that was nearly four times greater than on islands with deer. Deer florivory is also associated with lower clonal diversity and evenness. While deer herbivory has been linked to changes in plant community structure and declines of numerous species (Côté et al. 2004; Begley-Miller et al. 2014), to our knowledge this is one of the first studies that demonstrates a link between deer herbivory and shifts in reproductive mode (Tobler et al. 2006). As a natural experiment as opposed to a controlled experiment, the results of this study are correlative and not causal. While deer presence on the islands correlates well with our predictions regarding clonal structure, clonal reallocation could also be driven by pollen limitation or lack of compatible mates. Further studies would be required to determine the relative contribution of these factors.

The clonal structure of a patch is determined by the rate of recruitment of seeds, clonal introgression of genets from other patches, and the vegetative growth rates of genets already in the patch. Deer florivory can directly or indirectly influence all three of these. Seed recruitment is directly reduced by floral herbivory because a reduction in the number of flowers reduces the number of seeds produced. Introduction of new genets through clonal introgression from nearby patches is reduced because these patches are likely composed of fewer genets themselves. Indirectly, seed production may be further limited by the lack of compatible mates for flowers which survive consumption in species such as bluebead lily that have strong self-sterility, because many nearby flowers in the patch will be from the same clone. Finally, increased

vegetative growth rates may also alter clonal structure if established genets are able to reallocate resources not used in fruit and seed production.

Clonal richness simply measures the number of unique genotypes found in a patch, irrespective of their relative abundance. Clonal richness should reflect the relative contribution of seed recruitment versus vegetative growth occurring over time, because only recruitment through seed will introduce new genotypes into a population. Clonal richness was significantly lower on islands with deer, suggesting that the presence of deer constrains sexual reproduction and seed recruitment (Figure 3.2). If bluebead lilies reallocate resources not used on fruit and seed production (after flowers are eaten) into rhizomatous growth, it could allow large clones to proliferate and dominate in each patch. This would be reflected in reductions in measures of clonal diversity and clonal evenness.

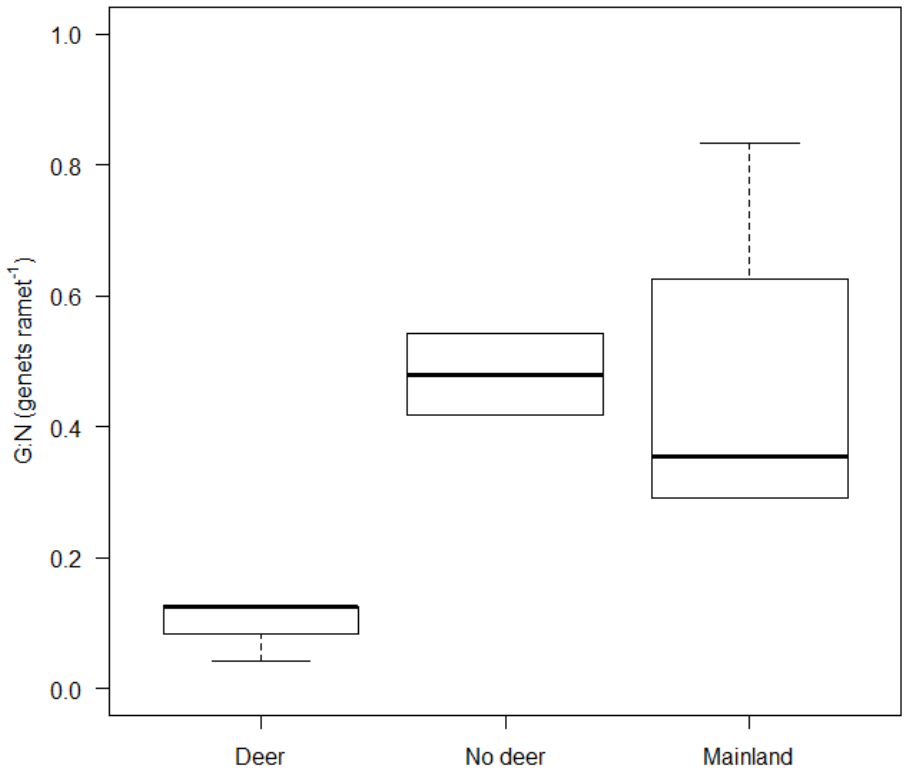

Figure 3.2 – Boxplot of G:N by deer presence on islands. Linear mixed effects models showed that islands without deer had a significantly higher mean G:N than islands with deer (ΔAICc = 3.94, Akaike weight = 0.878).

Clonal diversity, as measured by Simpson's complement index, was reduced in bluebead lily patches on islands with deer and reflects differences both in richness and relative abundance of clones in a patch. For clonal plants, the Simpson's complement index can be thought of as the probability that two randomly chosen ramets are members of different genets. I observed a two-fold difference in this index on islands without deer compared to islands with deer (Figure 3.3). Thus, geitonogamous mating is expected to be much more common when deer are present even

for plants that escape florivory for a season. Given the greatly reduced seed set under selfing reported for this species (Galen and Weger 1986; Barrett and Helenurm 1987, Dorken and Husband 1999; Galen, Plowright, and Thomson 1985), sexual reproduction will be further limited as a legacy effect of florivory.

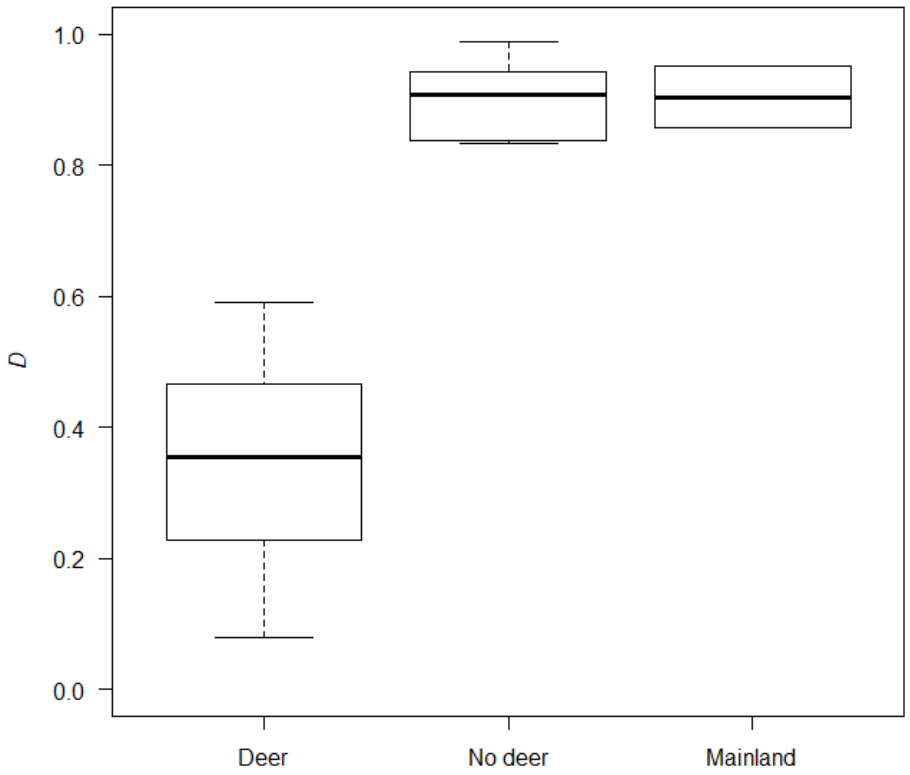

Figure 3.3 – Boxplot of clonal diversity as measured by Simpson's complement, *D*, by deer presence. Linear mixed effects models showed that islands without deer had a significantly higher clonal diversity than islands with deer (ΔAICc = 9.66, Akaike weight = 0.992).

While white-tailed deer on the mainland might be expected to similarly repress sexual reproduction, the mainland patches had clonal structure that was similar to islands without white-tailed deer (Figure 3.2). Further investigation would be required to verify why mainland plots are

less effected by deer, but I can suggest several reasons. Deer densities may be lower on the mainland due to hunting, predation by wolves, or other factors. The presence of wolves on the mainland may alter that feeding behavior of herbivores even without large mortality impacts, as shown for other ecosystems (Fortin et al. 2005). The patches I sampled on the mainland were also close (10 – 20 m) to heavily used hiking trails, and the presence of hikers rather than hunters or predators may alter the feeding behavior of deer or cause them to avoid the particular patches I sampled.

Deer density may have a non-linear effect on the reproductive mode of bluebead lily. Balgooyen and Waller (1995) found that on islands with deer densities above than 0.8 per km^2, bluebead lilies showed a reduction in scape height and number of pedicels per umbel. They suggest that deer densities above this threshold significantly lower the rate of sexual reproduction and reduce the density of bluebead lily populations. I developed an agent-based model (Chapter 2) that similarly identified a threshold; florivory rates needed to be higher than 30% to yield significant effects on G:N. The current study indicates that deer densities on islands are clearly above this threshold, but the lower densities of deer on the mainland may result in florivory rates below this threshold.

If sexual reproduction is suppressed on islands with white-tailed deer, as our results suggest, then new genets on those islands would only arise from the occasional flower which escapes herbivory and sets seed, or through seed dispersal from outside the island. Birds are thought to be the main seed dispersers, but there has been no field verification of the species involved (Ashmun and Pitelka 1985). If birds with small home ranges were the primary dispersers, they would disperse seeds locally and not between islands. If birds with large home ranges were common dispersers, they could readily transfer seeds between islands and between

the mainland and the islands. In the case of bluebead lily, asexual reproduction provides for the persistence of patches or populations for relatively long time spans, with genets persisting for 100 years or longer (Pitelka et al. 1985). Hence, even a very low rate of genet recruitment is sufficient to maintain ramet density and thus populations appear stable. The reduced genotypic diversity on the islands with deer would, however, reduce the landscape scale genetic diversity of the species.

Our results suggest that florivory drives the reallocation of resources into vegetative growth in bluebead lily, but other evolutionary and ecological responses are likely. For instance, bluebead lily might be reallocating resources toward increased floral production to promote sexual reproduction and reduce geitonogamy. Alternatively, bluebead lily might be increasing chemical defenses against florivory (Côté et al. 2004). Community responses are also possible; pollinator abundances on the islands with deer may decline and further reduce sexual reproduction through pollen limitation. Future studies might reveal a number of other direct and indirect effects of florivory.

The dramatic difference in clonal structure associated with the presence of deer has important implications for the bluebead lily populations that are subject to florivory and suggests that white-tail deer may impact reproductive modes of other understory plant species. It is worth noting that despite dramatic differences in clonal structure on islands with and without white-tailed deer, the bluebead lily patches were very similar in appearance, having similar ramet densities and plant sizes. Differences were only revealed after genotyping. Where clonal species are concerned, inferences of population structure from ramet distribution can be misleading. Local influences, such as community interactions (herbivores, florivores, pollinators) whose distribution may vary over the landscape, can have important but cryptic effects.

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CHAPTER 4: TRANSLOCATION OR LAGGING-EDGE ASSISTED MIGRATION: CONSIDERING THE LEGACY OF A SMALL, ISOLATED BLUEBEAD LILY POPULATION

4.1 Introduction

Human activity has led to the isolation and fragmentation of many natural habitats. Organisms in such habitats often have reduced population size and the increased distance between populations can make these small, isolated populations susceptible to genetic erosion (Ouborg et al. 2006). Genetic erosion can increase extinction risk due to the expression of deleterious alleles or due to the lack of genetic diversity needed to adapt to changing environmental factors (Frankel 1974). In order to know if a population is vulnerable to genetic erosion, population size must be determined. Determination of population size in many species is relatively straightforward since each individual is a unique reproductive unit. However, most plant species exhibit some form of clonal reproduction (Klimeš et al. 1997), so the number of genetically unique units (genets) does not necessarily correspond to the number of individual stems (ramets) (Palagi & Ashley, Chapter 3; Kim et al. 2015). The relationship between the number of ramets and the number of genets in a population can vary widely even within a species (Wilk et al. 2009; Meerow et al. 2011). Accurately describing population size and clonal diversity informs management choices intended to alleviate genetic erosion in small, isolated populations.

The conservation of a small, isolated population can be considered from two perspectives: the persistence of the population in its historic locale and the evolutionary potential of the population. Most conservation strategies focus on the former perspective in order to maintain local biodiversity. However, when an isolated population no longer exchanges alleles with conspecific populations, it no longer contributes to the evolution of the species (Honnay and

Bossuyt 2005). Any adaptations in the small, isolated population remain solely in that population. Preservation of the population's evolutionary potential might involve the assisted migration of individuals from the isolated population into larger, non-isolated populations.

Translocation has multiple meanings in conservation biology. Here I restrict its meaning as the introduction of individuals from large populations into small, isolated populations with the intention to offset the negative genetic effects of isolation and fragmentation (Menges 2008). However, translocation of new genotypes into a small, isolated population involves risks. If the small, isolated population has accumulated significant local adaptations, or if the population has been purged of deleterious alleles, progeny from matings between the local and introduced genotypes may have reduced fitness relative to the original population (Frankham 2005; Edmands 2007). The unintended introduction of non-adapted or deleterious alleles could result in outbreeding depression. Nonetheless, a recent meta-analysis of studies involving translocation have shown overwhelmingly positive results for the target populations (Frankham 2015).

The management value of translocating populations has been demonstrated for the genetic rescue of wild plant populations (Frankham 2005). Populations of both *Ipomopsis aggregata* and *Silene alba* which exhibited inbreeding depression due to limited gene flow responded positively to the pollen introduced from distant populations (Heschel and Paige 1995; Richards 2000). In the case of *Hymenoxys acaulis* var. *glabra,* the Lakeside daisy, habitat destruction and fragmentation had caused a population bottleneck which limited self-incompatibility alleles. The presence of only a single mating type effectively drove the last Illinois population to extinction (DeMauro 1993). Conservationists transplanted genotypes from distant populations of Lakeside daisy with efforts focused on maximizing S-allele diversity (DeMauro 1994).

Assisted migration is a management approach that seeks to accelerate the dispersal of plants across inhospitable landscapes with the idea of keeping species within hospitable bioclimatic zones (Hewitt et al. 2011). In general, climate change increases the rate plants need to disperse in order to stay within their bioclimatic envelope, and fragmentation increases the distance plants need to disperse (Vitt et al. 2010). The assisted migration discussion focuses largely on the leading edge of dispersal (e.g. generally in northern hemisphere populations, this would be the north side of the species distribution); transplanting species into novel environments where the climate has become or will soon become appropriate for the species. However, genetic adaptations of lagging-edge populations (e.g. generally in northern hemisphere populations, this would be the south side of the species distribution) – populations which are perhaps more adapted to warmer climates than the species on a whole – may prove advantageous (Hampe and Petit 2005). Further, conservation of remnant populations beyond the lagging-edge of the bioclimatic envelope will incur greater costs to maintain as the bioclimatic envelope shifts (Hällfors et al. 2017).

Here I quantify the population size and population genetics of a small, isolated population of *Clintonia borealis*, the bluebead lily, in order to make specific recommendations for the conservation of the population. Shirley Heinze Land Trust (SHLT) is a local conservation organization servicing in northwest Indiana that manages a reserve containing a bluebead lily population at the species southernmost distribution. Given concerns about the small size of the population, SHLT has expressed interest in translocating new genets into the Indiana bluebead lily population (Paul Quinlan, SHLT, pers. comm.). However, the translocation of new genotypes can have positive, neutral, or negative effects depending on the population and genetic characteristics of the Indiana population. In order to understand the potential ramifications any

translocations, the population must be characterized and compared to larger populations closer to the center of its range – e.g. Palagi & Ashley (Chapter 3*)*. The northwest Indiana bluebead lily population provides an important example of a small population with outcrossing management potential. Aside from an Appalachian peninsula (Kartesz 2011), northwest Indiana represents the southernmost extent of bluebead lily's species range.

The Indiana bluebead lily population is composed of only two patches and is roughly 70 km from the nearest known extant population. Based on excavation studies of bluebead lily (Pitelka et al. 1985), I hypothesized that the population would be composed of only two or three genets. Self-sterility has been observed in bluebead lily (Galen et al. 1985), so a lack of compatible genotypes may influence the reproductive potential of the population. If the population was found to be composed of only a few genets and fruit and seed set were likewise found to be low compared to published studies, then the Indiana population might persist only with the translocation of new genotypes. I characterized the number and distribution of genets in the northwest Indiana population using DNA microsatellite markers. I then compared its genet structure to naturally occurring populations of bluebead lily characterized in a parallel study (Palagi & Ashley, Chapter 3). I also quantified fruit and seed set in the population to investigate fecundity of the population.

4.2 Methods

Bluebead lily is a common understory herb of the boreal forest ecotype. The combination of the warming climate since the last glacial maxima and urban and agricultural development in the Midwestern states has resulted in isolated and fragmented native remnants. Since the range of the ecotype has moved northward since the last glacial maxima (Griffin and Barrett 2004), the northwest Indiana bluebead lily population resides at the southern extent of the current species

range for low altitudes. While it is a generalist forest species in more northern latitudes, bluebead lily is restricted to bogs and fens at the southern extent of its range (Swink, F, Wilhelm 1994; Reznicek, AA, Voss, EG, Walters 2011). Blubead lily is threatened in Maryland, endangered in Indiana and Ohio, and listed as special concern in Tennessee (United States Department of Agriculture). Due to its endangered status in Indiana, collections were restricted to one season.

One population of bluebead lily is known to exist in northwest Indiana, with the nearest known population being approximately 70 km away in Berrien County, MI (Swink, F, Wilhelm 1994). The population can be roughly categorized as three patches named for their locations relative to each other: east, west, and south. Volunteers from Shirley Heinze Land Trust (SHLT) counted the number of ramets and flowering ramets in the population from 1999 to 2012. Since bluebead lily is a preferred food of white-tailed deer (McCaffery et al. 1974; Balgooyen and Waller 1995; Augustine 1997), a protective fence was installed around the population in 2004 (Paul Quinlan, SHLT, pers. comm.). In order to visualize general trends, a linear model was fitted to the SHLT volunteer data on total number of ramets and total number of flowering ramets using R 3.3.2 (R Core Team 2016) and plotted using ggplot2 (Wickham 2009).

In 2011, the Indiana population was visited twice a week from May 1 to September 7, when all fruit had matured. Each individual ramet was labelled with an orange flag and assigned a unique number. In order to determine whether the population was reproducing sexually, fruits and seeds were collected. Each individual fruit was collected after the fruit had completely changed color from green to dark blue. Fruit set was determined by dividing the number of fruit harvested on each ramet by the number of flowers produced by that ramet. Seeds were removed from the fruits in the lab and categorized as viable or non-viable based on size. The total number of seeds in each fruit was counted to determine the number of ovules in each flower. Seed set

was computed as the number of viable seed divided by the number of ovlues. After all fruits had been harvested from the population, one leaf sample was taken from each ramet and dried with Drierite crystals ($CaSO_4$) to await DNA extraction. All ramets in the Indiana population were sampled except for two ramets belonging to the south patch.

To compare the Indiana population against non-isolated populations, I obtained leaf samples from Apostle Islands National Lakeshore (AINL) and Sleeping Bear Dunes National Lakeshore (SBDNL). Sampling was conducted on islands with historic populations of deer, islands without deer, and the Wisconsin mainland, as described in Chapter 3.

Total genomic DNA was extracted from leaf samples using Qiagen DNeasy plant kits and following manufacturer protocol (Qiagen, Valencia, California, USA). DNA concentration was quantified using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA) and samples were diluted to 10 ng/µL concentrations. The samples were amplified at 10 microsatellite loci: CLBO3_21, CLBO3_30, CLBO3_46, CLBO3_48, CLBO3_49, CLBO3_57, CLBO3_62, CLBO3_63, CLBO3_73, and CLBO3_78 (Palagi and Ashley, *in review*). Amplification was performed in 10 µL volumes with 10 ng of template DNA, 0.6 µM of reverse primer, 0.15 µM of forward primer, 0.25 µM each dNTP, 0.6 µL bovine serum albumin (BSA; 10% w/v), 1 µL 10x reaction buffer with 15 mM $MgCl_2$, and 0.2 µM florescent M13(-21) universal primer. The thermocycling profile consisted of an initial denaturation at 94°C for 5 min; then 35 cycles of 94°C for 30 s, T_a for 30 s (Palagi and Ashley, Chapter 3), 72°C for 30s; and a final extension at 72°C for 7 min. The PCR amplicons were analyzed on an ABI 3730 DNA analyzer with ALEXA custom size standard (Maddox and Feldheim 2014). Genotypes were scored using GeneMapper version 3.7 (Applied Biosystems, Foster City, CA, USA).

Multilocus genotypes (MLGs) in the Indiana population were identified using ALLELEMATCH in R 3.3.2 using an alleleMismatch parameter, $\hat{m}$, of 2, meaning that individuals can differ by up to 2 alleles and still be placed in the same MLG (Galpern et al. 2012). After combining all ramets belonging to each genet, I computed the following diversity indices: the complement of the Simpson's index corrected for small sample size, $D = 1 - \sum_i \frac{n_i}{N}\left(\frac{n_i-1}{N-1}\right)$ (Simpson 1949), the modified genet-to-ramet ratio, $G{:}N = \frac{G-1}{N-1}$ (Dorken and Eckert 2001), and the Hill's Simpson reciprocal, $Hill = \frac{1}{1-D}$ (Hill 1973). Harvested fruit and seed were identified through their mother ramets to individual genets. A generalized mixed effect model with random effects (GLMER) was used to determine the effect of fruit maturity data with seed set with the identity of the mother ramet and genet as random effects. I performed a generalized linear mixed effect model (GLMM) to analyze the effect of genet identity on seed set (Dray and Dufour 2007). I used Akaike's information criterion (AICc) to compare the fit of the GLMM against the null model (Anderson et al. 1998). Since seed set was recorded as the proportion of viable seed to ovules, I fitted the model with a binomial distribution. A post-hoc test was performed using the glht function of the multicomp R package which compared the effect of genet identity with Tukey's test (Bretz et al. 2010).

GenAlEx 6.5 was used to compute observed and expected heterozygosities (H_o, H_e) for all loci across all populations (Peakall and Smouse 2005). Allelic richness (A_r), inbreeding coefficient (F_{IS}), and private alleles (PALs) were determined with the POPPR package in R (Kamvar et al. 2014). F_{ST} and D_{JOST} were calculated in GenAlEx using 1000 bootstraps and 999 permutations to test for significance.

Population structure was analyzed using principal coordinates analysis and STRUCTURE 2.3.4. The principal coordinate analysis was performed in POPPR using Bruvo

distance transformed into Euclidean distance (Bruvo et al. 2004). STRUCTURE uses Baysian analyses to infer population structure of multilocus polymorphic genotypes. I used a protocol of 50K burnin with 100K MCMC with sampled populations as prior for a potential K of 1 to 8 at 30 repetitions each. I used STRUCTURE HARVESTER (Earl and Vonholdt 2012) to determine the best K by using the Evanno method (Evanno et al. 2005). I also used the find.cluster() function in adegenet to estimate K, retaining all principal components (Jombart 2008). The find.cluster function uses discriminant analyses of principle components (DAPC) to determine the Bayesian information criteria of different numbers of clusters, K (Jombart et al. 2010).

4.3 Results

Based on data collected by SHLT, the total number of ramets in the northwest Indiana population ranged from 41 to 86 over 13 years of monitoring (mean = 57.86, SE = 3.99). The number of flowering ramets ranged from 4 to 38 (mean = 15.7, SE = 2.98). Both total ramets and flowering ramets showed significant, positive slopes in linear models (total ramets: $F_{1,12} = 6.551$, p-value = 0.025, adj-$R^2 = 0.299$; flowering ramets: $F_{1,12} = 24.23$, p-value < 0.001, adj-$R^2 = 0.641$). Figure 4.1 shows the SHLT demographic data and the trends.

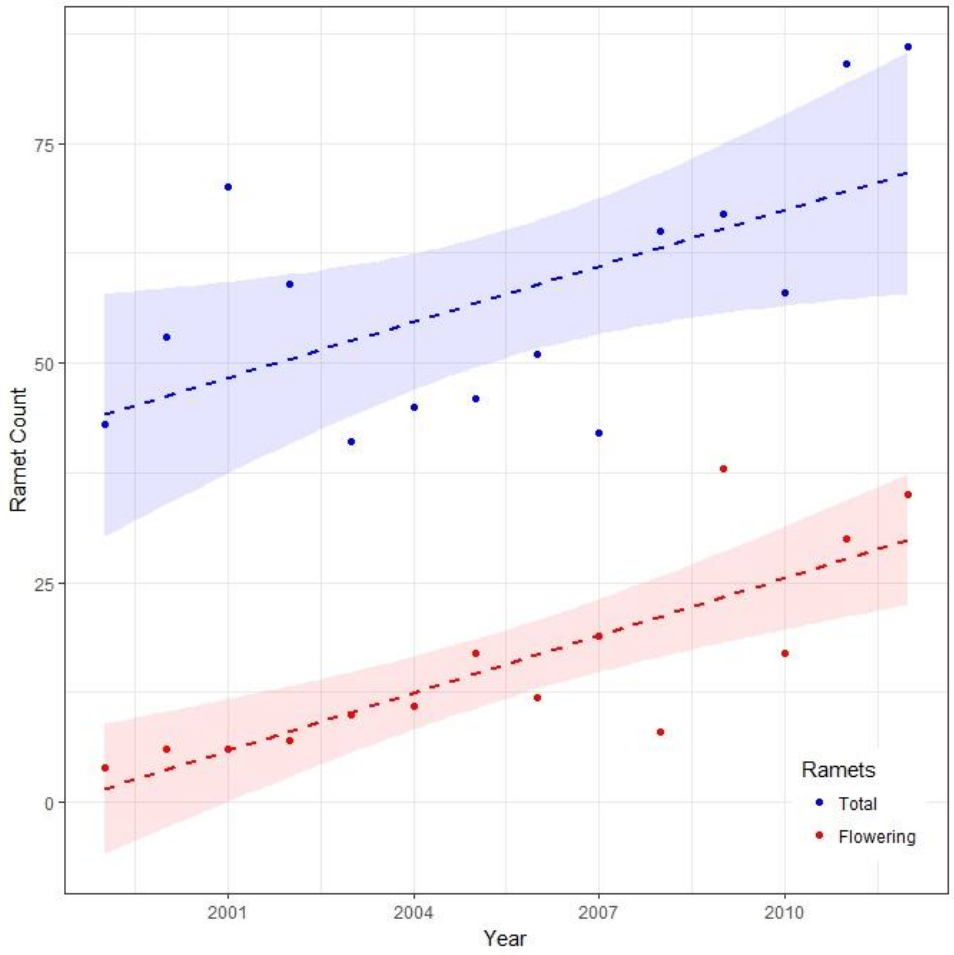

Figure 4.1 - Ramet counts of the northwest Indiana bluebead lily population from 1999 to 2012. Total ramets (blue) and flowering ramets (red) are shown with linear approximations of growth trends. Total ramets were growing at an annual rate of 2.12 (p-value = 0.250, adj-R^2 = 0.299). Flowering ramets were growing at an annual rate of 2.18 (p-value < 0.001, adj-R^2 = 0.641).

The Indiana population produced 101 flowers on 27 different ramets. The number of flowers produced by each ramet ranged from 2 to 7 (mean = 3.74, SE = 0.211). I collected a total of 56 fruit. While I observed that many of the fruit collected after August 1 took relatively longer to turn blue and never developed much in size, I considered these fruit in order to differentiate them from flowers which absolutely failed to produce fruit. Fruit set for individual ramets ranged from 0% to 85.71% (mean = 53.42%, SE = 5.463%. All fruit collected had 14 ovules, for a total of 1414 ovules for the harvested fruit. Collected fruit yielded 234 viable seeds. Seed set ranged

from 0% to 100% with an average seed set of 16.55%. Fruit with higher seed set matured earlier than fruit with low seed set based on the GLMER (p-value < 2e-16). No viable seeds were found in fruit that matured later than August 1 (Figure 4.2).

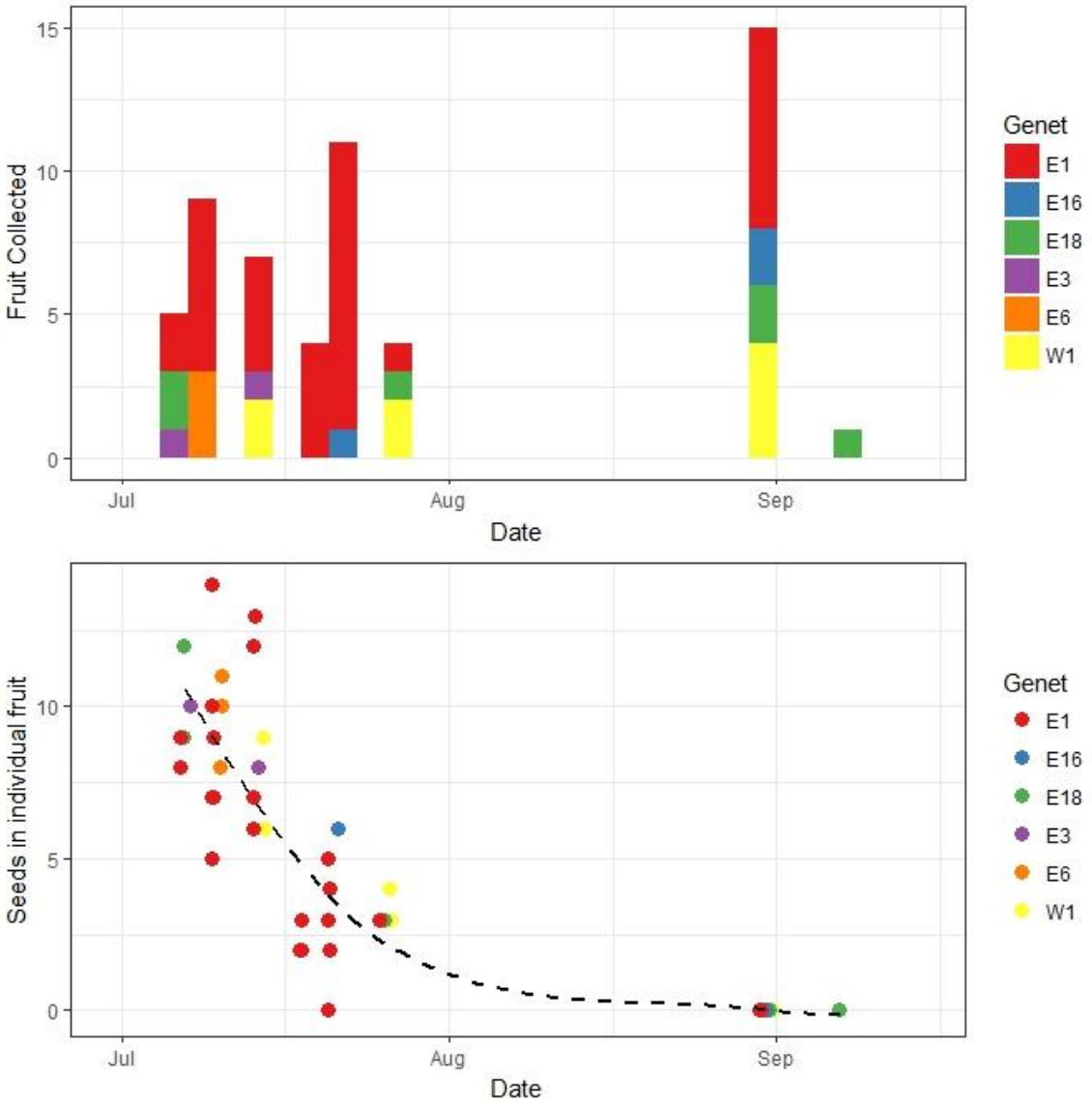

Figure 4.2 Fruit and viable seeds by date of harvest and genet identity from Indiana population. The upper plot shows the number of mature fruit collected on a particular date. The lower plot shows the number of viable seeds in the fruit collected on that date. The dashed line in the lower plot shows the trend of mean viable seeds harvested per fruit. No fruits had matured before July 7th.

Our census of the Indiana population ramets yielded 104 leaf samples, 80 ramets from the west patch and 24 ramets from the east patch. The two ramets of the south patch had prematurely senesced and were in a state of advanced decomposition when the leaf samples were taken from the other individuals, so they are not represented in any further analyses. Differences in number of ramets between volunteer data and leaf samples is due to the amount of effort taken to census the entire population. ALLELEMATCH identified 12 genets. The number of ramets belonging to each genet ranged from 1 to 34 (mean = 8.67, SE = 3.10), with two genets comprised of more than 20 ramets and eight genets comprised of fewer than ten ramets (Figure 4.3, Table IX). Genets varied widely in number of flowering ramets (0 to 14), number of flowers (0 to 54), fruit set (0 to 1, mean = 0.554, SE = 0.100), and seed set (0 to 0.690, mean = 0.298, SE = 0.070) (Table IX). Based on the number and genet identity of the ramets, G:N was 0.115, *D* was 0.807, and *Hill* was 5.181. The Indiana population was similar to islands with deer for G:N and *Hill*, shown in Table X (Palagi and Ashley, Chapter 3). The GLMM showed a significant influence of genet identity on seed set versus the null model (AIC_{genet} = 484; AIC_{null} = 444). Post-hoc analysis showed high significance for E3 and E6 against all the other genets (p-value < 0.02 for all comparisons), but not to each other (p-value > 0.99) (Table XI; Figure 4.4).

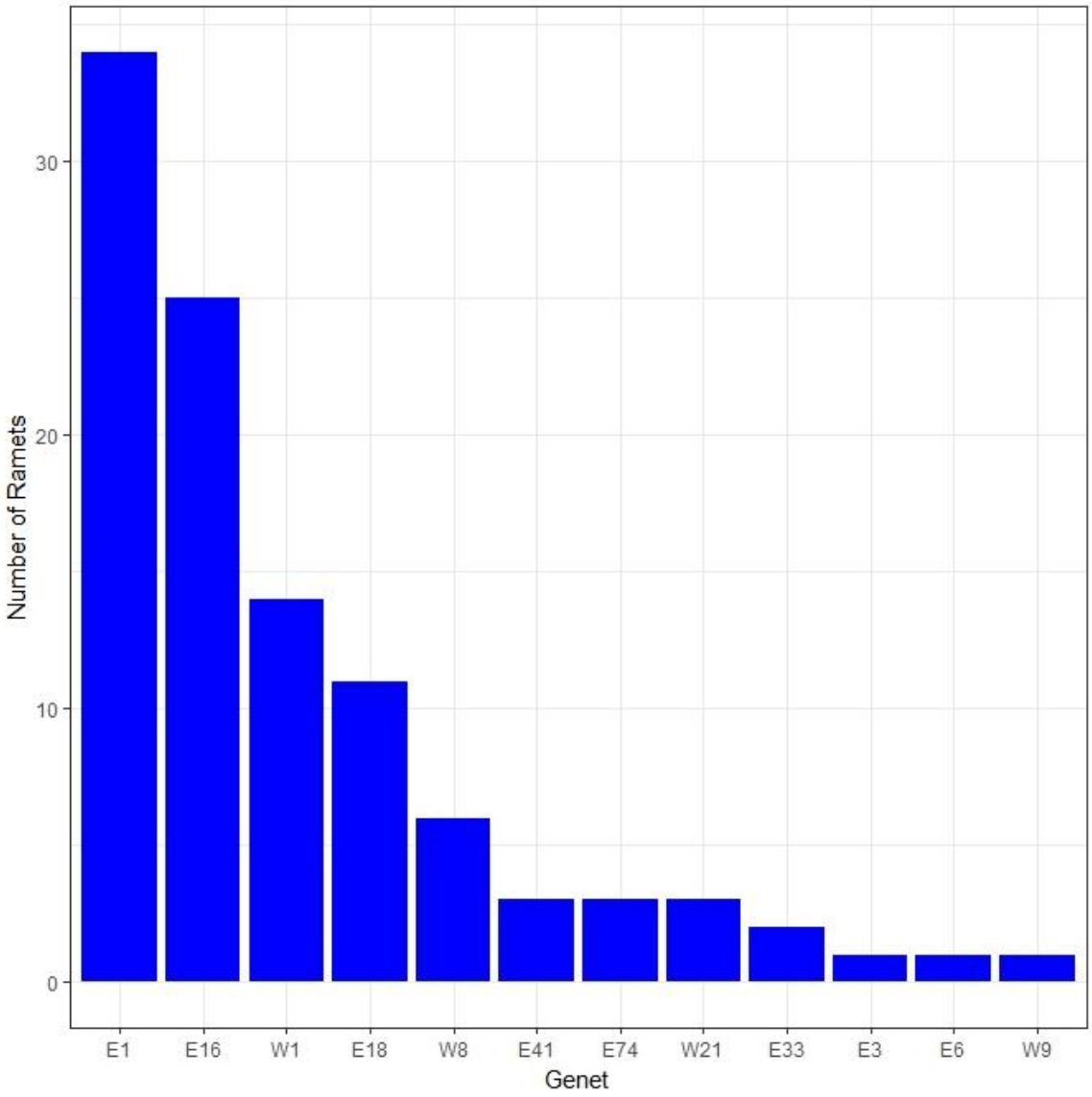

Figure 4.3 – The distribution of ramets per genet. Number of ramets on the ordinate inferred to belong to each individual genet indicated on the abscissa in the Indiana population.

Table IX – Compilation of fruit and seed data assigned to distinct genets. For each genet, data is given for the number of ramets, number of flowering ramets, the total number of flowers for the genet, the number of fruit produced, the number of viable seeds, rate of fruit and seed set, and p_{sex} of the genotype.

Genet	Ramets	Flowering Ramets	No. Flowers	No. Fruit	No. Seed	Fruit Set	Seed Set	p_{sex}
E1	34	14	54	34	147	0.630	0.309	9.74E-06
E3	1	1	5	2	18	0.400	0.643	9.81E-08
E6	1	1	5	3	29	0.600	0.690	2.70E-06
E16	25	2	5	3	6	0.600	0.143	8.50E-06
E18	11	2	6	6	24	1.000	0.286	7.88E-05
E33	2	0	-	-	-	-	-	2.89E-07
E41	3	1	6	0	0	0.000	0.000	5.67E-06
E74	3	0	-	-	-	-	-	6.25E-07
W1	14	6	20	8	22	0.400	0.196	3.74E-07
W8	6	0	-	-	-	-	-	2.30E-08
W9	1	0	-	-	-	-	-	1.22E-07
W21	3	0	-	-	-	-	-	5.68E-08
Totals	104	27	101	56	234	0.554	0.298	
					SE	*0.100*	*0.070*	

Table X - Diversity indices genet-to-ramet ratio, Simpson's complement index, and Hill's Simpson reciprocal for Indiana population compared against averaged data from Chapter 3. Except for Indiana, numbers represent means with the standard error below and in italics.

Treatment	G:N	*D*	*Hill*
Deer Islands	0.104	0.345	1.636
	0.014	*0.074*	*0.198*
No Deer Islands	0.458	0.903	21.399
	0.091	*0.025*	*11.239*
Mainland	0.480	0.904	13.497
	0.036	*0.027*	*3.755*
Indiana	0.115	0.807	5.181

Table XI - Results of post-hoc Tukey test comparing differences in mean seed set by genet. The significance of the difference between means is given in the last column and highlighted by the asterisks to the right.

Genets	Estimate	Std. Error	z value	Pr(>|z|)	
E3 - E1 == 0	1.3934	0.4067	3.426	0.00697	**
E6 - E1 == 0	1.608	0.3482	4.618	< 0.001	***
E16 - E1 == 0	-0.9861	0.452	-2.182	0.22611	
E18 - E1 == 0	-0.1107	0.2611	-0.424	0.99798	
W1 - E1 == 0	-0.6031	0.2577	-2.341	0.16184	
E6 - E3 == 0	0.2146	0.5167	0.415	0.99817	
E16 - E3 == 0	-2.3795	0.5916	-4.022	< 0.001	***
E18 - E3 == 0	-1.5041	0.4625	-3.252	0.01284	*
W1 - E3 == 0	-1.9966	0.4606	-4.335	< 0.001	***
E16 - E6 == 0	-2.5941	0.553	-4.691	< 0.001	***
E18 - E6 == 0	-1.7186	0.412	-4.172	< 0.001	***
W1 - E6 == 0	-2.2111	0.4098	-5.395	< 0.001	***
E18 - E16 == 0	0.8755	0.5028	1.741	0.47664	
W1 - E16 == 0	0.383	0.501	0.764	0.9696	
W1 - E18 == 0	-0.4925	0.339	-1.453	0.66992	

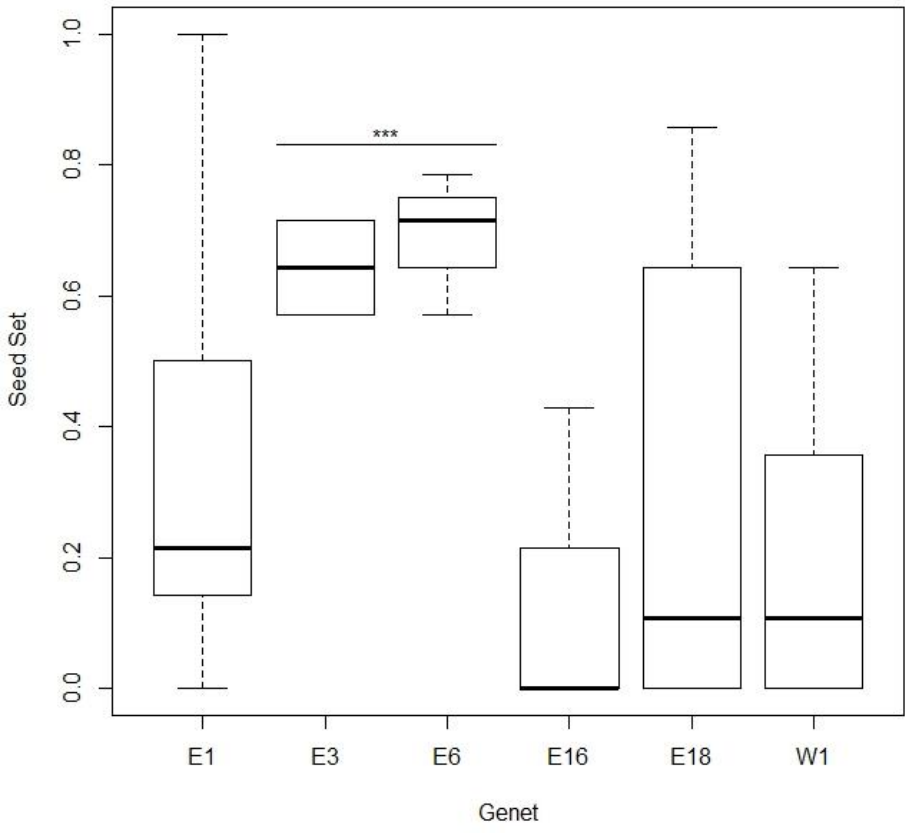

Figure 4.4 – Boxplot of seed set determined by genet identity in the Indiana population. An GLMM showed significant difference in seed set for genets E3 and E6 from all other genets. *** $p < 0.001$.

When the Michigan and Wisconsin populations were included, sample sizes ranged from 12 to 24. Observed heterozygosities (H_o) ranged from 0.550 to 0.675, and expected heterozygosities (H_e) ranged from 0.597 to 0.791. Inbreeding coefficient (F_{IS}) ranged from -0.0879 to 0.2813. Allelic richness (A_r) was lowest in the Indiana population, and ranged from 3.580 to 7.444 alleles/locus. Private alleles (*PALs*) ranged from 0 to 20, with SM having the most private alleles and the AINL mainland having no private alleles (Table XII). For both measures of population differentiation, F_{ST} and D_{EST}, the Indiana population showed large and significant differences, between 0.445 and 0.771 for D_{EST}, from the other population (Table XIII). South

Manitou island (SBDNL) showed the next largest differences, from 0.386 to 0.595 for D_{EST}. The only non-significant difference between islands for both indices was between Basswood Island and Oak Island (AINL).

Table XII - Descriptive statistics for bluebead lily populations. N = number of samples, H_o = observed heterozygosity, H_e = expected heterozygosity, F_{IS} = inbreeding coefficient, A_r = allelic richness, and PALs = private alleles. Horizontal lines group islands with and without deer.

Population	N	H_o	H_e	F_{IS}	A_r	PALs
IN	12	0.675	0.597	-0.0879	3.580	4
SM	17	0.608	0.791	0.2586	7.444	20
DV	24	0.645	0.689	0.0783	5.631	6
RI	24	0.552	0.709	0.2392	6.675	7
SI	20	0.667	0.703	0.0678	5.797	3
BW	24	0.574	0.719	0.2168	6.187	5
OI	15	0.550	0.732	0.2813	6.479	5
AM	21	0.584	0.733	0.2213	6.138	0

Table XIII – Genetic differentiation between populations of bluebead lily. Numbers below the diagonal are D_{EST} values while numbers above the diagonal are F_{ST} values. Bold indicates significance based on 999 permutations. Shading was added to emphasize differences in values.

	IN	SM	DV	RI	SI	BW	OI	AM
IN	0.000	**0.099**	**0.180**	**0.162**	**0.163**	**0.169**	**0.164**	**0.166**
SM	**0.445**	0.000	**0.088**	**0.086**	**0.097**	**0.089**	**0.069**	**0.089**
DV	**0.776**	**0.503**	0.000	**0.055**	**0.078**	**0.068**	**0.059**	**0.058**
RI	**0.708**	**0.521**	**0.223**	0.000	**0.041**	**0.049**	**0.042**	**0.041**
SI	**0.702**	**0.595**	**0.347**	**0.149**	0.000	**0.023**	**0.040**	**0.028**
BW	**0.766**	**0.558**	**0.304**	**0.205**	**0.051**	0.000	0.023	**0.023**
OI	**0.758**	**0.386**	**0.244**	**0.145**	**0.127**	0.029	0.000	**0.036**
AM	**0.771**	**0.575**	**0.250**	**0.157**	**0.073**	**0.052**	**0.112**	0.000

Principal coordinate analysis of variance (PCoA; Figure 4.5) shows tight clustering of most of the AINL islands with DV segregating along the second axis. The Indiana population clusters by itself along the first axis. The SM population overlaps slightly with the AINL populations. The two axes explain 8.35% and 5.28% of the variance, respectively. The Evanno method clustered the data into two populations (Figure 4.6) which correspond to Lake Michigan (IN and SM) and Lake Superior (others) populations. The DAPC method clustered the data into four populations (Figure 4.6), with IN and SM showing differentiation with a small amount of admixture and DV, the northernmost island of AINL, differentiating somewhat from the other AINL islands.

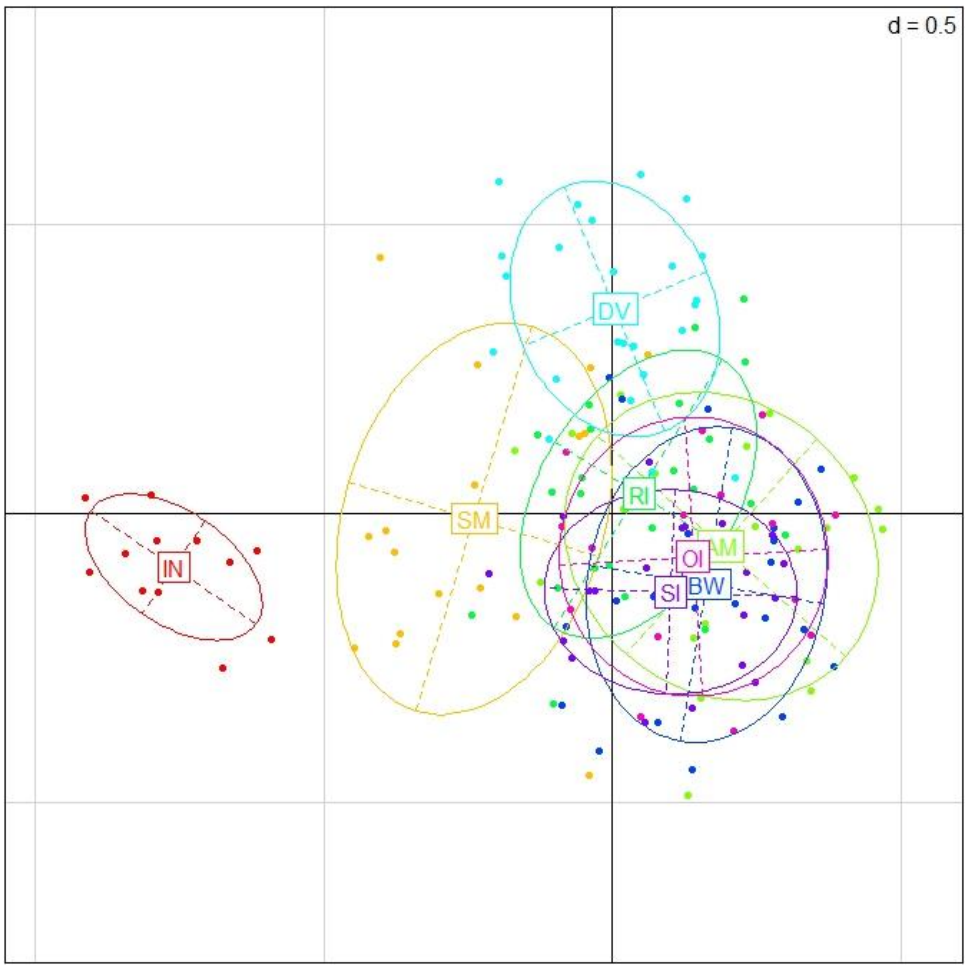

Figure 4.5 – Principal coordinate analysis of variance showing differentiation between bluebead lily populations. Principal coordinates 1 and 2 account for 8.35% and 5.28% of the variance respectively. Populations are denoted by color and initials.

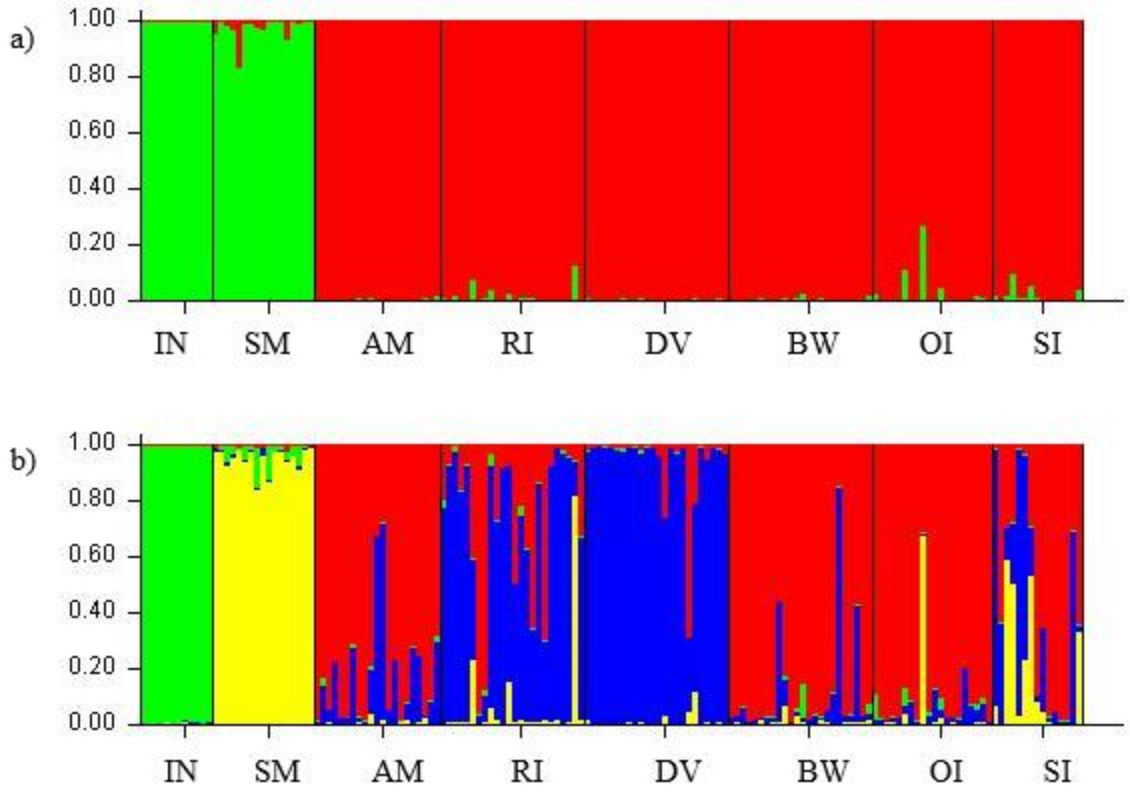

Figure 4.6 – STRUCTURE results of bluebead lily admixture proportions for each genetic cluster for individual plants (columns). Population are divided by vertical lines and labelled below the plot. Clustering for (a) is based on K = 2 per Evanno method. Clustering for (b) is based on K = 4 per DAPC method.

4.4 Discussion

The conservation of local biodiversity has become a complex issue due to the effects of climate change and habitat fragmentation. Isolated populations may suffer from genetic erosion and lose the potential to adapt to changing environmental factors. Translocation of novel genotypes is a conservation strategy intended to mitigate or negate the genetic erosion of isolated populations. Alternatively, adaptations that have developed in these small, isolated populations may be lost due to local extinction. Detailed knowledge of a population is necessary to understand when translocation or assisted migration may be useful to the preservation of the

population and the evolutionary trajectory of the species. I analyzed several aspects of an isolated bluebead lily population in northwest Indiana in order to consider appropriate conservation strategies.

Our analysis of the Indiana population discovered 12 genets. This is important information since the population could have been composed of one or a few genets, which could have led to severe inbreeding. Both the clonal diversity (G:N) and genet richness (Hill) indices of the Indiana population are similar to patches sampled on islands with deer (Table IX). Florivory by deer can have a dramatic influence on the reproductive mode of bluebead lily (Palagi and Ashley, Chapter 3). Suppression of floral reproduction due to deer prior to the construction of the protective fence in 2002 is probably a factor in the low clonal diversity in the Indiana population. The growth trends illustrated in Figure 4.2 could be indicative of a recovering population. The relatively high genet evenness (*D*) for the population is potentially a result of an evenness created by the conditions of no recent migration or seedling recruitment. While these conditions did not allow the formation of new genets, the few genets present were able to survive and multiply clonally. After the construction of the chain-link fence in 2002, deer could no longer consume the flowers and the population likely began to form new genets, perhaps those genets with low ramet counts in Figure 4.4.

The Indiana population was observed to be sexually reproducing as evidenced by the development of mature fruit and viable seed. Bluebead lily populations have shown various levels of self-sterility (Galen et al. 1985; Galen and Weger 1986; Barrett and Helenurm 1987; Dorken and Husband 1999). While the data in Table IX only represents one year of data, the fruit and seed set rates are similar to the rates reported for outcrossing bluebead lily flowers by Dorken and Husband (1999), 0.54 fruit set and 0.38 seed set. This similarity suggests that the

Indiana population may not be mate limited and that pollinator visitation does not seem to be a concern.

The population genetics analyses show high differentiation between populations. Using the Evanno method to calculate clusters, STRUCTURE plots show a marked difference between populations at AINL and Indiana/SBDNL (Figure 4.6). Geographically, this difference could be accounted for by AINL locations being in or near Lake Superior and Indiana/SBDNL locations being in or near Lake Michigan. Using DAPC to calculate clusters, STRUCTURE plots show four clusters that correlate with the PCoA plot. The Indiana and SM populations cluster individually and DV seems to be diverging from the rest of the AINL populations (Figure 4.5). The further differentiation of the Indiana population shown in the PCoA may indicate long term isolation and perhaps local adaptation. Allelic richness is lower in Indiana than in all the other sampled populations, possible showing evidence of a bottleneck. The differentiation and loss of alleles in the Indiana population provides some evidence for concern about the genetic diversity of the population.

Being that bluebead lily is a long-living perennial with strong tendencies for clonal growth, the Indiana population is likely to persist for decades (Pitelka et al. 1985). The population is composed of multiple genets with enough genetic diversity to produce viable seed. Translocation of new genotypes into the population is unlikely to harm the population due to outbreeding depression (Frankham 2015). Alternatively, since E3 and E6 have higher seed set than other genets, collection and careful germination of seeds from these genets could be used to augment the Indiana population with rare genotypes to maintain genetic diversity. The use of the rare genotypes from within the population minimizes the risk of swamping local adaptations while maintaining local genetic diversity.

Attention to various local environmental factors could aid the persistence of the bluebead lily population. Specifically, a reduction of interspecific competition by mechanically removing or inhibiting other species (even common natives) from the immediate area can provide more local habitat for the bluebead lily population. Since bluebead lily locally associates with *Betula alleghaniensis*, yellow birch, the encouragement of yellow birch saplings could have a positive effect on the bluebead lily population (Swink, F, Wilhelm 1994). Perhaps most importantly, the tree canopy must be maintained in order to protect the population from full sun conditions. Bluebead lily does not suvive without significant canopy cover (Ashmun and Pitelka 1985).

The Indiana bluebead lily population currently exists on the lagging edge of the bioclimatic envelope of the species. Given historic and current trends in climate change, the maintenance of the population will require increasing attention and resources in the future. Population differentiation of the Indiana population could be due to local adaptations, perhaps adaptations which have allowed the population to persist at the lagging edge of its bioclimatic envelope when other populations have gone extinct. Therefore consideration should be given to migrating offspring from the Indiana population to locations more centrally located within the species range. In its current location, neither pollen nor seed are likely to migrate via natural means to other populations of bluebead lily. The migration of Indiana offspring northward could confer adaptations for survival in slightly warmer conditions to other populations. Further, if the Indiana population were to go extinct sometime in the relatively near future, the assisted migration of offspring would preserve the genetic legacy of the population.

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