CLONAL STRUCTURE AND MATING PATTERNS IN A NATURAL POPULATION OF *SAGITTARIA LATIFOLIA*

A Thesis Submitted to the Committee on Graduate Studies in Partial Fulfillment of the Requirements for the Degree of Master of Science in the Faculty of Arts and Science

TRENT UNIVERSITY

Peterborough, Ontario, Canada

Environmental and Life Sciences M.Sc. Graduate Program

September 2022

ABSTRACT

CLONAL STRUCTURE AND MATING PATTERNS IN A NATURAL POPULATION OF *SAGITTARIA LATIFOLIA*

Samantha Jade Stephens

Increased plant size is expected to have negative consequences for mating by increasing pollen transfer among the same plant. However, recent theoretical studies have demonstrated that this may not be true for clonal plants. Instead, clonal expansion could enhance outcrossing opportunities without increasing selfing by reducing distances to potential mates. I investigated how the spatial structure of clones influences patterns of pollen dispersal, selfing rates and siring success in a natural population of *Sagittaria latifolia*. I found that pollen dispersal distances typically exceeded the spatial extent of clones and there was a positive association between clone size and the likelihood that clones were intermingled. Together, this resulted in a weak positive association between clone size and selfing rates, and a strong positive association between clone size and outcross siring success. This is the first empirical support for the theoretical expectation that any negative effects of selfing in large clones might be offset by increased siring success.

KEYWORDS

Fitness gain curve; dispersal kernel; clonal growth; sex allocation theory; plant mating; plant reproductive ecology; geitonogamy; *Sagittaria latifolia*

ACKNOWLEDGMENTS

First and foremost, I would like to thank my supervisor, Dr. Marcel Dorken for his guidance and patience throughout the course of my studies. I would also like to thank the other members of my committee, Dr. Neil Emery and Dr. Jeff Bowman, for their time and advice. Many colleagues generously assisted me with lab and field work or provided advice. Adele Bunbury-Blanchette began the fieldwork for this project, taught me fieldwork procedures, and continued to help me collect field data. Brandan Leroux, Rebecca Paton, and Danielle Carter also assisted me with the collection of field data. Laura Perry, Dr. Ryan Franckowiak, Dr. Sara Pieper, and Dr. Joanna Freeland provided advice on lab protocols and genotyping, and Hilary Sergeant and David Feairs assisted me with lab work. Thank you to lab technicians Caleigh Smith, Kristyne Wozney, Anne Kidd, and Matthew Harden for running my many plates. A special thank you to my lab sisters, Allison Kwok and Sara Pieper, for their advice, support and companionship.

I would not have made it to this point without the unwavering support of many friends and family. Mom and Dad, thank you for everything. Sarah Bonnett, Kat Cetinic, and Mark Basterfield—thank you for your friendship and support during the most challenging times. Tim Irvin—I am so thankful to have had a friend on a similar journey this entire time. Your support and motivation have made this far less lonely. Patrick Moldowan, thank you for your unlimited support, patience, and encouragement through the final stages.

TABLE OF CONTENTS

LIST OF FIGURES

Chapter 1

Chapter 2

have proportionally more self-ramets within their local mating neighbourhoods. However, this increase in the proportion of self-ramets was weak when I assumed that ramets occupied areas corresponding with the median pollen dispersal distance (i.e. tIRMD). Lines indicate the results of the linear mixed-effects model (a, c & e) and the GLM (b, d, & f) used for examining patterns of intermingling versus clustering.

Figure 3: Patterns of selfing as a function of genet size (number of ramets per MLL) in a natural population of *Sagittaria latifolia*. Point size is proportional to the number of seeds per MLL screened for selfing rates. The line indicates the slope of the weighted regression of average selfing rate on genet size, with the number of seeds screened per MLL used as the weighting variable.

Figure 4: Association between siring success and genet size (number of ramets) in a natural population of *Sagittaria latifolia*. Point size is proportional to the cumulative measure of confidence in siring (the sum of the probabilities of paternity per assigned sire, calculated using COLONY2). For plotting, the number of flowering ramets per genet was log-transformed, and the line indicates the slope of a weighted linear regression of siring success on the log(number of ramets per genet) using the iIRMD as the mating neighbourhood size.

Figure 5: Association between clone size (number of ramets per genet) and male investment (total number of male flowers per genet).

Figure 6: Comparison of the observed versus model-predicted patterns of siring success versus

male investment for the linear (red circles) and non-linear (power function; blue triangles) models. Symbol size was scaled by my cumulative measure of confidence in siring (the sum of the probabilities of paternity per assigned sire, calculated using COLONY2).

Figure 7: Association between the total investment in male function per genet (number of male flowers per MLL) and siring success in a natural population of *Sagittaria latifolia*. The nonlinear model (power function) was associated with a lower AIC score than the linear model. Lines were calculated from the fitted parameter values from each model. Point size is proportional to my cumulative measure of confidence in siring (the sum of the probabilities of paternity per assigned sire, calculated using COLONY2).

LIST OF TABLES

Chapter 2

CHAPTER 1: GENERAL INTRODUCTION

1.1 Mating Patterns in Plants

Mating patterns in plants are commonly more complex than those observed in animal groups (Meagher 1986). This complexity is attributed to the combined effects of modular growth, immobility, and the movement of gametes via third-party vectors of pollen dispersal. The angiosperms, or flowering plants, exhibit an impressive variety of forms and functions of their reproductive units, the flowers, as well as a variety of sexual systems (e.g., hermaphroditism versus dioecy) and mating strategies (e.g., selfing versus outcrossing; Barrett 2010). Most flowering plants have a hermaphroditic sexual system, with male and female reproductive functions appearing within a single individual. However, dioecious (separate male and female individuals), and trioecious (male, female, and hermaphrodite individuals), sexual systems occur as well. Even within hermaphroditic populations, the allocation of resources to male and female functions can be highly variable (i.e., female- or male-biased individuals).

Species may be predominantly selfing, outcrossing, or lie somewhere on the continuum between these strategies (Baker 1959; Barrett & Eckert 1990; Vogler & Kalisz 2001). The balance between promoting outcrossing and avoiding selfing is thought to be a major driver of reproductive adaptations in flowering plants (Barrett 2010). Traits affecting mating and sexual systems have evolved on numerous occasions rather than appearing in a single lineage. For example, dioecy occurs in only 6% of angiosperm species, however, this trait has evolved from hermaphroditism up to 5000 times (Renner 2014). Dioecy is one mechanism that enforces

outcrossing by limiting individuals to a single reproductive function. Indeed, a major driver of the transition from hermaphroditism to dioecy is the avoidance of inbreeding and its downstream effects on offspring fitness (inbreeding depression; Charlesworth & Charlesworth 1978; Barrett 2010).

1.2 Clonality

Clonality is an asexual, vegetative mode of reproduction in which an individual (genet) is composed of multiple connected shoots (ramets) that form from meristematic tissue (Harper 1977). Ramets may or may not remain physiologically connected to the rest of the genet and can be capable of independent growth and mating opportunities that ultimately contribute to the fitness of the entire genet (Van Drunen, van Kleunen & Dorken 2015). It is estimated that approximately 80% of angiosperm species are capable of clonal growth and these species occur in all major habitat types (Klimes et al. 1997). Clone architecture refers to how a clone is arranged in space. The phalanx strategy describes clones that are aggregated, whereas the guerilla strategy describes clones with larger distances between their ramets (Lovett-Doust 1981). When mates are scarce, such as in colonizing new areas, clonality may ensure propagation. It also allows a plant to forage for resources and spread the risk of death among ramets (Caraco & Kelly 1991; Silander 1985; Stuefer 1996). Clonality is an important feature of the life history of perennial plants, but its effects on plant fitness, particularly in terms of the effects of clonal growth on mating patterns, remains poorly understood.

Although clonality may provide ecological benefits, it also affects mating patterns, because clonality changes how reproductive units are distributed in space. Clonality allows an individual

to subdivide its reproductive effort by presenting various inflorescences over a larger area, rather than growing large and presenting all reproductive units in one place (Dorken & Van Drunen 2010). Pollen is most likely to be transferred to neighbouring ramets (Levin & Kerster 1974; Waser 1982) and therefore, if clones are isolated within a population (meaning individual ramets are surrounded by ramets belonging to the same genet), this could promote selfing (Handel 1985). However, if clones are intermingled among each other and ramets are instead surrounded by those of other genets, this could promote outcrossing (Van Drunen, van Kleunen & Dorken 2015). Clonality as a life-history trait can therefore have differing fitness outcomes dependent on the ecology of the population. The proportion of plants that are capable of clonal growth suggests that this life-history trait should have fitness benefits (Klimes et al. 1997).

1.3 Sex Allocation Theory (SAT)

Sex allocation theory (SAT) is the single unifying concept underlying the modern understanding of the evolution of reproductive systems and is one of the most successful theories in evolutionary biology (West 2009). SAT links organismal life histories to their fitness consequences (Charnov 1982) and explains the variation in mating systems observed in both plants and animals by predicting the conditions that allow different sexual systems, including various states of hermaphroditism and dioecy, to be evolutionarily stable. For hermaphrodites, it predicts the fitness consequences of allocation of resources to either male or female reproductive function. This theory attempts to predict the optimal sex allocation under different ecological conditions (Charnov 1982).

Central to this theory are fitness gain curves, which are applicable to mating systems in both plants and animals. These curves are a function of the investment in male or female reproductive effort and the resulting fitness gains. The fitness gain curve for male function is predicted to be decelerating, corresponding with the expectation that male fitness is subject to mate limitation. For plants, males produce more pollen than is needed to fertilize the ovules in a population, and because pollination vectors tend to move pollen to near neighbours, it is thought that males should tend to rapidly saturate their surrounding mating partners with pollen. This renders the production of additional pollen superfluous, leading to diminishing (fitness) returns on the production of pollen (Campbell 2000). By contrast, plants are generally assumed to experience linear returns on fitness through the production of ovules and seeds (Zhang 2006). Accordingly, the resources required to produce ovules and mature fruits are thought to be the primary limitation of fitness through female function (Fig. 1; Charnov 1982).

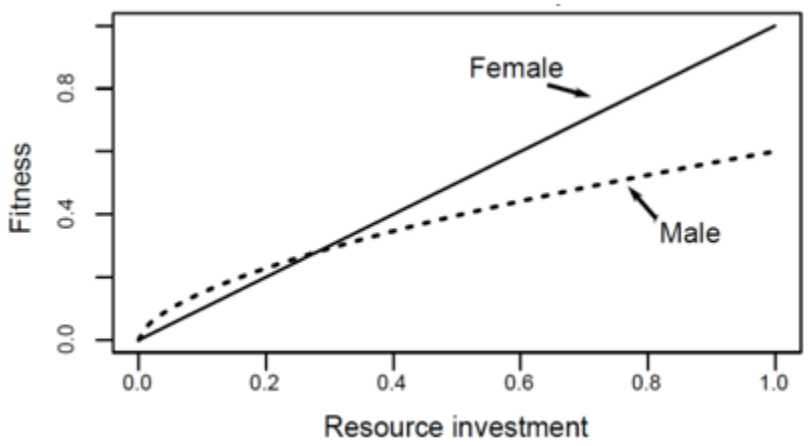

Figure 1. Shapes of the male and female gain curves predicted from Sex Allocation Theory

1.4 The Male Fitness Gain Curve & Mating Patterns

One ecological factor that may change the shape of the male gain curve is the temporal presentation of gametes (Wilson et al. 1994). This is because females produce a limited number

of ovules to be fertilized by males at a given time. The gradual presentation of pollen allows males to avoid saturating females by increasing the duration over which mating opportunities are available. The saturation of pollinators, which transfer pollen between male flowers and female ovules, can also be avoided, resulting in a higher proportion of pollen export. By investing only a proportion of resources into male reproduction at a time, males are able to spread out resource investment (Perry & Dorken 2011). In this way, each male flower can take advantage of the steepest portion of the gain curve, adding up to an overall more linear curve for the entire individual (Perry & Dorken 2011). This study by Perry and Dorken provides the clearest evidence to date that the gradual presentation of pollen yields a more linear gain curve, although no study has been able to directly measure the shape of the male gain curve for plants (Perry & Dorken 2011).

In addition to subdividing reproductive investment over time, plants can also subdivide their reproductive investment over space. As discussed above, most perennial plants are capable of clonal growth (Klimes et al. 1997). From the perspective of reproduction, the effect of clonality is to subdivide reproductive investment across the shoots (ramets) that make up a clone (Dorken & Van Drunen 2010). These ramets can act independently of one another during reproduction, attracting their own pollinators and thereby creating their own mating opportunities.

There are contrasting views however, of how clonality may affect the shape of the male gain curve. On one hand, increasing the number of shoots within a single individual increases the probability of pollen transferred among the flowers of the same genetic individual. When a

clone increases in size, the probability that two gametes drawn at random will belong to the same genet increases, and therefore theoretically increases selfing rates (geitonogamous selfing; Rademaker & de Jong 1998; Harder & Barrett 1995; Mori, Nagamitsu & Kubo 2009; Vallejo-Marín, Dorken & Barrett 2010). Selfingresults in pollen discounting, in which pollen is transferred within the same individual and produces an inbred seed at the expense of outcrossing. This effect might be especially important if the shoots of a clone are clumped together in a population, reducing the independence of mating opportunities across the shoots of a clone. In this scenario, clonal expansion is expected to yield a strongly diminishing gain curve for male function via local mate competition (Lloyd & Bawa 1984). On the other hand, if the mating opportunities of shoots within a clone are independent (e.g., if shoots are intermingled with other clones), this should yield a linear gain curve for male function (Dorken & van Drunen 2010; and see Albert, Raspé & Jacquemart 2008). By subdividing reproductive investment across shoots that act independently during reproduction, fitness gains for each ramet additively contribute to the total fitness of the clone, yielding a linear gain curve (Fig. 2).

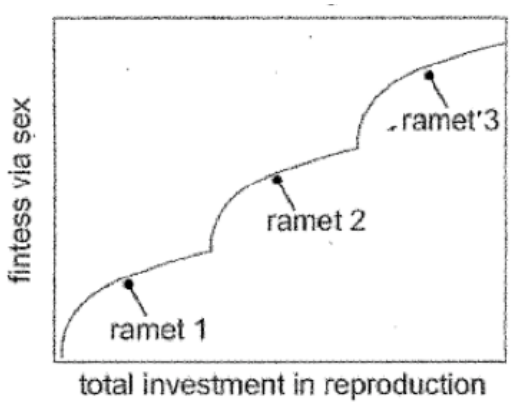

Figure 2. The subdivision of reproductive investment linearizes the male gain curve

Although there are some clear expectations from theory, the effects of clonality on fitness via male function are poorly studied. Currently, investigations in natural populations and artificial arrays of the effect of clonal growth on male fitness have only considered clumped distributions (Handel & Mishkin 1984; Routley, Kron & Husband 2004; Mori, Nagamitsu & Kubo 2009) or used indirect proxies of male fitness (Liao & Harder 2014). There are few studies that have mapped the distribution of clones within a population while also investigating how these distributions affect mating patterns, and therefore there remains a lack of empirical evidence for the effect of clonality on fitness. Matsuo et al. (2014) found no evidence for an association between clone size and selfing in a study of an intermingled population of dwarf bamboo. Moreover, and contrary to the expectations from Dorken and Van Drunen (2010) they inferred that fitness gains through pollen production are strongly diminishing. This could be in part because the study only included paternity assessment from a portion of the population, and larger clones are more likely to sire offspring outside of this area. If the spatial arrangement of clones is a key driver in the fitness gains resulting from clonality, mapping this spatial distribution is of primary importance.

1.5 Study System

Sagittaria latifolia (Alismataceae) is one of 11 species in the genus commonly known as arrowheads. It is a highly clonal, perennial, emergent aquatic plant commonly found in wetlands in North America (Dorken & Barrett 2003). It reproduces both asexually via corms and stolons, and sexually by seed. In Ontario, both monoecious and dioecious populations exist (Wooten 1971). In monoecious populations, individuals are hermaphroditic, and inflorescences display monoecious flowers allowing for unambiguous assignment of male and female

reproductive effort. Flowers are produced on racemes with whorls of three flowers at each node, with the number of whorls dependent on the size of the plant. Female flowers open first at the basal nodes, and male flowers open later at distal nodes, with each flower being receptive for one day. The flowering time between male and female flowers does not overlap within a single ramet, meaning that any selfing can be attributed as a cost of multiple ramets, i.e., clonality (Dorken, Friedman & Barrett 2002). The flowers are pollinated by several generalist pollinators including flies, wasps, bees, and butterflies (Muenchow & Delesalle 1994).

Microsatellite markers have already been developed for this species, allowing for genetic analysis (Yakimowski et al. 2009). On average, plants from monoecious populations produce approximately twice as many ramets and corms as dioecious populations (Dorken & Barrett 2003). One study compared the clonal structure of *S. latifolia* in monoecious versus dioecious populations and found that monoecious populations had larger clones than dioecious populations (Yakimowski & Barrett 2014). However, in both types of populations, there was little evidence for genetic sub-structuring; plants from neighbouring clones were no more likely to be genetically related to each other than pairs of clones positioned further away in the population (Yakimowski & Barrett 2014). Therefore, inbreeding, when it occurs in *S. latifolia*, should usually involve selfing and, because there is no overlap in the timing of when female and male flowers from the same inflorescence are open, selfing occurs between ramets from the same genet. Clonal growth is the primary mechanism by which selfing can occur in this plant and it provides a useful system with which to examine the mating effects of clonal growth.

1.6 Aims of Study

The aim of this study was to determine how the spatial arrangement of ramets within clones of *Sagittaria latifolia* affects patterns of mating in a natural population. The spatial arrangement of the entire natural population was mapped. Ramets were assigned to clones and selfing rates were estimated for each genet. Patterns of mating were revealed through paternity assignment, and the gain curve for male function was measured for each clone size (number of ramets). This study provides the first data assessing fitness consequences of clonality through male and female function. Understanding how ecological factors can influence the shape of the gain curve for male function has implications for our understanding of sex allocation theory.

CHAPTER 2: PATTERNS OF POLLEN DISPERSAL AND MATING IN A POPULATION OF THE CLONAL PLANT *SAGITTARIA LATIFOLIA*

2.1 Introduction

Flowering plants (angiosperms) exhibit more variety in their reproductive structures than any other group of animals, and this diversity is accompanied by an equally impressive variety of mating strategies and sexual systems (reviewed in Barrett 2010). This diversity—and resulting complexity—is due to a few key traits. First, plants are immobile and therefore cannot actively seek mates, relying instead on third-party vectors to distribute their gametes during sexual reproduction (Barrett & Harder 1996). Further complexity is introduced by their modular growth (Harper 1977)—allowing individuals to vary in size, shape, and reproductive effort—and hermaphroditism (Barrett & Harder 1996). For most plants, mating success through both male and female functions entails the efficient movement of pollen between plants (outcrossing), while avoiding self-fertilization (selfing; Lloyd & Schoen 1992; Barrett 2003). Indeed, selfing is typically associated with negative consequences for fitness via both male and female functions (reviewed in Barrett 2002). For female function, self-pollination can clog stigmas and 'usurp' ovules, reducing outcrossing opportunities. It can also result in inbreeding depression for selfed progeny (reviewed in Barrett 2002). For male function, selfing results in pollen discounting, whereby pollen transfer that results in selfing is 'wasted', or a missed opportunity for outcrossing (Harder & Barrett 1995). Investigating ecological factors and life-history traits that influence selfing and outcrossing rates can reveal what drives the diversity of plant mating systems.

Increased selfing and reduced siring success is driven by the number of (reproductive) modules per individual (i.e., overall size, e.g. Karron & Mitchell 2012). Clonality, a type of modular growth with few restrictions on overall plant size, might be expected to exacerbate these effects. An early empirical study by Handel et al. (1985) showed that genets with more ramets have higher rates of selfing, and since then the prevailing view has been that clonality has negative consequences for mating success (Charpentier 2002; Vallejo-Marín, Dorken & Barrett 2010). The population in Handel et al.'s study consisted of clonal plants that had little to no intermingling—the shoots (ramets) of each clone (genet) were clumped and isolated from the ramets of other genets in the population. For this early study, therefore, increased selfing was expected because pollen is usually transferred to neighbouring flowers (Levin & Kerster 1974). However, this spatial structure may not be broadly representative of clonal plants (Vallejo-Marín, Dorken & Barrett 2010), something few studies have investigated. Many clonal plants produce their shoot at the end of long rhizomes or stolons resulting in the intermingling of clones. Under these circumstances, nearest neighbours could provide outcrossing opportunities. Indeed, a more recent empirical study that considered a natural population with an intermingled spatial structure failed to demonstrate an association between clone size and selfing rates (Matsuo et al. 2014). Additionally, a study of bee foraging patterns in response to variation in clone structure using artificial flower arrays showed that increases in genet size do not necessarily lead to higher rates of selfing (Liao & Harder 2009). Flowers were presented either on one inflorescence (non-clonal) or three inflorescences spaced either closely (phalanx clonal structure) or widely (guerilla clonal structure). Results showed that bees visited fewer flowers per individual for clonal arrays because they often ignored one or more inflorescence. A

theoretical study showed similar results (Van Drunen, van Kleunen & Dorken 2015). Considering the variability that can exist in clonal structure, there are relatively few studies that have investigated the spatial structure of clones and the resulting effects on mating patterns, especially for natural populations. As a result, our understanding of how clonality influences mating patterns is very limited.

In addition to influencing the proportion of offspring generated via selfing versus outcrossing, the spatial structure of clones may also influence total mating success (Barrett 2015). Clonality may reduce the constraints on sexual reproduction that are induced by large size in non-clonal plants (Dorken & Van Drunen 2010; Vallejo-Marín, Dorken & Barrett 2010). Plants are limited to mating with the other individuals that are within range of the distance over which pollen is dispersed (Barrett & Harder 1996). Once the stigmas within this distance are saturated with pollen, investment in more pollen does not lead to additional siring success. As a result, a decelerating relationship between investment in pollen production and siring success is expected for most plants (i.e., the male fitness gain curve is decelerating; Lloyd 1984; Zhang 2006). But under clonal growth, individuals spatially subdivide their reproductive effort over multiple ramets, each of which can have a separate mating neighbourhood (the plants within typical pollen-dispersal distances). If so, clonal plants can avoid saturating the females within their pollen dispersal distance, resulting in a more linear fitness gain curve for the male function. This expectation is supported by the previously mentioned experimental study by Liao & Harder (2014) as well as a theoretical study by Dorken & Van Drunen (2010). However, only a single empirical study has investigated a natural population. Matsuo et al. (2014) found a

decelerating male fitness gain curve for a population of dwarf bamboo with high intermingling. But, because this study involved a relatively small sample of plants in a much larger population, siring events from the largest clones that involved plants outside the study zone may have been missed and it therefore remains somewhat unclear whether populations of intermingled clonal plants should experience linear or decelerating fitness gains for male function.

For most clonal plants, the spatial arrangement of clones within natural populations remains largely unknown, as well as the patterns of selfing and outcrossing in natural populations of clonal plants. The aim of my study was to assess the spatial structure of a natural population of a clonal plant and to investigate how this spatial structure influences mating patterns. I hypothesized that clonality can benefit mating success by both enabling increased intermingling with other individuals (i.e., potential mates) and by subdividing reproductive effort among ramets of a single genet (and yielding a linear association between male investment and siring). I tested the predictions that, first, clonal expansion enables greater outcrossed siring success by increasing the number of potential mating partners that occur within typical pollen dispersal distances. Second, I predicted that this increase in siring success occurs with minimal costs to mating arising from geitonogamous selfing due to intermingling. Lastly, I predicted that there will be a linear association between genet-level investment in male function and siring success. To test these predictions, I mapped all flowering ramets (n=506) in an isolated population of the monoecious clonal aquatic plant *Sagittaria latifolia*, a species that has the potential to be highly intermingled (Dorken & Barrett 2003). I genotyped these ramets using simple sequence repeat (SSR) loci to assign ramets to genets, and to assign paternity

(outcrossed and selfed) to a sample of seeds from each plant. I used these siring events to fit a pollen dispersal kernel that informed mating neighbourhoods for each ramet. I assessed the number of self and non-self ramets in each ramet's mating neighbourhood to understand potential selfing and outcrossing rates and compared that to observed selfing and outcrossing rates. Finally, I plotted the male fitness gain curve.

2.2 Methods

2.2.1 Study System

Sagittaria latifolia (Alismataceae) is a widespread emergent clonal aquatic plant commonly found in wetlands throughout eastern North America and naturalized in Europe (van Kleunen et al. 2019). Populations are common in the regions surrounding the Great Lakes in eastern North America where both monoecious (hermaphroditic plants with unisexual flowers) and dioecious (unisexual plants) populations occur, often in close geographic proximity (Dorken & Barrett 2004a). It reproduces both asexually via corms and stolons and sexually by seed and is self-compatible, with no compatibility barriers between plants from the two sexual systems (Dorken and Barrett 2004b). Ramets are formed throughout the growing season through the production of stolons, which average approximately 30 cm in length (Dorken & Barrett 2003); clonal growth, therefore, leads to the outward expansion of the genet by relatively large increments. Stolons disintegrate and the perennating corms are formed at the end of the growing season; they are the only vegetative biomass that remains over winter and can float. The population chosen for this study was a monoecious population isolated from other populations. In the area sampled, the flowering season occurs from July to September. Unisexual flowers open and are receptive for one day on whorled racemes of three and are pollinated by bees, flies, beetles,

butterflies and wasps (Muenchow & Delesalle 1994; Vamosi et al. 2006). Female flowers generally open first on lower whorls, with male flowers opening at least a day later, on upper whorls. There is no overlap in flowering time between the two sexes on a single ramet, and therefore any self-fertilization can take place only between two ramets of the same genet and not within a single ramet. This means that any observed selfing event is a consequence of clonal growth.

2.2.2 Field Observations

The monoecious population of *Sagittaria latifolia* used for this study was located in Peterborough, Ontario in a shallow area of Thompson Creek in Meadowvale Park (UTM17 0714256 4912404). Flowering ramets were patchily distributed over an area of approximately 25m x 60m and isolated from other populations of *S. latifolia* by at least 100m. Sampling began with the first flowering event on July 18, 2013 and continued daily until all plants were finished flowering on August 28, 2013. Each inflorescence was tagged, and flower phenology was recorded each day throughout the entire season. The pedicels of female flowers were tagged based on opening chronology to keep track of the corresponding date that each fruit was receptive, narrowing the pool of candidate fathers for paternity assignment. A leaf sample was collected for each reproductive ramet, for a total of 506 ramets. Leaf tissue was dried using silica and stored at -20 °C for genetic analysis. At the end of the season, all mature fruits were collected, dried in coin envelopes at room temperature and then stored at 4 °C. A total of 757 fruits were collected from 220 ramets, with an average of 2.7 fruits collected per ramet. We aimed to sample one seed per fruit for paternity analysis and selfing estimation; however not

all fruits matured, and viable DNA was obtained from 358 seeds representing 154 ramets. All ramets were mapped using triangulation (e.g., Ahee et al. 2015). To do this, I placed a series of reference poles (rebar) in the central channel of the creek spanning the length of the population. I used a measuring tape to measure the horizontal distance from each flowering shoot to two reference poles. These distances were used to calculate polar coordinates, which were converted to Cartesian coordinates. The resulting map was ground-truthed. Because of the large size of the mapped area (25 m by 60 m), we used 14 pairs of fixed reference points to map patches of plants. These reference points were then mapped to a pair of master reference points in the middle of the mapped area enabling the patches to be combined into a single map. Final spatial mapping was confirmed in the field.

2.2.3 Genotyping Parental Ramets

DNA amplification and SSR genotyping followed the methods outlined in Yakimowski et al. (2009). Dried leaf tissue was ground into a powder using a MM 300 Retsch mixer mill (Haan, Germany). DNA was then extracted using the E. Z. N. A. ^{TM} Plant DNA Mini Kit Spin Protocol (Omega Bio-Tek., Inc., Georgia, USA) following the manufacturer's protocol for dried specimens with the use of RNAse and a final elution of 100 µl. Extracted DNA concentration was evaluated using fluorometric quantitation and each sample was then diluted to approximately 50 ng/µl. Four nuclear SSR microsatellite loci (SL65, SL06, SL75 and SL27) were amplified individually and five (SL30, SL88, SL21, SL09, SL74) were amplified in two multiplexes by polymerase chain reaction (PCR) using fluorescently tagged primers developed by Yakimowski et al. (2009). For individual reactions the PCR master mix contained 0.2 µM of forward and reverse primer, 0.15

mM dNTPs, 1X PCR buffer, 2 mM MgCl2, 0.3 mM BSA and 0.05 U/rxn Taq DNA Polymerase (Invitrogen, Waltham, MA, USA ™). The PCR was run using an Eppendorf Mastercycler™ thermo cycler (Eppendorf). The cycling parameters were as follows: denaturing at 94 °C for 3 minutes, amplification repeated for 30 cycles of 94 °C for 30 seconds, annealing at 60-62 °C for 30 seconds and 72 °C for 45 seconds, with a final elongation at 72 °C for 45 minutes. All primers had an annealing temperature of 60 °C, except for SL65, which was annealed at 62 °C. Multiplexes were amplified in two reactions. Multiplex 1 contained SL21 forward and reverse primer at a concentration of 0.1 µM and SL30 forward and reverse primer at a concentration of 0.19 µM with the rest of the PCR master mix containing 0.15 mM dNTPs, 1X PCR buffer, 1.3 mM MgCl, 0.3 mM BSA, and 0.5 U/rxn GoTaq Flexi DNA polymerase (Promega, Madison, WI, USA). Multiplex 2 contained SL88 forward and reverse primer at a concentration of 0.18 µM, SL09 forward and reverse primer at a concentration of 0.1 µM and SL74 forward and reverse primer at a concentration of 0.16 µM with the PCR master mix containing 0.15mM dNTPs, 1X PCR buffer, 1.6 mM MgCl, 0.3 mM BSA and 0.5 U/rxn GoTaq Flexi DNA polymerase (Promega, Madison, WI, USA). PCR was run using the following cycling parameters: denaturing at 94 °C for 3 minutes, amplification repeated for 35 cycles of 94 °C for 45 seconds, annealing at 57 °C for multiplex 1 and 56.5°C for multiplex 2 for 45 seconds and 72 °C for 45 seconds, with a final elongation at 72 °C for 10 minutes. The quality of amplification products was evaluated on an agarose gel and then diluted 20X with ddH2O. 0.7 µl of this dilution was mixed with 9 µl of HiDi containing ROX 500 size standard (Applied Biosystems, Waltham, MA, USA) and run on an ABI3730 (Applied Biosytems, Waltham, MA, USA). The quality of amplification products was evaluated on an agarose gel and then diluted 20x using ddH2O. Then 0.7 µl of this dilution was

mixed with 9 µl of HiDi containing ROX 500 size standard (Applied Biosystems) and run on ABI3730 (Applied Biosystems). Genotypes were determined using Genemarker® software (v 1.6; Softgenetics®). Viable DNA was extracted and amplified from 508 reproductive ramets.

2.2.4 Clonal Assignment

From my sample of 506 ramets, I identified 210 unique multi-locus genotypes (MLGs). The probability that ramets sharing the same MLG arose from independent instances of sexual reproduction was calculated using the psex_Fis function in the RClone package (v. 1.0.2.; Bailleul, Stoeckle & Arnaud-Haond 2016) in R (v. 3.4.4.; R Core Team 2018). All but one of the calculated values of Psex, the probability that two identical MLGs arose from independent sexual reproductive events, was below 0.03. Accordingly, ramets with identical MLG assignments were assumed to have been produced via clonal growth. To account for scoring errors and somatic mutations, MLGs that differed by one allele at any locus were assigned to the same multi-locus lineage (MLL; Schnittler & Eusemann 2010), using the MLL_generator function from the RClone package. Using this approach, I identified 169 unique MLLs.

2.2.5 DNA Extraction & Amplification from Seeds

Air-dried seeds were soaked in deionized water at 4°C for a minimum of 1 day to loosen the seed from the achene and increase the effectiveness of grinding. Maternal tissues (including the achene and seed coat) were removed using needle-tipped forceps and seeds were crushed using standard forceps. DNA extraction was performed using QuickExtract ™ Seed DNA

Extraction Solution (Epicenter) following the manufacturer's protocol. DNA amplification and genotyping were carried out using the procedure described above for leaf tissue. Viable DNA was extracted from 358 seeds belonging to 154 ramets.

2.2.6 Paternity & Inter-Ramet Mating Distance

Paternity was inferred for seeds using the likelihood approaches implemented in CERVUS (v. 3.0.7; Kalinowski, Taper, & Marshall 2007; Marshall et al. 1998) and COLONY2 (v. 2.0.6.5; Jones & Wang 2010; Wang 2004; Wang 2018). Our sampling of potential sires in this isolated population was likely to have been nearly complete, and results from both CERVUS and COLONY2 were qualitatively similar. Below I report results from paternity assignments calculated in COLONY2. Both programs allow for errors in genotyping; for our COLONY2 run, we set per locus stochastic error rates from the output provided by a previous run of CERVUS. Calculations were made using a full likelihood (FL) option. Paternity analysis was conducted using MLG assignment to account for the possibility of somatic mutations within MLGs; these mutations could be passed on to offspring and could reduce assignment probabilities for MLLs comprised of ramets with different alleles at any one of the SSR loci used here. The resulting data were analyzed at the MLL level to enable clone-level calculations of siring success.

We calculated inter-ramet mating distances and used these values to infer the underlying pollen dispersal kernel using maximum likelihood. To do this, I largely followed the procedure outlined by Hardy et al. (2004) using a one-parameter dispersal kernel (the exponential decay function) and bootstrapping ramets from MLGs identified as the sire of a particular seed to

obtain ramet locations and male flower production (two-parameter dispersal kernels, including the exponential-power function used by Hardy et al. 2004 failed to converge). Only sires assigned with greater than 95% probability were retained for the analysis of pollen dispersal distances. For other analyses, the assignment probability for the most probable sire was used to weight the number of inferred siring events per MLL. However, including seeds that were assigned to sires with less than 95% probability in my calculations of pollen dispersal distances provided results that were qualitatively similar to those reported below. Including or excluding these seeds also yielded qualitatively similar results for analyses of clonal intermingling and siring patterns. The bootstrapping procedure resulted in a rough likelihood surface rendering standard optimization algorithms (e.g. derivative-based methods) unsuitable for identifying the maximum likelihood estimate. As a result, direct-search was used to estimate the maximum likelihood parameter value for the negative exponential distribution (Bolker 2008).

2.2.7 Genet Size & Intermingling

Our aim in quantifying patterns of clonal intermingling and the spatial clustering of genets was to characterize spatial relations in terms of their implications for mating patterns. Accordingly, ramets were defined as being intermingled with other genets if they were likely to disperse pollen to (or receive pollen from) other genets and clustered if they were likely to disperse pollen to other ramets from the same genet. Specifically, patterns of intermingling were quantified as the number of ramets from other genets (MLLs) versus the same genet (clustering) that occurred within the first 10^{th}, 25^{th}, and 50^{th} percentiles of the bootstrapped distribution of inter-ramet mating distances (as described above). These distances are referred

to below as the short, intermediate, and typical inter-ramet mating distances (sIRMD, iIRMD, tIRMD respectively). These counts of pairwise inter-ramet distances were then summed across ramets within a genet. Note that this method could result in the repeated counting of the same ramet (as many times as the ramet occurred within this minimum distance for each ramet per genet). This was done to account for the fact that the probability of overlap in sex phases between ramets increases with the number of ramets in local mating neighbourhoods of *Sagittaria* (Wang et al. 2012). Because these distances were used to scale the area spanned by a genet, in the results section below I refer to these distances in terms of patterns of occupancy—genets were assumed to span smaller versus larger areas under sIRMDs than under tIRMDs. For each IRMD, I calculated the index of clustering for each genet as the count of the number of ramets from the same genet within the IRMD. Similarly, the index of intermingling for each genet was given by counts of ramets involving different genets within the IRMD.

The size of genets was calculated in two ways. First, I tallied the number of ramets per genet (MLL). Second, I estimated the area occupied by each genet by assuming that each ramet occupied a fixed area and then summing the area occupied by all ramets per genet. For this calculation, I superimposed a rectangular grid of cells over the map of the population with resolution 30 cm X 30 cm. Ramets were assumed to occupy a square within this grid with sides equal to 2 X sIRMD. The area occupied per genet was then the sum of all grid cells occupied per genet. Overlapping cells occupied by more than one ramet per genet were only counted once. Using this approach, two genets with the same number of ramets but with different patterns of dispersion (e.g., a tightly clustered genet versus a more widely dispersed genet) would be

associated with different areal measurements. Calculations were repeated for the iIRMD and tIRMD.

2.2.8 Selfing Rates

All seven loci were used to estimate the multilocus outcrossing rate (t_m) at the ramet level using the multilocus estimation software MLTR (v. 3.4; Ritland 2002). Selfing rates were calculated as $1 - t_m$, and these values were then averaged across all ramets per genet (MLL) to estimate genet-level selfing rates. The mean number of offspring per ramet used for the calculations of t_m was 2 (range = 1-7). Because the genotype of the mother was known, selfing versus outcrossing events could be inferred from single progeny. Selfing rates were calculated for 148 families from a total of 353 seed genotypes.

2.2.9 Statistical Analyses

2.2.9.1 Clonal Expansion & Genet Intermingling

The indices of intermingling and clustering can only increase with genet size, as they are summed across ramets per genet (MLL), but the rates at which intermingling and clustering increase with genet size might differ. For example, larger genets might be more clustered and less intermingled than smaller genets. Accordingly, the association between genet size (the number of ramets per genet) and patterns of genet intermingling versus clustering was evaluated using a generalized linear mixed-effect model, calculated using the glmer function from the LME4 package (Bates et al. 2015) in R. The cumulative number of spatially overlapping ramet pairs was modelled as the interaction between the type of overlap (intermingling among ramets or clustering within genets; i.e., my indices of intermingling and clustering as defined

above) and the number of ramets per genet. The model was calculated using a Poisson error distribution with log link function. Because there were two measurements per genet (intermingling and clustering), which are not independent, I included genet as a random effect in the model. This model failed to converge when iIRMD and tIRMD were used to scale the area occupied by genets; for calculations of intermingling involving these spatial scales, I used a linear mixed model with log-transformed index values as the dependent variable. This model was calculated using the lmer function from the LME4 package (Bates et al. 2015) in R. Tests of significance for main effects were calculated using type III sums of squares implemented in the ANOVA function from the CAR pachage (Fox & Weisberg 2011). As a further test of the prediction that intermingling increases with clonal expansion, I also calculated a GLM for which the proportion of non-self versus self-ramets within the IRMD was the response variable and the number of ramets per genet was the predictor variable. The analysis was done using the quasibinomial setting to account for over-dispersion. To improve model fit, the number of ramets per genet was log-transformed. Both analyses included only genets comprised of more than one ramet. This was done to remove bias that might have arisen because genets comprised of only one ramet cannot spatially overlap with other ramets from the same genet but can overlap with ramets from other genets.

2.2.9.2 Genet Size & Selfing Rates

I evaluated the correspondence between genet size and rates of selfing using multiple regression. I was specifically interested in how measures of clone size were associated with selfing rates, but the two measures of clone size (number of ramets per genet and genet area)

were correlated with one another (Pearson r = 0.62, df = 53, p < 0.001, 95% CI = 0.42, 0.76). Therefore, to avoid collinearity among independent variables, I used the residuals of the regression of genet area on the number of ramets per genet in the analysis instead of genet area. Positive values of these residuals indicate genets that are more dispersed for a given number of ramets and negative values indicate genets that are more clustered. The independent variables for this analysis were the number of ramets per genet (MLL), the residuals described above and their interaction. The dependent variable was the selfing rate averaged across ramets per genet. Least-squares calculations for this model were weighted by the number of seeds screened per genet under the assumption that the accuracy of the estimates increased with family size. Because the calculation of genet area depended on my assumption of mating neighbourhood size (IRMD), and because I evaluated three different sizes of mating neighbourhoods (sIRMD, iIRMD, tIRMD), I report model results for each neighbourhood size. Tests of significance were carried out using type III sums of squares using the ANOVA function from the CAR package.

2.2.9.3 Genet Size, Male Investment & Siring Success

The total number of seeds sired within the sample per genet was modelled as the linear combination of the number of ramets per genet and the residuals of the regression of genet area on the number of ramets per genet, using Poisson regression. Residuals were used in this analysis for the same reasons as those given for the tests of the association between clone size and selfing rates. Because sires were assigned with varying degrees of confidence, I calculated the sum of the probabilities of assigned sire genets and used these sums to weight the Poisson

regression. The final model was calculated using the quasi-Poisson setting to account for overdispersion. Significance of the independent variables was calculated using type III tests using the ANOVA function from the CAR package. Because calculations of genet area depended on my assumption of neighbourhood size, I report model results for each neighbourhood size (sIRMD, iIRMD, tIRMD).

Genet size and male investment should be positively associated with one another. Accordingly, it was possible that the obtained association between genet size and siring success was driven by an underlying association with male investment. To account for this possibility, I compared the results of the Poisson regression between genet size and siring success outlined above with the results of a Poisson regression involving male investment as an independent variable. In particular, I modelled the association between the total number of seeds sired per genet as the linear combination of the total number of male flowers per genet and the residuals of the regression of genet area on the number of ramets per genet. As for the previous analysis, the sum of the probabilities for genets assigned as sires was used to weight the regression. Models involving genet size and male investment were compared using AIC scores.

To test whether the association between male investment and siring success was linear, we assumed that all male flowers of *S. latifolia* entail an equal investment in male reproductive function. Accordingly, the total number of male flowers produced per genet was used as the measure of male investment. To evaluate whether the association between total male investment (TMI) per genet and the index of siring success (as defined in the preceding

paragraph) was linear, as predicted, or non-linear, I fitted each model and compared their fits by plotting observed siring success per genet versus the siring success predicted by each model. Both models were calculated using the nls function in R. The linear model was calculated as siring success ~a + b x TMI, where a is a constant and b is the slope parameter. The nonlinear model was calculated as siring success ~a + TMIc, where c is the gain curve parameter (Charnov 1979). Both the linear and the nonlinear models were weighted by the index of confidence in siring per genet. Model results were calculated using nonlinear least squares using the nis function in R. I compared the results of each model by calculating AIC scores.

2.3 Results

2.3.1 Genet Size & Spatial Arrangement

The population consisted of 210 genets (MLLs). Genet size ranged from 1 to 35 ramets with a median genet size of 1 ramet. This median shows that the population consisted mostly of small genets, but also included a small number of large genets (Figure 1 inset). Genets tended to be spatially localized but interspersed with ramets from other genets (Figure 1). However, several genets spanned larger distances in the population, typically consisting of a cluster of ramets in one area and isolated ramet(s) elsewhere in the population. The maximum distance between two ramets within a single genet was 61.9 m; however, most genets spanned much smaller distances. Over short distances (<1.5 m), more than 20% of ramets were members of the same genet. However, at distances of 5 m fewer than 5% of ramets were part of the same genet, and at distances of 20 m fewer than 1% of ramets were part of the same genet.

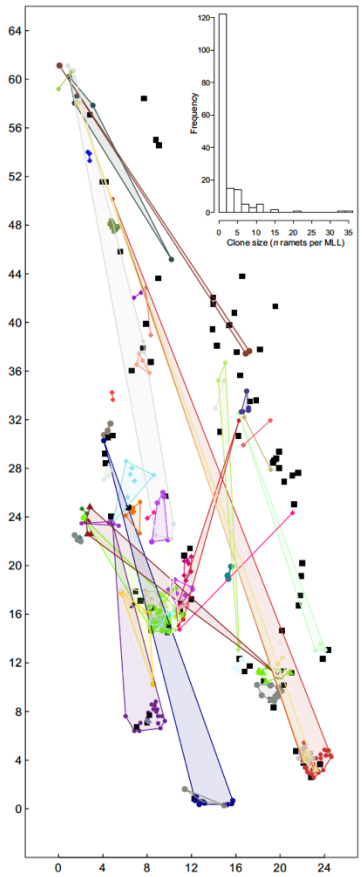

FIGURE 1. Spatial distribution of 506 ramets representing 210 genets in a natural population of *Sagittaria latifolia* inhabiting an area of 65 meters by 25 meters. Black squares correspond to genets consisting of a single ramet, whereas coloured shapes correspond to ramets belonging to genets with more than one ramet. Lines connect the ramets of genets to show whether genets are isolated or intermingled with other clones.

2.3.2 Paternity & Inter-Ramet Mating Distance

Of the 358 genotyped seeds, 301 were assigned to one or more sires. Most of these seeds (213 of 301) were assigned to a single sire with 100% estimated probability, and 216 of 301 seeds were assigned to a single siring MLL with a greater than 95% probability. The remaining 85 seeds were assigned to a siring MLL with probabilities ranging from 0.01 to 0.94 (M = 0.25 ± 0.22 SD). Pollen dispersal was inferred to have occurred over a broad range of distances. A separate analysis was used to estimate the median pollen dispersal distance based on

calculated inter-ramet mating distances that occurred between seeds with ≥95% assignment probability (Stephens et al. 2020). The mean observed inter-plant mating distance was 15.10 ± 0.95 m SE. Average inter-mating ramet distances, therefore, exceeded the typical distances separating ramets of the same genet, as described in the preceding section.

2.3.3 Genet Size & Intermingling

The shape of the pollen dispersal kernel was used to determine mating neighbourhoods corresponding to much shorter than average inter-ramet mating distance (sIRMD), intermediate inter-ramet mating distance (iIRMD) and typical inter-ramet mating distance (tIRMD). I examined intermingling and clustering under these three mating neighbourhood sizes. Not surprisingly, I found positive associations between the number of ramets per genet (i.e. clone or MLL size) and both clonal intermingling and spatial clustering of ramets belonging to the same MLL. This effect depended on the size of mating neighbourhoods. For sIRMAD neighbourhoods, smaller clones tended to be more intermingled than they were clustered, but for larger clones, the opposite pattern emerged; these clones tended to be more clustered than intermingled (Figure 2a). However, when considering tIRMD neighbourhoods, which represent the average mating distance in this population, clones were more intermingled than clustered, regardless of their size (Figure 2e). For all mating neighbourhood sizes considered, there was an interaction effect between genet size and the type of ramet pair (i.e., self vs. non-self, Table 1). There were steeper associations between genet size and clustering than between genet size and intermingling, which resulted in negative associations between genet size and the proportion of ramets intermingled with other genets (Figure 2a, c, e).

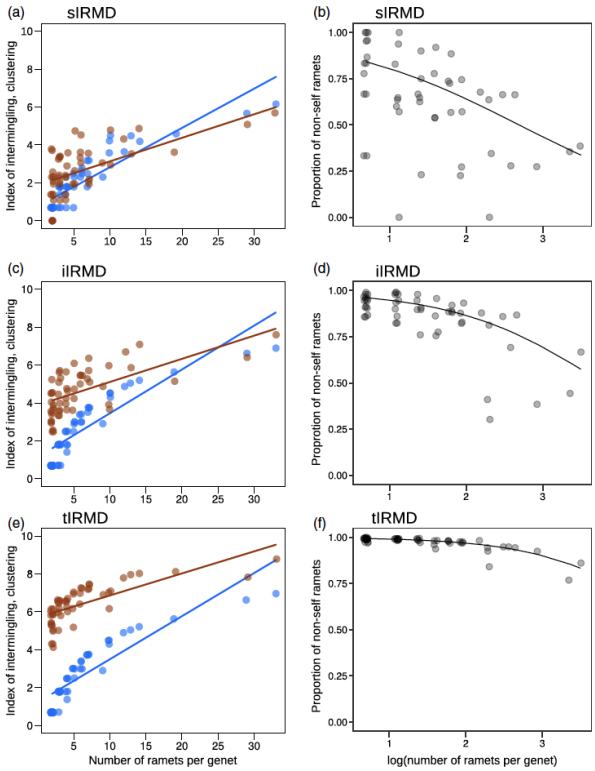

FIGURE 2. Patterns of clonal intermingling in a natural population of *Sagittaria latifolia*. Intermingling (red dots) was measured as the sum of the number of non-self ramets within the mating neighbourhoods of the ramets within each genet, where mating neighbourhoods were defined by the shape of the pollen dispersal kernel inferred from paternity analysis. Clustering (blue dots) was measured as the sum of the number of self-ramets within the mating neighbourhoods of the ramets within each genet. The spatial scales used to define the space occupied by ramets within genets were determined by the quantiles of inferred pollen dispersal distances (a & b: sIRMD = 1.06m, c & d: iIRMD = 4.13m, e & f: tIRMD = 13.9 m). Regardless of the spatial scales used to define the space occupied by ramets within genets, larger genets tended to have proportionally more self-ramets within their local mating neighbourhoods, although this increase in the proportion of self-ramets was weak when I assumed that ramets occupied areas corresponding with the median pollen dispersal distance (i.e. tIRMD). Lines indicate the results of the linear mixed-effects model (a, c & e) and the GLM (b, d, & f) used for examining patterns of intermingling versus clustering.

TABLE 1. The parameter estimates, their standard errors, and Wald chi-square test from a generalized linear mixed-effects model (sIRMD) and linear mixed-effects models (iIRMD and tIRMD). The dependent variable is the cumulative number of overlapping ramet pairs. For all mating neighbourhood sizes (short = sIRMD, intermediate = iIRMD, typical = tIRMD) there was a significant interaction between genet size (number of ramets) and the type of ramet pair (self vs. non-self).

	Short (sIRMD)		***Intermediate (iIRMD)***		***Typical (tIRMD)***	
Effect	**Estimate ± *SE***	χ^2	**Estimate ± *SE***	χ^2	**Estimate ± *SE***	χ^2
Intercept	1.87 ± 0.16	128.5***	3.88 ± 0.16	616.7***	5.70 ± 0.13	1.90 x 103***
Ramet type	–1.04 ± 0.07	243.9***	–2.74 ± 0.18	239.2***	–4.48 ± 0.12	1.51 x 103***
Genet Size	0.14 ± 0.02	57.9***	0.12 ± 0.02	42.9***	0.12 ± 0.02	55.2***
Ramet type x genet size	0.05 ± 0.00	282.9***	0.11 ± 0.02	26.6***	0.11 ± 0.01	65.6***

***p < 0.001.

2.3.4 Selfing Rates

Genet size (number of ramets) was positively associated with estimated rates of selfing for all mating neighbourhood sizes (Table 2), however, notably, this relationship was weak (sIRMD: R^2 = 0.12; iIRMD: R^2 = 0.11; tIRMD R^2 = 0.13). Furthermore, selfing rates for small genets were highly variable (Figure 3). There was no effect of the residuals from the regression of genet area on the number of ramets per genet for any of the mating neighbourhood sizes (Table 2), and there was no interaction between these residuals and the genet size (Table 2).

TABLE 2. Parameter estimates, their standard errors and *F*-statistics (with the numerator and denominator degrees of freedom) calculated using type III sums of squares are reported from linear models. Variation in estimated rates of selfing was influenced by genet size (number of ramets).

	Short (sIRMD)		***Intermediate (iIRMD)***		***Typical (tIRMD)***	
Effect	**Estimate ± *SE***	***F*-test**	**Estimate ± *SE***	***F*-test**	**Estimate ± *SE***	***F*-test**
Intercept	4.29×10^{-2} $\pm 4.48 \times 10^{-2}$	$F_{1,51} = 0.92$	7.14×10^{-2} $\pm 4.22 \times 10^{-2}$	$F_{1,51} = 2.8$	7.04×10^{-2} $\pm 3.74 \times 10^{-2}$	$F_{1,51} = 3.53$
Area (residuals)	-1.97×10^{-2} $\pm 1.47 \times 10^{-2}$	$F_{1,51} = 1.79$	-1.55×10^{-3} $\pm 1.80 \times 10^{-3}$	$F_{1,51} = 0.74$	-3.04×10^{-4} $\pm 2.02 \times 10^{-4}$	$F_{1,51} = 2.26$
Genet Size	1.27×10^{-2} $\pm 4.48 \times 10^{-3}$	$F_{1,51} = 8.10^{**}$	8.45×10^{-3} $\pm 3.88 \times 10^{-3}$	$F_{1,51} = 4.73^{*}$	8.34×10^{-3} $\pm 2.71 \times 10^{-3}$	$F_{1,51} = 9.48^{**}$
Area x genet size	1.52×10^{-3} $\pm 1.04 \times 10^{-3}$	$F_{1,51} = 2.12$	6.71×10^{-5} $\pm 1.60 \times 10^{-4}$	$F_{1,51} = 0.18$	2.55×10^{-5} $\pm 1.50 \times 10\text{-}5$	$F_{1,51} = 2.90$

* $p < 0.05$; ** $p < 0.001$

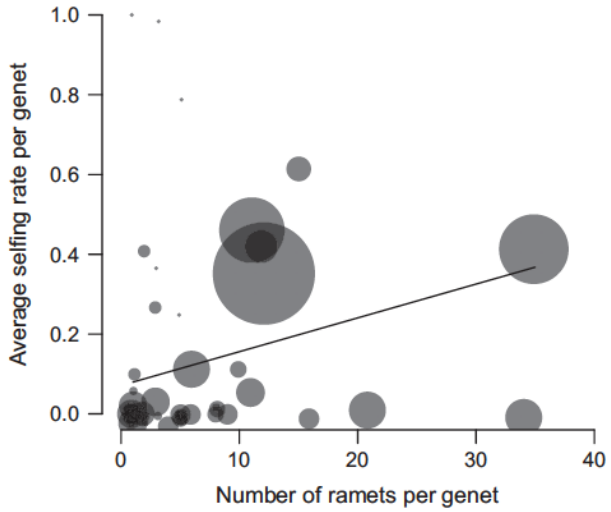

FIGURE 3. Patterns of selfing as a function of genet size (number of ramets per MLL) in a natural population of *Sagittaria latifolia*. Point size is proportional to the number of seeds per MLL screened for selfing rates. The line indicates the slope of the weighted regression of average selfing rate on genet size, with the number of seeds screened per MLL used as the weighting variable.

2.3.5 Genet Size & Siring Success

As predicted, I found a positive association between genet size and siring success for all mating neighbourhood sizes (sIRMD, iIRMD, and tIRMD; Table 3; Figure 4). Siring success was not

associated with the residuals from the regression of genet area on genet size (Table 3), or the interaction between these residuals and genet size (Table 3). This indicates that the increased siring success of large clones was driven mainly by genet size, the variable of interest, and not by the patterns of ramet dispersion.

TABLE 3. Siring success per genet in a natural population of the clonal plant *Sagittaria latifolia* was influenced by variation in genet size (number of ramets) for all mating neighbourhood sizes. Parameter estimates, their standard errors and likelihood-ratio test chi-square values are reported from Poisson regression. Parameter estimates for genet area, genet size and their interaction are scaled by 10^2.

	Short (sIRMD)		***Intermediate (iIRMD)***		***Typical (tIRMD)***	
Effect	**Estimate ± *SE***	χ^2	**Estimate ± *SE***	χ^2	**Estimate ± *SE***	χ^2
Intercept	1.05 ± 0.17	NA	1.27 ± 0.17	NA	1.24 ± 0.13	NA
Genet area (residuals)	–2.46 ± 4.19	0.35	–0.22 ± 0.42	0.28	–0.05 ± 0.04	1.73
Genet size	6.17 ± 1.60	15.60***	3.75 ± 1.58	5.42*	4.13 ± 0.81	21.7***
Area x size	0.52 ± 0.33	2.52	0.02 ± 0.05	0.15	0.00 ± 0.00	2.89

* $p < 0.05$; ** $p < 0.001$

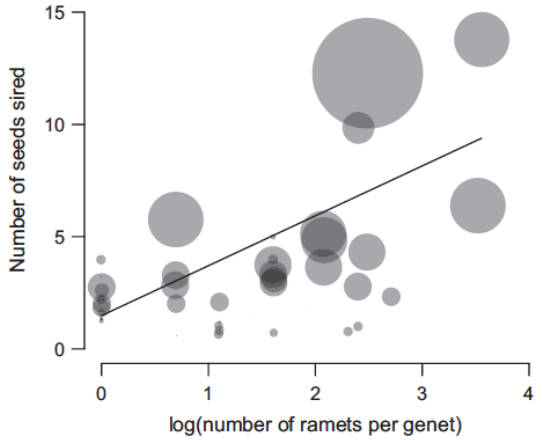

FIGURE 4. Association between siring success and genet size (number of ramets) in a natural population of *Sagittaria latifolia*. Point size is proportional to the cumulative measure of confidence in siring (the sum of the probabilities of paternity per assigned sire, calculated using COLONY2). For plotting, the number of flowering ramets per genet was log-transformed and the line indicates the slope of a weighted linear regression of siring success on the log(number of ramets per genet) using the iIRMD as the mating neighbourhood size.

Male investment was strongly correlated with genet size (linear model: male flowers per genet = 1.9 + 5.4 x number of ramets per genet, $R^2 = 0.88$, $F_{1,42} = 320.8$, $p < 0.001$; Figure 5), as an increase in flowering ramets would typically increase the number of male flowers produced per genet, although the number (and sex) of flowers per ramet is variable, so it is possible for two genets of the same size to differ in reproductive effort. Poisson regression between siring success and male investment resulted in substantially lower AIC scores than the Poisson regression reported above for genet size (ΔAIC = -12.7) and for all assumptions of spatial patterns of genet occupancy (sIRMD: ΔAIC = 26.71; iIRMD: ΔAIC = 12.5; tIRMD: ΔAIC = 10.7). This indicates that increased siring by large clones appears to have been driven by their greater investment in male function compared to smaller clones. Indeed, there was a positive linear association between male investment and siring success (siring success ~a + b x TMI; a = 3.0, t_a

= 4.61, $p_a < 0.001$, b = 0.04, $t_b = 5.34$, $p_b < 0.001$). However, the nonlinear model provided quantitatively similar fits to the data (Figure 6 & Figure 7) and was associated with substantially lower AIC (ΔAIC = -9.43). The model parameters for the nonlinear fit indicated strongly diminishing returns on male investment (siring success ~$a + TMI^c$; a = 0.19, t_a =-0.24, $p_a > 0.80$, c = 0.46, $t_c = 16.6$, $p_c < 0.001$). This result was driven by a single MLL with a TMI of 76 flowers that occurred in the highest density patch in the population. However, exclusion of this MLL from the analysis yielded equivocal evidence for a linear versus nonlinear association between male investment and siring success (ΔAIC = 0.02). Together these data indicate that large clones have greater siring success than small clones, but that this relationship might weaken as clones become very large.

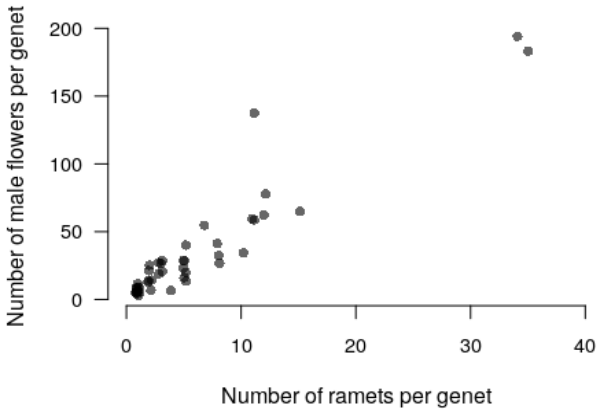

Figure 5. Association between clone size (number of ramets per genet) and male investment (total number of male flowers per genet).

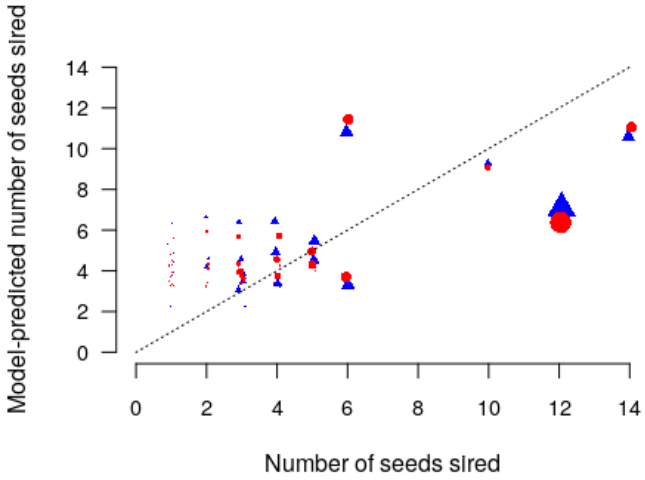

Figure 6. Comparison of the observed versus model-predicted patterns of siring success versus male investment for the linear (red circles) and non-linear (power function; blue triangles) models. Symbol size was scaled by my cumulative measure of confidence in siring (the sum of the probabilities of paternity per assigned sire, calculated using COLONY2).

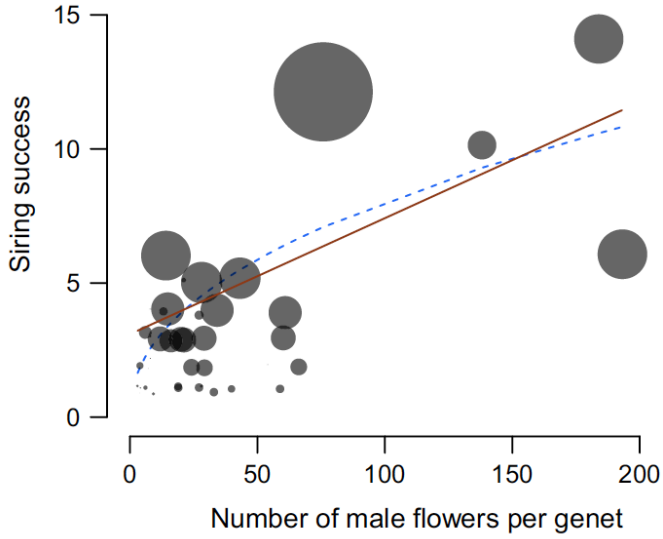

Figure 7. Association between the total investment in male function per genet (number of male flowers per MLL) and siring success in a natural population of *Sagittaria latifolia*. The nonlinear model (power function) was

associated with a lower AIC score than the linear model. Lines were calculated from the fitted parameter values from each model. Point size is proportional to my cumulative measure of confidence in siring (the sum of the probabilities of paternity per assigned sire, calculated using COLONY2).

2.4 Discussion

2.4.1 Spatial Structure, Pollen Dispersal Distance & Intermingling of Clones

In the natural population of *Sagittaria latifolia* studied here, the spatial distribution of plants within the study area was patchy. Ramets grew either along the shore, or in patches of vegetation within the shallow stream, where water flow was not as rapid and the conditions were perhaps better suited for the recruitment of seeds and establishment of clonal propagules. Not surprisingly, most genets were spatially confined to a single patch within the population, and this may have influenced the overall trend for the largest genets to be the most spatially clustered. There were, however, some ramets from larger clones that were spatially isolated, growing a large distance away (up to 61.9 m) from the other ramets of the genet. These ramets likely arose from the dispersal of clonal propagules. In *S. latifolia*, genets disintegrate annually, and clonal fragments are then capable of dispersing. Nevertheless, as mating neighbourhoods increased with genet size, so did the proportion of neighbours that belonged to the same genet. This positive association between clustering and genet size observed here is similar to findings from studies of species that, like *S. latifolia*, are capable of dispersing clonal fragments (e.g. *Cymodocea nodosa,* Ruggiero, Reusch & Procaccini 2005) or have a guerrilla-type clonal growth pattern (e.g. *Sasa veitchii* var. hirsute, Matsuo et al. 2014). Similar results were found in a study of *Dioscorea japonica*, where most nearest-neighbours were non-self ramets, but on a proportional basis, there was still a positive association between

genet size and clonal clustering (Mizuki et al. 2010). For populations that are dominated by one or a few large genets, this association is inevitable.

Clonal structure in a natural population is a product of sexual versus asexual recruitment. For *S. latifolia* the relative rates of sexual to asexual (clonal) recruitment in a natural population have not been investigated. In greenhouse conditions, genets of *S. latifolia* have grown in a single season to consist of more ramets than even the largest genet in the population studied here (Dorken & Barrett 2003), however, rates of clonal recruitment for *S. latifolia* were found to be much lower under more natural conditions (Dorken & Barrett 2003). The large number of single-ramet genets in the population studied here suggests that there has either been a recent bout of sexual recruitment, or possibly an event that caused population turnover. Indeed, population-level extirpation rates are reasonably high, particularly for monoecious populations of this species (Dorken & Barrett 2003). Furthermore, a study that later took place in this same population showed that a disturbance event resulted in nearly complete clonal turnover and an increase in patterns of clonal clustering (Holt, Kwok & Dorken 2019). This indicates that the age of a population, or time between disturbances, could be an important ecological factor influencing the degree of intermingling in natural populations. Indeed, given enough time even clonal plants with a phalanx-type growth pattern can become intermingled (e.g. Loh et al. 2015). The structure observed here is only from a single growing season, and how dynamic this structure is over time is largely unknown.

Mating neighbourhood size and the potential for selfing vs. outcrossing opportunities (degree of intermingling) is determined by the pollen dispersal distance. Previous studies of pollen dispersal distances in animal-pollinated herbaceous plants have found a broad range of distances. Some examples of distances include pasque flower (*Pulsatilla vulgaris*, mean within-population pollen dispersal distance estimated from seven populations = 4.98 m, range of within-population averages = 2.66—9.49 m; DiLeo, Holderegger & Wagner 2018), *Centaurea corymbosa* (mean = 21.6m; Hardy et al., 2004) and oxlip (*Primula elatior*, two within-population pollen dispersal estimates = 6.9, 32.4 m; Van Rossum et al. 2011). The mean pollen dispersal distance inferred in my study (15.1 ± 0.95 m), falls within the range of pollen dispersal distances for other animal-pollinated herbaceous plants. Mating neighbourhood size affects mating patterns by determining the number of available mates; as mating neighbourhood size increases, so do the number of potential mates. The mating benefits of clonality may accrue most strongly for plants that have small mating neighbourhoods, especially when pollen dispersal distances do not exceed the typical distance between ramets of the same genet (Van Drunen et al. 2015).

2.4.2 Clone Size, Selfing Rates & The Male Fitness Gain Curve

Larger genets in this study population tended to have moderately higher rates of geitonogamous selfing than smaller genets, as expected and consistent with modelling results in the study by Van Drunen et al. (2015). Since the early study by Handel (1985), the prevailing view has been that clonal expansion should provide increased opportunities for geitonogamous pollen transfer and, as a result, increased rates of self-fertilization (Barrett 2015; Charpentier

2002; Handel 1985; Silander 1985; Vallejo-Marín et al. 2010). However, this early study by Handel (1985) involved plants with a phalanx-type (clumped) growth pattern. Subsequent studies have demonstrated that larger genets do not necessarily have higher rates of selfing compared to smaller genets (Matsuo et al. 2014 and see also Liao et al. 2009). Increased selfing may inevitably be the outcome of larger plant size but, increases in selfing resulting from clonal expansion are expected to be lower than what would result from a comparative increase in plant size for a non-clonal plant (Liao & Harder 2014; Vallejo-Marín et al. 2010; Van Drunen et al. 2015). My results show that although there was a positive association between genet size and selfing rates, the relationship was weak and for small genets selfing rates were highly variable. This result suggests that genet size is not the only factor driving rates of geitonogamous selfing in clonal plants. Selfing rates may be influenced by a combination of other factors such as pollinator abundance and behaviour and spatial arrangement of flowering displays.

In this study population, larger genets were, in absolute terms, intermingled with a larger number of other genets. I predicted that this type of spatial arrangement should allow for ramets to have independent mating opportunities, and therefore linearize the male fitness gain curve. Indeed, larger clones, on average, had greater siring success than smaller genets. This evidence appears to support the 'mate-finding' hypothesis, that clonal expansion can increase the number of mates available to an immobile organism (Barrett 2015). Although realized returns on investment in reproduction began to diminish for the largest clones sizes in this population, larger clone size does result in more outcrossing opportunities without the level of

selfing increase that would occur for non-clonal plants. The results here are consistent with the results of Matsuo et al. (2014), however, my results show a less quickly decelerating male fitness gain curve. As suggested earlier, this may be because here I have included the entire population, whereas the study by Matsuo et al. (2014) did not sample seeds on the outside edges of their population, potentially underestimating siring success for the largest clones. If fitness through male function does not plateau with increased investment, this would allow more balanced sex allocations, and furthermore, and could allow for the evolution of male individuals from hermaphrodites.

2.4.3 Conclusions

Considering that the alternative to clonal growth is to increase in size without any spatial segregation among inflorescences, clonality may be a strategy to sub-divide reproduction investment over space, creating the possibility for more siring opportunities while avoiding an increase in negative mating consequences of larger sizes, such as selfing or pollen discounting. I predicted that larger clonal expansion would yield greater outcrossed siring success. As expected, it was shown that larger clones had an increased number of non-self ramets within their mating neighbourhood, and furthermore, the realized siring success of larger clones was greater than smaller clones. I also predicted that this increase in siring success would occur with minimal costs to mating arising from geitonogamous selfing. Although selfing rates did increase with larger clone size, this relationship was weak, and compared to the increase in selfing that may occur for a non-clonal plant increasing in size, this increase may be minimal. Lastly, I predicted a linear relationship between investment in male function and siring success. This

relationship was linear for small to moderate-sized genets, however, it became decelerating for clones of large size. It is possible still that this deceleration is less rapid than would be seen in a plant that was not capable of clonal growth. The results here are largely consistent with the predictions, demonstrating that larger clones have greater reproductive success, although this tapers off at a certain size. Taken together these results demonstrate that the idea that clonality is detrimental to reproductive success in plants is not the rule. Here I demonstrate that bigger—via clonality—is better, or at least better than it is for non-clonal plants (Vallejo-Marín, Dorken and Barrett 2010; Dorken & Van Drunen 2010). Under certain circumstances, such as intermingling, clonality may in fact instead be a life-history trait that allows plants to overcome mating challenges, especially those introduced by immobility.

CHAPTER 3: GENERAL DISCUSSION

The aim of my research was to determine how the spatial arrangement of ramets within clones of *Sagittaria latifolia* affects patterns of mating in a natural population. Very few studies have investigated the clonal structure of an entire natural population, so the results here provided one of only a few empirical examples of clone size and intermingling in a natural population. Furthermore, I looked at the mating patterns resulting from this clonal structure. I found that although selfing rates did increase with larger clone size, this relationship was weak, and larger clones also had increased siring success. Additionally, for clones of small to moderate size, there was a more linear relationship between investment in reproduction and siring success. These results demonstrate that the effect of clonality on mating patterns is complex, and that the prevailing view in the literature that clonality should theoretically lead to mating interference, and the limited empirical evidence to support this (Handel 1985), does not capture the complex ways in which clonality can vary and how this ultimately affects mating patterns.

3.1 Spatial Structure of Clones

Although this study revealed the spatial structure of a natural population of *Sagittaria latifolia*, it was limited to assessing this structure within a single growing and mating season. A subsequent study that assessed the clonal structure of this same population 4 years later, following a disturbance event (flooding), showed substantial changes in clonal structure (Holt, Kwok & Dorken 2019). Compared to the study here (conducted in 2013), by 2017 clones in this population were smaller (i.e., consisted of fewer ramets) and results from analyses of spatial

genetic structure indicated clones were more likely to be related to each other (Holt, Kwok & Dorken 2019). This indicates that clonal structure may not only vary between species and between populations of the same species but could also be substantially variable year-to-year even within a single population, especially in environments that are susceptible to disturbance.

My study showed that under the moderately intermingled structure of this population clonality can benefit siring success—to a point. Small to moderate sized clones had a more linear relationship between investment in reproduction and realized siring success but returns on investment became decelerating for clones of large size. In environments where disturbance events cause frequent turnovers in genets and result in clones of smaller size, it is possible that, depending on how frequently these disturbance events occur, clones may be prevented from reaching a large enough size to see decelerating return on reproductive investment, or if they do reach this large size, it may only be for a limited number of growing seasons before a disturbance event re-sets clonal structure. However, it was also shown that after the disturbance event, clones were more related to ramets within their mating neighbourhoods. Examining the extent to which disturbance events can keep clones from reaching large sizes, while also providing enough time between disturbance events for ramets to become intermingled would add more understanding to the effect of clonal structure in natural populations.

If clonal structure was conserved year-to-year, then a genet's reproductive success over its entire lifetime could be reasonably extrapolated from observations within a single growing

season. However, if clonal structure is highly variable in the short-term, as the study by Holt et al. (2019) suggests, or at least over the lifetime of a genet, siring success could also be highly variable year-to-year. Evolutionarily speaking, fitness of an individual is more meaningfully measured over its entire lifetime, rather than within one mating season. Since a genet's size and degree of intermingling, and therefore mating success, may be dynamic over its entire life, examining this over a single season limits our understanding.

3.2 Implications for Sex Allocation Theory (SAT)

Sex allocation theory (SAT) links organismal life histories to their fitness consequences (Charnov 1982) and explains the variation in mating systems observed in both plants and animals by predicting the conditions that allow different sexual systems, including dioecy and monoecy, to be evolutionarily stable. Fitness gain curves are central to this theory, as they measure the return on investment through the male or female function. In this study, I found evidence that clonality can lead to a more linear relationship between investment in male reproductive effort and resulting siring success for clones of a small to moderate size. This is one of only two studies to empirically measure the male fitness gain curve for clonal plants (see Matsuo et al. 2014) and the first time the male fitness gain curve has been measured for an entire population.

Understanding how ecological factors affect fitness gains through male and female functions is essential to understanding the ecological conditions under which dioecy may be an

evolutionarily stable strategy. Hermaphroditic individuals can gain fitness through both male and female functions, whereas individuals of a single sex can only gain fitness through the function of one sex. For dioecy to evolve from monoecy, genetic individuals must be capable of compensating for the loss of one function with equal gains in function through the other sex. Understanding the ecological conditions under which the fitness gain curves for either male or female function are linear, rather than decelerating, reveal the conditions under which dioecy may be an evolutionarily stable strategy because fitness gains through a single sex are not constrained. In this study, we showed that clonality influences the shape of the male fitness gain curve. Clones of small to moderate size had a more linear fitness gain curve, and therefore are less constrained in the potential for fitness gains through the male function. The life-history trait of clonality, combined with the ecological condition of intermingled genets, could therefore be a condition under which separate sexes could evolve.

3.3 Future Directions of Study

This study has provided a snapshot of clonal structure and resulting mating patterns for a single population during one growing and mating season. Future studies can expand on our understanding of clonal structure in natural populations by assessing different clonal species and different populations of the same species, as well as how clonal structure varies over time in a single population.

REFERENCES

Ahee, J.E., Van Drunen, W.E. & Dorken, M.E. 2015. Analysis of pollination neighbourhood size using spatial analysis of pollen and seed production in broadleaf cattail (*Typha latifolia*). Botany-Botanique, 93, 91-100.

Albert, T., Raspé, O., Jacquemart, A-L. 2008. Influence of clonal growth on selfing rate in *Vaccinium myrtillus* L. Plant Biology, 10, 643-649.

Bailleul, D., Stoeckel, S., & Arnaud-Haond, S. 2016. RClone: A package to identify multilocus clonal lineages and handle clonal datasets in R. Methods in Ecology and Evolution, 7, 966-970.

Baker, H.G. 1959. Reproductive methods as factors in speciation in flowering plants. Cold Spring Harbor Symp. Quant. Biol., 24, 177-191.

Barrett, S.C.H. 2003. Mating strategies in flowering plants: the outcrossing—selfing paradigm and beyond. Philosophical Transactions of the Royal Society B, 358(1434), 991-1004.

Barrett, S.C.H. 2010. Understanding plant reproductive diversity. Philosophical Transactions of the Royal Society of London Ser. B, 365, 351-368.

Barrett, S.C.H., 2002. Sexual interference of the floral kind. Heredity 88: 154-159.

Barrett, S.C.H. 2015. Influences of clonality on plant sexual reproduction. Proceedings of the National Academy of Sciences of the United States of America, 122:8859-8866.

Barrett, S.C.H. & Eckert, C. G. 1990. Variation and evolution of mating systems in seed plants. In *Biological approaches and evolutionary trends in plants* (ed. S. Kawano), pp. 229-254. London: Academic.

Barrett, S.C.H & Harder, L.D. 1996. Ecology and evolution of plant mating. Trends in Ecology and Evolution, 11(2), 73-79.

Bates, D., Mächler, M., Bolker, B. & Walker, S. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software, 67, 1-48.

Bolker, B.M. 2008. Ecological models and data in R. Princeton, NJ: Princeton University Press.

Campbell, D.R. 2000. Experimental tests of sex-allocation theory in plants. Trends in Ecology and Evolution, 15, 227-232.

Caraco, T. & Kelly, C. 1991. On the adaptive value of physiological integration in clonal plants. Ecology, 71, 81-83.

Charlesworth, B. & Charlesworth, D. 1978. A model for the evolution of dioecy and gynodioecy. The American Naturalist, 112(988), 975-997.

Charnov, E.L. 1979. Simultaneous hermaphroditism and sexual selection. Proceedings of the National Academy of Sciences of the United States of America, 76, 2480-2484.

Charnov, E. 1982. *The theory of sex allocation*. Princeton University.

Charpentier, A. 2002. Consequences of clonal growth for plant mating. Evolutionary Ecology 15: 521-530.

DiLeo, M.F., Holderegger, R., & Wagner, H.H. (2018). Contemporary pollen flow as a multiscale process: Evidence from the insect-pollinated herb, *Pulsatilla vulgaris*. Journal of Ecology, 106(6):2242-2255.

Dorken, M.E. & Barrett, S.C.H. 2003. Life-history differentiation and the maintenance of monoecy and dioecy in *Sagittaria latifolia* (Alismataceae). Evolution, 57(9), 1973-1988.

Dorken, M.E. & Barrett, S.C.H. 2004a. Chloroplast haplotype variation among monoecious and dioecious populations of *Sagittaria latifolia* (Alismataceae) in eastern North America. Molecular Ecology, 13, 2699-2707.

Dorken, M.E. & Barrett, S.C.H. 2004b. Sex determination and the evolution of dioecy from monoecy in *Sagittaria latifolia* (Alismataceae). Proceedings of the Royal Society of London Series B: Biological Sciences, 271, 213-219.

Dorken, M.E., Friedman, J. & Barrett, S.C.H. 2002. The evolution and maintenance of monoecy and dioecy in *Sagittaria latifolia* (Alismataceae). Evolution, 56(1), 31-41.

Dorken, M.E & Van Drunen, W.E. 2010. Sex allocation in clonal plants: might clonal expansion enhance fitness gains through the male function? Evolutionary Ecology, 24(6), 1463-1474.

Fox, J. & Weisberg, S. 2011. An R companion to applied regression. Thousand Oaks, CA: Sage.

Handel, S.N. 1985. The intrusion of clonal growth patterns on plant breeding systems. The American Naturalist, 125(3), 367-384.

Handel, S.N. & Mishkin, J.L.V. 1984. Temporal shifts in gene flow and seed set: evidence from an experimental population of *Cucumis sativus*. Evolution, 38, 1350-1357.

Harder, L.D. & Barrett, S.C.H. 1995. Mating cost of large floral displays in hermaphrodite plant. Nature, 373, 512-515.

Hardy, O.J., González-Martínez, S.C., Fréville, H., Boquien, G., Mignot, A., Colas, B., & Olivieri, I. 2004. Fine-scale genetic structure and gene dispersal in Centaurea corymbose (Asteraceae) I. Pattern of pollen dispersal. Journal of Evolutionary Biology, 17, 795-806.

Harper, J.L. 1977. *Population biology of plants.* London: Academic Press.

Holt, R., Kwok, A., & Dorken, M.E. (2019). Increases spatial-genetic structure in a population of the clonal aquatic plant *Sagittaria latifolia* (Alimataceae) following disturbance. Heredity, 124(3):514-523.

Jones, O. & Wang, J. 2010. COLONY: A program for parentage and sibship inference from multilocus genotype data. Molecular Ecology Resources, 10, 551-555.

Kalinowski, S.T., Taper, M.L. & Marshall, T.C. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Molecular Ecology, 16, 1099-1106.

Karron, J.D., and Mitchell, R.J., 2012. Effects of floral display size on male and female reproductive success in *Mimulus ringens*. Annals of Botany 109: 563-570.

Klimes, L., Klimesova, J., Hendricks, R., & van Groenendael, J. 1997. Clonal plant architecture: a comparative analysis of form and function. P. 1-29 in H. de Kroon and J. van Groenendael (eds.). The ecology and evolution of clonal plants. Backhuys Publishers, Leiden, The Netherlands.

Levin, D.A. & Kerster, H.W. 1974. Gene flow in seed plants. Evolutionary Biology, 7, 139-220.

Liao, W-.J. & Harder, L.D. 2014. Consequences of multiple inflorescences and clonality for pollinator behavior and plant mating. The American Naturalist, 184(5), 580-592.

Liao, W.-J., Hu, Y., Zhu, B.-R., Zhao, X.-Q., Zeng, Y.-F. & Zhang, D.-Y. 2009. Female reproductive success decreases with display size in monkshood, *Aconitum kusnezoffii* (Ranunculaceae). Annals of Botany, 104, 1405-1412.

Lloyd, D.G. & Bawa, K.S. 1984. Modification of the gender of seed plants in varying conditions. Evolutionary Biology, 17, 255-388.

Lloyd, D.G., & Schoen, D.J. 1992. Self- and cross-fertilization in plants. I. Functional Dimensions. International Journal of Plant Sciences, 153(3), 358-369.

Loh, R., Scarano, F.R., Alves-Ferreira, M., & Salgueiro, F. (2015). Clonality strongly affects the spatial genetic structure of the nurse species *Aechmea nudicaulis* (L.) Griseb. (Bromeliaceae). Botanical Journal of the Linnean Society, 178:329-341.

Lovett-Doust, L. 1981. Population dynamics and local specialization in a clonal perennial (*Ranunculus repens*). I. The dynamics of ramets in contrasting habitats. Journal of Ecology, 69, 743-755.

Marshall, T., Slate, J., Kruuk, L. & Pemberton, J. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. Molecular Ecology, 7, 639-655.

Matsuo, A., Tomimatsu, H., Suzuki, J.-I., Saitoh T., Shibata, S., Makita, A. & Suyama, Y. 2014. Female and male fitness consequences of clonal growth in a dwarf bamboo population with a high degree of clonal intermingling. Annals of Botany, 114, 1035-1041.

Meagher, T.R. 1986. Analysis of paternity within a natural population of *Chamaelirium luteum*. 1. Identification of most-likely male parents. The American Naturalist 128(2).

Mizuki, I., Ishida, K., Tani, N., & Tsumura, Y. (2010). Fine-scale spatial structure of genets and sexes in the dioecious plant *Dioscorea japonica*, which disperses by both bulbils and seeds. Evolutionary Ecology, 24:1399-1425.

Mori, Y., Nagamitsu, T. & Kubo, T. 2009. Clonal growth and its effects on male and female reproductive success in *Prunus ssiori* (Rosaceae). Population ecology, 51, 175-186.

Muenchow, G.E. & Delesalle, V.A. 1994. Pollinator response to male floral display size in two *Sagittaria* (Alismataceae) species. American Journal of Botany, 81, 568-573.

Perry, L.E. & Dorken, M.E. 2011. The evolution of males: support for predictions from sex allocation theory using mating arrays of *Sagittaria latifolia* (Alismataceae). Evolution, 65(10), 2782-2791.

Rademaker, M.C.J. & de Jong, T.J. 1998. Effects of flower number on estimated pollen transfer in natural populations of three hermaphroditic species: an experiment with fluorescent dye. Journal of Evolutionary Biology, 11, 623-641.

Renner, S.S. 2014. The relative and absolute frequencies of angiosperm sexual systems: dioecy, monoecy, gynodioecy, and an updated online database. American Journal of Botany, 101(10), 1588-1596.

Ritland, K. 2002. Extensions of models for the estimation of mating systems using n independent loci. Heredity, 88, 221.

Routley, M.B., Kron, P., Husband, B.C. 2004. The consequences of clone size for paternal and maternal success in domestic apple (*Malus x domestica*). American Journal of Botany, 91, 1326-1332.

Ruggiero, M.V., Reusch, T.B. & Procaccini, G. 2005. Local genetic structure in a clonal dioecious angiosperm. Molecular Ecology, 14, 957-967.

Schnittler, M. & Eusemann, P. 2010. Consequences of genotyping errors for estimation of clonality: A case study on *Populus euphratica* Oliv. (Salicaceae). Evolutionary Ecology, 24, 1417-1432.

Silander, J.A. 1985. Microevolution in clonal plants. In J.B. Jackson, L.W. Buss, and R.E. Cook [eds.], Population biology and evolution of clonal organisms, 107-152. Yale University Press, New Haven.

Stephens, S., van Kleunen, M. & Dorken, M.E. 2020. Data from: Patterns of pollen dispersal and mating in a population of the clonal plant *Sagittaria latifolia*. Dryad Digital Repository.

Stuefer, J.F. 1996. Separating the effects of assimilate and water integration in clonal fragments by the use of steam-girdling. Abstracta Botanica, 19, 75-81.

Vallejo-Marín, M., Dorken, M.E., Barrett, S.C.H. 2010. The ecological and evolutionary consequences of clonality for plant mating. Annual Review of Ecology, Evolution and Systematics, 41, 193-213.

Vamosi, J.C., Vamosi, S.M., Barrett S.C.H. 2006. Sex in advertising: dioecy alters the net benefits of attractiveness in *Sagittaria latifolia* (Alismataceae). Proceedings of the Royal Society of London B, 273, 2401-2407.

Van Drunen, W.E., van Kleunen, M. & Dorken, M.E. 2015. Consequences of clonality for sexual fitness: Clonal expansion enhances fitness under spatially restricted dispersal. Proceedings of the National Academy of Sciences, 112(29), 8929-8936.

van Kleunen, M., Pyšek, P., Dawson, W., Essl, F., Kreft, H., Pergl, J., … Lenzner, B. 2019. The Global Naturalized Alien Flora (GloNAF) database. Ecology 100: e02542.

Van Rossum, F., Stiers, I., Van Geert, A., Triest, L., & Hardy, O.J. (2011). Fluorescent dye particles as pollen analogues for measuring pollen dispersal in an insect-pollinated forest herb. Oecologia, 165(3):663-674.

Volger, D.W. & Kalisz, S. 2001. Sex among the flowers: the distribution of plant mating systems. Evolution, 55, 202-204.

Wang, J. 2004. Sibship reconstruction from genetic data with typing errors. Genetics, 166, 1963-1979.

Wang, J. 2018. Estimating genotyping errors from genotype and reconstructed pedigree data. Methods in Ecology and Evolution, 9, 109-120.

Wang, X., Zhou, W., Lu, J., Wang, H., Xiao, C., Xia, J. & Lui, G. 2012. Effects of pollination size on synchronous display of female and male flowers and reproductive output in two monoecious *Saggitaria* species. PLoS ONE, 7, e48731.

Waser, N.M. 1982. A comparison of distances flown by different visitors to flowers of the same species. Oecologica, 55, 251-257.

West, S. 2009. *Sex Allocation*. Princeton University Press, New Jersey.

Wilson, P., Thomson, J.D., Stanton, M.L. & Rigney, L.P. 1994. Beyond floral Batemania: gender biases in selection for pollination success. American Naturalist, 143, 283-296.

Wooten, J.W. 1971. The monoecious and dioecious conditions in *Sagittaria latifolia* L. (Alismataceae). Evolution, 25(3), 549-553.

Yakimowski, S.B., Rymer, P.D., Stone, H., Barrett, S.C.H. & Dorken, M.E. 2009. Isolation and characterization of 11 microsatellite markers from *Sagittaria latifolia* (Alismataceae). Molecular Ecology Resources, 9(2), 579-581.

Yakimowski, S.B. & Barrett, S.C.H. 2014. Clonal genetic structure and diversity in populations of an aquatic plant with combined versus separate sexes. Molecular Ecology, 23, 2914-2928.

Zhang, D.-Y. 2006. Evolutionary stable reproductive investment and sex allocation in plants. Pp 41-60 in L.D. Harder, and S.C.H. Barrett, eds. Ecology and evolution of flowers. Oxford University Press, Oxford.