SEXUAL SELECTION, SEX ALLOCATION AND STOCHASTICITY: A STUDY OF MATING PATTERNS IN *SAGITTARIA LATIFOLIA*

A dissertation submitted to the Committee on Graduate Studies

in partial fulfilment of the requirements for the degree of

Doctor of Philosophy

in the Faculty of Arts and Science.

TRENT UNIVERSITY

Peterborough, Ontario, Canada

Environmental and Life Sciences Ph.D. Graduate program

January 2025

ABSTRACT

Sexual selection, sex allocation and stochasticity: A study of mating patterns in *Sagittaria latifolia*

Allison Kwok

Diversity of flowers in flowering plants is understood to facilitate mating success, and yet understanding the relationships between plant sexual diversity and mating patterns remains a challenge. In this thesis, I used *Sagittaria latifolia*, an aquatic plant with unique sexual systems, to investigate relationships between plant sexual diversity, mating patterns, and reproductive success and to understand their evolutionary consequences. First, I measured the magnitude of sexual selection in a dioecious and a monoecious population of *S. latifolia*, while accounting for size variation. I showed that sexual selection does operate in natural populations of plants. Estimates of sexual selection were similar in both populations, possibly due to the temporal separation of female and male flowering in hermaphroditic *S. latifolia*, enabling individuals to act temporarily as unisexuals. Second, I examined how seasonal differences in sex allocation and flowering time can affect male mating opportunities, and the effect on reproductive success in hermaphroditic *S. latifolia*. I found that shifting allocations to be more male-biased with earlier flowering increased male mating opportunities. However, greater mating opportunities did not equate to higher rates of siring, indicating that factors other than temporal overlap contribute to male reproductive success. Finally, I determined the factors affecting mating and reproductive success of males and male-function hermaphrodites, distinguishing between the predictable effects of plant-level traits and population-level features, and unpredictable effects of stochastic factors. Across both populations, we found that plant-level traits had a greater impact on mating and reproductive success than population-level features. Nevertheless, these effects were frequently masked by stochastic factors. Furthermore, unexplained variation in mating and reproductive success may stem from additional plant traits affecting post-pollination gametophyte-sporophyte interactions. My findings indicate that while sexual selection does operate in natural populations of *S. latifolia* as expected under Bateman's principles, temporal overlap in flowering, plant traits and population features did not explain patterns of male mating or reproductive success, suggesting that post-pollination factors may be influencing mating outcomes.

KEYWORDS

Mating, reproduction, sex allocation, phenology, sexual selection, dioecy, monoecy, plant reproduction, stochasticity, *Sagittaria latifolia*

PREFACE

This dissertation has been written in manuscript format. Chapters 2 and 3 have been published in the journals Proceedings of the Royal Society B: Biological Sciences and Royal Society Open Science, respectively. Each of those chapters is presented in the style of the journal in which it was published. I used the plural 'we' in each of the published chapters as they are presented as they appear in the journals and I use 'I' in the remaining chapters. However, all manuscript chapters (2-4, inclusive) are the result of collaborations and collaborators are indicated at the beginning of each chapter.

ACKNOWLEDGEMENTS

First and foremost, I am grateful to my supervisor, Dr. Marcel Dorken, for your guidance and dedicated support throughout my Ph.D. Your enthusiasm and encouragement have been a balm during the many inevitable struggles of a thesis. I greatly appreciate your patience and thoughtful mentorship during my time at Trent. I have grown as a scientist; I thank you for it.

Thank you to my committee members Drs. Joanna Freeland and Erica Nol for your time and advice. Your insight and support during my thesis have always helped to push me forward.

Funding for this work was provided by Trent University and the National Science and Engineering Research Council (NSERC) through grants to Dr. Dorken and a Queen Elizabeth II Graduate Scholarship in Science and Technology to me. I would also like to thank the French American Charitable Trust Graduate Scholarship for financial assistance.

To all those who assisted me in the lab and field, your help was invaluable through the data collection. Lindsay Bond, Sarah Rousselle, Jacob Houpt, Ryan Holt, and Christina Steinecke; you all stepped up to help in the lab and field, your assistance was very much appreciated. Thank you to lab technicians Caleigh Smith, Kristyne Wozney, Anne Kidd, and Matthew Harnden for your help with genotyping.

A special thank you to my lab sisters, Sam Stephens and Sara Pieper, your friendships have been incomparable. Sam, our long chats about *Sagittaria* and life were beyond helpful. Sara, I always look forward to our science talks and mutual love of Star Wars. You always seem to swoop in to catch me when I'm falling from a bad day, thank you.

To my friends and family, your support has been everything. My parents, Mariam Hoosen and John Kwok, your love and support have been paramount. Sara Klapstein, Sarah Baldwin, Eryn McFarlane, and Tianna Burke, your cheerleading and enthusiasm have helped to celebrate my victories and pull me through my challenges. Our chats always give me a new perspective and inspire new ideas, I am grateful for your positive presence.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

1. CHAPTER 1: GENERAL INTRODUCTION

Flowers, the reproductive organs of flowering plants, exhibit a high level of diversity. This diversity in flowers is reflected not just in floral morphology but also in the sex allocation of flowers (Brunet & Charlesworth 1995; Campbell 2000), the timing of flowering (Kudo 2006), the spatial distribution of flowers and shoots (Diggle 2003), and pollination modes (Fenster et al. 2004; Morgan 2006). All this diversity in plant sexual reproduction is to facilitate mating success (Barrett 2002). Plant sexual diversity has been long documented (Darwin 1877) and much theory is devoted to explaining its evolution and maintenance (Lloyd & Webb 1977; Charlesworth & Charlesworth 1978; Brunet 1996; Pannell 2002). Plants evolved diverse strategies for sexual reproduction to counteract the limitations due to their sessile nature (Harder & Barrett 2006). And yet, understanding why the promotion of a single function, mating success, requires such diversity remains unclear. In my thesis, I look to understand, in part, this relationship between plant sexual diversity and mating patterns.

Life-history traits, specifically reproductive traits, influence evolutionary patterns by determining mating patterns. Plant mating is determined by maternal and paternal mating success, whether from outcrossed or self-fertilization events (Barrett, 2003). Mating regulates the transmission of genes from one generation to the next. By shaping the interactions between mating partners and influencing which genetic traits are passed on, mating patterns contribute to patterns of genetic diversity in plant lineages over time. For instance, opportunities for self-fertilization can result in inbreeding depression, the negative fitness consequences due to inbreeding, and is considered a fundamental selective force that drives the evolution of plant mating strategies (Charlesworth & Charlesworth 1987; Barrett 2002). Changing mating patterns are also associated with the evolution of gender strategies in plants (Levin 1971), patterns of sexual dimorphism (Moore & Pannell 2011), variation in floral traits (Delph & Ashman 2006), and the regulation of pollination (Arnold 1994; Murphy 1998), among many other processes that shape plant diversity. Thus, a fundamental part of understanding plant diversity is understanding mating patterns, their dynamics, and the factors influencing their change.

A crucial aspect of understanding the relationship between life-history traits and mating patterns is quantifying fitness. Mating is fundamentally important in determining organismal fitness. Due to their sessile nature, modularity, and reliance on third-party vectors for mating, plants have complex life histories that make measuring fitness challenging (Barrett & Harder 2006). Reproductive opportunities in plants can occur concurrently and for extended periods via the production of multiple flowering shoots, which can each vary in the production of flowers, pollen, and other reproductive traits (Diggle 2003). The timing and spatial distribution of flowering within a plant can vary, affecting pollinator visitations (Herrera 2009) and, consequently fitness. For example, in *Mimulus ringens*, large floral displays of multiple flowers were associated with greater overall fitness but also increased self-fertilization (Karron & Mitchell 2012). Mating opportunities from flowers of the same individual can alter mating patterns and affect fitness by reducing the number of offspring sired by males and increasing the production of inbred offspring by females (Van Drunen et al. 2015). The difficulty in measuring fitness in plants is that they potentially have numerous mating events with many unknown mates at any given time.

Plant sexual strategies are highly diverse and can result in complex mating patterns. Gender variation in flowering plants is a spectrum of sex expression and can involve various combinations of female, male, and hermaphroditic flowers. Typically, gender strategies are categorized into two distinct groups: ‘gender monomorphism’, wherein most individuals serve as both maternal and paternal parents and populations have continuous variation in female and male sex functions, and ‘gender dimorphism’, where individuals typically serve as either the maternal or paternal parent and populations consist of two sex morphs (Sakai & Weller 1999). Whether individuals are monomorphic or dimorphic can have implications on mating patterns, reproduction and evolutionary trajectories. For monomorphic individuals, fitness gains through female or male functions affect mating patterns and have consequences for the evolution of dimorphic sexual systems (Charnov 1979). Mating patterns and sexual systems are intimately tied, the evolution of dimorphic sexual systems is linked to selection against self-fertilization (Charlesworth & Charlesworth 1987; Karron et al. 2012). Sexual selection is known to shape plant mating traits (Moore & Pannell 2011; Skogsmyr & Lankinen 2002) with scope in both monomorphic and dimorphic systems (Anthes et al. 2010; Péllisié et al. 2011). Understanding the selective forces that drive the evolution of these diverse sexual systems and their consequences on mating is a fundamental area of study in plant evolutionary biology.

The allocation of resources between male and female functions shapes hermaphrodite mating opportunities and fitness. For hermaphroditic individuals, sex allocation refers to how resources are distributed between female and male sex functions and when each of those sex functions are deployed (Charnov 1979). Different allocations to female or male functions determine how a plant gains fitness via maternal and paternal contributions, and sex allocation theory predicts the optimal allocations to each sex depending on ecological contexts (Charnov 1979). For sequential hermaphrodites, where sex functions occur one after the other, changes in allocations to sex functions also determine what mating opportunities are available and when (Brunet & Charlesworth 1995). Individuals that allocate resources to a particular sex function at suboptimal times can lose mating opportunities (Brunet & Charlesworth 1995, and see Brookes

& Jesson 2000). Sex allocation for simultaneous hermaphrodites, where sex functions co-occur, can impact selfing and outcrossing and have consequences for the evolution of separate sexes (Charnov 1979). Hypotheses on the evolution of dioecy (individuals with separate sex functions) from hermaphroditism involve reallocations from one sex to the other leading to gradual gender specialization (Charlesworth & Charlesworth 1978; Charlesworth 2003). Ultimately, the allocation of resources between male and female functions shapes the mating opportunities and overall fitness of hermaphrodites, which in turn can drive further changes in sex allocation.

Bateman's principles assert that male reproductive success is mate limited and female success is resource limited (Bateman 1948) and has been increasingly recognized as applicable to sexual selection in flowering plants. Under Bateman's second principle, the expectation is that there should be greater variance in male mating success compared to female mating success. Plant sexual selection, once a contentious concept, is now widely acknowledged as fundamental in understanding the evolutionary significance of floral characteristics (Hodgins & Barrett 2008; Delph & Herilihy 2012; Cocucci et al. 2014), pollination strategies (Huang et al. 2006), shaping patterns of sexual dimorphism (Moore & Pannell 2011; Tonnabel et al. 2002) and its role in male and female reproductive strategies (Lankinen et al. 2007; Lankinen et al. 2017; Aljiboury & Friedman 2022). Mating success is a fundamental aspect of sexual selection and despite its importance, rarely is it included in studies of sexual selection in plants (Tonnabel et al. 2019). Although there are several plant studies that examined selection on traits that may be under sexual selection (for examples see: Marshall & Ellstrand, 1986; Møller et al. 1995; Carlson, 2007), due the difficulty of accounting for mating success, there are few attempts at quantifying the strength of sexual selection (Tonnabel et al. 2019) and none that do so in natural populations of plants. With the broad acceptance that sexual selection operates as an evolutionary force in plants, there is a need to understand how it operates in plants in natural environments.

Sexual reproduction involves genetic contributions from both maternal and paternal parents. The majority of studies examining plant mating and reproduction emphasize the female role in plant sexual fitness. Typically, there is a focus on how mating dynamics affect fruit set and seed set, both measures of female mating and reproductive success (Totland et al. 1998; Matsuhisa & Ushimaru 2015). In contrast, success through male function has been more difficult to quantify because direct observations of mating events are challenging as mating occurs via third-party pollen vectors and reliance on the use of molecular markers to identify paternity (Minnaar et al. 2019). With current molecular techniques (Flanagan and Jones 2019), we are beginning to gain an understanding of male mating success, including who has mated with whom and how often (e.g., Tomaszewski et al. 2018; Santos Del Blanco et al. 2019; Tonnabel et al. 2019; Christopher et al. 2020). Despite the importance of male fitness to floral evolution, the focus on female contributions over male contributions means our understanding of male mating and reproduction is lacking.

Study system *Sagittaria latifolia*

Sagittaria latifolia Willd. is a clonal aquatic plant, commonly found in wetlands throughout North America. It reproduces sexually and asexually via clonal propagation. Plants grow from a rosette of emergent leaves; plants produce clones from new ramets during the growing season (Dorken & Barrett 2003) and can be vegetative or reproductive. Corms are produced as perennating structures at the end of the season (Van Drunen & Dorken 2012) and because of their large size do not typically have long-distance dispersal to other wetlands (Dorken & Barrett 2003). Both ramets and corms are produced at the terminal ends of stolons.

Three sex phenotypes exist in *S. latifolia* –hermaphrodite, female, and male (Figure 1.1). Across its range populations can be monoecious or dioecious (Wooten 1971). Monoecious populations consist of hermaphroditic individuals while dioecious population are made up of individuals that are either female or male. In Southern Ontario, Canada, the flowering season lasts from June to mid-September, flowers are produced on racemes with three flowers at each node. Flowering in *S. latifolia* is diclinous, where individual flowers are either female or male. For monoecious populations of S. *latifolia*, female flowers occupy basal nodes and male flowers occupy distal nodes, inflorescences are protogynous with basal flowers maturing first. In dioecious populations, sex is genetically determined and ramets produce only female or male flowers (Dorken & Barrett 2004). For both monoecious and dioecious populations, female flowers open simultaneously while male flowers open sequentially, typically one whorl at a time. Flowers are open for approximately one day and are visited by generalist insect pollinators, including bees, flies, wasps and butterflies (Muenchow & Delesalle 1994; Glaettli & Barrett 2008). In both monoecious and dioecious populations, male flowers have larger petals and are wider than female flowers, but female floral displays are larger due to synchronous flowering (Yakimowski et al. 2011). Plants are self-compatible but due to synchronous dichogamy, self-fertilization within ramets is unlikely (Dorken et al. 2002). Fruits are winged achenes found in globular clusters (Fig 1.2) (Voss & Reznieck 2012), when fruit is dried, achenes dehisce and are dispersed via water.

Populations of *S. latifolia* in eastern North America are typically either monoecious or dioecious (Wooten 1971; Dorken et al. 2002) and differ slightly in ecological and life history strategies. Monoecious populations are found in wetland habitats including shorelines, ponds, creeks and roadside ditches, habitats that tend to be ephemeral and are characterized by greater disturbance (Dorken et al. 2002). Dioecious populations are typically found in large stable wetlands or edges of lakes and rivers. This differentiation in habitats is associated with differences in life-histories. In monoecious populations, ramets are smaller and flower earlier in comparison to ramets in dioecious populations (Dorken & Barrett 2003). Differences in life history and habitat, reinforce reproductive isolation between the sexual systems (Dorken et al. 2002), however, individuals in monoecious and dioecious populations are fully inter-fertile (Dorken & Barrett 2004).

In this thesis, my primary goal is to understand factors that influence patterns of male mating and reproduction in *S. latifolia* and their consequences. In Chapter 2, I investigate sexual selection in natural populations of *S. latifolia*. Traits that impact mating and reproductive success have been intensely studied in plants and often times, sexual selection is invoked as an explanation of any observed patterns. There are several plant studies that examined selection on traits that may be under sexual selection (for examples see: Marshall & Ellstrand 1986; Møller et al. 1995; Carlson 2007). Most studies have focused on assessing natural selection by measuring reproductive success, instead of sexual selection by measuring mating success. As a result, most previous studies conflate sexual selection with fecundity selection. Few studies quantify sexual selection (Tonnabel et al. 2019) and none have done so in natural populations. Natural populations can be complex and make measuring sexual selection challenging (Anthes et al. 2016). Measures of fitness often scale with size and is one factor that can confound patterns of sexual selection. In Chapter 3, I examine how variation in sex allocation and flowering time determines availability of mating opportunities. For hermaphroditic plant species, the assignment of resources to either female or male functions, as well as the scheduling of these functions, determines the scope of available mating opportunities. Ill-timed presentation of gametes can be problematic if the deployment of sexual structures occurs when there are limited opportunities for mating or if the environment is highly competitive. I examined how differences in sex allocation and timing of flowering in *S. latifolia* can affect the mating environment and in turn mating opportunities. In Chapter 4, with greater understanding of the effect of flowering time on mating, I look to determine the drivers of male mating and reproductive success. Plants have intrinsic traits and extrinsic features that promote the movement of pollen among plants and should have predictable effects on mating and reproduction. However, stochastic events during pollination can disturb the predictable effects of these traits and features. For example, environmental conditions may prevent pollinator visitations thereby disrupting pollination events. Pollen is often displaced during delivery or lost during grooming by pollinators. There are multiple stages along the pathway to paternity where stochasticity can act (Minnar et al. 2019). I investigate the effect of intrinsic traits, extrinsic features and stochasticity in explaining patterns of male mating and reproduction. Finally, in Chapter 6, I summarize my findings and interpret them together to determine how the complexity of mating in plants affects male mating patterns and its implications for our understanding of mating and reproduction in plants.

1.2 Figures

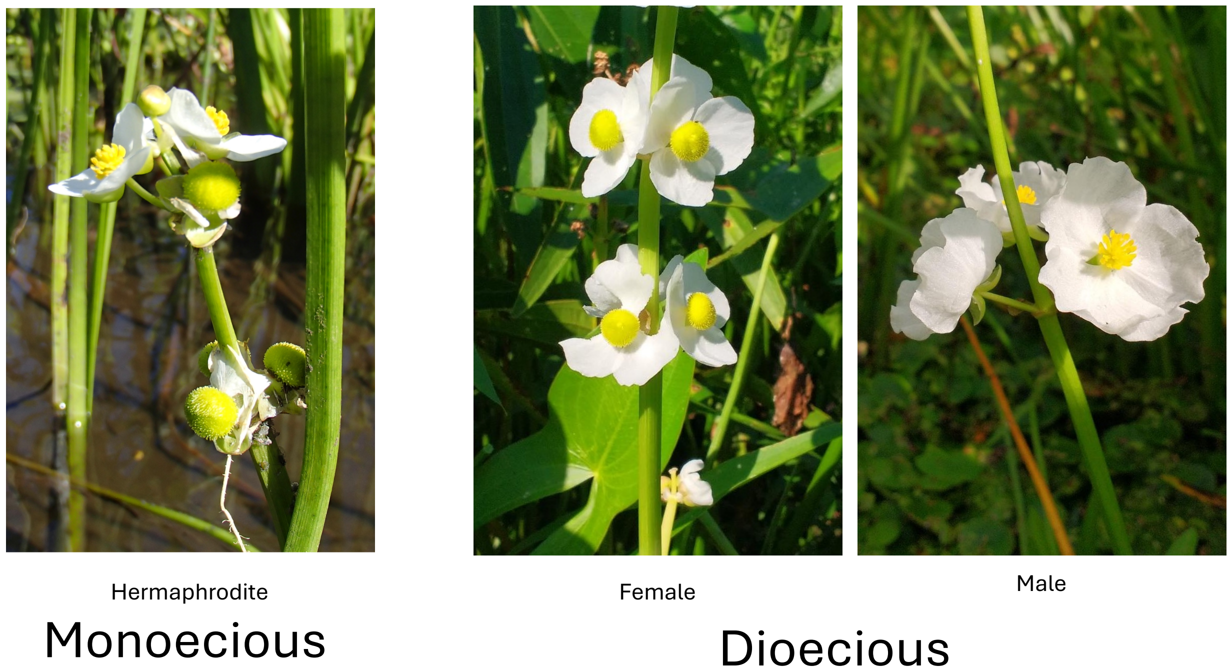

Figure 1.1 Inflorescences of monoecious and dioecious populations of Sagittaria latifolia. Monoecious populations are composed of individuals that produce both female and male flowers (left). Note the open male flowers at the top of the ramet and the lower female flowers have dehisced, showing that female and male flowering do not overlap within an inflorescence. While dioecious populations have individuals that exclusively produce female flowers (centre) or male flowers (right)

Figure 1.2 Fruits of Sagittaria latifolia, globular clusters of achenes are shown. Single achene is indicated with an arrow. When fruits are dried, they dehisce into water and are dispersed via waterways.

2. CHAPTER 2. SEXUAL SELECTION ON MALE BUT NOT FEMALE FUNCTION IN MONOECIOUS AND DIOECIOUS POPULATIONS OF BROADLEAF ARROWHEAD (*SAGITTARIA LATIFOLIA*)

A version of this chapter has been published:

Kwok, A., and Dorken, M.E. 2022. Sexual selection on male but not female function in monoecious and dioecious populations of broadleaf arrowhead (*Sagittaria latifolia*). Proceedings of the Royal Society B: Biological Sciences 289: 20220919

2.1 Abstract

Direct measures of sexual selection in plants are rare and complicated by immobility and modular growth. For plants, instantaneous measures of fitness typically scale with size, but covariances between size and mating success could obscure the detection of sexual selection. We measured the magnitude of sexual selection in a monoecious and a dioecious population of the clonal plant *Sagittaria latifolia* using Bateman gradients (*ßss*). These gradients were calculated using parentage analysis and residual regression to account for the effects of shoot and clone size on mating and reproductive success. In both populations, (i) there was greater promiscuity via male function than via female function and (ii) *ßss* were positive, with significant associations between mating and reproductive success for male but not female function. Moreover, estimated *ßss* were similar for the monoecious and dioecious populations, possibly because non-overlapping female and male sex phases in hermaphroditic *S. latifolia* reduced the scope for interference between sex functions during mating. This study builds on previous studies of selection on plant mating traits, and of sexual selection under experimental conditions, by showing that sexual selection can operate in natural populations of plants, including populations of hermaphrodites.

Keywords: Bateman gradients, clonal growth, dioecy, modularity, monoecy, sexual systems

2.2 Introduction

Sexual selection arising from fitness differences in reproductive success via nonrandom variation in mating success is a key process affecting organismal evolution in anisogamous lineages, including animals (Kokko et al. 2008) and plants (Marshall & Ellstrand 1985; Moore & Pannell 2011). Mating regulates the transmission of genes from one generation to the next, so sexual selection can have important consequences not only for individual fitness, but also for patterns of genetic diversity within populations (Marshall & Ellstrand 1985; LaMunyon et al. 2007), reproductive isolation (Boughman 2001) and speciation (Seehausen & van Alphen 1999; Panhuis et al. 2001). Several lines of evidence point to the importance of sexual selection in shaping patterns of sexual dimorphism in dioecious plants (i.e. populations of distinct female and male individuals (Tonnabel et al. 2022)), variation in floral traits (Delph & Herlihy 2011; Cocucci et al. 2014; Tonnabel et al. 2022) and the processes regulating pollination (Huang et al. 2006) and fertilization success (Lankinen et al. 2007; Delph & Herlihy 2011; Cocucci et al. 2014; Lankinen et al. 2017; Aljiboury & Friedman 2022). However, very few studies of sexual selection in plants have included measures of mating success (i.e. number of mates (Lankinen et al. 2017; Tonnabel & Pannell 2019)). As a result, studies directly quantifying the strength of sexual selection in plants remain rare (Marshall & Ellstrand 1985; Tonnabel & Pannell 2019).

The measurement of sexual selection in plants has lagged studies of animals, at least in part because the occurrence of sexual selection in plants has been contentious (2 Marshall & Ellstrand 1985). Mate choice is an important driver of sexual selection (Kokko et al. 2002; Jones & Ratterman, 2009; Kekäläinen & Evans 2018) and plants, which rely on third-party vectors for pollination, lack the ability to directly choose mates during pollination (Marshall & Ellstrand 1985; Skogsmyr & Lankinen, 2002; Barrett & Hough 2013). However, sexual selection via mate choice does not require the active choosing of mates (Simmons 2014), and several aspects of plant reproduction can yield sexual selection (Marshall & Ellstrand 1985), including male–male competition in sporophytic (during pollination) (Dorken & Perry 2017) and gametophytic (post-pollination) phases of the life cycle (McCallum & Change 2016; Swanson et al. 2016; Dorken & Perry 2017). Indeed, there may even be scope for female choice in plants via sporophyte–gametophyte interactions (Marshall & Ellstrand 1985). Nevertheless, sexual selection may be a weaker force in hermaphroditic than in dioecious populations if there is reciprocal mating and/or male and female sex functions are expressed simultaneously (Anthes et al. 2015). These long-standing issues notwithstanding, sexual selection is now widely considered to be an important evolutionary force in plants, even if the magnitude of sexual selection may be weaker in hermaphroditic compared to dioecious populations.

An additional and less widely discussed problem with the measurement of sexual selection in plants is that measures of fitness usually scale with plant size. Although individual size is considered a sexually selected trait for some unitary organisms (Höglund & Säterberg 1989; Lindenfors & Tullberg 1998; Székely et al. 2000), size variation poses a challenge to the inference of sexual selection for modular organisms like plants. For plants, as for some unitary organisms (McDowall 1994; Ousterhout et al. 2015), size varies with age and reflects ongoing trade-offs in the allocation of resources to growth versus survival and reproduction (Reznick 1985; Obeso 2002). For unitary animals with indeterminate growth, these trade-offs make size a sensible trait when considering the operation of sexual selection (i.e. there may be heritable variation in size among individuals within age cohorts (Shimada et al. 2011)). Although (non-heritable) variation in local resource conditions should contribute to variation in size for plants and animals, immobility further exacerbates these effects for plants. Moreover, modularity and

opportunities for independent resource capture among modules might weaken trade-offs between growth and reproduction. Variation in plant size may therefore obscure patterns of sexual selection whenever plant size covaries with reproductive and mating success. In particular, underlying covariances between plant size and reproductive and mating success should yield positive measures of the strength of sexual selection even under random mating—in this scenario, some phenotypes (large plants) can be expected to have high reproductive and mating success, while other phenotypes (small plants) have low reproductive and mating success. Accordingly, rather than considering plant size a sexually selected trait, studies of sexual selection in plant populations control for variation in size among individuals (e.g. by including size as a covariate when estimating the strength of sexual selection (Dai & Galloway 2013), or via the use of standardized growth conditions for same-aged plants
(Tonnabel & Pannell 2019).

A variety of metrics for estimating the strength of sexual selection exist, but they vary in their utility (Klug et al. 2010; Jennions et al. 2012). For example, one of the most widely used metrics of sexual selection, *Is* (the opportunity for sexual selection), measures the variance in mating success but, among other problems (Jennions et al. 2012), cannot be scaled to take variation in plant size into account. Selection gradients and/or selection differentials are useful when specific traits are hypothesized to be subject to sexual selection (Jennions et al. 2012). But by being trait-specific these metrics do not estimate the total strength of sexual selection within populations (Krakauer et al. 2011). Bateman gradients are one of the most direct methods for quantifying the overall strength of sexual selection (Krakauer et al. 2011; Anthes et al. 2015). They are calculated from the slope of the association between mating success and reproductive success for each sex function, and under Bateman's third principle (the relationship between mating and reproductive success is stronger for males than for females (Arnold 1994)), are expected to be steeper for male than for female function (Janicke et al. 2016). Bateman gradients have been used to examine the strength of sexual selection in a range of animal groups (Anthes et al. 2015; Janicke et al. 2016; Johnson & Shaw 2016), and more recently in plants (Anthes et al. 2015; Johnson & Shaw 2016). Here, we investigated patterns of sexual selection in natural populations of the clonal aquatic plant *Sagittaria latifolia*. Populations of *S. latifolia* may be either monoecious (populations of hermaphroditic plants with separate female and male flowers) or dioecious. Previous studies of this plant have indicated that reproductive traits, such as male floral display size, might be subject to selection (Glaettli & Barrett 2008), including sexual selection (Dorken & Perry 2017). Moreover, the duration of flowering and number of flowers produced vary between the sexes (or between sex functions in monoecious populations) in a manner that might affect the magnitude of sexual selection. Using direct measures of reproductive success and indirect measures of mating success determined using parentage analysis from the segregation of simple-sequence repeat (SSR) alleles, we estimated the degree of promiscuity, the opportunity for sexual selection and the magnitude of Bateman gradients for females and males in a dioecious population, and for female and male function in a monoecious population. We expected mating and reproductive success to be strongly size-dependent for *S. latifolia* (Stephens et al. 2020). Accordingly, we adjusted our primary measure of sexual selection (the Bateman gradients) for variation in plant size and compared this size-adjusted measure to unadjusted measures that were based purely on patterns of mating and reproductive success (I_s and an index of promiscuity).

2.3 Methods

2.3.1 Study Species

Sagittaria latifolia Willd. is an emergent clonal aquatic plant, commonly found in wetland habitats across North America. Shoots (ramets) are produced from axillary stolons during the growing season and can be vegetative or reproductive. Corms are also produced from axillary stolons and function as perennating structures (Dorken & Barrett 2004). Genetic individuals (genets) may be composed of multiple ramets that are temporarily interconnected by stolons, which, along with all above-ground vegetative tissues, deteriorate at the end of each growing season. Variation in plant size is driven by clonal expansion of genets (Van Drunen & Dorken 2012; Stephens et al. 2020) and by variation in ramet size that is primarily determined by local growing conditions (Dorken & Barrett 2004). Flowers are produced on racemes with three flowers at each node. In monoecious populations, female flowers occupy basal nodes and male flowers occupy distal nodes (7.1 Appendix, figure A7.1.1).

Flowers open basipetally, remain open for a single day, and nodes with male flowers mature later than nodes with female flowers, resulting in inflorescences that are synchronously protogynous (i.e. there is usually no overlap between female and male sex phases (Van Drunen & Dorken 2012)). In dioecious populations, sex is genetically determined and ramets produce male or female flowers (Dorken & Barrett 2004). For both monoecious and dioecious ramets, female flowers tend to open simultaneously, while male flowers open sequentially, usually one whorl at a time. In Southern Ontario, flowering occurs from late June through to mid-September and flowers are pollinated by a variety of insects (Glaettli & Barrett 2008). Seed set is not pollen-limited in monoecious populations (Yakimowski et al. 2011). In both monoecious and dioecious populations, male flowers have larger petals than female flowers, but female floral displays are larger due to greater floral synchrony (Sarkissian et al. 2001).

In dioecious populations, the number of flowers produced per ramet is size-dependent for both sexes (Sarkissian et al. 2001; Dorken & Barrett 2003). In monoecious populations, only female flower production is size-dependent (Sarkissian et al. 2001; Dorken & Barrett 2003). Plants are self-compatible, but due to synchronous protogyny, self-fertilization within ramets is unlikely (Dorken et al. 2002). Selfing between flowering ramets of the same genet can occur. However, as clones increase in size, they tend to intermingle with ramets from unrelated genets such that selfing rates—at least in the monoecious population studied here—may be only weakly associated with variation in genet (clone) size (Stephens et al. 2020).

2.3.2 Study site and floral sampling

To survey flowering in *S. latifolia*, we sampled a monoecious and a dioecious population for each of five consecutive days. The monoecious population was located in a shallow area of Thompson Creek in Meadowvale Park, Peterborough, ON, Canada (44° 200 N, 78° 180 W). Flowering shoots at this site were sampled from 8 to 12 August 2016 in a 13× 17 m area of the population. The dioecious population occupied a 13 × 4 m area of a shallow roadside ditch beside an agricultural field near Stoney Point, ON (44° 170 N, 82° 280 W). Plants at this site were sampled between 21 and 26 August 2016. Due to rain and the absence of pollinators, there was no sampling on 23 August. On the first day of sampling at each site, study zones were delimited to areas of approximately 100 flowering ramets and open flowers were marked with tags. At the monoecious site, the study zone was separated from the rest of the population by natural gaps between patches of *S. latifolia* and ramets outside the study zone were prevented from mating with plants in the study zone by removing all male flowers on plants outside the zone. At the dioecious site, all flowering ramets in the population were included in the study.

Within each population, open female flowers were tagged and labelled each day to enable tracking of the date of pollination. The numbers of open female and male flowers were recorded each day for all flowering ramets in the study zone. The mean numbers of flowering ramets per day were similar for the monoecious (71.60 ± 15.14 SD flowering ramets per day) and dioecious populations (68.80 ± 22.54 SD). A total of 167 flowering ramets were tracked at the monoecious site, and 183 flowering ramets were tracked at the dioecious site.

Developing fruits were collected approximately one month after initial sampling. The one-month gap between floral sampling and fruit collection resulted in numerous fruits not being recovered due to disturbances from flooding and foraging by wildlife. Because of these disturbances, the total sample of fruits obtained was a subset of the total number of ramets with female flowers in each population (33/113 in the monoecious population; 38/54 in the dioecious population). Once collected, fruits were allowed to dry at room temperature and then stored in air-tight containers at 4°C. Fruits were stored individually in coin envelopes and labelled with the ramet ID and flowering date. Prior to DNA extraction, a random subset of the seeds from each fruit were soaked in water to enable the removal of maternally derived tissues around the embryo. A total of nine seeds per fruit per ramet were subsequently sampled for genotyping and parentage analysis.

2.3.3 Genet and paternity assignment

Leaf and seed DNA were extracted and genotyped using microsatellite loci following procedures outlined in the Appendix (7.1 Appendix: DNA extraction and SSR genotyping; and see (Stephens et al. 2020)). Ramets were assigned to genets (here defined as multi-locus lineages, MLLs) (7.1 Appendix: Genet and paternity assignments). Ramets with multi-locus genotypes (MLGs) that differed by one allele were assigned to the same MLL to avoid subdividing genets with slightly different MLG scores because of potential genotyping errors or somatic mutations (Arnaud-Haond et al. 2007). Seeds were assigned paternity using software that groups parents and offspring into paternal and maternal sibships by maximizing the likelihood of the probability of these sibships based on their MLGs (Wang & Santure 2009; Jones & Wang 2010; Wang 2017). The paternal parent of each seed was assumed to be the MLL with the greatest probability of paternity (as estimated by COLONY2 (Jones & Wang 2010), the probability of paternity is indicated as π in the calculations below), and calculations of the reproductive success via male function (paternity share) for each MLL were weighted by π. Further details on genet and paternity assignments are provided in the Appendix (7.1 Appendix: Genet and paternity assignments).

2.3.4 Reproductive and mating success

Reproductive success via female function (RS^f) was estimated as the total number of seeds produced per MLL i:

$$RS_i^f = \sum_j \sum_k N_{jk}^S,$$

where N^S was the estimated number of seeds produced per pollination date k by each ramet j within MLL i. Because ramets typically produce thousands of seeds, we estimated N^S as the quotient of the total mass of all fruits per ramet and the average mass of individual seeds (achenes). Paternity share (RS^m) was calculated as the estimated number of outcrossed seeds sired per MLL i:

$$RS_i^m = \sum_j \sum_k \left[\left(\frac{\sum_l \pi_{ijkl}}{N_{jk}^G}\right) \times N_{jk}^S\right],$$

where *πijkl* is the probability that each genotyped seed l per maternal ramet *j* was sired by MLL *i* on pollination date *k*, N^S is the estimated total number of seeds per maternal ramet and N^G is the total number of seeds genotyped per maternal ramet. Self-fertilized seeds were excluded from calculations of mating and reproductive success. There is substantial inbreeding depression in monoecious populations of *S. latifolia* (Dorken et al. 2002), reducing the expected contribution of selfed offspring to reproductive success.

We estimated the genetic mating success for female and male function in each population as the total number of mates obtained per MLL via each sex function, as identified from parentage analysis. We refer to these estimates as proxies of mating success because we inferred mating events from the products of fertilization rather than from direct observation. For female function, the proxy of mating success in each population (MS^f) was calculated as the number of unique sire MLLs (N^m) with seeds on maternal MLL *i* summed across its *j* ramets:

$$MS_i^f = \sum_j N_j^m.$$

Male mating success (MS^m) was estimated by summing the number of unique maternal MLLs (N^f) with seeds sired by each MLL *i* across its *j* ramets as follows:

$$MS_i^m = \sum_j N_j^f.$$

For the calculation of Bateman gradients, mating success and reproductive success were each mean standardized (Tonnabel et al. 2019; Jones & Ratterman 2009). To quantify promiscuity, we calculated an index of mate diversity using the parentage assignments of our sample of genotyped seeds. Mate diversity (*M*) was calculated for each sex (or sex function) as the number of mating partners per MLL *i* (MS_i), divided by *e*, the total number of seeds for which an MLL was assigned as the parent (i.e. the known maternal parent or the inferred paternal parent):

$$M_i = \frac{MS_i - 1}{e_i - 1}.$$

We subtracted one from both the numerator and the denominator so that the index varied between 0 and 1, where a value of 1 indicated that all mating events involved different MLLs. The minimum value of 0 was not possible in this study—we only tracked mating and reproductive success for MLLs that produced seeds and/or were involved in one or more siring events.

2.3.5 Quantifying sexual selection

To estimate the magnitude of sexual selection in each population, we calculated (i) the opportunity of selection (I) – the relative variance in reproductive success; (ii) the opportunity for sexual selection (I_s) – the relative variance in mating success and(iii) the Bateman gradient (β_{ss}) – the slope of the association between mating and reproductive success (7.1 Appendix: Bateman gradients— calculated using residual regression). Both I and I_s are standardized and unitless, enabling comparisons between sexes, and across studies (Krakauer et al. 2011). However, I and I_s cannot be calculated in a way that accounts for size effects—if there are underlying associations between size and reproductive and mating success, values of I and I_s might be subject to a positive bias if there is variation in plant size. Moreover, these two measures can—at least in principle— provide misleading inferences regarding the strength of sexual selection (Klug et al. 2010) and are most useful when reported alongside other measures of the strength of sexual selection (Willson 1994; Jones & Ratterman 2009; Krakauer et al. .2011; Jennions et al. 2012).

We estimated I and I_s by first dividing the variance of reproductive and mating success by their respective squared means for each sex (for the dioecious population: I_m and I_f, and I_{sm} and I_{sf}) or sex function (for the monoecious population: I_{mm} and I_{ff}, and I_{smm} and I_{sff}). Binomial errors were calculated for Is to account for an extra binomial error that can arise from sampling effects (Janicke et al. 2016). Sex differences in the opportunity of selection (I) and sexual selection (I_s) were calculated as $I_m - I_f$ and $I_{sm} - I_{sf}$ for the dioecious population; $I_{mm} - I_{ff}$ and $I_{smm} - I_{sff}$ for the monoecious population (Jones & Ratterman 2009).

Bateman gradients were calculated using residual regression to control for the effects of ramet and genet size. We first calculated least-squares multiple regressions of mating and reproductive success as functions of genet size (number of flowering ramets per MLL) and ramet size (average mid-vein length per ramet per MLL). In these models, relative mating success or relative reproductive success were the dependent variables, and ramet size and log_{10}(genet size) were the independent variables (genet size was log-transformed to account for right-skew in the data). Bateman gradients were then calculated separately for each sex (or sex function; 7.1 Appendix: Bateman gradients calculated using residual regression) as the slope of the least-squares regression of standardized residual reproductive success on standardized residual mating success as follows:

$$RS^{f}_{resid} = \alpha + \beta_f MS^{f}_{resid} + \varepsilon$$

$$RS^{m}_{resid} = \alpha + \beta_m MS^{m}_{resid} + \varepsilon$$

The slope of each regression was then the sex-specific Bateman gradient (β_f and β_m for the dioecious population; β_{ff} and β_{mm} for the monoecious population) that describes how variation in mating success contributed to reproductive success and ε is an (non-constant) error term. For each population, the sex difference in sexual selection ($\Delta\beta_{ss}$) was calculated using separate least-squares multiple regression models that included a (residual) mating success × sex interaction term, where $\Delta\beta_{ss}$ corresponds to the slope coefficient for the interaction term. Combined with significant values of β_m or β_f, values of $\Delta\beta_{ss}$ indicate whether sexual selection is stronger in one sex (or sex function) than the other (Krakauer et al. 2011). All regression analyses were calculated using the lm function in R (R Core Team, 2022). We present alternative analyses that accounted for variation in plant size (multiple regression (Freckleton 2002)) or that involved subsampling of equal-sized MLLs via bootstrapping in the appendix. Finally, we also estimated Bateman gradients without accounting for variation in plant size and contrast the findings with those from residual regression (7.1 Appendix: Bateman gradients uncorrected for plant size).

In monoecious populations, reproductive success via one sex function might be affected by mating events that involve the other sex function. For example, if the presence of female sex organs reduces pollen export, reproductive success via male function may be positively associated with mating success through male function but negatively associated with mating success via female function (Barrett 2002). For this reason, cross-sex effects should be considered when estimating Bateman gradients in populations of hermaphrodites (Anthes et al. 2015). However, we recovered an insufficient number of hermaphrodites that were inferred to have been both sires and mares to calculate these cross-sex effects (n = 4 MLLs). Despite this, we expect these cross-sex effects to be minimal for monoecious *S. latifolia*—hermaphrodites in monoecious populations of *S. latifolia* are synchronously protogynous with unisexual flowers, meaning that at any point in time hermaphroditic ramets are functionally dioecious.

2.4 Results

2.4.1 Flowering and genet size

Out of the 140 MLLs in the monoecious population, the vast majority were small, with 90% comprising a single flowering ramet. The largest MLL was composed of 14 flowering ramets. In the dioecious population, where 110 MLLs were detected, genets were also skewed towards smaller sizes and 87% of MLLs were composed of a single flowering ramet. However, the largest MLLs were bigger in the dioecious populations than in the monoecious population, with one MLL having a total of 28 flowering ramets. There were 14 multi-ramet genets in both the monoecious and dioecious populations (7.1 Appendix: figure A7.1.2).

In the monoecious population, the average numbers of open female and male flowers per day were approximately equal (mean daily floral sex ratio = 0.52 ± 0.03 SE, calculated as proportion of males/total number of male and female flowers; figure 2.1a), but in the dioecious population there were, on average, more open male flowers than female flowers (mean daily floral sex ratio = 0.60 ± 0.03 SE; figure 2.1b). In the monoecious population more ramets flowered in male-phase than in female-phase per day (mean daily proportion male-phase ramets = 0.69 ± 0.03 SE, calculated as the proportion of male-phase ramets versus total flowering ramets per day; figure 2.1a). The same was true in the dioecious population where more male than female ramets flowered each day (mean daily proportion male ramets = 0.75 ± 0.03 SE; figure 2.1b).

2.4.2 Size effects on mating and reproduction

In both populations, larger genets produced more flowers (7.1 Appendix: table A7.1.2 and figure A7.1.3) and unsurprisingly, had greater mating and reproductive success (Appendix 7.1, table A7.1.6). In the monoecious population, genet size was positively associated with mating and reproductive success for both female and male function (multiple linear regression: female function MS = 0.32 + (1.49 × genet size) + (0.03 × ramet size), $F_{2,22} = 7.30$, $p < 0.01$, $R^2 = 0.34$; female function RS = 0.10 + (4.01 × genet size) + (0.03 × ramet size), $F_{2,22} = 26.20$, $p < 0.01$, $R^2 = 0.68$; male function MS =0.95 + (7.36 × genet size) – (0.02 × ramet size), $F_{2,15} = 78.21$, $p \leq 0.001$, $R^2 = 0.90$; male function RS = 1.11 + (8.75 × genet size) – (0.03 × ramet size), $F_{2,15} = 37.39$, $p < 0.001$, $R^2 = 0.81$; Appendix 7.1, table A7.1.6). Patterns were the same in the dioecious population, where the association between genet size and mating and reproductive success was positive for both sexes (multiple linear regression: female MS= 0.80 + (1.20 × genet size) + (0.003 × ramet size), $F_{2,13}$ = 12.19, p < 0.01, R^2 = 0.60; female RS = −0.41 + (3.80 × genet size) + (0.06 × ramet size), $F_{2,13}$ = 142.10, p < 0.001, R^2 = 0.95; male MS = −0.13 + (1.38 × genet size) + (0.06 × ramet size), $F_{2,6}$=6.67, p = 0.03, R^2 = 0.59; male RS = −0.05 + (1.15 × genet size) + (0.05 × ramet size), $F_{2,6}$ = 4.50, p = 0.06, R^2 = 0.47; 2.11 Appendix 7.1, table A7.1.6).

2.4.3 Promiscuity and the opportunity for sexual selection

Mating was highly promiscuous in both populations, particularly via male function. In the monoecious population, MLLs had nearly twice the mate diversity via their male function (M_{mm} = 0.52 ± 0.09 SE) than via their female function (M_{ff} = 0.27 ± 0.03 SE). The same was true in the dioecious population, where males had an average mate diversity of M_m = 0.40 (± 0.05 SE), compared to M_f = 0.22 (± 0.03 SE) for females. High levels of promiscuity were associated with substantial variance in mating and reproductive success in both populations.

In the monoecious population, the variance in RS was more than three times greater for male function (I_{mm} = 3.12 ± 0.03 SE) than for female function (I_{ff} = 0.93 ± 0.04 SE), yielding a greater opportunity for selection via male function (ΔI = 2.19; table 2.1). Similarly, the opportunity for sexual selection was more than seven times greater for male function (I_{smm} = 1.93 ± 0.03 SE) than for female function (I_{sff} = 0.26 ± 0.04 SE), leading to a positive value of ΔI_s (1.67; table 2.1). By contrast, in the dioecious population, the opportunity for selection was greater for females (I_f = 1.84 ± 0.09 SE) than for males (I_m = 0.57 ± 0.04 SE), yielding a negative value of ΔI (−1.27). However, the opportunity for sexual selection was greater in males (I_{sm} = 0.69 ± 0.04 SE) than in females (I_{sf} = 0.26 ± 0.09 SE), leading to a positive value of ΔI_s (0.43).

2.4.4 Bateman gradients

In the monoecious population, there was a significant, positive association between mating and reproductive success for male function (β_{mm} = 1.02 ± 0.35 SE; table 2.1, figure 2.2a; 7.1 Appendix: table A7.1.8). For female function, this association was weaker and non-significant (β_{ff} = 0.42 ± 0.26 SE). As a result, the sex difference in Bateman gradients was positive in the monoecious population ($\Delta\beta_{ss}$ = 0.60 ± 0.42 SE; 7.1 Appendix: table A7.1.9). Patterns of sexual selection were similar in the dioecious population, where there was a significant positive gradient for males (β_m = 0.95 ± 0.15 SE) and a weaker, non-significant gradient for females (β_f = 0.20 ± 0.25 SE; table 2.1, figure 2.2b; 7.1 Appendix: table A7.1.8). These sex-specific values of the Bateman gradient yielded a positive value of the sex difference in Bateman gradients in the dioecious population ($\Delta\beta_{ss}$ = 0.76 ± 0.30 SE; table 2.1). Alternative calculations of Bateman gradients that accounted for variation in plant size (multiple regression and bootstrapping) yielded qualitatively similar results, with evidence for stronger sexual selection in males (or male function) than in females (or female function; 7.1 Appendix:). By contrast, calculations of Bateman gradients that did not account for variation in plant size yielded larger values of β for female function than for male function (monoecious population: β_{ff} = 1.20 ± 0.30 SE, β_{mm} =1.17 ± 0.10 SE; dioecious population: β_f = 2.16 ± 0.42 SE, β_m = 0.88 ± 0.09 SE; 7.1 Appendix: table A7.1.3) leading to negative values of $\Delta\beta_{ss}$ in both populations (monoecious population: $\Delta\beta_{ss}$ = −0.03 ± 0.30 SE; dioecious population: $\Delta\beta_{ss}$ = −1.28 ± 0.45 SE; 7.1 Appendix: table A7.1.4).

2.5 Discussion

Our data reveal strong sexual selection operating on male function in natural populations of *S. latifolia*. Patterns of sexual selection were similar in the monoecious and dioecious populations studied here and might reflect the fact that—at the inflorescence level—plants in monoecious populations are temporally unisexual, limiting the kinds of interference that might otherwise restrict the magnitude of sexual selection in hermaphrodites. Our analysis indicated that sexual selection did not operate on female function in either population. However, total mating and reproductive success were size dependent, with similar patterns of size dependency for both female and male function in each population. On one hand, these patterns underscore the size dependency of instantaneous measures of fitness in modular organisms (Primack & Kand 1989). On the other, because size variation in natural populations is probably driven by non-heritable differences in plant age and local growing conditions (Geber & Griffen 2003), these data also highlight the insights that can be gained by accounting for variation in plant size when considering forms of selection that affect one phase of the life cycle. Below, we discuss our findings and interpretations in more detail and consider their implications for life-history evolution in *S. latifolia*.

In the two natural populations of *S. latifolia* studied here, sexual selection occurred via male but not female function. Similar patterns have been observed for dioecious *Mercurialis annua* (Tonnabel et al. 2019) and are consistent with the results of studies of selection on floral traits that might affect mating success via male function in plants (e.g. selection on anther dimorphism (Thompson 2001; Zhou et al. 2020) or petal size (Stanton & Preston 1988; Zhou et al. 2020). Indeed, a previous study involving mating arrays of *S. latifolia* found evidence for sexual selection on male function that operated via variation in floral display size (the number and size of open flowers (Dorken & Perry 2017)). In general, pollinators prefer to visit larger floral displays (Fritz & Nilsson 1996; Harder & Barrett 1996; Kudo & Harder 2005), but for members of the genus *Sagittaria*, which is characterized by plants with unisexual flowers, a positive association between display size and pollinator visitation appears to apply to male but not female sex functions (Glaettli & Barrett 2008) (and see Muenchow & Deladalle 1994)). For female function, the association between display size and pollinator visitation is weaker, and females in dioecious populations are not mate (pollen)-limited (Glaettli & Barrett 2008), indicating that any associations between display size and reproductive success are driven by resource—instead of mate-limitation. Moreover, for congeneric *Sagittaria trifolia*, greater pollinator visitation is associated with increased pollen removal from male flowers but independent of pollen receipt by female flowers (Huang et al. 2006). Together, these patterns of pollinator visitation provide a mechanistic explanation for the operation of sexual selection in *S. latifolia*, with variation in floral display size driving variation in mating success for males (and male function in monoecious populations) but not females (and female function in monoecious populations).

Using non-size-corrected data would have yielded steeper Bateman gradients for female than for male function in both populations and negative $\Delta\beta_{ss}$ values, a result that would have been consistent with sexual selection on female function. Indeed, our estimates of If, the magnitude of the opportunity for selection via female function, were comparable to those from a study reporting sexual selection on the female function of hermaphroditic *Passiflora incarnata* (Dai & Galloway 2013). However, in both this study and the study of *Passiflora*, size was a major predictor of total reproductive success via female function. For the *Passiflora* study, size variation was driven by the application of different levels of a nutrient fertilizer treatment. For *S. latifolia*, size variation is expected to emerge via differences in clone age (Holt et al. 2020) and local growing conditions (Dorken et al. 2002). Variation resulting from differences in age and local conditions are largely non-heritable (Samson & Werk 1986) and the need to correct for plant size when measuring selection on reproductive traits has been recognized in previous studies (Morgan & Schoen 1997).

Our estimates of Bateman gradients in monoecious and dioecious populations indicated that sexual selection operated similarly in both populations. The magnitude of sexual selection can depend on the extent to which sex roles are separated during mating (Arnold 1994; Anthes et al. 2015). When mating via female and male function occurs simultaneously, mating success for male and female sex functions may covary. As a result, any cross-sex effects that arise from that covariance weaken the sex-specific dependence of reproductive success on mating success, reducing the scope for sexual selection to operate on mating success via male roles without also affecting mating success via female roles (and vice versa). For example, the freshwater snail *Biomphalaria glabrata* typically engages in reciprocal mating via the alternation of sex roles during mating encounters, and the estimation of cross-sex effects indicates that selection on mating success is sexually mutualistic (mating benefits both sex roles; (Anthes et al. 2015)). As a result, reproductive success via male function depends not only on the mating success of snails via their male function, but also on their mating success via female function (Anthes et al. 2015). Conversely, for hermaphroditic organisms that deploy their sex functions at different times (e.g. individuals are temporally unisexual), mating via male and female roles involves separate (and at least partly independent) mating events, reducing the magnitude of cross-sex effects (Pélissié et al. 2012; Anthes et al. 2015). This is the case for *S. latifolia*—inflorescences are functionally unisexual when they are visited by pollinators. Thus, there should be reduced the scope for covariance in mating success between sex roles, something that could be confirmed in a study focused on estimating cross-sex effects in monoecious *S. latifolia*.

The magnitude of sexual selection is influenced by the amount of time each sex is available for mating, in part via effects on operational sex ratios (Kokko et al. 2012). All else being equal, selection on the frequency of mating events is stronger on the sex that spends less time out of the mating pool (i.e. the sex with the shorter 'dry' time (Kokko et al. 2012)). For dioecious *S. latifolia*, males produce more flowers and open those flowers more gradually than females (Perry & Dorken 2011), yielding strongly male-biased operational (floral) sex ratios during peak flowering (Perry & Dorken 2011, this study) and a shorter 'dry time' for males compared to females. Short dry times for males should be associated with selection for high mating frequency (i.e. a positive Bateman gradient (Kokko et al. 2012)) and favour plants with greater investment in traits that promote mating success via male function (Kokko et al. 2012). Flower size is strongly sexually dimorphic in dioecious populations of *S. latifolia*, and there is a positive association between pollinator visitation and the degree of flower size dimorphism between females and males (Glaettli & Barrett. 2008). Total floral display size (the number and size of flowers) has previously been shown to be subject to sexual selection in mating arrays comprised of male and hermaphrodite plants (Dorken & Perry 2017). Our study of a natural population of dioecious *S. latifolia* complements these previous findings by revealing a positive Bateman gradient for males.

Floral sex ratios were less strongly biased in the monoecious population studied here—they were nearly 50:50—but does this mean that sexual selection should necessarily be a weaker force in monoecious than in dioecious populations of *S. latifolia* where floral sex ratios were male-biased? As long as male fitness is solely determined by mating rate, the answer should be 'no' (Kokko et al. 2012). Two features of *S. latifolia* should make mating rate the primary driver of fitness via male function, in spite of unbiased floral sex ratios. First, and most obviously, the positive Bateman gradient in the monoecious population studied here indicates that mating success drives reproductive fitness through male function. Second, life-history trade-offs arising from investment in male function appear to be weaker than those for the investment in female function (Van Drunen & Dorken 2012). In particular, whether there are any negative fitness consequences for the production of male flowers may be context-dependent (Wright & Dorken 2014). Investment in female function by *S. latifolia* plants has strong and direct consequences for plant (clonal) growth and survival (Dorken & Barrett 2004). Although there appear to be some costs associated with the production of male flowers (via reductions in total plant nitrogen content (Van Drunen & Dorken 2012)), in natural populations, these costs do not appear to directly affect measures of plant growth (including total size (Dorken & Barrett 2004)), perhaps because the costs associated with male investment can be recovered more easily than those associated with female investment, at least in some environments (Wright & Dorken 2014). Together, a positive Bateman gradient and weaker life-history trade-offs for male function should favour selection on traits that increase male mating success.

Measurements of the magnitude of sexual selection in natural populations of plants are rare—certainly in comparison with the numbers of studies of animal populations. Here we found a positive association between mating and reproductive success for males in a dioecious population and for the male function of hermaphrodites in a monoecious population of *S. latifolia*. By contrast, and after accounting for the effects of plant size on fecundity, mating success was not associated with reproductive success for female function. Accordingly, sexual selection appeared to operate on male but not female function in the two populations studied here. Should similar results also apply to other plants? In general, the answer seems to be 'yes'

(Arnold 1994; Willson 1994; Skogsmyr & Lankinen 2002; Moore & Pannell 2011), but whether that answer applies to any particular plant species depends on the processes generating variation in mating success. For *S. latifolia*, there is not only considerable variance in mating success; there is also a positive, linear association between investment in male function and siring success (Perry & Dorken 2011; Dorken & Perry 2017). However, this kind of association is not thought to apply to most plants, which are usually considered to be subject to a decelerating association between investment in male function and reproductive fitness (i.e. there is a saturating male gain curve (Skogsmyr & Lankinen 2002; Zhang 2006)). Specifically, increased investment in pollen production is not expected to yield commensurate increases to mating or reproductive success, in part because plant immobility and localized pollinator foraging should generally result in diminishing returns on investment in male function via local mate competition (Charnov 1979). Indeed, local mate competition is expected to have strong effects on the strength of sexual selection (Lehtonen 2022; Henshaw et al. 2022). Moreover, many plants are thought to be pollen limited (Knight et al. 2005), which could lead to sexual selection on female function via positive associations between mating and reproductive success (Dai & Galloway 2013). Thus, although sexual selection can operate in plant populations, many more studies from natural populations will be required before any general statements about the importance of sexual selection in plants can be made.

2.6 Acknowledgements

The authors thank S. Rousselle for field assistance, L. Bond, J. Houpt, K. Minor, C. Steinecke for assistance with lab work, S. Stephens and J. Freeland for advice, and two anonymous reviewers for helpful suggestions.

2.7 Tables

Table 2.1: Sexual selection metrics for female-function MLLs (*n* = 25) and male function MLLS (*n* = 18) in a monoecious population and for female MLLs (*n* = 16) and male MLLs (*n* = 9) in a dioecious population of *Sagittaria latifolia*.

	I (SE)	I_s (SE)	β (SE)
Monoecious			
Female function	0.93 (0.04)	0.26 (0.04)	0.42 (0.26)
Male function	3.12 (0.03)	1.93 (0.03)	1.02 (0.35)
Δ (male – female)	2.19	1.67	0.60 (0.42)
Dioecious			
Female	1.84 (0.09)	0.26 (0.09)	0.20 (0.25)
Male	0.57 (0.04)	0.69 (0.04)	0.95 (0.15)
Δ (male – female)	-1.27	0.43	0.75 (0.30)

2.8 Figures

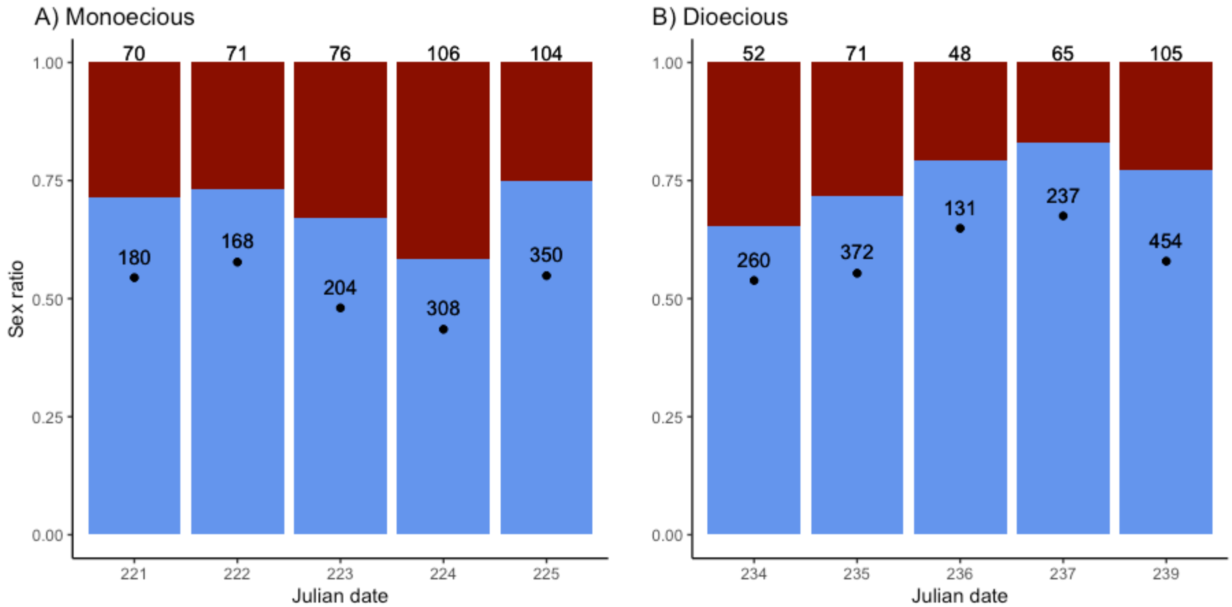

Figure 2.1. Daily ramet and floral sex ratios in a monoecious and a dioecious population of *Sagittaria latifolia*. Daily ramet sex ratios are indicated by height of the segments in each bar (lower segment, male; upper segment, female). (a) In the monoecious population, ramet sex ratios reflect the proportion of ramets with open male or open female flowers. (b) In the dioecious population, genet sex ratios reflect the proportion of male versus female ramets flowering on that day. Floral sex ratios (number of open male flowers divided by the total number of open flowers per day) are indicated by the location of the black circle within each bar. Numbers above each bar indicate the number of ramets with open flowers per day and numbers in the bars represent the number of open flowers per day.

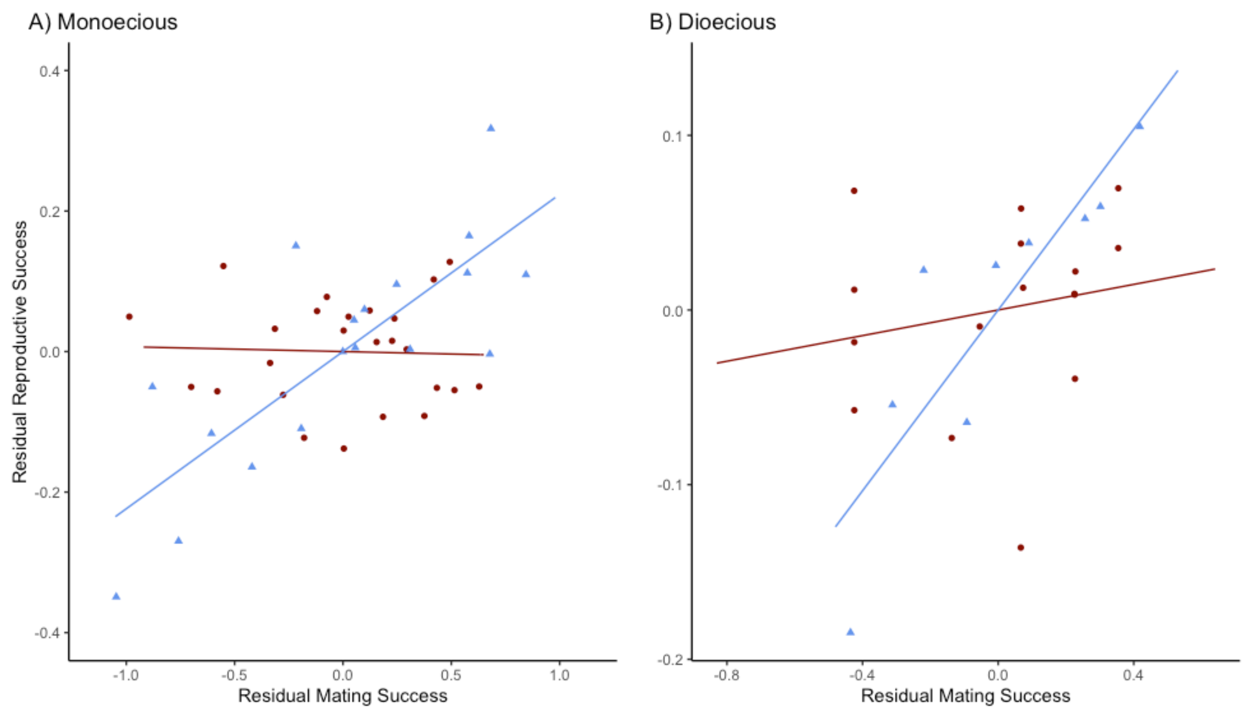

Figure 2.2. Bateman gradients estimated using residual regression in a monoecious and a dioecious population of *Sagittaria latifolia*. Residuals were calculated from the multiple regression of the number of ramets and the average size of each ramet (measured as average mid-vein length) per MLL onto reproductive success and mating success per MLL via its female or male function. Fitted Bateman gradients are shown for female (solid red lines) and male (dashed blue lines) function in each population. Dots represent the residual reproductive success and residual mating success for each MLL per population (using circles for female function and triangles for male function). (a) In the monoecious population, these values represent the residual reproductive and mating success of MLLs via their female and their male sex functions. (b) In the dioecious population, these values are the residual reproductive and mating success of female or male MLLs.

3.CHAPTER3. MALE REPRODUCTIVE SUCCESS IS NOT STRONGLY AFFECTED BY PHENOLOGICAL CHANGES IN MATE AVAILABILITY IN MONOECIOUS *SAGITTARIA LATIFOLIA*

A version of this chapter has been published:

Kwok, A., Stephens, S. and Dorken, M.E. 2023. Male reproductive success is not strongly affected by phenological changes in mate availability in monoecious *Sagittaria latifolia*. Royal Society Open Science 10: 231117

3.1 Abstract

Many plants express their female and male sex roles at different times (dichogamy), with important consequences for mating. Dichogamy can yield mate limitation via biased floral sex ratios, particularly at the beginning and end of the flowering season when many plants simultaneously function as the same sex. This form of mate limitation should be reduced if plants adjust their allocations to female versus male sex functions in a manner that tracks seasonal variability in mating opportunities. For example, under protogyny (i.e. dichogamy with female function expressed first) plants with male-biased sex expression should have enhanced mating opportunities early in the flowering season as other plants begin to flower (in female sex phase). We quantified seasonal changes in sex allocation, patterns of mate availability and realized siring success in a population of protogynous *Sagittaria latifolia*. Our results were consistent with previous findings that seasonal changes in sex allocation should compensate for lost mating opportunities under the temporally variable mating environments caused by dichogamy. However, patterns of siring success in the population were inconsistent with this interpretation. We suggest that realized siring success might depend more strongly on spatial than on temporal aspects of mate availability.

Keywords: dichogamy, flowering phenology, mating environment hypothesis, monoecy, sex allocation, protogyny

3.2 Introduction

The timing of life-history events has important consequences for organismal fitness via effects on reproduction, survival and growth. For plants, flowering time affects fitness via effects on mate availability (Brunet & Charlesworth 1995). However, there are important constraints on the timing of flowering (Kochmer &Handel 1986; Davies et al. 2013), and in particular the timing that floral organs associated with female function (i.e. the gynoecium) versus those associated with male function (the androecium) are actively engaged in reproduction. On one hand, and all else being equal, mass flowering should maximize mate availability. On the other hand, because most plants are hermaphroditic, mass flowering can have negative consequences for mate acquisition from the repeated transfer of pollen grains among flowers of the same plant (i.e. geitonogamy), deleteriously affecting reproductive fitness via the female and male functions of plants (Lloyd 1992). For example, in mass flowering *Rhododendron ferrugineum* outcrossing was determined by the local availability of mates, with high selfing rates when pollinators transferred self pollen within patches of low mate availability (Delmas et al. 2015).

Many plants can avoid the negative consequences of self pollen transfer between flowers via the temporal segregation of their female and male function (i.e. dichogamy). Dichogamy separates the timing of pollen export and pollen receipt, thereby reducing self-interference within flowers (e.g. reduced pollen export and/or increased self-fertilization via the presentation of pollen and receptive stigmas in the same flower at the same time; Lloyd & Webb 1986) and reducing geitonogamous pollen transfer (e.g. by reducing the frequency that pollinators move between flowers in complementary sex phases Harder et al. 2000). This reduction in geitonogamous pollen transfer is expected whether dichogamy is synchronous (no temporal overlap in the expression of female and male sex functions within plants) or asynchronous (some temporal overlap in the expression of sex functions) (Harder et al. 2000). The reduction in self interference from dichogamy can improve the efficiency of pollen export with fitness benefits to plants via their male function (i.e. as pollen donors; Routley & Husband 2003). There are two main classes of dichogamy: protandry (male function is expressed before female function) and protogyny (female function is expressed before male function). Dichogamy is a widespread feature of outcrossing angiosperms and appears to occur in most species (Bertin & Newman 1993).

The positive effects of dichogamy on mating are counterbalanced by skewed floral sex ratios that limit mate availability (Sargent et al. 2006). Under protogyny, the first plants to flower may be pollen limited if most other plants are also expressing the female sex phase. Similarly, the last plants to flower are also expected to be mate limited—in this case by the frequency of plants expressing male sex phase. However, these negative effects of mate limitation under dichogamy should be mitigated if plants adjust their sex allocations across the flowering period such that (gametophytic) sex ratios are re-balanced (Brunet & Charlesworth 1995). For example, plants in protogynous populations with increased allocations to male function early in the flowering period (and decreased allocations to male function towards the end of the flowering period) have greater mating opportunities than plants that do not adjust their sex allocations (figure 3.1). These enhanced mating opportunities arise because biased floral sex ratios under dichogamy favour plants that express the minority sex. Accordingly, adjustments of sex allocation towards the minority sex at any time in the season are expected to enhance mating success via a plant's female and/or male sex functions (Brunet & Charlesworth 1995).

That plants might adapt to temporal changes in floral sex ratios via evolutionary adjustments to floral sex allocations in early- versus late-developing flowers is referred to as the mating environment hypothesis (Huang et al. 2004). Empirical support for the mating environment hypothesis exists in the form of evidence that the female function of plants (e.g. fruit set) in protandrous populations (Brookes & Jesson 2010), but whether benefits also accrue to male function via enhanced siring has not been demonstrated empirically. There is therefore a gap in the testing of the mating environment hypothesis, which is built around the idea that adjustments to floral sex allocations over time in dichogamous plants yield enhanced pollen transfer probabilities (Brunet & Charlesworth 1995).

Here, we test the hypothesis that variation in patterns of sex allocation over the duration of the flowering season affect reproductive success via male function (i.e. siring success) in protogynous *Sagittaria latifolia*. Flowers of *S. latifolia* are unisexual and in monoecious populations inflorescences produce a combination of female and male flowers (Dorken et al. 2002). Flowers open for a single day and inflorescences are synchronously protogynous—female flowers occupying basal positions of the inflorescence open first and after female phase is over male flowers begin to open. As a result, on a given day, inflorescences are functionally unisexual. Because of monoecy, adjustments to sex allocation occur at the inflorescence level—a given number of male flowers, plants can increase or decrease their sex allocation via adjustments to the production of female flowers (or vice versa Sarkissian et al. 2001, Dorken & Barrett 2003). If protogyny affects the availability of female flowers for outcross-pollen transfer, we expected (1) that opportunities for siring will change over the flowering season. In particular, these opportunities should decline towards the end of the season as fewer new inflorescences begin to flower (and therefore no new female flowers become available). We further expected (2) that plants adjust their sex allocations to reflect these changes in the availability of female flowers, with greater allocations to male flower production early in the season when there are many open female flowers. Finally, we expected (3) that the joint effects of changes in siring opportunities over the season and changes to floral sex allocations affected patterns of siring. In particular, we expected that pollen-transfer probabilities—estimated from temporal changes in the availability of mating partners and competition for mating events (Brunet & Charlesworth 1995)—were positively associated with siring success. To evaluate these expectations, we tracked flowering phenology and inflorescence-level sex allocations over an entire flowering season to calculate pollen-transfer probabilities and conducted parentage analysis to estimate siring success via male function.

3.3 Methods

3.3.1 Study species

Sagittaria latifolia Willd. is an emergent clonal aquatic plant, commonly found in wetland habitats across North America. Daughter ramets are produced at the ends of stolons during the growing season and can be vegetative or reproductive (Dorken & Barrett 2003). Corms are produced at the end of stolons and are the perennating structures (Van Drunen & Dorken 2012). Across the species range, populations of *S. latifolia* can be either dioecious or monoecious (Wooten 1971). In monoecious populations of *S. latifolia*, flowers are produced on racemes with three flowers at each node. Female flowers occupy basal nodes and male flowers occupy distal nodes. Flowers open basipetally, both female and male flowers remain open for a single day, and nodes with male flowers mature later than nodes with female flowers, resulting in inflorescences that are synchronously protogynous (Dorken & Barrett 2003). Female flowers tend to open simultaneously, while the opening of male flowers is staggered (Delesalle & Muenchow 1992). Male flowers tend to be larger, while female floral displays are larger (Glaettli & Barrett 2008). Flowering in *S. latifolia* in southern Ontario occurs from late June through to mid-September and flowers are pollinated by a variety of pollinators, including solitary and social bees, wasps, flies, beetles and lepidopterans, that forage for both pollen and nectar (Glaettli & Barrett 2008). Larger flower size and floral display size have been shown to positively affect pollinator visitation (Glaettli & Barrett 2008). Plants are fully self-compatible (Dorken et al. 2002; Dorken & Barrett 2004), but temporal separation of female and male flowers makes self-fertilization within a ramet unlikely (Dorken et al. 2002, Stephens et al. 2020). However, because of clonality, selfing between flowering ramets of the same genet can occur, and selfing rates in monoecious populations can vary substantially (range in selfing rates across six monoecious populations: 0.13–0.63 (Dorken et al. 2002)).

3.3.2. Study site and phenological sampling

To examine flowering phenology and sex allocation in *S. latifolia*, a monoecious population in Peterborough, ON (44° 200 N, 78° 180 W) was visited daily for the duration of the flowering season (between 18 July 2013 and 28 August 2013). The study site was in a shallow part of Thompson Creek in Meadowvale Park, Peterborough Ontario, covering approximately 25 × 60 m, and separated from other populations of *S. latifolia* by at least 100 m. Flowering ramets were distributed throughout the site, with some ramets grouped in patches and others more spatially isolated. The phenological data were collected from the same population as in Stephens et al. (2020) and Holt et al. (2020). Each day, newly-opened inflorescences were tagged, and flowering dates were recorded for a total of 943 flowering ramets. The numbers of female and male flowers open on each ramet were also recorded. A sample of leaf tissue from the youngest leaf of each flowering ramet was collected in labelled coin envelopes and dried using Sorbead silica beads (eCompressedair, OK, USA). Once dry, the leaf tissue was stored at −20°C . Developing fruits in the population were monitored and collected as they matured. A total of 757 fruits were collected from 220 ramets (average = 2.7 fruits collected per ramet). Our goal was to sample one seed per fruit for paternity analysis, however, not all fruits were fully mature at the time of collection, and we were therefore unable to extract viable DNA from all samples; in the end 358 seeds from 154 ramets were used for genetic analyses.

3.3.3 DNA extraction and SSR genotyping

We assigned ramets to genets and sires to seeds so that variation in realized siring success could be assessed at the whole-plant (genet) level. These procedures are outlined in detail in Stephens et al. (2020). Briefly, genomic DNA extraction for genet assignment involved approximately 5 mg of dried leaf tissue, homogenized using a MM 300 Retsch mixer mill (Haan, Germany). DNA was extracted using the E.Z.N.A. Plant Extraction kit (Omega Bio-Tek Inc, GA, USA) and eluted to a final volume of 100 µl. To extract seed DNA, seeds were soaked in distilled water for 24 h, after which they were separated from the surrounding fruit tissues and the seed coat. Seed DNA was extracted using the QuickExtract Plant Seed DNA Extraction Solution (Lucigen, Madison WI) following the manufacturer's protocol, and eluted to a final volume of 50 µl.

Samples were genotyped at seven microsatellite loci (SL06, SL09, SL21, SL30, SL74, SL75 and SL88; Yakimowski et al. 2009). Fluorescent labels FAM (SL06, SL27, SL30, SL74 and SL75) and HEX (SL21, SL09 and SL88) were applied to the forward primers. Polymerase Chain Reaction (PCR) amplifications were performed as either singleplexes (SL06, SL27 and SL75) or as multiplexes (SL09/SL74/SL88; SL21/SL30). The singleplexes were performed in a total reaction volume of 10 µl including approximately 10 ng of DNA, 1 × PCR buffer, 2 mM MgCL2, 0.15 mM of dNTPs, 0.3 mg ml−1 BSA, 0.2 µM of each forward and reverse primer, 0.5 U/reaction of Taq DNA polymerase (Invitrogen, Waltham, MA, USA) and additional ddH2O.

The singleplex PCR conditions consisted of initial denaturation step of 94°C for 3 min; 30 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 45 s; and a final extension of 72°C for 45 min. Multiplex reactions were conducted in total reaction volumes of 10 µl. Each 10 ul SL21/30 reaction included approximately 10 mg of DNA, 1 × PCR buffer, 1.3 mM of MgCl2, 0.3 mM of DNTPs, 1 mg ml−1 BSA, 0.1 µM each of SL21 forward and reverse primers, 0.19 µM each of SL30 forward and reverse primers, 0.5 U GoTaqFlexi DNA polymerase (Promega, Madison, WI, USA) and additional ddH2O. For the SL09/74/88 multiplex, the reaction volume of 10 µl included 1 × PCR buffer, 1.6 mM MgCl2, 0.3 mM DNTPs, 1 mg ml−1 BSA, 0.2 µM each of SL88 forward and reverse primers, 0.1 µM each of SL09 forward and reverse primers, 0.16 µM each of SL74 forward and reverse primers, and 0.5 U GoTaqFlexi DNA polymerase (Promega, Madison, WI, USA) and additional ddH2O. The PCR conditions for the multiplex reactions were as follows: initial denaturation at 94°C for 2 min; 35 cycles of 94°C for 45 sec, annealing temperature of 57°C for Sl21/ 30; 56.5°C for Sl09/74/88 for 45 sec, 68°C for 45 sec; and a final extension of 68°C for 8 min. Each PCR included a negative control using ddH2O and 10% of samples were re-amplified to ensure consistency. Amplifications were carried out on a MasterCyclerepGradient thermocycler (Eppendorf, Hamburg, Germany). PCR products were screened using gel electrophoresis in a 1.5% agarose gel and 10% SYBRGreen (Sigma Aldrich, St. Louis, MO, USA) at 90 V; fluorescently labelled products were scored using an automated sequencer (Applied Biosystems 3730 DNA analyzer) with a ROX size standard (Applied Biosystems, Waltham, MA, USA) and analysed using GeneMarker (SoftGenetics, State College, PA, USA).

3.3.4 Genet and paternity assignment

Using the SSR data we grouped ramets into multi-locus genotypes and then assigned them to clones (genets) using the RClone package (v. 1.0.1.1, Bailleul et al. 2016) in R (v. 3.4.4; R Core Team 2023) following procedures outlined in Holt et al. (2020) and Stephens et al. (2020). Ramets with multi-locus genotypes (MLGs) that differed by up to one allelic difference at any locus were grouped into multi-locus lineages (MLLs) using the MLG_list and MLL_generator2 functions from the RClone package (for additional details on MLL assignment, see Holt et al. 2020). This procedure yielded 210 MLGs, and 169 unique MLLs (for more details, see Stephens et al. 2020). Genets (MLL) comprised between 1 and 35 ramets. The majority of genets (40%) were made up of single ramets, with a few large genets. Assignment of paternity to one or more of these genets was conducted using a combination of Cervus (v. 3.0.7; Marshall et al. 1998) and COLONY2 (v. 2.0.6.5; Wang 2004; Jones & Wang 2010; Wang 2018). Paternity analysis was conducted using MLG assignments and these MLG-level assignments were then mapped to the corresponding MLL. To conduct the analysis, we used the full-likelihood method for determining the most likely full- and half-sib families using COLONY2. Runs in COLONY2 were made with medium-length runs and with high-likelihood precision, incorporating per locus stochastic error rates that were determined by comparing expected and observed heterozygosity in parental genotypes per locus using Cervus. Ploidy was set as diploid and the mating system was set as polygamous for both males and females, without inbreeding (parental inbreeding coefficients in monoecious populations are low, ranging between 0 - 0.2 from a sample of six populations; Dorken et al. 2002). Because we knew the identity of the maternal parents for each seed, we specified maternal sibships for the analysis. Of the 358 seeds that were genotyped 312 were assigned paternity to one or more MLGs. The seeds had siring probabilities ranging from 0.01 to 0.94 (mean = 0.25 ± 0.22 SD), most seeds (72%) were assigned to sires with 95% or greater confidence, with 24 seeds having siring probabilities with less than 10%. A total of 52 MLLs were assigned as sires to one or more seeds.

3.3.5 Male reproductive success

The MLG assigned as the most probable paternal parent of a seed was treated as the sire and for any given seed the probability of paternity π was used to estimate total siring per MLL. These probabilities were summed across all the seeds assigned to that MLL as the paternal parent such that total reproductive success via male function *RSm* per MLL *i* was estimated as:

$$RS_i^m = \sum_j \pi_{ijk}$$

where π is the probability that each genotyped seed *j* was sired by MLL *i* on pollination date *k*. Self-fertilized seeds were excluded from calculations of $RS^m{}_i$.

3.3.6 Mating opportunities

The calculation of pollen transfer probabilities (Brunet & Charlesworth 1995) yields a numerical description of opportunities for plants to obtain mates via their male function (see also Brunet 1996). Our approach involves a minor modification of the one taken by Brunet and Charlesworth (1995). In their study the focus was on the probability of pollen transfer for flowers occupying different positions in a sequence (and see Brookes & Jesson 2010). Here, the focus is on the probability of pollen transfer for ramets with male flowers that are open on a given day in the flowering period. We refer to the metric that describes opportunities for pollen transfer as the index of pollen transfer opportunity (the metric is not bound by 0 and 1). We estimated total relative pollen transfer opportunities per genet from matrices of female and male flower production per day for the ramets comprising a genet. We first calculated the daily fraction of open male flowers per ramet as:

$$f_j^m = \frac{N_j^m}{\sum_{l \neq j}^{n} N_l^m},$$

where $N^m{}_j$ corresponds with the total number of open male flowers on a ramet j and $N^m{}_1$ corresponds with the number of open male flowers on the n other ramets on that day. To estimate the relative pollen transfer opportunity per ramet, we multiplied the fraction f_m by the total number of female flowers ($N^f{}_i$) open on the same day on all non-self ramets i (i.e. on all ramets other than those that were part of genet g):

$$K_{ij} = f_{j \subseteq g}^m \times \sum_{i \notin g}^{n} N_i^f.$$

Daily values of K_{ij} were summed across all the j flowering ramets from the same genet g to estimate the per genet pollen transfer opportunity as: $K_g = \sum_{j \in g} K_{ij}$.

3.3.7 Seasonality of sex allocation

Because *S. latifolia* is monoecious and inflorescences include a combination of female and male flowers, the smallest unit of organization at which sex allocation is expressed is the inflorescence. To examine whether patterns of allocation to male and female flowers varied over the flowering season, we calculated the proportion of male flowers per inflorescence (referred to here as the inflorescence sex ratio) (n = 298 inflorescences) and used this as the measure of sex allocation. We used a generalized additive mixed model (GAMM), as implemented by the gamm4 function from the gamm4 package (v 0.2-6 Wood & Scheipl 2020) in R (v. 4.3.1; R Core Team 2023). GAMM was chosen because we had no a priori expectation regarding linear versus nonlinear changes in sex allocation over time. The relaxed assumptions of linearity in GAMMs involve the calculation of nonlinear smoothing splines, enabling the estimation of linear or nonlinear associations between response and predictor variables (Zuur et al. 2009). A significant parameter estimate for the smoothing function indicates that a nonlinear model provides a better fit. In the GAMM, inflorescence sex ratio (a composite variable of the number of female and male flowers) was the response variable and the date of first flowering per inflorescence was the predictor variable. The model was specified using a binomial error distribution and a logit link function. Because inflorescences and MLLs were sampled across multiple days, inflorescence and MLL were specified as random effects, with inflorescence nested within MLL. The default base function of thin plate regression spline was used to estimate the degree of smoothing because of the small number of predictor variables.

3.3.8 Seasonal changes in mating opportunities.

To evaluate seasonal changes in mating opportunities via the male function, we again used a GAMM. For this model, daily pollen transfer opportunity was the response variable and date was the independent variable and treated as a fixed effect. Because pollen transfer opportunities were calculated at the genet level (n = 123 genets) and repeated sampling of MLLs occurred over multiple dates, MLL was included as a random effect. The GAMM was specified using a Gaussian error distribution with an identity link function and calculated using the gamm4 function from the gamm4 package in R. The gam.check function from the mgcv package (Wood 2004) was used to ensure that this model and the one described in the previous paragraph (changes in infloresence-level sex ratios over time) were not over-fitted. For both GAMM models we examined whether the inclusion of a smoothing function improved model fit by comparing GAMM results with those from linear and generalized linear mixed-effects models with the same dependent and independent variables using the lmer functions from the lmerTest package (v 3.1-3 Kuznetsova et al. 2017) and glmer functions from the lme4 package (v 1.1-33 Bates et al. 2015) in R and then compared AIC values between models (Quinn & Keough 2002).

In both cases, the smoothing term was associated with a significant parameter estimate and the GAMMs were assigned lower AIC values than the linear models. Accordingly, here we report the GAMM results and linear model results are reported in the Appendix (7.2 Appendix: tables A7.2.1. Sex allocation GLME and A7.2.2. Pollen transfer opportunity LME).

3.3.9 Seasonal changes in mating opportunities and siring success

To evaluate the prediction that siring success was positively associated with pollen transfer opportunity, we used a linear mixed-effects (LME) model using the lmer function, with siring success (n = 39 genets) as the dependent variable and pollen transfer opportunity and siring date as independent variables, each treated as fixed effects. The siring success data were right skewed with many near-zero values. Accordingly, siring success was log10-transformed. As with previous analyses, MLL was treated as a random effect. We used step-wise model selection to determine the effect of siring date and pollen transfer opportunity as separate predictors and together (Quinn & Keough 2002).

3.3 Results

3.3.1 Flowering phenology

Flowering started on 18 July 2013 (Julian date = 199) and ended on 28 August 2013 (day 240). Only eight inflorescences had open flowers over the first 12 days of the season. Starting August 3rd (day 215) a minimum of two different genets flowered each day with at least one genet in male phase and another in female phase until August 28th (day 239; i.e. mating events were continually possible over this period). Peak flowering, the day with the greatest number of open inflorescences, occurred on August 18th (day 230; figure 3.2 and see 7.2 Appendix: figure A7.2.1 Phenogram of sex allocation). Peak male phase (254 male flowers on 107 inflorescences from 67 genets) occurred on August 18th (day 230), one day after peak female phase on August 17th (day 229; 117 female flowers on 36 inflorescences from 25 genets).

3.3.2 Sex allocation

After August 3rd (day 215; i.e. during the period of continuous flowering), inflorescence-level sex allocations tended to be male-biased (figure 3.3; 7.2 Appendix: figure A7.2.2 Inflorescence sex allocation for full flowering season). By contrast, after peak flowering (August 18th, day 230), inflorescences tended to be female-biased. This change in inflorescence-level sex allocations was associated with a significant nonlinear association between date and floral sex ratios (table 3.1 model A). The smoothing term of the nonlinear model was significant and the GAMM provided a better fit compared to the GLME model (7.2 Appendix: table A7.2.1 Sex allocation GLME).

3.3.3 Pollen transfer opportunity

In the first ten days of the flowering season there were so few flowering plants that pollen transfer opportunities were at or close to zero (7.2 Appendix: figure A7.2.3 Pollen transfer opportunity). After July 28th (day 219) pollen transfer opportunity values began to increase, with male-phase plants having the greatest opportunities for siring between Aug. 7th–18th (day 220 to 230; figure 3.4). After August 18th, pollen transfer opportunities began to decrease, indicating declining siring opportunities towards the end of the season. These changes in pollen transfer opportunity over time were associated with a significant smoothing term for flowering date in the GAMM, indicating a nonlinear association between pollen transfer opportunity and date (table 3.1 model B; cf. 7.2 Appendix: table A7.2.2. Pollen transfer opportunity LME).

3.3.4 Siring success

Patterns of siring success did not vary over time (figure 3.5a) and the association between siring success and date was not significant ($F_{1,96.13} = 1.03 \times 10^{-2}$, $p = 0.92$, table 3.2 model A; figure 3.5a). There was also no relationship between siring success and pollen transfer opportunities ($F_{1,100.03} = 1.41$, $p = 0.24$; table 3.2 model B; figure 3.5b), contrary to our prediction that pollen transfer opportunities reflected realized opportunities for obtaining mates as a male parent.

3.4 Discussion

Changes in inflorescence-level sex allocations over the course of the flowering season coincided with changes in mating opportunities via male function, but not realized siring success in the population of studied here. Early and mid-season inflorescences were male-biased, but inflorescences became increasingly female-biased towards the end of the season. The timing of these changes in sex allocation in the population occurred in the manner expected under the mating environment hypothesis: when plants began to flower at the start of the season (in female phase), plants tended to have male-biased allocations. Similarly, towards the end of the season as fewer new plants began to flower, plants became increasingly female-biased in their allocations. These changes in sex allocation should have affected mating opportunities through male versus female function: siring opportunities (measured as pollen transfer opportunity) were highest during peak flowering, coinciding with the period over which allocations were most strongly male biased. In so far as we have demonstrated that patterns of sex allocation change in a manner that should increase siring opportunities, our data are consistent with the mating environment hypothesis and provide a similar degree of support as previous tests of the hypothesis (Brunet &

Charlesworth 1995; Huang et al. 2004; Brookes & Jesson 2010). However, pollen transfer opportunities were not clearly associated with realized patterns of siring success, and so this study fails to provide direct support for the mating environment hypothesis. Processes not accounted for in this study might have had stronger effects on variation in siring success than seasonal changes in inflorescence-level sex allocations, including (1) sexual selection (Kwok & Dorken 2022) and (2) spatially restricted pollen dispersal (Stephens et al. 2020). (3) Patterns of size-dependent sex allocation in *S. latifolia* also appear to be confounded with expectations from the mating environment hypothesis. Below we discuss how these alternative processes might have affected patterns of mating, obscuring the expected association between pollen transfer opportunities and siring success.

3.4.1. Sexual selection

The calculation of pollen transfer opportunities assumes that the transfer of pollen to female flowers is determined by a plant's production of male flowers. However, this assumption is violated if opportunities for pollen transfer are unequal. At least two processes can generate such unequal patterns of pollen movement. The first of these is sexual selection, defined as non-random variation in mating success (Kokko & Jennions 2008) and has been shown to operate in the population studied here (Kwok & Dorken 2022). Previous studies have implicated variation in floral-display size as a potential mechanism underlying sexual selection in *S. latifolia* Dorken & Perry 2017). For other plants, the contributions of individual flowers to fitness can decline on a per-flower basis, even if total siring is greater for larger compared to smaller inflorescences (Karon & Mitchell 2012). However, smaller per-flower contributions to siring success on larger inflorescences may be caused by the transfer of self-pollen within inflorescences (Dorken & Perry 2017)—something that is not possible under synchronous protogyny in *S. latifolia*. Indeed, there appears to be a direct association between flower production and siring success (Perry & Dorken 2011). Perhaps as a result of this linear association between male flower production and siring success, individuals with large male floral displays might have sired a disproportionate number of seeds in our study, regardless of seasonal changes in mate availability.

3.4.2 Spatially restricted pollen movement

The second process generating non-random mating is spatially restricted pollen dispersal. Realized patterns of pollen transfer are determined not only by the proportions of flowers in female versus male phase but also by the distances between mating partners. Pollination tends to occur between near neighbours (Lvin & Kerster 1974; Fenster 1991; Karron et al. 1995) and to decline with distance (Carvalheiro et al. 2010). Indeed, distances between mates combined with overlap in flowering time determine patterns of realized mating (e.g. *Echinacea angustifolia* (Ison &Wagenius 2014)). However, the calculation of pollen transfer opportunities does not incorporate these spatial effects and might therefore miss an important aspect of mating in plants. Indeed, a previous study of *S. latifolia* found that realized patterns of pollen dispersal follows a negative exponential distribution, with mating subject to some degree of spatial restriction (Stephens et al. 2020). Studying the joint effects of phenology and spatial distributions may provide greater insight into realized mating opportunities in plants.

3.4.3 Size-dependent sex allocation

Sex allocation in plants can be strongly size-dependent and a variety of adaptive processes underlying patterns of size-dependent sex allocation have been proposed (Klinkhamer et al. 1997). These mechanisms include changes in the expression of reproductive trade-offs between female and male function for plants of different sizes (Lloyd & Bawa 1984; Mazer & Dawson 2001); and changes in the strength of local mate competition and/or local resource competition (Klinkhamer et al. 1997; Lloyd & Bawa 1984) as plants increase in size. For monoecious populations of *S. latifolia*, larger ramets tend to produce more female flowers than smaller ramets (Sarkission et al. 2001). However, the production of male flowers tends to be independent of ramet size, resulting in female-biased allocations for larger ramets (Sarkissian et al. 2001). This pattern of size-dependent sex allocation in *S. latifolia* has been interpreted as a mechanism to prevent the over-production of pollen in large shoots that might otherwise result in increased geitonogamous selfing (Sarkissian et al. 2001). Although we cannot rule out the operation of these processes in the population studied here, patterns of size-dependent sex allocation in *S. latifolia* are also consistent with expectations under the mating environment hypothesis. In particular, synchronous protogyny should favour male-biased allocations early in the flowering season (when ramets are small) and female-biased allocations later in the season (as ramets increase in size). Indeed, because ramets increase in size over the flowering season, size may be a reliable cue for adaptive adjustments in sex allocation under dichogamy.

3.5 Conclusion

Dichogamy—and synchronous dichogamy in particular—has the effect of limiting the availability of mating partners. Synchronized protogyny results in functionally unisexual inflorescences and, all else being equal, only half of the other shoots in the population are potential mating partners at any moment in time. These negative consequences of dichogamy are well known and might be more than compensated by the avoidance of self-fertilization and increased pollen-transfer efficiency via reduced sexual interference within flowers (Sargent et al. 2006; Barrett 2002). They are also thought to be compensated by seasonal changes to floral—or as for *S. latifolia*—inflorescence sex allocations that re-balance mating opportunities (Brunet &

Charlesworth 1995). Previous studies have demonstrated that these seasonal adjustments to sex allocation can promote mating opportunities for dichogamous plants. Although our observational data largely mirror the results of those previous studies, paternity analysis failed to provide direct support for the mating environment hypothesis.

3.6 Acknowledgements

The authors thank J. Freeland for advice, A. Bunbury-Blanchette for assistance in the field, and two anonymous reviewers for helpful comments

3.7 Tables

Table 3.1. Generalized additive mixed-effects models (GAMMs) of temporal changes in inflorescence sex allocation (A) and pollen transfer opportunity (B) in a monoecious population of *Sagittaria latifolia*. Values are the parameter estimates, and associated *t*- and *p*-values for the modeled average sex allocation (model intercept), the effective degrees of freedom (*edf*) and associated test statistics (model A: χ^2, model B: *F*-test) for the smoothing term, and model Akaike Information Criterion values (AIC).

Model	term	Estimate	SE	*t*	*p*	*edf*	χ^2/F	*p*	AIC
A	(Intercept)	0.42	0.04	10.41	<0.001***				1058.25
	s(First flowering date)					3.3	13.19	<0.01**	
B	(Intercept)	0.30	0.04	6.93	<0.001***				11423.11
	s(Date)					7.39	54.55	<0.001***	

Note:
Significance *p < 0.05, **p < 0.01, ***p < 0.001

Table 3.2. Linear mixed effects models of siring success in a monoecious population of *Sagittaria latifolia*. Independent variables were pollen transfer opportunities (PTO; A) and siring date (B). Values are the parameter estimates, and associated *t*-test and *p*-values. We also report measures of overall fit for each model, including *F*-tests and their numerator and denominator degrees of freedom, and model AIC values

Model	term	Estimate	SE	*t*	*p*	*F*	*df*	*p*	AIC
A	(Intercept)	0.16	0.97	0.17	0.87	0.01	1, 96.13	0.92	-39.21
	Date	4.36×10^{-4}	4.29×10^{-3}	0.10	0.92				
B	(Intercept)	0.24	0.03	7.88	<0.001***	1.41	1, 100.03	0.24	-41.87
	PTO	9.58×10^{-3}	8.10×10^{-3}	1.19	0.25				

Note:
Significance *p < 0.05, **p < 0.01, ***p < 0.001

3.8 Figures

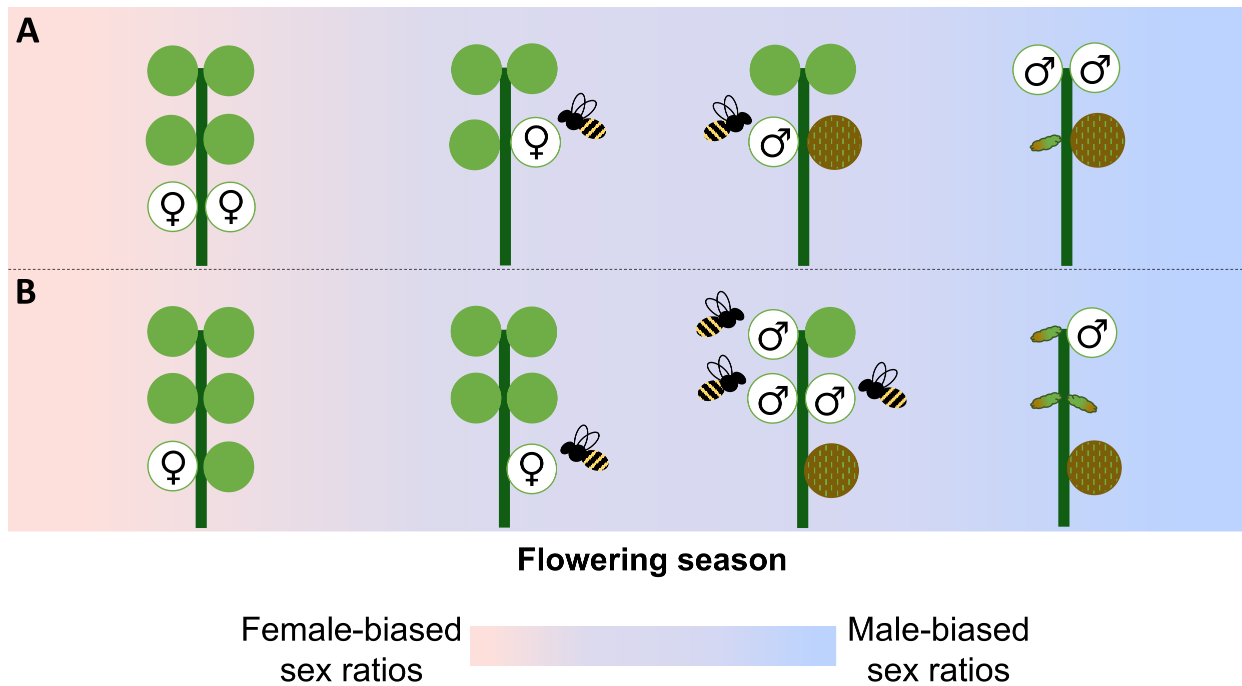

Figure 3.1. Under protogyny, floral sex ratios are female-biased at the beginning of the season and male-biased at the end of the season, with potential consequences for mate availability. Each panel is composed of maturing inflorescences with flower buds (green circles), gradually maturing female or male flowers (open circles with female or male symbols), and either maturing fruits (spotted green and brown circles) or senesced male flowers. (A) A plant that allocates resources equally between its female and male sex functions may lose mating opportunities due flowering synchrony with other plants in the population. (B) By adjusting sex allocations to its male function earlier, a plant might have additional mating opportunities to transfer pollen to open female flowers than the plant in A. Mate limitation is indicated by flowers that are not visited by pollinators.

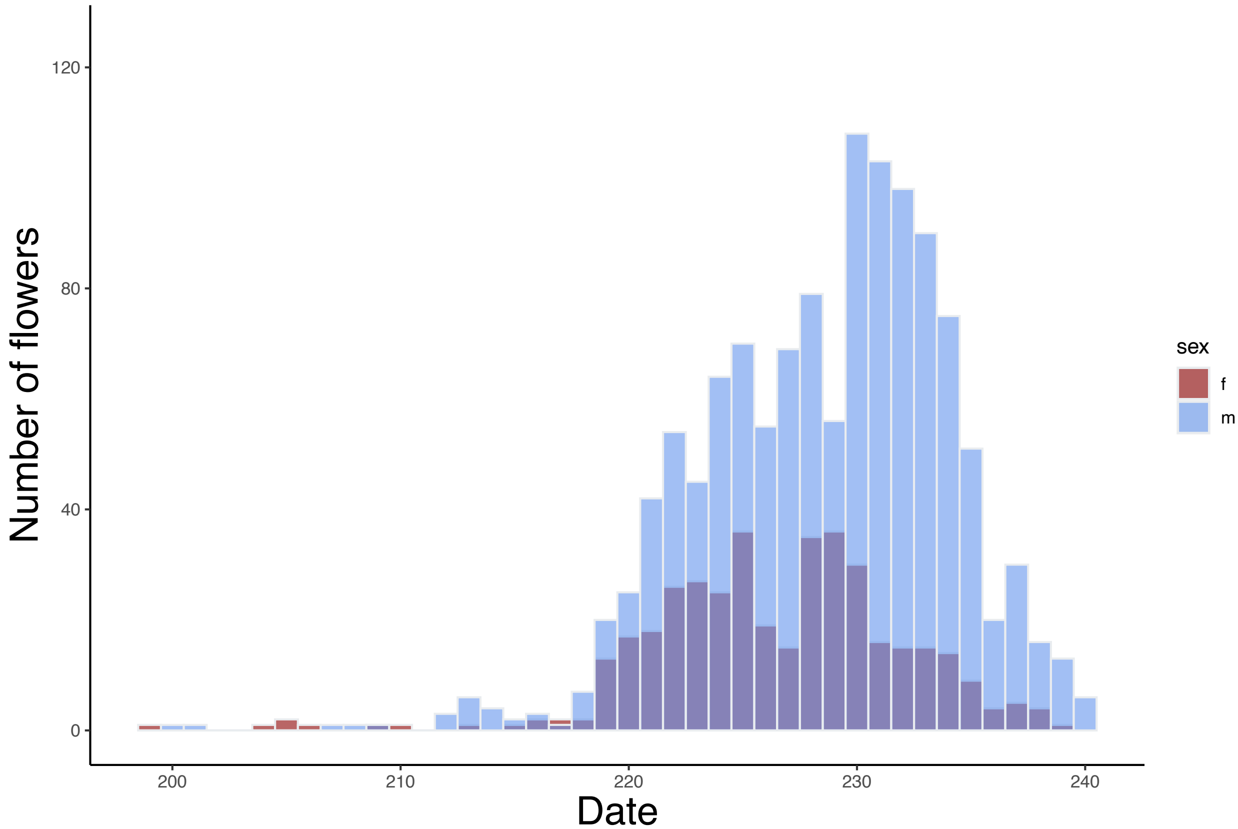

Figure 3.2. Total numbers of open female and male flowers per day in a monoecious population of *Sagittaria latifolia* from July 18th (day 199) to August 28th, 2013 (day 240).

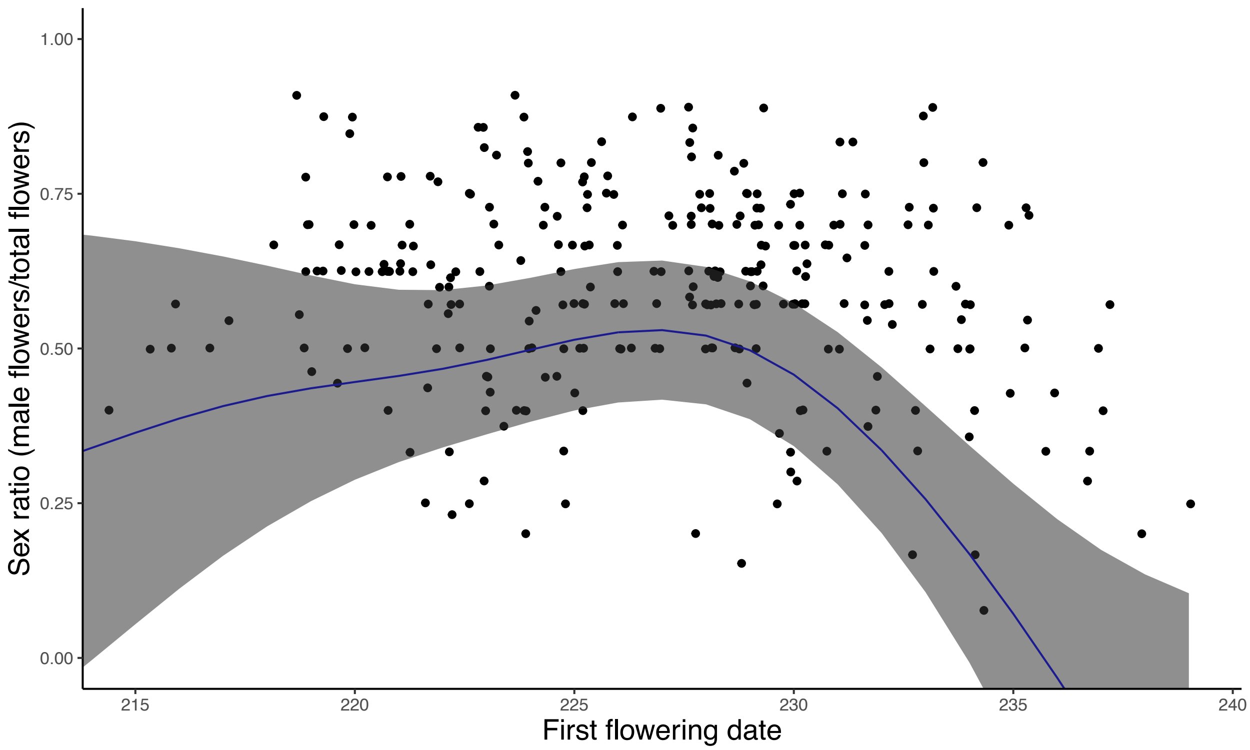

Figure 3.3. First flowering date and the corresponding inflorescence sex ratio (proportion of male flowers) for each ramet in a monoecious population of *Sagittaria latifolia* between August 3rd (day 215) to August 28th, 2013 (day 240). The solid line represents the estimated trend in the date of first flowering from a generalized additive mixed-effects model and the shaded area represents the 95% confidence intervals.

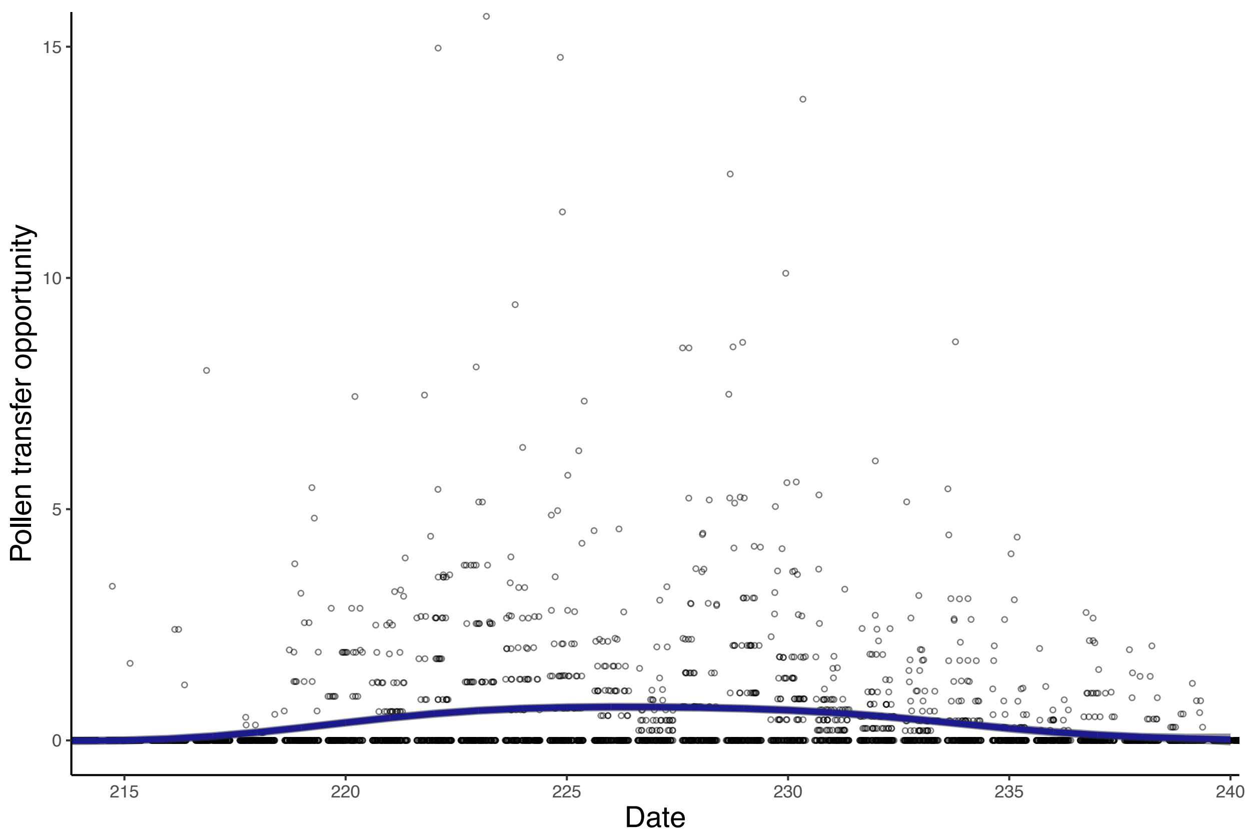

Figure 3.4. Temporal trend of mating opportunities per ramet in a monoecious population of *Sagittaria latifolia* between August 3rd (day 215) to August 28th, 2013 (day 240). Mating opportunities were measured as the daily pollen transfer opportunity per MLL. The solid line indicates the daily trend in pollen transfer opportunities (PTOs) from a generalized additive mixed effect model. PTO values (open dots) are jittered so that overlapping values can be more easily seen.

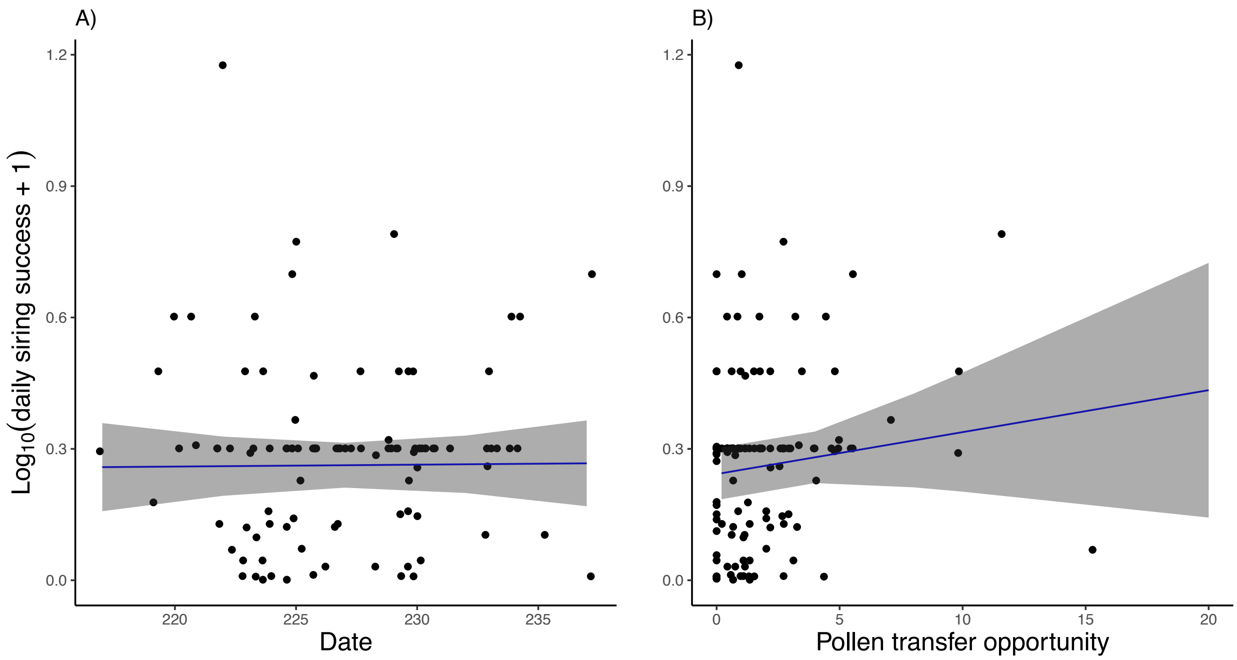

Figure 3.5. Linear regression of daily siring success ($\log_{10}$ transformed) with (A) siring date and (B) pollen transfer opportunity per genet (MLL) in a monoecious population of *Sagittaria latifolia*. Blue lines are the least-squares regression lines and the shaded areas represent the 95% confidence intervals.

4. CHAPTER 4. PLANT TRAITS AND STOCHASTICITY DRIVE VARIATION IN MALE MATING AND REPRODUCTIVE OUTCOMES IN MONOECIOUS AND DIOECIOUS POPULATIONS OF *SAGITTARIA LATIFOLIA*

Authorship: Allison Kwok and Marcel Dorken

4.1 Abstract

Because each plant has a maternal and paternal parent, average female and male contributions to mating and reproductive success within populations are constrained to be equal, but there have been few studies of these male contributions, particularly in terms of mating success (the number of mating partners). Traits that facilitate the movement of pollen among plants (e.g. plant height in wind pollinated plants (Bickle & Freeman 1993)) might to some extent, have predictable effects on mating success via male function. However, stochastic events occurring during pollination can disrupt these predictable effects and contribute to population-level variance in male mating success. We attempted to partition predictable versus unpredictable components of mating and reproductive success (the number of offspring sired) via male function in two populations of the diclinous clonal plant *Sagittaria latifolia* (one monoecious population and one dioecious population). We did this by estimating the relative contributions of plant-level traits (e.g. daily floral display size), population-level features (e.g., the size of daily pollination neighbourhoods measured as the number of plants within the average pollen-dispersal distance), and stochasticity (i.e., the joint contributions of measurement error and unmeasured effects on variation in mating and reproductive success for male-function). To understand how spatial patterns influenced mating patterns, we also compared observed and predicted amounts of pollen deposition from a spatially explicit model parameterized using spatial data from each population. We found that in the monoecious population, the combined effects of area, flower size, flower number, and the size of mating neighbourhoods explained more than half (60%) of

the variation in the mating success of plants via their male sex role, while these same characteristics explained less than half of variation in their reproductive success (43%). In the dioecious population, the joint effects of the plant-and population-level characteristics measured here accounted for much less of the total variation in male mating (10%) and reproductive success (18%). Moreover, our spatially-explicit model of pollen movement did not predict realized pollen movement in either population. In general, plant-level traits had a greater influence on mating and reproductive success than population-level features in both populations, but these effects tended to be overshadowed by stochastic effects. Other plant traits affecting plant mating and reproductive success, including those affecting post-pollination gametophyte-sporophyte interactions, might have contributed to the unaccounted-for variation in in mating and reproductive success in plants observed here.

Keywords: Mating patterns, stochasticity, pollen movement, male fitness, spatial patterns

4.2 Introduction

Sexual reproduction in anisogamous lineages involves genetic contributions from individuals via their male and female sex roles. However, studies of variation in mating and reproductive success, particularly for plants, have usually not accounted for male contributions to sexual fitness. In part, this is because mating events via the male sex role are difficult to observe directly – plants are sessile and rely on pollinators to enact mating. Because pollinators may carry pollen from a number of plants on their bodies identifying the source of pollen grains deposited on any given stigma is impractical. This difficulty has meant that few studies have assessed variation in reproductive success via the male sex role (Marshall & Ellstrand 1985; Conner et al. 1996; Conner, 2006; Kulbaba & Worley 2012; Setsuko et al. 2013; Runquist et al. 2017; Tonnabel et al. 2019), and even fewer have attempted to measure mating success via male function (Tonnabel et al. 2019; Aljiboury & Friedman 2021). Indeed, typically only components of female fitness, such as fruit or seed set (e.g. Totland et al. 1998; Matsuhisa & Ushimaru 2015), are used when measuring fitness, and so, at least for outcrossing plants, by ignoring male components of fitness, the vast majority of fitness estimates in plants account for no more than 50% of total fitness.

The quantification of male fitness components has often involved indirect measures such as pollen removal (Young & Stanton 1990; Marshall & Ellstrand 1985; Campbell et al. 1991; Johnson et al. 1995; Matsuhisa & Ushimaru 2015) or pollinator visitation rates (Broyles & Wyatt 1990; Inoue et al. 1995; Conner & Rush 1996). However, pollen removal is not equal to pollen export (Wilson & Thompson 1991; Harder & Johnson 2008; Gong & Huang 2014) with the majority of pollen lost during transport between pollen donors and recipients (Gong & Hunag 2014; Minnaar et al. 2019). Additionally, pollinator visitation may increase the chances of pollen export, albeit there is no guarantee that pollinators will visit a conspecific. Genotyping of individual pollen grains on pollinators has been used to investigate pollen carry over and infer mating systems in one study of *Castenea crenata* (Hasegawa et al. 2015), however pollen was removed from pollinators before ever reaching stigmas. The most direct way to estimate mating and reproductive success would be tracking the movement of pollen from donors to recipients and determining the resulting number of successful fertilizations. Pollen tracking has been used to learn about pollen transfer and plant mating systems (Minnaar & Anderson 2019; Villamil et al. 2022) but few individuals can be tracked simultaneously, limiting its use. Inference-based measures of siring success from genetic markers have been used (e.g. Kulbaba &Worley 2012; Irwin & Brody 2000), but they are difficult to obtain and studies that use them remain rare. Statistical biases exist when using genetic estimates of mating success that may result from overestimation of the paternal share of fertilized seeds, as successful matings with unsuccessful fertilizations are not counted (Tonnabel et al. 2019). However, genetic estimates of male mating and reproductive success provide the most complete estimates of variance in components of male fitness. Mating and reproductive success are different components of male fitness, it is necessary to have distinct and accurate quantifications of each for a more comprehensive understanding of sexual reproduction in plants.

Plants have a considerable diversity in vegetative and reproductive traits that can affect mating and reproductive success. In animal-pollinated plants, competition for pollinators can drive selection on traits affecting attraction of pollinators (Mosquin 1971; Schemske et al. 1978; Waser 1983), including floral display size (Augspurger 1980; Thomson 1988; Cruzan et al. 1988; Robertson & McNair 1995), flower size (Young & Stanton 1990; Campbell 1991; Eckhart 1991), colour (de Jager et al. 2016), shape (Gómez et al. 2006), and scent (Kessler et al. 2008; Larue et al. 2015). Vegetative traits can also directly impact mating and reproduction via size-dependency of flower and fruit production, and indirectly via effects on pollinators. Larger plants tend to have greater reproductive output because they produce more flowers (Younginger et al. 2017). Larger floral displays are also more attractive to pollinators which garner more mating opportunities (Grindeland et al. 2006). For example, large plants of *Echium vulgare* produced more flowers and had more pollinator visitors than small plants (de Jong & Klinkhamer 1994). Similarly, taller plants may be more visible to pollinators and across 44 species showed that the height of flowers affected pollinator visitation rates (Fornoff et al. 2016). Phenotypic traits of individuals can modify pollinator behaviours, influencing mating and reproduction.

While plant-level traits undoubtedly affect patterns of mating and reproduction, features of populations that affect mate availability are also influential. For example, high densities of *Mimulus ringens* had positive effects on pollinator visitation and outcrossing rates in experimental arrays (Karron et al. 1995). Similarly, for *Lychnis viscaria*, larger population sizes were linked to greater pollinator visitation (Mustajärvi et al. 2001). Population densities and sizes determine the distance to potential mates, because pollinators tend to fly short distances between plants (Schmitt 1980; Ramussen & Brødsgard 1992) and plant mating tends to involve near neighbours (Sowring, 1989; Ghazoul 2005). Population-level features have a clear impact on mating patterns and can be important determinants of mating and reproductive success.

Unpredictable and stochastic aspects of mating may have contrasting effects on male and female fitness. Under anisogamy, fitness via male function tends to be mate limited while female function tends to be resource limited (Bateman 1948). Nevertheless, there has been theoretical support that the greater production of male gametes can drive mate limitation (Lehtonen 2022). If only one sex role is mate limited and stochastic events disproportionately affect mate acquisition, stochasticity could have stronger effects on male fitness. In spite of this and somewhat counterintuitively, studies of plant mating have typically focused on the effects of mate limitation on female function (pollen limitation; Burd, 1994; Hansen & Totland, 2006). However, the local movement of pollinators and its effects on opportunities for pollen transfer (Stanton 1994; Ghazoul 2005) mean that male function should be subject to strong mate limitation (i.e., the number of potential mating partners for a pollen-producing plant should generally greatly exceed the number of actual mates) (Willson 1979; Willson 1990; Willson 1994). If male function is more strongly mate-limited than female function and male reproductive success is determined primarily by events during the pollination phase, then stochastic events during pollination will impact male function more strongly than female function. Although, we expect that mate limitation will have a stronger effect on males compared to female fitness we know surprising little about the effects of mate limitation on male function.

The purpose of this study was to investigate processes affecting variation in mating and reproduction in a monoecious and a dioecious population of *Sagittaria latifolia*. We considered plant-level and population-level features expected to affect mating and reproductive success, including features previously shown to affect reproductive success in *S. latifolia* (Sarkissian et al. 2001; Glaettli & Barrett, 2008; Perry & Dorken, 2012; Stephens et al. 2020). We attempted to compare the contributions of these plant and population-level features to those from stochastic effects on variation in mating and reproductive success. Past studies of *S. latifolia* have identified traits that may affect mating and reproductive success: flower size is associated with pollinator visitation (Glaettli & Barrett, 2008) and appears to be subject to sexual selection (Dorken & Perry 2017 and see Kwok & Dorken 2022); and floral display size is positively associated with the number pollinator visitations per inflorescence (Glaettli & Barrett, 2008; and see Huang et al.

2006). Additionally, in dioecious populations, flower production is size dependent for both females and males (Sarkissian et al. 2001). Plant (genet) size has been found to positively affect male reproductive success in *S. latifolia*, large genets consisting of more ramets (shoots) had larger mating neighbourhoods and were associated with greater siring success (Stephens et al. 2020). While those studies showed that plant-level traits and population-level features influence mating in *S. latifolia*, their joint contributions, together with stochastic variation has not been considered for this plant species. Here, we test the hypothesis that plant-level traits have predictable effects on mating outcomes and reproductive success. We predicted that variation in male mating and reproductive success can be modeled by accounting for variation in plant traits (vegetative and reproductive) and population-level features that affect mate availability. We measured, mapped, and genotyped ramets and their offspring in two populations of *S. latifolia.* And used two approaches to test our predictions; first a statistical modeling approach to partition the contributions of plant-traits, population features and stochasticity, and second a separate spatially explicit modeling approach that compared the predicted versus observed patterns of pollen deposition.

4.3 Methods

We used plant-level traits and a population-level feature that impacts mate availability to predict variation in male mating and reproductive success. Plant-level traits included both vegetative traits and reproductive traits. Ramet size, clone size, and area of a genet were considered vegetative traits, and reproductive traits were comprised of number of flowers and mean flower diameter. The population-level feature was daily local mating neighbourhoods of each genet estimated using a pollen dispersal kernel based on inter-ramet mating distances.

4.3.1 Study Species

Sagittaria latifolia Willd. is an emergent clonal aquatic plant, commonly found in wetland habitats across North America. Shoots (ramets) are produced from axillary stolons during the growing season and can be vegetative or reproductive. Corms are also produced from axillary stolons and function as perennating structures (Dorken & Barrett 2004). Genetic individuals (genets) may be composed of multiple ramets that are temporarily interconnected by stolons, which, along with all above-ground vegetative tissues deteriorate at the end of each growing season. Variation in plant size is driven by clonal expansion of genets (Yakimowski & Barrett 2014; Stephens et al. 2020) and by variation in ramet size that is primarily determined by local growing conditions (Dorken & Barrett 2003).

Populations of *S. latifolia* are either monoecious or dioecious. In monoecious populations, plants are protogynous, where in an inflorescence female flowering occurs before male flowering. Female flowers tend to open simultaneously, while male flowers open sequentially, usually one whorl at a time. In dioecious populations of *S. latifolia*, sex is genetically determined and ramets produce female or male flowers (Dorken & Barrett 2004). Flowers are produced on racemes with three flowers at each node. In our study area in Southern Ontario, flowering occurs from late June through to mid-September and flowers are pollinated by a variety of insects (Glaettli & Barrett 2008). Seed set is not pollen limited in dioecious populations (Glaettli & Barrett 2008). Male flowers have larger petals than female flowers, but female floral displays are larger due to greater floral synchrony (Yakimowski et al. 2011).

Spatial processes have been shown to effect mating patterns in *S. latifolia*. For genets with multiple ramets, the spatial arrangement of ramets can impact mating patterns, by affecting self-pollen transfer between inflorescences on the same plant (Albert et al. 2008; Liao & Harder

2014). In the same monoecious population of *S. latifolia* studied here, genet size was found to have a strong positive relationship with siring success and clonal intermingling had a positive effect on siring success (Stephens et al. 2020). However, because of the aggregated arrangement of clones in this population, neighbouring ramets were often from the same genet, contributing to moderately higher selfing rates in larger genets (Stephens et al. 2020). Genets expand outward through ramet growth, allowing for aggregated clone structure. Clonal propagules can disperse when ramets disintegrate at the end of the growing season and during hydrological disturbances, allowing for intermingling of genets. Inter-ramet spacing can also contribute to intermingling, in a field transplant experiment daughter ramets were spaced approximately 30 cm from parent plants (Dorken & Barrett 2003).

4.3.2 Study sites and floral sampling

To survey flowering in *S. latifolia*, we sampled a monoecious and a dioecious population each over five consecutive days. The monoecious population was located in a shallow area of Thompson Creek in Meadowvale Park, Peterborough, Ontario, Canada (44° 20' N, 78° 18' W). Flowering shoots at this site were sampled from August 8th to 12th 2016 in a 13 m × 17 m area of the population. The dioecious population occupied a 13 m × 4 m area of a shallow roadside ditch beside an agricultural field near Stoney Point, Ontario, Canada (44° 17' N, 82° 28' W). Plants at this site were sampled between August 21st and 26th, 2016. Due to rain and the absence of pollinators there was no sampling on August 23rd. On the first day of sampling, study zones were delimited to areas of approximately 100 flowering ramets and open flowers were marked with tags. The study zone of the monoecious site was separated from the rest of the population by natural gaps between patches of *S. latifolia.* Additionally, male flowers from outside the study zone were removed to prevent matings from pollen sources outside the study zone, effectively isolating the study population from other sources of *S. latifolia* pollen. All flowering ramets in the dioecious population were included in this study. The mean number of flowering ramets per day was similar in the monoecious (71.60 ± 15.14 SD) and the dioecious populations (68.80 ± 22.54 SD). A total of 167 ramets were tracked at the monoecious site and 183 ramets were tracked at the dioecious site. To assess the spatial distribution of flowering ramets, the location of all flowering ramets were measured using triangulation. Polar coordinates of each ramet were then converted to Cartesian coordinates (*e.g.* Ahee et al. 2015; Stephens et al. 2020).

In each population, the number of open female and male flowers was recorded each day for all flowering ramets in the study zone. Female flowers were in both populations were tagged and labelled each day to enable the tracking of pollination days. Developing fruits were collected approximately one month after initial sampling. The one-month gap between floral sampling and fruit collection resulted in numerous fruits not being recovered due to disturbances from flooding and foraging by wildlife. Because of these disturbances, the total sample of fruits obtained was a subset of the total number of ramets with female flowers (monecious: 33 ramets with fruits from an original of 113 ramets that produced female flowers; dioecious: 38 ramets with fruits from an original count of 54 flowering female ramets). Once collected, fruits were allowed to dry at room temperature and then stored in air-tight containers at 4 ºC. Fruits were stored individually in coin envelopes and labelled with the ramet ID and flowering date.

4.3.3 Floral measurements and ramet size

Vegetative and reproductive traits were measured to examine their contributions to patterns of mating and reproductive success. We estimated mean flower size by measuring the width of the largest flower at each inflorescence node using digital calipers (to the nearest 0.01mm). Ramets produced only a single inflorescence. Daily average flower size was calculated per genet and daily floral display size was estimated as the number of open flowers per genet. To account for variation in ramet size, we measured leaf mid-vein length of the largest leaf (cm) each day, a measure that is highly correlated with other estimates of ramet size (Dorken et al. 2001).

4.3.4 DNA extraction and genotyping

Leaf and seed DNA were extracted and genotyped using microsatellite loci following procedures described in Chapter 2 (Chapter 2: Appendix: Genet and Paternity Assignments) and in previous studies of *S. latifolia* (Holt et al. 2017; Stephens et al. 2020). Leaf DNA was extracted using E.Z.N.A. Plant Extraction kits (Omega Bio-Tek Inc., GA, USA) following the manufacturer's plant DNA dried sample protocol and eluted to a final volume of 100 µl. For seeds, prior to DNA extraction, a random subset of the seeds from each fruit were soaked in water to enable removal of maternally derived tissues around the embryo. Seed DNA was extracted using the QuickExtract Plant Seed DNA Extraction Solution (Lucigen, Madison WI) following the manufacturer's protocol, and eluted to a final volume of 50 µl. Not all fruits were fully mature and some yielded insufficient DNA for our analyses. A total of 350 seeds from 33 ramets from the monoecious population and 448 seeds from 36 ramets from the dioecious population were used for the parentage analysis.

Leaf samples were genotyped at seven SSR microsatellite loci (SL06, SL09, SL21, SL21, SL65, SL74, and SL75) (Yakimowski et al. 2009). We screened 166 ramets from the monoecious population and 174 ramets from the dioecious population. Seeds were genotyped at either three (SL27, SL74, SL75 for the monoecious population and SL65, SL74, SL75 for the dioecious population) or all seven loci. Nine seeds per ramet per day per population were genotyped; three of these nine seeds were genotyped at all seven loci and the rest at the three loci listed above (total sample of seeds genotyped at seven loci: 113 seeds from the monoecious population, 151 seeds from the dioecious population; 3 loci: 247 seeds from the monoecious population, 297 seeds from the dioecious population).

4.3.5 Genet and paternity assignment

The assignment of ramets to genets was conducted using the methods outlined in Chapter 2 (Chapter 2: Appendix 7.1: materials, Genet and Paternity Assignments). Briefly, we used SSR data from leaf samples to group ramets into multi-locus genotypes (MLGs) using the RClone package (v.1.0.2 (Bailleul et al. 2016)) using R (v.4.3.1(R Core Team 2023)). To account for scoring errors, MLGs that differed by up to one allelic difference at any locus was grouped into multi-locus lineages (MLLs) using the MLG_list and MLL_generator2 function from the Rclone package. Siring success was calculated following the use of parentage analysis using COLONY2 (v. 2.0.6.5; Jones & Wang 2010; Wang 2004, 2018). Paternity assignment was conducted using the procedures detailed in Chapter 2 (Chapter 2: Appendix 7.1: Genet and Paternity Assignments). However, separate paternity analyses were run for each day, limiting candidate sires to MLGs that were flowering on the same day as the recorded pollination date of the seed. Paternity assignments were made using MLGs, however siring success was summed per MLL so that siring success could be tabulated for each genet. Assignment to one or more sires was made for 197 seeds in the monoecious population and 205 seeds in the dioecious population. Siring probabilities for seeds genotyped at seven loci ranged from 0.002 to 1.00 (mean = 0.83 ± 0.001 SE) for the monoecious population and 0.002 to 0.76 (mean = 0.55 ± 0.01 SE) for the dioecious population. For seeds genotyped at three loci, siring probabilities ranged from 0.002 to 1.00 (mean = 0.69 ± 0.001 SE) for the monoecious population and 0.002 to 0.68 (mean = 0.44 ± 4.8×10^{-4} SE) for the dioecious population. Each seed was assigned to a single sire (MLG), based on the MLG with the highest siring probability. Because some seeds were assigned to sires with less than 100% probability, we tabulated total siring per multi-locus lineage (MLL) as the sum of each siring event per MLL multiplied by the siring probability.

4.3.6 Inter-ramet mating distances

Average inter-ramet mating distances were estimated by calculating the distance between maternal and siring ramets. The most probable siring ramet of a MLG was used as the siring ramet. Sires were identified as mostly single ramet MLLs, the exception of one multi-ramet MLL sire in the monoecious population. Because paternity assignments are made using MLGs, it is impossible to directly identify which ramet of a multi-ramet MLL was the sire. Other studies have used bootstrapping procedures to identify specific ramets of multi-ramet sires in order to calculate inter-ramet mating distances (Stephens et al. 2020), but analyses for the monoecious population did not converge. However, as there was only one multi-ramet sire identified in the monoecious population and two multi-ramet sires in the dioecious population, they were excluded from calculations of inter-ramet mating distances. Instead, we directly estimated the parameters of the underlying pollen dispersal kernel for both populations. We first identified the locations of maternal and paternal ramets of genotyped seeds and calculated the distance between parents, selfed mating events were excluded. We used a maximum likelihood method to parameterize the fit of the pollen dispersal kernel based on the mating distances of known dames and sires using the fitdistr function in the MASS package (7.3-60; Venables & Ripley, 2002) in R. A gamma distribution provided the best fitting function for the pollen dispersal kernel for both populations. Estimation of the pollen dispersal kernel used mating distances from all 5 days of sampling.

4.3.7 Genet size and local mating neighbourhoods

To investigate contributions of plant size and location to mating and reproductive success, we combined patterns of genet size (the number of flowering ramets belonging to the same MLL) and genet intermingling to estimate the size of mating neighbourhoods. Because flowers open for a single day, and females (and female-function of hermaphrodites) tend to open a large proportion of their flowers at once, mating neighbourhoods are temporally dynamic. As a result, for the calculations presented below we use daily estimates of genet size, including only ramets with open flowers for the calculation of genet size. Because genet size also has a spatial component, we also estimated the total area occupied per genet per day. Following methods from Stephens et al. (2020), we estimated area of a genet by assuming that each ramet of a genet occupied a fixed area and summed areas of all ramets belonging to the same genet. Calculations of area started with superimposing a grid (grain resolution = 30 cm × 30 cm) onto the population and assuming that each ramet occupied a 1 m^2 square within the grid, with daily estimates of genet area (area) equal to sum of the area occupied by each ramet per genet per day. If ramets had overlapping cells within the grid, overlapping areas were only counted once. Therefore, based on the degree of spatial overlap of ramets within a genet, two genets with the same number of flowering ramets could have different estimates of genet area (see Stephens et al. 2020). To determine how spatial relations between ramets affected mating patterns, we quantified variation in the daily size of local mating neighbourhoods for each male-phase MLL using methods for estimating intermingling outlined in Stephens et al. (2020). Briefly, the typical daily mating neighbourhood sizes for each MLL were determined as sum of the number of unrelated female-phase ramets within the 50th percentile of the distribution of inter-ramet mating distances calculated from inter-ramet mating distances over all days. Daily mating distributions were used because flowers are open for a single day and stochastic variations in the pollination environment would be different day to day. We assumed daily mating neighbourhoods for male-phase ramets included only female-phase ramets belonging to non-self genets, as female-phase ramets belonging to the same genet would not count as potential mates due to selfing.

4.3.8 Estimates of reproductive and mating success

Mating and reproductive success were calculated as daily estimates to ensure that daily changes in the spatial arrangement of flowering ramets and the mating environment were captured. First daily reproductive success via female-function was estimated as the total number of seeds produced per ramet j:

$$RS_j^f = N_{jk}^s,$$

where N^s was the estimated sum of the number of seeds produced per ramet j per pollination date k. Because ramets typically produce 1000s of seeds, we estimated N^s as the product of the total mass of all fruits per ramet and the average mass of individual seeds (achenes).

Daily reproductive success via male function (RS^m) was calculated as the number of outcrossed seeds sired per MLL i as:

$$RS_i^m = \sum_j \left(\frac{\sum_l \pi_{ijkl}}{N_{jk}^G} \times N_{jk}^S \right)$$

where π is the probability that each genotyped seed l per maternal ramet j was sired by MLL i on pollination date k, and the sum of these probabilities was scaled by the total number of seeds that were genotyped per maternal ramet j per pollination date k (N_{jk}^G). This value was multiplied by the total number of seeds produced per maternal ramet (N^S) and summed across ramets of MLL i for each pollination date to estimate total male reproductive success. Self-fertilized seeds were excluded from the calculations of RS^f and RS^m. There is substantial inbreeding depression in monoecious populations of *S. latifolia (*Dorken et al. 2002) reducing the contribution of selfed offspring to reproductive success.

Male mating success (MS^m) was estimated as the number of female mating partners (N^f) per paternal MLL *i* summed across ramets *j* on day *k*:

$$MS_i^m = \sum_{j \neq i,} N_{jk}^f,$$

such that our measure of male mating success was intended to reflect the number of female-phase MLLs in the population that produced offspring after mating with each male-phase MLL.

4.3.9 Predictors of male mating and reproductive success

We evaluated the contribution of plant-level traits and population-level features to daily mating and reproductive success using linear models. All explanatory variables, excluding flowering date, were standardized with a mean of 0 and a standard deviation of 1 prior to analysis so that the units of the regression coefficients are the same. For both populations, to explain variation in daily mating success, we used a multiple generalized linear regression of daily mating success as a function of daily vegetative traits, reproductive traits, a population-level feature and to account for temporal differences pollination date was also included. Plant-level traits consisted of ramet size (average mid-vein length per ramet per MLL per day), genet size (daily number of flowering ramets per MLL), genet area (area per MLL), number of flowers and mean flower size. Daily mating neighbourhood was used as the population feature. Interaction terms included were mating neighbourhood × clone size, mating neighbourhood × ramet size, mating neighbourhood × plant size and flower number × flower size. All plant traits, population-level feature and pollination date were treated as independent variables with daily mating success as the dependent variable. For both populations, we used a generalized linear multiple regression of daily reproductive success as a function of the same predictor variables listed above. Male reproductive success for both populations was right skewed and was $\log_{10}$ transformed prior to analysis. The lm function from the stats package in R was used to analyze all linear models. Backwards model selection was used to determine the best fitting models, where parameters with the smallest and non-significant partial F-statistic is dropped (Quinn & Keough, 2002). For all models, remaining variation was attributed to stochasticity, measurement error and unmeasured variables (Cintrón-Berdecia & Tremblay 2006).

To understand how much each predictor contributed to explaining variation in mating and reproductive, we calculated the relative importance of independent variables to each model. Relative importance analysis determines the proportional contribution of each predictor to the R^2 in relation to other variables (Budescu 1993; Azen & Budescu 2003; Baltes et al. 2004; Tonidandel & LeBreton 2010) and therefore its relative importance to explaining variation in a response variable. For all linear models, the calc.relaimpo function in the relaimpo package (v 2.2-6, Gromping, 2007) in R was used to determine the relative contribution of each predictor to the R^2.

4.3.10 Observed and predicted pollen dispersal

The gynoecium on female flowers of *S. latifolia* is composed of many (> 100) unfused carpels, each with a single stigma and ovule. To estimate the amount of pollen received per female flower, a fraction of the stigmas per flower were sampled from each female ramet by collecting stigmas from a single female flower on the second whorl of the inflorescence. For each female inflorescence, stigmas from the sampled flower were collected from the center of the gynoecium using fine-tipped forceps and mounted onto a microscope slide in the field using basic-fuchsin glycerin jelly modified to exclude the use of phenol (Beatie 1971; Kearns &

Inoyue, 1993). The total number of pollen grains in each sample of stigmas was counted using a light microscope (Nikon Eclipse E200) at 40 × magnification and divided by the number of sampled stigmas (mean 23.48 stigmas per ramet per day ± 9.17 SD) to estimate the average number of pollen grains per stigma. This average was then extrapolated to estimate the total number of pollen grains deposited per inflorescence per day by multiplying it by the mean number of stigmas per flower (calculated from the average weight of a whole fruit divided by the average weight of a single achene from the whole sample) and the number of open female flowers on the inflorescence that day.

To understand how plant-level traits and accounting for population-level features and stochastic spatial-temporal variation influence mating outcomes, we used a spatially explicit model to predict patterns of pollen movement in the both the monoecious and dioecious populations. We then compared the patterns of modelled pollen dispersal to observed pollen dispersal to determine the accuracy of the model. To predict pollen dispersal, we used the pollen dispersal kernels to determine how far pollen was moving in each population and we estimated the number of pollen grains deposited by other ramets in the population based on spatial and temporal patterns of flowering using methods outlined in Stephens et al. (2020). Briefly, we first predicted the amount of pollen that could be transferred to ramets with female flowers given the overlap in flowering time of ramets with male-flowers that were within local mating neighbourhoods, as estimated from the inferred pollen dispersal kernel. Our predictions were based on the assumptions that all male flowers had the same pollen production and that they were all equal in their ability to attract pollinators. All calculations were completed at the ramet level. We assumed the total amount of pollen reaching stigmas of ramets were sourced from ramets belonging to the same genet and unrelated genets. The amount of self pollen reaching stigmas was proportional to the number of female and male flowers with overlapping flowering on pairs of ramets of the same genet within mating neighbourhoods as estimated from the gamma distribution of the pollen dispersal kernel (Clark et al. 1998, Stephens et al. 2020). The amount of outcross pollen dispersed to stigmas was proportional to the number of open female and male flowers from pairs of ramets from different genets within the same mating neighbourhood, estimated from the same gamma distribution. Additional details of calculations and equations can be found in Stephens et al. (2020). To compare how well the pollen dispersal model predicted pollen movement in the populations, both the observed and predicted pollen deposition were standardized with a mean of 0 and standard deviation of 1. To determine how well the modelled pollen deposition predicted realized pollen deposition we used a linear regression using the lm function from the stats package (3.6.2; R Core Team 2023), with observed pollen deposition as the dependent variable and predicted pollen deposition as the independent variable.

4.4 Results

4.4.1 Plant- and population-level characteristics

The mean ramet size (measured as mid-vein length of the largest leaf) in the monoecious population was 12.79 cm ± 0.35 SE and 15.58 cm ± 0.21 SE in the dioecious population. There were few multi-shoot genets in either population that were involved in siring events. In the monoecious population mean genet size of siring genets was 1.06 ramets ± 0.04 SE, while the mean genet size of siring genets in the dioecious population was 2.92 ramets ± 1.36 SE. Mean male flower size (flower diameter) in the monoecious population was 22.10 mm ± 0.23 SE, while in the dioecious population mean flower size was 32.14 mm ± 0.36 SE. Siring ramets in both populations produced similar number of flowers each day (monoecious: 2.11 flowers ± 0.07 SE; dioecious: 3.30 flowers ± 0.14 SE). The mating neighbourhood size in the monoecious population included an average of 52.5 ramets ± 11.7 SE but only 29.9 ramets ± 5.68 SE in the dioecious population.

4.4.2 Predictors of male mating and reproductive success

The final linear model for male mating success in the monoecious population included mating neighbourhood, genet area, the number of male flowers, and male flower size as predictors ($F_{4,11} = 4.08$, $p = 0.03$, $R^2 = 0.59$) (table 4.1, model A). Genet area had a significant, positive effect on male mating success, but male flower production, mating neighbourhood and male flower size did not (table 4.1, model A). Together, plant-level traits explained 47% of the variation in mating success. Mating neighbourhood size - a population-level feature - explained 13% of the variation (figure 4.1a), leaving 40% of the variation in male mating success unattributed to the independent variables in the model. For male reproductive success in the monoecious population, the best fitting model included the size of mating neighbourhoods, genet area, and the number of male flowers per ramet as predictor variables ($F_{3,12} = 2.98$, p =0.07, R^2 = 0.43; table 4.1, model B). The population-level feature, size of mating neighbourhoods, explained 12% of the variation in reproductive success, while plant-level traits (genet area and number of flowers) contributed 31% of the variation (figure 4.1b). This left the majority of the variation in reproductive success unattributed to the independent variables in the model (57%). For the dioecious population, the best fitting model for mating success included the effects of genet size, ramet size, the number of male flowers per ramet, and size of mating neighbourhoods. However, the mating neighbourhood size did not account for a significant fraction of the variance in mating patterns ($F_{4,8} = 0.23$, p=0.91, $R^2 = 0.10$; table 4.2, model A). Only a small portion (2%) of the variation in mating success was explained by the effect of the size of mating neighbourhoods, while plant-level traits (genet size, ramet size and number of flowers) explained only 8% of the variation (figure 4.2a). This left the majority of the variation in mating success unattributed to the independent variables in the model (90%). In the dioecious population, the final model for reproductive success included, ramet size and mating neighbourhood size as explanatory variables ($F_{4,\,8}$ = 0.44, p =0.77, R^2 = 0.18; table 4.2, model B). Here, the plant-level traits and the population-level feature explained 13% and 5% of the variation in reproductive success, respectively, with 82% of the remaining variation unattributed to the independent variables in the model (figure 4.2b).

4.4.3 Observed and predicted pollen dispersal

In the monoecious population, ramets received on average of 8.9 ×10^3 ± 800 SE pollen grains per day and a mean observed number of pollen grains per stigma of 8.13 ± 0.48 SE, while mean daily pollen deposition in the dioecious population was 2.0 ×10^4 ± 2000 SE pollen grains per ramet per day and a mean of 12.15 ± 0.84 SE pollen grains observed per stigma. We inferred that pollen was dispersed 4.13 m ± 0.26 SE on average, in the monoecious population, but only and 2.47 m± 0.10 SE in the dioecious population. Based on these average dispersal distances and the number of open male flowers per day in each population, we expected a total of 9.27 pollen grains to be deposited per ramet per day (SE ± 1.49) in the monoecious population and a mean of 11.74 pollen grains per ramet per day (SE 1.28) in the dioecious population. However, these expectations were inconsistent with observed patters of pollen deposition in each population, with no patterns between observed and predicted pollen deposition in either population (monoecious: $F_{1,16}$ = 0.30, p = 0.59; dioecious: $F_{1,33}$ = 2.65, p = 0.07) (table 4.3, figure 4.3).

4.5 Discussion

We found that both plant-level traits and population-level features accounted for some of the variation in mating and reproductive success in a monoecious and dioecious population of *S. latifolia*. Our results revealed that plant- and population-level characteristics explained most of the variation in mating success and a substantial proportion of the variation in reproductive success via male function in the monoecious population. However, in the dioecious population, only a small portion of the variation in mating and reproductive success was explained by our statistical models, none of which were significant, leaving the majority of the variation in mating and reproductive success unexplained. Our probabilistic model of pollen movement also did not predict realized patterns of pollen deposition. Together, these results indicate that while plant-level traits and population-level features contribute to patterns of mating and reproduction, stochasticity and contributions from unmeasured processes affecting mating and reproduction played a large role, particularly in the dioecious population where much of the variation was unexplained. We explore further how processes that impact traits, features, stochasticity, and other variables can influence patterns of mating and reproduction in plants.

The joint effect of genet area (the cumulative sum of grid cells occupied by ramets for each genet) had the strongest effect on mating patterns, positively affected mating success in the monoecious population. The area over which a genet was able to disperse pollen increased the number of mating events, so a genet that has a larger area would have more mating opportunities than a genet with smaller areas. Pollen movement can be locally restricted, as seen in *Trillium cuneata* (Gonzales et al. 2006) where maternal plants were most likely to be pollinated by a few plants within short distances. For bees, foraging strategies are optimal when they only need to fly short distances between flowers (Sowig, 1989; Smithson & Macnair 1997). For male fitness, the greater number of potential mates within short distances increases the likelihood of successful pollen export, resulting in greater mating and reproductive success. Plants in the monoecious population were more spread out, with large patches of plants that were dense or sparse. Plants that were able to move pollen over larger areas had greater mating success. Contrary to expectations that the more potential mates that a plant spatially overlaps with the greater the mating opportunities it would have, mating neighbourhood did not affect mating success in either population. However, the mating neighbourhood did have a negative effect on reproductive success in the monoecious population. The spatially explicit pollen dispersal model did not predict the realized movement of pollen in either population, indicating factors other than the spatial overlap between potential mates contributed to the movement of pollen. While the number of potential mates available is affected by temporal overlap, this is accounted for in our daily surveys of flowering ramets and more specifically in Kwok et al. (2023). Traits and features associated with the spatial distribution of plants (area and mating neighbourhood) did not affect mating success in the dioecious population. The more even distribution of plants in the dioecious population may have negated the benefits of a plant having a large area. Plant-level traits, such as area, may only have impacts on mating in the context of the population it is found.

Floral traits did not significantly explain variation in mating and reproductive success, inconsistent with past findings that suggest sexual selection may operate on flower size and floral display size in *S. latifolia* (Glaettli & Barrett 2008; Dorken & Perry 2017). Together with the finding that sexual selection operates on male function in both of the populations studied here (Kwok & Dorken 2022), we expected floral and other genet-level traits to affect mating success . However, flower size and number explained only 10% of the variation in mating success in the monoecious population studied here, and even less in the dioecious population. Moreover, and contrary to expectations, flower size was negatively associated with mating success in the monoecious population. These findings contrast with previous studies showing that larger floral displays are generally more attractive to pollinators (Fritz & Nilsson 1996; Harder & Barrett 1996; Kudo & Harder 2005), and accordingly are under strong selection (Delph & Herilihy 2012; Hodgins & Barrett 2008; Parachnowitsch & Kessler 2010; Brunet et al. 2021). Larger floral displays lead to greater mating success. In spite of these patterns in other plants (and the previous studies of *S. latifolia* mentioned above), the operation of sexual selection in the populations studied here appears not to have been driven by variation in floral display size. One possible explanation for this is that sexual selection could be acting on post-pollination processes in *S. latifolia* (and see Tonnabel et al. 2021). In both populations studied here, multiple pollen grains deposited on each stigma (mean across both populations = 10.90 pollen grains per stigma ± 0.99 SE) and because there is only one ovule per stigma there would have been competition among pollen grains for mating success. Indeed, once pollen grains arrive on stigmas, direct competition among them might lead to greater opportunities for the operation of sexual selection than does indirect competition among plants for the attraction of pollinators (Dorken and Perry 2017; Beaudry et al. 2020; Tonnabel et al. 2021). In *Viola tricolor,* pollen tube growth rates have been shown to vary among individuals and are heritable (Skogsmyr & Lankinen 2000), showing the potential for selection to affect the evolution of male gametophytes. Biochemical interactions between pollen and pistils that drive variation in fertilization success have been suggested as potential evidence of female choice in plants (Higashiyama & Hamamura 2008; Skogsmyr & Lankinen 2000; Immler & Otto, 2018), however direct evidence of intersexual selection in plants remains understudied. While we did not see evidence of sexual selection acting on traits that impact the pollination phase, sexual selection may be acting on traits that influence fertilization success in the post-pollination phase.

An alternative explanation for evidence of the operation of sexual selection in these populations of *S. latifolia* (Kwok & Dorken 2022) despite no apparent associations between floral traits and mating success, is that commonly use metrics of sexual selection (the opportunity for sexual selection - I_s, Bateman gradients) often overestimate the strength of sexual selection (Klug et al. 2011), because they measure the potential for sexual selection to operate in a population (Jones 2009; Krakauer et al. 2011). For example, if mating success were stochastic (and therefore not subject to sexual selection) and males were more mate limited than females, both I_s and Bateman gradients would still indicate that sexual selection is operating (Klug et al. 2010). While these metrics of sexual selection are useful in determining if sexual selection is able to operate in a population, to understand how sexual selection actually operates in a population, it is necessary to know how traits affect mating success (Jones 2009; Klug et al. 2010; Krakauer et al. 2011). However, for *S. latifolia* and other plants, we still do not know which traits are impacting mating success (Tonnabel et al. 2021). Until such time, by accounting for stochasticity in mating and reproductive success we can determine if mating success is non-random and a reflection that sexual selection is operating.

Despite plant traits explaining some of the variation in mating and reproductive success, much of the remaining variation could be attributed to stochasticity. Although plant- and population level features are known to affect plant reproduction, several aspects of mating are fundamentally uncertain and may have important effects on realized mating and reproductive success. In *Lavandula latifolia*, stochastic variation in spatial and temporal access to sunlight in a population influenced pollinator composition and quality (Herrera 1995). Stochastic variation in extrinsic environmental factors, such as sunlight, can be more important to pollinator assemblages and plant fitness than traits intrinsic to the plants (Lechowicz & Bell 1991; Herrera 1995). Heterogenous environments allow for more opportunity for stochasticity to operate, in particular, due to pollinator responses to environmental factors that are highly variable (Kilkenny & Galloway 2005). Variation in pollinator environments can vary considerably annually, seasonally, daily, and within days. Here we measured mating and reproductive success daily because the availability of flowers for pollination in *S. latifolia* lasts a single day. Within a day stochastic variation in the pollination environment can change to impact plant fitness. Variation in pollen dispersal is determined by the type of pollinators, number of visits, and stochasticity during pollen dispersal (Richards et al. 2009). While plant-level traits and population-level features can influence pollinator visitations and pollinator types (Ghazoul 2006; Ishii & Harder 2006; Richards et al. 2009), there are many avenues where unpredictable events can disrupt pollen dispersal and pollination. Stochasticity may have the opportunity to act during pollen transfer, but there are fewer opportunities for disruption once pollination has occurred and the first phase of mating has taken place (Minnaar et al. 2019). However, the stochastic nature of pollen delivery can impact patterns of mating. In *Hellebore foetidus*, pollen tube formation on stigmas was highly variable between flowers and within stigmas of the same flower due to stochastic variation in pollen loads among flowers and styles (Herrera 2002). Fine-scale variation between flowers and stigmas within plants explained more variation in pollen tube formation than population or regional differences. Stochastic variation in pollen loads on stigmas impacted the competitive environment in which pollen tubes are formed. Sources of stochastic effects on mating and reproductive success can range from broad-scale extrinsic factors to fine-scale intrinsic factors.

The unexplained variation in mating and reproductive success can be attributed to stochasticity in part. Variables that were not measured and measurement error likely also contributed. While we measured many plant-level traits, other traits have been shown to affect mating and reproduction in plants. Both the volume (Delph & Lively 1992) and concentration (Kulbaba & Worley 2012) of nectar have been shown to affect pollination and specifically to have an effect on male (or male function of hermaphrodites) fitness. Pollen traits may also affect patterns of pollen transfer or fertilization (Opedel et al. 2023). Pollen grain size can affect successful pollination, pollinators that forage on pollen may select for smaller grains that are less likely to be consumed and more likely to successfully reach stigmas (Hao et al. 2020).
In *Fusarium oxysporum,* pollen size was negatively associated with pollen tube growth rate (Tejaswini 2002). The stickiness of pollen is suggested as another trait that may maximize pollen delivery (Hasegawa et al. 2021). Differences in daily flowering phenology can influence pollination success, depending on when a flower opens during the day can determine how successful a plant is at donating pollen (Miyake & Yahara 1999). Environmental factors that may have undue effects on pollinator behaviour (Kilkenny & Galloway 2005; Sanchez-Lafuente et al. 2005). There are many plant-level traits and environmental factors that could explain variation in mating and reproductive success.

Our findings suggest that plant-level traits and a population-level feature explained some of the variation in mating and reproductive success in a monoecious population of *S. latifolia*. However, stochastic effects and unmeasured traits are likely culprits of much of the observed variation. Additionally, there are opportunities for sexual selection to act on post-pollination traits. The dynamics of mating and reproduction in plants may have some predictability, but they are also contextual and dependent on more than intrinsic plant-level traits or extrinsic population-level features.

4.4 Tables

Table 4.1. Linear models of mating success on determinants of mating success and reproductive success on determinants of reproductive success in a monecious population of *Sagittaria latifolia*. Explanatory variables are genet area, number of flowers, flower size, and local mating neighbourhood. Significant values are in bold. Values are the parameter estimates, and associated *t*-test and *p*-values. we also report measures of overall fit for each model, including associated *F*-test statistics, their numerator and denominator degrees of freedom, and R^2.

	Estimate (SE)	*t*	*p*	*F*	*p*	*df*	R^2
A. Mating success							
Intercept	4.55 (0.41)	11.14	**<0.001**	4.08	**0.03**	4, 11	0.60
Mating neighbourhood	-2.39 (1.23)	-1.94	0.08				
Genet area	2.87 (0.89)	3.20	**<0.01**				
Number of flowers	0.51 (0.65)	0.78	0.45				
Flower size	-0.58 (0.63)	0.93	0.34				
B. Reproductive success							
Intercept	3.29 (0.14)	23.48	**<0.001**	2.98	0.07	3, 12	0.42
Mating neighbourhood	-0.83 (0.36)	-2.35	**0.03**				
Genet area	0.63 (0.29)	2.16	0.05				
Flower number	0.47 (0.22)	2.13	0.05				

Table 4.2. Multiple linear regression of mating and reproductive success on determinants of mating and reproductive success in a dioecious population of *Sagittaria latifolia*. Explanatory variables are genet size (number of ramets), ramet size (mid-vein length), number of flowers, flower size (flower diameter), and local mating neighbourhood. Values are the parameter estimates, and associated *t*-test and *p*-values. we also report measures of overall fit for each model, including *F*-tests and their numerator and denominator degrees of freedom, R^2 for the whole model and the relative R^2 contributions of each explanatory variable.

	Estimate (SE)	*t*	*p*	*F*	*p*	*df*	R^2
A. Mating success							
Intercept	4.46 (0.75)	5.94	**<0.001**	0.23	0.91	4,8	0.10
Genet size	-4.52 (7.11)	-0.63	0.54				
Mating neighbourhood	4.01 (11.73)	0.34	0.74				
Ramet size	-0.28 (1.21)	-0.23	0.82				
Number of flowers	0.09 (8.55)	0.01	0.99				
B. Reproductive success							
Intercept	3.39 (0.17)	20.53	**<0.001**	0.44	0.77	4, 8	0.18
Genet size	-2.21 (2.21)	-1.00	0.35				
Ramet size	-0.17 (0.23)	-0.72	0.49				
Mating neighbourhood	2.15 (2.25)	0.95	0.37				
Number of flowers	0.20 (0.31)	0.64	0.54				

Table 4.3. Linear regression of observed pollen deposition on predicted pollen deposition in a monoecious and dioecious population of *Sagittaria latifolia*. Response and explanatory variables were observed and predicted pollen deposition, both were standardized by dividing by their mean values. Values are the parameter estimates, and associated *t*-test and *p*-values. We also report measures of overall fit for each model, including *F*-tests and their numerator and denominator degrees of freedom, R^2 for the whole model.

	Estimate (SE)	t	p	F	p	df	R^2
A. Monoecious							
Intercept	-2.15×10^{-16} (0.24)	0.00	1.00	0.30	0.59	1, 16	0.02
Predicted pollen deposition	-1.35 (0.25)	-0.55	0.59				
B. Dioecious							
Intercept	2.20×10^{-17} (0.17)	0.00	1.00	2.65	0.11	1, 33	0.07
Predicted pollen deposition	-0.27 (0.17)	-1.67	0.11				

4.5 Figures

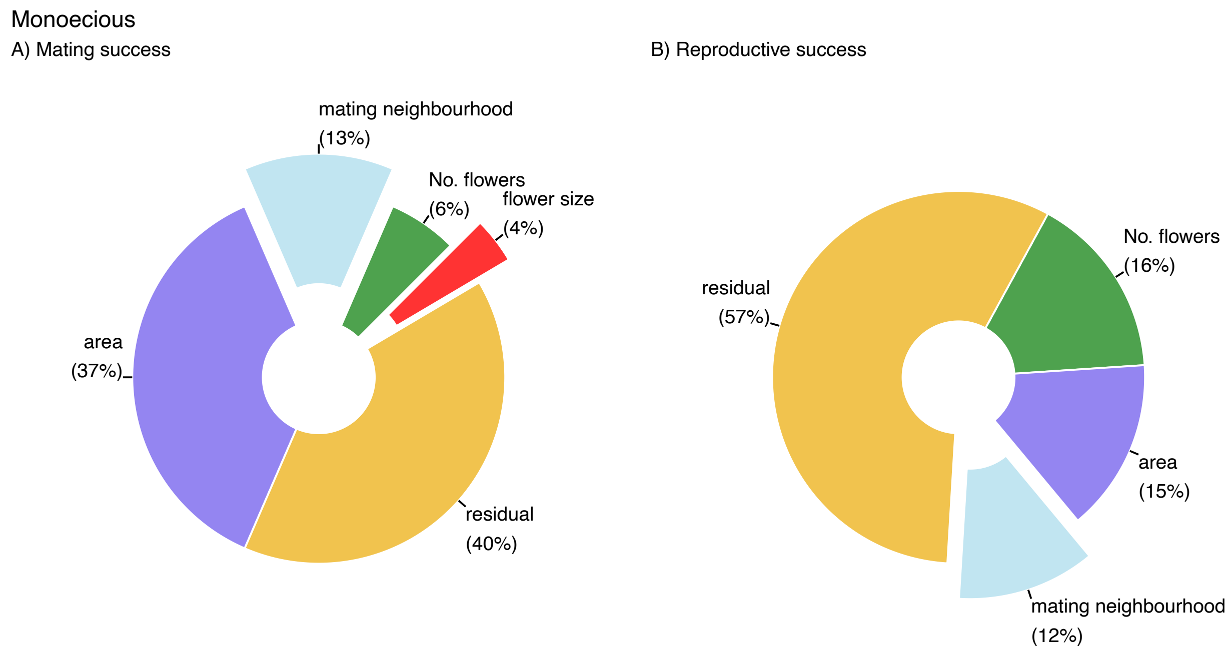

Figure 4.1: The relative contribution of plant-level traits, population-level feature and stochasticity to patterns of male mating (A) and reproductive success (B) in a monoecious population of *Sagittaria latifolia*. Withdrawn segments indicate predictors that had a negative effect.

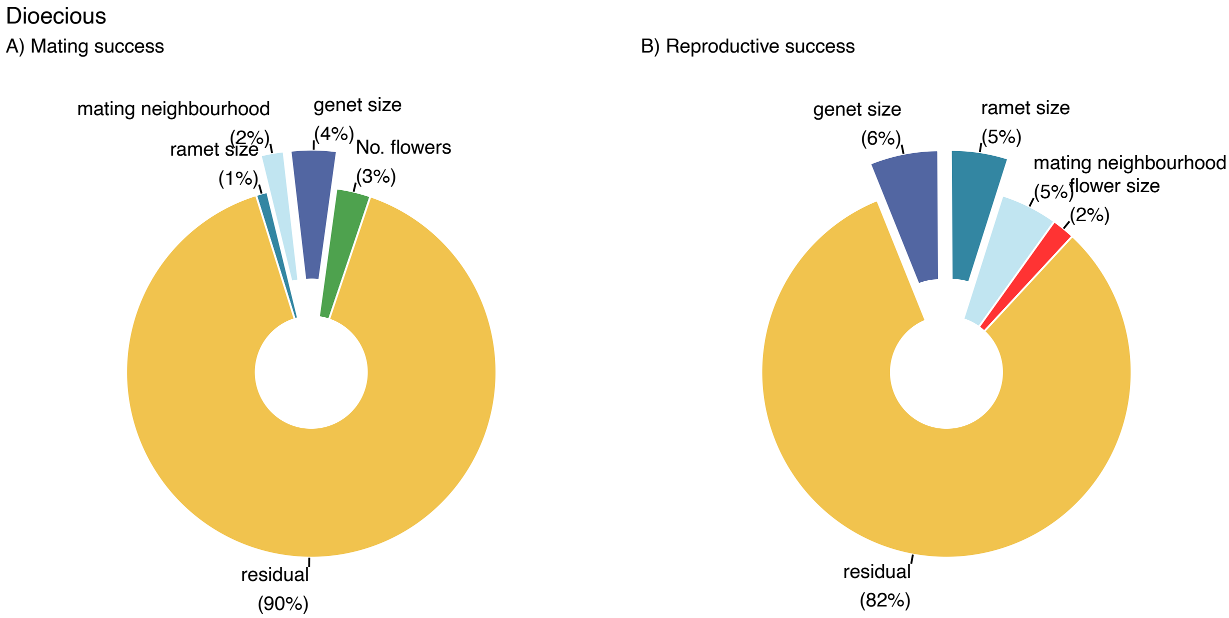

Figure 4.2: The relative contribution of plant-level traits, population-level feature and stochasticity to patterns of male mating (A) and reproductive success (B) in a dioecious population of *Sagittaria latifolia*. Withdrawn segments indicate predictors that had a negative effect.

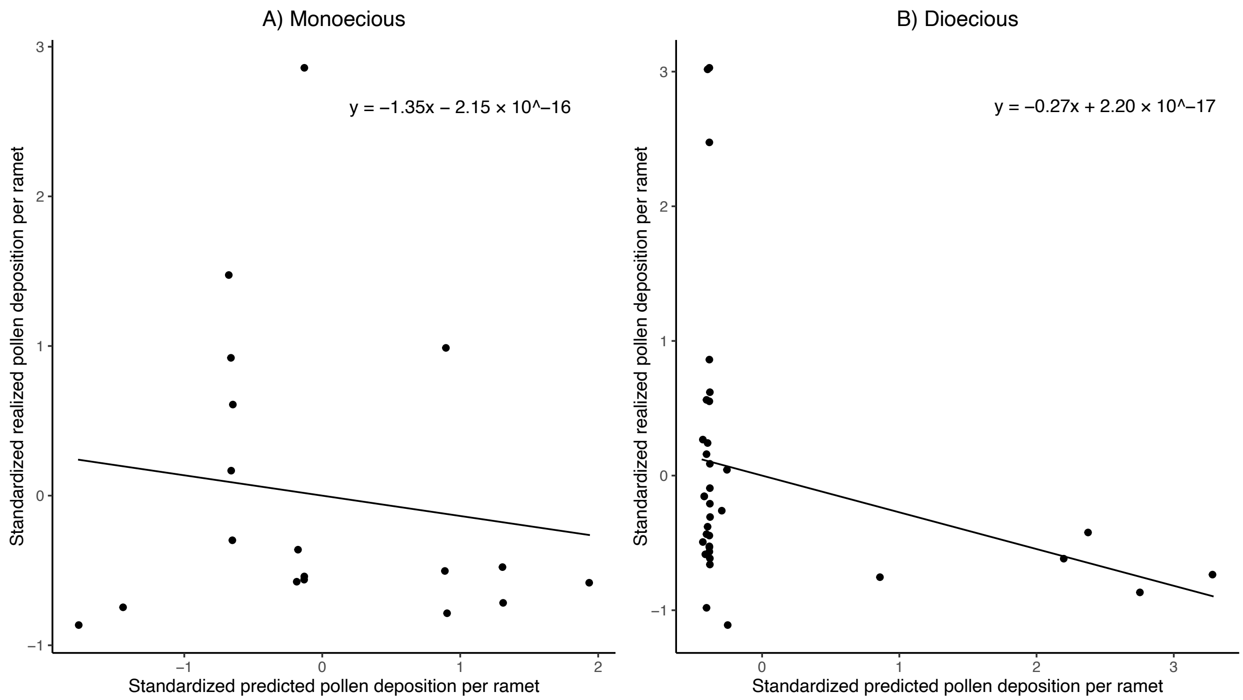

Figure 4.3: Relationships between predicted pollen deposition and realized pollen deposition per ramet in populations of *Sagittaria latifolia*. Points represent standardized daily predicted and realized pollen deposition per ramet. The line represents the slope of the regression of realized vs. predicted pollen deposition, shaded area is the 95% CI.

5. CHAPTER 5. GENERAL DISCUSSION

Mating in plants is complex and has far reaching consequences for the evolution of plant sexual diversity. Understanding plant mating patterns and the forces that drive variation in how mating is achieved is an important area of research in the evolutionary biology of plants. While numerous studies examine plant reproductive patterns, few investigate mating patterns using mating success. This thesis contributes to our understanding of plant mating and reproduction, bringing greater insight into patterns of male mating and reproductive success and their evolutionary consequences. The variation in gender strategies of *Sagittaria latifolia* provided a unique opportunity to study how different sexual systems affect plant mating. I found that in natural populations of *S. latifolia*, male mating success was associated with reproductive success more so than female mating and reproductive success, that shifts in sex allocation were not associated with greater siring in hermaphrodites, and that the predictable effects of plant traits and population features on male mating and reproductive success were obscured by stochastic factors. My results show that sexual selection does operate in natural populations of plants, validating its broad acceptance as an important evolutionary force in plants. However, mating in plants is highly complex and while adjustments to sex allocation or plant traits may influence mating opportunities, there is still much about mating dynamics and their outcomes that remain unclear.

5.1 Sexual Selection in natural populations

In Chapter 2, I quantified sexual selection in natural populations of *S. latifolia* using Bateman gradients. I found that the relationship between mating and reproductive success in males and male-function of hermaphrodites was greater than that of females and female-function of hermaphrodites, indicating that sexual selection is operating in these two populations.

Variation in size is often a trait that is subject to sexual selection in unitary organisms (Höglund & Säterberg 1989). In freshwater snails, *Physa acuta*, body size was found to have positive effect on mating and fitness for both sex functions, with a greater effect on male mating (Pélissié et al. 2011). For modular organisms, such as the case for plants, size variation may instead be due to non-heritable variation in local-resource conditions and plant-age. And plants that are larger or older may have more reproductive capability than plants that are smaller and younger. For *Plantago laceolata*, larger plants had greater reproductive output, and plants that were larger and older had a higher probability of flowering (Baden et al. 2020). I found that without accounting for size variation, patterns of sexual selection could be masked. However, once size was accounted for strong sexual selection was found to be operating in both monoecious and dioecious populations, in accordance with predictions made under Bateman's principles (Bateman 1948) and patterns of sexual selection found in the animal literature (Seehausen et al. 1999; Boughman 2001).

In addition, I found that pattens of sexual selection were similar between monoecious and dioecious populations. Differences in patterns of sexual selection were expected due to cross-sex effects associated with hermaphroditism (Anthes et al. 2015). However, in the case of *S. latifolia*, female and male sex functions are separated during mating and may allow for greater independence. In accordance with a study on freshwater snails, *P. acuta*, found that there were minor cross-sex effects despite being simultaneous hermaphrodites (Pélissié et al. 2011). The snails were found to preferentially outcross over selfing, in this case mating behaviours of the snails allowed for independence of sex functions which may have reduced any cross-sex effects. The temporal separation of sexes granted plants temporary phases of unisexuality, which tempered the cross-sex effects on mating success in *S. latifolia*.

This chapter joins other research (Dai & Galloway 2013; Cocucci et al. 2014; Tonnabel et al. 2019; Barbot et al. 2023; Lavaut et al. 2023) in the application of sexual selection theory to plants. Specifically, this research contributes to the broader literature in being the first to measure the strength of sexual selection in natural populations of plants and comparing patterns of sexual selection in a species with variable sexual systems. Next steps would be to determine if Bateman gradients can be used more broadly and to determine the extent to which sexual selection is operating in pre- and post-pollination phases.

5.2 Sex allocation and flowering phenology

In Chapter 3, I measured seasonal changes in sex allocation, mating environment and siring success in a population of hermaphroditic *S. latifolia*. I found that changes in sex allocation across the season could offset missed mating opportunities due to dichogamy. Plants that flowered earlier allocated more to male function than plants that flowered later. The patterns of allocation were as predicted by the mating environment hypothesis (Brunet & Charlesworth 1995) and mating opportunities for male function (as measured by pollen transfer opportunities) corresponded to this pattern. However, pollen transfer opportunities were not associated with siring success. The results in this chapter align with other studies in showing that temporal shifts in sex allocation can alter mating opportunities in dichogamous plants. However, I did not find evidence that seasonal adjustments to sex allocation affected siring success. Despite the importance of temporal overlap in flowering for determining mating opportunities, other processes were responsible for shaping patterns of siring.

The mating environment hypothesis assumes that the probability of transferring pollen to potential mates is equal among flowers based only on the temporal overlap between female and male flowers. However, the ability to transfer pollen is not equal between plants, nor between flowers of the same plant. Sexual selection is linked to differential pollen transfer based on a variety of traits, including but not limited to floral morphology, scent, nectar production, flower duration, and flower height (Delph & Ashman, 2006). Pollen and flower production can be affected by variable local environmental conditions (Delph et al. 1997). For example, soil fertility (Lau & Stephenson 1993; Lau & Stephenson 1994), herbivory (Quesada et al. 1995; Delph et al. 1997) and mycorrhizal infections (Stephenson et al. 1994) can all affect pollen production and quality, which in turn affects the likelihood of pollen being transferred successfully. Additionally, if pollen dispersal is spatially restricted in a population, patterns of pollen transfer probabilities may not be realized as siring success. Pollination typically occurs between nearest neighbours (Levin & Kerster 1974), pollen is less likely to be transferred to potential mates that are farther away. For *Raphanus sativus*, pollen dispersal and siring were negatively affected with increasing distance (Devlin et al. 1992). In the case of *S. latifolia*, past studies have found that there is some spatial restriction in pollen dispersal (Stephens et al. 2023). While temporal overlap undoubtedly contributes to the mating environment, incorporating a spatial component can further our understanding of the mating environment and subsequent mating patterns. To further explore the mating environment hypothesis, as *S. latifolia* is a clonal plant, accounting for both temporal and spatial variation may provide further insight to how changes in sex allocation can affect mating opportunities and siring patterns.

5.3 Stochasticity and predictors of male mating and reproductive success

In Chapter 4, I examined the effect of intrinsic traits, extrinsic features and stochasticity on male mating and reproductive success. While both intrinsic plant-level traits and extrinsic population-level features of plants can have predictable effects on patterns of mating and reproduction (Bickle & Freeman 1993; Karron et al. 1995), there is inherent stochasticity to plant mating that can disrupt these predictable effects (Minnaar et al. 2019). I found that while plant-level traits and population-level features explained some of the variation in mating and reproductive success, much of the variation was attributed to stochasticity and unmeasured variables. Despite evidence of sexual selection occurring in both these populations (Chapter 2), I did not find evidence that floral traits were sexually selected for. While sexual selection is most often studied during pre-pollination phases, sexual selection can also operate post-pollination.

The scope for sexual selection during the post-pollination phase through pollen-pistil interactions remains a possibility (Tonnabel et al. 2021). Continued interactions between female and male functions in the pistil has been proposed as an arena for female choice, another mode of sexual selection. During the pre-pollination phase, competition is indirect between competing males. However, the first direct contact between female and male mating partners occurs during the post-pollination phase, providing an opportunity for intrasexual competition. Pistils are responsible for nutritive molecules that promote pollen tube growth, while pollen grains are also able to harvest nutrients from pistils (Iwano et al. 2014). Additionally, tissues in the style guide pollen tube formation towards ovules (Chae & Lord 2011). Pollen grains have numerous cellular processes that affect pollen tube performance, for example, reorganization of actin filaments to stabilize pollen tube growth (Zhang et al. 2010), expression of molecule transporters that regulate metabolic activity of pollen tubes (Cheng et al. 2015) and navigation through the style (Yanagisawa et al. 2017). These processes provide opportunity for both female choice and male-male competition in the post-pollination phase. Further study on both pollen and pistil traits, and their effects on mating and reproductive success is needed.

While stochasticity had a large effect in both populations of *S. latifolia*, this study was a snapshot of the flowering season. Flowering for *S. latifolia* in S. Ontario, typically lasts from

June to mid-September. It is possible that stochastic processes had a smaller contribution when considering the whole of the reproductive season. Past studies have found that flower size and floral display size in *S. latifolia* may be under sexual selection (Glaettli & Barrett 2008; Dorken & Perry 2017). Sexual selection may be operating in these two populations but has been obscured by stochastic processes for the short duration of this study. To have a more complete understanding of how predictive and stochastic processes affect mating and reproduction patterns, observations of the entire mating season would be valuable.

This chapter joins other research on understanding the role of intrinsic traits (Schemske et al. 1978; Eckhart 1991; Robertson & McNair 1995) and population features (Karron et al 1995; Mustajärvi et al. 2001; Ramussen & Brødsgard 1992; Sowring, 1989; Ghazoul 2005) in examining patterns of mating and reproduction. However, this study provides additional consideration of the contributions of stochastic processes. Stochasticity has effects, whether through error and measurement bias or through chance alone, it is a non-selective process that can affect mating and reproduction. Accounting for it, is a necessary part of having a more complete understanding of how predictive processes impact patterns of plant mating and reproduction

5.4 Conclusions and summary

In summary, these findings suggest that in natural populations of *S. latifolia*: sexual selection operates on male function, even in monoecious populations despite the potential for cross-sex effects (Chapter 2), that temporal changes to sex allocation in hermaphrodites can result in more mating opportunities, however siring success is dependent on more than the mating opportunities garnered from temporal overlap in flowering (Chapter 3), and while intrinsic traits and extrinsic features contribute to male mating and reproductive success, stochastic factors play a large role in determining patterns of mating and reproduction (Chapter 4).

My thesis has helped to shed light on male mating patterns in plants. Sexual selection operates in plants as predicted by Bateman's Principles (Bateman 1948), males and male function of hermaphrodites experience greater variation in mating and reproductive success. Despite this, I failed to find patterns of selection acting on traits acting on traits that would impact pollination. This leads to the conclusion that the post-pollination phase may be an arena where sexual selection could operate, through pollen competition, female choice or more likely some combination of the two. For hermaphroditic plants, how a plant allocates resources to each sex function and when affects mating opportunities. Yet, temporal overlap and the potential for mating alone cannot explain the patterns of siring I observed. There are many steps between flowering and siring. The complexity of mating in plants makes it difficult to predict interactions between potential mating partners and subsequent reproductive patterns. Despite extensive studies on pollination and floral trait selection, further research into post-pollination processes, sporophyte-gametophyte interactions may provide more insight to plant mating patterns. This underscores the need for further investigation into factors influencing plant mating patterns, including those occurring after pollination.

6. REFERENCES

Ahee JE, Van Drunen WE, Dorken ME. 2015 Analysis of pollination neighbourhood size using spatial analysis of pollen and seed production in broadleaf cattail (*Typha latifolia*). *Botany* **93**, 91–100. (doi:10.1139/cjb-2014-0169)

Albert T, Raspé O, Jacquemart A-L. 2008 Influence of clonal growth on selfing rate in *Vaccinium myrtillus* L. *Plant Biol.* **10**, 643–649. (doi:10.1111/j.1438-8677.2008.00067.x)

Aljiboury AA, Friedman J. 2022 Mating and fitness consequences of variation in male allocation in a wind-pollinated plant. *Evolution* **76**, 1762-1775 (doi:doi:10.1111/evo.14544)

Anthes N, David P, Auld JR, Hoffer JNA, Harne P, Koene JM, Kokko H, Lorenzi MC, Pélissié B, Sprenger D, Staikou A, Schärer L. 2010 Bateman gradients in hermaphrodites: an extended approach to quantify sexual selection. *Am. Nat. 8* **176**, 249–263. (doi:10.1086/655218)

Anthes N, Häderer IK, Michiels NK, Janicke T. 2017 Measuring and interpreting sexual selection metrics: evaluation and guidelines. *Methods Ecol. Evol.* **8**, 918–931. (doi:10.1111/2041-210X.12707)

Anthes N, Putz A, Michiels NK. 2015 Bateman gradients in hermaphrodites: an extended approach to quantify sexual selection. *Am. Nat*. **176**, 249–263. (doi:10.1086/655218)

Arnaud-Haond S, Duarte CM, Alberto F, Serrão EA. 2007 Standardizing methods to address clonality in population studies. *Mol. Ecol*. **16**, 5115–5139. (doi:10.1111/J.1365-294X.2007.03535.X)

Arnold SJ. 1994 Bateman's principles and the measurement of sexual selection in plants and animals. *Am. Nat.* **144**. (doi:10.1086/285656)

Augspurger CK. 1980 Mass-flowering of a tropical shrub (*Hybanthus prunifolius*): influence on pollinator attraction and movement. *Evolution* **34**, 475–488. (doi:10.2307/2408217)

Azen R, Budescu DV. 2003 The dominance analysis approach for comparing predictors in multiple regression. *Psychol. Methods* **8**, 129–148. (doi:10.1037/1082-989X.8.2.129)

Baden M, Roach D, Schweingruber FH, Reitzel K, Lundsgreen K, Dalgren JP. The effects of age on the demography of a perennial plant depende on interactions with size and environment. *J. Ecol*. **2021**, 1068-1077

Bailleul D, Stoeckel S, Arnaud-Haond S. 2016 RClone: a package to identify MultiLocus Clonal Lineages and handle clonal data sets. *R. Meth. Ecol. Evol.* **7**, 966–970. (doi:10.1111/2041-210X.12550)

Baltes BB, Parker CP, Young LM, Huff JW, Altmann R. 2004 The practical utility of importance measures in assessing the relative importance of work-related perceptions and organizational characteristics on work-related outcomes. *Organ. Res. Methods* **7**, 326–340. (doi:10.1177/1094428104266014)

Barbot E, Dufay M, De Cauwer I. 2023. Sex-Specific selection patterns in a dioecious insect-pollinated plant. *Evolution*.**77**, 1578-1590

Barrett SCH, Hough J. 2013 Sexual dimorphism in flowering plants. *J. Exp. Bot*. **64**, 67–82. (doi:10.1093/JXB/ERS308)

Barrett SCH. 2002 Sexual interference of the floral kind. *Heredity* **88**, 154–159. (doi:10.1038/sj.hdy.6800020)

Barrett SCH. 2002 The evolution of plant sexual diversity. *Nat. Rev. Genet.* **3**, 274–284. (doi:10.1038/nrg776)

Bateman AJ. 1948 Intra-sexual selection in *Drosophila*. *Heredity* **2**, 349–368. (doi:10.1038/HDY.1948.21)

Bates D, Mächler M, Bolker B, Walker S. 2015 Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67, 1–48. (doi:10.18637/jss.v067.i01)

Beattie A. 1971 A technique for the study of insect-borne pollen. *Pan-Pac. Entomol.* **47**, 82.

Beaudry FEG, Rifkin JL, Barrett SCH, Wright SI. 2020 Evolutionary genomics of plant gametophytic selection. *Plant Commun.* **1**, 100115. (doi:10.1016/j.xplc.2020.100115)

Bertin RI, Newman CM. 1993 Dichogamy in angiosperms. Bot. Rev. 59, 112–152. (doi:10.1007/BF02856676)

Bickel AM, Freeman DC. 1993 Effects of pollen vector and plant geometry on floral sex ratio in monoecious plants. *Am. Midl. Nat.* **130**, 239–247. (doi:10.2307/2426124)

Boughman JW. 2001 Divergent sexual selection enhances reproductive isolation in sticklebacks. Nature 411, 944–948. (doi:10.1038/35082064)

Brookes RH, Jesson LK. 2010 Do pollen and ovule number match the mating environment? An examination of temporal change in a population of *Stylidium armeria*. Int. J. Plant Sci. 171, 818–827. (doi:10.1086/655770)

Broyles SB, Wyatt R. 1990 Paternity analysis in a natural population of *Asclepias exaltata*: multiple paternity, functional gender, and the “pollen-donation hypothesis”. *Evolution* **44**, 1454–1468. (doi:10.1111/j.1558-5646.1990.tb03838.x)

Brunet J, Charlesworth D. 1995 Floral sex allocation in sequentially blooming plants. Evolution 49, 70–79. (doi:10.1111/j.1558-5646.1995.tb05959.x)

Brunet J. 1996 Male reproductive success and variation in fruit and seed set in *Aquilegia caerulea* (Ranunculaceae). *Ecology* **77**, 2458–2471. (doi:10.2307/2265746)

Brunet J, Flick AJ, Bauer AA. 2021 Phenotypic selection on flower color and floral display size by three bee species. *Front. Plant Sci.* **11**. (doi:10.3389/fpls.2020.587528)

Budescu DV. 1993 Dominance analysis: A new approach to the problem of relative importance of predictors in multiple regression. *Psychol. Bull.* **114**, 542–551. (doi:10.1037/0033-2909.114.3.542)

Burd M. 1995 Ovule packaging in stochastic pollination and fertilization environments. *Evolution* **49**, 100–109. (doi:10.1111/j.1558-5646.1995.tb05962.x)

Campbell DR. 1991 Effects of floral traits on sequential components of fitness in *Ipomopsis aggregata*. *Am. Nat.* **137**, 713–737. (doi:10.1086/285190)

Campbell DR. 2000 Experimental tests of sex-allocation theory in plants. *Trends Ecol. Evol.* **15**, 227–232. (doi:10.1016/S0169-5347(00)01872-3)

Carlson JE. 2007 Male-biased nectar production in a protandrous herb matches predictions of sexual selection theory in plants. *Am. J. Bot.* **94**, 674–682. (doi:10.3732/AJB.94.4.674)

Carvalheiro LG, Seymour CL, Veldtman R, Nicolson SW. 2010 Pollination services decline with distance from natural habitat even in biodiversity-rich areas. J. Appl. Ecol. 47, 810–820. (doi:10.1111/j.1365-2664.2010.01829.x)

Charlesworth B, Charlesworth D. 1978 A model for the evolution of dioecy and gynodioecy. *Am. Nat.* **112**, 975–997.

Charlesworth D, Charlesworth B. 1987 Inbreeding depression and its evolutionary consequences. *Annu. Rev. Ecol. Syst.* **18**, 237–268.

Charlesworth D. 1999 Theories of the evolution of dioecy. In *Gender and Sexual dimorphism in Flowering Plants* (eds Geber, Monica A., Dawson, Todd D., Delph, Lynda F.), pp33-56. Germany: Springer-Verlag Berlin Heidelberg.

Charnov EL. 1979 Simultaneous hermaphroditism and sexual selection. *Proc. Natl. Acad. Sci.* **76**, 2480–2484. (doi:10.1073/PNAS.76.5.2480)

Charnov EL. 1982 *The Theory of Sex Allocation*. Princeton, New Jersey: Princeton University Press.

Christopher DA, Mitchell RJ, Karron JD. 2020 Pollination intensity and paternity in flowering plants. *Ann. Bot.* **125**, 1–9. (doi:10.1093/aob/mcz159)

Cintrón-Berdecía ST, Tremblay RL. 2006 Spatial variation in phenotypic selection on floral characteristics in an epiphytic orchid. *Folia Geobot.* **41**, 33–46. (doi:10.1007/BF02805260)

Clark JS, Silman M, Kern R, Macklin E, HilleRisLambers J. 1999 Seed dispersal near and far: patterns across temperate and tropical forests. *Ecology* **80**, 1475–1494.

Cocucci AA, Marino S, Baranzelli M, Wiemer AP, Sérsic A. 2014 The buck in the milkweed: evidence of male–male interference among pollinaria on pollinators. *New Phytol.* **203**, 280–286. (doi:10.1111/NPH.12766)

Conner JK, Rush S, Kercher S, Jennetten P. 1996 Measurements of natural selection on floral traits in wild radish (*Raphanus raphanistrum*). II. selection through lifetime male and total fitness. *Evolution* **50**, 1137–1146.

Conner JK, Rush S. 1996 Effects of flower size and number on pollinator visitation to wild radish, *Raphanus raphanistrum*. *Oecologia* **105**, 509–516. (doi:10.1007/BF00330014)

Conner JK. 2003 Artificial selection: a powerful tool for ecologists. Ecology 84, 1650–1660. (doi:10.1890/0012-9658(2003)084[1650:ASAPTF]2.0.CO;2)

Cruzan MB, Neal PR, Willson MF. 1988 Floral display in *Phyla incisa*: consequences for male and female reproductive success. *Evolution* **42**, 505–515.

Dai C, Galloway LF. 2013 Sexual selection in a hermaphroditic plant through female reproductive success. J. Evol. Biol. 26, 2622–2632. (doi:10.1111/JEB.12254)

Darwin C. 1877 *The Different Forms of Flowers on Plants of the Same Species*. Cambridge University Press.

Davies TJ et al. 2013 Phylogenetic conservatism in plant phenology. J. Ecol. 101, 1520–1530. (doi:10.1111/1365-2745.12154)

de Jager M, Newman E, Theron G, Botha P, Barton M, Anderson B. 2016 Pollinators can prefer rewarding models to mimics: consequences for the assumptions of Batesian floral mimicry. *Plant Syst. Evol.* **302**, 409–418. (doi:10.1007/s00606-015-1276-0)

de Jong TJ, Klinkhamer PGL. 1994 Plant size and reproductive success through female and male function. *J. Ecol.* **82**, 399. (doi:10.2307/2261307)

Delesalle VA, Muenchow GE. 1992 Opportunities for selfing and inbreeding depression in *Sagittaria* congeners (Alismataceae) with contrasting sexual systems. Evol. Trends Plants 6, 81–91.

Delmas CEL, Escaravage N, Cheptou P-O, Charrier O, Ruzafa S, Winterton P, Pornon A. 2015 Relative impact of mate versus pollinator availability on pollen limitation and outcrossing rates in a mass-flowering species. Plant Biol. 17, 209–218. (doi:10.1111/plb.12200)

Delph LF, Ashman T-L. 2006 Trait selection in flowering plants: how does sexual selection contribute? *Integr. Comp. Biol.* **46**. (doi:10.1093/icb/icj038)

Delph LF, Herlihy CR. 2012 Sexual, fecundity and viability selection on flower size and number in a sexually dimorphic plant. *Evolution* **66**, 1154–1166. (doi:10.1111/j.1558-5646.2011.01510.x)

Delph, LF, Johannsson MH, Stephenson AG. 1997. How Environmental factors affect pollen performance: Ecological and evolutionary perspectives. Ecology. 78, 1632-1639

Delph LF, Lively CM. 1992 Pollinator visitation, floral display, and nectar production of the sexual morphs of a gynodioecious shrub. *Oikos* **63**, 161–170. (doi:10.2307/3545374)

Devlin, B., J. Clegg, N. C. Ellstrand. 1992. The effect of flower production on male reproductive success in wild radish populations. Evolution 46:1030–1042

Diggle PK. 2003 Architectural effects on floral form and function: a review. *Regnum Veg.* , 63–80.

Dorken ME, Barrett SCH. 2003 Life-history differentiation and the maintenance of money and dioecy in *Sagittaria latifolia* (Alismataceae). *Evolution* **57**, 1973–1988. (doi:10.1111/J.0014-3820.2003.TB00378.X)

Dorken ME, Barrett SCH. 2004 Phenotypic plasticity of vegetative and reproductive traits in monoecious and dioecious populations of *Sagittaria latifolia* (Alismataceae): a clonal aquatic plant. *J. Ecol.* **92**, 32–44. (doi:10.1111/J.1365-2745.2004.00857.X)

Dorken ME, Barrett SCH. 2004 Sex determination and the evolution of dioecy from monoecy in *Sagittaria latifolia* (Alismataceae). Proc. R. Soc. Lond. B 271, 213–219. (doi:10.1098/rspb.2003.2580)

Dorken ME, Friedman J, Barrett SCH. 2002 The evolution and maintenance of monecy and dioecy in *Sagittaria latifolia* (Alismataceae). *Evolution* **56**, 31–41. (doi:10.1111/J.0014-3820.2002.TB00847.X)

Dorken ME, Perry LE. 2017 Correlated paternity measures mate monopolization and scales with the magnitude of sexual selection. J. Evol. Biol. 30, 377–387. (doi:10.1111/JEB.13013)

Eckhart VM. 1991 The effects of floral display on pollinator visitation vary among populations of *Phacelia linearis* (Hydrophyllaceae). *Evol. Ecol.* **5**, 370–384.

Fenster CB, Armbruster WS, Wilson P, Dudash MR, Thomson JD. 2004 Pollination syndromes and floral specialization. *Annu. Rev. Ecol. Evol. Syst.* **35**, 375–403. (doi:10.1146/annurev.ecolsys.34.011802.132347)

Fenster CB. 1991 Gene flow in *Chamaecrista fasiculata* (Leguminosae) i. Gene dispersal. Evolution 45, 398–409. (doi:10.1111/j.1558-5646.1991.tb04413.x)

Flanagan, Sarah P., Jones Adam G. 2019 The future of parentage analysis: from microsatellites to SNPS and beyond. *Mol. Ecol.* **28**, 544–567.

Fornoff F, Klein A-M, Hartig F, Benadi G, Venjakob C, Schaefer HM, Ebeling A. 2017 Functional flower traits and their diversity drive pollinator visitation. *Oikos* **126**, 1020–1030. (doi:10.1111/oik.03869)

Freckleton RP. 2002 On the misuse of residuals in ecology: regression of residuals vs. multiple regression. J. Anim. Ecol. 71, 542–545. (doi:10.1046/j.1365-2656.2002.00618.x)

Fritz AL, Nilsson LA. 1996 Reproductive success and gender variation in deceit-pollinated orchids. In Floral biology: studies on floral evolution in animal pollinated plants (eds DG Lloyd, SCH Barrett), pp. 319–333. New York, NY: Chapman & Hall.

Geber MA, Griffen LR. 2003 Inheritance and natural selection on functional traits. Int. J. Plant Sci. 164, 21–42. doi:10.1086/368233)

Ghazoul J. 2005 Pollen and seed dispersal among dispersed plants. *Biol. Rev.* **80**, 413–443. (doi:10.1017/S1464793105006731)

Glaettli M, Barrett SCH. 2008 Pollinator responses to variation in floral display and flower size in dioecious *Sagittaria latifolia* (Alismataceae). *New Phytol.* **179**, 1193–1201. (doi:10.1111/J.1469-8137.2008.02532.X)

Gómez JM, Perfectti F, Camacho JPM. 2006 Natural selection on *Erysimum mediohispanicum* flower shape: insights into the evolution of zygomorphy. *Am. Nat.* **168**, 531–545. (doi:10.1086/507048)

Gong Y-B, Huang S-Q. 2014 Interspecific variation in pollen–ovule ratio is negatively correlated with pollen transfer efficiency in a natural community. *Plant Biol.* **16**, 843–847. (doi:10.1111/plb.12151)

Gonzales E, Hamrick JL, Smouse PE, Dyer RJ. 2006 Pollen-mediated gene dispersal within continuous and fragmented populations of a forest understorey species, *Trillium cuneatum*. *Mol. Ecol.* **15**, 2047–2058. (doi:10.1111/j.1365-294X.2006.02913.x)

Grindeland JM, Sletvold N, Ims RA. 2005 Effects of floral display size and plant density on pollinator visitation rate in a natural population of *Digitalis purpurea*. *Funct. Ecol.* **19**, 383–390.

Grömping U. 2007 Relative mportance for linear regression in R: the package. *Am. Stat.* **61**, 139–147.

Hansen V-I, Totland Ø. 2006 Pollinator visitation, pollen limitation, and selection on flower size through female function in contrasting habitats within a population of *Campanula persicifolia*. *Can. J. Bot.* **84**, 412–420.

Hao K, Tian Z-X, Wang Z-C, Huang S-Q. 2020 Pollen grain size associated with pollinator feeding strategy. *Proc. R. Soc. B Biol. Sci.* **287**, 20201191. (doi:10.1098/rspb.2020.1191)

Harder LD, Barrett SCH, Cole WW. 2000 The mating consequences of sexual segregation within inflorescences of flowering plants. Proc. R. Soc. Lond B. 267, 315–320. (doi:10.1098/rspb.2000.1002)

Harder LD, Barrett SCH. 1996 Pollen dispersal and mating patterns in animal-pollinated plants. In Floral biology: studies on floral evolution in animal pollinated plants (eds DG Lloyd, SCH Barrett), pp. 149–190. New York, NY: Chapman & Hall.

Harder LD, Barrett SCH. 2006 *Ecology and Evolution of Flowers*. OUP Oxford.

Harder LD, Johnson SD. 2008 Function and evolution of aggregated pollen in angiosperms. *Int. J. Plant Sci.* **169**, 59–78.

Hasegawa Y, Suyama Y, Seiwa K. 2015 Variation in pollen-donor composition among pollinators in an entomophilous tree species, *Castanea crenata*, revealed by single-pollen genotyping. *PLOS ONE* **10**, e0120393. (doi:10.1371/journal.pone.0120393)

Henshaw JM, Jones AG, Schärer L. 2022 Anisogamy explains why males benefit more from additional matings. Nat. Commun. 13, 3893. (doi:10.1038/s41467-022-31620-w)

Herrera CM. 1995 Microclimate and individual variation in pollinators: flowering plants are more than their flowers. *Ecology* **76**, 1516–1524. (doi:10.2307/1938153)

Herrera CM. 2002 Censusing natural microgametophyte populations: variable spatial mosaics and extreme fine-graininess in winter-flowering *Helleborus foetidus* (Ranunculaceae). *Am. J. Bot.* **89**, 1570–1578. (doi:10.3732/ajb.89.10.1570)

Herrera CM. 2009 *Multiplicity in Unity, Plant Subindividual Variation and Interaction with Animals*. Chicago: The University of Chicago Press.

Higashiyama T, Hamamura Y. 2008 Gametophytic pollen tube guidance. *Sex. Plant Reprod.* **21**, 17–26. (doi:10.1007/s00497-007-0064-6)

Hodgins KA, Barrett SCH. 2008 Natural selection floral traits through male and female function in wild populations of heterstylous daffodil *Narcissus triandrus*. *Evolution* **62**, 1751–1763. (doi:10.1111/J.1558-5646.2008.00404.X)

Höglund J, Säterberg L. 1989 Sexual selection in common toads: correlates with age and body size. J. Evol. Biol. 2, 367–372. (doi:10.1046/J.1420-9101.1989.2050367.X)

Holt R, Kwok A, Dorken ME. 2019 Increased spatial genetic structure in a population of the clonal aquatic plant *Sagittaria latifolia* (Alismataceae) following disturbance. Heredity 124, 514–523. (doi:10.1038/s41437-019-0286-z)

Huang S-Q, Tang L-L, Sun J-F, Lu Y. 2006 Pollinator response to female and male floral display in a monoecious species and its implications for the evolution of floral dimorphism. *New Phytol.* **171**, 417–424. (doi:10.1111/J.1469-8137.2006.01766.X)

Huang SQ, Tang LL, Yu Q, Guo YH. 2004 Temporal floral sex allocation in protogynous *Aquilegia yabeana* contrasts with protandrous species: Support for the mating environment hypothesis. Evolution 58, 1131–1134. (doi:10.1111/j.0014-3820.2004.tb00446.x)

Immler S, Otto SP. 2018 The evolutionary consequences of selection at the haploid gametic stage. *Am. Nat.* **192**, 241–249. (doi:10.1086/698483)

Inoue K, Maki M, Masuda M. 1995 Different responses of pollinating bees to size variation and sexual phases in flowers of *Campanula*. *Ecol. Res.* **10**, 267–273. (doi:10.1007/BF02347852)

Irwin RE, Brody AK. 2000 Consequences of nectar robbing for realized male function in a hummingbird-pollinated plant. *Ecology* **81**, 2637–2643.

Ishii HS, Harder LD. 2006 The size of individual *Delphinium* flowers and the opportunity for geitonogamous pollination. *Funct. Ecol.* **20**, 1115–1123. (doi:10.1111/j.1365-2435.2006.01181.x)

Ison JL, Wagenius S. 2014 Both flowering time and distance to conspecific plants affect reproduction in *Echinacea angustifolia*, a common prairie perennial. J. Ecol. 102, 920–929. (doi:10.1111/1365-2745.12262)

Janicke T, Häderer I, Lajeunesse MJ, Anthes N. 2016 Darwinian sex roles confirmed across the animal kingdom. Sci. Adv. 2, e1500983. (doi:10.1126/sciadv.1500983)

Jennions MD, Kokko H, Klug H. 2012 The opportunity to be misled in studies of sexual selection. J. Evol. Biol. 25, 591–598. (doi:10.1111/j.1420-9101.2011.02451.x)

Johnson MG, Shaw AJ. 2016 The effects of quantitative fecundity in the haploid stage on reproductive success and diploid fitness in the aquatic peat moss *Sphagnum macropyllum*. Heredity 116, 523–530. (doi:10.1038/hdy.2016.13)

Johnson SG, Delph LF, Elderkin CL. 1995 The effect of petal-size manipulation on pollen removal, seed set, and insect-visitor behavior in *Campanula americana*. *Oecologia* **102**, 174–179. (doi:10.1007/BF00333249)

Jones AG, Ratterman NL. 2009 Mate choice and sexual selection: what have we learned since Darwin? Proc. Natl Acad. Sci. USA 106, 10 001–10008. (doi:10.1073/PNAS.0901129106)

Jones AG. 2009 On the opportunity for sexual selection, the Bateman gradient and the maximum intensity of sexual selection. *Evolution* **63**, 1673–1684. (doi:10.1111/J.1558-5646.2009.00664.X)

Jones OR, Wang J. 2010 COLONY: a program for parentage and sibship inference from multilocus genotype data. Mol. Ecol. 10, 551–555. (doi:10.1111/j.1755-0998.2009.02787.x)

Karron JD, Ivey CT, Mitchell RJ, Whitehead MR, Peakell R, Case AL. 2012 New perspectives on the evolution of plant mating systems. *Ann. Bot.* **109**, 493–503.

Karron JD, Mitchell RJ. 2012 Effects of floral display size on male and female reproductive success in *Mimulus ringens*. *Ann. Bot.* **109**, 563–570. (doi:10.1093/AOB/MCR193)

Karron JD, Thumser NN, Tucker R, Hessenauer AJ. 1995 The influence of population density on outcrossing rates in Mimulus ringens. Heredity. 75, 175–180. (doi:10.1038/hdy.1995.121)

Kearns CA, Inouye DW. 1993 *Techniques for Pollination Biologists*. University Press of Colorado. See https://upcolorado.com/university-press-of-colorado/item/1694-techniques-for-pollination-biologists.

Kekäläinen J, Evans JP. 2018 Gamete-mediated mate choice: towards a more inclusive view of sexual selection. Proc. R. Soc. B 285, 20180836. (doi:10.1098/rspb.2018.0836)

Kessler D, Kallenbach M, Diezel C, Rothe E, Murdock M, Baldwin IT. 2015. How scent and nectar influence floral antagonists and mutualists. *eLife* **2015**, e077641.

Kilkenny FF, Galloway LF. 2008 Reproductive success in varying light environments: direct and indirect effects of light on plants and pollinators. *Oecologia* **155**, 247–255. (doi:10.1007/s00442-007-0903-z)

Klinkhamer PGL, de Jong TJ, Metz H. 1997 Sex and size in cosexual plants. Trends Ecol. Evol. 12, 260–265. (doi:10.1016/S0169-5347(97)01078-1)

Klug H, Heuschele J, Jennions MD, Kokko H. 2010 The mismeasurement of sexual selection. J. Evol.Biol. 23, 447–462. (doi:10.1111/J.1420-9101.2009.01921.X)

Klug H, Heuschele J, Jennions MD, Kokko H. 2010 The mismeasurement of sexual

Knight TM. Steets JA, Vamosi JC, Mazer SJ, Burd M, Campbell DR, Dudash MR, Johnston MO, Mitchell RJ, Ashman T-L. 2005 Pollen limitation of plant reproduction: pattern and process. Annu. Rev. Ecol. Evol. Syst. 36, 467–497. (doi:10.1146/annurev.ecolsys.36.102403.115320)

Kochmer JP, Handel SN. 1986 Constraints and competition in the evolution of flowering phenology. Ecol. Monogr. 56, 303–325. (doi:10.2307/1942549)

Kokko H, Brooks R, McNamara JM, Houston AI. 2002 The sexual selection continuum. Proc. R. Soc. B 269, 1331–1340. (doi:10.1098/RSPB.2002.2020)

Kokko H, Jennions MD, Brooks R. 2006 Unifying and testing models of sexual selection. Annu. Rev. Ecol. Evol. Syst. 37, 43–66. (doi:10.1146/annurev.ecolsys. 37.091305.110259)

Kokko H, Jennions MD. 2008 Parental investment, sexual selection and sex ratios. J. Evol. Biol. 21, 919–948. (doi:10.1111/j.1420-9101.2008.01540.x)

Kokko H, Klug H, Jennions MD. 2012 Unifying cornerstones of sexual selection: operational sex ratio, Bateman gradient and the scope for competitive investment. Ecol. Lett. 15, 1340–1351. (doi:10.1111/J.1461-0248.2012.01859.X)

Krakauer AH, Webster MS, Duval E. H, Jones A. G., Shuster S. M. 2011 The opportunity for sexual selection: not mismeasured, just misunderstood. *J. Evol. Biol.* **24**, 2064–2071. (doi:10.1111/J.1420-9101.2011.02317.X)

Kudo G, Harder LD. 2005 Floral and inflorescence effects on variation in pollen removal and seed production among six legume species. *Funct. Ecol.* **19**, 245–254. (doi:10.1111/J.1365-2435.2005.00961.X)

Kudo G. 1996 Flowering phenologies of animal-pollinated plants: reproductive strategies and agents of selection. In *Ecology and Evolution of Flowers* (eds Lloyd, David G., Barrett, Spencer C.H.), pp. 139–158. New York, NY: Chapman & Hall.

Kulbaba MW, Worley AC. 2012 Selection on floral design in *Polemonium brandegeei* (Polemoniaceae): female and male fitness under hawkmoth pollination. *Evolution* **66**, 1344–1359. (doi:10.1111/j.1558-5646.2011.01536.x)

Kuznetsova A, Brockhoff PB, Christensen RHB. 2017 lmerTest package: Tests in linear mixed effects models. J. Stat. Softw. 82, 1–26. (doi:10.18637/jss.v082.i13)

Kwok A, Dorken ME. 2022 Sexual selection on male but not female function in monoecious and dioecious populations of broadleaf arrowhead (*Sagittaria latifolia*). *Proc. R. Soc. B Biol. Sci.* **289**, 20220919. (doi:10.1098/rspb.2022.0919)

LaMunyon CW, Bouban O, Cutter AD. 2007 Postcopulatory sexual selection reduces genetic diversity in experimental populations of *Caenorhabditis elegans*. J. Hered. 98, 67–72. (doi:10.1093/JHERED/ESL052)

Lankinen A, Armbruster WS, Antonsen L. 2007 Delayed stigma receptivity in *Collinsia heterophylla* (Plantaginaceae): genetic variation and adaptive significance in relation to pollen competition, delayed self-pollination, and mating-system evolution. *Am. J. Bot.* **94**, 1183–1192. (doi:10.3732/ajb.94.7.1183)

Lankinen A, Hydbom S, Strandh M. 2017 Sexually antagonistic evolution caused by male–male competition in the pistil. Evolution 21, 2359–2369. (doi:10.1111/evo.13329)

Larue A-AC, Raguso RA, Junker RR. 2016 Experimental manipulation of floral scent bouquets restructures flower–visitor interactions in the. *J. Anim. Ecol.* **85**, 396–408.

Lau TC, Stephenson AG. 1993. Effects of soil nitrogen on pollen production, pollen grain size, and pollen performance in *Cucurbita pepo* (Cucurbitaceae). *AJB* **80**, 763–768.

Lau TC, Stephenson AG. 1994. Effects of soil phosphorus on pollen production, pollen size, pollen phosphorus content, and the ability to sire seeds in *Cucurbita pepo* (Cucurbitaceae). *Sexual Plant Reproduction* **7**, 215–220.

Lavaut E, Valero M, Mauger S, Guillemin ML, Destombe C, Dufay M. 2023. Sexual selection in seaweed? Testing Bateman's principles in red alga *Gracilaria gracilis*. Proc. R. Soc. B. **290**, 20231668

Lechowicz MJ, Bell G. 1991 The ecology and genetics of fitness in forest plants. II. microspatial heterogeneity of the edaphic environment. *J. Ecol.* **79**, 687–696.

Lehtonen J. 2022 Bateman gradients from first principles. *Nat. Commun.* **13**, 3591.

Levin DA, Kerster HW. 1974 Gene flow in seed plants. Evol Biol. 7, 139–220. (doi:10.1007/978-1-4615-6944-2_5)

Levin DA. 1971 The origin of reproductive isolating mechanisms in flowering plants. *Taxon* **20**, 91–113.

Liao W-J, Harder LD. 2014 Consequences of multiple inflorescences and clonality for pollinator behavior and plant mating. *Am. Nat.* **184**, 543–694.

Lindenfors P, Tullberg BS. 1998 Phylogenetic analyses of primate size evolution: the consequences of sexual selection. Biol. J.Linn. Soc. 64, 413–447. (doi:10.1111/J.1095-8312.1998.TB00342.X)

Lloyd DG, Bawa KS. 1984 Modification of the gender of seed plants in varying conditions. Evol. Biol. 17, 255–338. (doi:10.1007/978-1-4615-6974-9_6)

Lloyd DG, Webb CJ. 1977 Secondary sex characters in plants. *Bot. Rev.* **43**, 177–216. (doi:10.1007/BF02860717)

Lloyd DG, Webb CJ. 1986 The avoidance of interference between the presentation of pollen and stigmas in angiosperms. I. dichogamy. N. Zeal J. Bot. 86, 135–162. (doi:10.1080/0028825X.1986.10409725)

Lloyd DG. 1992 Self- and cross-fertilization in plants. II. The selection of self-fertilization. Int. J. Plant Sci. 153, 370–380. (doi:10.1086/297041)

Marshall DL, Ellstrand NC. 1986 Sexual Selection in *Raphanus sativus*: Experimental Data on nonrandom fertilization, maternal choice, and consequences of multiple paternity. *Am. Nat.* **127**, 446–461. (doi:10.1086/284494)

Marshall DL, NC Ellstrand. 1985 Proximal causes of multiple paternity in wild radish, *Raphanus sativus*. *Am Nat* **126**, 596–605. (doi:10.1086/284441)

Marshall TC, Slate J, Kruuk LEB, Pemberton JM. 1998 Statistical confidence for likelihood-based paternity inference in natural populations. Mole. Ecol. 7, 639–655. (doi:10.1046/J.1365-294X.1998.00374.X)

Matsuhisa S, Ushimaru A. 2015 Sexual dimorphism in floral longevity and flowering synchrony in relation to pollination and mating success in three dioecious *Ilex* species. *Am. J. Bot.* **102**, 1187–1197. (doi:10.3732/AJB.1500073)

Mazer SJ, Dawson KA. 2001 Size-dependent sex allocation within flowers of the annual herb *Clarkia unguiculata* (Onagraceae): Ontogenetic and among-plant variation. Am. J. Bot. 88, 819–831. (doi:10.2307/2657034)

McCallum B, Chang SM. 2016 Pollen competition in style: effects of pollen size on siring success in the hermaphroditic common morning glory, *Ipomoea purpurea*. Am. J. Bot. 103, 460–470. (doi:10.3732/ajb.1500211)

McDowall RM. 1994 On size and growth in freshwater fish. Ecol. Fres. Fish 3, 67–79. (doi:10.1111/J.1600-633.1994.TB00108.X)

Minnaar C, Anderson B. 2019 Using quantum dots as pollen labels to track the fates of individual pollen grains. *Methods Ecol. Evol.* **10**, 604–614. (doi:10.1111/2041-210X.13155)

Minnaar C, de Jager M, Anderson B. 2019 Intraspecific divergence in floral-tube length promotes asymmetric pollen movement and reproductive isolation. *New Phytol.* **224**, 1160–1170.

Miyake T, Yahara T. 1999 Theoretical evaluation of pollen transfer by nocturnal and diurnal pollinators: When Should a Flower Open? *Oikos* **86**, 233–240. (doi:10.2307/3546441)

Møller AP, Eriksson M, Moller AP. 1995 Pollinator preference for symmetrical flowers and sexual selection in plants. *Oikos* **73**, 15. (doi:10.2307/3545720)

Moore JC, Pannell JR. 2011 Sexual selection in plants. *Curr. Biol.* **21**, R176–R182. (doi:10.1016/J.CUB.2010.12.035)

Morgan MT, Schoen DJ. 1997 Selection on reproductive characters: floral morphology in *Asclepias syriaca*. Heredity 79, 433–441. (doi:10.1038/hdy.1997.178)

Morgan MT. 2006 Selection on reproductive characters: conceptual foundations and their extension to pollinator interactions. In *Ecology and Evolution of Flowers* (eds Lloyd, David G., Barrett, Spencer C.H.), pp. 25–40. New York, NY: Chapman & Hall.

Mosquin T. 1971 Competition for pollinators as a stimulus for the evolution of flowering time. *Oikos* **22**, 398–402. (doi:10.2307/3543864)

Muenchow G, Delesalle V. 1994 Pollinator response to male floral display size in two *Sagittaria* (Alismataceae) species. *Am. J. Bot.* **81**, 568–573. (doi:10.1002/J.1537-2197.1994.TB15486.X)

Murphy CG. 1998 Interaction-independent sexual selection and the mechanisms of sexual selection. *Evolution* **52**. (doi:10.1111/j.1558-5646.1998.tb05133.x)

Mustajärvi K, Siikamäki P, Rytkönen S, Lammi A. 2001 Consequences of plant population size and density for plant-pollinator interactions and plant performance. *J. Ecol.* **89**, 80–87.

Obeso JR. 2002 The costs of reproduction in plants. New Phyt. 155, 321–348. (doi:10.1046/J.1469-8137.2002.00477.X)

Opedal ØH, Pérez-Barrales R, Brito VLG, Muchhala N, Capó M, Dellinger A. 2023 Pollen as the link between floral phenotype and fitness. *Am. J. Bot.* **110**, e16200. (doi:10.1002/ajb2.16200)

Ousterhout BH, Anderson TL, Drake DL, Peterman WE, Semlitsch RD. 2015 Habitat traits and species interactions differentially affect abundance and body size in pond-breeding amphibians. J. Anim. Ecol. 84, 914–924. (doi:10.1111/1365-656.12344)

Panhuis TM, Butlin R, Zuk M, Tregenza T. 2001 Sexual selection and speciation. Trends Ecol.Evol. 16, 346–371. (doi:10.1016/S0169-5347(01)02160-7)

Pannell JR. 2002 The evolution and maintenance of androdioecy. *Annu. Rev. Ecol. Evol. Syst.* **33**, 397–425. (doi:10.1146/annurev.ecolsys.33.010802.150419)

Parachnowitsch AL, Kessler A. 2010 Pollinators exert natural selection on flower size and floral display in *Penstemon digitalis*. *New Phytol.* **188**, 393–402. (doi:10.1111/j.1469-8137.2010.03410.x)

Pélissié B, Jarne P, David P. 2012 Sexual selection without sexual dimorphism: Bateman gradients in a simultaneous hermaphrodite. *Evolution* **66**, 66–81. (doi:10.1111/J.1558-5646.2011.01442.X)

Perry LE, Dorken ME. 2011 The evolution of males: support for predictions from sex allocation theory using mating arrays of *Sagittaria latifolia* (Alismataceae). Evolution 65, 2782–2791. (doi:10.1111/j.1558-5646.2011.01344.x)

Primack RB, Kang H. 1989 Measuring fitness and natural selection in wild plant populations. Annu. Rev. Ecol. Syst. 20, 367–396. (doi:10.1146/annurev.es.20.110189.002055)

Quesada, M., K. Bollman, and A. G. Stephenson. 1995. Leaf damage decreases pollen production and hinders pollen performance in *Cucurbita texana. Ecology* **76**, 437–443.

Quinn G, Keough M. In press. *Experimental design and data analysis for biologists.* Cambridge University Press.

R Core Team. 2023 R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/

Rasmussen IR, Brødsgaard B. 1992 Gene flow inferred from seed dispersal and pollinator behaviour compared to DNA analysis of restriction site variation in a patchy population of *Lotus corniculatus* L. *Oecologia* **89**, 277–283. (doi:10.1007/BF00317228)

Reznick D. 1985 Costs of reproduction: an evaluation of the empirical evidence. Oikos 44, 257–267. (doi:10.2307/3544698)

Richards SA, Wlliams NM, Harder LD. 2009 Variation in pollination: causes and consequences for plant reproduction. *Am. Nat.* **174**, 382–398.

Robertson AW, MacNair MR. 1995 The effects of floral display size on pollinator service to individual flowers of *Myosotis* and *Mimulus*. *Oikos* **72**, 106–114. (doi:10.2307/3546044)

Routley MB, Husband BC. 2003 The effect of protandry on siring success in *Chamerion angustifolium* (Onagraceae) with different inflorescence sizes. Evolution 57, 240–248. (doi:10.1111/j.0014-3820.2003.tb00259.x)

Runquist RDB, Geber MA, Pickett-Leonard M, Moeller DA. 2017 Mating system evolution under strong pollen limitation: evidence of disruptive selection through male and female fitness in *Clarkia xantiana*. *Am. Nat.* **189**, 549–563. (doi:10.1086/691192)

Sakai AK, Weller SG. 1999 Gender and sexual dimorphism in flowering plants: a review of terminology, biogeographic patterns, ecological correlates, and phylogenetic approaches. In *Gender and Sexual Dimorphism in Flowering Plants* (eds MA Geber, TE Dawson, LF Delph), pp. 1–31. Berlin, Heidelberg: Springer. (doi:10.1007/978-3-662-03908-3_1)

Samson DA, Werk KS. 1986 Size-dependent effects in the analysis of reproductive effort in plants. Am. Nat. 127, 667–680. (doi:10.1086/284512)

Sánchez-Lafuente AM, Guitián J, Medrano M, Herrera CM, Rey PJ, Cerdá X. 2005 Plant traits, environmental factors, and pollinator visitation in winter-flowering *Helleborus foetidus* (Ranunculaceae). *Ann. Bot.* **96**, 845–852. (doi:10.1093/aob/mci236)

Santos del Blanco L, Tudor E, Pannell JR. 2019 Low siring success of females with an acquired male function illustrates the legacy of sexual dimorphism in constraining the breakdown of dioecy. *Ecol. Lett.* **22**, 486–497.

Sargent RD, Mandegar MA, Otto SP. 2006 A model of the evolution of dichogamy incorporating sex-ratio selection, anther-stigma interference, and inbreeding depression. Evolution 60, 934–944. (doi:10.1111/j.0014-3820.2006.tb01172.x)

Sarkissian TS, Barrett SCH, Harder LD. 2001 Gender variation in *Sagittaria latifolia* (Alismataceae): is size all that matters? *Ecology* **82**, 360–373. (doi:10.1890/0012-9658)

Schemske DW, Willson MF, Melampy MN, Miller LJ, Verner L, Schemske KM, Best LB. 1978 Flowering ecology of some spring woodland herbs. *Ecology* **59**, 351–366. (doi:10.2307/1936379)

Schmitt J. 1980 Pollinator foraging behavior and gene dispersal in *Senecio* (Compositae). *Evolution* **34**, 934–943. (doi:10.2307/2407999)

Schnittler M, Eusemann • P, Eusemann ÁP. 2010 Consequences of genotyping errors for estimation of clonality: a case study on *Populus euphratica* Oliv. (Salicaceae). Evol. Ecol. 24, 1417–1432. (doi:10.1007/s10682-010-9389-y)

Seehausen O, van Alphen JJM. 1999 Can sympatric speciation by disruptive sexual selection explain rapid evolution of cichlid diversity in Lake Victoria? Ecol. Lett. 2, 262–271. (doi:10.1046/j.1461-0248.1999.00082.x)

Setsuko S, Nagamitsu T, Tomaru N. 2013 Pollen flow and effects of population structure on selfing rates and female and male reproductive success in fragmented *Magnolia stellata* populations. *BMC Ecol.* **13**, 10. (doi:10.1186/1472-6785-13-10)

Shimada Y, Shikano T, Kuparinen A, Gonda A, Leinonen T, Merilä J, Liu Z. 2011 Quantitative genetics of body size and timing of maturation in two nine-spined stickleback (*Pungitius pungitius*) populations. PLoS ONE 6, e28859. (doi:10.1371/journal.pone.0028859)

Simmons LW. 2014 Sexual selection and genital evolution. Aust. Ento. 53, 1–17. (doi:10.1111/aen.12053)

Skogsmyr I, Lankinen Å. 2000 Potential selection for female choice in *Viola tricolor*. *Evol. Ecol. Res.* **2**, 965–979.

Skogsmyr I, Lankinen Å. 2002 Sexual selection: an evolutionary force in plants? *Biol. Rev.* **77**, 537–562. (doi:10.1017/S1464793102005973)

Smithson A, MacNair MR. 1997 Negative frequency-dependent selection by pollinatros on artificial flowers without rewards. *Evolution* **51**, 715–723.

Sowig P. 1989 Effects of flowering plant's patch size on species composition of pollinator communities, foraging strategies, and resource partitioning in bumblebees (Hymenoptera: Apidae). *Oecologia* **78**, 550–558.

Stanton ML, Preston RE. 1988 Ecological consequences and phenotypic correlates of petal size variation in wild radish, *Raphanus sativus* (Brassicaceae). Am. J. Bot. 75, 528–539. (doi:10.1002/j.1537-2197.1988.tb13471.x)

Stanton ML. 1994 Male-male competition during pollination in plant populations. *Am. Nat.* **144**, S40–S68. (doi:10.1086/285652)

Stephens S, Kleunen M van, Dorken ME. 2020 Patterns of pollen dispersal and mating in a population of the clonal plant *Sagittaria latifolia*. *J. Ecol.* **108**, 1941–1955. (doi:10.1111/1365-2745.13399)

Stephenson, A. G., C. W. Erickson, T-C. Lau, M. R. Quesada, and J. A. Winsor. 1994. Effects of growing conditions on the male gametophyte. Pages 220–229 in A. G. Stephenson and T-h. Kao, editors. Pollen–pistil interactions and pollen tube growth. Volume 12. Current Topics in Plant Physiology. An American Society of Plant Physiologists Series, Rockville, Maryland, USA.

Swanson RJ, Hammond AT, Carlson AL, Gong H, Donovan TK. 2016 Pollen performance traits reveal prezygotic nonrandom mating and interference competition in *Arabidopsis thaliana*. Am. J. Bot.103, 498–513. (doi:10.3732/ajb.1500172)

Székely T, Reynolds JD, Figuerola J. 2000 Sexual size dimorphism in shorebirds, gulls and alcids: the influence of sexual and natural selection. Evolution 54, 1404–1413. (doi:10.1111/J.0014-3820.2000.TB00572.X)

Tejaswini. 2002 Variability of pollen grain features: a plant strategy to maximize reproductive fitness in two species of *Dianthus*? *Sex. Plant Reprod.* **14**, 347–353. (doi:10.1007/s00497-002-0130-z)

Thompson JD. 1988 Effects of variation in inflorescence size and floral rewards on the visitation rates of trap lining pollinators of *Aralia hispida*. *Evol. Ecol.* **2**, 65–76.

Thompson JD. 2001 How do visitation patterns vary among pollinators in relation to floral display and floral design in a generalist pollination system? Oecologia 126, 386–394. (doi:10.1007/S004420000531)

Tomaszewski CE, Kulbaba MW, Harder LD. 2018 Mating consequences of contrasting hermaphroditic plant sexual systems. *Evolution* **72**, 2114–2128. (doi:10.1111/evo.13572)

Tonidandel S, LeBreton JM. 2010 Determining the relative importance of predictors in logistic regression: an extension of relative weight analysis. *Organ. Res. Methods* **13**, 767–781. (doi:10.1177/1094428109341993)

Tonnabel J, Cosette P, Lehner A, Mollet JC, Mlouka MAB, Grladinovic L, David P, Pannel JR. 2022 Rapid evolution of pollen and pistil traits as a response to sexual selection in the post-pollination phase of mating. Curr. Biol. 32, 1–8. (doi:10.1016/j.cub.2021.10.007)

Tonnabel J, David P, Janicke T, Lehner A, Mollet J-C, Pannell JR, Dufay M. 2021 The scope for postmating sexual selection in plants. *Trends Ecol. Evol.* **36**, 556–567. (doi:10.1016/j.tree.2021.02.013)

Tonnabel J, David P, Pannell JR. 2019 Do metrics of sexual selection conform to Bateman's principles in a wind-pollinated plant? *Proc. R. Soc. B* **286**. (doi:10.1098/RSPB.2019.0532)

Totland Ø, Andersen HL, Bjelland T, Dahl V, Eide W, Houge S, Pedersen TR, Vie EU. 1998 Variation in pollen limitation among plants and phenotypic selection on floral traits in an early-spring flowering herb. *Oikos* **82**, 491–501. (doi:10.2307/3546370)

Van Drunen WE, Dorken ME. 2012 Trade-offs between clonal and sexual reproduction in *Sagittaria latifolia* (Alismataceae) scale up to affect the fitness of entire clones. *New Phytol.* **196**, 606–616. (doi:10.1111/J.1469-8137.2012.04260.X)

Van Drunen WE., van Kleunen M, Dorken ME. 2015 Consequences of clonality for sexual fitness: clonal expansion enhances fitness under spatially restricted dispersal. *Proc. Natl. Acad. Sci.* **112**, 8929–8936.

Venables WN, Ripley BD. 2002 Random and mixed effects. In *Modern Applied Statistics with S* (eds WN Venables, BD Ripley), pp. 271–300. New York, NY: Springer. (doi:10.1007/978-0-387-21706-2_10)

Villamil N, Boege K, Stone GN. 2022 Defensive mutualists affect outcross pollen transfer and male fitness in their host plant. *Oikos* **2022**, e08788.

Wang J, Santure AW. 2009 Parentage and sibship inference from multilocus genotype data under polygamy. *Genetics* **181**, 1579–1594. (doi:10.1534/genetics.108.100214)

Wang J. 2004 Estimating pairwise relatedness from dominant genetic markers. *Mol. Ecol.* **13**, 3169–3178. (doi:10.1111/J.1365-294X.2004.02298.X)

Wang J. 2004 Sibship reconstruction from genetic data with typing errors. Genetics 166, 1963 1979. (doi:10.1093/genetics/166.4.1963)

Wang J. 2012 Computationally Efficient Sibship and Parentage Assignment from Multilocus Marker Data. Genetics 191, 183–194. (doi:10.1534/GENETICS.111.138149)

Wang J. 2017 Estimating genotyping errors from a genotype and reconstructed pedigree data. *Meth. Ecol. Evol.* **9**. 109–120. (doi:10.1111/2041-210X.12859)

Wang J. 2019 Pedigree reconstruction from poor quality genotype data. *Heredity* **122**, 719–728. (doi:10.1038/s41437-018-0178-7)

Waser NM, Price MV. 1983 Pollinator behaviour and natural selection for flower colour in *Delphinium nelsonii*. *Nature* **302**, 422–424. (doi:10.1038/302422a0)

Willson MF. 1979 Sexual selection in plants. *Am. Nat.* **113**, 777–790. (doi:10.1086/283437)

Willson MF. 1990 Sexual selection in plants and animals. *Trends Ecol. Evol.* **5**, 210–214. (doi:10.1016/0169-5347(90)90133-X)

Willson MF. 1994 Sexual selection in plants: perspective and overview. *Am. Nat.* **144**, S13–S39. (doi:10.1086/285651)

Wilson P, Thomson JD. 1991 Heterogeneity among floral visitors leads to discordance between removal and deposition of pollen. *Ecology* **72**, 1503–1507. (doi:10.2307/1941124)

Wood S, Scheipl F. 2020 gamm4: Generalized additive mixed models using 'mgcv' and 'lme4' [Internet]. See https://CRAN.R-project.org/package=gamm4.

Wood SN. 2004 Stable and efficient multiple smoothing parameter estimation for generalized additive models. J Am Stat Assoc. 99, 673–686. (doi:10.1198/016214504000000980)

Wooten JW. 1971 The monoecious and dioecious conditions in *Sagittaria latifolia* L. (Alismataceae). *Evolution* **25**, 549–553. (doi:10.2307/2407354)

Wright VL, Dorken ME. 2014 Sexual dimorphism in leaf nitrogen content but not photosynthetic rates in *Sagittaria latifolia* (Alismataceae). Botany 92, 109–112. (doi:10.1139/cjb-2013-0246)

Yakimowski SB, Barrett SCH. 2014 Clonal genetic structure and diversity in populations of an aquatic plant with combined vs. separate sexes. *Mol. Ecol.* **23**, 2914–2928. (doi:10.1111/MEC.12791)

Yakimowski SB, Glaettli M, Barrett SCH. 2011 Floral dimorphism in plant populations with combined versus separate sexes. *Ann. Bot.* **108**, 765–776. (doi:10.1093/AOB/MCR025)

Yakimowski SB, Glaettli M, Barrett SCH. 2011 Floral dimorphism in plant populations with combined versus separate sexes. *Ann. Bot.* **108**, 765–776. (doi:10.1093/AOB/MCR025)

Yakimowski SB, Rymer PD, Stone H, Barrett SCH, Dorken ME. 2009 Isolation and characterization of 11 microsatellite markers from *Sagittaria latifolia* (Alismataceae). *Mol. Ecol. Resour.* **9**, 579–581. (doi:10.1111/j.1755-0998.2008.02400.x)

Young HJ, Stanton ML. 1990 Influences of floral variation on pollen removal and seed production in wild radish. *Ecology* **71**, 536–547.

Younginger BS, Sirová D, Cruzan MB, Ballhorn DJ. 2017 Is biomass a reliable estimate of plant fitness? *Appl. Plant Sci.* **5**, 1600094. (doi:10.3732/apps.1600094)

Zhang DY. 2006 Evolutionarily stable reproductive investment and sex allocation in plants. In Ecology and evolution of flowers (eds LD Harder, SCH Barrett), pp. 41–60. Oxford, UK: Oxford University Press.

Zhou J, Reynolds RJ, Zimmer EA, Dudash MR, Fenster CB. 2020 Variable and sexually conflicting selection on Silene stellata floral traits by a putative moth pollinator selective agent. Evolution 74, 1321–1334. (doi:10.1111/evo.13965)

Zuur A, Ieno EN, Walker N, Saveliev AA, Smith GM. 2009 Mixed effects models and extensions in ecology with r. New York, NY: Springer.

7. APPENDIX

7.1 Appendix: Sexual selection on male but not female function in monoecious and dioecious populations of Broadleaf Arrowhead (*Sagittaria latifolia*)

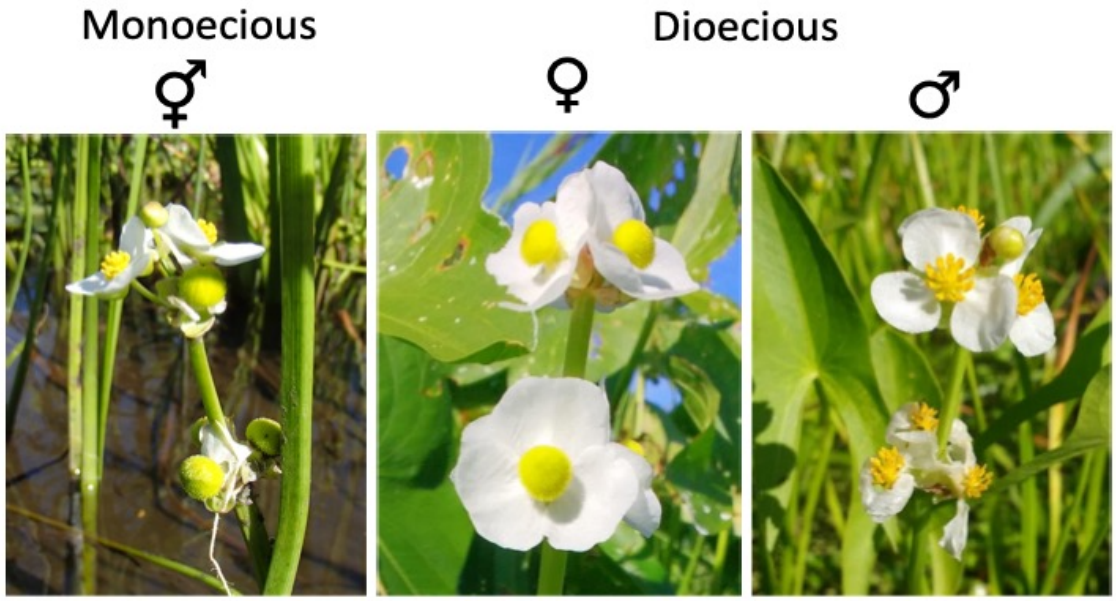

Figure A7.1.1 Photos of inflorescences in the monoecious and dioecious populations of *Sagittaria latifolia* studied here. Left: a hermaphrodite inflorescence from the monoecious population showing senesced female flowers at basal nodes and open male flowers at distal nodes. Center and right: Female (center) and male (right) inflorescences from the dioecious population.

DNA extraction and SRR genotyping

Leaf tissue was dried using silica and then stored at 4 °C. To extract genomic DNA for assignments of ramets to genets, we homogenized approximately 5 mg of dried leaf tissue using a MM 300 Retsch mixer mill (Haan, Germany). We followed procedures for leaf DNA extraction used in previous studies of *S. latifolia* (Holt et al. 2020; Stephens et al. 2020).

Leaf DNA was extracted using E.Z.N.A. Plant Extraction kits (Omega Bio-Tek Inc., GA, USA) following the manufacturer's plant DNA dried sample protocol and eluted to a final volume of 100 µl. To extract seed DNA, we first separated seeds from maternally derived tissues after soaking them in water. We sampled a total of nine seeds per fruit from each ramet for subsequent genotyping. Seed DNA was extracted using the QuickExtract Plant Seed DNA Extraction Solution (Lucigen, Madison WI) following the manufacturer's protocol, and eluted to a final volume of 50 µl. Not all fruits were fully mature and produce insufficient progeny DNA for our analyses. A total of 350 seeds from 33 ramets from the monoecious population and 448 seeds from 36 ramets from the dioecious population were used for parentage analysis.

Leaf samples were genotyped at eight SSR microsatellite loci (SL06, SL09, SL21, SL 27, SL30, SL65, SL74, and SL75) (Yakimowski et al. 2009) and seeds were genotyped at either three (SL27, SL74, SL75 for the monoecious population and SL65, SL74, SL75 for the dioecious population) or all eight loci. Nine seeds per ramet per day per population were genotyped; three of these nine seeds were genotyped at all eight loci and the rest at the three loci listed above (total sample of seeds genotyped at eight loci: 113 seeds from the monoecious population, 151 seeds from the dioecious population; 3 loci: 247 seeds from the monoecious population, 297 seeds from the dioecious population). Our initial aim for genotyping additional seeds at 3 loci was to enable the calculation of outcrossing rates and correlated paternity. Those data are not presented here, however, seeds genotyped at three loci were also useful for paternity assignment and are included here. Each sample of DNA was amplified at each of the loci specified above and sequenced following methods in (Yakimowski et al. 2009).

Successfully amplified DNA samples were genotyped using an automated sequencer (Applied Biosystems 3730 DNA analyzer). Each run included a ROX 500 size standard (Applied biosystems, Waltham, MA, USA) and results were scored manually using GeneMarker® (SoftGenetics). Locus SL30 appeared to have a high frequency of null alleles and this locus was not included in the analyses presented below.

Genet assignment & paternity analysis

We used the SSR data from leaf samples to group ramets into multi-locus genotypes (MLGs). Assignment of ramets to MLGs was conducted to enable measurement of mating and reproductive success at the whole-plant (genet) level. Analyses of genet size were based on samples that amplified at all seven loci (i.e. all loci excluding SL30) using the RClone package (v. 1.0.2., Bailleul et al. 2016) using R (v. 3.6.1., R Core Team 2019) following procedures outlined in (Holt et al. 2020; Stephens et al. 2020). Samples with missing data were excluded because RClone package cannot process missing data. One ramet from the monoecious population and nine ramets from the dioecious population were excluded due to incomplete genotyping data, a total of 166 ramets from the monoecious population and 174 ramets from the dioecious population were included for further analysis. The probability that ramets with the same multi-locus genotypes (MLG) arose from independent mating events was estimated using the 'psex_Fis' function from the Rclone package (v. 1.0.2. Bailleul et al. 2016). We identified 160 unique MLGs from the monoecious population and 153 MLGs from the dioecious population, all of which had calculated values of P_{sex} less than 0.0001, indicating that ramets with identical MLGs were likely the result of clonal growth. To account for scoring errors and somatic mutations, we grouped MLGs that differed by up to one allelic difference at any locus into multi-locus lineages (MLLs) (Arnaud-Haond et al. 2007). Ramets were assigned to MLLs using the 'MLG_list' and 'MLL_generator2' functions from the Rclone package, which assign MLLs using MLG scores and a threshold value of pairwise genetic differences (see Holt et al. 2020, for details of a similar analysis using the same SSR loci for *S. latifolia*). Together 140 MLLs and 110 MLLs were identified for the monoecious and dioecious populations, respectively.

Seed paternity was determined by parentage analysis using COLONY2 (v. 2.0.6.5 Jones & Wang 2010; Wang 2012). Paternity assignments involved MLG- level data to account for any potential somatic mutations within MLGs that might have been passed to offspring. MLG-level assignments were then converted to the MLL level so that siring success could be tabulated for each genet. COLONY2 was run after selecting the following options: polygamy for both males and females with inbreeding; species was selected to be dioecious and diploid; and a full-likelihood analysis method was used as it provides the greatest accuracy in parental assignments (Wang 2012). The monoecious option was used for the parentage analysis of the monoecious population, where all potential dams are also potential sires. While female and male flowers on the same ramet do not typically open at the same time, it is possible for female and male flowers on different ramets that are from the same MLG to be open at the same time and therefore act as both dam and sire. For each run, we specified maternal sibships based on the MLG of the maternal parent. In both populations, MLGs that produced male flowers were included in the list of potential sires. Runs of COLONY2 were conducted separately for each population using medium run lengths with high likelihood precision. Each run also allowed for stochastic per locus genotyping error rates, values for which were estimated by comparing expected and observed heterozygosity in parental genotypes at each locus using CERVUS 3.0.7 (Marshall et al. 1998) (table A7.1.1).

Assignment to one or more sires was made for most of the genotyped seeds from the monoecious (218 of 247) and dioecious (233 of 297) populations. For seeds genotyped at all seven loci, siring probabilities for individual sire MLGs ranged from 0.42 to 1.00 (mean = 0.89 ± 0.02 SE) for the monoecious population and from 0.46 to 1.00 (mean = 0.58 ± 0.01 SE) for the dioecious population. For seeds genotyped at three loci, siring probabilities ranged from 0.42 to

1.00 (mean = 0.86 ± 0.03 SE) for the monoecious population and 0.46 to 1.00 (mean = 0.72 ± 0.01 SE) for the dioecious population. Each seed was assigned a single sire corresponding to the MLL with highest siring probability. To account for uncertainty in siring assignments when calculating male reproductive success, we tabulated total siring per MLL as the sum of the siring events associated with each MLL weighted by their estimated probability.

Selfed seeds were excluded from counts of mating and reproductive success. Only two MLLs in the monoecious population produced any selfed seed but selfing rates were quite high in those two MLLs. The proportion of selfed seed for the two MLLs involved in selfing events was 0.63 and 0.89 respectively, and because one of these MLLs was among the largest in the population, the overall population average selfing rate was 0.36 ± 0.07 SE. No females in the dioecious population were inferred to have produced selfed seeds.

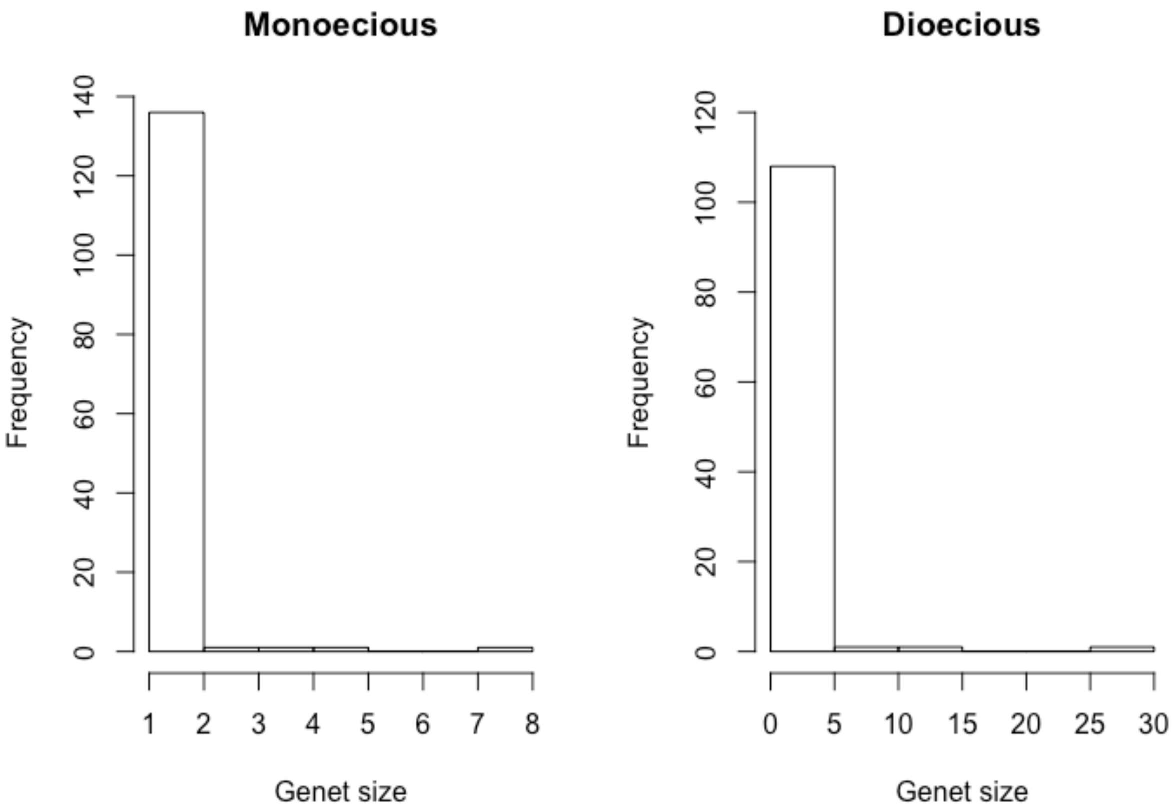

Figure A7.1.2 Frequency distribution of genet (MLL) sizes in the monoecious (left panel) and dioecious populations (right panel) of *Sagittaria latifolia* for which patterns of sexual selection were studied.

Table A7.1.1 Per-locus genotyping error rates for the dioecious and monoecious populations of *Sagittaria latifolia* for which patterns of sexual selection were studied, calculated using CERVUS (v 3.0.7).

Population	SL06	SL09	SL21	SL27	SL65	SL74	SL75
Dioecious	0.04	0.01	0.02	0.04	0.05	0.06	0.01
Monoecious	0.08	0.02	0.00	0.00	0.01	0.09	0.02

Size-dependency of flower production

A least-squares linear regression was used to examine the relationship between clone size and number of flowers produced for each sex in both monoecious and dioecious populations. Flower number was used as the dependent variable and clone size, measured as the number of ramets, was used as the independent variable for each sex. Regression analyses were calculated using *lm* function in R (R Core Team 2019).

For both populations, genet size had a significant positive association with flower production in both sexes (or sex functions of hermaphrodites; table A7.1.2, figure A7.1.3). Genets composed of many clones had more flowers than genets composed of few clones.

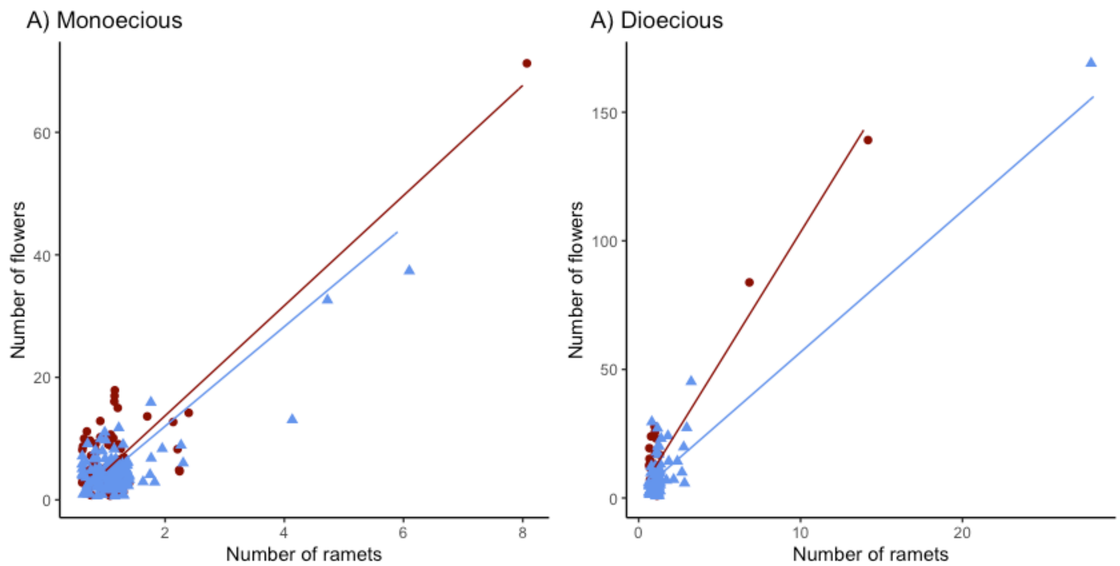

Figure A7.1.3 Number of flowers as a function of genet size calculated as number of ramets in *Sagittaria latifolia* for each sex (red circles for female function, blue triangles for male function) in a (A) monoecious and (B) dioecious population from a linear model.

Table A7.1.2 Linear regression parameters, standard errors, *t*- and *P*-values for the association between the number of flowers per genet and genet size (number of ramets) for each sex function in the monoecious and dioecious population of *Sagittaria latifolia* studied here.

	Estimate (SE)	*t*	*P*	*F*	*df*	*P*	R^2
Monoecious							
n female flowers							
(intercept)	-4.25 (0.70)	-6.06	**<0.001**	297.80	1, 98	**<0.001**	0.75
Genet size	8.98 (0.52)	17.26	**<0.001**				
n female flowers							
(intercept)	-1.88 (0.49)	-3.79	**<0.001**	242.60	1, 123	**<0.001**	0.66
Genet size	5.76 (0.37)	15.56	**<0.001**				
Dioecious							
n female flowers							
(intercept)	10.17 (1.59)	1.10	0.27	370.00	1, 28	**<0.001**	0.93
Genet size	10.17 (0.53)	19.24	**<0.001**				
n female flowers							
(intercept)	1.23 (0.88)	1.39	0.17	533.10	1, 78	**<0.001**	0.87
Genet size	6.02 (0.26)	23.09	**<0.001**				

Bateman gradients uncorrected for plant size

Least-squares linear regressions were used to model the relationship of reproductive success against mating success to determine Bateman gradients for each sex function without accounting for ramet or genet size. Standardized reproductive success was used as the dependent variable and standardized mating success was the independent variable.

$$RS^f = \alpha + \beta_f \, MS^f + \varepsilon$$

$$RS^m = \alpha + \beta_m \, MS^m + \varepsilon$$

The sex difference in Bateman gradients was calculated using separate least-squares multiple regression with a mating success × sex interaction term. Specifically, the sex-difference in Bateman gradients was estimated from:

$$RS = \alpha + \beta_1 \times MS + \beta_2 \times Sex + \beta_3 \times MS \times \mathrm{Sex} + \varepsilon,$$

where β_3 corresponds with $\Delta\beta_{SS}$.

Using this uncorrected approach for the monoecious population there was a significant positive association between mating and reproductive success for both female (β_{ff} = 1.20 ± 0.30 SE) and male function (β_{mm} = 1.17 ± 0.10 SE) of hermaphrodites (table A7.1.3). Accordingly the value for the sex-difference in Bateman gradients was small ($\Delta\beta ss$ = -0.03 ± 0.30, table A7.1.4). By contrast, in the dioecious population, there was a stronger positive association between mating and reproductive success for females (β_f = 2.16 ± 0.42 SE) than for males (β_m = 0.88 ± 0.09 SE, table A7.1.3), and the sex-difference in Bateman gradients was negative ($\Delta\beta_{ss}$ = -1.28 ± 0.45, table A7.1.4). Together, this analysis - that do not take variation in plant size into account – provided values suggesting marginal sex-differences in sexual selection in the monoecious populations and stronger sexual selection in females compared to males in the dioecious population.

Table A7.1.3 Bateman gradient analysis for female and male function of hermaphrodites in the monoecious population, and females and males of the dioecious population of *Sagittaria latifolia*, uncorrected for variation in ramet or genet size. Relative mating success was the number of genetic mating partners, standardized by dividing by their mean values. Significant values are in bold. Parameter estimates for mating success via female function (β_{ff}), male function (β_{mm}), and for females (β_f) and males (β_m). Parameter estimates are reported alongside their associated standard errors, test-statistic and *P*-values. Model-wide *F*-statistics (with numerator and denominators degrees of freedom), *p*-values, and R^2 are reported for each population.

	Estimate (SE)	*t*	*P*	*F*	*df*	*P*	R^2
Monoecious							
Female function Relative RS							
(intercept)	-0.20 (0.33)	-0.61	0.55	16.54	1, 23	**<0.001**	0.39
Relative MS	1.20 (0.30)	4.07	**<0.001**				
Male function Relative RS							
(intercept)	-0.17 (0.17)	-1.00	0.33	130.30	1, 17	**<0.001**	0.88
Relative MS	1.17 (0.10)	11.42	**<0.001**				
Dioecious							
Female Relative RS							
(intercept)	-1.16 (0.46)	-2.5	**0.03**	27.05	1, 14	**<0.001**	0.63
Relative MS	2.16 (0.42)	5.20	**<0.001**				
Male Relative RS							
(intercept)	0.12 (0.11)	1.10	0.31	102.50	1, 7	**<0.001**	0.93
Relative MS	0.88 (0.09)	10.12	**<0.001**				

Table A7.1.4 Bateman gradient analysis for female and male function of hermaphrodites in the monoecious population, and females and males of the dioecious population of *Sagittaria latifolia*, uncorrected for variation in ramet or genet size. Relative mating success was the number of genetic mating partners, standardized by dividing by their mean values. Significant values are in bold. Coefficients for the interaction term were used to estimate the sex-difference in Bateman gradients ($\Delta\beta_{ss}$). Parameter estimates are reported alongside their associated standard errors, test-statistic and *P*-values. Model-wide *F*-statistics (with numerator and denominators degrees of freedom), *p*-values, and R^2 are reported for each population.

	Estimate (SE)	*t*	*P*	*F*	*df*	*P*	R^2
Monoecious							
Relative RS							
(intercept)	-0.20 (0.30)	-0.66	0.51	38.99	3, 40	**<0.001**	0.73
Relative MS	1.20 (0.27)	4.31	**<0.001**				
Sex (males)	0.03(0.36)	0.09	0.98				
Relative MS: males	-0.03 (0.30)	-0.11	0.91				
Dioecious							
Relative RS							
(intercept)	-1.16 (0.38)	-3.02	**<0.01**	16.18	3, 21	**<0.001**	0.65
Relative MS	2.16 (0.34)	6.27	**<0.001**				
Sex (males)	1.28 (0.53)	2.41	**0.03**				
Relative MS: males	-1.28 (0.45)	-2.85	**0.01**		1		

Bateman gradients calculated using multiple regression

Multiple regression is an alternative to residual regression for controlling for confounding variables (Freckleton 2002). For each population, we calculated least-squares linear multiple regression of relative reproductive success using relative mating success, genet size (number of clones), and ramet size (measured as height), subset for each sex function, as the independent variables:

$$relative\ RS = \alpha + \beta_1(relative\ MS) + \beta_2\,(log_{10}clone\ size) + \beta_3(ramet\ size) + \varepsilon,$$

where β_1 corresponds with the Bateman gradient for each sex (or sex function). Genet size was log-transformed due to right skew. The results of the multiple regression analyses indicated that there were significant positive effects of mating success on reproductive success via male function in both populations (monoecious β_{mm} = 1.01 ± 0.37 SE; dioecious: β_m = 0.95 ± 0.18 SE; table A7.1.5). By contrast, there were no significant associations between mating and reproductive success via female function in either population (monoecious β_{ff} = 0.42 ± 0.27 SE; dioecious β_f = 0.20 ± 0.27; table A7.1.5). Together, these values are consistent with the results presented in the main text indicating that sexual selection was more likely to operate on male compared to female function (i.e., once ramet and clone size are taken into account).

Table A7.1.5 Multiple regression of relative reproductive success on relative mating success ramet size (measured as mid-vein length) and genet size (number of ramets) from a monoecious and a dioecious population of *Sagittaria latifolia.* Genet size was $\log_{10}$ transformed because of right skew. Significant values are in bold. Values are the parameter estimates, and associated *t*-test and *p*-values. We also report measures of overall fit for each model, including *F*-tests and their numerator and denominator degrees of freedom, and R^2.

	Estimate (SE)	*t*	*p*	*F*	*df*	*p*	R^2
Monoecious							
female RS							
(intercept)	-0.03 (0.45)	-0.07	0.94	19.40	3, 21	**<0.001**	0.70
relative MS	0.42 (0.27)	1.55	0.14				
$\log_{10}$(genet size)	3.38 (0.67)	5.00	**<0.001**				
ramet size	0.02 (0.03)	0.82	0.42				
male RS							
(intercept)	0.15 (0.52)	0.28	0.78	38.21	3, 14	**<0.001**	0.87
relative MS	1.01 (0.37)	2.73	**0.02**				
$\log_{10}$(genet size)	1.27 (2.86)	0.44	0.67				
ramet size	-0.02 (0.03)	-0.64	0.53				
Dioecious							
female RS							
(intercept)	-0.57 (0.54)	-1.04	0.32	91.56	3, 12	**<0.001**	0.95
relative MS	0.20 (0.27)	0.73	0.48				
$\log_{10}$(genet size)	3.56 (0.40)	9.01	**<0.001**				
ramet size	0.06 (0.03)	1.86	0.09				
male RS							
(intercept)	0.08 (0.41)	0.19	0.86	25.90	3, 5	**<0.01**	0.90
relative MS	0.95 (0.18)	5.30	**<0.01**				
$\log_{10}$(genet size)	-0.15 (0.30)	-0.51	0.63				
ramet size	-9.08×10^{-5} (0.03)	3.00×10^{-3}	1.00				

Associations between plant size and mating and reproductive success

Plant size, and clone size in particular, was strongly associated with both mating and reproductive success in the two populations of *S. latifolia* included in our study (table A7.1.6). In both the monoecious and the dioecious population, mating success and reproductive success was positively associated with genet size (the number of ramets per MLL) but not with ramet size (the average mid-vein length of ramets within MLLs; table A7.1.6). This was true for both sexes in the dioecious population, and for both sex functions in the monoecious population.

To determine how the effect of variation in plant size affected our raw estimates of reproductive success (i.e., before taking size variation into account), we evaluated the association between female reproductive success (at the MLL level) and number of genotyped seeds using a linear model. We calculated a least-squares linear regression of relative reproductive success as a function of number of seeds genotyped for each population.

For both populations, the number of genotyped seeds had a significant positive effect on raw estimates of female reproductive success (table A7.1.7). Larger genets (from which more seeds were sampled) tended to have higher female reproductive success (figure A7.1.4).

Table A7.1.6 Multiple regression of mating and reproductive success on ramet size (measured as mid-vein length) and $\log_{10}$ transformed genet size (number of ramets) in a monoecious and a dioecious population of *Sagittaria latifolia*. Significant values are in bold. Values are the parameter estimates, and associated *t*-test and *p*-values. We also report measures of overall fit for each model, including *F*-tests and their numerator and denominator degrees of freedom, and R^2.

	Estimate (SE)	*t*	*p*	*F*	*df*	*p*	R^2
Monoecious							
$\log_{10}$(female MS)							
(intercept)	0.32 (0.35)	0.92	0.37	7.30	2, 22	**<0.01**	0.34
$\log_{10}$(genet size)	1.49 (0.43)	3.48	**0.02**				
ramet size	0.03 (0.02)	1.70	0.10				
$\log_{10}$(male MS)							
(intercept)	0.95 (0.27)	3.53	**<0.01**	78.21	2, 15	**<0.001**	0.90
$\log_{10}$(genet size)	7.36 (0.59)	12.51	**<0.01**				
ramet size	-0.02 (0.02)	-1.03	0.32				
$\log_{10}$(female RS)							
(intercept)	0.10 (0.46)	0.22	0.83	26.20	2, 22	**<0.01**	0.68
$\log_{10}$(genet size)	4.01 (0.56)	7.14	**<0.01**				
ramet size	0.03 (0.02)	1.39	0.18				
$\log_{10}$(male RS)							
(intercept)	1.11 (0.46)	2.40	**0.03**	37.39	2, 15	**<0.001**	0.81
$\log_{10}$ genet size)	8.75 (1.01)	8.63	**<0.001**				
ramet size	-0.03(0.03)	-1.15	0.26				
Dioecious							
$\log_{10}$ female MS)							
(intercept)	0.80 (0.52)	1.53	0.15	12.19	2, 13	**<0.01**	0.60
$\log_{10}$(genet size)	1.20 (0.24)	4.91	**0.01**				
ramet size	3.10×10^{-3} (0.03)	0.10	0.92				
$\log_{10}$(male MS)							
(intercept)	-0.13 (0.95)	-0.14	0.89	6.67	2, 6	**0.03**	0.59
$\log_{10}$(genet size)	1.38 (0.39)	3.50	**0.01**				
ramet size	0.06 (0.06)	0.93	0.39				
$\log_{10}$(female RS)							
(intercept)	-0.41 (0.49)	-0.83	0.42	142.10	2, 13	**<0.001**	0.95
$\log_{10}$(genet size)	3.80 (0.23)	16.50	**<0.001**				
ramet size	0.06 (0.03)	1.92	0.07				
$\log_{10}$(male RS)							
(intercept)	-0.05(0.98)	-0.05	0.96	4.50	2, 6	0.06	0.47
$\log_{10}$(genet size)	1.15(0.41)	2.84	**0.03**				

ramet size	0.05 (0.06)	0.86	0.43

Table A7.1.7 Linear regression of female reproductive success on the number of genotyped seeds from a monoecious and dioecious population of *Sagittaria latifolia*. Values are the parameter estimates with the associated *t*-test and *p*-values. We include measures of overall fit for each model, including *F*-test, their numerator and denominator degrees of freedom and R^2.

.	*Estimate (SE)*	*t*	*P*	*F*	*df*	*P*	R^2
Monoecious							
(intercept)	0.25 (0.12)	2.03	0.05	84.5	1, 23	**<0.001**	0.78
Number of genotyped seeds	0.05 (0.01)	9.19	**<0.001**				
Dioecious							
(intercept)	-0.14 (0.13)	-1.04	0.32	160.0	1, 14	**<0.001**	0.91
Number of genotyped seeds	0.05 (0.00)	12.7	**<0.001**				

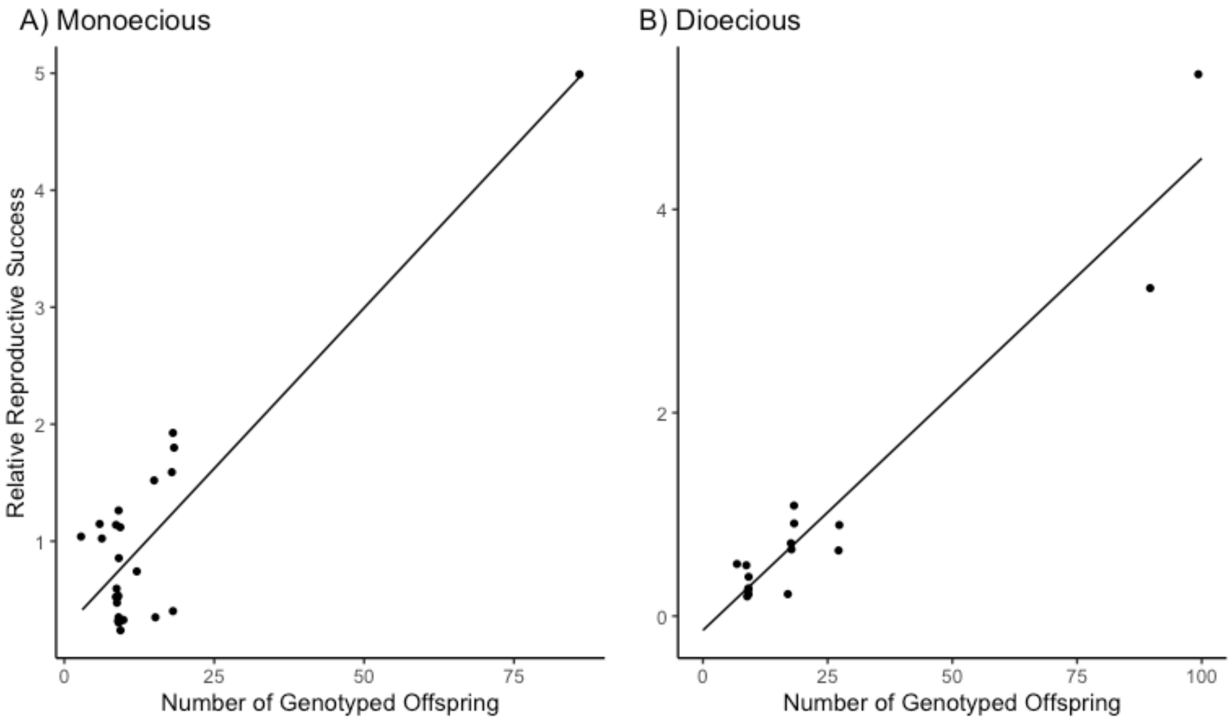

Figure A7.1.4 Linear regression of relative reproductive success of females on number of ovules genotyped for a monoecious and dioecious population of *Sagittaria latifolia*. Points represent the relative reproductive success of seed-producing MLLs (females in the dioecious population, and hermaphrodites in the monoecious populations) versus the number of genotyped seeds per MLL in each population. Points are jittered to enable visualization of points from MLLs with a similar number of genotyped seeds.

Table A7.1.8 Summary of linear (residual) regression of reproductive success on mating success for female and male function from a monoecious and a dioecious population of *Sagittaria latifolia*. Values are the parameter estimates, and associated *t*-test and *p*-values. We also report measures of overall fit for each model, including *F*-tests and their numerator and denominator degrees of freedom, and R^2.

	Estimate (SE)	*t*	*P*	*F*	*df*	*P*	R^2
Monoecious							
Female resid. RS							
(intercept)	6.30×10^{-17} (0.10)	0.00	1.00	2.65	1, 23	0.11	0.06
Resid. MS	0.42 (0.26)	1.63	0.11				
Male resid. RS							
(intercept)	-2.61×10^{-17} (0.14)	0.00	1.00	8.60	1, 16	**<0.01**	0.31
Resid. MS	1.02 (0.35)	2.93	**<0.01**				
Dioecious							
Female resid. RS							
(intercept)	-1.39×10^{-17} (0.07)	0.00	1.00	0.63	1, 14	0.44	-0.03
Resid. MS	0.20 (0.24)	0.79	0.44				
Male resid. RS							
(intercept)	1.67×10^{-17} (0.07)	0.00	1.00	39.36	1, 7	**<0.001**	0.83
Resid. MS	0.95 (0.15)	6.27	**<0.01**				

Table A2.9. Summary of multiple linear (residual) regression of reproductive success on mating success for female and male function from a monoecious and a dioecious population of *Sagittaria latifolia*. Sex-differences in the Bateman gradient ($\Delta\beta_{ss}$) were estimated from the interaction term between (residual) mating success and sex. Values are the parameter estimates, and associated *t*-test and *p*-values. We also report measures of overall fit for each model, including *F*-tests (with numerator and denominator degrees of freedom), and R^2.

	Estimate (SE)	*t*	*P*	*F*	*df*	*P*	R^2
Monoecious							
Relative RS							
(intercept)	5.52×10^{-17} (0.11)	0.00	1.00	4.17	3, 39	**0.01**	0.18
Relative MS	0.42 (0.27)	1.51	0.14				
Sex (males)	-1.35×10^{-16} (0.17)	0.00	1.00				
Relative MS: males	0.60 (0.42)	1.42	0.17				
Dioecious							
Relative RS							
(intercept)	3.89×10^{-17} (0.07)	0.00	**1.00**	7.81	3, 21	**0.001**	0.46
Relative MS	0.20 (0.22)	0.87	0.39				
Sex (males)	-2.03×10^{-17} (0.11)	0.00	1.00				
Relative MS: males	0.76 (0.30)	2.52	**0.02**				

Bateman gradients calculated using bootstrapping

Bateman gradients for female function are subject to sampling biases if different numbers of offspring are sampled across females. Sampling different numbers of offspring per female can lead to covariances between measures of reproductive success and – if females have opportunities to mate multiple times – and measures of mating success, leading to positive estimates of the female Bateman gradient even in the absence of sexual selection. Our study design involved the sampling of an equal number of seeds per fruit. However, our calculations of the strength sexual were made at the genet (MLL) level, and genets varied in their number of ramets (and fruits). Our use of size-corrected estimates of Bateman gradients via residual regression represents one approach to correct for underlying covariances between plant (clone) size and the sample sizes used to estimate reproductive and mating success to affect inferences regarding the strength of sexual selection. An alternative approach is to randomly sample the same number of seeds from same-sized MLLs using bootstrapping.

We calculated Bateman gradients for both sexes using clustered bootstrapping of the seed data set. From each maternal MLL, 5 seeds were randomly sampled with replacement from a single, randomly sampled ramet per MLL. Random samples were replicated 1000 times using the '*replicate*' function in base R (R Core Team 2019). Each of these seed datasets were used to estimate mating and reproductive success for female and sex functions in each population, and from these values calculate the sex-specific Bateman gradients using least squares multiple regression. Sex-differences in sex Bateman gradients ($\Delta\beta_{ss}$) were calculated using separate multiple regressions that included a mating success sex interaction, as described above for the data presented in table A7.1.4. The regression model was estimated with 1000 replicates and

95% confidence intervals were calculated from the distribution of estimates from these replicate analyses. Regression models were calculated using the lm function in R (R Core Team 2019).

Using this bootstrapping approach we found positive Bateman gradients for both female and male function, but with much larger values for female than for male function in the monoecious population (mean β_{mm} = 3.82 ± 0.02 SE, β_{ff} = 0.33 ± 0.01 SE; table A7.1.10, Figure A7.1.5A). As a result, the sex-difference in Bateman gradients was strongly positive ($\Delta\beta_{ss}$ = 3.49 ± 0.72 SE, table A7.1.11), consistent with stronger sexual selection on male than on female function. We obtained a similar result in the dioecious population, with a strongly positive association between mating and reproductive success for males, but not females (mean β_m = 1.06 ± 0.05 SE, β_f = -0.11 ± 0.01 SE; table A7.1.10, Figure A7.1.5B). Again, this yielded a positive sex-difference in Bateman gradients for the dioecious population ($\Delta\beta_{ss}$ = 1.17 ± 1.59 SE, table A7.1.11) indicating much stronger sexual selection in males compared to females.

Table A7.1.10: Bateman gradient analysis for each sex function in a monoecious and a dioecious population of *Sagittaria latifolia*. Bootstrapped analyses each with 1000 replicates were performed by sampling seeds from a single ramet per MLL. Bateman gradients were calculated for each replicate using relative mating success was the number of genetic mating partners, standardized by dividing by their mean values. Values shown are the bootstrapped mean parameter estimates and 95% confidence intervals.

	Mean estimate (SE)	*95% CI*
Monoecious		
Female relative RS		
(intercept)	0.34 (0.01)	(-0.43, 1.04)
Relative MS	0.33 (0.01)	(-0.42, 1.14)
Male relative RS		
(intercept)	-2.25 (0.01)	(-3.19,-1.52)
Relative MS	3.82 (0.02)	(2.70, 5.19)
Dioecious		
Female relative RS		
(intercept)	1.81 (0.06)	(-1.69, 5.06)
Relative MS	-0.11 (0.05)	(-3.13, 3.01)
Male relative RS		
(intercept)	-0.48 (0.01)	(-0.87, -0.20)
Relative MS	1.06 (0.01)	(0.78, 1.43)

Table A7.1.11. Summary of multiple linear regression of reproductive success on mating success for female and male function from a monoecious and a dioecious population of *Sagittaria latifolia*. Bootstrapping with 1000 replicates were performed on seed sampling with MLLs as a clustering factor. Both male and females were included in this model with sex as an interaction term. The estimate for the interaction was used to estimate sex-differences in the Bateman gradient ($\Delta\beta_{SS}$). Values are the bootstrapped mean parameter estimates and 95% confidence intervals.

	Mean estimate (SE)	*95% CI*
Monoecious		
(intercept)	0.34 (0.66)	(-0.43, 1.04)
Relative MS	0.33 (0.64)	(-0.42, 1.15)
Sex (male)	-2.59(0.78)	(-3.7, -1.49)
Relative MS: males	3.49 (0.72)	(2.07, 5.04)
Dioecious		
(intercept)	1.81 (1.64)	(-1.69, 5.06)
Relative MS	-0.11 (1.40)	(-3.13, 3.02)
Sex (male)	-2.29 (2.05)	(-5.58, 1.24)
Relative MS: males	1.17 (1.59)	(-2.00, 4.13)

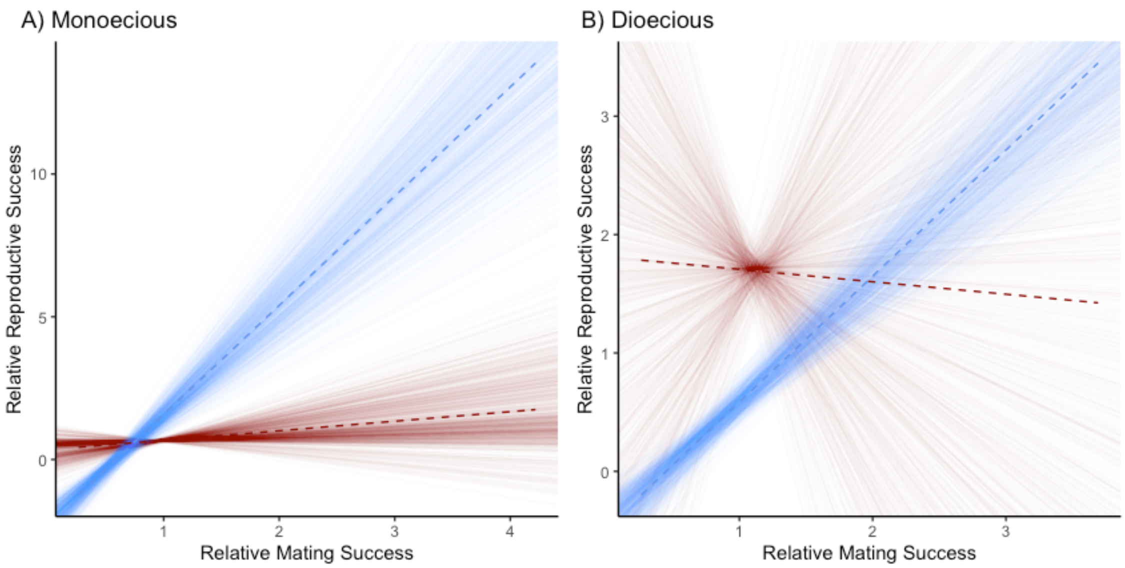

Figure A7.1.5: Sex-specific Bateman gradients in a monoecious (A) and dioecious (B) population of *Sagittaria latifolia*. Gradients for both sexes were calculated using linear models with clustered bootstrapping, regressing relative mating success onto reproductive success of ramets at the MLL level. The bootstrapped mean regressions are shown for males (and male function; blue dashed line) and for females (and female function; red dashed line).

7.2 Appendix: Male reproductive success is not strongly affected by phenological changes in mate availability in monoecious *Sagittaria latifolia*

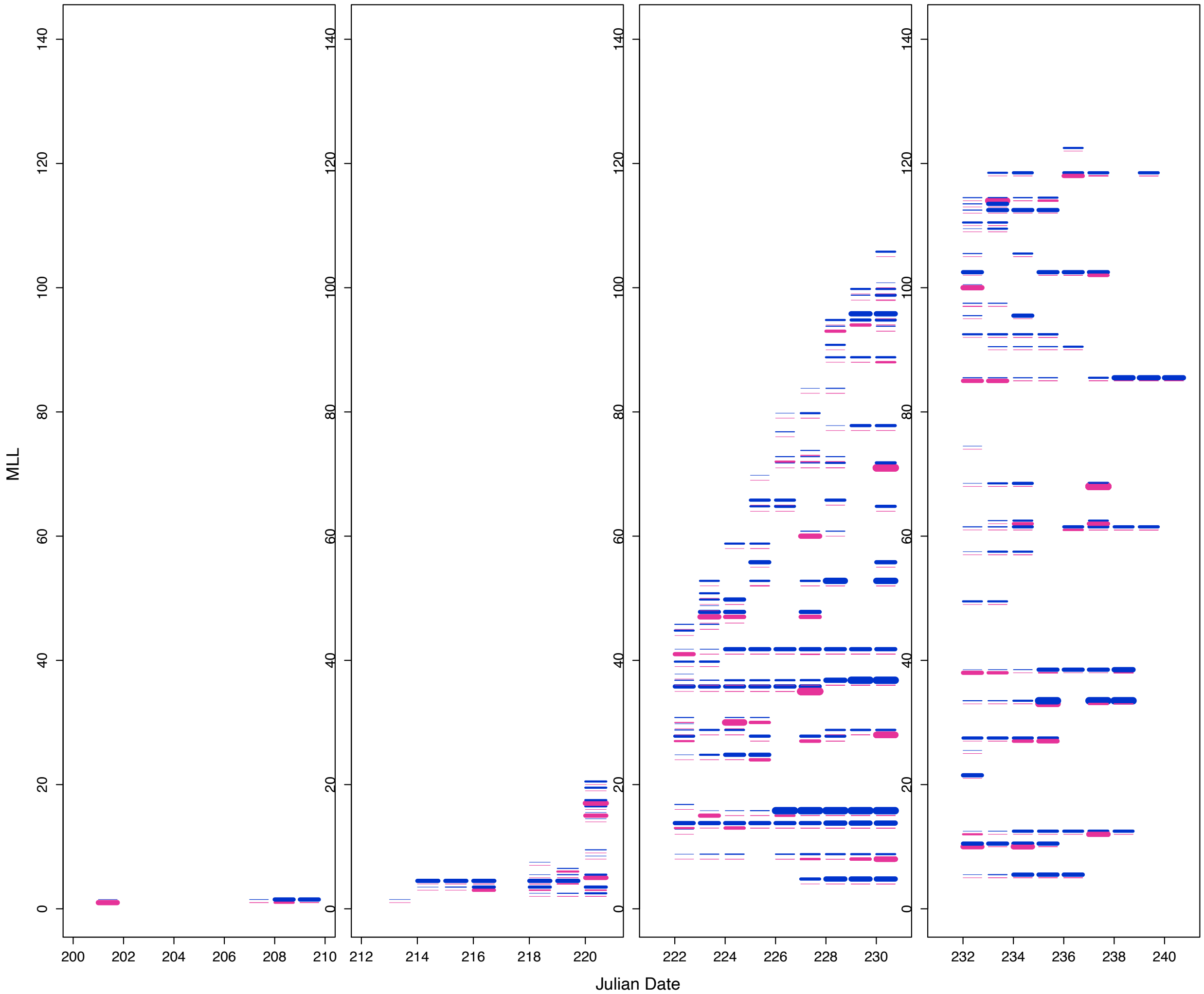

Figure A7.2.1 Male and female flowering days for each ramet of *Sagittaria latifolia*, sampled July 18th (day 199) to Aug 28, 2013 (day 240) in Peterborough, Ontario. Each panel represents an approximate 10 day period of the 41 day flowering season. Each row of paired pink and blue bars represents the pistillate and staminate phases of a single genet (MLL), with pink bars representing female phase and blue bars representing male phase. The bar width is scaled by number of female and male flowers open per day.

Seasonality of Inflorescence sex allocations

To examine whether patterns of allocation to male and female flowers varied over the flowering season, we calculated the inflorescence sex ratio (n = 298 inflorescences) and used this as the measure of sex allocation. We compared model fits of a generalized additive mixed model (GAMM) (as specified in the main text) and a generalized linear mixed effect (GLME) model. For inflorescence sex ratio (a composite variable was the response variable of female and male flowers, and the first flowering date per inflorescence was the predictor variable. The GLME model was specified using a binomial error distribution and a logit link function. Because inflorescences and MLLs were sampled across multiple days, inflorescence and MLL were specified as random effects, with inflorescence nested within MLL. using the glmer function from the lme4 package (Bates et al. 2015) in R (v. 4.3.1; R Core Team 2023). The AIC values of the GAMM and GLME models were used to assess the best fitting model (Quinn and Keough 2002).

Table A7.2.1 Generalized linear mixed effects (GLME) model of inflorescence-level sex allocations a combination of female and male flowers per inflorescence in a monoecious population of *Sagittaria latifolia*. The model used a binomial error distribution and included random effects of MLL and ramet ID nested within MLL. Significant model terms are in bold. Values are the parameter estimates, associated z-score and p-values, χ^2 and p-values of the model test statistics and model Akaike Information Criterion value (AIC).

Model	*Estimate (SE)*	*z*	*p*	χ^2	*df*	*p*	*AIC*
Sex allocation ~ date of first flowering							
(intercept)	2.92 (1.70)	1.72	0.09	2.15	1	0.14	1061.70
First flowering date	-0.01 (7.50×10^{-3})	-1.47	0.14				

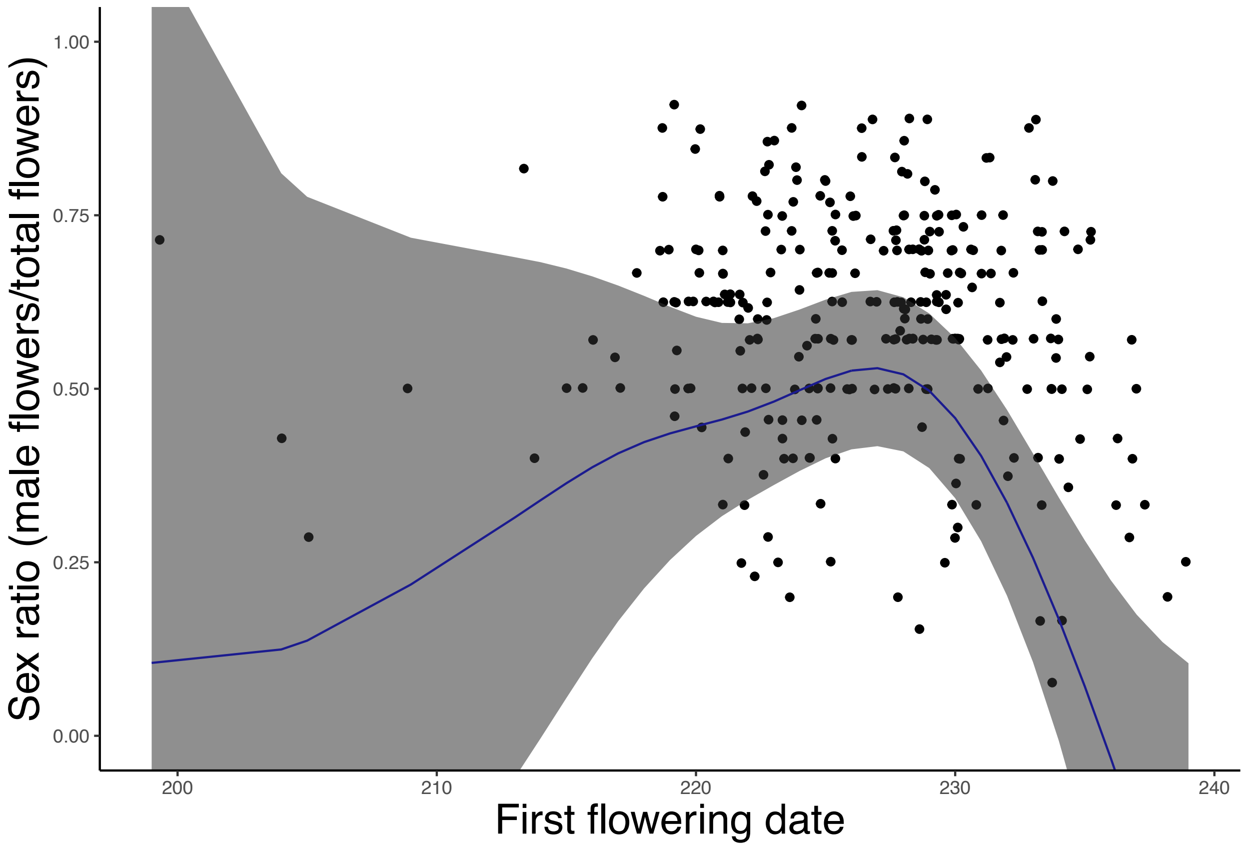

Figure A7.2.2 Estimated trends of the first flowering date and the sex ratios (proportion of male flowers) of each inflorescences in a population of *S. latifolia*, July 18th (day 199) to August 28th, 2013 (day 240). The blue line represents the trend of date of first flowering as a predictor of sex ratio estimated using a generalized additive mixed effect model. The shaded area represents the 95% confidence intervals.

Seasonality of the mating environment

To evaluate seasonal changes in mating opportunities via the male function, we again compared a GAMM (as described in the main text) and linear mixed effect model (LME) results to examine daily changes in pollen transfer opportunities over time. For the LME model, daily pollen transfer opportunity was the response variable and date was the independent variable and treated as a fixed effect using the lmerTest package (v 3.1-3, Kuznetsova et al. 2017). Because pollen transfer opportunities were calculated at the genet level (n =123 genets) and repeated sampling of MLLs occurred over multiple dates, MLL was included as a random effect. The GAMM and LME were compared using AIC to determine the best fitting model (Quinn & Keogh 2002).

Table A7.2.2 Linear mixed effects (LME) model of pollen transfer opportunity (PTO) in a monoecious population of *Sagittaria latifolia*. PTO was measured at the genet-level and so MLL was included as a random effect. Significant model terms are in bold. Values are the parameter estimates, associated *t*-value and *p*-values, *F*-test and *p*-values for the overall fit of the model and model Akaike Information Criterion values (AIC).

Model	*Estimate (SE)*	*t*	*p*	*F*	*df*	*p*	*AIC*
PTO ~ date							
(intercept)	-2.48 (0.23)	-10.99	**<0.001**	149.17	1, 5042	**<0.001**	13691.86
Date	0.01 (1.02×10^{-3})	12.21	**<0.001**				

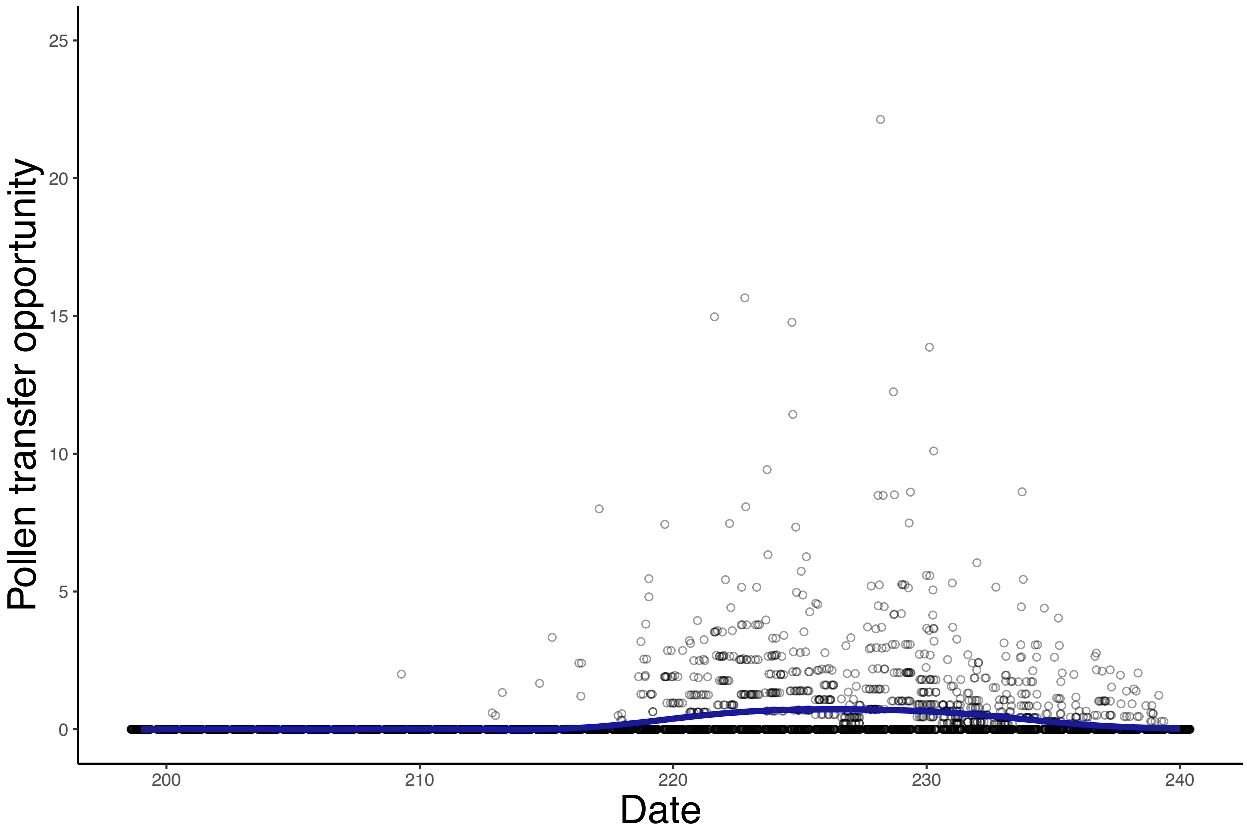

Figure A7.2.3 Estimated trend of the mating opportunity for each flowering ramet for everyday of the flowering season in a population of *Sagittaria latifolia* sampled July 18th (day 199) to August 28th, 2013 (day 240). Mating opportunity as measured as the pollen transfer opportunity (PTO) of each MLL per day (n=123 MLLs). The blue line denotes the trend of the day as a predictor of PTO estimated using a generalized additive mixed effect model. PTO values (open dots) are jittered so that overlapping values can be more easily seen.