A Clone Together: Exploring the Causes and Consequences of Range Divergence Between Sexual and Asexual Easter Daisies

by

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A Clone Together: Exploring the Causes and Consequences of Range Divergence Between Sexual and Asexual Easter Daisies

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Abstract

Sexual and asexual organisms exhibit a wide variety of biological differences that can impact their ecological and evolutionary trajectories. One result of these differences is that closely related sexual and asexual taxa often exhibit range divergence, with asexuals typically having larger geographic ranges and being found at higher latitudes and elevations. This pattern, termed "geographical parthenogenesis", has been documented in numerous plant and animal systems and a variety of potential mechanisms have been proposed. Hypotheses relate to differences in reproductive assurance (asexuals can reproduce without mates, while most sexuals require mates), genetic consequences of sexuality vs asexuality, selection on clonal lineages, ecological impacts of sexuality and asexuality, and demographic differences between reproductive modes. In this thesis, I explore several of these potential drivers of geographical parthenogenesis in *Townsendia hookeri*, a subalpine perennial flowering plant in the Asteraceae that has diploid sexual and polyploid apomictic (reproducing asexually through seed) forms with divergent but overlapping ranges. Population genomic analyses of apomicts in *T. hookeri* revealed largely monoclonal populations and geographically widespread clones, suggesting that apomictic range expansion may have been aided by "general-purpose genotypes" that can withstand an array of environmental conditions. Results from a large-scale, multi-year reciprocal transplant garden experiment showed that sexual populations had comparable performance when planted

into the apomictic range as into their own, but that fitness of apomictic individuals generally declined in sexual regions. This provides evidence that while sexuals are likely limited by dispersal (they cannot reach suitable habitat outside of their current range), apomicts are not well adapted to the ecological conditions (biotic and/or abiotic) in the sexual range. When comparing early life history traits between sexuals and apomicts, apomicts were found to have increased germination success and improved seed dispersal traits in comparison to sexuals. These traits are expected to have given apomicts a colonization advantage, which (along with reproductive assurance) has likely contributed to their increased range size. Overall, the work presented in this thesis highlights the intricate nature of geographical parthenogenesis in *Townsendia hookeri*, and underscores the need to investigate complex biological phenomena using a diverse suite of approaches.

Lay Summary

Most organisms reproduce sexually (with sperm and egg from two mates fusing to form a new individual), but many reproduce asexually without mates (forming clones of themselves). In nature, we often find that closely related sexuals and asexuals are found in different places, with asexuals usually having broader geographic ranges than sexuals. Biologists have several ideas for why this happens, but because organisms are complicated and diverse, no single explanation adequately addresses the pattern as a whole. In this thesis, I investigate this pattern in Townsend's Easter daisies (*Townsendia hookeri*), a flowering plant with sexual and asexual forms that have different ranges. Using several approaches, including DNA sequencing and field experiments, I found evidence that the spread of asexual Easter daisies was likely aided by particularly successful clones, seed traits that help them start new populations, and their ability to reproduce without mates. In other words: "it's complicated".

Preface

All of the work presented in this thesis was conducted in the field or in the Biodiversity Research Centre at the University of British Columbia, Point Grey campus.

I was the lead investigator for all chapters and was responsible for conceptualization, data collection, data analysis, and manuscript writing. Several undergraduates and colleagues contributed to the data collection. Chris Lee, a PhD student in the Whitton Lab, collected plant material that was sequenced for Chapter 2. Jaime Grimm and Katya Hernandez (undergraduates) as well as Adam Wilkinson, Rachel Wilson, Erin Warkman, Ryan Cologne, Lynn Riedel, and Bianca Hersh (research assistants) contributed to the collection of data presented in Chapter 3. Alberto Ruiz-Larrera, an undergraduate in the Whitton Lab, contributed seed dispersal trait data that was presented in Chapter 4. Amy Angert, Sally Otto, Loren Rieseberg, and Dolph Schluter contributed manuscript edits and helped with the interpretation of results for all chapters. Jeannette Whitton was my PhD supervisor, and was involved at a fundamental level in all aspects of this thesis work, including conceptualization, data collection, interpretation of results, and manuscript edits.

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My garden experiment would not have happened without the generosity of the Blake family, Lynn Riedel, and Daniel Tinker, who donated their land (or land they were responsible for) to the cause of science. I owe my undying gratitude to Adam Wilkinson, who helped me get my experiment off the ground and taught me everything there is to know about "the rules of the road", as well as Julia Costa and Marco Todesco, who patiently and effectively gave me a crash course on molecular biology. I must also thank Jamie Fenneman, Chris Lee, and Shona Ellis for being the best darned teaching team this side of the Georgia Strait, and for making me a better botanist and educator.

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Chapter 1

Introduction

As humans and scientists, we are obsessed with exploring, documenting, and (hopefully) understanding the natural world. Life on Earth presents many of the most complex systems - and most tantalizing mysteries - for us to unravel. In the past 200 years alone we've achieved incredible insight into biological processes large and small, but each discovery spawns a cascade of new questions that unfurl infinitely in all directions, whether at the microscopic scale of genomes or from the planet-wide perspective of interlocking ecosystems. Our individual investigations often focus on one species at a time, one question at a time, and what we continue to learn is that everything is connected - genes and morphology, individuals and populations, past and future. Grappling with this dizzying complexity and diversity is the great charge (and challenge) of ecology and evolutionary biology.

Active areas of research in ecology and evolution reflect this endeavor to connect the large with the small, and use known patterns to inform what we can expect in the future. Recent advancements in sequencing technology along with rapidly reducing costs have given rise to the widespread use of genomics, which has allowed researchers to investigate population genetic processes at greater depth and more easily tie them

to ecological questions. Similarly, the increase in publicly available climate data has allowed biologists to gain deeper and broader insight than previously possible into the interface between biotic and abiotic forces. In addition, the increased use of programming, particularly open-source languages like R and Python, has made advanced statistical analyses and bioinformatics widely available to anyone who is willing to put in the time and effort to learn. All of these tools have helped push the scope and breadth of ecology and evolution research programs that aim to address big questions with granular data. The work presented in this thesis focuses on investigating a pattern that sits at the intersection of several important research areas, including the evolution of sex, drivers of species range limits, local adaptation, the evolutionary impacts of polyploidy, and the co-evolution of mating system and dispersal traits.

Geographical parthenogenesis (GP), as the name implies, describes an often-found motif where closely related sexual and asexual (parthenogenic) taxa inhabit different geographic ranges. The emerging question is simple: why? It might seem straightforward at first, but patterns of GP are the result of multiple interacting ecological and evolutionary forces that are bound to differ depending on the unique biological contexts in which the pattern manifests. The fact that so many potential drivers coincide can make GP research challenging, but also extremely rewarding in the insights that we gain into each of the processes involved.

1.1 The history of geographical parthenogenesis and its proposed explanations

The term "geographical parthenogenesis" was first introduced by Albert Vandel in the 1920's, who was investigating geographic trends in certain species of arthropods where males were more rare at higher latitudes. He realized that these latitudinal trends were due to an increased prevalence of obligately parthenogenic forms of the same

species, which could be applied to similar patterns found in other groups. He defined GP as the phenomenon where sexual and asexual forms of the same species occupy distinct (though potentially overlapping) geographic ranges. Several decades later, cases of GP were reviewed in animals by Glesener & Tilman (1978) and in plants by Bierzychudek (1985), from which some refinements of the pattern emerged. Modern definitions of GP propose that asexuals generally have broader distributions, occur at higher latitudes, and are found at higher elevations than their sexual progenitors (Tilquin & Kokko 2016). Along with these, several models imply (sometimes indirectly) that parthenogens are also found more frequently in "marginal" or disturbed habitats (Vrijenhoek & Parker 2009). A number of potential mechanisms have been proposed for GP, and while many of them overlap in some aspects, they tend to focus on different ecological, evolutionary, and/or genetic components that may affect the ranges of closely related sexuals and asexuals.

1.1.1 Uniparental reproduction

Parthenogenesis is a derived trait that is found in all major groups of eukaryotes, and is generally defined as a form of asexual reproduction where a zygote is formed from an unfertilized female gamete (Tilquin & Kokko 2016). Parthenogens are capable of uniparental reproduction, meaning that (in most cases) they have full reproductive assurance and can proliferate without mates. Parthenogenic organisms are therefore assumed to have a demographic advantage (all else being equal) over those that reproduce sexually, because sexuals are largely dependent on mates (Bell 1982). This advantage forms the basis for one of the most obvious and intuitive potential explanations for GP. Parthenogens with full reproductive assurance are expected to have a colonization advantage over sexuals, because it only takes a single propagule to establish a new population, whereas sexuals require two propagules *and* successful mating

in order to successfully establish (Baker 1955). Uniparental reproduction is clearly advantageous during colonization, but also in other contexts where mate-limitation is likely (i.e., low density populations or, for plants, areas with low pollinator availability; Gascoigne *et al.* 2009), for example in highly disturbed environments or previously glaciated areas (Hörandl 2009). This implies that unless dispersal/demographic barriers are too high to overcome, sexuals should be able to catch up given enough time, which points to other drivers interacting with uniparentality in maintaining stable range divergence between sexuals and asexuals.

Support for the effects of reproductive assurance can be gleaned from "Baker's Law" (Baker 1955), which predicts that self-compatible plants will be more likely to have larger ranges than those that are not capable of selfing (Pannell *et al.* 2015). The Baker's Law literature is vast, and a recent large-scale study suggests that selfing species do have larger ranges than outcrossing species (Grossenbacher *et al.* 2015), but comparisons of selfing and parthenogenesis should be made with caution. Selfing results from the fusion of two gametes and thus will lead to an increase in homozygosity. The transition to selfing is therefore often associated with inbreeding depression, while parthenogens avoid inbreeding depression as a result of frozen levels of heterozygosity (Haag & Ebert 2004). We would therefore expect different long-term trajectories of selfing and parthenogenic populations due to the two reproductive strategies having different genetic consequences. While inbreeding depression can lead to decreased fitness and potential extirpation in selfing populations, selfers also have the ability to purge deleterious mutations (Schemske & Lande 1985) that are expected to accumulate in asexuals and hinder their long-term persistence (Muller 1964).

The evolution of dispersal and mating system traits is related to the idea of Baker's Law effects, but is curiously rare in the GP literature. Given that parthenogens often

have larger ranges than sexuals, it is possible that they may also possess traits (beyond reproductive assurance) that benefit dispersal and/or colonization ability. Besides a few recent examples (see Coughlan *et al.* 2014 and Chrtek *et al.* 2018), differences in dispersal ability *per se* between sexuals and asexuals have rarely been explored in a GP context (but see O'Connell & Eckert 2001 who investigated differences between sexual and apomictic diaspores in *Antennaria parlinii*). This is an important open research area within GP, as dispersal in time and space can both aid range expansions and allow escape from pathogens or environmental instability (Wilson 2011).

1.1.2 Polyploidy and hybridization correlate with parthenogenesis

Given that parthenogens originate from an incredibly diverse range of taxa, it is no surprise that it functions differently depending on the evolutionary context in which it arose. Still, many parthenogens share common features; for example, most parthenogens are polyploids of hybrid origin (Bengtsson 2009). Because of this association, some proposed hypotheses for GP focus on the effects that ploidy and hybridity have on driving patterns of range divergence. Polyploidy itself, as well as many of the traits associated with polyploidy, have been hypothesized to bestow advantages, including larger cells, increased genetic expression, protection from deleterious mutations, and increased evolutionary potential (Comai 2005). Polyploidy has also been linked with increased invasive ability (Te Beest *et al.* 2012), though some large-scale studies suggest that polyploids do not consistently show range shifts relative to their diploid progenitors (Martin & Husband 2009). Similar to Baker's Law, the literature concerning the ecological and evolutionary impacts of polyploidy is expansive, and several reviews and meta-analyses have been published in recent years that often appear to take opposing stances; for example, Glennon *et al.* (2014) found that poly-

ploids do not consistently show niche shifts in comparison to diploids, while Baniaga *et al.* (2020) propose that polyploids show faster rates of niche evolution. Because polyploidy is so pervasive (especially in plants; Otto & Whitton 2000), the predicted effects of polyploidy on patterns of GP will differ depending on the evolutionary context in each system. For example, genome duplication can occur within a single species (autopolyploidy) or as a result of hybridization between two close relatives (allopolyploidy), and the latter will be influenced by the interacting effects of both hybridization and polyploidy.

Viewed on its own, hybridization between two sexual species is often deleterious (Johnson 2010), but in some cases can result in hybrid vigour (Rieseberg *et al.* 1999; Baack & Rieseberg 2007; Chen 2013). The infusion of genetic diversity from hybridization may aid plants during colonization scenarios, and such benefits would be conserved by parthenogenesis (Kearney 2005). However, because most of the well-studied GP systems are allopolyploids (Bengtsson 2009), it can be difficult to disentangle the relative contributions of reproductive assurance, polyploidy, and hybridity in driving the pattern. These potential combinatory effects highlight the importance of viewing GP as the result of an interacting suite of traits (especially given that they are commonly associated) as opposed to attempting to identify the single most important component in driving the pattern across a diverse array of taxa.

1.1.3 Selection on clonal lineages

Some models for GP focus on the ways in which selection acts differently on sexuals and asexuals, and how different types of selective pressures can affect clonal diversity. As new clonal lineages emerge, those with a favorable set of attributes will succeed and be able to establish and spread. Depending on the frequency of origins of new clones and their fitness, a variable number of clonal lineages may become established,

and those that do will be protected (by parthenogenesis) from the breakdown of beneficial allele combinations that would otherwise occur in sexual populations (Lynch 1984). This idea lead to the formulation of two opposing (but not mutually exclusive) hypotheses: the general-purpose genotype hypothesis (Baker 1965; Parker *et al.* 1977) and the frozen niche variation hypothesis (Vrijenhoek 1979; evidence for both reviewed in Vrijenhoek & Parker 2009).

The general-purpose genotype hypothesis predicts that selection on clones in highly variable environments will give rise to lineages that are tolerant of a wide-array of environmental conditions. Populations consisting of these "general-purpose" clonal lineages are expected to be better able to persist in abiotically unpredictable areas than more specialized sexual genotypes, but would be outcompeted by sexuals (or specialized clones) in parts of the range that are stable enough for populations to become locally adapted (Tilquin & Kokko 2016). This pattern is in line with the descriptions of Baker (1965), who likened parthenogens to "weedy" species that are excluded to the range margins where they are safe from competitors.

The frozen niche variation hypothesis instead predicts that a diverse array of specialized clones will be better able to partition the available niche space than a population of sexuals, because sex can pull divergent phenotypes back to the mean of the distribution (Weeks 1993). This hypothesis assumes that a variety of clones will arise within populations, and competition (either among clones or with sexual progenitors) will eliminate clonal lineages whose niches overlap, leaving only specialized clones that are "frozen" in their respective niche spaces. While the general-purpose genotype hypothesis is concerned with fitness fluctuations in time (i.e., due to variable environmental conditions), the frozen niche variation hypothesis focuses more on fitness variation in space (i.e., heterogenous resource availability within habitats; Vrijenhoek & Parker 2009).

Broadly speaking, the general-purpose genotype hypothesis predicts the establishment of a few geographically widespread clones, while the frozen niche variation hypothesis predicts the establishment of a diverse array of local, specialized clones. More specific predictions about clonal diversity within and among populations are somewhat unclear, however. For example, it seems possible that marginal populations may consist of multiple general-purpose genotypes, and a specialized clone may still become widespread if it is able to disperse to distant locations that exhibit similar niche characteristics. In addition, both patterns may become apparent within a system if clones in different parts of the range are subject to varying selective pressures (Kenny 1996). Like with other models of GP, it remains important to evaluate system-specific parameters (i.e., population genetic diversity, mode of clonal origin, and niche characteristics) in order to assess how selection has influenced the geographic spread of clonal lineages.

1.1.4 Ecological impacts on the benefits of sex vs asexuality

Another realm of GP theory relates to how the benefits of sex/asex vary in different ecological conditions. The two most prominent candidates here are the Red Queen hypothesis and the Tangled Bank hypothesis. The Red Queen hypothesis, which was verbally presented by Glesener & Tilman (1978), suggests that sexuals will be better able to adapt in the face of rapidly changing biotic pressures (e.g. pathogens and competition) than asexuals due to the benefits of genetic recombination. Sexuals will therefore be able to persist in more ecologically complex areas, while parthenogens will be excluded to more marginal habitats (e.g. higher elevations and latitudes) where biotic interactions are assumed to be less intense (Louthan *et al.* 2015). The Tangled Bank hypothesis focuses more on competition over shared resources, and predicts that the phenotypic diversity of sexuals will allow them to better parse re-

sources in heterogeneously structured environments than a homogeneous set of clones (Bell 1982). Similar to the Red Queen hypothesis, the Tangled Bank expects that parthenogens will be favoured in environments that are unpredictable over space and time, but their demographic advantage over sexuals will vanish in areas of greater diversity (in this case resource diversity as opposed to biotic diversity).

As with all models, there are important assumptions to keep in mind about both the Red Queen and Tangled Bank models. The Red Queen hypothesis assumes the long-held belief that biotic interactions decrease in intensity and frequency with latitude, but that has been challenged by recent large-scale reviews (Moles *et al.* 2011b; Hargreaves *et al.* 2020). In addition, both the Red Queen and the Tangled Bank models assume that asexuals exhibit reduced phenotypic diversity in comparison to sexuals (Tilquin & Kokko 2016), but as explained in the previous section, competition among clones may lead to a diverse array of clonal lineages that may exhibit greater diversity (at least in terms of niche breadth) than local sexuals. The processes and predictions of these two models also bear a conspicuous resemblance to those of the general-purpose genotype and frozen niche variation hypotheses. For example, the Red Queen and general-purpose genotype hypotheses both predict that parthenogens will be relegated to marginal environments. The Tangled Bank and frozen niche variation hypotheses both discuss the ability to partition niche space, but the Tangled Bank assumes that *sexuals* will have the advantage in this context while the frozen niche expects *parthenogens* to have the advantage (assuming the diversity of clones is high enough). Taken together, the overlap among models of GP (and in some cases dissonant expectations) underscores the need to take a comprehensive approach to investigations of the pattern in each system, as the drivers described above may compound or counteract each other in ways that are difficult to predict without sufficient organismal context.

1.2 Geographical parthenogenesis in *Townsendia hookeri*

Some of the most well-developed GP systems are in plants. Plants provide excellent model systems for GP in part due to their great diversity of ploidies and reproductive modes. Angiosperms display a wide range of reproductive strategies, including vegetative reproduction, selfing, and apomixis (parthenogenic seed production), all of which can vary among and even within species. Polyploidy is also quite common in flowering plants (Otto & Whitton 2000; Jiao *et al.* 2011), with some genera exhibiting a range of ploidies, for example, North American *Crepis* range from diploid to as high as decaploid (Sears & Whitton 2016). This widespread variation, coupled with the immense diversity of flowering plants in general (∼300,000 species; Christenhusz & Byng 2016), provides all but limitless contexts in which to test the myriad potential drivers of GP. This thesis employs one of the classic plant GP systems included in the seminal review of Bierzychudek (1985), *Townsendia hookeri* (Townsend's Easter Daisies; figure 1.1), to explore several of the most prominent theories described above.

Townsendia is a charismatic genus in the sunflower family (Asteraceae). First described as a monotypic genus by Hooker (1840), *Townsendia* expanded over the decades with the botanical work of Gray (1888), Larsen (1927), and Beaman (1957b). Beaman's work constitutes the first comprehensive investigation of the genus from an evolutionary perspective, and incorporates field studies, crossing experiments, herbarium specimens, and important cytological work. Beaman was the first to extensively characterize ploidy and mating system variation in *Townsendia*, finding widespread polyploidy and asexual seed production via apomixis amongst many of the species in the genus (Beaman 1954). In addition, he was responsible for clarifying confusing taxonomy concerning *T. exscapa* and *T. sericea* (they were included together in a

mixed herbarium voucher), which resulted in him renaming *T. sericea* to *T. hookeri* in honour of William Hooker (Lee 2015).

Figure 1.1: *Townsendia hookeri* growing in its natural habitat.

As with a number of other species in *Townsendia*, Beaman (1957b) found that *T. hookeri* comprised diploid and polyploid forms, with the diploids being obligately outcrossing (self-incompatible) and the polyploids reproducing via autonomous gametophytic apomixis (producing seed asexually without the need for pollen). He also inferred that polyploids were of autopolyploid origin, and that parthenogenic embryo development was precocious, suggesting that apomixis is obligate without much chance for sexually produced ovules. Through his field and herbarium work, Beaman

was the first to identify that the divergent ranges of sexuals and apomicts in *T. hookeri* exhibit a classic pattern of GP. *T. hookeri* is a long-lived perennial, and its two forms are macro-morphologically indistinguishable despite having different ploidies. The species as a whole occurs primarily along subalpine zones surrounding the Rocky Mountains of North America, with a small disjunct range in the Yukon Territory. Populations typically consist of scattered individuals over somewhat open areas, such as rocky outcrops and hogback ridges, and often co-occur with succulents (*Coryphantha* and *Sedum* spp.), *Phlox* spp., sagebrush (*Artemisia* spp.), and other tough perennials. While sexual populations are situated along Colorado and Wyoming's Front Range, apomictic populations are distributed from southern Wyoming to as far north as British Columbia. Though the sexual and apomictic ranges overlap, no mixed populations had been identified before the work of this thesis began (see Chapter 2). Since Beaman's (1957b) monograph, *Townsendia* was not subject to much conceptual or experimental work (aside from the addition of new species) until it was revitalized by Jeannette Whitton and her lab members in the early 2000's.

Building on the foundation laid down by Beaman, the Whitton lab has investigated patterns of GP in *Townsendia hookeri* using several approaches. Using chloroplast sequence data from populations across the species range, Thompson & Whitton (2006) confirmed that apomixis has originated multiple times and subsequently spread from sexual populations that were likely isolated to glacial refugia, reflecting a pattern that is seen in many apomictic plants (Brochmann *et al.* 2003). Utilizing crossing experiments, Garani (2014) confirmed that pollen from apomicts can successfully fertilize sexual ovules, and that these "heterospecific" crosses can negatively impact sexual seed set. Furthermore, flow cytometric analyses of the offspring from these crosses suggest that both diploids and polyploids were produced, which (along with the negative impact on seed set) points to the potential role of asymmetrical reproductive

interference in limiting sexual range expansion (Kyogoku 2015). Most recently, Lee (2015) used rangewide occurrence records and environmental niche modeling to characterize the niches of sexual and apomictic populations in *T. hookeri*. Their niche models predict that while the occurrence of apomicts is largely in agreement with their current distribution, suitable habitat for sexuals exists within the apomictic range that they are not inhabiting. These results suggest that dispersal limitation may play a role in driving patterns of GP in the system.

1.3 Thesis outline and objectives

The work presented in this thesis continues the excellent work achieved by my labmates and further explores the drivers of geographical parthenogenesis in *Townsendia hookeri*. I evaluate several potential mechanisms for range divergence between sexual and apomictic forms, guided by the rich realms of theory discussed above. Rather than focusing on identifying the single most important driver of GP, I take particular care throughout to consider the ways in which all of these processes may interact to influence the distributional patterns we see today. My hope is that the following chapters will inform the greater biological community beyond the view of *Townsendia hookeri*, and help inspire more integrative approaches to investigating complex ecological and evolutionary processes.

In Chapter 2, I use novel population genomic techniques to analyze the structure of several sexual and apomictic populations across the range of *Townsendia hookeri*. In particular, assessing the diversity of clones within and among apomictic populations (e.g. the number of clonal lineages per population and the occurrence of widespread clonal lineages that are found in multiple populations) helps us draw conclusions about how different selective pressures may have shaped the historical spread of apomicts in the system.

In Chapter 3, I employ modern reciprocal transplant experimental design to explore potential drivers of range divergence between sexuals and apomicts. Dispersal limitation is an important putative factor for limiting sexual ranges in a GP context, while apomicts may be limited more by ecological/biotic factors. Transplanting sexuals and apomicts into each others' ranges and assessing their fitness allows us to compare within vs beyond the range performance for both mating types, which aids in evaluating the factors that limit their respective ranges.

In Chapter 4, I investigate differences in early life history traits between sexuals and apomicts that may contribute to patterns of GP. Given that apomicts have a much broader geographic range than sexuals, it is possible that they possess traits important for colonization (i.e., improved germination success and dispersal ability of seeds in comparison to sexuals), though these may trade-off with traits that are important for establishment and competition.

Chapter 2

Clonal Population Genomic Structure of Polyploid Apomictic Easter Daisies

2.1 Introduction

Sex is prevalent in nature despite its costs (i.e. recombination load, the cost of producing males, the two-fold cost of sex), while asexuality is rare despite its demographic and genetic advantages (Bell 1982; West *et al.* 1999). A widely accepted explanation for this paradox is that meiotic recombination provides benefits to adaptive potential that eventually overcome the costs of sex, while the genetic inflexibility of asexuals limits their response to selection and results in them being "evolutionary dead-ends". However, in systems that have closely related sexuals and asexuals, asexuals often have larger ranges (i.e. geographical parthenogenesis; Vandel 1928; Glesener & Tilman 1978; Bierzychudek 1985). This indicates that asexuals have the ability to establish on the landscape and out-perform their sexual progenitors, at

least in the short term (though there are examples of long-lived parthenogenic lineages; Schon *et al.* 1998; Welch & Meselson 2000). Systems that show patterns of geographical parthenogenesis (GP) are therefore especially valuable for determining the conditions that allow asexuals to flourish where sexuals are not found.

The primary explanations for patterns of GP focus to varying degrees on the demographic benefits of uniparentality (Baker's Law), ecological differentiation between sexuals and asexuals, the genetic consequences of parthenogenesis, and differences in how sexuals and parthenogens respond to selection (Tilquin & Kokko 2016). Distinguishing among these explanations requires teasing apart the ecological and genetic components contributing to the range advantage of asexuals. For example, contemporary clone structure and diversity can give indications of how selection has acted on clonal lineages. We might expect that surviving asexual lineages succeed because they perpetuate trait combinations that are well suited to their environments; an equally fit sexual genotype would be subject to recombinational breakdown of their beneficial suite of alleles. The idea that asexuals are "protected" from this genetic cost of sex (Lynch 1984) has given rise to two opposing (but not mutually exclusive) hypotheses concerning the success of parthenogens in GP contexts: frozen niche variation and general-purpose genotypes (Vrijenhoek & Parker 2009). The frozen niche variation hypothesis asserts that as multiple clonal lineages arise from a diverse sexual background, selection favours clones that best partition the available niche space, resulting in the fixation of an array of specialized clones that have reduced niche overlap with already established sexuals and asexuals (Vrijenhoek 1979). On the other hand, the general-purpose genotype hypothesis states that selection in a fluctuating environment will fix a clonal genotype that is successful in many different conditions, an idea first introduced by Baker (1965) when describing traits associated with weedy plant species.

Though these predictions do not appear to be explicit, researchers often interpret the presence of widespread clones (and generally low clonal diversity) as evidence for general-purpose genotypes, and high clonal diversity (both within and among populations) as evidence for frozen niche variation. There is mixed empirical support for these hypotheses in the literature, however (reviewed in Vrijenhoek & Parker 2009). Providing direct support for either hypothesis is difficult, as one must show both population genetic (i.e., presence of either widespread or partitioned clones) and ecological (i.e., clones with wide vs narrow niches) support. To make matters more complicated, there is no widely agreed-upon definition of a "clone", and researchers use different clonal concepts and make different interpretations depending on the resolution of the marker system used (Martens *et al.* 2009). The fact that both widespread clones and polyclonal populations have been found indicates that these two hypotheses are not competing hypotheses that preclude each other, but instead represent two extremes of a continuum along which asexuals fall depending on each system's unique attributes. Frequency of asexual origins, proximity to sexual progenitors, dependence on sperm or pollen, and the competitive regime facing newly arisen asexuals are all thought to be important factors shaping the diversity and spread of parthenogenic lineages (Vrijenhoek & Parker 2009).

The frozen niche variation model focuses on the importance of direct competition with sexual progenitors (and highly similar clones) in influencing niche diversification, and hence requires a varying array of clonal genotypes for selection to act on (Roughgarden 1974; Vrijenhoek 1979; Bell 1982). For this reason, frozen niche variation is often associated with the need for recurring origins of asexual lineages from extant sexual progenitors in order to efficiently partition the available niche space and withstand strong competition (though the generation of too many clones is predicted to lead to exclusion of sexuals; Weeks 1993). The general-purpose genotype model, on

the other hand, is associated with parthenogens that escape competition with their sexual progenitors; none of the widespread clones reviewed by Vrijenhoek & Parker (2009) required co-occurring sexuals (i.e., were sperm dependent). Instead, these widespread clones are typically considered weak competitors, shunted into "marginal" habitats where biotic interactions are generally less intense (Baker 1965), but where conditions vary over time. In these environments, genotypes that can survive in a range of conditions may be able to persist, whereas highly specialized clones might be eliminated under fluctuating conditions (Parker *et al.* 1977). Under models of GP, while the generation of variable clonal genotypes is still required, the assumption is that only a few successfully establish and become widespread. Given that GP systems often comprise a broad geographic range (with sexuals inhabiting only a small portion), it stands to reason that both patterns (i.e., the existence of both widespread generalist and narrow specialist clones) may be evident in different parts of the range depending on the competitive regime and proximity to sexual populations.

Other genetic attributes are thought to play important roles in patterns of GP, including polyploidy, hybridization, and levels of heterozygosity (Bierzychudek 1985; Haag & Ebert 2004; Kearney 2005). Polyploidy has been linked to rapid diversification and the production of genotypes that may have increased invasive potential (Comai 2005; Te Beest *et al.* 2012). In general, benefits conferred by polyploidy or hybridization will be conserved by asexual reproduction, suggesting that these processes may work in concert to generate patterns of GP. Similarly, asexuals are hypothesized to be protected from inbreeding depression, maladapted gene flow, and loss of heterozygosity (Haag & Ebert 2004), all of which (when combined with the demographic benefits of uniparentality) may contribute to the distributional success of asexual lineages.

In plant GP systems, the majority of parthenogens are polyploid apomicts (pro-

ducing seeds asexually) often with hybrid ancestry (Whitton *et al.* 2008). To date, few studies of GP in plants have investigated the structure and diversity of apomictic populations, but instead focused on comparing the ranges/niches of sexuals and apomicts, the distribution of apomictic cytotypes, or deeper phylogenetic origins of apomictic lineages. Existing evidence, however, seems to provide greater support for frozen niche variation than general purpose genotypes. Literature surveys of clonal diversity in plants (though mainly from vegetative parthenogens) indicate that most asexual populations are polyclonal and that widespread clones are rare (Ellstrand & Roose 1987; Widén *et al.* 1994; Horandl & Paun 2007; Silvertown 2008). Silvertown (2008) found that while apomicts did have lower clonal diversity than vegetative clones, they rarely consisted of monoclonal populations. Similarly, surveys of clonal diversity in model plant GP systems such as *Taraxacum* (Lyman & Ellstrand 1984; Menken *et al.* 1995; Van Der Hulst *et al.* 2003) and *Ranunculus* (Cosendai *et al.* 2013) suggest that populations are rarely if ever monoclonal, but rather have remarkably high levels of genotypic diversity. Support for general-purpose genotypes, on the other hand, has largely come from ecological studies showing that apomicts have a broader niche breadth than sexual progenitors (e.g. in *Antennaria*; Bierzychudek 1989), though Coughlan *et al.* (2017) interpret the presence of widespread clones (but not monoclonal populations) in *Crataegus* as evidence for general-purpose genotypes. Regardless of the trends found in the few well-studied plant GP systems, more evidence is required before generalizations can be made about the diversity and spread of apomictic lineages.

Townsendia hookeri (Asteraceae) is a perennial flowering plant species with diploid sexual and polyploid apomictic forms that display a classic pattern of GP (Bierzychudek 1985). While the apomictic range dwarfs that of the sexuals, the two forms come into contact in a small region of range overlap (Lee 2015). Sexuals are obligately

outcrossing, and apomicts set seed autonomously (i.e., they are non-pseudogamous and therefore do not require pollination to initiate embryo development). As a result, they can colonize new sites without the need for mates or pollinator services (Beaman 1957a; Garani 2014). As part of a broader research program that aims to determine the conditions under which apomicts established a distinct range alongside their sexual progenitors, we used double-digest restriction-site associated DNA (ddRAD) sequencing to explore the genetic structure of sexual and apomictic populations sampled throughout the range in order to address the following questions: (1) Is there evidence for widespread clones with monoclonal populations or an array of clones with polyclonal populations (or both)? (2) What are the relationships between sexual and apomictic populations, and can we infer apomictic origins from sexual populations/regions? (3) Does genetic diversity differ between sexuals and apomicts? We use our findings to explore the predictions of frozen niche variation and general-purpose genotype hypotheses in the context of how selective pressures have shaped clonal diversity in *Townsendia hookeri*.

2.2 Materials and methods

2.2.1 Study system

Townsendia hookeri consists of sexual individuals that are self-incompatible (obligately outcrossing) and apomicts that set seed autonomously (non-pseudogamous). Sexual populations have a smaller range than apomicts and primarily occur between Boulder, CO and Laramie, WY, while apomictic populations range from southern WY along the eastern Rocky Mountains to British Columbia (Lee 2015). A small disjunct distribution of diploid-sexual and polyploid-apomictic populations occurs in the Yukon Territory (Thompson & Whitton 2006; Garani 2014). The majority of

apomictic populations are triploid, with the only known tetraploid populations occurring in the Yukon territory. Before this study, no mixed populations containing both sexual and apomictic individuals had been detected, though there is a range of overlap (centered around Laramie, WY) where both types of populations can be found. The genome size is estimated to be quite large in *T. hookeri* (diploids $\approx$ 7.3 gb). While not much was known previously about the species' population genetic structure, phylogenetic analysis of plastid haplotype variation indicates a minimum of four origins of apomixis (Thompson & Whitton 2006).

2.2.2 Population sampling

We sampled leaf tissue from 27 populations across the range of *T. hookeri*, including 12 populations previously identified as sexual diploids and 15 identified as apomictic polyploid populations (Table 2.1). We sampled populations most densely around the core of the species range (centered around Laramie, WY), but also included populations on the periphery in ND, BC, SK, and YT. Because of our focus on describing the structure and distribution of clones, we aimed to sample 5 individuals per apomictic population (except for populations L41 and S03, for which only 4 individuals were available), and 3 individuals per sexual population, for a total of 114 individuals. We sampled individuals somewhat haphazardly within populations, but made sure to spread our sampling spatially across each site. We dried field-collected leaf tissue using silica gel, and then stored it in a -80°C freezer. The ploidy level of populations used in this study was either known previously or confirmed using flow cytometry (Lee 2015, unpublished). Mixed-ploidy populations were previously thought not to occur, but flow cytometric analyses of one of the populations used in this study (L62; previously characterized as diploid only) revealed that it harbors polyploids at low frequencies. Ploidy assignments were verified using SNP data (see

Section 2.2.5 “Data analysis” below).

2.2.3 ddRAD library preparation

We extracted DNA from frozen leaf tissue using a modified version of the protocol of Murray & Thompson (1980). We assessed the quality of DNA using a NanoDrop spectrophotometer and DNA quantity using a Qubit 2.0 flourometer (Thermo Fisher Scientific, Waltham, MA, USA). We used this DNA to make one ddRAD (a.k.a. genotype-by-sequencing) library containing 114 individuals. We included DNA from each polyploid-apomictic individual twice in the library in order to increase the sequencing coverage, as higher read depth is required to confidently identify variants in polyploids (Dufresne *et al.* 2014); this had the added benefit of providing replicates which allowed us to estimate sequencing and genotyping error rates (see Section 2.2.5). We created the *PstI-MspI* ddRAD library using a modified version of the protocol described in Poland *et al.* (2012). In summary, we first digested the DNA with HF-*PstI* and *MspI* at 37°C for 5 hours, then ligated barcoded adapters and common adapters to digested DNA at 22°C for 3.5 hours. Following ligation, we cleaned and concentrated samples using SPRI magnetic beads, then amplified by PCR using KAPA HiFi Hotstart master mix (Kapa Biosystems, Wilmington, MA, USA). We then pooled all samples together into a single library using normalized concentrations, and selected DNA fragments between 300-450 bp using gel size selection. We checked the quality of the completed library using qPCR and Bioanalyzer (Agilent, Santa Clara, CA, USA) before sequencing on one lane of Illumina HiSeq 2500 paired-end 125bp platform at Génome Québec (Montréal, Québec, Canada). The sequencing resulted in ∼270 million reads with an average quality score of 35.

2.2.4 *De novo* assembly and SNP calling

We de-multiplexed the raw reads, concatenated the polyploid-apomictic replicate files, and performed *de novo* assembly and SNP calling with dDocent v2.7.8 (Puritz *et al.* 2014). dDocent is a bioinformatic pipeline that combines several existing software packages and is designed specifically for efficient assembly and SNP calling of paired-end RAD data in non-model organisms. Notably, dDocent uses freebayes (Garrison & Marth 2012) to call SNPs, which is capable of processing both diploid and polyploid data. In order to reduce the potential for recently derived paralogs to influence the quality of the assembly, we only included previously identified diploid-sexual individuals when constructing the reference catalogue. We also excluded the YT sexual population (C59) from the assembly, because preliminary analyses showed it to be highly divergent from the other sexual populations. We used a reference optimization script provided by Puritz (2019) to choose assembly parameters, using only reads that had a depth of at least 4 within individuals and were found in at least 4 individuals, and a clustering similarity of 90%. After creating the reference catalogue, we used it to call SNPs on all individuals, outputting the results in a variant call file (VCF). We re-ran the pipeline using un-concatenated polyploid-apomict files in order to estimate error rates between replicate samples.

We filtered the resulting VCFs using bcftools (Li *et al.* 2009) and vcflib (Garrison 2019) following the dDocent SNP filtering tutorial (Puritz 2019). First, we filtered SNPs to a minimum quality score of 30, minor allele count of 3, minor allele frequency of 5%, and only kept SNPs genotyped in 95% of the individuals. We also filtered SNPs with an average depth below 20 (set high to ensure confident SNP calling in polyploids) and above 132.5 (exluding paralogs and multicopy loci). We performed further filtering steps to remove SNPs likely to be the result of sequencing errors,

paralogs, multicopy loci or artifacts of library preparation. These include: filtering based on allele balance (removed loci for which the less common SNP variant was below a frequency of 0.125 and above 0.875), removing SNPs found on both forward and reverse reads, filtering based on ratio of mapping qualities between reference and alternate alleles (below 0.9 and above 1.05), and filtering based on ratio of locus quality score and depth (removing any locus that has a quality score below 1/4 of the depth). We removed indels and other complex variants, and only kept bi-allelic SNPs. Our filtering steps resulted in a final VCF containing 16,573 SNPs.

2.2.5 Data analysis

We analyzed clone structure in the SNP dataset using the packages POPPR v.2.8.3 (Kamvar *et al.* 2014, 2015) and ADEGENET v.2.1.2 (Jombart 2008; Jombart & Ahmed 2011) in R v.3.6.1 (R Core team 2019). We imported the VCF into R using the VCFR package (Knaus & Grünwald 2016, 2017), and converted the data into a "genind" object (used by ADEGENET and POPPR), which allows individuals to be coded as different ploidy levels. We calculated pairwise prevosti distances (Prevosti *et al.* 1975) between each individual and used POPPR's *mlg.filter* function to determine the number of multi-locus genotypes (MLGs) present in the dataset. This is accomplished by visualizing the distribution of pairwise genetic distances and choosing a distance threshold indicated by a gap in the distribution; we chose a distance cutoff of 0.1, which grouped individuals into the same MLG (i.e., clone) if they had a genetic distance of 0.1 or less (Figure 2.1A). This cutoff is somewhat higher than the average pairwise distance between apomictic replicate samples (0.027), which served as our estimate of genotyping error rate. Error rates between replicates were largely below 0.05, but four replicate pairs had especially high error rates (between 0.075 and 0.10). As discussed below (Section 2.3.1), whether we exclude these samples as outliers and

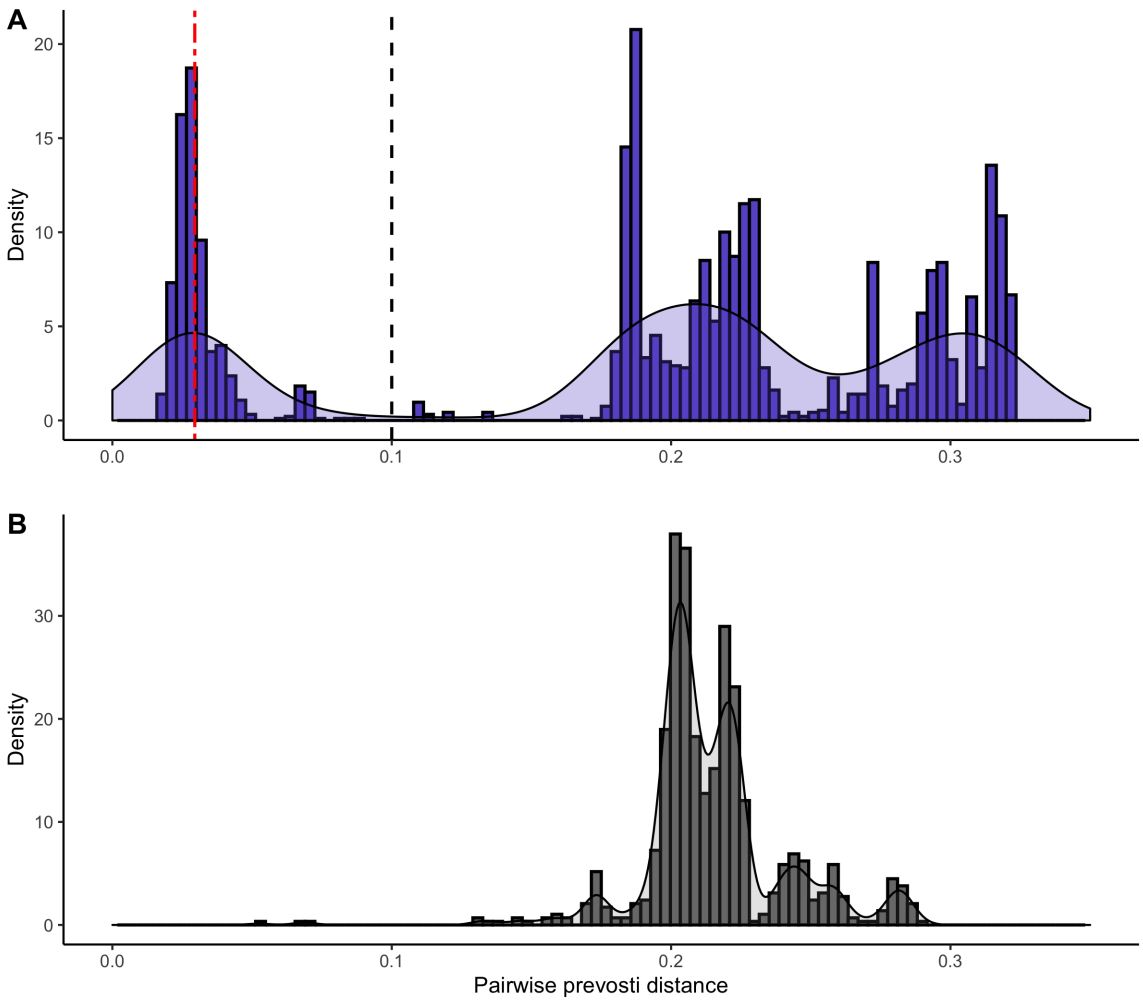

Figure 2.1: Density plots of pairwise prevosti distances (A) between all apomictic individuals and (B) between all sexual individuals. In panel (A), the red line indicates the mean distance between replicates (our estimation of genotyping error rate) and the black line indicates our genetic distance threshold for MLG designations (where any individuals with a genetic distance less than 0.1 were grouped into the same MLG).

use a lower cutoff, or include these samples and set a higher cutoff, does not change our interpretation of the number of MLGs.

We used discriminant analysis of principal components (DAPC; Jombart *et al.* 2010) in ADEGENET to investigate patterns of genetic structure and compare clustering of apomictic individuals to the distance-based MLG designations. First, we

estimated the number of groups within the apomicts using K-means clustering, which uses principal components analysis (PCA) and then determines clusters using discriminant analysis *without* prior population membership. To accomplish this, we used the *find.clusters* algorithm to identify clusters and explored a range of K-values (corresponding to the number of clusters) that had the lowest Bayesian information criterion (BIC). We also used DAPC *with* prior population membership to explore genetic diversity between all populations using the *dapc* function. We used the *xvalDapc* function to select the optimal number of principal components (PCs) to include in this analysis.

To further explore the relationships between sexual and apomictic populations, we built neighbor-joining trees of individuals and populations. We used prevosti distance to construct a tree displaying all individuals, and Nei's distance (Nei 1972) to show relationships among populations. We used POPPR's *aboot* function to generate trees with 1000 bootstraps each and used the APE package (Paradis & Schliep 2019) to visualize.

We used the HIERFSTAT package (Goudet 2005) to calculate overall and pairwise *Fst* values for sexual populations and tested for structure between populations using the G-statistic test with 1000 bootstraps. We used POPPR to calculate observed heterozygosity and a Kruskal-Wallis test to test for differences in observed heterozygosity between mating systems.

We confirmed the ploidy of individuals using a method described by Knaus & Grünwald (2018), where distributions of allele balances of heterozygotes are compared to expected patterns at each ploidy level. A peak in an allele balance histogram at 0.5 is expected for diploids, while peaks at 0.33/0.66 are expected for triploids and 0.25/0.5/0.75 for tetraploids. One individual that was originally thought to be polyploid (L45_1, in an otherwise polyploid population in the apomictic part of the

range) was identified as diploid from allele balance plots (Figure 2.7).

2.3 Results

2.3.1 Clone structure

Genetic distance thresholds delineated 10 MLGs amongst the 73 apomictic individuals in the dataset. Most populations were inferred to be monoclonal, with just two populations (L39 and L16) having representatives from two MLGs (Figure 2.2). Two clonal genotypes were widespread, each occurring in multiple, geographically clustered populations. The most commonly detected MLG was spread across 7 populations in central WY and MT (shown in blue on Figure 2.2) and comprised 33 sampled individuals. Mixed populations (consisting of multiple MLGs or both sexual and apomictic individuals) were mostly located in the region where the ranges of sexuals and apomicts overlap. Four individuals had higher within-MLG pairwise distances than the others (see the small distribution between $\sim$ 0.05 and 0.1 in Figure 2.1A) due to having higher error rates. Excluding these samples as outliers and setting the MLG cutoff to 0.055 resulted in the same number of MLGs as the current setting of 0.1.

K-means clustering analyses largely support the distance-based MLG designations, with apomictic individuals from the same MLG being clustered, and generally showing the same geographically structured groups (Figure 2.3C). Notably, the two widespread clones (blue and sky blue) were recovered under values of K from 8-11 (at K=12 individual L16-3 is assigned its own group). Posterior membership probabilities were mostly 1.0, except for the large widespread MLG (blue, K=9-12). This may indicate sub-structure within that clonal lineage, though it more likely reflects that those values of K were too high and that individuals were being assigned to two

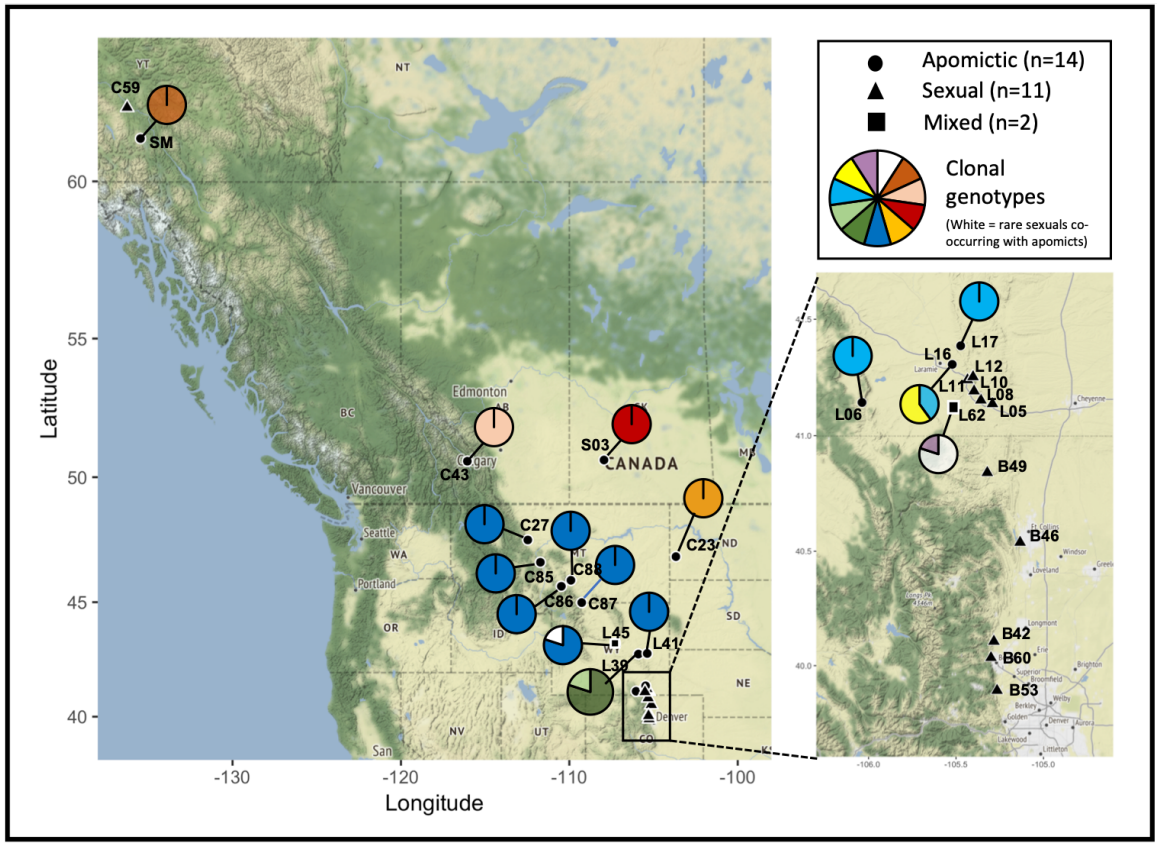

Figure 2.2: Map displaying populations sampled and the clonal structure of apomictic populations in *T. hookeri*. Circles represent apomictic populations, squares represent mixed sexual-apomictic populations, and triangles represent sexual populations. Colored pies indicate the MLG designation based on genetic distance thresholds (MLG cutoff = 0.1). White shading in pies L45 and L62 indicate sexual individuals in mixed populations.

groups that were not substantially different from one another (see overlap of blue, lilac, and purple groups when K=9-12; Figure 2.10).

DAPC of all individuals showed 7 distinct clusters of apomictic individuals, and overall depicted a similar pattern to the genetic distance and K-means results (Figure 2.5). Individuals from the two widespread clones formed distinct groups, and all other monoclonal populations were well-differentiated from each other. Individuals from populations designated as polyclonal by genetic distance thresholds were grouped together in the DAPC, which suggests that these clonal lineages share a

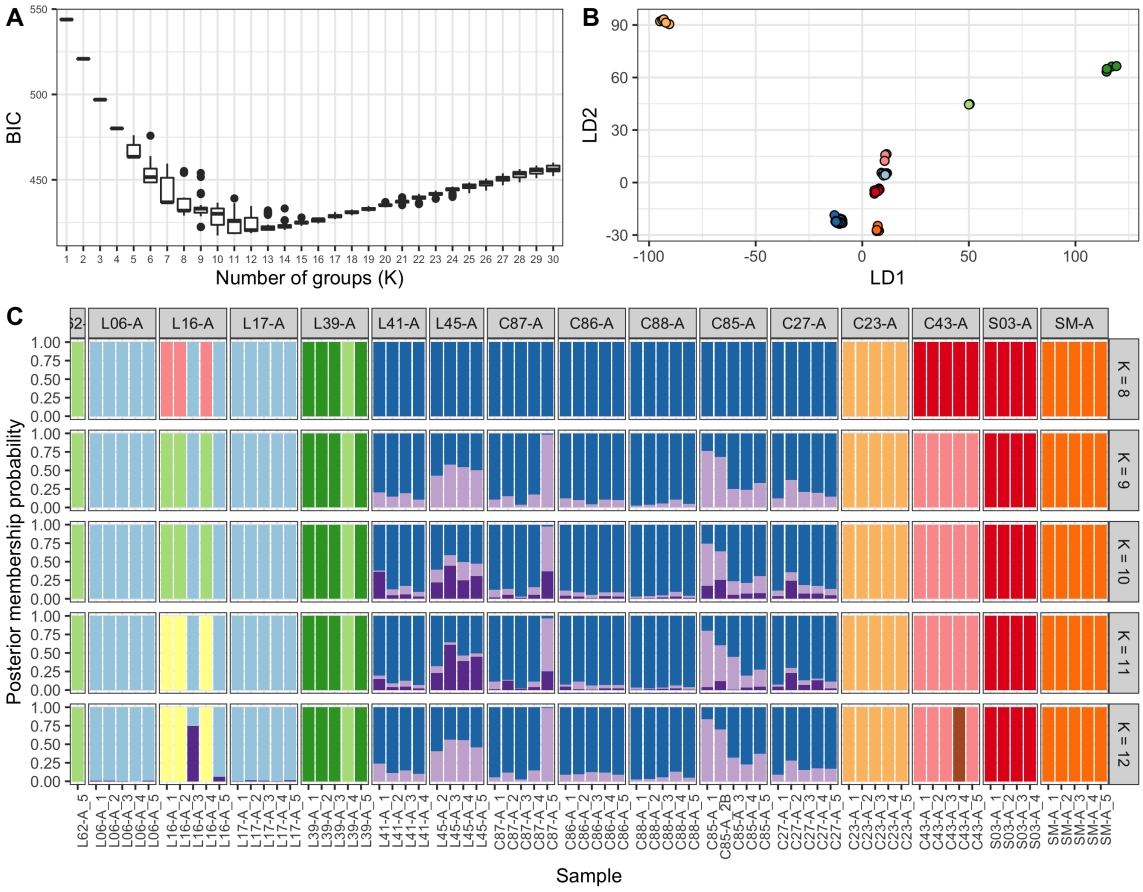

Figure 2.3: K-means clustering results for apomictic populations. (A) Plot of BIC values under a range of K values. (B) DAPC showing differentiation of clusters when K=8. (C) Plots of posterior membership probabilities of group assignments for K=8-12. Individuals are grouped by population, and shared colors indicate individuals with the same group membership.

recent evolutionary history. L62-5 (an apomictic individual from a majority-sexual population) grouped closely with the individuals in population L39, despite being much closer geographically to populations L06, L16, and L17 (Figure 2.2).

2.3.2 Genetic structure of sexual populations and relationships with apomictic lineages

Sexual individuals generally had greater pairwise genetic distances between individuals within populations than we observed between apomicts within MLGs, the one

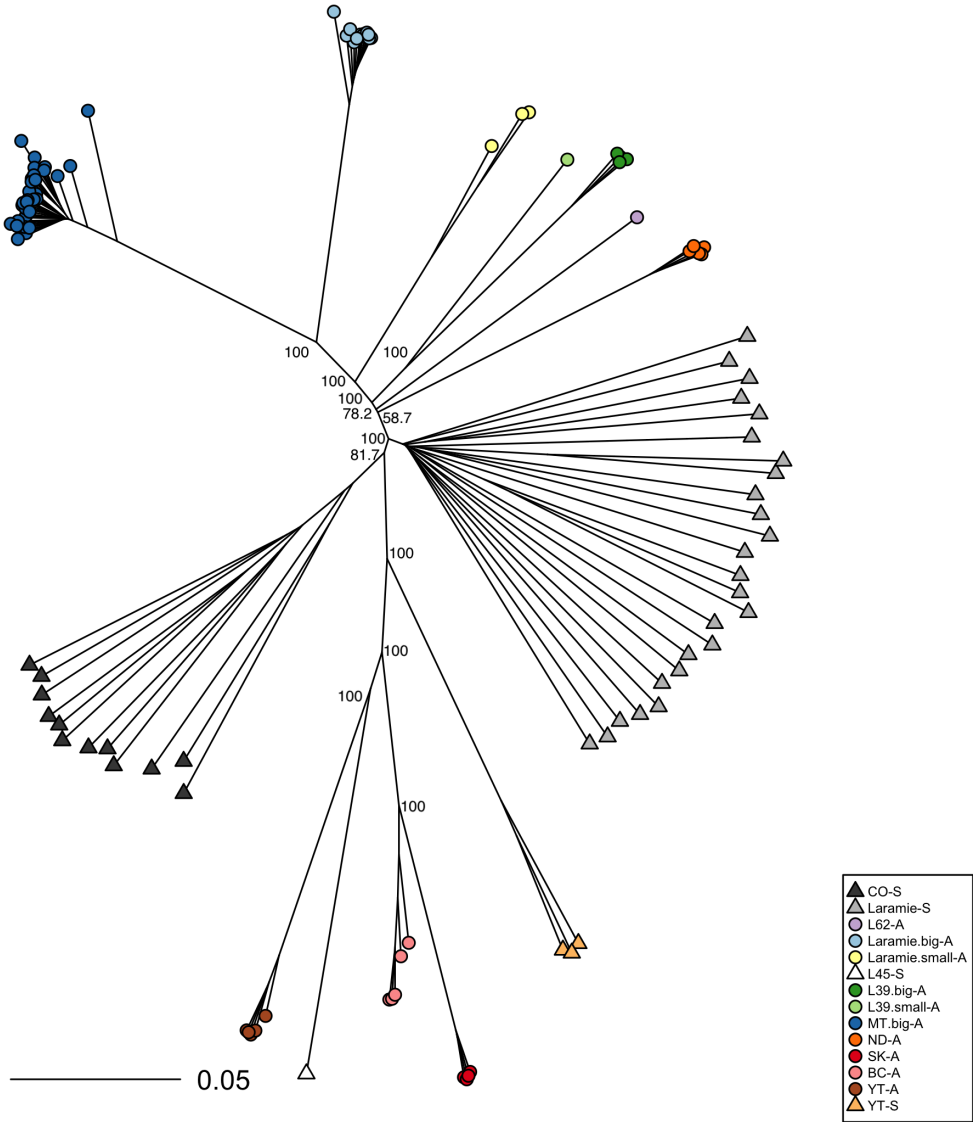

Figure 2.4: Neighbor-joining tree of all sexual and apomictic individuals based on pairwise prevosti distances. Apomictic individuals are represented by circles which are colored by their MLG designations. Sexuals are represented by triangles which are colored according to whether the populations are in Colorado, near Laramie, WY., or the Yukon Territory. Bootstrap values are displayed for the nodes along the backbone of the tree.

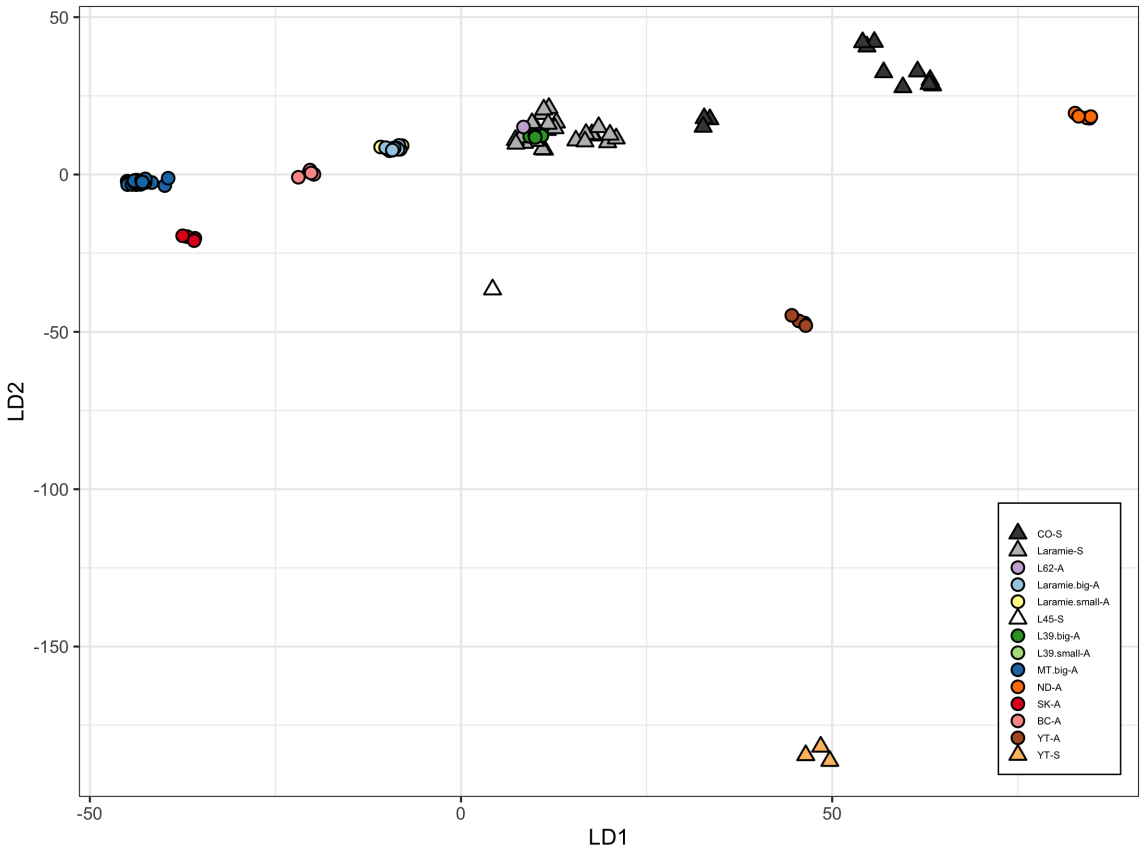

Figure 2.5: Discriminant analysis of principal components (DAPC) of all sexual and apomictic individuals. Linear disciminants 1 & 2 are plotted. Circles represent apomictic individuals, triangles represent sexual individuals. Individuals are colored by their MLG designations (apomicts) or their geographic location (sexuals).

exception involving the disjunct sexual population found in YT (C59), which had lower pairwise distances ($\sim$ 0.05-0.07; Figure 2.1B). Sexual populations were differentiated into 3 groups geographically situated around Boulder, CO (black), Laramie, WY (gray), and YT (gold) (Figure 2.2, Figure 2.4, Figure 2.5). Pairwise *Fst* among sexual populations averaged 0.166 and the *G*-statistic test indicated significant population structure ($P = 0.01$). Pairwise *Fst* values ranged between 0.087 and 0.371 (Figure 2.9, Table 2.2), with the higher values being between the YT (C59) and the populations in the core of the range.

Neighbor-joining trees suggest at least two origins of apomictic lineages, with one being most closely related to the lineage represented by the YT sexual population and the other emerging from the Laramie sexual clade (Figure 2.4, Figure 2.8). The peripheral apomictic populations found in Canada (and the YT sexual population) were well-differentiated from the others but most closely related to the Boulder sexuals (albeit with lower bootstrap support; 81.7). The clonal lineages in the apomictic interior range were more closely related to the sexuals found in Laramie.

2.3.3 Genetic diversity of sexuals and apomicts

Average observed heterozygosity was higher amongst apomictic individuals (0.425) than amongst sexual individuals (0.215; χ^2=76.831, P=2.2x10^{-16}, Figure 2.6). YT sexual individuals (C59) had the lowest observed heterozygosities, while individuals with the highest values were part of MLGs spread across multiple clonal lineages.

2.4 Discussion

2.4.1 Clone identification

The identification of clonal lineages requires careful consideration of several factors, including the marker system used, the number of markers, the use of distance/similarity cutoffs, and what clonal concept is being employed (Martens *et al.* 2009). Marker systems vary in sensitivity, such that the type of marker used (e.g., dominant vs codominant) and their rates of evolution (e.g., AFLPs vs microsatellites) can have a large impact on the estimation of clonal diversity (Horandl & Paun 2007). In addition, if clones are being defined as individuals with 100% identity in whatever marker system is used, then the number of clonal genotypes inferred is expected to rise with the number of markers scored. Given some probability for scoring errors (Douhovnikoff

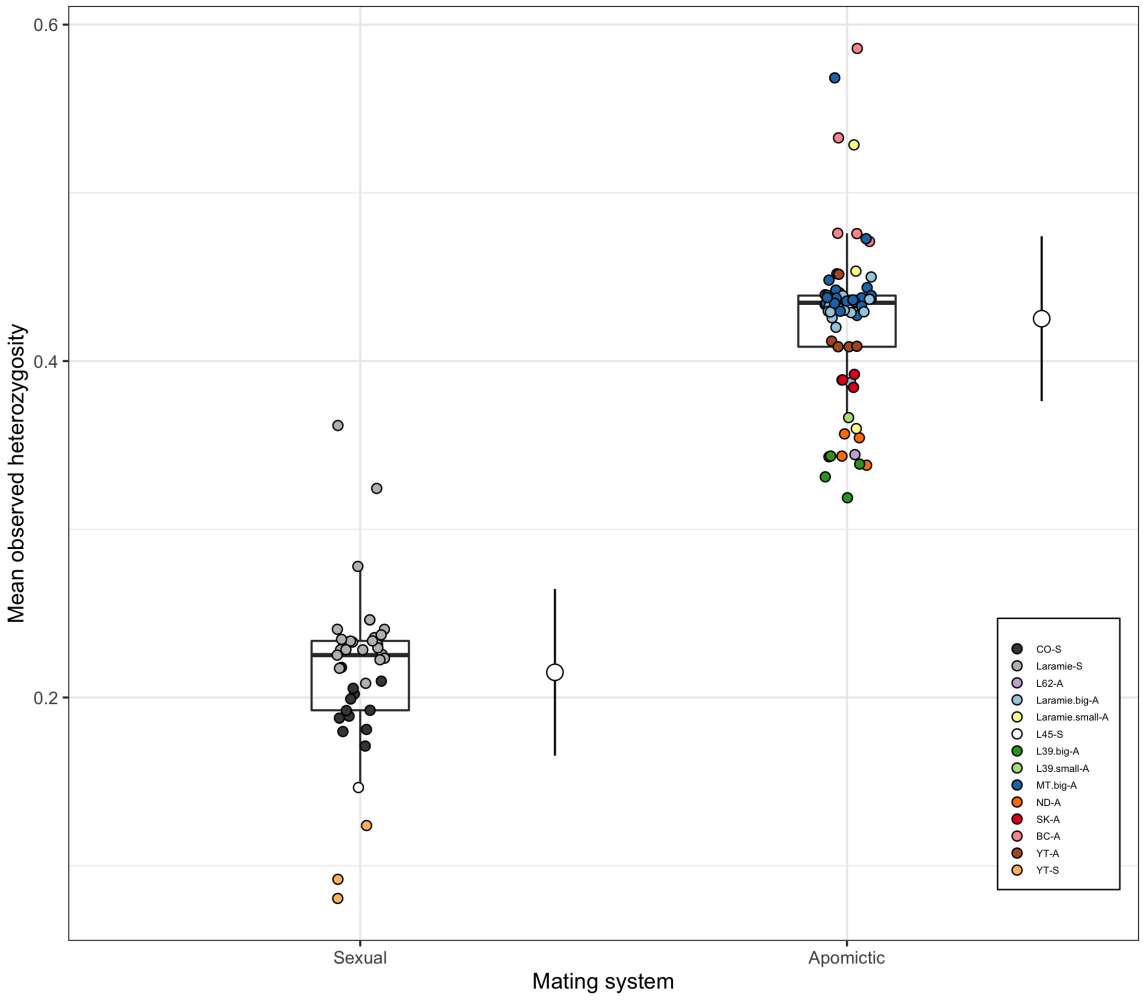

Figure 2.6: Boxplots of observed heterozygosity in sexual and apomictic individuals. Jittered points represent individual means, and points with error bars (to the right) indicate means (± s.d.) for each mating system.

& Dodd 2003), the possibility of overestimating the number of clones will also increase with the number of markers used (Arnaud-Haond *et al.* 2007). For this reason, Arnaud-Haond *et al.* (2007) recommend using cutoffs to allow small amounts of genetic distance when designating clones; this is done by creating histograms of pairwise genetic distances between individuals and using a "hump" in the distribution (representing individuals with very low distances) to guide the cutoff. The use of cutoffs for clone identification is crucial when using high-throughput sequencing (HTS) ap-

proaches such as ddRAD, because the elevated number of markers (and associated error-rates) would cause every individual to be assigned a unique MLG without the careful application of distance thresholds. However, we note that in taking this approach, we will likely group together clones that are differentiated by a small number of somatic mutations. As a result, MLGs as we define them might be best thought of as clonal lines (sometimes referred to as multi-locus lineages) rather than strict clones. With that in mind, our replicate sequencing of apomictic individuals helped us verify which humps were due to sequencing artifacts or genotyping errors and which ones represent true genetic differences.

Due to the variation in marker systems and statistical methodology used, it is difficult to compare measures of clonal diversity between studies. The fact that some researchers use a cutoff approach while others do not highlights a definitional problem, in that some use a "molecular" clonal concept where clones are defined as groups of individuals with 100% sequence similarity, while others use a "phylogenetic" clonal concept where a clone is defined as a monophyletic cluster of individuals that are genetically very similar (see Martens *et al.* 2009 for a review of clonal concepts). Somatic mutations are common in plants (Horandl & Paun 2007), which must be considered when analyzing asexual populations using modern marker systems that generate thousands of SNPs. To our knowledge, the use of HTS to investigate the population genetic structure of clonal plants has been limited, and so far does not appear to have been applied to apomictic plants (but see Bock *et al.* 2018 who used ddRAD to identify clones in vegetatively propogating *Helianthus tuberosus* using similar cut-off methods). Given the volume of data and potential for somatic mutations and genotyping error rates, researchers should strongly consider the use of appropriate cutoffs when using HTS to investigate clone structure. Our study shows that ddRAD can provide the resolution to identify clones with a degree of certainty that may not

be possible with other marker systems (see Figure 2.1A which shows a multimodal distribution of pairwise distances with a clear distinction between humps). The cost of ddRAD and other HTS technologies continues to decrease, allowing the generation of very rich datasets for non-model organisms (Matz 2018). For those with access to a reference genome, HTS allows the investigation of in-depth patterns of genome evolution that are of interest to those studying apomictic plants (e.g., the accumulation of mutations in *Boechera*; Lovell *et al.* 2017).

2.4.2 Clone structure in *Townsendia hookeri*

All of our analyses point to the presence of widespread clones and largely monoclonal populations in apomictic *T. hookeri*, a pattern that appears to be rare in asexual plant populations. Consistent with expectations under general-purpose genotype models, we found that 13 out of 15 populations were monoclonal, and that over 60% of our sampled apomictic individuals belonged to one of two widespread clones. Perhaps most striking is our finding of one geographically widespread clone (found in 7 out of 15 populations and 33 out of 73 individuals), which was the only clonal lineage detected in all populations sampled in Montana and northern Wyoming. While the sample sizes from apomictic populations were relatively small (5 individuals) and denser sampling may have unveiled additional clonal lineages, the general pattern of low intrapopulation diversity (as well as the broad spatial patterns) are likely to be robust to additional sampling.

Townsendia hookeri has multiple characteristics that have been hypothesized to be associated with conditions that would favor the general-purpose genotype model. Apomicts are autonomous and have no dependence on pollen or pollinators, attributes which have likely allowed them to disperse long distances and escape competition with their sexual progenitors. Evidence provided by Beaman (1957a) indicates that

embryo development is precocious in *Townsendia*, which suggests that apomixis is obligate and that the potential for sexually produced ovules is low. All else being equal, obligate apomicts are expected to have lower clonal diversity than facultative apomicts (Horandl & Paun 2007). In addition, the apomictic range of *T. hookeri* is consistent with high abiotic stress, low competition (populations are mainly found on rocky soils with sparse vegetation), and small population sizes in comparison to sexuals, all of which is in line with Baker's (1965) assertion that general-purpose genotypes are likely to be weak competitors excluded to "marginal" environments.

Our finding of broadly distributed clones stands in stark contrast with what has been found in other apomictic species. While monoclonal populations have been found in several well-studied apomicts, most populations have been found to be polyclonal, with the vast majority of genotypes being restricted to a single site (Horandl & Paun 2007). As discussed above, some of this incongruence may be due to the adoption of different clonal concepts and variation in marker systems, but these studies remain our only point of comparison. In a similar study to our own, Cosendai *et al.* (2013) found that almost every sampled apomictic individual of *Ranunculus kuepferi* had a unique MLG. Although the two systems have many similarities (both have apomictic autopolyploids and self-incompatible sexuals with comparatively small ranges), there are some important differences, most notably that *R. kuepferi* is a pseudogamous facultative apomict (with partial sexuality found in $\sim 1/3$ of sampled seeds; Cosendai & Hörandl 2010). Cosendai *et al.* (2013) suggest that the high clonal diversity in *R. kuepferi* is the result of facultative sexuality and multiple long-distance dispersal events. Similarly, occasional sex is believed to have led to the high genotypic diversity observed within some apomictic microspecies of *Taraxacum* (Van Der Hulst *et al.* 2003), though there appears to be considerable variation in clonal diversity between microspecies (Majeský *et al.* 2015). These studies highlight how the rates of sex

(which are linked to the frequency with which new clonal lineages are generated) can impact the clonal diversity of apomictic plants.

2.4.3 Origins and spread of apomictic lineages

Diploid-sexual individuals clustered into 3 groups geographically, corresponding to populations situated around Boulder, CO, Laramie, WY, and YT. Our neighbor joining tree (Figure 2.4) indicates that apomictic lineages in the core of the range (WY, MT, and ND) were most closely related to the sexuals found near Boulder and Laramie, while some of the apomictic lineages found in the range periphery (SK, BC, and YT) were most closely related to the YT sexuals. These results suggest at least two deep origins of apomictic lineages in *Townsendia hookeri*, with clones originating in YT spreading south and those originating in Boulder/Laramie spreading north. This is consistent with the previously proposed scenario involving post-glacial dispersal of apomictic lineages originating from sexual populations found in glacial refugia (Thompson & Whitton 2006), a pattern that is often associated with GP (Bierzychudek 1985; Kearney 2005). Apomicts were likely able to colonize previously glaciated habitat relatively quickly due to reproductive assurance (i.e., Baker's Law effects) and improved dispersal potential (Chapter 4), while sexual expansion was arrested due to dispersal limitation and/or being excluded from habitat already colonized by apomicts (Mogie 1992; Hewitt 2004; also see evidence for asymmetrical reproductive interference between sexuals and apomicts in *T. hookeri*; Garani 2014). Demographic bottlenecks that would likely have occurred as the wave of colonization proceeded would not have led to genetic bottlenecks in apomicts, given that apomicts are protected from loss of heterozygosity (Figure 2.6). In contrast, for colonizing sexuals, bottlenecks could easily have resulted in inbreeding, with the expectation (given a history of obligate outcrossing) of at least moderate inbreeding depression (Haag

& Ebert 2004). The disjunct sexual population found in YT (C59) had the lowest levels of observed heterozygosity, which may indicate a history of inbreeding caused by genetic bottlenecks or the breakdown of self-incompatiblity mechanisms (Hörandl 2010).

It may not be possible to tie the origins of particular apomictic lineages to sexual populations given that the progenitor sexual populations may no longer exist or may not have been sampled in this study. In our neighbor-joining tree (Figure 2.4), the only instance of sexuals and apomicts grouping together involves the lone diploid-sexual individual (diploidy confirmed via allele balance plot; figure 2.7) found in population L45, an otherwise apomictic population in a part of the range where sexuals are normally not found. This putative sexual individual shares branches with the peripheral apomictic lineages - a somewhat paradoxical result given that it appears to be quite distantly related to the apomictic individuals found in the same population. *K*-means clustering analysis (Figure 2.3) grouped apomictic lineages found in BC and SK together (K=8, populations C43 and S03), as well as lineages from populations L16, L39, and L62 (all from polyclonal populations found near the core of the range; K=9-10, light green), which may indicate common origins. The latter were also tightly grouped in DAPC analyses (Figure 2.5) and overlapped with sexual individuals from Laramie, which suggests these apomicts originated from Laramie sexual populations.

2.4.4 Beyond frozen niches and general-purpose genotypes

Various features of lineages with parthenogenic organisms may influence their diversity and geographic structure, including the frequency of origins, proximity to sexual ancestors, dependence on sperm, and the competitive regimes that clonal lineages face in time and space. Vrijenhoek & Parker (2009) point out that portraying frozen

niche variation and general-purpose genotypes as mutually exclusive hypotheses fails to acknowledge that the two models focus on different sources of fitness variation: the frozen niche variation hypothesis is often considered in the context of spatial fitness variation, while general-purpose genotype models focus on fitness fluctuations in time. This is likely an oversimplification - fitness can vary in both space and time - which underscores the need to move away from the false dichotomy of general-purpose genotypes vs frozen niche variation and towards more nuanced models of geographical parthenogenesis (and asexual diversity in general).

The predictions that general-purpose genotype and frozen niche variation hypotheses make about the population genetic structure of clones are unclear, at least in part because the two terms are inconsistently applied. Support for these hypotheses tends to come in the form of either ecological or population genetic evidence, but rarely both. To add to the confusion, the term "general-purpose genotype" was first used to describe the characteristics of weedy plant species (Baker 1965), so this term is also used in non-parthenogenic contexts. The existence of higher-than-expected clonal diversity in many systems is invoked as evidence against general-purpose genotypes and support for frozen niche variation, even when the results are not consistent with either model. Cosendai *et al.* (2013) state that, while their findings are more consistent with frozen niche variation than general-purpose genotypes, the fact that apomicts in *Ranunculus kuepferi* do not show clonal population structure (i.e. every individual was a different clone) suggests that ecological niches are not being "frozen" by specialized apomictic lineages. This begs the question: what are the specific predictions that frozen niche variation makes about the population genetic structure of asexuals? It seems that either multiple clones per population *or* monoclonal populations (but no widespread clones) have been interpreted as consistent with frozen niche variation. This is consistent with the definition of the model, provided that each clone occupies

a different niche. With the niche breadth lens, a widespread clone that occupies a narrow but broadly available niche would also fit the frozen niche variation model, but in the absence of the evaluation of niche breadth this pattern would likely be erroneously interpreted as a general-purpose genotype (Vrijenhoek & Parker 2009).

Despite the opacity of the general-purpose genotype vs frozen niche variation discussion, it can nonetheless be helpful to think about the conditions that are expected to give rise to each pattern. In *T. hookeri*, the patterns of clonal diversity found in the apomictic range indicate that a small number of lineages were successfully able to establish and spread (and/or that clones originate infrequently). The existence of a very widespread clone spread across several monoclonal populations (colored blue; Figure 2.2) is consistent with the hypothesis that this region was colonized by a single successful genotype. On the other hand, we found greater clonal diversity near the core sexual range, which could suggest that in this region, clones are successful over narrower conditions. Given the proximity of sexual populations, this distribution pattern also supports an origin of many of the apomictic lineages in this southern region of their range. Thus we see patterns of diversity consistent with general-purpose genotypes in one part of the range and (at least as assessed by clonal diversity) frozen niche variation in the other. In the north, the lack of clonal diversity could reflect a history of strong selection excluding genotypes incapable of persisting in the harsh conditions that characterize their habitat. Such "hard selection" is expected to lead to the evolution of a broad niche width, resulting in genotypes that can persevere in varying abiotic conditions but are sensitive to biotic pressures (Kenny 1996). In the south, less variable environments with more intense competition may have led to the the fixation of multiple clones, which would be predicted to have comparatively narrow niches.

Patterns of geographical parthenogenesis are the result of complex ecological and

evolutionary dynamics between sexuals and their asexual descendants. General-purpose genotype and frozen niche variation models are complimentary frameworks that, when taken together, provide predictions about how clonal diversity is shaped by the frequency of origins and the subsequent selective pressures that clonal lineages face. Characterizing the genetic structure of clonality provides necessary insight into the history of asexual spread, but these results can only be fully appreciated when placed within a context that includes information about the ecological performance and dispersal potential of sexual and asexual forms.

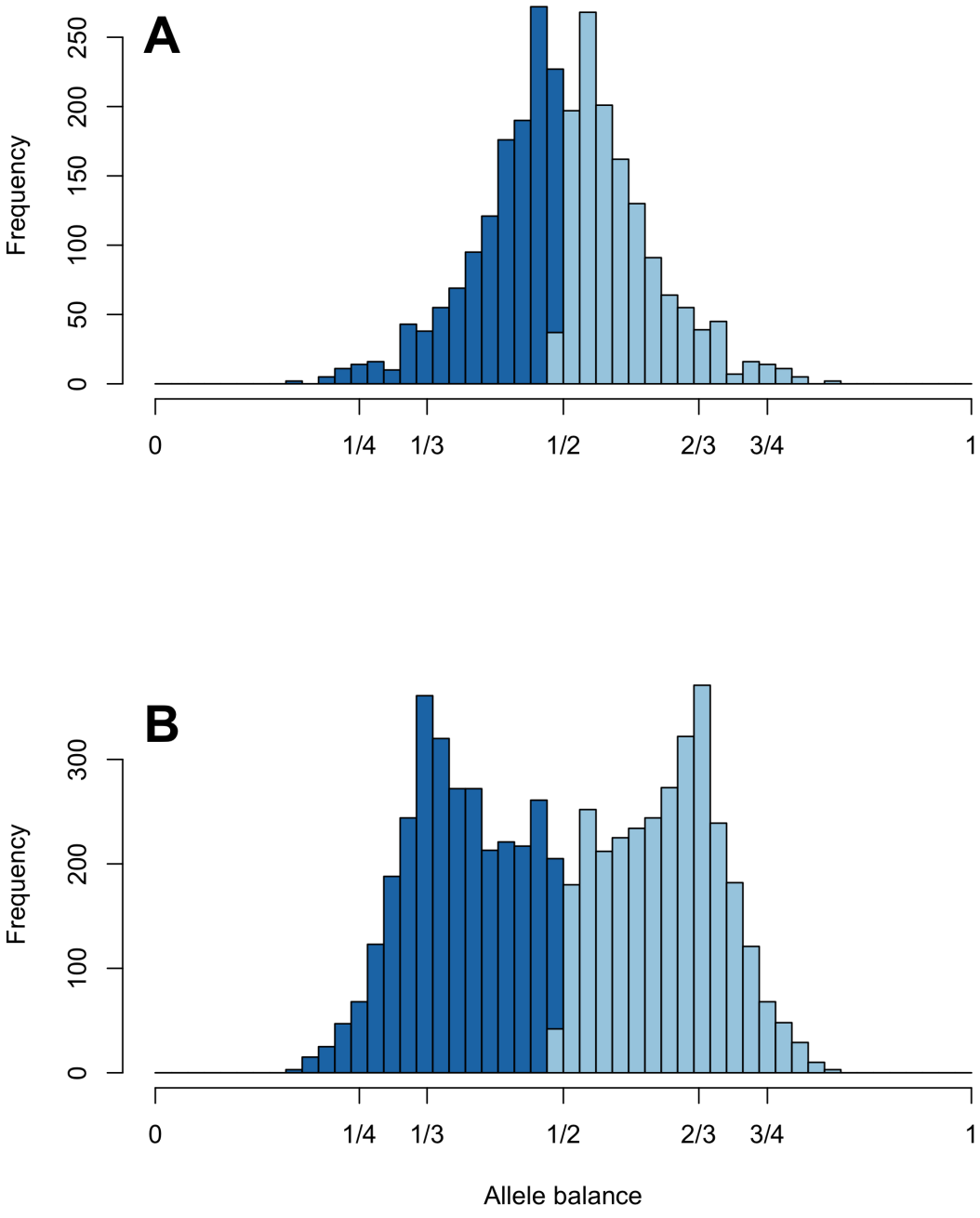

Figure 2.7: Histograms showing allele balance of heterozygous loci. A) Individual L45-1; the peak centered around 1/2 indicates that this individual is diploid. B) Individual L45-2; the peaks centered around 1/3 and 2/3 indicate that this individual is triploid.

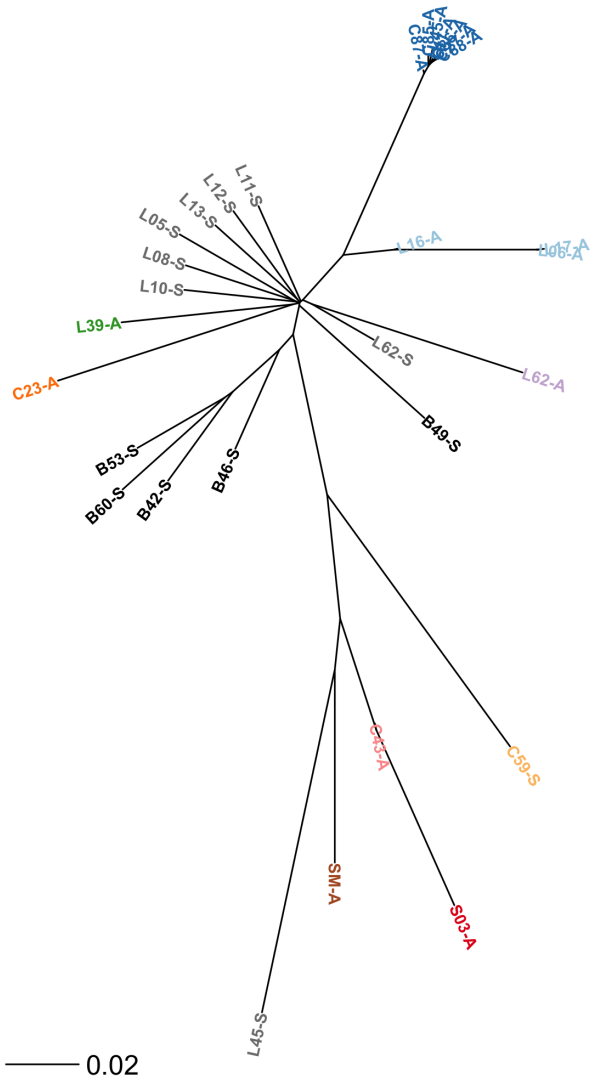

Figure 2.8: Neighbor-joining tree based on pairwise Nei's distance between all populations.

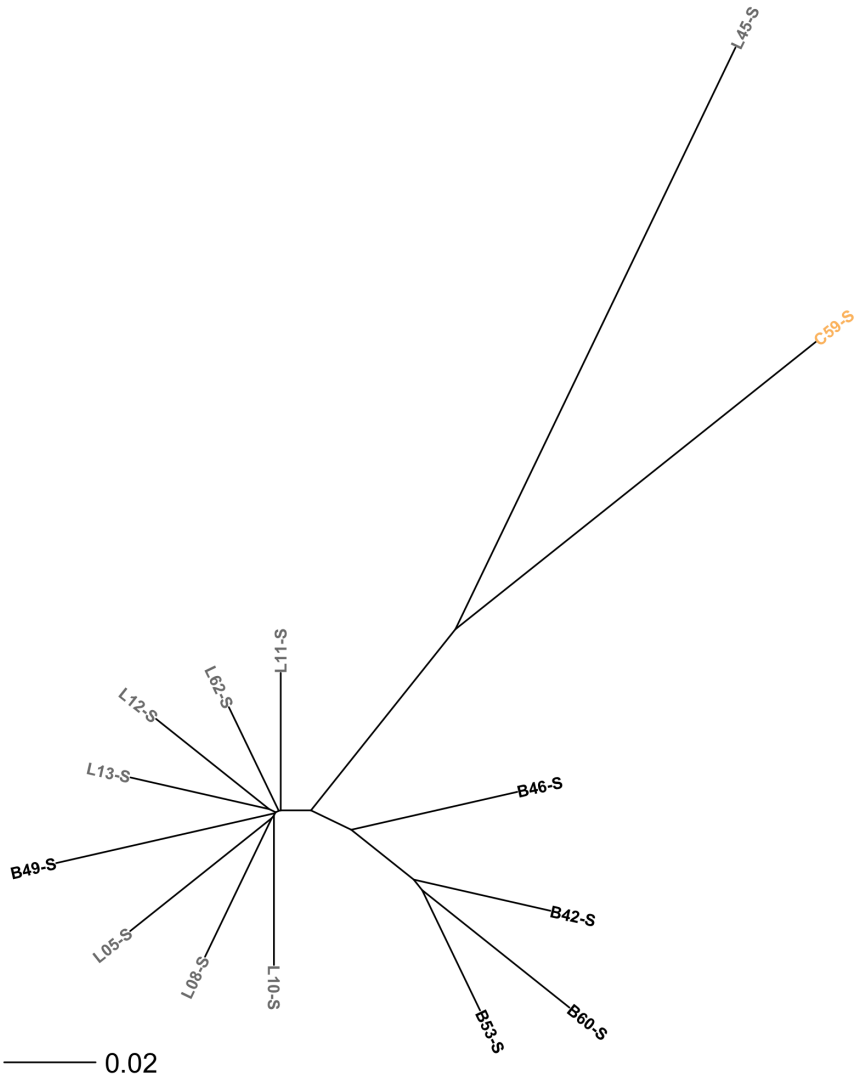

Figure 2.9: Neighbor-joining tree based on pairwise Nei's distance between sexual populations.

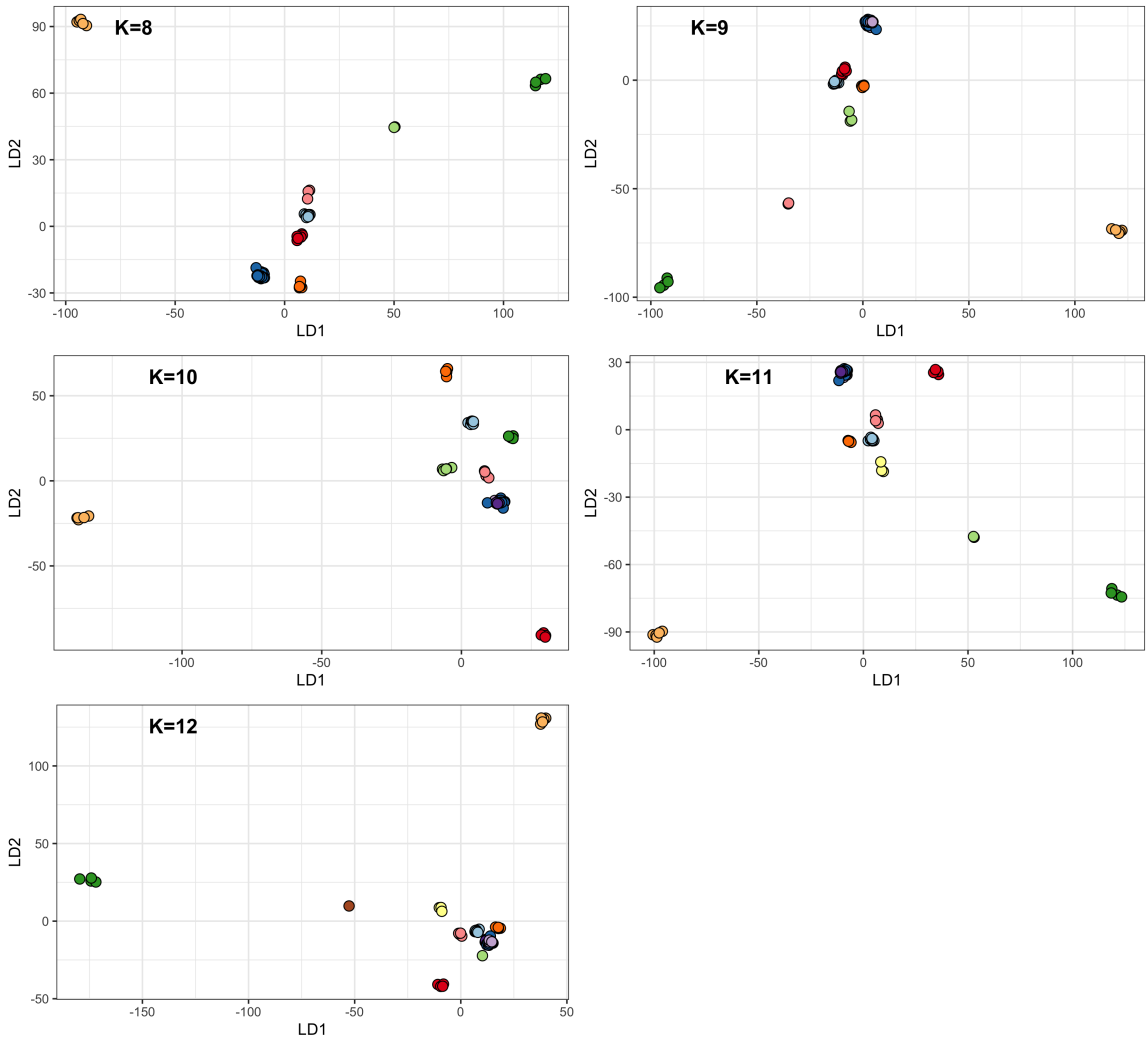

Figure 2.10: DAPC plots showing differentiation of groups under different values of K from K-means clustering analysis of apomictic populations. When K=9-12, blue, lilac, and purple groups overlap, indicating that these groups are not well differentiated.

Table 2.1: List of *Townsendia hookeri* populations sampled.

Mating System	Population	Ploidy	State/Province	Latitude	Longitude	Number sampled
Sexual	B53	2	CO	39.8913	-105.2655	3
	B60	2	CO	40.0352	-105.3001	3
	B42	2	CO	40.1070	-105.2836	3
	B46	2	CO	40.5384	-105.1335	3
	B49	2	CO	40.8403	-105.3214	3
Mixed	L62	2/3	WY	41.1140	-105.5118	4/1
	L05	2	WY	41.1372	-105.2916	3
	L08	2	WY	41.1529	-105.3578	3
	L10	2	WY	41.1919	-105.3967	3
	L11	2	WY	41.2400	-105.4342	3
	L12	2	WY	41.2529	-105.4062	3
	C59	2	YT	62.1235	-136.2575	3
Apomictic	L06	3	WY	41.1430	-106.0375	5
	L16	3	WY	41.3065	-105.5216	5
	L17	3	WY	41.3864	-105.4735	5
Mixed	L39	2/3	WY	42.7833	-105.9000	1/4
	L41	3	WY	42.8087	-105.3778	4
	L45	3	WY	43.2566	-107.2798	5
	C87	3	MT	44.9827	-109.2657	5
	C86	3	MT	45.6619	-110.4752	5
	C88	3	MT	45.9108	-109.9078	5
	C85	3	MT	46.6505	-111.7200	5
	C23	3	ND	46.8788	-103.6716	5
	C27	3	MT	47.5500	-112.4667	5
	C43	3	BC	50.6057	-116.0595	5
	S03	3	SK	50.6526	-107.9437	4
	SM	4	YT	61.2461	-135.4625	5

Table 2.2: Pairwise *Fst* values between diploid-sexual populations of *Townsendia hookeri*.

	B42	B46	B49	B53	B60	C59	L05	L08	L10	L11	L12	L13	L45	L62
B42														
B46	0.165													
B49	0.203	0.180												
B53	0.132	0.161	0.199											
B60	0.156	0.188	0.226	0.146										
C59	0.346	0.328	0.343	0.337	0.371									
L05	0.192	0.171	0.159	0.189	0.217	0.319								
L08	0.177	0.155	0.148	0.177	0.204	0.306	0.126							
L10	0.167	0.146	0.139	0.166	0.191	0.295	0.121	0.111						
L11	0.163	0.141	0.139	0.160	0.189	0.297	0.119	0.106	0.102					
L12	0.176	0.152	0.145	0.173	0.199	0.301	0.125	0.119	0.111	0.105				
L13	0.177	0.156	0.143	0.175	0.201	0.302	0.127	0.115	0.109	0.105	0.107			
L45	0.272	0.257	0.257	0.265	0.295	0.293	0.242	0.229	0.219	0.208	0.230	0.229		
L62	0.152	0.133	0.125	0.153	0.173	0.272	0.111	0.098	0.094	0.087	0.094	0.096	0.185	

Chapter 3

Investigating Drivers of Geographical Parthenogenesis in *Townsendia hookeri* Using a Reciprocal Transplant Experiment

3.1 Introduction

Closely related sexual and asexual taxa often have disparate ranges, with asexuals tending to be more geographically widespread and/or found at higher elevations and latitudes than related sexuals (Bierzychudek 1985). This pattern, termed geographical parthenogenesis (GP), has been found in numerous plant and animal systems, and its proposed explanations interface with several open areas of ecology and evolution research. These include: the nature of sex, the evolutionary impacts of polyploidy, clone selection, dispersal limitation, and range limits / local adaptation (see Tilquin & Kokko 2016 for an excellent review of GP). Most models of GP propose that the

effects of parthenogenesis (or its correlates, e.g., polyploidy) influence the ecology of asexuals in a way that allows them to expand their range beyond that of the sexuals, or alternately, that the benefits of sexual recombination allow sexuals to persist in areas where asexuals cannot. Given that range shifts between close relatives occur frequently (Weber & Strauss 2016), it is not surprising that patterns of GP may be the result of several different interacting mechanisms that require explorations of both genetic and ecological attributes of sexuals and asexuals in each system.

The defining feature of parthenogenic organisms is their ability to reproduce without mates (i.e., reproductive assurance), and this is often invoked as one of the primary explanations for GP. First described by Baker (1955) in the context of self-compatible plant species, "Baker's law" predicts that plants capable of uniparental reproduction (i.e., have reproductive assurance) will be better able to colonize new habitats than plants dependent on mates, because only a single propagule is required to establish a new population (see Pannell *et al.* 2015 for a modern review of Baker's law). Baker's law provides an intuitive explanation for GP, but it is not without its caveats. All else being equal, reproductive assurance would provide an asexual range advantage in the short term, but sexuals should be able to catch up given enough time. Asexual range advantage would only persist in the long term if sexuals suffer from mate limitation (Gascoigne *et al.* 2009), for example in habitats characterized by high mortality and low pollinator-availability. Another possibility is that asexuals occupy the sexuals' available niche space after a wave of early colonization, preventing sexual range expansion through competitive exclusion or reproductive interference (Britton & Mogie 2001; Kyogoku 2015; Hersh *et al.* 2016).

While Baker's law emphasizes advantages conferred by parthenogenesis as the primary explanation for range disparity between sexuals and asexuals, some models propose that sexual recombination gives sexuals an adaptive advantage, allowing

them to respond to selective pressures more effectively than asexuals (who are assumed to suffer from reduced adaptive potential; Tilquin & Kokko 2016). The Red Queen hypothesis proposes that asexuals escape to "marginal" habitats where biotic interactions are less intense, whereas sexuals are able to adapt to constantly evolving pressures such as pathogens and interspecific competition (Glesener & Tilman 1978). It is important to note that the Red Queen hypothesis still supposes a demographic advantage to asexuality, and predicts that parthenogens will have the advantage in areas that are absent of parasites, predators, and competitors. The Red Queen hypothesis points towards asexuals being less suited to (and likely to be excluded from) environments with intense or frequent biotic interactions but reaping the benefits of their demographic advantage in habitats where abiotic stress is the dominant selective pressure. Sexuals are expected to have higher fitness than asexuals in areas subjected to frequent biotic pressures, and may also exhibit adaptations (e.g., resistance to herbivores or increased plant size) depending on which biotic interactions are influencing selection.

Implicit within the definition of GP is the fact that sexuals and asexuals have different ranges, and therefore different range limits. Given that they occupy different ranges, an obvious question to ask is whether sexuals and asexuals are adapted to different niches. Ecological niche models provide one way to assess whether species range limits coincide with their niche limits (reviewed in Lee-Yaw *et al.* 2016), but ideally, the best method to test range limits is by using reciprocal transplant experiments. Species' range limits (RL) are largely thought to be a reflection of their niche limits (NL) (Sexton *et al.* 2009), and recent surveys of reciprocal transplant studies indicate that fitness does often decline when species are transplanted beyond their range (Hargreaves *et al.* 2014; Lee-Yaw *et al.* 2016). When fitness does not decline beyond the range (i.e. RL $\neq$ NL), this is often seen as evidence for dispersal limitation

(Pulliam 2000). When local populations have higher fitness than populations from other regions ("home-site advantage"), this provides some evidence for local adaptation (Kawecki 2008), though strict definitions of local adaptation require reciprocal home site advantage (i.e., local populations have the highest fitness in every site *and* perform better at home than away; Blanquart *et al.* 2013). The framework of testing range limits through reciprocal transplant experiments is particularly well suited for investigations of GP, because it allows us to simultaneously evaluate two important alternatives: (1) that sexuals are dispersal limited (evidenced by comparable performance when planted within and beyond their range, supporting Baker's law), and (2) that the two mating types are ecologically adapted to their respective ranges, evidenced by a decline in fitness (due to abiotic and/or biotic factors) when either type is planted beyond their range. While ecological niche models have recently been applied to investigate patterns of GP (Lee 2015; Kirchheimer *et al.* 2018), few have applied modern reciprocal transplant experimental design within a GP context.

In addition to a review of transplant experiments beyond the species range, Hargreaves *et al.* (2014) provide an excellent framework to guide the design of reciprocal transplant experiments and interpret their results. The authors propose several recommendations for effective experimental design: (1) include both core and range edge sites and source populations in order to determine whether populations are locally adapted to different parts of the range. (2) Include multiple transplant sites beyond the range to detect gradients in habitat quality. (3) Estimate lifetime fitness instead of single life-stage components in order to get the best indication of whether populations are self-sustaining. (4) Conduct experiments under natural conditions in order to assess the impacts of biotic interactions on fitness.

Most broadly, results from transplant experiments can be sorted into two categories: RL = NL (decline in fitness beyond the range) or RL $\neq$ NL (fitness does

not decline beyond the range *or* fitness declines within the range; Hargreaves *et al.* 2014). Ideally, interpretations of whether range limits and niche limits coincide are supported by lifetime fitness estimates, which allow researchers to assess whether populations are self-sustaining ($\lambda \geq 1$). However, because lifetime fitness estimates are often difficult to obtain, many rely on comparisons of relative fitness within and between sites. In cases where RL < NL, that could be due to dispersal limitation - i.e., suitable habitat exists outside of the current range where populations can be self-sustaining, but they have not been able to colonize these areas. Cases where RL > NL, for example at a range edge site, could indicate that the range edge comprises sink populations being maintained by dispersal from populations within the range interior. It is important to note that this framework is somewhat (perhaps necessarily) simplistic and that niche limitation can interact with and contribute to dispersal limitation. For example, declines in fitness driven by niche limitation will contribute to dispersal limitation by reducing mate availability and seed set. These effects are important to keep in mind in a GP context, as they are more likely to affect sexuals than asexuals due to having comparatively reduced reproductive assurance.

Townsendia hookeri (Asteraceae) is a long-lived perennial plant species that displays a classic pattern of GP (Bierzychudek 1985). It has two forms: diploid sexuals that are self-incompatible, and polyploid autonomous apomicts that produce seeds asexually without the need for pollen. The apomictic range is much larger than the sexual range and there is a range of overlap where populations of both types can be found. Given that sexuals are obligately outcrossing and that apomicts have full reproductive assurance, it is possible that sexual range expansion was limited by dispersal. Sexual dispersal limitation may be compounded by environmental and/or reproductive barriers; for example, it is possible that apomicts colonized new habitat first, and then prevented subsequent sexual range expansion through reproductive

interference. Apomictics have the potential to reduce seed set and produce apomictic offspring when pollinating sexual flowers, but apomicts are unaffected by pollen produced by sexuals (Garani 2014). On the other hand, the distinctness of the two ranges indicates a clear possibility that sexuals and apomicts are ecologically differentiated, which is supported by ENMs suggesting significant niche divergence between the two forms (Lee 2015).

These patterns provide an excellent backdrop to test for local adaptation and dispersal limitation using reciprocal transplant experiments. The ranges of the two forms are generally situated from south (sexual range) to north (apomictic range), with overlap in the middle. This allows us to establish a mirrored version of the classic reciprocal transplant design, where interior sites for one mating type can act as within range for itself and beyond the range sites for the other, with sites in the overlap zone representing range edge sites. We used this design, following the recommendations provided by Hargreaves *et al.* (2014) as closely as possible, to address the following questions: (1) Do the ranges of sexuals and apomicts coincide with their ecological niches (RL = NL), such that there is a decline in fitness when planted into the range of the opposing type? (2) Does either mating type show evidence of dispersal limitation (RL < NL), as indicated by comparable performance when planted in sites within and beyond the range? While it is possible that both sexuals and apomicts may be dispersal limited, we expect this to be more likely in sexuals due to being mate and pollinator dependent. (3) Are there any trait differences that may indicate that sexuals and apomicts adopt different strategies in response to biotic stress (e.g., competition)?

3.2 Materials and methods

3.2.1 Reciprocal transplant experimental design

In 2013 and 2014, we collected achenes (hereafter referred to as "seeds") from 6 sexual and 6 apomictic source populations distributed throughout the core of the range of *T. hookeri* (Figure 3.1; Table 3.1). We chose three source populations from each of four regions that correspond to the interior and edge of the geographic ranges of the sexual and apomictic forms: the interior sexual range (S_s, located in the southern-most portion of the range), the sexual "edge" where the sexual and apomictic ranges overlap (SO_s), the apomictic "edge" in the zone of overlap (AO_s), and the interior apomictic range (A_s, located in the north). We chose 11 maternal plants (hereafter referred to as "moms") from each source population, and 32 viable seeds (based on criteria described in Hersh Ch. 4) from each mom and germinated them in agar-filled petri dishes. We grew the resulting seedlings in growth chambers, and hardened them in the greenhouse before transplanting them into field sites (see Chapter 4 for detailed methods on germination and greenhouse experiments).

In early September of 2014, we reciprocally transplanted seedlings from all moms and populations into two gardens (separated by $\sim$ 2 km) in each of the four regions described above over the course of two weeks (garden regions labelled: S_g, SO_g, AO_g, A_g; Figure 1). The placement of the gardens allowed us to evaluate the performance of populations when planted in the range interior, range edge, just beyond the range, and far beyond the range of each mating type as recommended by Hargreaves *et al.* (2014). We randomized the seedlings and planted them in rows of 10, with 10 cm spacing between plants and a small path down the middle of each plot to facilitate data collection. We transplanted 250-350 seedlings into each garden, with the larger gardens being located in the overlap regions (we anticipated higher mortality in the

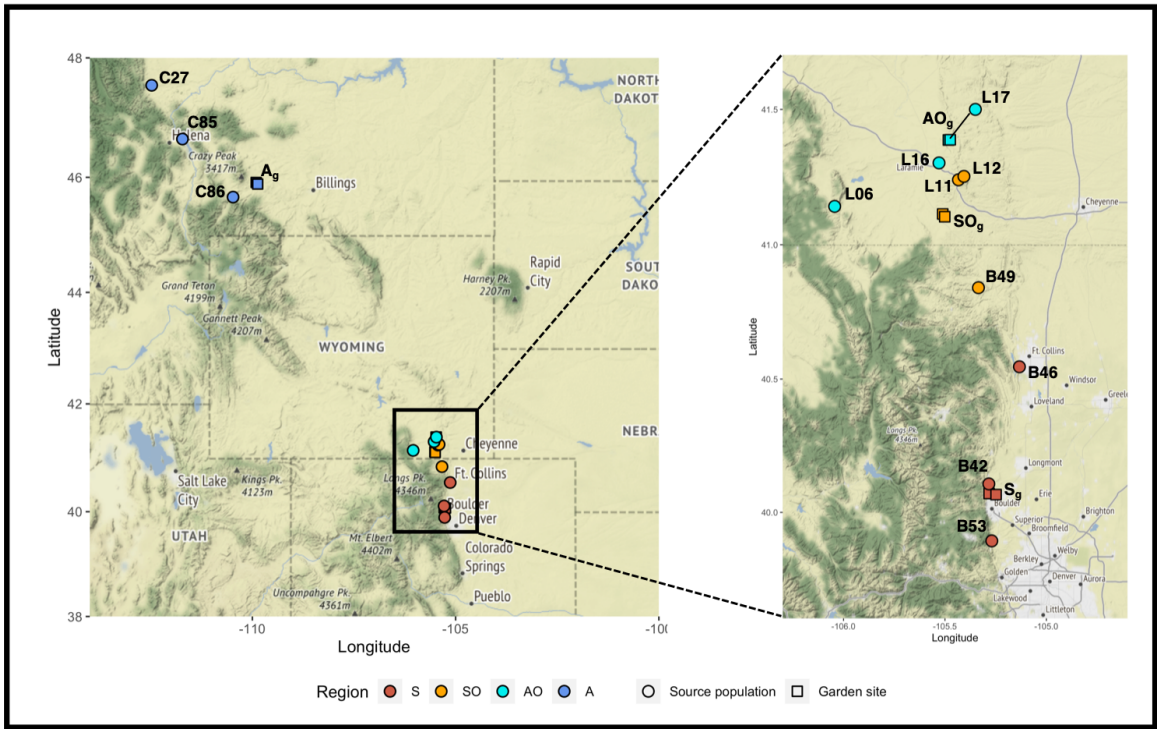

Figure 3.1: Map of source populations and garden sites. Circles represent source populations and square represent garden sites. Points are coloured based on range location: A = apomictic range (blue), AO = apomictic overlap range (sky blue), SO = sexual overlap range (orange), S = sexual range (red).

overlap regions based on observations from multiple field seasons), for a total of 2,531 plants (Table 3.5). We chose garden sites based on proximity to known natural populations and qualititative attributes based on our experience with the habitats containing *T. hookeri* in each part of the range (i.e., typically rocky soils with sparse vegetation, but higher vegetation cover in S_g which is characteristic of populations found there). Natural individuals were found within 500 m of each garden site, which supports the suitability of the sites. We planted seedlings directly without removing vegetation or conditioning the soil in order to expose the plants to conditions that closely reflect the area's natural ecology. Aside from watering the seedlings right after planting, we did not install irrigation or provide any supplemental water to the gardens.

At each garden site, we installed a small "establishment" plot with two seeds from each of the families represented in the gardens in order to capture the germination and early seedling establishment life history stages (totalling 480 seeds per garden region; Table 3.3). Each establishment plot consisted of a small plastic grid, with the seeds randomly placed on the soil in each cell of the grid (one seed per cell). Before installing each plot, we cleared the soil of vegetation and scraped away the top layer of soil in order to reduce competition with the natural seed bank. We covered each plot with thrip-proof mesh to protect the seeds from predation and from being scattered by strong winds that are typical in sites where *T. hookeri* is found.

We returned to the garden sites every spring from 2015-2019 to collect data on survival, growth (number of leaves, leaf length - measuring one haphazardly chosen representative leaf per individual) and reproduction (the number of inflorescences, hereafter referred to as "buds") produced per individual. We also checked the establishment plots to score the presence of juvenile individuals in 2015 and 2016 (though 95% of recruits emerged in the first year). The first flower buds were seen in 2016. Our permitting requirements included the need to mitigate the release of alien seed and pollen in the sexual range. Therefore, to address this in a consistent manner, we removed flower buds before they opened at all garden sites. In order to quantify potential differences in seed set between mating types, in 2017 we obtained permission to allow individuals in the gardens to flower. We bagged the apomicts before the flowers opened, and the sexuals after allowing enough time for pollination to occur. We also collected mature seed heads from natural individuals in source populations to complement the seeds collected from the gardens, in case sexuals in the gardens were pollen limited (due to having few compatible individuals concurrently flowering).

We assessed vegetation cover in our gardens in 2017. We used 1x1 meter quadrats to estimate percent vegetation cover at each site. At the end of the experiment (2019),

we harvested surviving individuals and estimated plant size by water displacement. We immersed below ground parts in a graduated cylinder filled with water, and then used a graduated pipette to estimate below ground volume. A similar approach was used for whole plants, allowing us to estimate an index of above vs below-ground biomass.

3.2.2 Data analysis

We analyzed establishment success in the field using generalized linear mixed models (GLMMs; logit link - binomial) in R version 3.6.1 (R Core team 2019), including source region, garden region, and their interaction as fixed effects, and originally including garden, population, and mom (nested within population) as random effects. After full mixed models did not converge, we dropped the random effects of population and mom, which allowed the models to converge successfully. Damage to one of the establishment plots installed in A_g led to a data gathering error which resulted in a partial loss of data; this reduced the sample size and likely contributed to slightly larger predicted confidence intervals in that garden region.

Given that we collected data from our reciprocal transplant experiment over several years and that lifetime bud production was strongly zero inflated, we used ASTER models (ASTER package, *reaster()* function; Geyer *et al.* 2007, 2013) in R to analyze our garden data. Our ASTER models incorporate survival, flowering, and number of buds produced over the course of the experiment, and are hierarchically structured so that, for example, survival in year 5 is contingent upon that individual surviving in years 1-4 as well (see ASTER model diagram; Figure 3.3). We modeled survival and flowering as Bernoulli variables (coded as 0 or 1), and yearly bud production as zero-truncated Poisson variables. Individuals did not become reproductive until year 2, so we removed the year 1 flowering and bud production nodes from our model

structure. We tested the fixed effects of source region, garden region, and their interaction, and originally included garden, population, and mom as random effects. The random effects of garden and population resulted in model singularities (random effect variances estimated as zero), and were dropped from the model. We visualized bud production over 5 years (our estimate of lifetime fitness) by plotting predicted values with 95% confidence intervals from fixed effects models (there is no predict() function for random effects ASTER models).

Because we were not able to incorporate our seedling establishment data into our ASTER models (because they measure different individuals), we used a random sampling approach to combine the two datasets. We separately randomly sampled each dataset 1000 times and calculated the mean establishment success and mean number of buds produced per individual planted over the course of the experiment in each source region and garden region. We multiplied these values for each randomly-sampled dataset and calculated means and 95% confidence intervals.

We analyzed leaf length and leaf number in the gardens using mixed effects models. We tested the effects of source region, garden region, and their interaction as fixed effects and originally included garden, population, and mom (nested within population) as random effects, modelling the data from each year separately. When models did not converge, we removed random effects until models converged. We modeled leaf length using linear mixed effects models (LMMs) and leaf number using GLMMs (log link - poisson).

We analyzed root to shoot volume ratio of individuals surviving to year 5 using LMMs, including mating system as a fixed effect and population as a random effect. We analyzed proportion of viable seed and total number of seeds per bud (collected in 2017) in the gardens and source populations using LMMs, including mating system as a fixed effect and population as a random effect. We analyzed percent vegetation

cover in the gardens using LMMs, with garden region as a fixed effect and garden (nested within region) as a random effect.

All mixed models (besides ASTER) were performed using the LME4 package (Bates *et al.* 2015) in R. We tested the effects of all models using likelihood ratio tests and visualized predicted means and confidence intervals using the GGEFFECTS package (Lüdecke 2018) in R. We inferred statistical differences between groups when both the likelihood ratio test *P*-values were below 0.05 and confidence intervals from model predictions were largely non-overlapping.

In order to compare mean annual temperature (MAT) and mean annual precipitation (MAP) during experiment years to historical averages, we pulled climate data from 1901-2018 at each garden site using ClimateNA v.5.10 (Wang *et al.* 2016).

3.3 Results

3.3.1 Establishment success

Establishment success varied among source regions (labelled S_s, SO_s, AO_s, and A_s) and garden regions (labelled S_g, SO_g, AO_g, and A_g), and there was an interaction between these two effects (Table 3.2). Despite statistical support for an interaction based on LRTs, visualization of confidence intervals suggests a lack of difference in establishment success among source regions within garden regions (Figure 3.2A). Overall, populations did not have higher establishment success when planted into their own region, and therefore our results are not consistent with local adaptation at early life stages. For all source regions, establishment success was highest in AO_g (averaging 77%) and lowest in S_g (averaging 14%; Table 3.3).

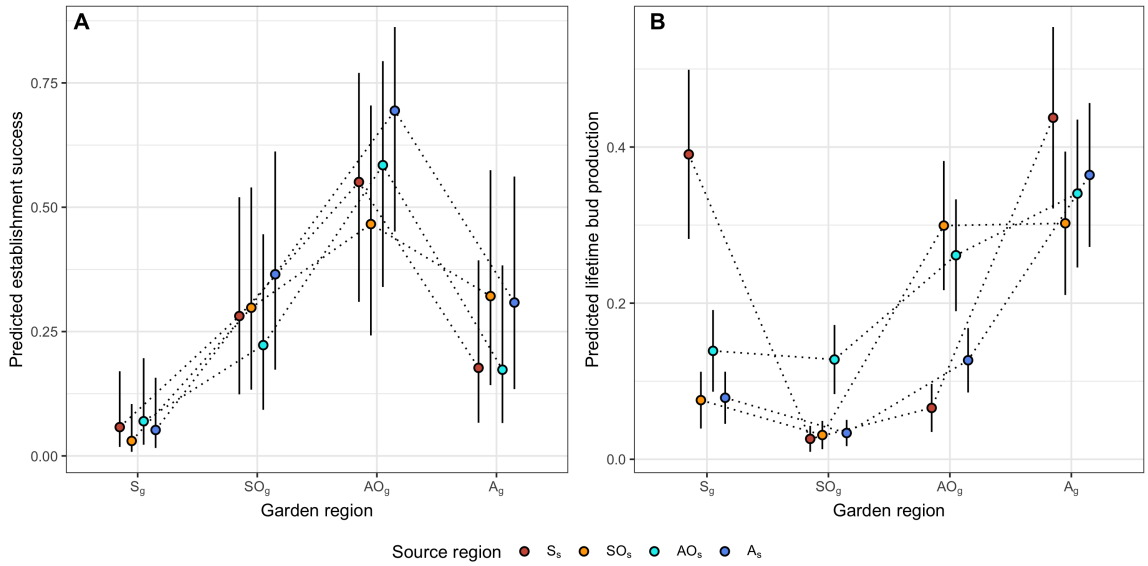

Figure 3.2: A) Predicted establishment success by source region and garden from GLMMs. B) Predicted estimates of lifetime bud production by source region and garden region from ASTER models. Error bars represent 95% confidence intervals.

3.3.2 Reciprocal transplant experiment

ASTER model analyses showed that lifetime bud production differed among garden regions and that there was an interaction between source region and garden region, but no effect of source region overall (Table 3.4). While the fitness of apomicts (A_s and AO_s) decreased when transplanted into the sexual range (S_g and SO_g), sexual fitness (S_s and SO_s) was similar or increased when transplanted into the apomictic range (A_g and AO_g; Figure 3.2B). Some source regions show a pattern that is (at least partially) consistent with local adaptation. For example, A_s had the highest fitness in its own region (though performance in A_g did not differ among source regions) and had low fitness in other garden regions. S_s performed well and had the highest fitness of all source regions in S_g, but it had equally high (if not higher) fitness in A_g, which is the region farthest away from its own source region. AO_s performed best in the apomictic regions, had lower fitness in sexual regions, and was the source region with

the most consistent performance across sites. SO_S, on the other hand, had low fitness in the sexual regions and considerably higher fitness in the apomictic garden regions. Overall, fitness was lowest in SO_g and highest in A_g (Table 3.5).

Combining establishment and bud production per individual planted (using the random sampling approach) shows similar patterns to lifetime bud production from ASTER models, but with wider confidence intervals and less pronounced differences in performance between source regions (Figure 3.4). Most notably, when establishment and bud production are combined, the fitness of S_s was considerably reduced in S_g and A_g due to low establishment success in both regions.

Leaf number and leaf length were affected by source region, garden region, and their interaction, but these effects differed in significance and magnitude by year (Table 3.6, Table 3.7). However, plant size was largely comparable among source regions within garden regions and years (Figure 3.5, Figure 3.6). Leaf number tended to be higher in A_g (and higher amongst apomicts within that garden region), while leaf length was consistently high in S_g with individuals from S_s often having the longest leaves.

The total number of seeds produced per bud did not differ by mating system in either the gardens or in source populations. Nonetheless, apomicts tended to have a higher proportion of viable seeds within gardens and source populations (Table 3.8, Figure 3.7), though this effect was weak in source populations (as indicated by overlap in confidence intervals and slightly higher P-value from likelihood ratio tests (P=0.0716)). In the plants surviving to the end of the experiment (year 5), apomicts had a higher root to shoot volume ratio than sexuals (df=4, $\chi^2 = 3.883$, $P = 0.0488$; Figure 3.8), though partial overlap in predicted confidence intervals indicate that this is not a particularly strong effect.

3.3.3 Site characteristics

S_g had considerably higher vegetation cover than the other garden regions, which did not differ from each other (df=6, $\chi^2 = 22.424$, $P = 5.3 \times 10^{-5}$; Figure 3.9). When comparing mean annual temperature and mean annual precipitation during experiment years to historical averages, experiment years largely fall within the historical distributions for both climate variables (Figure 3.10). Mean annual temperature was higher in S_g gardens (SS1 and SS2) both historically and during the experiment, while mean annual precipitation was similar across sites.

3.4 Discussion

The results from our reciprocal transplant experiment show that, in comparison to their home ranges, the fitness of sexuals was similar or increased when transplanted into the apomictic range (indicating dispersal limitation), but the fitness of apomicts largely decreased when transplanted into the sexual range (indicating that their range limits coincide with their niche limits). Differences in fitness appear to be driven primarily by survival and reproduction, as establishment success did not differ among source regions within garden regions. Source populations from the sexual interior had markedly higher performance in their home range than populations from other regions, which indicates that they are locally adapted. The sexual interior gardens also had considerably warmer temperatures and higher vegetation cover than the other garden regions, which hints at the potential influence of biotic interactions in limiting the fitness of populations from other regions. Overall, these patterns are consistent with Baker's law effects limiting the sexuals' northern range limit and apomicts' being constrained by unsuitable habitat beyond their southern range limit in *Townsendia hookeri*, though there are several alternative interpretations which we

discuss below.

3.4.1 Sexual range expansion is limited by dispersal

ASTER model analyses incorporating survival and bud production over 5 years indicate that sexual populations from both the sexual range interior (S_s) and the sexual range edge (SO_s) perform as well or better when transplanted beyond their range as when planted into their home regions (Figure 3.2B). S_s had equally high fitness in its home region (S_g) as in A_g, despite A_g being more than 600 km north of the sexual range edge. SO_s performed best when transplanted into the apomictic range and somewhat surprisingly had the lowest fitness in its home region. This suggests that, at least over the years of our study, suitable conditions exist beyond the current range of sexuals that they have not been able to colonize (RL<NL). This type of pattern is typically interpreted as evidence for dispersal limitation (Hargreaves *et al.* 2014).

Given that in *Townsendia hookeri* sexuals are self-incompatible and that apomicts are autonomous with full reproductive assurance, it seems plausible that mate or pollen limitation could play an important role in limiting sexual range expansion. Apomicts produced a higher proportion of viable seeds in both the garden experiment and in natural populations (Figure 3.7). In particular, the reduced seed set of sexuals in the gardens (which likely had fewer concurrently flowering individuals than would be found in natural populations) suggests that recruitment is likely to suffer in sexual populations when there is a low density of potential mates (Gascoigne *et al.* 2009). The fact that sexuals and apomicts produced a similar number of seeds overall indicates that sexuals are capable of comparable seed set to apomicts in good years (i.e., given sufficient mates and pollinator services), though increased germination success under lab conditions and improved dispersal architecture (Chapter 4) may still give apomicts a colonization advantage even when seed set is equal.

While species range limits often coincide with their niche limits (Hargreaves *et al.* 2014; Lee-Yaw *et al.* 2016), there are numerous examples from transplant studies where fitness does not decline beyond the range edge. Samis *et al.* (2016) found that in almost 1/3rd of the within- vs beyond-range transplant studies reviewed by Hargreaves *et al.* (2014), fitness actually increased beyond the range limit. Inferences about dispersal limitation are dependent on mating system and life-history characteristics, however. For example, Stevens & Emery (2015) found that asexual gametophytes of the fern *Vittaria appalachiana* had similar or better performance when transplanted beyond the range as compared to within. The authors interpreted their results as evidence of dispersal limitation because asexual fern gametophytes have no method of long-distance dispersal (which, in ferns, is primarily accomplished by sexually produced spores). Similarly, Samis *et al.* (2016) found that fitness of the dune plant *Camissoniopsis cheiranthifolia* continually increased when transplanted toward and beyond the species range limit. In this case, the authors acknowledged that their results were in line with dispersal limitation, but the mechanism was unclear because *C. cheiranthifolia* is self-compatible and population density did not decrease at the northern range edge (i.e., dispersal limitation was not likely driven by mate limitation). Instead, dispersal limitation may have been driven by metapopulation dynamics (Holt & Keitt 2000); *C. cheiranthifolia* seeds lack dispersal architecture and may suffer from reduced dispersal between habitat patches at the range edge (Samis & Eckert 2009). These studies highlight how mating system, dispersal mechanism, life history, and demographics can all play important roles in delimiting where species are found on the landscape.

The comparatively increased colonization potential of asexuals due to reproductive assurance (Baker's law effects) is often considered one of the primary drivers of patterns of GP (Tilquin & Kokko 2016). Supporting evidence for this mechanism

comes in part from an over-abundance of apomictic taxa in previously glaciated habitats (Brochmann *et al.* 2003), which points to rapid asexual colonization from glacial refugia (Hörandl 2009). GP systems are complicated, however, and the importance of reproductive assurance in giving apomicts a colonization advantage over sexuals will vary in each system depending on the interacting suite of traits possessed by each mating type. For example, many apomicts require pollen to fertilize the endosperm in order to set seed (pseudogamy), while others set seed autonomously (Whitton *et al.* 2008). Pseudogamous apomicts that are self-incompatible would not be expected to have the same colonization advantage as an autonomous apomict, because they are still dependent on pollen from another individual and would require two individuals to establish a new population. Apomicts in *T. hookeri* set seed autonomously while sexuals are self-incompatible, making Baker's law effects more likely to be important in this system than in others where there may be less of a discrepancy in reproductive assurance between mating types. In the *Boechera* agamic complex, for example, sexual progenitors are self-compatible and populations are highly selfing. Sexuals in *Boechera* therefore have full reproductive assurance and are at less of a demographic disadvantage in comparison to apomicts, which may help explain why the genus does not show a strong pattern of GP (Mau *et al.* 2015). This is a rare case, however, as the sexual progenitors of most apomicts are self-incompatible (Asker & Jerling 1992), and pseudogamous apomicts often benefit from the breakdown of self-incompatibility (so that self-pollen can trigger endosperm development; Hörandl 2010), which points to Baker's law effects as being a plausible driver in most plant GP systems.

3.4.2 Habitat is not suitable for apomicts beyond their southern range limit

In contrast with sexuals, the fitness of apomicts generally decreased when transplanted beyond their range (Figure 3.2B) indicating that the apomicts' southern range limit and niche limit coincide (RL=NL). This suggests that their southern range limit is caused by a gradient in habitat quality (Hargreaves *et al.* 2014), which could mean that a shift in abiotic conditions, biotic interactions, or some combination of these is important in limiting the range (Gaston 2003; Case *et al.* 2005; Normand *et al.* 2009). While we do not have lifetime fitness estimates and therefore cannot be certain that apomictic populations would not be self-sustaining in the sexual range (i.e., λ may still be greater than 1), apomicts had comparatively lower fitness than local sexual populations in the sexual interior (S_g), which provides additional support that apomicts are not well adapted to this region. One possible cause for this reduction in fitness is that apomicts are simply not well adapted to the environmental (abiotic) conditions in the southern portion of their range. This pattern is consistent with environmental niche models, which predicted that, despite overlap in the environmental niches of the two mating types, the apomicts' niche did not extend fully into the sexual range (Lee 2015). As for individual abiotic factors that impact their respective niches, Lee (2015) found that temperature variables were more important for sexuals and precipitation variables were more important for apomicts. Populations from the sexual interior had the highest fitness in their own region (S_g), which also had historically warmer temperatures (Figure 3.10) than the other garden regions. This provides some evidence that sexuals are better suited to warm climates than apomicts, and may indicate that the southern range limits of apomicts is limited by temperature (and/or other factors that correlate with temperature).

Temperature is linked with several other factors, including many biotic interac-

tions (Burnside *et al.* 2014). Biotic interactions have traditionally been thought to be more intense at lower latitudes (Louthan *et al.* 2015), though it should be noted that some large-scale analyses have challenged this assumption; Moles *et al.* (2011a) found that herbivory and plant defenses are not greater at lower latitudes, and Hargreaves *et al.* (2020) found that biotic interactions may affect fitness but often fail to drive local adaptation. In the case of *T. hookeri*, the manner in which biotic interactions are thought to contribute to southern range limits is consistent with how the Red Queen hypothesis is thought to contribute to patterns of GP, allowing sexuals (through their superior ability to adapt to biotic pressures) to persist in areas where asexuals are excluded. Apomictic fitness was lowest in the southernmost garden region (sexual interior; S_g), where vegetation cover was considerably higher (Figure 3.9) and temperatures were warmest (SS1 and SS2; Figure 3.10). Populations from the sexual interior (S_s) had considerably higher performance in their home region (S_g) than populations from other regions (Figure 3.2B), a pattern indicative of local adaptation (Kawecki 2008). In addition, all source regions had longer leaves in S_g than in other garden regions, and populations from S_s had the longest leaves in that region in most years (Figure 3.6). While this increase in leaf size among all source regions is likely a response to higher temperatures in the sexual interior (Peppe *et al.* 2011), the fact that local source populations tended to have the longest leaves and the highest fitness indicates that they may be better adapted to respond to competition than populations originating from areas with lower vegetation cover (Novoplansky 2009).

By transplanting seedlings directly into the plots without removing local vegetation, we were able to detect potential biotic effects (i.e., low apomictic fitness and high vegetation cover in S_g) which would have been obscured had we standardized sites like most transplant studies have done historically (Hargreaves *et al.* 2014). However, our experiment was not explicitly designed to test for the effects of biotic interac-

tions, and we are therefore somewhat limited in what conclusions we can draw about how/which biotic interactions affected fitness, or indeed, whether abiotic factors alone would produce the same results. Studies that have explicitly investigated Red Queen effects within the context of GP in plants provide mixed empirical support. Using population surveys and greenhouse experiments in *Taraxacum officinale*, Verhoeven & Biere (2013) found that soil pathogens and seed-eating weevils were more common in southern (largely sexual) populations than in northern apomictic populations. On the other hand, Herman *et al.* (2017) investigated seed predator intensity and vegetation density in *Hieracium alpinum* but found no differences in the prevalence of either biotic pressure between sexual and apomictic populations. Given that the Red Queen hypothesis is consistently invoked in the conversation surrounding the evolution and ubiquity of sex, investigations of the relative effects of biotic interactions in more GP systems will provide important insight into this potential mechanism.

3.4.3 Drivers of geographical parthenogenesis in *Townsendia hookeri*

Results from our reciprocal transplant garden experiment show that the fitness of apomicts decreased when transplanted into the sexual range, but sexual fitness was comparable (or increased) when transplanted into the apomictic range (Figure 3.2B). This suggests that while apomicts have largely been able to occupy their available niche space (RL=NL), sexual range expansion is likely limited by dispersal and establishment (RL< NL) in *Townsendia hookeri*. These results corroborate what was found by previous niche models, which indicated that the apomictic range was suitable for both mating types, but the sexual range was suitable only for sexuals (Lee 2015). Our results are in line with apomicts being excluded from the sexual range due to ecological differentiation, possibly driven by their inability to persist in the

face of increased competition beyond their southern range limit.

Our results provide evidence for Baker's law effects (i.e. dispersal limitation due to lack of reproductive assurance) limiting the sexuals expansion northward and Red Queen effects (i.e. lack of adaptation to biotic pressures) preventing the apomicts from moving south, but other processes are likely at play as well. Sexual populations had quite low fitness in the gardens placed at their northern range edge (SO_g), which may indicate that this area exists outside of the sexual niche (RL<NL). This inhospitable part of the range could act as an environmental barrier preventing sexuals from dispersing to northern regions that are more suitable (AO_g and A_g; Figure 3.2B). Interestingly, populations from the sexual edge (SO_s) performed as well as (if not better than) the apomictic edge populations (AO_s) in the apomictic edge garden region (AO_g). This indicates that the sexual edge populations are well poised to expand their range further into the apomictic edge region (as predicted by Hargreaves *et al.* 2014), but they apparently have not done so despite being in relatively close proximity (SO_g and AO_g are only separated by ∼30 km). Given that mixed sexual-apomictic populations are exceedingly rare (Chapter 2), it is possible that asymmetric reproductive interference from apomicts (i.e., apomictic pollen reducing sexual seed set and/or siring apomictic offspring; Garani 2014), contributes to limiting the sexuals' expansion, and may even cause the sexual range to shrink over time (Britton & Mogie 2001). Populations from the apomictic edge (AO_s) performed best in the sexual overlap region (SO_g), which suggests that they may be well-situated to invade the sexual range. In fact, apomicts were detected at low frequencies in a population situated at the sexual range edge (population L62; Chapter 2), which points to the possibility that a "frozen" apomictic lineage has emerged that is able to persist in this otherwise unsuitable environment.

We have emphasized how biotic interactions may be excluding apomicts from the

southern portion of the range, and this is likely influenced by the fact that sexuals and apomicts appear to embody different ecological strategies. While sexuals tended to have longer leaves (particularly in the sexual interior; Figure 3.6), apomicts tended to produce more leaves (Figure 3.5), which hints at the possibility of trade-offs in resource allocation to vegetation vs reproduction - each leaf is typically associated with an axillary bud which has the potential to become a flower, so plants with more leaves have a larger "bud bank" (Kleiman & Aarssen 2007). In addition, apomicts appear to devote relatively more energy to root growth than shoot growth in comparison to sexuals (Figure 3.8). Changes in allocation to root mass are typically associated with reduced water or nutrient availability, but can also be positively correlated with wind intensity (Poorter *et al.* 2012), which may indicate that apomicts are better adapted to open or disturbed environments (Reynolds & Pacala 1993). These patterns echo the predictions from various hypotheses (general-purpose genotypes, Lynch 1984; asexuals as "weedy" species, Baker 1965; establishment vs dispersal tradeoffs, discussed in Hersh Ch. 4) which liken apomicts to being "r-selected" taxa that can best express their demographic advantages in marginal environments that are dominated more by environmental stochasticity than biotic pressures. This highlights how the poor establishment performance in the sexual interior (S_g, Figure 3.2A) mitigates the apomicts' demographic strength, which likely contributes significantly to their inability to invade the sexual range.

Many investigations into GP focus either on the geographic success of asexuals *or* the adaptive benefits of sexuality, but in reality these alternatives are opposite sides of the same coin. Tilquin & Kokko (2016) sagely wrote that any model for GP should address: "why sex (here) and why asex (there)". In this study we have harnessed the power of modern reciprocal transplant experimental design and adapted it to investigate the performance of both sexuals and apomicts across the species range, which

has enabled us to address both sexual and asexual aspects of the GP question. In fact, this "mirrored" reciprocal transplant design can be used to address similar questions about dispersal limitation and local adaptation in any pair of sister species with parapatric ranges. Although GP is housed within a complex sexual-asexual framework, the questions it addresses transcend the idiosyncrasies of the (often understudied) systems the work is done in and can inform contemporary conversations surrounding plant mating systems, range limits, dispersal, and the dynamic interplay of ecological and evolutionary processes.

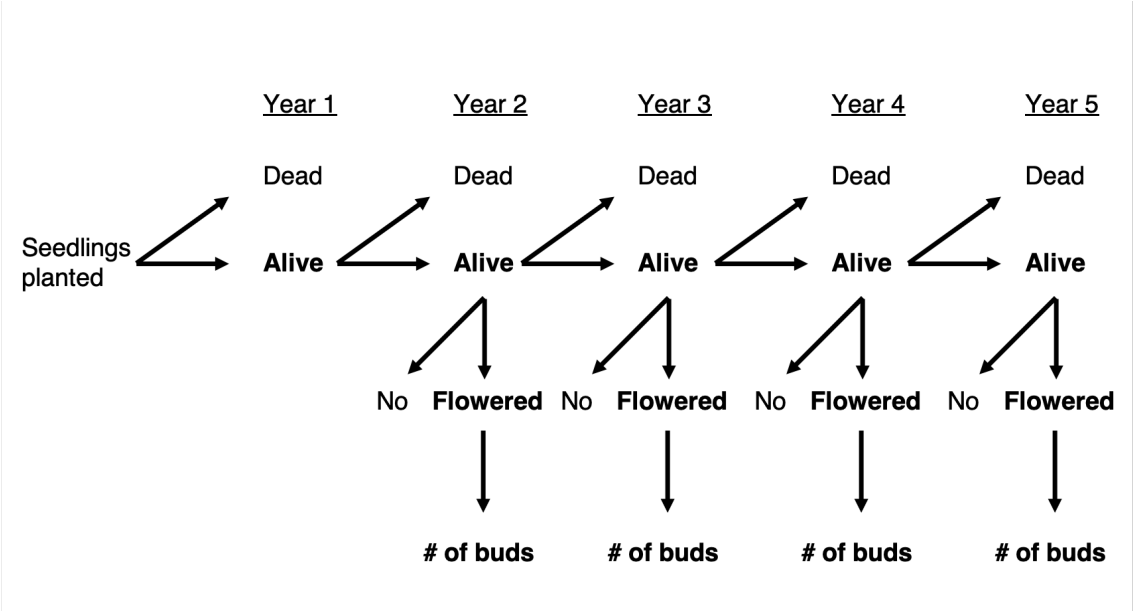

Figure 3.3: Hierarchical structure of ASTER models used to analyze lifetime fitness in *Townsedia hookeri* over the course of a 5-year reciprocal transplant experiment. Survival is contingent upon survival in previous years, and the number of buds produced is contingent upon flowering (that year) and survival in previous years. Because no individuals flowered in year 1, we removed those variables from the model.

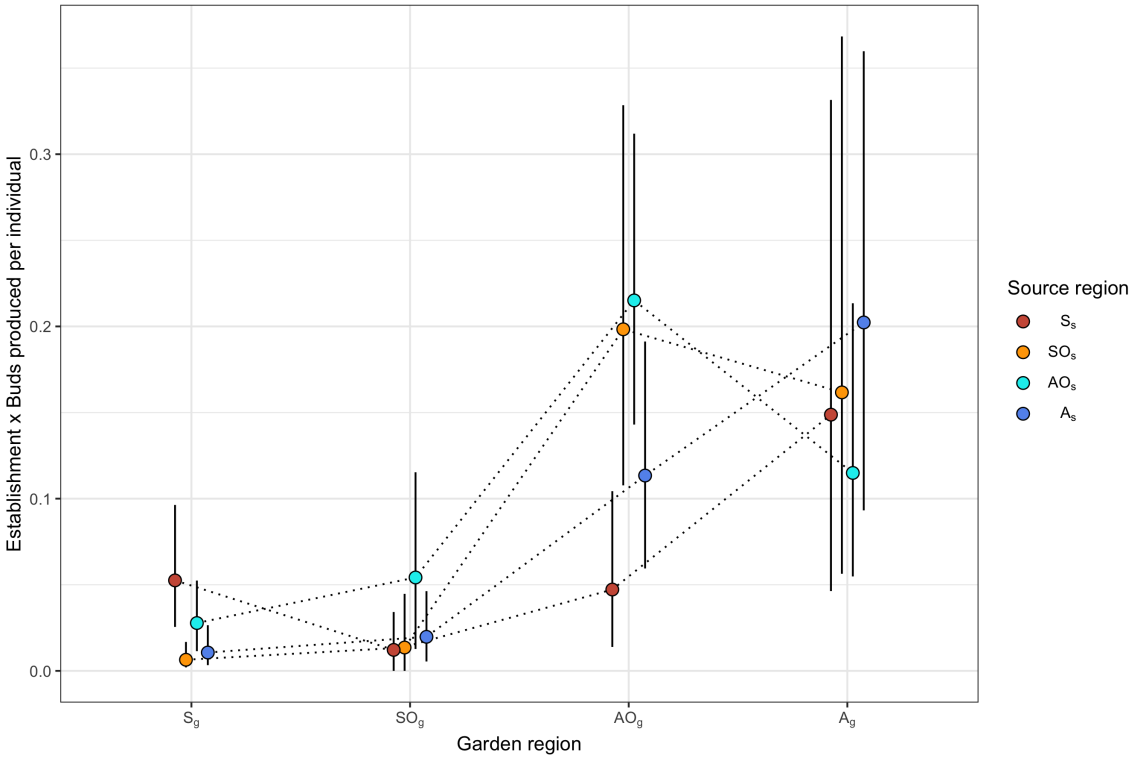

Figure 3.4: Means and 95% confidence intervals of establishment success x buds produced per individual calculated by randomly sampling each dataset 1000 times.

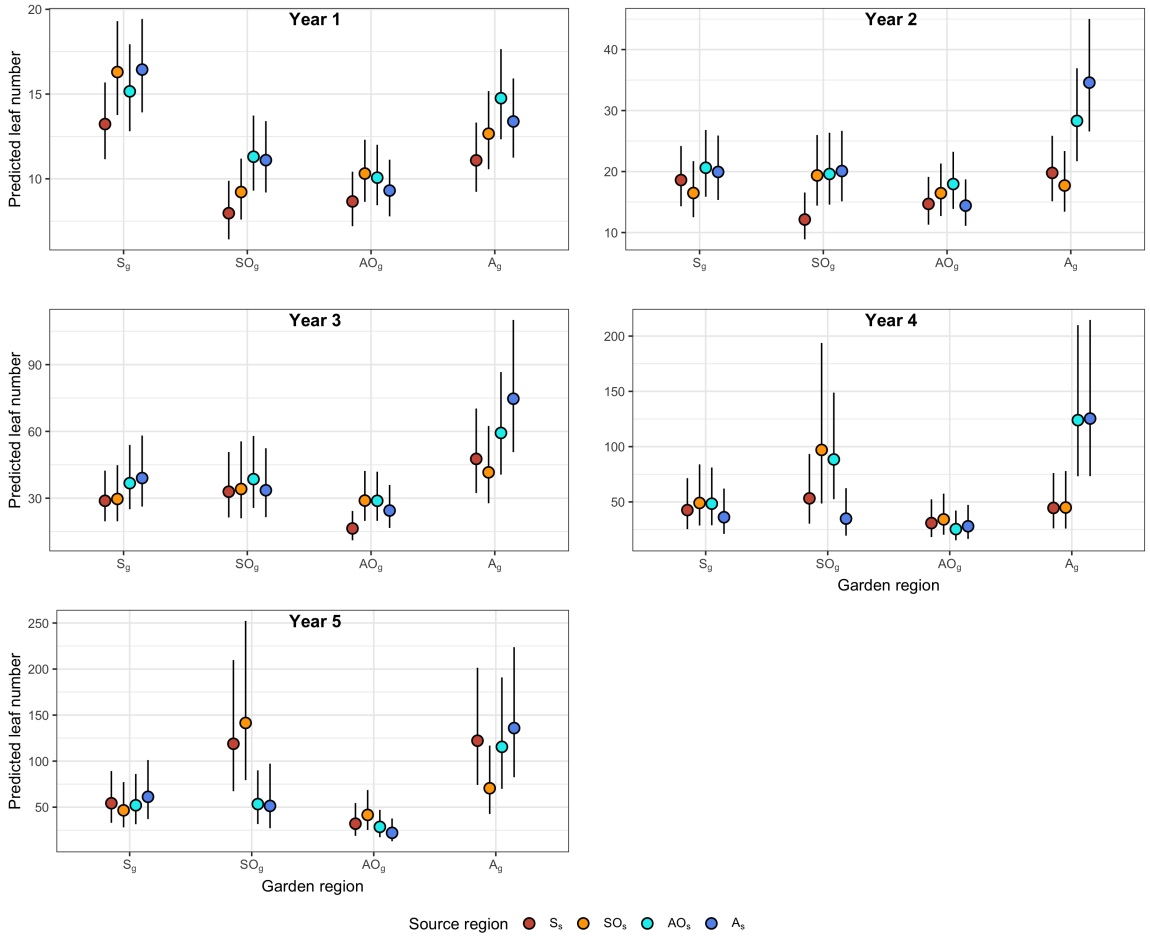

Figure 3.5: Predicted leaf number by source region and garden region in each year of the reciprocal transplant garden experiment. Points represent means from model predictions, and error bars represent 95% confidence intervals.

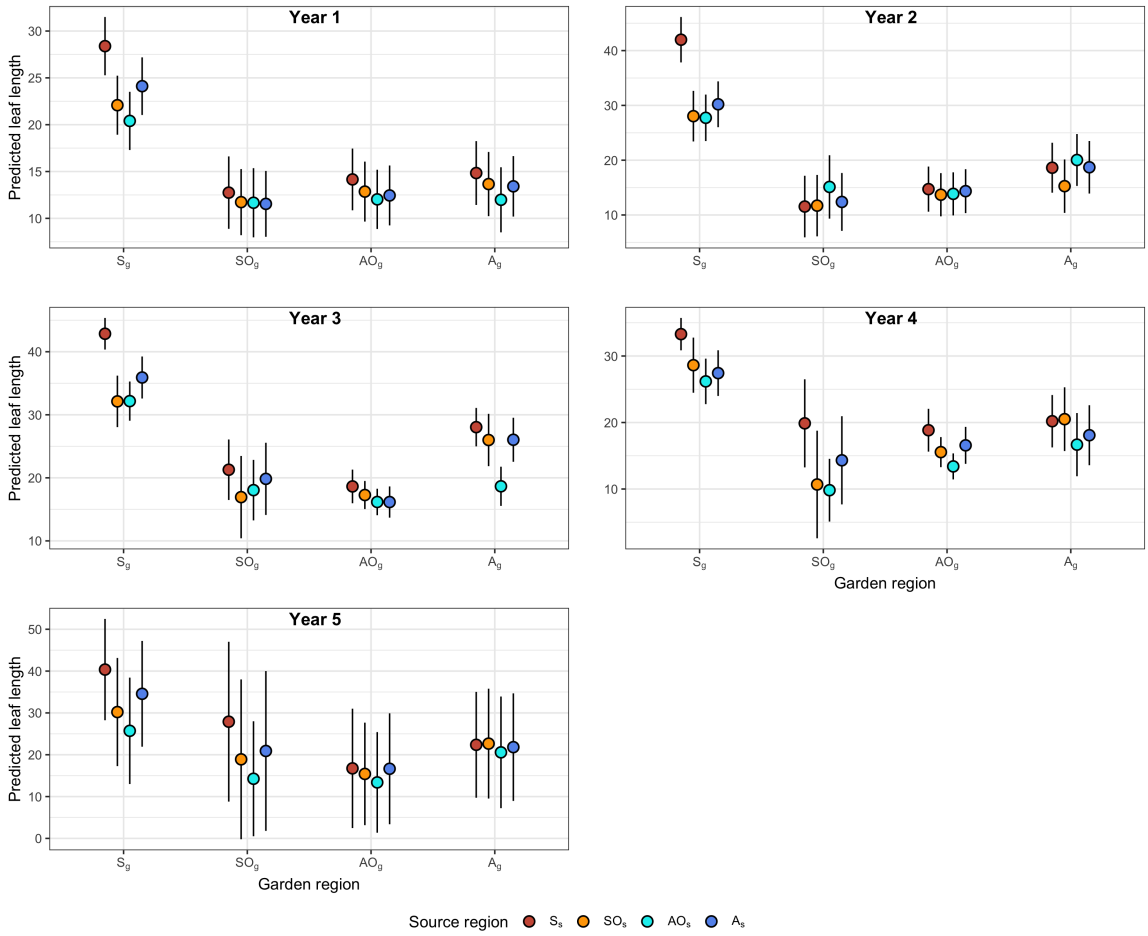

Figure 3.6: Predicted leaf length by source region and garden region in each year of the reciprocal transplant garden experiment. Points represent means from model predictions, and error bars represent 95% confidence intervals

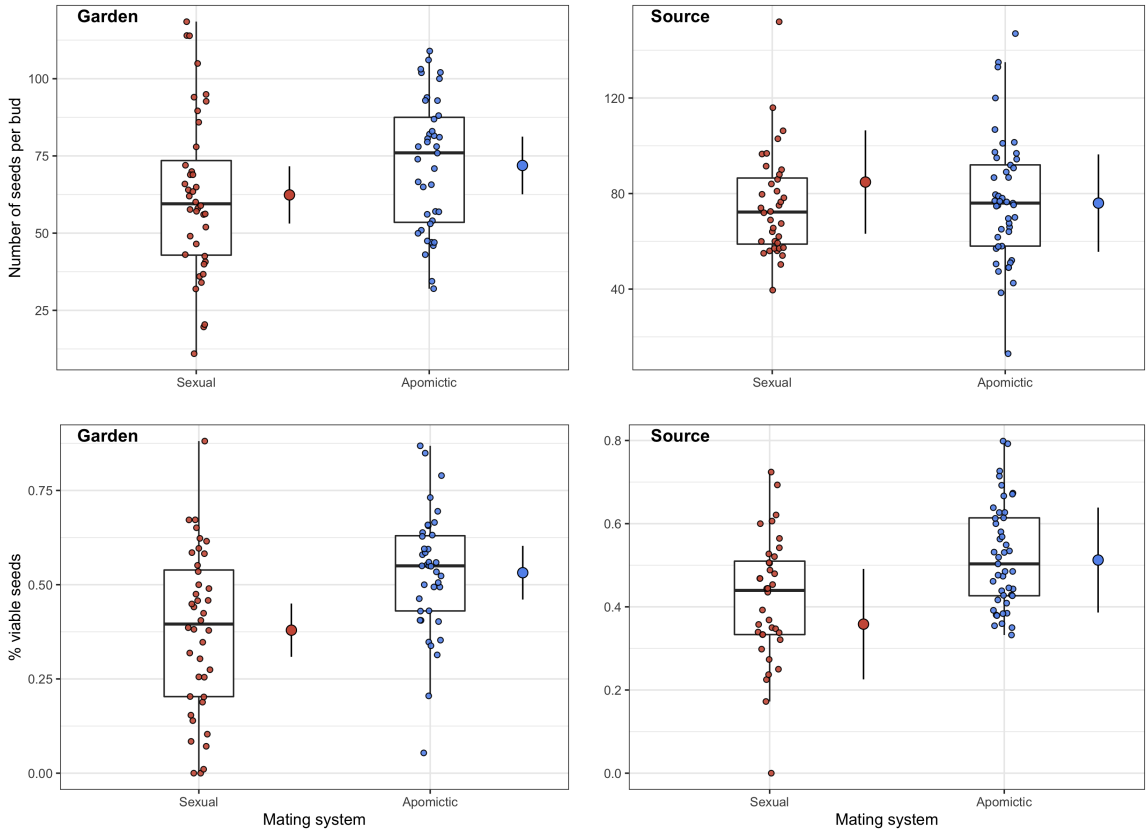

Figure 3.7: Seed set (number of seeds per bud and proportion viable) in reciprocal transplant gardens and source populations by mating system. Boxplots with jittered points represent values for each bud, and points with confidence intervals (to the right) indicate model predictions.

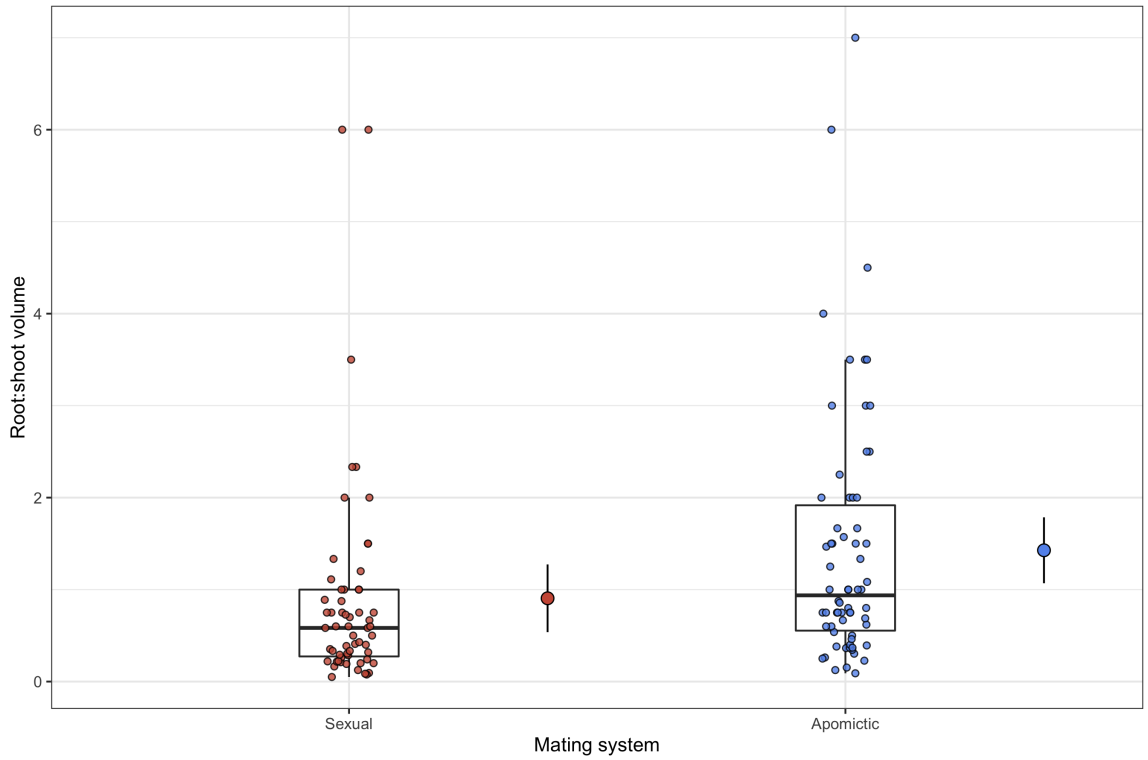

Figure 3.8: Root to shoot volume ratio of plants surviving to the end (year 5) of reciprocal transplant garden experiment. Boxplots with jittered points represent values for each individual measured, and points with confidence intervals (to the right) indicate model predictions.

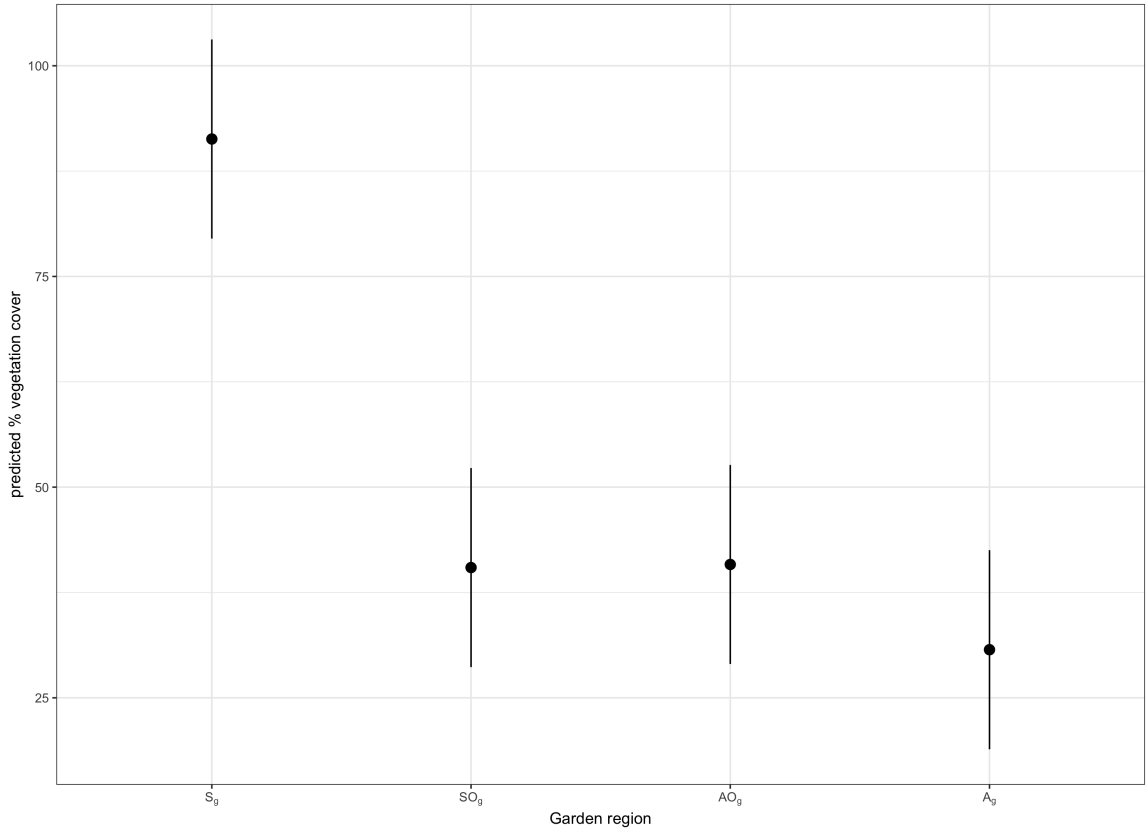

Figure 3.9: Predicted % vegetation cover in each garden region in the reciprocal transplant garden experiment.

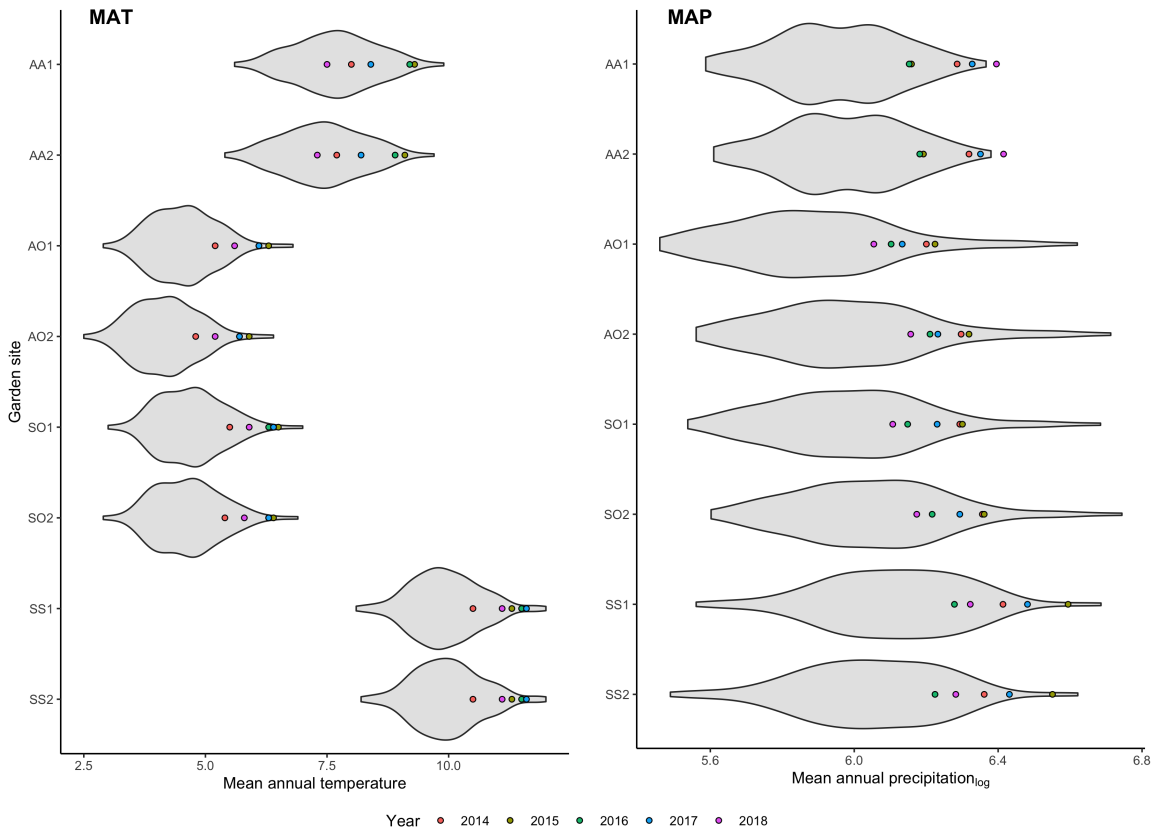

Figure 3.10: Mean annual temperature (MAT) and log mean annual precipitation (MAP) at each garden site. Violin plots represent historical distribution of the two climate variables from 1901 - 2012, and colored points represent values during reciprocal transplant experiment years.

Table 3.1: Source population and garden locations. For mating system, S = sexual and A = apomictic.

	Site	Region	Mating system	Latitude	Longitude
Source Populations	B53	S	S	39.89244	-105.26918
	B42	S_s	S	40.10705	-105.28365
	B46	S_s	S	40.54588	-105.13292
	B49	SO_s	S	40.84053	-105.33504
	L11	SO_s	S	41.24026	-105.43345
	L12	SO_s	S	41.25297	-105.40644
	L06	AO_s	A	41.14204	-106.04387
	L16	AO_s	A	41.30268	-105.52930
	L17	AO_s	A	41.38666	-105.47291
	C86	A_s	A	45.66205	-110.47504
	C85	A_s	A	46.65055	-111.72013
	C27	A_s	A	47.54790	-112.47641
Gardens	SS1	S_g		40.07072	-105.28236
	SS2	S_g		40.06680	-105.24803
	SO1	SO_g		41.11396	-105.51121
	SO2	SO_g		41.10375	-105.50114
	AO1	AO_g		41.38728	-105.48304
	AO2	AO_g		41.38809	-105.47413
	AA1	A_g		45.91201	-109.90557
	AA2	A_g		45.88509	-109.86897

Table 3.2: Likelihood ratio test statistics for the fixed effects of source region, garden region, and their interaction on establishment success.

Effect	df	χ^2	P
Source region	8	13.745	0.0033
Garden region	8	10.320	0.0160
Source region x Garden region	17	18.395	0.0309

Table 3.3: Summary statistics for establishment success.

Garden region	Source region	Number of seeds	Mean establishment success
S_g	S_s	120	0.133
	SO_s	120	0.083
	AO_s	120	0.200
	A_s	120	0.133
SO_g	S_s	120	0.467
	SO_s	120	0.433
	AO_s	118	0.424
	A_s	120	0.583
AO_g	S_s	120	0.717
	SO_s	120	0.667
	AO_s	120	0.800
	A_s	120	0.900
A_g*	S_s	76	0.342
	SO_s	88	0.523
	AO_s	88	0.341
	A_s	84	0.548

* Damage to one of the establishment plots installed in this garden region lead to a data gathering error which resulted in a loss of data.

Table 3.4: Likelihood ratio test statistics for the fixed effects of source region, garden region, and their interaction on lifetime bud production from ASTER models.

Effect	df	χ^2	P
Source region	19	2.558	0.465
Garden region	19	50.68	5.7×10^{-11}
Source Region x Garden Region	28	31.664	0.0002

Table 3.5: Raw data for the reciprocal transplant garden experiment.

Garden region	Source region	# planted	# surviving year 5	# of flowering individuals	total # of buds produced
S_g	S_s	128	19	19	50
	SO_s	132	8	6	10
	AO_s	144	9	9	20
	A_s	165	10	6	13
SO_g	S_s	153	1	2	4
	SO_s	161	1	2	5
	AO_s	180	5	6	23
	A_s	208	1	5	7
AO_g	S_s	152	3	6	10
	SO_s	157	14	18	47
	AO_s	176	23	28	46
	A_s	205	5	14	26
A_g	S_s	128	8	11	56
	SO_s	129	5	9	39
	AO_s	141	6	16	48
	A_s	162	6	12	59

Table 3.6: Likelihood ratio test statistics for leaf number in the reciprocal transplant garden experiment. Tests were performed separately for each year.

	Effect	df	χ^2	P
Year 1	Source region	9	13.809	0.0032
	Garden region	9	10.921	0.0122
	Source Region x Garden Region	18	25.309	0.0027
Year 2	Source region	9	4.579	0.2054
	Garden region	9	7.353	0.0615
	Source Region x Garden Region	18	117.78	2.2×10^{-16}
Year 3	Source region	10	4.6	0.2036
	Garden region	10	8.705	0.0335
	Source Region x Garden Region	19	76.955	6.5×10^{-13}
Year 4	Source region	10	1.213	0.75
	Garden region	10	11.045	0.0115
	Source Region x Garden Region	19	207.84	2.2×10^{-16}
Year 5	Source region	9	2.0424	0.5637
	Garden region	9	12.134	0.007
	Source Region x Garden Region	18	189.38	2.2

Table 3.7: Likelihood ratio test statistics for leaf length in the reciprocal transplant garden experiment. Tests were performed separately for each year.

	Effect	df	χ^2	P
Year 1	Source region	10	16.405	0.0009
	Garden region	10	19.559	0.0002
	Source Region x Garden Region	19	23.687	0.0048
Year 2	Source region	10	14.931	0.0019
	Garden region	10	25.42	1.3×10^{-5}
	Source Region x Garden Region	19	65.338	1.2×10^{-10}
Year 3	Source region	10	19.697	0.0002
	Garden region	10	29.491	1.8×10^{-6}
	Source Region x Garden Region	19	29.686	0.0005
Year 4	Source region	9	30.468	1.1×10^{-6}
	Garden region	9	29.252	2.0×10^{-6}
	Source Region x Garden Region	18	4.586	0.8688
Year 5	Source region	9	19.064	0.0003
	Garden region	9	7.36	0.0613
	Source Region x Garden Region	18	9.978	0.3523

Table 3.8: Likelihood ratio test statistics for seed set by mating system in reciprocal transplant gardens and source populations.

Seed trait (effect of mating system)	df	χ^2	P
Seed per bud (natural)	4	0.2103	0.6465
Proportion viable seed (natural)	4	3.247	0.0716
Seed per bud (garden)	4	2.229	0.1355
Proportion viable seed (garden)	4	7.761	0.0054

Chapter 4

Differences in Early Life History Traits between Diploid Sexual and Polyploid Apomictic Easter Daisies

4.1 Introduction

Geographical parthenogenesis (GP) describes a pattern in which closely related sexual and asexual taxa exhibit differences in geographic distributions, with asexuals often having larger ranges and occurring at higher latitudes and elevations than their sexual ancestors (Stebbins 1940; Glesener & Tilman 1978; Bierzychudek 1985). Despite the consistency of the pattern, the contexts in which GP occurs vary wildly across taxa, encompassing several forms of asexuality and origins of parthenogenesis (for a thorough review of GP in plants and animals, see Tilquin & Kokko 2016). While the specific causes of the pattern may be context-dependent in each system, it remains important to investigate the common conditions that allow both sexual and asexual reproduction to be maintained despite, on the one hand, the costs of sex and, on the

other, the prevalence and presumed benefits of sex in nature.

Apomictic complexes in flowering plants provide classical examples of GP. Asexuality in these systems typically comes in the form of apomixis (asexual reproduction through seeds), and the vast majority of apomictic plants are polyploids that are often of hybrid origin (i.e. allopolyploids) (Hörandl 2006; Whitton *et al.* 2008). Because polyploidy and hybridization can both affect plant species' ranges and are often coincident with apomixis, disentangling their influences on patterns of GP has been challenging. One of the central questions around the existence of GP involves distinguishing between the relative contributions of enhanced colonization potential that apomicts derive from uniparental reproduction ("Baker's Law" effects; Baker 1955) and the ecological and trait shifts that further enhance dispersal and establishment. Reproductive assurance of apomicts can contribute to range expansion, but this also means that individuals have to disperse to new areas and successfully become established. As a result, traits that enhance dispersal and the likelihood of establishment are expected to be associated with range expansion (Chuang & Peterson 2016). While uniparental reproduction can give asexuals a head start on range expansion (e.g. post-glaciation; Kearney 2005), it alone cannot explain why sexuals would not catch up; therefore, characterizing variation in other dispersal/establishment traits between mating types may help clarify whether sexuals would be capable of matching the ranges of their asexual counterparts given enough time. To date, few studies have considered changes in dispersal and establishment traits (other than uniparental reproduction) when investigating GP (but see Coughlan *et al.* 2014 and Chrtek *et al.* 2018).

It might seem that given a pattern in which apomicts have established a range beyond their sexual relatives, they would necessarily possess traits that favoured colonization. However, traits that enhance plant dispersal ability are often thought

to trade off with traits promoting establishment (e.g., seed number vs seed size) (Coomes & Grubb 2003). In wind-dispersed species of Asteraceae, dispersal potential depends on the diaspore (seed) mass and the size of the dispersal architecture (pappus) (Matlack 1987). Several studies have demonstrated an interaction between seed mass, pappus size, and drop time, where small seeds with a long pappus have a lower terminal velocity (staying aloft longer) than large seeds with a short pappus (O'Connell & Eckert 2001; Soons & Heil 2002; Gravuer *et al.* 2003; Riba *et al.* 2005). On the other hand, larger seeds often have higher germination success and more rapid seedling growth (Dolan 1984; Westoby *et al.* 1996; Turnbull *et al.* 2004). This suggests a trade-off in seed traits, where capacity for long distance dispersal comes at the cost of reduced establishment success. Additionally, some researchers have reported a negative relationship between seed size and germination speed, where smaller seeds tend to germinate more rapidly than larger seeds (Grime *et al.* 1981; Hendrix 1984; Bu *et al.* 2016, but see also McKersie *et al.* 1981; Piper 1986; Eriksson 1999 who found no relationship). The combination of these seed trade-offs suggest the possiblity of contrasting dispersal syndromes: one with large, slow-germinating seeds that have high germination success but low dispersal potential, and one with small seeds capable of rapid germination and long distance dispersal but with reduced germination success.

These divergent "establishment" and "dispersal" oriented life histories may contribute to patterns of GP if closely related sexuals and asexuals have divergent strategies, but inferring the role of selection in generating any observed differences is complicated by the association of apomixis with polyploidy and hybrid ancestry. While polyploidy is typically invoked to explain phenotypic shifts in GP contexts, the effects of apomixis may also contribute to differences in dispersal and establishment traits. Because of this, there is some tension in what mechanisms and outcomes

would be expected when considering both GP and life history trade-off theory. Polyploidy alone may result in a shift to either an establishment strategy (e.g., via the production of larger seeds that improve germination success in new environments) or a dispersal strategy (e.g., by producing seeds with a larger pappus:seed ratio that are better able to achieve long distance dispersal). While allopolyploids are historically considered to experience more extreme transformations due to heterosis (Rieseberg *et al.* 1999; Chen 2010; Paun *et al.* 2011), there is some evidence that autopolyploids (originating from a single parent species) can also experience phenotypic shifts that may significantly impact niche divergence (Paterson 2005; Ramsey 2011; Spoelhof *et al.* 2017).

Apomixis is not thought to result in phenotypic shifts directly, but it may indirectly contribute to differences in dispersal and establishment traits through clone selection. For example, high-dispersal sexual genotypes may be "frozen" by apomixis and, if successful, spread across the landscape into previously uncolonized regions (Lynch 1984; Vrijenhoek & Parker 2009). Similarly, clonal lineages may be under selection to escape to marginal habitats where biological interactions are less intense, favoring genotypes with strong colonization potential (i.e., Red Queen hypothesis; Asker & Jerling 1992). Having high dispersal ability may also be favored in marginal environments with high levels of mortality and environmental disturbance, allowing clones to maintain metapopulations in the face of repeated local extinctions while avoiding the deleterious effects of inbreeding depression (due to frequent genetic bottlenecks) that would occur in sexual metapopulations (Haag & Ebert 2004).

Townsendia hookeri provides an excellent system in which to test for differences in early life history traits within the context of GP. It is a wind-dispersed species with diploid outcrossing and autopolyploid apomictic forms that exhibit a classic pattern of GP (Bierzychudek 1985). *T. hookeri* provides a broad geographic range over

which to examine differences in dispersal and establishment traits between sexuals and apomicts. In this study, we ask the following questions: (1) Are there differences in dispersal and/or establishment traits between the two forms of *T. hookeri*? (2) If there are differences, are these consistent with the existence of opposing strategies (establishment vs dispersal) as predicted by life history trade-off theory? (3) Overall, do apomicts show evidence of enhanced colonization ability relative to their sexual progenitors? To address these questions, we conducted lab and greenhouse experiments to test for differences in early life history traits (seed mass, pappus morphology, terminal velocity of diaspores, germination success and speed, and seedling growth) between diploid sexual and polyploid apomictic forms of *T. hookeri* using natural populations sampled throughout their ranges.

4.2 Materials and methods

4.2.1 Study system

Townsendia hookeri is a diminutive perennial member of the sunflower family (Asteraceae) with two forms: diploids that reproduce sexually and autopolyploids (mainly triploid) that reproduce via gametophytic apomixis (Beaman 1957a). Sexual individuals are self-incompatible, while apomicts set seed autonomously without the need for pollen to fertilize the endosperm. *T. hookeri* is assumed to exhibit a generalist pollination syndrome like most species of Asteraceae (Mani & Saravanan 1999), and a variety of pollinators (including bees, flies, and beetles) have been seen visiting *Townsendia* species in the field (personal observation; Tepedino *et al.* 2004). Despite differing in ploidy and reproductive mode, the two forms are morphologically indistinguishable in the field. Polyploids can be identified by pollen staining (polyploids produce larger pollen grains than diploids and have much lower pollen viability) or by

using flow cytometry to estimate genome size; to date, information from more than 90 populations throughout the range reveal a nearly perfect association between low pollen viability and high genome size (Thompson & Whitton 2006; Garani 2014; Lee 2015), suggesting that diploids are sexual and polyploids are apomictic (Thompson & Whitton 2006; Thompson *et al.* 2008).

Sexual populations have a much smaller range than the apomicts and primarily occur between Boulder, CO and Laramie, WY (Lee 2015). Apomictic populations extend from southern WY along the eastern side of the Rocky Mountians as far north as British Columbia. A small number of diploid-sexual and polyploid-apomict populations can also be found in a disjunct distribution in the Yukon territory (Thompson & Whitton 2006; Garani 2014).

4.2.2 Seed collection

In order to compare diaspore traits between reproductive modes, we used wind-dispersed diaspores (technically achenes, but from now on referred to as "seeds") collected between 2008 and 2013 from five diploid sexual and seven polyploid apomictic populations from across *T. hookeri*'s range, including northern populations in British Columbia and the Yukon territory (Table 4.1). For the germination and greenhouse experiments, we collected seeds from six diploid sexual populations and six polyploid apomict populations in the spring of 2013 and 2014 (Table 4.1). Seeds were stored at room temperature in paper coin envelopes. The ploidy level of each population used in this study was assessed previously by flow cytometry (Lee 2015).

4.2.3 Seed dispersal traits and terminal velocity measurements

We selected two seeds per maternal plant from up to ten moms per population, using only filled and darkly coloured seeds (traits that indicate viability; Garani 2014) with an intact pappus. In order to restore the pappus to a comparably open state (relative to the somewhat flattened state that resulted from storage in the collection envelopes), we placed each seed on wet filter paper in a sealed petri dish for ∼24 hours, then removed them and allowed them to air-dry for another 24 hours. At this point the pappus had achieved a more regular form consistent with what is seen at the time of field collection. For each seed, we estimated the mass using an analytical balance, recorded the number of bristles, and measured the length of two bristles from the center-apex using an ocular micrometer. We measured the angle of attack, the maximum angle across the open pappus bristles centered on their point of attachment to the seed proper, using a protractor.

We estimated terminal velocity by dropping individual seeds (=diapores) down a clear 120 cm long plexiglass tube. Seeds were dropped by holding the pappus with tweezers and releasing. We recorded the bottom 50 cm with a video camera (shooting at 30 frames per second), thus allowing the seeds to reach terminal velocity over the first 70 cm of the drop. We calculated the terminal velocity of each seed by dividing the drop time by drop distance (50 cm). We took great care throughout not to damage or disturb the structure of the pappus.

4.2.4 Germination in the lab

We chose 11 maternal plants (hereafter known as "moms") from each population, and 32 "viable" seeds (based on the same criteria as above) from each mom. We assessed germination in agar-filled petri dishes (hereafter referred to as "plates"). Each plate

was divided into four quadrants, and four seeds from a single mom were randomly assigned to each quadrant. Seeds were arranged within quadrants to avoid contact. Due to the large number of seeds, we filled the plates over the course of three days (beginning on June 18, 2014). We stacked the plates on the lab bench at room temperature (21°C) away from direct sunlight, and re-ordered the stacks every 3-4 days. We checked the plates every 1-2 days until no new seeds had germinated for 10 days, and scored germination as successful based on the emergence of both a radicle and a pair of cotyledons.

4.2.5 Seedling traits

We transplanted seeds from agar into racks of cone-tainer pots (Proptek - Watsonville, California) 2-3 days after germination over a period of two weeks. We filled the pots with a well-draining soil mixture (4 potting soil: 2 sand: 1 perlite) and placed the seedling racks in growth chambers (Conviron, various models). Growth chambers were set for 12h light (20°C) / 12h dark (10°C) cycles. We hand-watered seedlings daily in the week following transplanting, and every two days after that. We rotated the racks between chambers once per week to account for potential differences among growth chamber models. After most seedlings reached a height of at least 2 cm (early July 2013), we hardened the seedlings in a greenhouse at the UBC farm. The greenhouse environment was regulated by Argus Controls (12h light - 20°C / 12h dark - 12°C), and the seedlings were bottom-watered on flood tables. Because we subsequently transplanted the seedlings into a common garden experiment, we brought them outside two weeks before transplanting to harden them to UV light (3-5 hours per day). Just prior to moving to field sites, we censused all seedlings for survival and estimated plant size using two leaf measurements: total number of leaves, and the leaf length of one haphazardly chosen representative leaf per seedling. Thus, our

measurement of seedling size reflects the size achieved from the day of germination until the census approximately two months later. While not every seed germinated on the same day (and therefore did not have equal time to grow until the census), 75% of seeds that germinated did so within a five day period, so we expect the variation in total growth time had minimal effects on our growth estimates.

4.2.6 Data analysis

We analyzed differences in seed traits (terminal velocity, angle of attack, bristle length, number of bristles, and seed mass) between mating systems using mixed effects models. Terminal velocity, angle of attack, and bristle length were analyzed using linear mixed effect models (LMMs), and number of bristles was analyzed used generalized linear mixed effects models (GLMMs) with a log link function for poisson data. Mating system was a fixed factor; population and mom (nested within population) were included as random factors. We used linear models to test the effects of each seed trait on terminal velocity separately. Traits were centered and scaled (using the *scale()* function in R), and included as a fixed effect in each model without random effects.

We used GLMMs to analyze differences in germination success (logit link - binomial) and germination speed (log link - poisson, due to speed being represented by day counts) between mating systems. Mating system was a fixed factor; plate, population, and mom (nested within population) were included as random factors. We also used GLMMs to analyze differences between mating systems for seedling survival (logit link - binomial) and leaf number (log link - poisson), and LMMs for leaf length. Mating system was a fixed factor; rack, population, and mom (nested within population) were included as random factors. Including rack as a random effect resulted in model singularities for the leaf length and leaf number models, so we removed it from those analyses. Because populations were the main level of replication, we

used subsequent mixed models to determine whether there were differences between populations, with population as a fixed effect and the same random factors as above.

We performed the mixed-effect analyses using the lmer and glmer functions implemented in the lme4 package (Bates *et al.* 2015) and fixed effect analyses using the lm function in R version 3.6.1 (R Core team 2019). We evaluated the strength of effects by using a combination of likelihood ratio tests (LRTs; comparing fully fitted model to a model with the tested term removed) and model predictions using the ggeffects package (Lüdecke 2018). Statistical differences between groups were inferred when both the LRT P-values were below 0.05 and confidence intervals from model predictions were largely non-overlapping.

4.3 Results

4.3.1 Seed dispersal traits and terminal velocity

Variation in terminal velocity was associated with variation in each of the four seed traits, with bristle length and angle of attack having the strongest effects (Table 4.2; Figure 4.1). Apomictic seeds had longer pappus bristles and a wider angle of attack than sexual seeds, but there were no differences in seed mass or bristle number between mating systems (Table 4.3; Figure 4.3). Terminal velocity differed between mating systems (Table 4.3; Figure 4.1A) and populations (df=14, χ^2=31.697, P=7.74x10^{-08}), with apomicts having lower terminal velocity (0.91 cm/s) than sexuals (1.38 cm/s; Table 4.4), indicating increased dispersal ability of apomicts over sexuals. Despite differences in trait mean intercepts, the slopes of traits and terminal velocity did not differ between sexuals and apomicts (Figure 4.1), suggesting that the relationships between traits and terminal velocity do not differ between mating types.

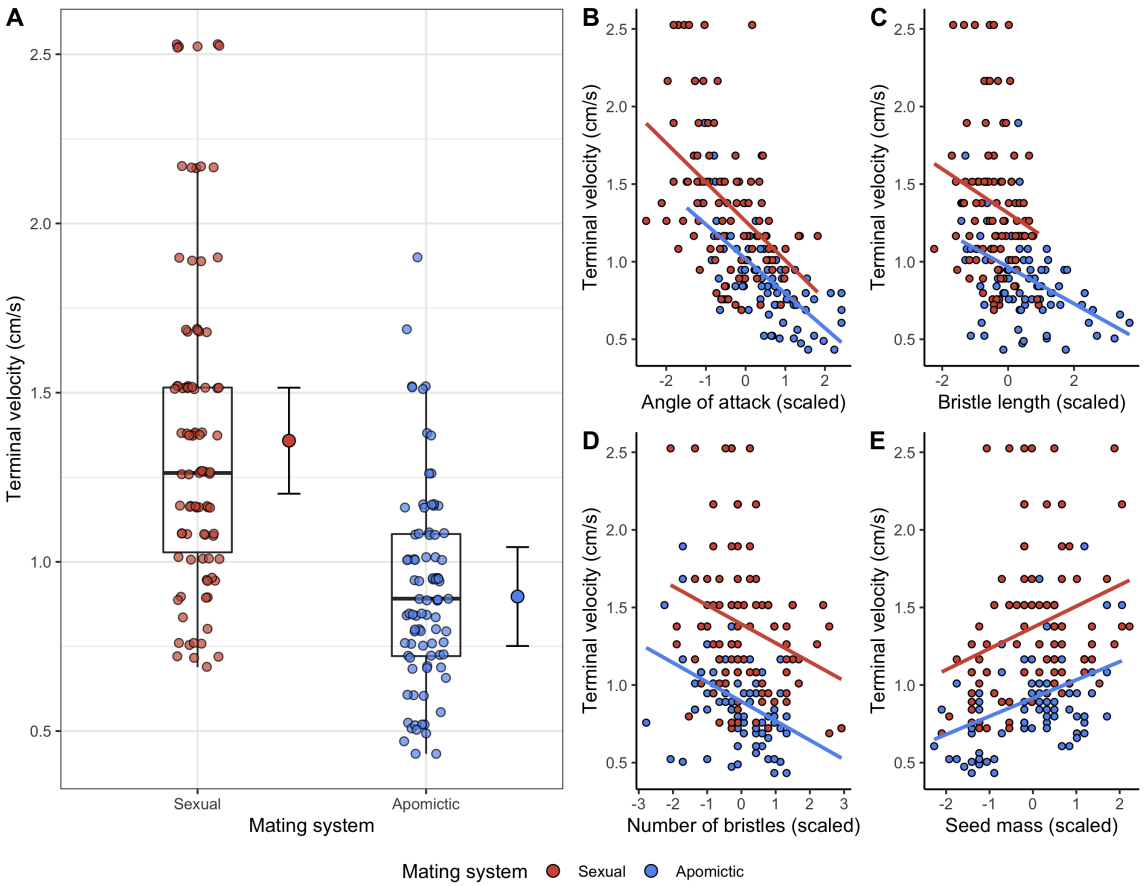

Figure 4.1: Seed dispersal traits. A) Terminal velocity by mating system. Boxplots provide summaries of the data, with jittered points representing individual seeds. To the right of the boxplots, points and error bars represent predicted means and their 95% confidence intervals. B-E) The relationship between terminal velocity and measured seed dispersal traits by mating system. The slopes are shown for data from sexuals and apomicts separately.

4.3.2 Germination traits

Germination success differed between mating systems and among populations (Table 4.5; Figure 4.2A, Figure 4.4B), with apomicts having much higher germination success (78%) than sexuals (59%; Table 4.6). Germination speed (mean days to germination) did not differ between mating systems (Figure 4.2B), but there were differences among populations, with three of the sexual populations germinating slower than the ma-

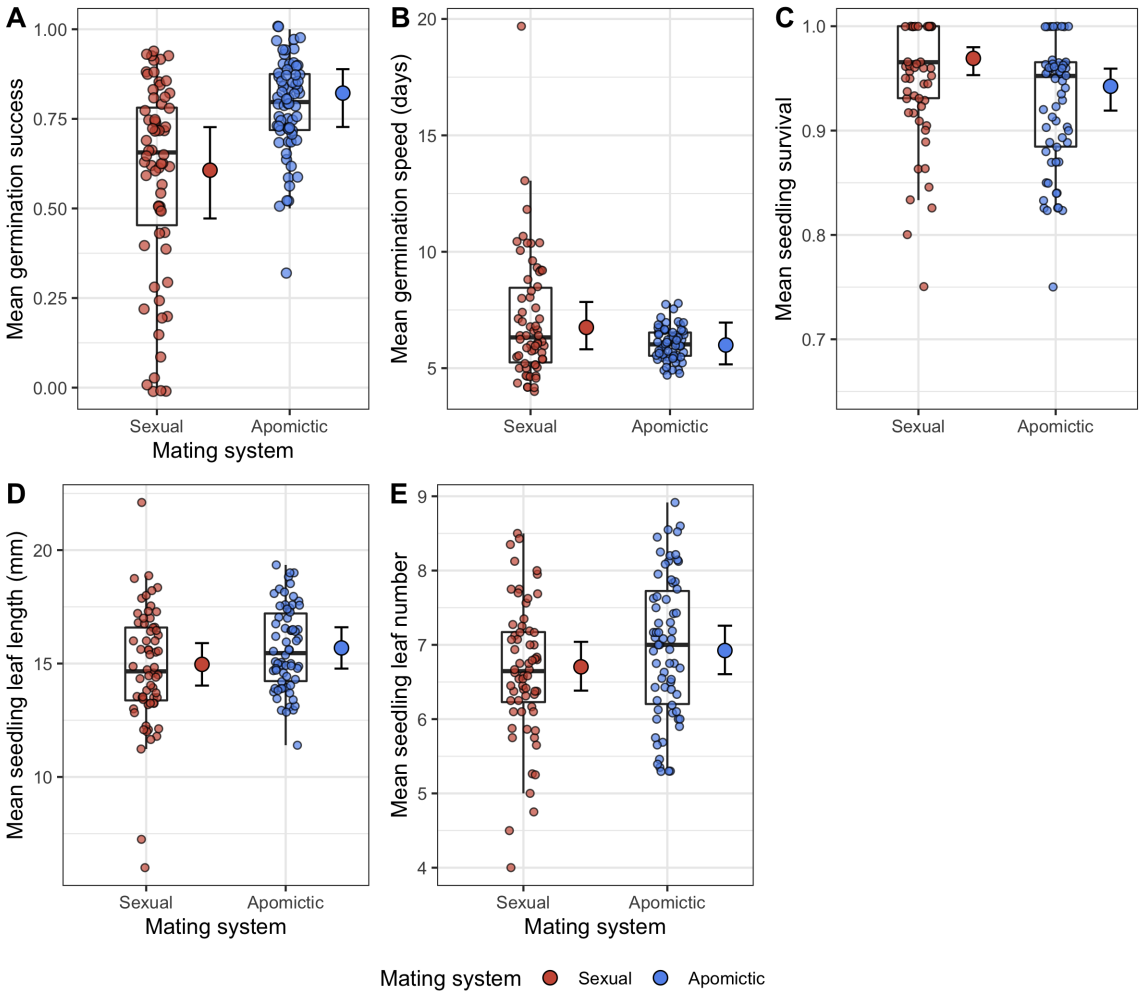

Figure 4.2: Germination and seedling traits by mating system. Boxplots provide summaries of the data, with jittered points representing averages for each mom for each trait. To the right of the boxplots, points and error bars represent predicted means and their 95% confidence intervals.

jority of the apomictic populations (B53, B42, and B49; Table 4.5, Table 4.7; Figure 4.4C). Apomicts had lower variance than sexuals in both germination traits (Table 4.7, Table 4.6; Figure 4.2A&B).

4.3.3 Seedling traits

Seedling survival was high overall (>90% with the exception of population L06), as expected for greenhouse-raised plants. Seedling survival differed between mating systems and among populations, with apomicts having slightly lower survival (92.4%) than sexuals (95.7%; Table 4.5, Table 4.8; Figure 4.2C, Figure 4.4D). While LRTs demonstrate that mating system affected survival, there was partial overlap in the model prediction confidence intervals, indicating that this is not a particularly strong effect. Neither leaf length or number of leaves differed between mating systems, but both differed among populations (Table 4.5, Table 4.9, Table 4.10; Figure 4.2D&E, Figure 4.4E&F).

4.4 Discussion

We found that the seeds of apomicts had both lower terminal velocity and higher germination success than sexual seeds, indicating that apomicts have a distinct advantage in dispersal ability and colonization success. This advantage is offset by lower survival of apomicts at the seedling stage, but the survival difference is much smaller than the differences found in terminal velocity and germination success. In combination with reproductive assurance conferred by apomixis, these differences in early life history traits likely contribute to the apomicts having a much broader range than sexuals in *T. hookeri*. Interestingly, our results are largely *not* in line with what would be expected under life history trade-off theory, as the apomicts' dispersal advantage coincided with increased germination success as opposed to a decrease (though the cost to seedling survival is in the expected direction).

As seen in other wind-dispersed diaspores (O'Connell & Eckert 2001; Soons & Heil 2002; Gravuer *et al.* 2003; Riba *et al.* 2005), terminal velocity was affected by all of

the seed traits measured, with longer bristles and wider angle of attack contributing the most to reducing drop times. These two dispersal traits were also the only ones to differ between sexuals and apomicts. The apomicts' increased pappus volume resulted in a ∼50% decrease in terminal velocity, indicating a considerable dispersal advantage in comparison to sexuals. Though seed mass did not differ by mating system (Figure 4.3D, Table 4.4), we did observe a positive relationship between mass and terminal velocity (Figure 4.1E) as found in other systems (Greene & Johnson 1993; Greene & Quesada 2005). The fact that there were no appreciable differences in seed mass between mating types indicates that a large increase in pappus volume comes with a relatively small cost to maternal investment in *T. hookeri*, at least on a per-seed basis.

Apomicts had much higher germination success than sexuals, once again suggesting increased colonization potential. Life history trade-off theory predicts that this improvement would be associated with an increase in seed mass, but that was not the case in our study, as we found no appreciable differences in seed mass between sexuals and apomicts. While germination speed did not differ overall between mating types, three sexual populations took longer than others to germinate, indicating that apomicts were more consistent in their ability to germinate rapidly than sexuals. It is important to note that rapid and increased germination success is not always advantageous, as delayed germination or dormancy can provide a temporal escape from unfavourable environmental conditions (Finch-Savage & Leubner-Metzger 2006). However, we have seen very little evidence that *T. hookeri* forms a seed bank or displays consistent dormancy (95% of established seedlings germinated in the first year in Chapter 3), which suggests that germination success is driven primarily by seed viability. As these seeds were collected in nature, we cannot discount the possibility that environmental and/or genetic maternal effects contributed to the trait

differences that we found (Roach & Wulff 1987), but it is notable that apomicts showed considerably less variance than sexuals in both germination traits. This finding suggests that germination performance is influenced at least to some extent by genotype. Following this logic, it is not surprising that we found more variability among outcrossing populations than among clonal populations when germinating under uniform and benign conditions.

Seedling survival was quite high in the greenhouse experiment for both mating systems, but slightly lower for apomicts. This is the one result from this study that diminishes the colonization advantage of apomicts and lends partial support to a trade-off between dispersal and competitive ability. On the other hand, we found no differences in leaf number or leaf length between mating systems at the seedling stage, so whatever contributed to the reduction in survival did not seem to affect growth rates (which we might expect if apomicts had reduced maternal investment). While the differences in seedling survival were quite small, these effects might be amplified under field conditions. If so, the reduced seedling survival of apomicts might counteract their germination advantage in nature, which could help explain why we found no overall differences in seedling establishment plots (which incorporate germination success and seedling survival) in the field (Chapter 3).

As is true of most GP studies, we are hampered in our power to disentangle the effects of ploidy and apomixis in *T. hookeri*, though in our case we can disregard the effect of hybridization because apomicts are autopolyploids (Thompson & Whitton 2006). While we cannot discount the possibility that polyploidy has affected the anatomy and physiology of apomicts as found in some polyploids (Ramsey & Schemske 2002; Nuismer & Cunningham 2005; Cohen *et al.* 2013; Eliášová & Münzbergová 2014; Gao *et al.* 2016), until now, no consistent morphological differences between diploids and polyploids have been documented in *T. hookeri* (Beaman

1957a; Reveal 1970). The differences that we document in two seed traits (angle of attack and bristle length) are the first indication of macro-morphological differences between the mating types. In general, polyploids that benefit from increased germination success also have larger seeds (Beaulieu *et al.* 2007; Haouala *et al.* 2009; Hahn *et al.* 2013), so it is surprising that polyploid *T. hookeri* have such a large germination advantage without any obvious differences in seed size. The interaction between ploidy, seed size, and germination performance may be complex, however. Bretagnolle (1995) compared seeds of equal mass between diploid and polyploid *Dactylis glomerata* and found that polyploid seeds both germinated faster and had higher germination success than diploid seeds, indicating that the effects of ploidy on germination may not be simply due to increased seed size. However, it is also important to point out that despite the long recognized importance of polyploidy in the diversification of angiosperms, there remain few studies of life history differences between cytotypes in nature or in common gardens from which we might attempt to extract general predictions. Additionally, some broad-scale studies indicate that polyploids do not consistently show niche shifts (Glennon *et al.* 2014) or range shifts (Martin & Husband 2009) in comparison to related diploids (though see Baniaga *et al.* 2020 and Prentis *et al.* 2008 for opposing trends), which suggests that other traits besides genome duplication may play a role in patterns of GP.

The differences in colonization potential found in this study may be more easily explained by the joint effects of apomixis and polyploidy (as opposed to the effects of polyploidy alone). Assuming that apomicts originated from sexual populations and subsequently spread, selection may have favored those clonal lineages with enhanced colonization ability. In fact, given that our study necessarily surveys clones that have successfully established, it is in some ways unsurprising that we find enhanced colonization and establishment traits. We expect that as clones arise, those that succeed

in colonizing new areas will be the lineages that have a successful suite of traits, protected (by apomixis) from the action of recombination, which would breakdown beneficial allele combinations (Lynch 1984). This is consistent with our finding that apomicts had reduced variance in germination speed, germination success, and terminal velocity in comparison to sexuals (Table 4.7, Table 4.6, Table 4.4). While this reduced variance gives apomicts improved colonization trait performance on average, their values largely lie within the range of sexual traits. This pattern is particularly noticeable in the germination traits; some sexual populations/families have the ability to germinate rapidly and with high success, but sexuals have considerably more variance than apomicts in both traits. This interpretation is in line with the fact that apomictic populations and regions are largely monoclonal (Chapter 2), as we would expect less variation among individuals within a clone than among siblings from an outcrossing mom.

An open question is whether reproductive assurance alone is enough to account for range differences found in plant GP systems. Chrtek *et al.* (2018) investigated diaspore differences within a GP context in *Hieracium alpinum*, which, like *T. hookeri*, also has self-incompatible diploids and autonomously apomictic autopolyploids. While they did find slight differences in terminal velocity favoring apomicts, they also found that sexuals had much higher germination success than apomicts, which is opposite to what we found. This highlights that dynamics can differ significantly even between otherwise very similar systems. Regardless of the mechanism in other systems, it is clear that in *T. hookeri* apomicts have early life history traits that give them an advantage in dispersal and colonization potential. This advantage is compounded by the fact that apomicts already benefit from full reproductive assurance, and that sexuals are self-incompatible and entirely dependent on local mate availability and pollinator services. This study provides some of the only evidence

thus far that dispersal ability can differ between sexual and asexual propagules in a GP context (but also see O'Connell & Eckert 2001; Coughlan *et al.* 2014). This is interesting, because dispersal can provide an escape (in the absence of sexual recombination) from biotic stressors (i.e., Red Queen hypothesis; Glesener & Tilman 1978; Judson 1997; Hartfield & Keightley 2012) and environmental instability (Haag & Ebert 2004). Comparisons of dispersal and colonization traits in other systems will provide much needed context, and may provide an alternative (or complementary) explanation to patterns of GP driven by reproductive assurance.

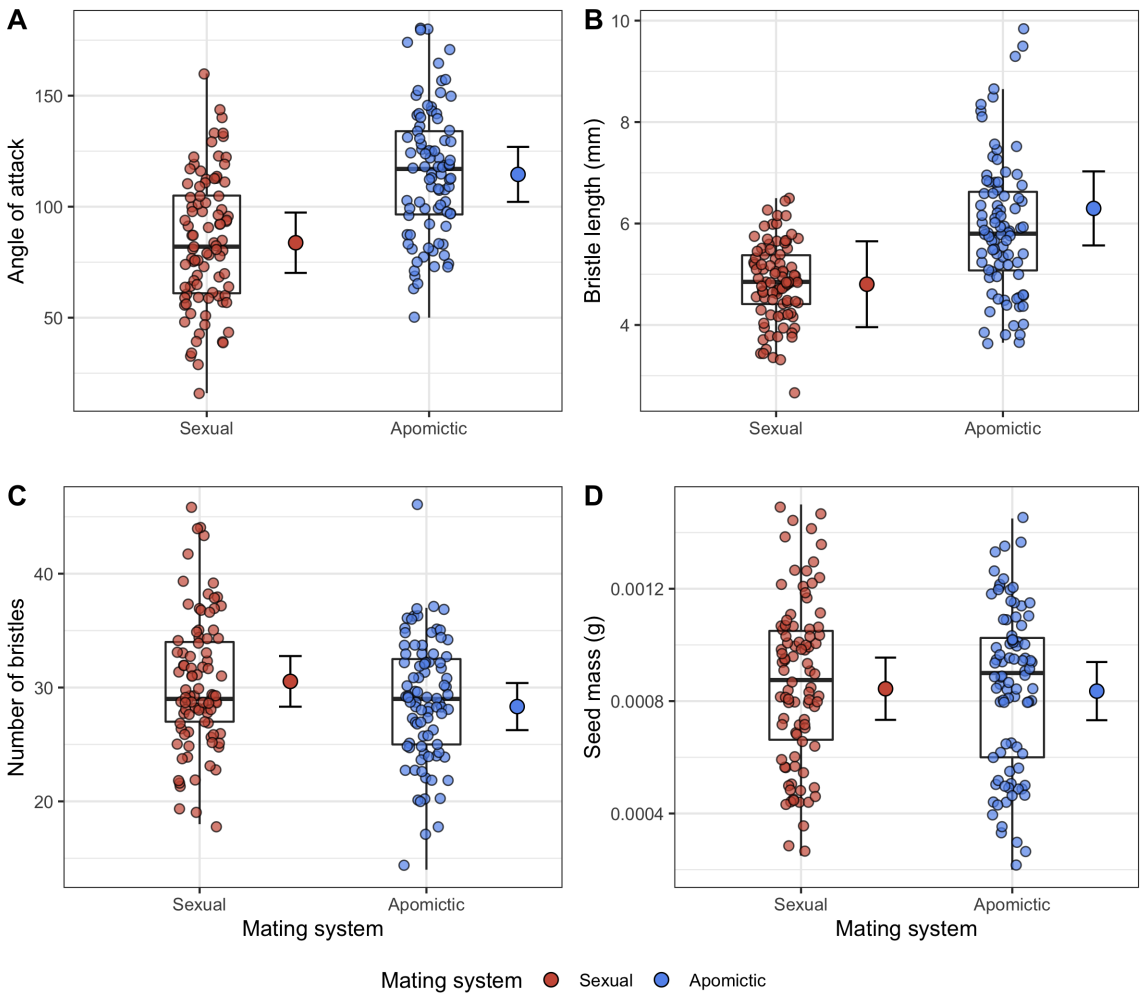

Figure 4.3: Seed dispersal traits by mating system. Boxplots and jitter represent individual seeds, while points and error bars (right) represent predicted means with 95% confidence intervals.

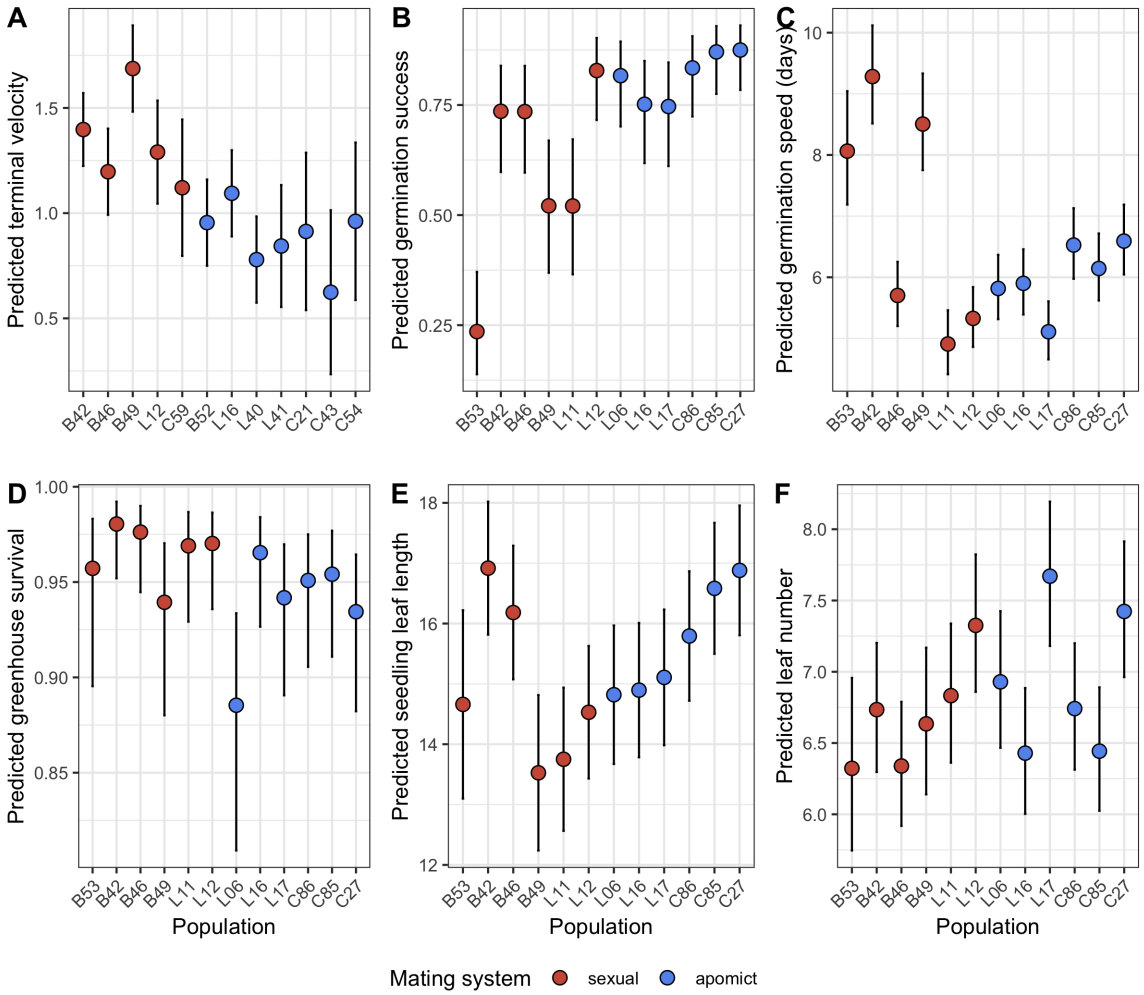

Figure 4.4: Terminal velocity, germination, and seedling traits by population. Points and error bars represent predicted means with 95% confidence intervals. Within each reproductive type, populations are arranged from south (on the left) to north.

Table 4.1: List of populations used in this study. 'Experiment' indicates the experiment in which each population was used; S = seed, G = germination / greenhouse.

Mating system	Population	Ploidy	State/Province	Latitude	Longitude	Experiment
Sexual	B53	2	CO	39.8913	-105.2655	G
	B42	2	CO	40.1070	-105.2836	S,G
	B46	2	CO	40.5384	-105.1335	S,G
	B49	2	CO	40.8403	-105.3214	S,G
	L11	2	WY	41.2400	-105.4342	G
	L12	2	WY	41.2528	-105.4062	S,G
	C59	2	YT	62.1235	-136.2575	S
Apomictic	B52	3	CO	40.9410	-106.0499	S
	L06	3	WY	41.1430	-106.0375	G
	L16	3	WY	41.3064	-105.5216	S,G
	L17	3	WY	41.3864	-105.4735	G
	L18	3	WY	41.5478	-110.5527	S
	L20	3	WY	41.6802	-110.6119	S
	L40	3	WY	42.7973	-105.8814	S
	L41	3	WY	42.8087	-105.3778	S
	C86	3	MT	45.6619	-110.4752	G
	C21	3	MT	46.3329	-111.5563	S
	C85	3	MT	46.6505	-111.7200	G
	C27	3	MT	47.5500	-112.4667	G
	C43	3	BC	50.6057	-116.0595	S
	C54	4	YT	60.8360	-135.9143	S

Table 4.2: Relationships between seed traits and terminal velocity.

Trait	df	Estimate (slope)	R^2	F	P
Angle of attack (scaled)	1	-0.2943	0.4112	122.2	2.00E-16
Bristle length (scaled)	1	-0.2042	0.198	43.21	5.44E-10
Number of bristles (scaled)	1	-0.0885	0.0372	6.753	0.0102
Mass (scaled)	1	0.1352	0.0868	16.63	6.88E-05

Table 4.3: Likelihood ratio test statistics for the fixed effect of mating system on seed traits.

Trait (seed)	Source of variation	df	χ^2	P
Terminal velocity	Mating system	5	12.163	0.0005
Angle of attack		5	8.7348	0.0031
Bristle length		5	6.3478	0.0118
Number of bristles		4	2.1093	0.1464
Weight		5	0.0247	0.8751

Table 4.4: Terminal velocity - summary statistics by mating system and population.

Mating system	Population	n (seeds)	Raw mean (cm/s)	SE	Predicted means (cm/s)	Lower CI	Upper CI
Sexual		90	1.3800	0.0503	1.3581	1.2014	1.5147
Apomictic		87	0.9120	0.0311	0.8975	0.7514	1.0436
Sexual	B42	28	1.3974	0.0883	1.3974	1.2242	1.5706
	B46	20	1.1968	0.0602	1.1968	0.9919	1.4018
	B49	20	1.6873	0.1228	1.6873	1.4823	1.8922
	L12	14	1.2901	0.1354	1.2901	1.0451	1.5351
	C59	8	1.1208	0.1146	1.1208	0.7967	1.4449
Apomictic	B52	20	0.9550	0.0485	0.9550	0.7500	1.1600
	L16	20	1.0943	0.0722	1.0943	0.8893	1.2992
	L40	20	0.7792	0.0765	0.7792	0.5743	0.9842
	L41	10	0.8438	0.0654	0.8438	0.5539	1.1337
	C21	6	0.9131	0.0273	0.9131	0.5389	1.2874
	C43	5	0.6100	0.0463	0.6241	0.2343	1.0139
	C54	6	0.9613	0.0660	0.9613	0.5871	1.3356

Table 4.5: Likelihood ratio test statistics for the separate fixed effects of mating system and population on germination and seedling traits.

Trait (germination/seedling)	Source of variation	df	χ^2	P
Germination success	Mating system	5	6.0279	0.01408
Germination speed		5	1.1727	0.2789
Seedling survival		5	5.8538	0.0155
Seedling leaf number		4	0.8434	0.3584
Seedling leaf length		5	1.3304	0.2487
Germination success	Population	14	68.26	$2.61\text{x}10^{-10}$
Germination speed		14	123.82	$2.20\text{x}10^{-16}$
Seedling survival		14	22.121	0.0235
Seedling leaf number		13	31.833	0.0008
Seedling leaf length		14	39.701	$4.03\text{x}10^{-05}$

Table 4.6: Germination success - summary statistics by mating system and population.

Mating system	Population	n (seeds)	Raw mean	SE	Predicted means	Lower CI	Upper CI
Sexual		2112	0.5885	0.0107	0.6067	0.4720	0.7269
Apomictic		2112	0.7822	0.0090	0.8219	0.7273	0.8887
Sexual	B53	352	0.3125	0.0247	0.2355	0.1385	0.3711
	B42	352	0.7074	0.0243	0.7357	0.5977	0.8391
	B46	352	0.6960	0.0246	0.7351	0.5964	0.8390
	B49	352	0.5142	0.0267	0.5210	0.3689	0.6694
	L11	352	0.5284	0.0266	0.5207	0.3654	0.6722
	L12	352	0.7727	0.0224	0.8283	0.7158	0.9023
Apomictic	L06	352	0.7813	0.0221	0.8166	0.7012	0.8942
	L16	352	0.7330	0.0236	0.7518	0.6179	0.8501
	L17	352	0.7244	0.0238	0.7466	0.6112	0.8467
	C86	352	0.7869	0.0219	0.8343	0.7238	0.9063
	C85	352	0.8182	0.0206	0.8706	0.7751	0.9293
	C27	352	0.8494	0.0191	0.8749	0.7842	0.9308

Table 4.7: Germination speed - Summary statistics by mating system and population.

Mating system	Population	n (seeds)	Raw mean (days)	SE	Predicted means (days)	Lower CI	Upper CI
Sexual		1204	6.8900	0.1010	6.7509	5.8104	7.8436
Apomictic		1607	6.0800	0.0489	5.9938	5.1632	6.9579
Sexual	B53	104	8.1731	0.2848	8.0632	7.1891	9.0437
	B42	237	9.5316	0.3233	9.2823	8.5150	10.1188
	B46	240	5.8292	0.1283	5.7032	5.2020	6.2526
	B49	171	8.3918	0.2298	8.5059	7.7525	9.3325
	L11	183	4.9945	0.1591	4.9095	4.4141	5.4606
	L12	269	5.3532	0.1127	5.3279	4.8608	5.8398
Apomictic	L06	263	5.8555	0.1270	5.8174	5.3152	6.3672
	L16	253	5.8340	0.1158	5.9009	5.3915	6.4584
	L17	250	5.2320	0.1117	5.1097	4.6571	5.6063
	C86	268	6.4701	0.1196	6.5273	5.9754	7.1301
	C85	278	6.2518	0.1056	6.1440	5.6206	6.7162
	C27	295	6.7017	0.1167	6.5922	6.0458	7.1879

Table 4.8: Seedling Survival - summary statistics by mating system and population.

Mating system	Population	n (seedlings)	Raw mean	SE	Predicted means	Lower CI	Upper CI
Sexual		1297	0.957	0.00565	0.9692	0.9531	0.9798
Apomictic		1595	0.924	0.00663	0.9424	0.9191	0.9594
Sexual	B53	127	0.9449	0.0203	0.9572	0.8954	0.9832
	B42	257	0.9728	0.0102	0.9805	0.9520	0.9922
	B46	247	0.9676	0.0113	0.9762	0.9447	0.9900
	B49	188	0.9255	0.0192	0.9394	0.8801	0.9704
	L11	213	0.9577	0.0138	0.9690	0.9292	0.9868
	L12	265	0.9585	0.0123	0.9702	0.9358	0.9865
Apomictic	L06	265	0.8642	0.0211	0.8854	0.8092	0.9337
	L16	248	0.9556	0.0131	0.9654	0.9266	0.9841
	L17	248	0.9234	0.0169	0.9418	0.8906	0.9698
	C86	276	0.9420	0.0141	0.9508	0.9055	0.9750
	C85	280	0.9429	0.0139	0.9541	0.9108	0.9769
	C27	278	0.9173	0.0166	0.9344	0.8822	0.9645

Table 4.9: Seedling leaf number - Summary statistics by mating system and population.

Mating system	Population	n (seedlings)	Raw mean	SE	Predicted means	Lower CI	Upper CI
Sexual		1142	6.7723	0.0846	6.7040	6.3836	7.0405
Apomictic		1388	6.9561	0.0799	6.9240	6.6050	7.2578
Sexual	B53	104	6.3462	0.2478	6.3226	5.7460	6.9570
	B42	231	6.7576	0.2033	6.7345	6.2968	7.2026
	B46	227	6.3612	0.1609	6.3389	5.9186	6.7890
	B49	151	6.6755	0.2199	6.6346	6.1399	7.1692
	L11	189	6.8571	0.2144	6.8327	6.3620	7.3382
	L12	240	7.3542	0.1991	7.3254	6.8592	7.8234
Apomictic	L06	208	6.9327	0.2032	6.9293	6.4665	7.4253
	L16	222	6.4595	0.1820	6.4292	6.0027	6.8859
	L17	216	7.7037	0.2198	7.6707	7.1811	8.1936
	C86	253	6.7668	0.1968	6.7418	6.3130	7.1997
	C85	242	6.4504	0.1577	6.4432	6.0248	6.8906
	C27	247	7.4575	0.1997	7.4233	6.9624	7.9148

Table 4.10: Seedling leaf length - Summary statistics by mating system and population.

Mating system	Population	n (seedlings)	Raw mean (mm)	SE	Predicted means (mm)	Lower CI	Upper CI
Sexual		1142	15.1226	0.2125	14.9640	13.0280	15.9000
Apomictic		1388	15.7421	0.1837	15.6882	14.7771	16.5993
Sexual	B53	104	14.7212	0.6650	14.6596	13.0986	16.2206
	B42	231	16.9740	0.5694	16.9179	15.8158	18.0201
	B46	227	16.2467	0.4629	16.1825	15.0752	17.2899
	B49	151	13.5364	0.5304	13.5261	12.2383	14.8139
	L11	189	13.7196	0.4844	13.7504	12.5638	14.9370
	L12	240	14.5542	0.4022	14.5292	13.4308	15.6277
Apomictic	L06	208	14.8077	0.4448	14.8202	13.6725	15.9678
	L16	222	14.9189	0.4344	14.8963	13.7830	16.0096
	L17	216	15.1250	0.4504	15.1072	13.9823	16.2320
	C86	253	15.8379	0.4708	15.7929	14.7199	16.8660
	C85	242	16.5703	0.4223	16.5830	15.4975	17.6685
	C27	247	16.8988	0.4508	16.8808	15.8058	17.9559

Chapter 5

Conclusion

A multitude of factors interact to contribute to patterns of geographical parthenogenesis. These factors take a different emphasis depending on both the biological context (i.e., system-specific differences between sexuals and asexuals) *and* the theoretical context (i.e., proposed explanations and their specific ecological/evolutionary lens) being explored. In my thesis, I have explored GP in *Townsendia hookeri* with a diversity of approaches in order to reflect the diversity of models that have been advanced to explain the pattern. Each chapter views GP in the system through a different lens, examining population genetic diversity (Chapter 2), ecological differentiation (Chapter 3), and life history traits (Chapter 4). I believe that this is an effective approach to take in *any* system with *any* question, but it is particularly important for complex biological phenomena such as GP.

One of the primary goals of this thesis was to assess what I consider the "default" explanation for patterns of GP: that asexuals have larger ranges than sexuals due to the colonization advantages imparted by reproductive assurance ("Baker's Law effects"). In Chapter 3, we found that habitat suitable for sexuals exists within the apomictic range, which provides considerable evidence that the sexual range is limited

by dispersal. This is underscored by the results of Chapter 4, which indicate that apomicts have early life history traits that likely give them a colonization advantage in comparison to sexuals. The biology of *T. hookeri* also points to the likelihood of Baker's Law effects being a driver for GP. Sexuals and apomicts lie on polar opposite ends of the mating system spectrum, with sexuals being self-incompatible (thus dependent on mates and pollinator services) and apomicts being autonomous (thus having no dependence on mates or even pollen). Given these attributes, it would be surprising if divergent colonization potential did *not* play a role in GP in *T. hookeri*. While most apomicts are pseudogamous (requiring pollen to fertilize the endosperm and set seed) and autonomous apomixis is relatively rare (but common in Asteraceae), most pseudogamous apomicts are self-fertile and are therefore still capable of uniparental reproduction (Hörandl 2010). This, coupled with the fact that the sexual progenitors of most apomicts are self-incompatible (Asker & Jerling 1992), suggests that Baker's Law effects are likely to play at least some role in most plant GP systems due to the inherent dichotomy between their reproductive modes.

Baker's law effects are unlikely to be the sole cause of GP in *Townsendia hookeri*, however. Given enough time, sexuals should be able to expand into suitable habitat in the apomictic range. The fact that they have not suggests that there are barriers at their northern range limit preventing, or at least greatly slowing their expansion. Habitat at the sexuals' northern range edge appears to be unsuitable for sexuals (as evidenced by the generally poor performance in the SO_g region; Chapter 3), which could mean that sexuals are unable to push past this region into more favorable environments further north in the apomictic range. However, natural sexual populations in this region are some of the largest we have seen (personal observation), which goes against the interpretation that the habitat is unsuitable. Although it is possible that these populations are demographic sinks being maintained by high dispersal

from the sexual interior (RL>NL; Hargreaves *et al.* 2014), it seems unlikely that sink populations would be larger than sources if habitat is less suitable in sink sites.

A more likely scenario is that suitable sites just beyond the sexual edge (AO_g) that are occupied by apomicts can exclude sexuals through reproductive interference. Mixed sexual/apomictic populations are exceedingly rare (we have only detected two instances; Chapter 2), and apomicts appear capable of reducing sexual seed set (and even producing apomictic offspring) when acting as pollen donors for sexual ovules (Garani 2014). Apomicts exhibit reduced pollen viability in comparison to sexuals (Garani 2014), which can favor stable coexistence between sexuals and apomicts (Britton & Mogie 2001), but even modest asymmetrical reproductive interference can, over time, lead to sexual extirpation in mixed populations (Mogie 2011). Given this, the sexual invasion of sites where apomicts have precedence seems unlikely. As it stands, apomicts seem well-primed to push southward into the sexual range; apomictic edge populations performed best in the sexual edge region (Chapter 3), and one of the natural populations that we studied in this region, although first identified as sexual, appears to harbor apomicts at low frequencies (population L62; Chapter 2; unpublished flow cytometry data). If apomicts are able to establish in this region in sufficient numbers, they may displace sexuals over time, leading to sexual range contraction (Mogie 1992).

Assuming apomicts have considerable colonization and demographic advantages over sexuals, strong barriers must be in place that are preventing their southern expansion. The poor performance of apomictic populations in the sexual interior (S_g; Chapter 3) suggests that habitat is not suitable for apomicts there, but the cause of the gradient in habitat quality is not entirely obvious. The sexual interior garden sites had higher vegetation cover and historically warmer temperatures than the other sites, suggesting that interspecific competition (and potentially other biotic factors)

is more intense in this region. In addition, populations from the sexual interior had higher fitness in their home region than any of the other source regions, and tended to have longer leaves as well. These results fit quite well with the expectations of the Red Queen hypothesis, which predicts that sexuals will be able to adapt and persist in the face of biotic pressures (perhaps by growing longer leaves to compensate for increased light competition?), while asexuals will be excluded due to their inability to adapt (Glesener & Tilman 1978). Our experiment was not explicitly designed to test for biotic interactions, but our data suggest that Red Queen effects make for a promising candidate theory warranting further investigation.

Another interpretation is that apomicts are simply not well adapted to the environmental (abiotic) conditions in the southern portion of the range. This fits with the predictions from ecological niche models, which suggest that the climatic niche of apomicts does not extend into the southernmost (sexual interior) portion of the range (Lee 2015). Though there was some overlap in niche characteristics between sexuals and apomicts in *T. hookeri*, Lee (2015) found that some important climatic variables differed between regions occupied by the two types. While precipitation in the wettest quarter was important for both sexuals and apomicts, niche models put greater emphasis on temperature variables for sexuals and precipitation variables for apomicts. These results hint at potential environmental characteristics that are important in limiting the apomicts southern range, but additional experiments will be required to pinpoint whether apomicts are affected by abiotic factors *per se* or instead by ecological factors (including biotic factors) that correlate with climate.

It is important to tie ecological and life history differences to patterns of clonal diversity, because attributes benefitting range expansion are expected to be conserved within apomictic lineages (Lynch 1984). The results from Chapter 2 reveal largely mono-clonal populations, including two widespread clonal lineages - one of which

encompasses much of the apomictic interior range, and another that occurs at the apomicts' southern range edge. In Chapter 4, we present evidence that apomictic seeds have improved germination success and seed dispersal architecture in comparison to sexuals, and several of the populations investigated were found to be members of the two widespread clonal lineages characterized in Chapter 2. Assuming that these early life history traits benefitting colonization were conserved within apomictic lineages, they likely contributed to the geographic expansion of apomictic populations.

The pattern of a single widespread clone occupying much of the range is in line with the general-purpose genotype hypothesis, which predicts that varying abiotic conditions will select for the rare clonal lineage that is resilient to environmental unpredictability (Vrijenhoek & Parker 2009). Improved colonization ability (along with reproductive assurance conferred by apomixis) is likely to be a boon to apomicts subjected to intense abiotic pressures, as they will be better able to re-establish populations and bounce back from low densities after the frequent disturbances that are assumed to occur in "marginal" environments (Baker 1965). It is important to note that while several of the proposed hypotheses for GP suggest that asexuals will dominate in "marginal" environments, Tilquin & Kokko (2016) point out that "marginality" is a vague concept, and is defined somewhat differently across the GP literature. That being said, our anecdotal experience with these plants in the field (particularly in the apomictic range) certainly agrees with several of the defined features of marginal habitats, including low amount and diversity of resources, low population productivity/density, and high habitat openness and vacancy.

The pattern of clonal diversity is somewhat different in the southern portion of the range (around the southern apomictic edge), where we found a greater diversity of apomictic lineages (Chapter 2). These results are partially in line with the expectations of the frozen niche variation hypothesis, which predicts that competitive

interactions will select for an array of specialized clonal lineages that partition the resource space in order to avoid niche overlap with sexual progenitors and other clones (Vrijenhoek & Parker 2009). If this is the case, this may help explain why we find primarily monotypic sexual and apomictic populations in the overlap range despite being in relatively close proximity, though this would depend on the scale of environmental variation in this region. In addition, given that apomicts originate from sexuals (Whitton *et al.* 2008), it is not surprising that we find increased apomictic diversity closer to the sexual range.

Discussions of the diversity of clones and frequency of origins brings to mind the long-term dynamics and trajectories of sexuals and apomicts in *T. hookeri*. While the demographic benefits of asexuality may be advantageous in the short-term, the accumulation of deleterious mutations (Muller 1964) and inability to adapt to a changing environment are predicted to lead to the demise of asexual lineages over the long-term. In fact, there are no known "ancient" apomictic taxa, and all apomicts appear to have closely-related sexual progenitors (van Dijk 2009). This highlights that apomicts are likely dependent on their sexual progenitors for much-needed injections of genetic diversity, whether it be from *de novo* origins of new clonal lineages directly from sexuals, or via occasional hybridization between sexuals and apomicts.

Thompson & Whitton (2006) found that apomixis likely originated multiple times in *T. hookeri*, and that apomicts most likely spread from glacial refugia (in Colorado and the Yukon territory) into their current distribution post-glaciation. Relationships between sexual populations and apomictic lineages (Figure 2.4, Chapter 2) are largely consistent with this interpretation. Given the challenges to long-term persistence of apomictic lineages, we might predict cyclical extinctions of individual clones and their periodic replacement by clones originating from sexual parts of the range. The widespread clone occupying large swaths of the apomictic range has proven to

be quite successful so far, but it would likely be vulnerable to major environmental shifts or the advent of a new predator/pathogen. If this clonal genotype were to be extirpated, it would leave a large portion of the range unoccupied, which could take a long time to be recolonized considering there are no nearby sexual populations to supply a replacement general-purpose genotype. It is somewhat ironic that apomicts are dependent on the existence of sexuals over the long run, but at the same time may be contributing to sexual range contraction over time (Mogie 1992) via reproductive interference and "contagious" apomixis (Garani 2014). Whatever is limiting the apomicts' southern expansion (and potential decimation of sexual populations) may turn out to be the salvation of the system in the long run.

It seems clear that no single proposed model for GP is sufficient to capture the range of possibilities across the diverse array of taxa in which the pattern occurs. Within plants alone, apomixis can take various forms and exists within strikingly different evolutionary contexts. GP in *Townsendia hookeri* is set against a backdrop of autonomous apomixis vs obligate outcrossing, autopolyploidy, rare co-occurrence, and putatively infrequent origins from sexual populations. We can use this information (along with carefully designed experiments) to make predictions about the causes of range divergence between sexuals and apomicts. In another system that is characterized by (for example) pseudogamous apomixis, allopolyploidy, mixed populations, and frequent origins through hybridization, we should expect very different dynamics and mechanisms underlying GP. This is *not* to say that it is futile to attempt broad generalizations for complex eco-evolutionary phenomena, but rather that predictions should be made using a comprehensive compendium of theory and biological context, interspersed with plenty of caveats. Perhaps we would benefit from framing our hypotheses within a nested set of "if-then" statements, much like a dichotomous key but for concepts instead of species. Trying to speak the cryptic language of geographical

parthenogenesis can be maddening at times, but I prefer this reality to the alternative. If biological phenomena were easy to explain, there would far less intrigue, and far fewer labyrinthine systems to devote entire theses to exploring...and where would be the fun in that?

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