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ECOLOGICAL AND EVOLUTIONARY FACTORS AFFECTING POPULATION
PERSISTENCE OF A GLOBALLY THREATENED WETLAND PLANT,
POLEMONIUM VANBRUNTIAE (POLEMONIACEAE)

A Dissertation Presented

by

Laura Hill Bermingham

to

The Faculty of the Graduate College

of

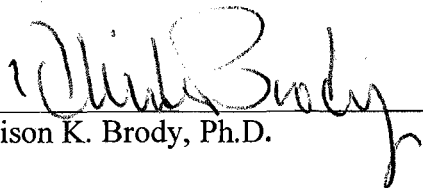
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for the Degree of Doctor of Philosophy
Specializing in Biology

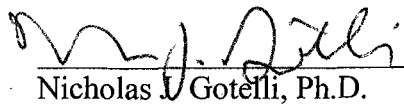
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Accepted by the Faculty of the Graduate College, The University of Vermont, in partial fulfillment of the requirements for the degree of Doctor of Philosophy, specializing in Biology.

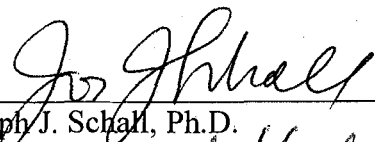
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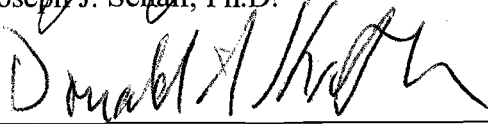
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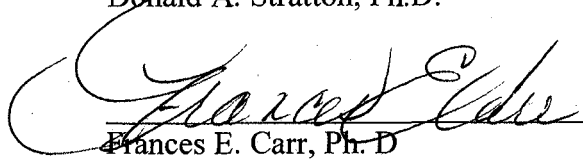
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ABSTRACT

Ecological and evolutionary factors affecting threatened and endangered species may compromise a population's ability to persist through time. Here, I determined how plant mating system, pollination biology, pollen source, habitat type, and white-tailed deer (*Odocoileus virginianus*) herbivory affected the persistence of a rare wetland endemic plant, *Polemonium vanbruntiae* (Eastern Jacob's ladder).

In contrast to several of the more common species of *Polemonium*, I found no pollen limitation in populations of *P. vanbruntiae*. The lack of pollen limitation was best explained by the capacity for *P. vanbruntiae* to self-fertilize. However, pollinators play an important role as inter- and intra-plant pollen vectors in this system because female reproductive fitness was greatly reduced when pollinators were excluded. These results support the reproductive assurance hypothesis, whereby the ability to self assures fertilization for plants in small populations. A mixed-mating strategy, including the ability for clonal reproduction, may explain the ability for this rare species to persist in small, fragmented populations. However, mixed mating strategies may incur both costs and benefits. The ability to self-fertilize may provide reproductive assurance that when pollinators and/or potential mates are scarce. Yet, selfing is potentially costly when the result is a reduction in offspring quantity and quality. I found that the relative performance of selfed offspring was lower than outcross offspring in terms of germination and offspring vigor. When pollen is received from a distant site, offspring exhibit heterosis with increased vigor in terms of more leaves, larger leaf area and height. Although I was unable to follow offspring survival to flowering, enhanced germination success and more vigorous growth suggest that gene flow among populations may increase plant vigor and enhance genetic variation within small, isolated populations of *P. vanbruntiae*.

For long-lived organisms such as perennial plants, it is often difficult to determine which environmental factors will have the largest effects on long-term population dynamics. I incorporated the effects of habitat type and white-tailed deer herbivory into a population viability model for *P. vanbruntiae* to determine the effects of both habitat and herbivory on long-term ramet dynamics. *Polemonium vanbruntiae* ramets in wet meadow habitats are expected to increase at a faster rate than ramets in the forest seep habitats, but *P. vanbruntiae* is expected to increase over time in both habitat conditions. White-tailed deer preferentially browsed adult ramets, primarily those occurring in forest seep sites. Deer browsing significantly decreased the predicted population growth rate under stochastic conditions, although *P. vanbruntiae* ramets are expected to increase at a slow rate in the future under herbivore pressure. Herbivory also increased the extinction risk to a detectable level. Deer browsing shifted the potential for younger life histories stages to change future population growth. Instead, survival and stasis of large vegetative ramets became the vital rates having the largest potential impact on future population growth. Thus, active long-term management of deer populations appears necessary for the guaranteed persistence of populations of rare, endemic herbs such as *P. vanbruntiae*.

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CHAPTER 1: GENERAL INTRODUCTION

1.1 Introduction

The biodiversity of the planet is in sharp decline as species extinctions are occurring at an alarming rate (Brook et al. 2003, Koh et al. 2004). Specifically, endemic species that have restricted ranges, occupy a small number of sites, and have poor mechanisms of dispersal to new sites are especially vulnerable to extinction (Terborgh and Winter 1980, Hanski et al. 1982, Diamond 1984, Simberloff and Gotelli 1984, Pimm et al. 1988, Cowling and Bond 1991, Gaston 1994). An examination of the ecological and evolutionary factors that affect rare and endemic species persistence is paramount in understanding the mechanisms that drive species rarity and threaten extinction.

Rare species are frequently found in fragmented landscapes, where important ecological interactions, such as mutualistic relationships between plants and pollinators, are likely to be disrupted (McKey 1989, Rathcke and Jules 1993, Aizen and Feinsinger 1994). Larger populations of plant species attract more pollinators, resulting in higher visitation rates and therefore greater levels of pollination success (Sih and Baltus 1987, Ågren 1996). Conversely, small, isolated plant populations may experience reduced pollinator visitation and, therefore, pollen limitation may limit reproductive success (Lloyd 1980, Byers 1998). As a result, reduced pollinator visitation to rare plant populations may affect plant species abundance and compromise population viability (Lloyd 1992, Lennartsson 2002, Ashman et al. 2004).

Pollen limitation can be alleviated by employing a mixed mating strategy, which includes the potential for plants to outcross when pollen is abundant and self-fertilize when outcross pollen is unavailable (Goodwillie et al. 2005). When pollinators and/or potential mates are scarce, the ability to self-fertilize may provide reproductive assurance that ensures seed production (Jennersten, 1988, Lamont et al. 1993, Ågren 1996, Fischer and Matthies 1998). Yet, selfing is potentially costly when the result is a reduction in offspring quantity and quality (Buza et al. 2000, Kéry et al. 2000, Goodwillie et al. 2005). Inbred offspring may suffer from reduced vigor, growth, and fitness when compared to outcrossed offspring (Jennersten 1988, Lamont et al. 1993, Ågren 1996, Fischer and Matthies 1998), which may increase risk of extinction for rare plants (Barrett and Kohn 1991, Ellstrand and Elam 1993, Frankham 2005). Whether differences exist between the relative performance of outcrossed and selfed offspring has been an important question since the days of Darwin (1876), and is especially important for rare plant populations and our understanding of mixed mating system evolution and rare plant population dynamics.

Population viability analysis provides a powerful tool in projecting population dynamics of rare species. Population viability analysis allows the estimation of population growth rate, assessment of extinction risk, and the identification of the life history stages that most significantly affect future population growth (Morris and Doak 2002). For long-lived organisms such as perennial plants, population viability analyses provide a means to determine which environmental factors will have the largest effects on population dynamics (Knight 2004).

Population viability analyses are especially informative when they are conducted in different habitat types, because the results can guide future conservation management activities (Oostermeijer et al. 1996). For example, many rare plants require canopy-opening events such as the creation of tree-gaps to persist through time (Eisto et al. 2000, Lennartsson and Oostermeijer 2001). Woody vegetation inhibits sunlight from reaching the herbaceous layer, thus leading to a decrease in plant fitness, offspring recruitment, and survival (Gross 1980, de Jong and Klinkhamer 1988). Most herbaceous plants experience increased growth and reproduction in response to increased light levels (Collins and Pickett 1988, Neufeld and Young 2003). However, increased light levels may be partially offset by the negative effects of interspecific competition due to an increase in species diversity in open-canopy habitats (Hughes 1992). If rare plants occur in different habitat types, it is informative to understand how habitat affects long-term population growth.

The type of habitat where a rare plant population exists can also influence species interactions. For example, white-tailed deer (*Odocoileus virginianus*) are particularly notorious herbivores in forested habitats in part due to their increasing abundance and potential to modify natural vegetative communities (Côté et al. 2004). Many studies have shown that herbivores can have strong deleterious effects on plant growth, survival, and reproduction (Crawley 1988, Louda 1989, Gange 1990, Doak 1992, Marquis 1992, Strauss and Zangrel 2002). In addition, deer herbivory can have a measurable impact on long-term population dynamics, especially for small plant populations (Augustine and Frelich 1998, Rooney and Gross 2003, Knight 2004,

McGraw and Furedi 2005). Stochastic events, such as habitat disturbance and herbivore browsing, can significantly affect future rare plant population dynamics, and may increase the probability of extinction for threatened and endangered plant species. Therefore, it is important to empirically determine the effect of disturbance to inform management strategies to ensure future population persistence.

The objective of my dissertation was to examine the various ecological and evolutionary factors affecting population persistence of the globally threatened endemic plant, *Polemonium vanbruntiae* (Eastern Jacob's ladder, Polemoniceae). Specifically, I examined how pollinators, herbivores, and habitat type affected the populations of *P. vanbruntiae*.

1.2 Dissertation Overview

Objective 1: To characterize the mating system of *P. vanbruntiae*, examine the degree of pollen limitation, and determine the effect of geitonogamous (within-plant) and autonomous (within-flower) self-fertilization on female reproductive success (Chapter 2).

I tested whether lack of pollen receipt reduced female reproductive fitness by experimentally supplementing a subset of treatment plants with outcross pollen. I compared female reproductive fitness (i.e., percent fruit set, seeds/fruit, total seed production, and seed mass) between open-pollinated and supplemental outcross plants to test for pollen limitation. Experimental hand-pollinations were conducted in 4 natural

populations for 2 years. To describe the mating system of *P. vanbruntiae* and to test whether plants could use self-pollen to set viable fruits and seed, I employed 4 hand-pollination treatments: (1) geitonogamous selfing (within-plant self-pollination), (2) autonomous selfing (within-flower self-pollination), (3) fully outcrossed, and (4) open-pollinated controls. I compared the aforementioned estimates of female reproductive fitness among all 4 pollination treatments using statistical analyses.

In contrast to several of the more common species of *Polemonium*, I found no pollen limitation in any of the four populations of *P. vanbruntiae* in either year. The lack of pollen limitation was best explained by the capacity for *P. vanbruntiae* to both geitonogamously and autonomously self-fertilize, unlike some of its more common congeners. Geitonogamously selfed flowers set equivalent numbers of seeds when compared to purely outcrossed and open-pollinated flowers. However, autonomously selfed flowers produced significantly fewer seeds, demonstrating that pollinators play an important role as inter- and intra-plant pollen vectors in this system. These results support the reproductive assurance hypothesis, whereby the ability to self assures fertilization for plants in small populations. However, I still did not know how pollen source affected the quality of selfed and outcrossed offspring.

Objective 2: To determine the effect of pollen source on the quantity and quality of offspring and to examine whether outcrossed offspring exhibit heterosis for early life-history traits (Chapter 3).

It is relevant to our understanding of the evolution of mixed mating systems and rare plant population dynamics to determine whether differences exist between outcrossed and selfed offspring. To test how self-fertilization affected offspring quality, I germinated seeds produced from plants receiving geitonogamous-self pollen, autonomous-self-pollen, intrasite outcross pollen (i.e., pollen delivered from plants within the same site), intersite outcross pollen (i.e., pollen delivered from plants from a distant site), and open-pollinated controls. I measured probability of germination, number of true leaves, leaf area (mm^2), and seedling height (cm) for selfed and outcrossed offspring. Additionally, I calculated the “relative performance of crosstypes” (RP) of outcrossed and selfed offspring (Ågren and Schemske 1993), and compared the mean performance of intrasite and intersite progeny using a calculation for heterosis.

Germination and cumulative quality (i.e., seed mass \times probability of germination) of selfed seeds was significantly lower than outcrossed seeds. However, the number of seeds per fruit and seed mass was significantly lower for intersite-outcross seeds compared to intrasite-outcross progeny. Yet, intersite-outcross progeny had a significantly higher RP index for germination, indicating heterosis for this early offspring trait. Outcross offspring had more leaves, larger leaf area and were taller than selfed offspring. Although I was unable to follow offspring survival to flowering, enhanced

germination success and more vigorous growth suggest that increasing gene flow among populations may increase plant vigor and genetic variation within small populations of *P. vanbruntiae*.

These results suggest that pollinators play an important role in maintaining female reproductive fitness and offspring quality by moving pollen among *P. vanbruntiae* plants. However, it remained unknown whether populations of *P. vanbruntiae* were expected to persist, or if risk of extinction was high. Additionally, it was imperative to determine how environmental stochasticity affected long-term population dynamics.

Objective 3: To describe the life cycle and life history transition probabilities of *P. vanbruntiae* ramets, (2) to utilize matrix population modeling to predict future population growth, (3) to determine how habitat type or white-tailed deer herbivory affect long-term population dynamics, (4) to project probability of ramet extinction under each habitat and deer browsing scenario, and (5) to evaluate the importance of each life history stage transition using sensitivity and elasticity analysis (Chapter 4).

To determine population viability under various scenarios, I followed a cohort of over 2000 *P. vanbruntiae* ramets for 3 annual transitions (2004-2007) at 9 sites to estimate survival, growth, and fitness. *Polemonium vanbruntiae* has the ability to reproduce both sexually and vegetatively. Because *P. vanbruntiae* is a clonal plant, it is important to define the ramet and genet. A genet is the inclusive genetic individual originating from one zygote, and the ramet is the functional, yet potentially independent,

part of the genet (Eriksson 1994). In non-clonal species, the ramet and genet are equivalent.

I described the life cycle of *P. vanbruntiae* and designated ramets into 6 classes, based on plant stage and size. The life cycle graph characterized the life history transitions for *P. vanbruntiae* informed construction of the transition matrix models. I drew comparisons between the 2 habitat types (i.e., wet meadow and forest seep) and examined whether habitat type translated into differences in future population growth. Additionally, a subset of ramets were browsed by white-tailed deer (*Odocoileus virginianus*) during the demographic study, which allowed me to examine the effect of herbivory on long-term population dynamics by comparing the demography of plants that were browsed and not browsed.

An important caveat to this demographic analysis is that the matrix population models constructed here examined the life history of *P. vanbruntiae* ramets, rather than genets, and the life cycle graph focuses on sexual reproduction. Few studies have addressed the demography of genets due to difficulties of deciphering a true genet in the field, but many have been performed on ramets (Harper 1977, Hartnett and Bazzaz 1985, de Steven 1989, Falinska 1995, Damman and Cain 1998, Fair et al. 1999, Jongejans and de Kroon 2005). Additionally, matrix population modeling of ramets provides a valid estimate of population growth rate (Caswell 1985), and, thus, ramet-based analyses will ultimately inform studies of clonal plants.

The population vital rates (i.e., survival, growth, and fitness) were used to parameterize 4 types of matrix population models: (1) forest seep habitat, (2) wet

meadow habitat, (3) “browse” (i.e, ramets that experienced white-tailed deer herbivory), and (4) “no browse.” These matrices were iterated to solve for the deterministic population growth rate (λ) with both the raw data and with corrected mean and variance estimates, which removed the effect of sampling variation. I used matrix model simulations to solve for the stochastic growth rate (λ_S), which provides a more realistic estimate of future population growth in a variable environment (Caswell 2001). I also used model simulations to solve for the cumulative probability of extinction under each habitat and browsing scenario. Finally, I examined the sensitivity and elasticity of each life history transition, which identifies the vital rates and life history transitions that contribute most to future population growth rate.

Under all scenarios, *P. vanbruntiae* ramets are expected to increase over time. Ramets in the wet meadow habitat are expected to increase at a significantly faster rate when compared to forest seep sites, although habitat type is not expected to largely influence future population growth. White-tailed deer preferentially browsed vegetative and reproductive adult ramets over younger life history stages. Only ramets in the forest sites experienced white tailed deer herbivory. Herbivory significantly increased the probability of small vegetative ramet survival, and also increased the probability that small and large vegetative ramets will remain vegetative the following year. Herbivory also significantly reduced reproductive adult fertility due to the direct consumption of inflorescences. Differences in vital rates between the “browse” and “no browse” ramets resulted in a significant decrease in the predicted population growth rate under of browsed ramets, although *P. vanbruntiae* ramets are expected to increase at a slow rate in

the future under herbivore pressure. Herbivory also increased the extinction risk to a detectable level, although the probability of ramet extinction is still quite low. Deer browsing shifted the potential for younger life histories stages, such as seedlings, to change future population growth. Instead, survival and stasis of large vegetative ramets became the vital rates having the largest potential impact on future population growth for ramets that were browsed. Overall, the most important factor affecting long-term population dynamics of *P. vanbruntiae* is white-tailed deer herbivory.

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CHAPTER 2: MATING STRATEGIES AND POLLEN LIMITATION IN A GLOBALLY THREATENED PERENNIAL *POLEMONIUM VANBRUNTIAE*

2.1 Abstract

Knowledge of the factors that limit reproduction is critical to an understanding of plant ecology, and is particularly important to predicting population viability for threatened species. Here, we investigated the pollination biology of a globally threatened plant, *Polemonium vanbruntiae*, using hand-pollination experiments in four natural populations to determine the degree of pollen limitation. In addition, we investigated the mating system and extent to which plants can self-fertilize by comparing geitonogamously and autonomously self-fertilized plants with purely outcrossed and open-pollinated plants. In contrast to several of the more common species of *Polemonium*, we found no pollen limitation in any of the four populations of *P. vanbruntiae* over two years. The lack of pollen limitation was best explained by the capacity for *P. vanbruntiae* to both geitonogamously and autonomously self-fertilize, unlike some of its more common congeners. Geitonogamously selfed flowers set equivalent numbers of seeds when compared to purely outcrossed and open-pollinated flowers. However, autonomously selfed flowers produced significantly fewer seeds, demonstrating that pollinators play an important role as inter- and intra-plant pollen vectors in this system. Our results support the reproductive assurance hypothesis, whereby the ability to self assures fertilization for plants in small populations. Self-compatibility in *Polemonium vanbruntiae* may decrease extinction risk of isolated populations experiencing a stochastic pollinator pool within a

restricted geographic range. In addition, a mixed-mating strategy, including the ability for clonal reproduction, may explain the ability for this rare species to persist in small, fragmented populations.

2.2 Introduction

Plants that exist in small populations and rely on animals to vector pollen are at an inherent disadvantage, because pollinators often bypass small, relatively unrewarding plant populations for larger and more resource-rich populations (Charnov, 1982; Sih and Baltus, 1987; Waites and Ågren, 2004). Thus, these plants are likely to be pollen limited. Rare plants may be especially prone to pollen limitation as they often occur in fragmented landscapes where pollination is disrupted (McKey, 1989; Rathcke and Jules, 1993; Aizen and Feinsinger, 1994), and pollen quality or quantity may be insufficient for maximal seed set (Ashman *et al.*, 2004). When plants persist in small, fragmented habitats, and conspecifics are patchy throughout the landscape, pollen is often wasted due to failure to reach conspecific stigmas (Koenig and Ashley, 2003). The result is higher amounts of heterospecific pollen receipt and a subsequent reduction in quality of the pollen pool (Rathcke, 1983). In addition, animal-pollinated, self-incompatible species existing in a rare state are likely to be especially susceptible to pollen limitation when pollinators are in short supply (Larson and Barrett, 2000). These factors may greatly limit the success of many rare plant species.

Over the long term, the potentially negative fitness effects of sessile organisms persisting in a small population may be partially ameliorated through the ability to self-

fertilize. Selfing taxa often occupy habitats with low pollinator services, such as those occurring in a restricted geographic range (Lloyd, 1980). Natural selection may favor self-compatible individuals in the face of low pollinator service, even at a cost of reduced heterozygosity and/or lower offspring quality or quantity, if it provides the plant with reproductive assurance (“reproductive assurance hypothesis” sensu Baker, 1955; Stebbins, 1957; also see Charnov, 1982). However, populations with increased rates of self-fertilization may be prone to inbreeding depression, which may result in lower seed set and fitness when compared to populations receiving large amounts of outcross pollen (Jennersten, 1988a; Lamont *et al.*, 1993; Ågren, 1996; Fischer and Matthies, 1998). Inbreeding depression may, in turn, have potentially serious negative consequences for population persistence (Oostermeijer, 2000) as genetic diversity and fitness is reduced over time (Ellstrand and Elam, 1993). Despite the risks selfing imposes through a reduction in genetic variation and offspring vigor, it can provide a means for persistence when pollinators are scarce.

Here, we examined the degree of pollen limitation and compatibility system for a rare herbaceous perennial, *Polemonium vanbruntiae* Britton. Although the genus is well-studied, the pollination biology, reproductive strategies and mating system of *Polemonium vanbruntiae* were unknown. Because plant mating systems affect population genetic diversity (Hamrick and Godt, 1989), and potentially population persistence, an initial step in the development of an effective management plan for rare plants should be an understanding of the taxon’s reproductive biology (Hamrick *et al.*, 1991). Indeed, the

lack of data on rare species biology is repeatedly cited as a shortcoming of threatened and endangered species' recovery plans (Schemske *et al.*, 1994; Clark *et al.*, 2002).

The genus *Polemonium* contains about 35 taxa (Porter and Johnson, 2000) characterized by a diverse assemblage of floral structures, mating systems, and pollinators (Grant and Grant, 1965). Self-incompatibility and self-compatibility both exist within the genus *Polemonium*. It is posited that *P. pulcherrimum* may have partial self-compatibility (Grant and Grant, 1965), but *P. viscosum* and *P. foliosissimum* are entirely self-incompatible (Galen, 1985; Zimmerman, 1980). *Polemonium vanbruntiae* is in the same clade as the European *P. caeruleum* (A.C. Worley, *pers. communication*), and *P. caeruleum*'s mating system has been characterized as a facultatively xenogamous and autogamous (Ostenfeld, 1923; Plitmann, 1994). *Polemonium vanbruntiae* was reported self-incompatible (Popp, 1990), but without an experimental test of this assumption.

Overall, we were interested in elucidating the mating system of *P. vanbruntiae* and determining whether insufficient levels of pollen receipt may be one of several factors contributing to rarity. Given the small, fragmented nature of *P. vanbruntiae* populations, we hypothesized that populations would be pollen-limited and increased levels of outcrossing would result in an increase in female reproductive success. In particular, we sought to (1) characterize the mating system of *P. vanbruntiae* (2) examine the degree of pollen limitation both spatially and temporally, and (3) determine the effect of geitonogamous and autonomous self-fertilization on female reproductive success when compared to purely outcrossed and open-pollinated plants. Our study provides critical data on the reproductive biology and possible factors limiting the persistence of *P.*

vanbruntiae, which can inform further studies on the life history and population dynamics of this globally threatened species.

2.3 Materials and Methods

2.3.1 Study system

Polemonium vanbruntiae is listed as a globally threatened perennial plant species which is extirpated from New Brunswick (NatureServe, 2005), but small populations persist in southern Quebec, eastern Maine, Vermont, and New York, south to Maryland and West Virginia (Fernald, 1950; Gleason and Cronquist, 1991; Crow and Helquist, 2000).

Polemonium vanbruntiae occurs in wetland habitats, including shrub swamps, marshes, wooded floodplains, forested swamps, and moist roadsides, at elevations generally above 330m in the northeastern U.S. The species has a global conservation rank of G3 (“vulnerable to extirpation or extinction;” NatureServe, 2005), and fewer than 100 populations remain worldwide.

Polemonium vanbruntiae flowers are protandrous and herkogamous, which may signify a reliance on pollinators to transfer gametes. The stamens become functionally mature prior to maturation of the pistil and, as the corolla opens, the unreceptive stigma is exerted beyond the stamens, surpassing the corolla by 5 – 7 mm (Gleason and Cronquist, 1991; **Figure 1**). Upon fertilization, flowers mature into fruits comprised of a capsule with three locules containing 1-10 seeds per locule (Thompson 1991). The capsule is surrounded by the calyx, which becomes papery as it matures. Seeds require a period of winter dormancy in order to germinate successfully (Brumback, 1989). Seeds are

passively dispersed around the parent plant and may be dispersed long distances by winter winds and spring floodwaters (Sabourin, 2002). *Polemonium vanbruntiae* can also reproduce vegetatively via rhizomes and may be capable of forming large, interconnected clones, although the degree of clonal diversity in natural populations has yet to be assessed (Deller, 2002).

2.3.2 Study sites

In 2004, we conducted pollen limitation studies in 3 Vermont populations: Blue Banks (BB), Abbey Pond (AP), and Forest Road 233 (FR). In 2005, we conducted pollen limitation and self-compatibility experiments in a single, large Vermont population in Camel's Hump State Forest (CHSF). In 2006, self-compatibility experiments were conducted Lordsland Preserve (LP) in Otsego County, New York. All Vermont populations occur in the northern Green Mountain National Forest in Addison County, Vermont. The habitats are characterized as minerotrophic wetlands which receive both groundwater and overland water inputs (Bridgham *et al.*, 2001).

In 2004, we counted all reproductive adult plants within 1m² quadrats at five locations within Vermont populations CHSF, BB, and AP and estimated population size. We measured total population area (m²) with a Sonin® Electronic Distance Measurer. The FR population is small, and *P. vanbruntiae* is patchily distributed in the FR site. Populations CHSF, AP, and BB are comparatively large and plants are more evenly spatially distributed. For the FR population, we traversed the entire population along 2 transects and counted every reproductive adult. For sites CHSF, AP, and BB, we

estimated total effective population size by calculating the number of reproductive adults per m² quadrat averaged over the 5 quadrats per site, and multiplying the mean by the total population area (m²).

2.3.3 Pollination experiments

We tested whether seed production of *Polemonium vanbruntiae* was pollen limited during the summers of 2004-2005. In 2004, we selected a total of 110 flowering plants evenly divided among three populations (BB, AP, and FR) and randomly divided the plants into either a supplemental outcross pollen or open-pollinated control treatment. Pairs of plants were matched for height and number of flower buds and then randomly assigned to one of the two treatment groups. We measured the height of each flowering stalk to the nearest cm. Flowers of supplemental treatment plants received outcross pollen collected from a single donor plant 1-5m away from the recipient plant. However, because pollen collected close to a recipient plant could potentially come from the same genet, in 2005 we collected a mixture of outcross pollen from a group of at least 10 donor plants that were 10m or further from the recipient plant. Mixing pollen should have also more closely mimicked natural pollination while increasing the diversity of pollen delivered (Ashman *et al.*, 2004). For clarity, from here on we will refer to the supplemental pollen treatment in both 2004 and 2005 as the “supplemental outcross” treatment. In 2005, the pollen limitation treatments were repeated on 30 plants in a single, large population (CHSF). In both years, we collected dehiscing anthers in a small glass vial and delivered pollen to the receptive stigmas of the treatment flowers using a fine-

tipped paintbrush. Flowers of control plants were manipulated with a clean paintbrush in a manner analogous to the supplemental outcross treatment plants, but no pollen was transferred and the flowers were left to be open-pollinated. We performed pollination treatments twice per week on all open female-phase flowers throughout the blooming period. This ensured that the majority of flowers received a pollen treatment as *P. vanbruntiae* flowers remain receptive for an average of 3-5 days. After applying pollen, we marked the calyx with indelible ink and later collected those fruits to score female reproductive success.

We collected fruits from all treatment plants as they matured in late summer 2004 and 2005. In 2004 at site FR, white-tailed deer (*Odocoileus virginianus*) browsed the flowering stalks of 14 of our experimental treatment plants, thus reducing our sample size to 9 control plants and 7 supplemental outcross plants for this population. We calculated the following for all available treatment plants: (1) percent fruit set (2) seed set per fruit, (3) total seed production per plant, and (4) seed mass (g). We kept 10% of the seeds in the lab for offspring germination experiments. The remaining seeds were returned to the original populations and dispersed as naturally as possible around the parent plant. To test the effect of supplemental pollen on offspring germination and growth, the seeds underwent a 40-day stratification period at 4°C and were germinated in the lab under fluorescent lights. We calculated percent germination, seedling survival at 60 days, and height of plant and number of leaves at 100 days as measures of offspring quality.

2.3.4 Self compatibility experiments

To determine if *P. vanbruntiae* is self-compatible, in 2005 we randomly assigned 15 plants at site CHSF to a self-pollen treatment group. We staked and bagged whole flowering stalks containing unopened flower buds with breathable, organdy fabric to exclude pollinators. When flowers were fully open, we applied self-pollen from a mixture of several flowers to all receptive stigmas within the same plant. We then marked the calyx with indelible ink and these fruits were later used to score fruit and seed set.

To more closely examine the degree of self-compatibility of *P. vanbruntiae*, and to examine the effect of selfing on female reproductive success, in 2006 we randomly assigned flowers of 53 plants at the LP population to one of four hand-pollination treatments: (1) *geitonogamous selfing* - bagged, emasculated, self-pollinated by hand with a mixture of within-plant self-pollen, (2) *autonomous selfing* - bagged, no emasculation, no hand-pollination, (3) *fully outcrossed* - bagged, emasculated, hand-pollinated with a mixture of purely outcross pollen collected from >10 donor plants at least 10m away from the recipient plant and (4) *open-pollinated controls*. As in previous years, we marked the calyx of each treatment flower with indelible ink and these fruits were later used to score seed set per fruit and seed mass (g).

2.3.5 Pollinator observations

Pollinator observations were conducted during peak flowering over 4-days in populations FR and BB in 2004. Population BB was our largest experimental site and FR was our

smallest. During each observation, we flagged 10 target plants, recorded number of open flowers, and observed all insect visitors during a 15-minute observation period. During the pollinator observations, we counted the number of pollinator visits, the number of flowers visited within a plant, and the “result” of visit (i.e., insect either visited another *P. vanbruntiae* flower, visited a heterospecific flower, or flew out of the population).

Individual insects were collected for field identification, identified to species in the field, and then released. Insects that could not be identified in the field were placed in a glass jar with 95% ethyl alcohol and brought to the lab for identification.

2.3.6 Statistical analysis

To examine the importance of pollen limitation to female reproductive success in 2004, we compared total seed set per plant, percent fruit set, seed set per fruit, and seed mass (g) using separate analysis of variance (ANOVA) models with our pollination treatments as a fixed effect, site as a random factor, and a treatment \times site interaction term. Before analysis, we examined normality of the data. Seed set and seed mass (g) data were normally distributed and thus were not transformed. Percent fruit set was arcsine square-root transformed, and seeds/fruit was natural log-transformed in 2004 and square root-transformed in 2005 to meet normality assumptions. All data were tested for homogeneity of variance with Levene’s test, and variances among treatment groups of all response variables were equal. We determined whether insect visitation rates and number of open flowers in 2004 differed significantly between populations BB and FR by using

one-way ANOVAs with total insect visits and number of open flowers (both square root-transformed) as our response variables.

We tested pollen limitation in years 2004 and 2005 separately as methods and sites differed between the two years. In 2005, we used separate ANOVAs to test the effect of supplemental pollen on the response variables: percent fruit set (arcsine square root-transformed), seeds/fruit (square root-transformed), total seed set per plant, and seed mass (natural log-transformed).

To analyze whether supplemental, outcross pollen affected offspring quality in 2004, we used MANOVA with hand-pollination treatment (supplemental outcross vs. open-pollinated) as our independent variable and germination rate (arcsine square-root transformed), seedling survival at 60d (natural log-transformed), plant height (cm) at 100d and number of leaves (100d) as our response variables.

To determine the relationship between seed mass and seed number and whether this relationship differed between hand-pollination treatments and among populations in 2004, we used ANCOVA with seed mass (mg) as the dependent variable, # seeds as the covariate, site and treatment as random and fixed effects, respectively, and # seeds \times site and # seeds \times treatment as our interaction terms.

For our 2006 hand-pollination experiments, we calculated the average seeds/fruit and seed mass for each pollination treatment within each plant as individual flowers within a plant are not statistically independent. We then used separate one-way ANOVAs to analyze mean seeds/fruit (natural log-transformed) and mean seed mass (mg) separately with hand-pollination treatment as the predictor variable. We then used

Tukey's HSD to test whether there were significant differences in mean fitness values among hand-pollination treatments. All statistical analyses were carried out using JMP version 4.0.2 (SAS Institute, 2000).

2.4 Results

Reproductive *P. vanbruntiae* adults have an erect flowering stem ranging in height from 0.4 - 1.0 m. The leaves are alternate, pinnately compound, and consist of 15 to 21 leaflets. Flowers are campanulate, arranged in a compact terminal raceme, and each inflorescence produces a range of 5-37 flowers per plant with an average of 15 flowers per plant. Flowering within inflorescences is basipetalous. The flowers are protandrous, and the anthers dehisce pollen for 1-2 days before the stigma becomes receptive.

2.4.1 Pollen limitation experiments

Supplemental outcross pollen did not significantly increase female reproductive success of *P. vanbruntiae* in any of the populations in any year (**Figure 2**). Open-pollinated control plants set an average (± 1 s.d.) of 169.87 ± 79.6 seeds and plants supplemented with outcross pollen set 164.06 ± 84.3 seeds ($F_{1,85} = 0.36$, $p = 0.55$). Open-pollinated control plants set an average of 10.8 ± 3.9 seeds/fruit and supplemental outcross plants set 10.1 ± 2.5 seeds/fruit ($F_{1,85} = 1.21$, $p = 0.27$). Percent fruit set was $88.9\% \pm 10.9\%$ for control plants and $89.1\% \pm 10.6\%$ for supplemental outcross plants ($F_{1,85} = 0.49$, $p = 0.49$). Seed mass averaged $0.874 \pm .13$ mg for control plants and $0.895 \pm .15$ mg for

supplemental outcross plants ($F_{1, 85} = 1.24, p = 0.27$). Site was significant for each measure of female reproductive success except for percent fruit set (seed set: $F_{2, 85} = 20.6, p < 0.0001$; percent fruit set: $F_{2, 85} = 1.1, p = 0.32$; seeds/fruit: $F_{2, 85} = 4.4, p = 0.01$; seed mass: $F_{2, 85} = 9.0, p = 0.0003$). There was no significant treatment \times site interaction in 2004. Again in 2005, we detected no significant differences in female reproductive success between open-pollinated controls and plants receiving supplemental outcross pollen (seed set: $F_{1, 27} = 0.01, p = 0.91$; percent fruit set: $F_{1, 27} = 0.26, p = 0.62$; seeds/fruit: $F_{1, 27} = 0.40, p = .54$; seed mass: $F_{1, 27} = 0.03, p = .86$).

Although we did not detect differences in female reproductive success between hand-pollination treatments, we did detect differences among sites AP, BB, FR, and CHSF. Experimental sites differed in the number of plants (**Table 1**), and those plants differed in total seed set ($F_{3, 125} = 8.48, p < 0.0001$), percent fruit set ($F_{3, 120} = 3.96, p = 0.01$), number of seeds/fruit ($F_{3, 121} = 5.59, p = 0.0013$), and seed mass ($F_{3, 125} = 3.93, p = 0.01$). The largest population (BB) produced the highest number of seeds per fruit and total seeds per plant, but at the expense of decreased mean seed mass (mg). Population FR had the lowest mean value of female reproductive success in terms of fruit and seed production, although plants at FR produced the heaviest seeds.

As plants produced more offspring, seed mass significantly decreased (**Figure 3**). Both hand-pollination treatment and site were non-significant, and there was no interaction between treatment and number of seeds. However, there was a significant seed set \times site interaction (**Table 2**).

We found no significant difference in offspring quality between open-pollinated controls and supplemental outcross plants ($F_{4,44} = 1.72, p = 0.16$). The germination rate (± 1 s.d.) of outcrossed seeds was $23.7 \pm .18\%$ compared to $23.8 \pm .23\%$ for open-pollinated seeds. The number of seedlings that survived to 60 days was 5.8 ± 6.8 for control seeds and 4.0 ± 4.1 for outcrossed seeds. Open-pollinated seedlings averaged 16.4 ± 3.1 cm height with 8.9 ± 1.4 leaves compared to 15.1 ± 2.9 cm height and 8.0 ± 1.1 leaves on outcross seedlings.

2.4.2 Self compatibility experiments

Geitonogamously self-fertilized plants at CHSF in 2005 produced an average (± 1 s.d.) of 86.79 ± 66.5 seeds per plant with an average seed mass of 0.83 ± 0.27 mg. Fifty-three-percent ($\pm 23\%$) of all selfed flowers in population CHSF set seed with an average seed production of 10.44 ± 0.66 seeds/fruit.

In 2006 when we further tested the ability for *P. vanbruntiae* to self-fertilize, both autonomously-selfed flowers and geitonogamously selfed flowers set viable fruits and seeds. However, selfing treatments differed significantly in amount of seeds/fruit at site LP ($F_{3,156} = 6.66, p = 0.0003$). Autonomously-selfed flowers set the lowest number of seeds/fruit and differed significantly from geitonogamously selfed flowers ($F_{1,42} = 12.70, p = 0.0009$), outcrossed flowers ($F_{1,45} = 5.03, p = 0.03$), and open-pollinated controls ($F_{1,64} = 5.36, p = 0.02$). Seed mass (g) did not differ significantly among selfing treatments ($F_{3,156} = .12, p = 0.95$). At site LP, autonomously-selfed flowers set the lowest average

number of seeds (± 1 s.d.) with 1.8 ± 3.3 seeds/fruit, geitonogamously-selfed flowers tended to have the highest number of seeds with 7.6 ± 6.2 seeds/fruit, and open-pollinated control flowers and flowers receiving purely outcross pollen set equivalent numbers of seeds with 5.8 ± 5.2 seeds/fruit and 5.9 ± 5.5 seeds/fruit, respectively (**Figure 4**).

2.4.3 Pollinator observations

Polemonium vanbruntiae attracted a variety of potential pollinators, including honeybees (*Apis mellifera*), bumblebees (*Bombus* spp.), halictid bees, andrenid bees, large carpenter bees (*Xylocopa virginica*), yellow jackets (*Vespula maculifrons*), Eastern tiger swallowtails (*Papilio glaucus*), Atlantis fritillaries (*Speyeria atlantis*), hoverflies (*Metasyrphus americanus*), and ruby-throated hummingbirds (*Archilochus colubris*). Bees were the most common insect visitors in populations BB and FR, comprising 66.7% and 54.8% of the insect visits in BB and FR, respectively. The number of flowers visited by bees on a plant was correlated with the number of open flowers ($F_{1,36} = 5.35$, $p = .03$). Syrphid fly visits were more common in FR (45.2% of total insect visits) when compared to syrphid fly visits at site BB (28.7%). Butterflies constituted only 4.6% of insect visitors in population BB and no butterflies were observed visiting flowers in FR. During a 15-minute observation, plants at site BB received an average (± 1 s.d) of 3.2 ± 2.9 insect visitors and plants at site FR received an average of 2.1 ± 1.1 insect visitors. However, we detected no significant differences in the number of flower visitors to BB and FR in 2004 ($F_{1,36} = 2.59$, $p = 0.11$). The statistical non-significance may be due to

low sample size for our insect visitation observations. Insects visited an average of 4.9 flowers (± 5.0) per plant before moving onto foraging on another flowering stalk.

Twenty-one percent of the insects left the population after visiting a single *P. vanbruntiae* flower, whereas 79% of the insects that remained in the population visited another *P. vanbruntiae* plant within the population.

2.5 Discussion

Contrary to expectation, we found no pollen limitation in female reproductive success or offspring quality in *Polemonium vanbruntiae*. We also determined that *P. vanbruntiae* is fully self-compatible, which is contrary to previous reports regarding this rare plant's mating system. Supplementing plants with outcross pollen did not enhance any measures of female reproductive success, nor did it improve offspring quality over open-pollinated controls. *Polemonium vanbruntiae*'s mixed mating system may help to explain how this species has been able to persist over time in small, fragmented populations along its narrow geographic range. The lack of pollen limitation for the populations and years we tested may be attributable to *P. vanbruntiae*'s ability to both self-fertilize and clonally reproduce.

The "reproductive assurance hypothesis" (sensu Baker, 1955; Stebbins, 1957) implies that if self-fertilization produces viable offspring, then self-incompatible and self-compatible species are expected to differ in the magnitude of pollen limitation. Over a broad range of selfing and outcrossing taxa, Burd (1994) detected a correlation between levels of pollen limitation and plant mating system. Typically, self-compatible species are

not pollen-limited, whereas self-incompatible species are commonly limited by pollen receipt (Burd, 1994; Goodwillie, 2001). Pollen limitation in self-compatible species can be half the pollen limitation observed in self-incompatible species (Larson and Barrett, 2000). The genus *Polemonium* includes both self-compatible and self-incompatible species. Although the ability to self-fertilize would provide reproductive assurance for the strongly pollen limited *P. foliosissimum* and *P. viscosum*, these species remain obligate outcrossers (Zimmerman, 1980; Zimmerman and Pyke, 1988; Galen and Newport, 1988). Thus, our finding a high degree of self-compatibility and lack of pollen limitation in *P. vanbruntiae* suggests that the ability to self may provide additional reproductive assurance for this rare plant persisting in small, isolated populations.

We detected significant differences in female reproductive success among populations, and the smallest experimental population (FR) had the lowest average flower production, which in turn resulted in fewer pollinator visits, and subsequent fruit and seed production. Even so, increased delivery of outcross pollen did not increase seed set in FR. An explanation is that even when a consistent amount of pollen is delivered to receptive *P. vanbruntiae* stigmas, variation in resource availability may dictate the potential for reproduction when ample pollen is delivered (Goodwillie, 2001). These results suggest that populations of *Polemonium vanbruntiae* may be resource- rather than pollen- limited. Pollen- and resource-limitation are not mutually exclusive (Haig and Westoby 1988; Zimmerman and Pyke 1988; Campbell and Halama, 1993), and resource limitation in this system remains to be experimentally tested. However, some evidence of resource limitation may be inferred by the apparent trade-off between seed number and seed size.

Reduced seed size may, in turn, affect the population dynamics of *P. vanbruntiae*. Plants producing more seeds at the expense of seed size may be at a competitive disadvantage (Venable, 1992). In other studies, seed size had little impact on offspring fitness at low sowing densities, but at high density seed size may be particularly important in determining offspring success (Gross, 1984; Stanton, 1984). *Polemonium vanbruntiae* seeds are passively dispersed (Sabourin and Paquette, 1994) and, at high densities around the maternal plants, intraspecific competition among seedlings may be important.

The capacity to produce viable offspring under environmental conditions that reduce the probability of outcrossing (i.e., small population size, low pollinator visitation) can be an important selective force favoring clonality and self-fertilization (Schoen *et al.*, 1996; Vallejo-Marin and O'Brien, 2007). If populations of *P. vanbruntiae* experienced chronic pollen limitation, there is likely to have been strong selection favoring mutations that confer the ability to self-fertilize or reproduce clonally (Lloyd, 1992; Lennartsson, 2002). Indeed, *P. vanbruntiae* can produce clonal ramets, and plants in close spatial proximity may be members of the same genet. Therefore, clonal growth may further enhance the ability to self-fertilize, as outcross pollen receipt is likely to be greatly reduced (Handel, 1985; Ritland, 1989; Back *et al.*, 1996).

Aside from providing assurance of offspring production, increased reliance on methods of self-fertilization may reduce genetic diversity. High rates of self-fertilization and clonal growth may increase inbreeding depression, increase genetic load, and perpetuate loss of genetic diversity through drift, resulting in genetically-differentiated populations each with low heterozygosity (Charnov, 1987; Barrett and Kohn, 1991).

However, theory predicts that selfing plant populations harboring relatively low levels of genetic variation will evolve towards self-compatibility, whereas species with high genetic loads evolve self-incompatibility systems to maintain high levels of outcrossing (the “genetic load-developmental selection hypothesis” sensu Wiens *et al.*, 1987; also see Klekowski, 1988; Morales and Galetto, 2003). Yet, inbreeding depression may facilitate the maintenance of self-incompatibility if levels of inbreeding depression are high or, alternatively, promote its breakdown if levels of inbreeding depression are low (Charlesworth and Charlesworth, 1979; Steinbachs and Holsinger, 1999). The amount of genetic variation and degree of inbreeding depression in *P. vanbruntiae* is currently unknown and remains to be experimentally tested.

Ultimately, whether selfing is favored or not depends on its costs and benefits. Geitonogamy clearly has a fitness advantage over autonomy in *P. vanbruntiae*, and clonality may explain why geitonogamously-selfed flowers set more seeds/fruit when compared to autonomously-selfed flowers. Perhaps the number of available outcross pollen grains may have been reduced because *P. vanbruntiae* has been repeatedly exposed to geitonogamy due to its clonal habit. Even if pollinators visit multiple flowering stalks within a population, these may be ramets of the same genet, thus leading to an increase of geitonogamous self-pollen transfer among ramets. Multiple studies have shown that geitonogamy may be a widespread cause of self-fertilization in animal-pollinated plants (Schoen and Lloyd, 1992; Leclerc-Potvin and Ritland, 1994; Eckhert, 2000). In addition, the spatial separation of stigmas and anthers within flowers coupled with protandry is likely to limit the degree of autonomous selfing. These factors may

also limit the amount of autogamy caused by pollinators. However, some studies have shown that autonomous selfing capacity may be unrelated to pollinator activity (Kron *et al.*, 1993; Zink and Wheelwright, 1997; Herrera *et al.*, 2001; Morales and Galetto, 2003). Nonetheless, autonomous self-fertilization can assure that some fertilization occurs when pollinators are scarce and neither outcross nor self-pollen is delivered to receptive stigmas (Kalisz *et al.*, 2004). Then again, reproductive assurance as a selective force for the evolution of autonomous selfing has gained little support (Morales and Galetto, 2003). This may be especially true for *P. vanbruntiae*, because autonomous selfing results in a significant decrease in female reproductive success. Therefore, we can conclude that pollinators are important for successful gamete transfer and offspring production in rare plant *P. vanbruntiae*. Future studies might reveal whether autonomous selfing rates increase in *P. vanbruntiae* populations that experience a chronic scarcity of pollinators, and whether autonomy occurs facultatively in the face of a lack of pollen receipt. Overall, reproductive assurance provided by autonomous selfing is relatively low for *P. vanbruntiae*, and the role of insect-facilitated pollination seems to be an important mechanism for plant fitness and subsequent population persistence.

2.6 Conclusions

Here, we provide evidence for a mixed-mating system in *Polemonium vanbruntiae*, a rare plant of high conservation concern whose reproductive biology has remained virtually unknown to the scientific community. Overall, our results did not support our initial hypothesis that small, fragmented populations of *Polemonium vanbruntiae* are limited by pollen receipt. We characterized the mating system of *P. vanbruntiae* as self-compatible, contrary to previous reports of self-incompatibility. Compensatory reproductive mechanisms, such as clonal growth and autonomous self-pollination, may allow for population persistence of *P. vanbruntiae*. However, reproductive assurance provided by autonomous self-fertilization may be limited, implying that pollinators are serving a crucial role in fruit and seed production in this system. These results should serve to inform conservation management strategies for remaining populations of this globally threatened species.

2.7 References

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Table 1: Among-population differences in female reproductive success. The values represent the mean of each fitness estimate \pm standard errors of the mean (SEM). Values represent pooled fitness values from both open-pollinated and hand-pollinated plants as there were no significant differences between treatments. Abbreviations refer to Vermont study sites: AP, Abbey Pond; BB, Blue Banks; FR, Forest Rd 233; CHSF, Camel's Hump State Forest.

Site	Population size	Plant height (cm)	No. flowers	Fruit set (%)	Seeds/plant	Seeds/fruit	Seed mass (mg)
AP	155	76.58 \pm 1.9	15.9 \pm 1.4	90.7 \pm 1.7	158.94 \pm 10.02	10.16 \pm 0.62	0.868 \pm .02
BB	225	84.73 \pm 2.3	21.3 \pm 1.2	89.9 \pm 1.7	210.63 \pm 10.53	10.85 \pm 0.67	0.845 \pm .02
FR	75	79.81 \pm 1.8	13.2 \pm 1.3	82.7 \pm 2.6	47.99 \pm 11.29	6.05 \pm 0.88	1.01 \pm .04
CHSF	205	90.57 \pm 2.2	16.7 \pm 1.8	66.5 \pm 5.1	119.59 \pm 17.17	10.02 \pm 0.37	0.948 \pm .16

Table 2: ANCOVA table results. Seed mass is the response variable, total seed production per plant is a covariate, and hand-pollination treatment, experimental site, and interaction terms are predictor variables.

Source	DF	Sums of Squares	F	P
# seeds	1	9.0e-8	6.13	.02
Site	2	6.2e-9	0.20	.82
Treatment	1	1.0e-8	0.72	.40
Site × # seeds	2	1.0e-7	3.31	.04
Treatment × # seeds	1	2.0e-8	1.51	.22
Error	84	1.3e-6	—	—

2.8 Figure Legends

Figure 1: Root, shoot, and reproductive components of *Polemonium vanbruntiae*. The rhizomatous roots send up new vegetative shoots as a mode of asexual reproduction. Adult plants may reproduce sexually via the production of a flowering stalk. When the protandrous flowers become reproductively mature, the stamens dehisce pollen prior to the opening of the sticky 3-lobed stigma. As the corolla opens, herkogamy (spatial separation of male and female reproductive parts) is evident as the unreceptive stigma is exerted beyond the stamens. Illustration courtesy of Réjean Roy.

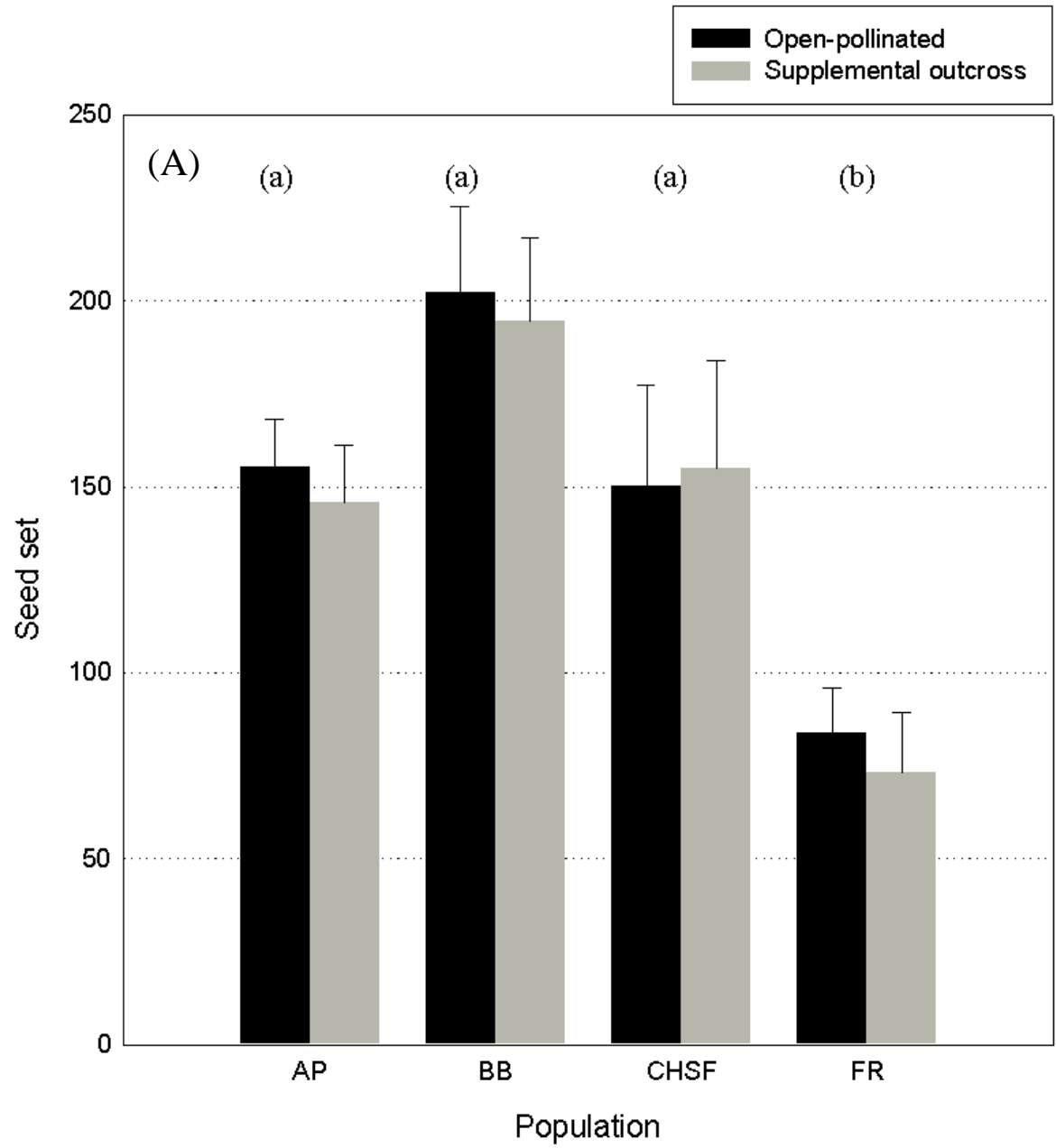
Figure 2: Comparison of female reproductive success: total seed production/plant (A), percent fruit set (B), number of seeds/fruit (C), and seed mass (D) in open-pollinated and pollen-supplemented plants across four sites of *Polemonium vanbruntiae*. Different letters denote significant differences among experimental sites. There were no significant differences between hand-pollination treatments. Error bars represent the standard error of the mean (SEM). The x-axis contains abbreviations for study sites located in Addison County, Vermont: AP, Abbey Pond; BB, Blue Banks; CHSF, Camel's Hump State Forest; FR, Forest Rd 233.

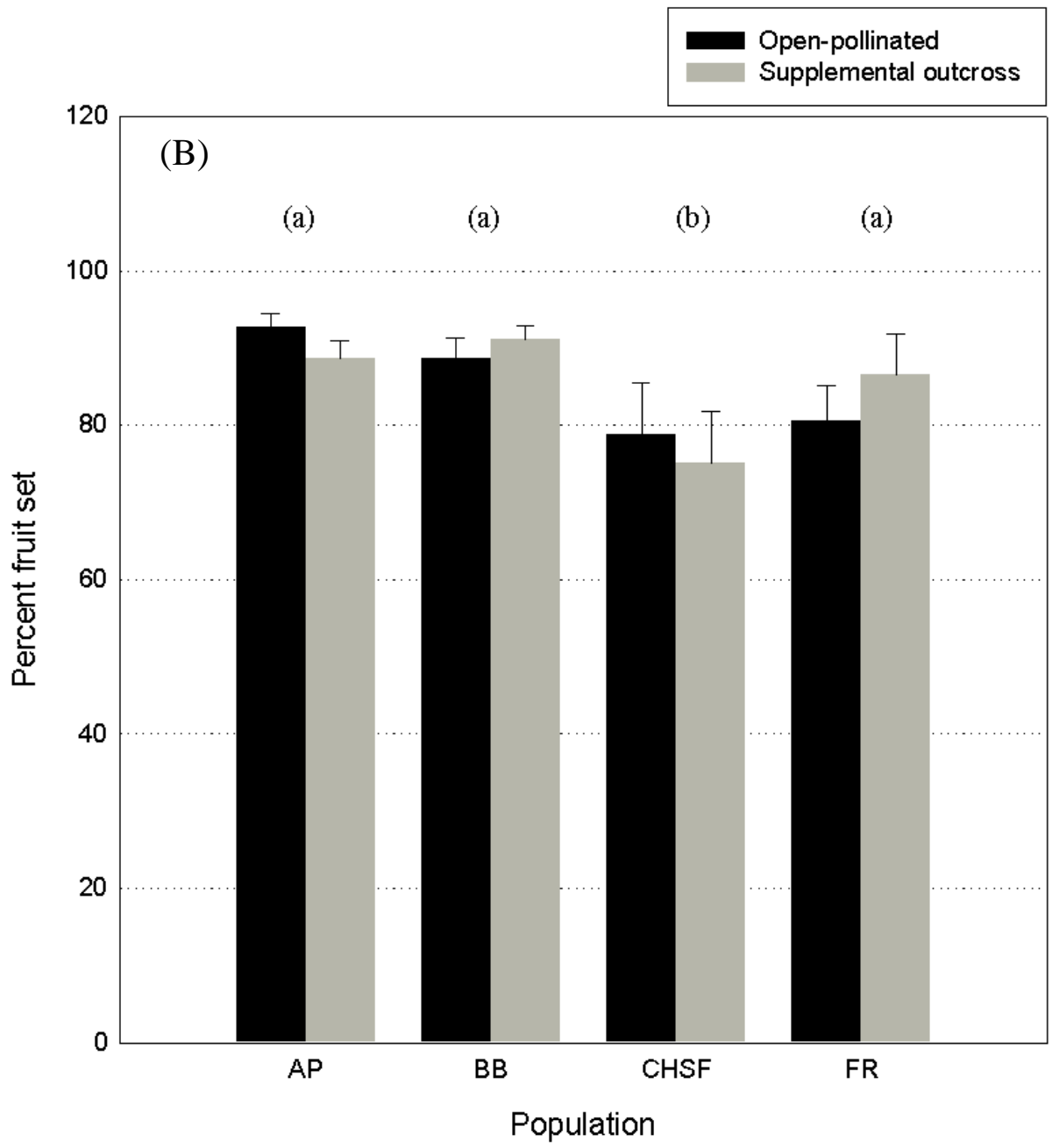
Figure 3: Individual seed size as a function of total seed production. Seed number and mass (mg) are significantly negatively correlated ($F_{1, 91} = 6.13$, $p = .02$; $r^2 = .09$).

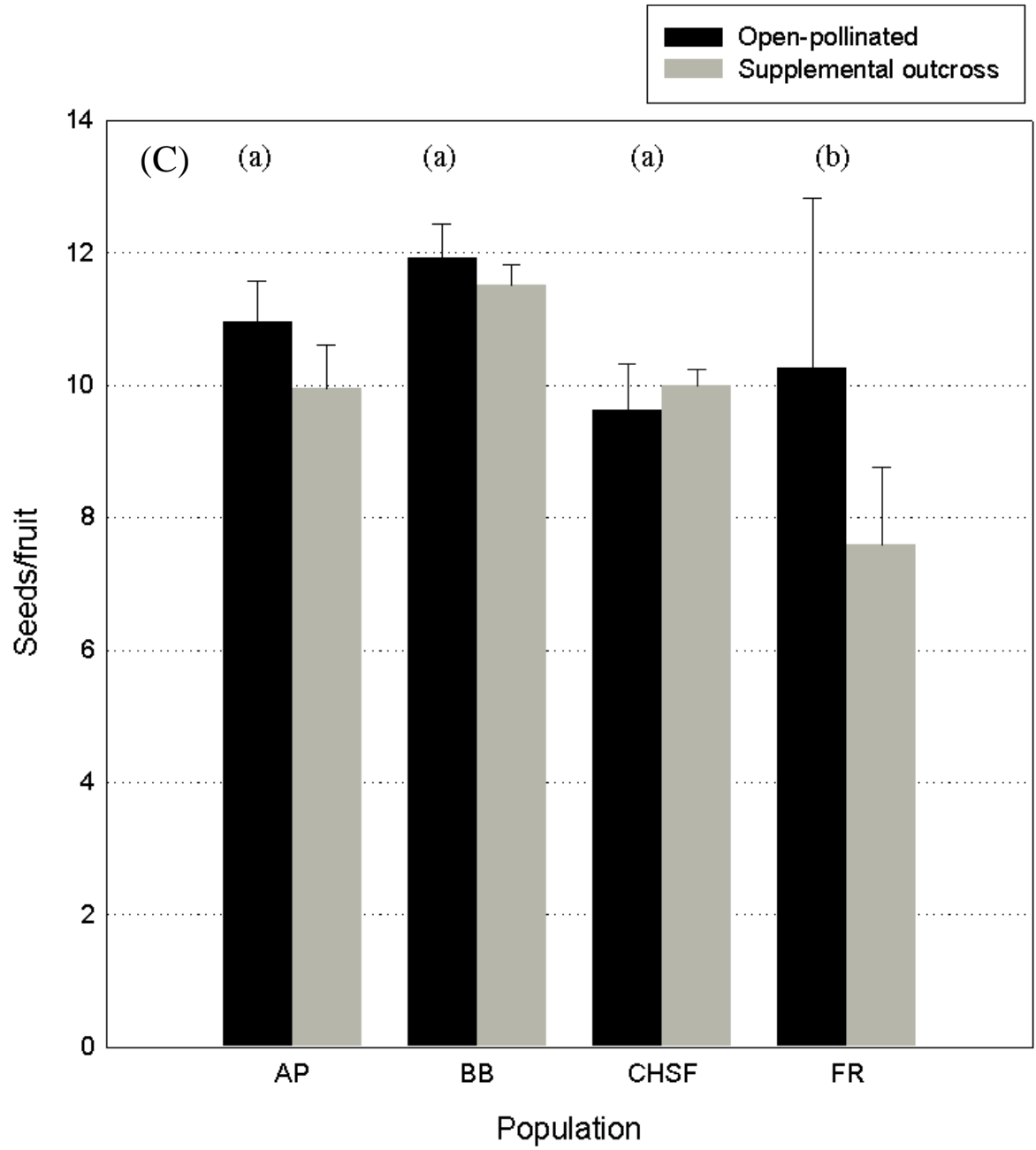
Figure 4: The effect of geitonogamous and autonomous self-fertilization on female reproductive success compared to purely outcrossed and open-pollinated flowers. Different letters denote significant differences among hand-pollination treatments. Error bars refer to SEM. Abbreviations refer to 2006 hand-pollination treatments: C, open-pollinated controls; O, outcross hand-pollination; GS, geitonogamous self-pollination; AS, autonomous self-pollination.



Figure 1: Root, shoot, and reproductive components of *Polemonium vanbruntiae*. The rhizomatous roots send up new vegetative shoots as a mode of asexual reproduction. Adult plants may reproduce sexually via the production of a flowering stalk. When the protandrous flowers become reproductively mature, the stamens dehisce pollen prior to the opening of the sticky 3-lobed stigma. As the corolla opens, herkogamy (spatial separation of male and female reproductive parts) is evident as the unreceptive stigma is exerted beyond the stamens. Illustration courtesy of Réjean Roy.







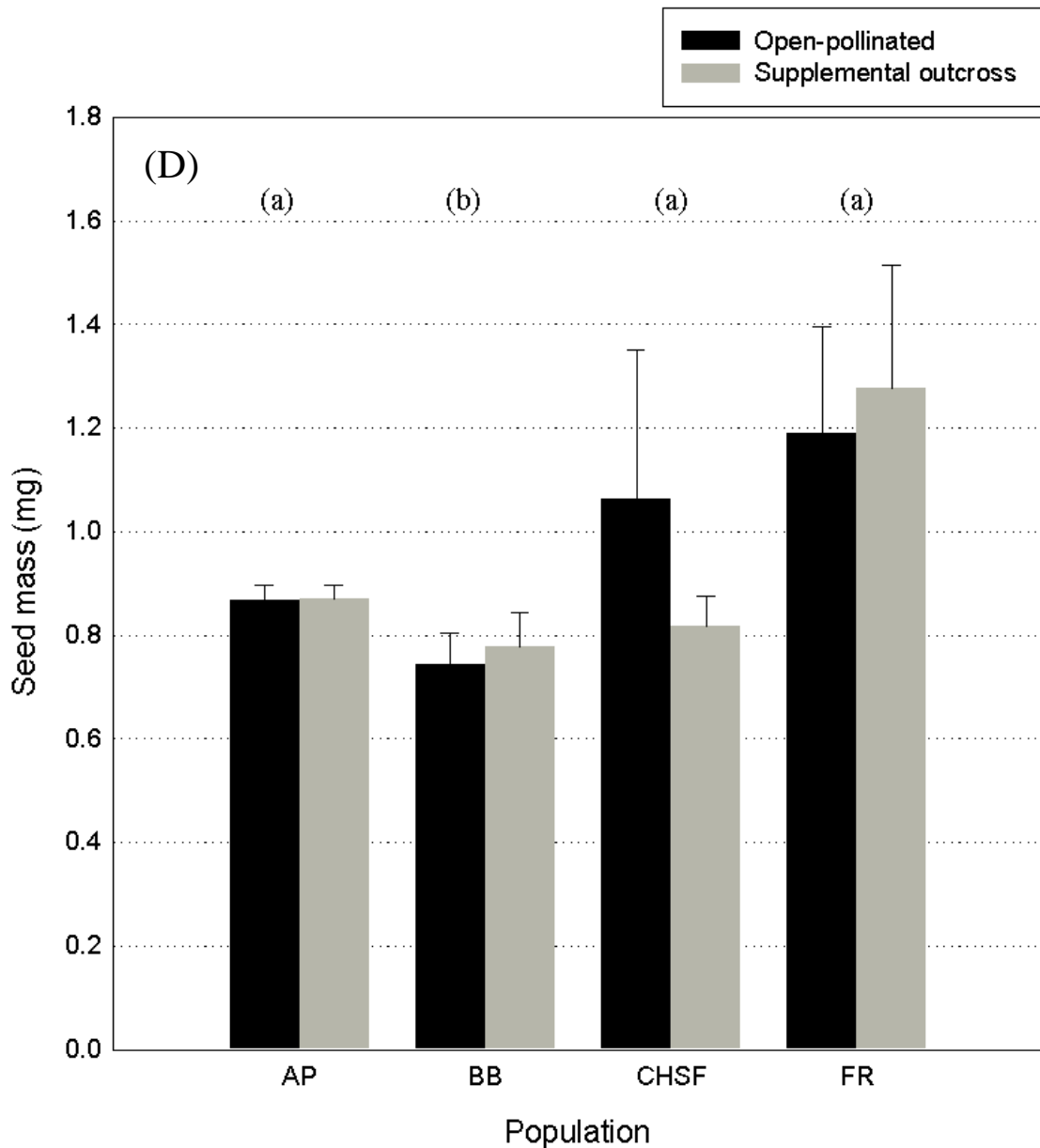


Figure 2: Comparison of female reproductive success: total seed production/plant (A), percent fruit set (B), number of seeds/fruit (C), and seed mass (D) in open-pollinated and pollen-supplemented plants across four sites of *Polemonium vanbruntiae*. Different letters denote significant differences among experimental sites. There were no significant differences between hand-pollination treatments. Error bars represent the standard error of the mean (SEM). The x-axis contains abbreviations for study sites located in Addison County, Vermont: AP, Abbey Pond; BB, Blue Banks; CHSF, Camel’s Hump State Forest; FR, Forest Rd 233.

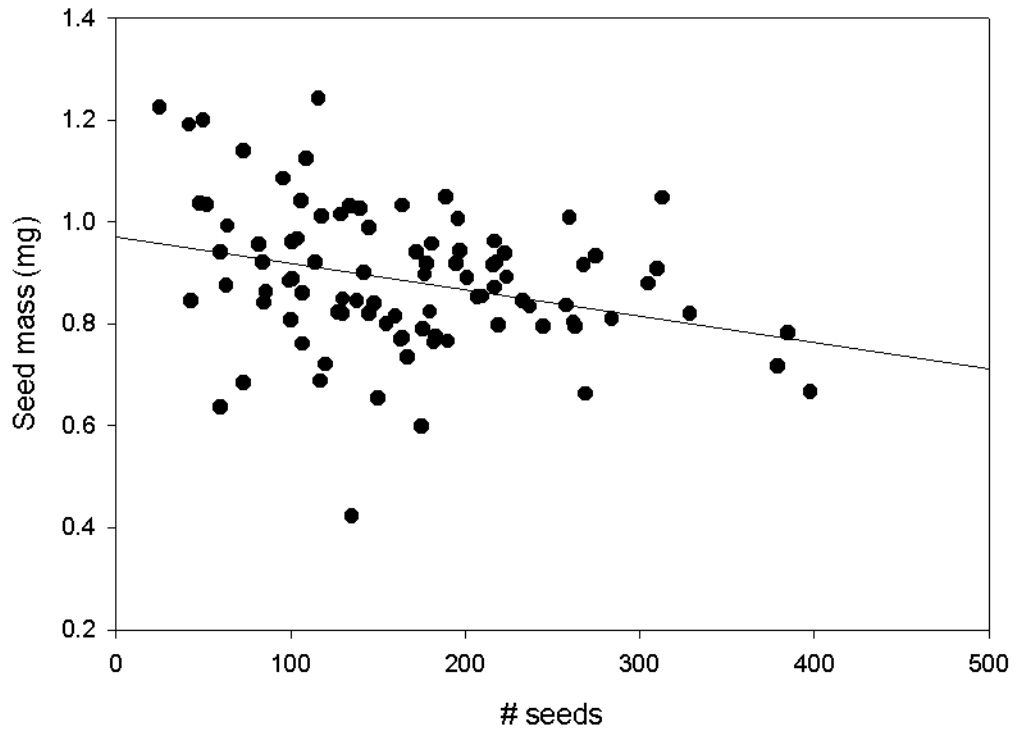


Figure 3: Individual seed size as a function of total seed production. Seed number and mass (mg) are significantly negatively correlated ($F_{1,91} = 6.13, p = .02; r^2 = .09$).

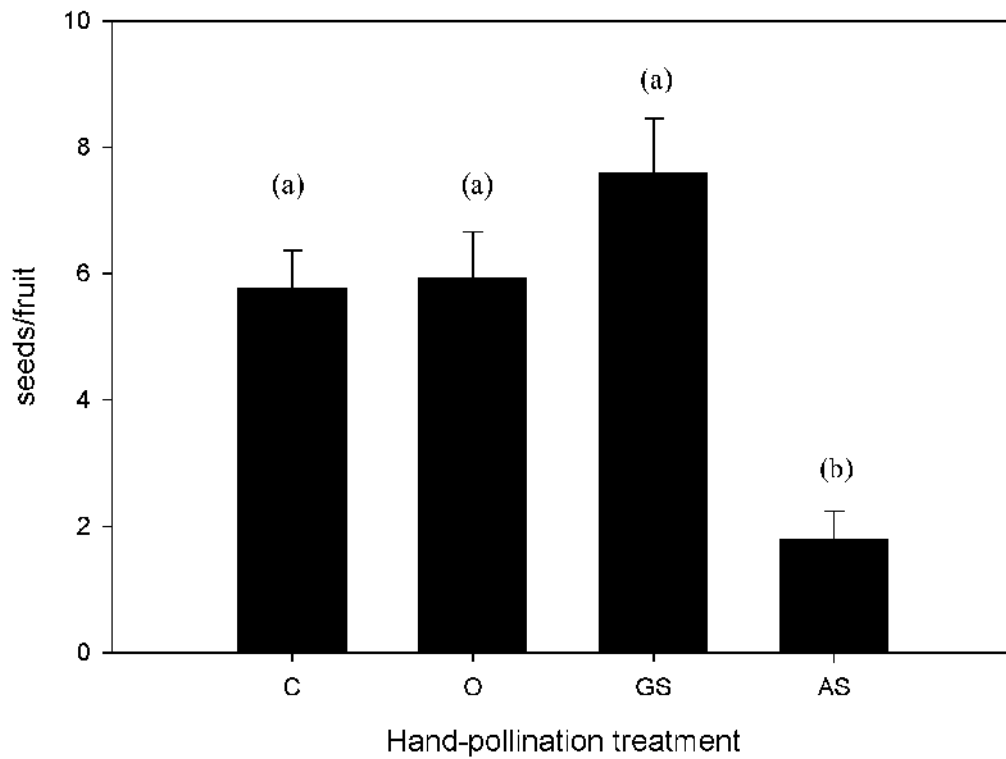


Figure 4: The effect of geitonogamous and autonomous self-fertilization on female reproductive success compared to purely outcrossed and open-pollinated flowers. Different letters denote significant differences among hand-pollination treatments. Error bars refer to SEM. Abbreviations refer to 2006 hand-pollination treatments: C, open-pollinated controls; O, outcross hand-pollination; GS, geitonogamous self-pollination; AS, autonomous self-pollination.

**CHAPTER 3: RELATIVE PERFORMANCE OF RARE PLANT PROGENY:
HOW POLLEN SOURCE AFFECTS EARLY OFFSPRING TRAITS IN
POLEMONIUM VANBRUNTIAE (POLEMONIACEAE)**

3.1 Abstract

Mixed mating strategies may incur both costs and benefits. When pollinators and/or potential mates are scarce, the ability to self-fertilize may provide reproductive assurance that ensures seed production. Yet, selfing is potentially costly when the result is a reduction in offspring quantity and quality. It is important to assess the effects of mixed mating strategies on offspring quality of rare plants. In the field and in the laboratory, we established experimental pollen crosses, including geitonogamous-self, autonomous-self, intrasite-outcross, intersite-outcross, and open-pollinated controls, to determine the effects of pollen source on early offspring traits in a listed threatened plant, *Polemonium vanbruntiae*. Seed set did not differ statistically among geitonogamous-self, intrasite-outcross, and open-pollinated flowers. However, germination of selfed seeds was significantly lower than outcrossed seeds, as was the cumulative quality estimate of seed mass \times probability of germination. Number of seeds per fruit and seed mass was significantly lower for intersite-outcross seeds compared to intrasite-outcross progeny. However, intersite-outcross progeny had a significantly higher relative performance index for germination, indicating heterosis for this early offspring trait. Outcross offspring had more leaves, larger leaf area and were taller than selfed offspring. Although

we were unable to follow offspring survival to flowering, enhanced germination success and more vigorous growth suggest that increasing gene flow among populations may increase plant vigor and genetic variation within small, isolated populations of rare plant *P. vanbruntiae*.

3.2 Introduction

Mixed mating systems have evolved frequently among plants, the relative benefits and costs of which vary spatially and temporally. The advantages of mixed mating strategies include the potential for plants to outcross when pollen is abundant and self-fertilize when outcross pollen is unavailable (Goodwillie et al. 2005). Because of this, mixed mating systems have evolved frequently in angiosperms (Goodwillie et al. 2005), particularly in clades that are primarily animal-pollinated (Wolfe and Barrett 1988, Eckert 2002). Plants existing in small, fragmented populations may rely on selfing, because pollinators often prefer larger populations with more pollen and nectar resources (Charnov 1982, Sih and Baltus 1987, Waites and Ågren 2004). Self-fertilization is potentially costly if selfing results in a reduction in offspring vigor and viability (Herlihy and Eckert 2002). The potential costs and benefits of mixed mating systems may therefore be of particular importance for rare plant populations of conservation value.

Self-fertilization in plants can result from both within-flower (autogamous) and between-flower (geitonogamous) pollen transfer (Herlihy and Eckert 2002). Within-flower selfing may be either spontaneous (“autonomy”) or pollinator-facilitated (“facilitated autogamy”), but geitonogamous pollination always requires a pollen vector

in animal-pollinated taxa (Richards 1986, Schoen and Lloyd 1992). When pollen is not limiting, autonomous selfing can result in pollen discounting (i.e., the reduction of pollen available for outcrossing, sensu Harder and Wilson 1998). However, if populations are limited by pollen, only autonomous selfing provides reproductive assurance, thus allowing offspring to be produced even under little to no pollinator service (Baker 1955, Stebbins 1957, Charnov 1982, Schoen and Brown 1991, Lloyd 1992). The capacity for autonomous selfing may be adaptive for rare plant populations existing in small, fragmented populations receiving stochastic pollinator service (Kennedy and Elle 2008).

In contrast, geitonogamy offers no reproductive assurance for populations experiencing pollinator scarcity (Goodwillie et al. 2005). Additionally, geitonogamy may also cause pollen discounting. For example, if a pollinator has already picked up the pollen for outcrossing, but the pollinator transfers pollen to another flower on the same plant, the result is geitonogamous selfing (Lloyd 1992). However, geitonogamous selfing may be an inevitable consequence of plants producing large floral displays to attract pollinators (de Jong et al. 1993), especially for clonal plants that produce multiple flowering ramets of the same genet (Harder and Barrett 1995). The sex allocation theory posits that increased plant size and large floral displays should increase male reproductive success (i.e., pollen deposition) and these fitness gains will decrease with plant size (Charnov 1982). However, female reproductive success (i.e., seed production) may decrease with plant size if geitonogamously-produced offspring are of lesser quality (de Jong et al. 1993). It is important to discern the effects of geitonogamous selfing on

offspring fitness to predict patterns of selection on plant reproductive and life history traits as well as determine the effects on offspring quantity and quality.

Self-fertilization may reduce offspring quality (Buza et al. 2000, Kéry et al. 2000, Goodwillie et al. 2005), because inbred offspring may suffer from reduced vigor, growth, and fitness when compared to outcrossed offspring (Jennersten 1988, Lamont et al. 1993, Ågren 1996, Fischer and Matthies 1998). A reduction in offspring fitness may increase risk of extinction, especially for threatened and endangered species (Barrett and Kohn 1991, Ellstrand and Elam 1993, Frankham 2005). Whether differences exist between the relative performance of outcrossed and selfed offspring in rare plant populations with mixed mating systems is relevant to our understanding of the evolution of mixed mating systems and rare plant population dynamics.

Small, isolated plant populations that have undergone substantial rates of self-fertilization and reduced gene flow may have experienced a history of genetic isolation, genetic drift, and local site adaptation. If the genetic distance among fragmented populations is large and dispersal among populations is low, a decrease in fitness may also occur from long-distance matings due to local site adaptation and the introduction of foreign genetic material (Price and Waser 1979, Waser 1993). However, if heterozygous individuals exhibit a fitness advantage over homozygotes (i.e., heterosis), then hybridization between fragmented populations may rescue a population from reduced genetic variation and drift by introducing new genetic material, thereby enhancing reproductive success and offspring performance (Lande and Schemske 1985, Lynch 1991, Hufford and Mazer 2003). Heterosis has been reported from a variety of plant

populations (Oostermeijer et al. 1995, Byers 1998, Fenster and Galloway 2000). Artificial gene flow among fragmented populations of rare plants may be a potential management activity in populations of threatened and endangered species exhibiting heterosis (Oostermeijer et al. 1995, Sheridan and Karowe 2000). However, it is increasingly important to discern the effects of pollen source on rare plant populations prior to management activities, as there may be both positive and negative effects of crossing individuals from fragmented populations (van Groenendael et al. 1998).

Many studies on small populations of perennial plants with mixed mating strategies have examined the costs and benefits of selfing by estimating the relative performance of selfed and outcrossed offspring. For example, in small populations of *Aquilegia canadensis* experiencing stochastic pollinator service and limited outcrossing opportunities, self-fertilization resulted in decreased seed set and offspring quality of selfed progeny (Herlihy and Eckert 2002). Mooney and McGraw (2007) did not detect negative effects of self-fertilization at the germination stage of rare plant *Panax quinquefolius*. However, they did detect differences in outcrossed and selfed progeny for early offspring traits, including seedling leaf area and stem height, and early size influences long-term survival of *P. quinquefolius* (Mooney and McGraw 2007). When a series of small to large populations of *Lupinus perennis* were examined for relative performance of various life stages of first-year plants (i.e., seed production, seedling survival, and seedling growth), a reduction in offspring quality was detected in selfed offspring although population size did not influence relative performance (Michaels et al. 2008). Generally, differences among outcrossed and selfed offspring are likely to

increase over time as the expression of the offspring genome overrides environmental variation and maternal effects (Wolfe 1993, Husband and Schemske 1996). A reduction in relative performance of early offspring traits may imply significant inbreeding depression; however, complete studies of inbreeding depression must involve an estimation of offspring fitness.

Here, we studied the effects of pollen source on seed production, germination, and seedling growth in the threatened perennial plant *Polemonium vanbruntiae* Britton (Polemoniaceae). *Polemonium vanbruntiae* is a wetland habitat specialist and is patchy throughout the landscape. *Polemonium vanbruntiae* has a mixed mating system, including the capacity for autonomous and geitonogamous self-fertilization as well as vegetative reproduction (Hill et al. 2008). Our main objective was to determine the consequences of a mixed mating system on offspring quality in *P. vanbruntiae*. To do this, we measured the quantity and quality of offspring resulting from 5 pollen crosses (autonomous-self, geitonogamous-self, intrasite-outcross, intersite-outcross and open-pollinated controls) by comparing seed production, seed mass, and germination among all pollen crosses. Additionally, we compared offspring vigor (leaf number, leaf size, and seedling height) between intersite-outcross and geitonogamous-self progeny. We asked two main questions: (1) Does the quantity and quality of offspring vary depending on pollen source? (2) Do outcrossed offspring exhibit heterosis for early life-history traits?

3.3 Materials and Methods

3.3.1 Study species

Polemonium vanbruntiae Britton (Eastern Jacob's ladder) is a globally threatened clonal perennial plant in the Phlox family (Polemoniaceae). *Polemonium vanbruntiae* is endemic to wetland regions in southern Québec, eastern Maine, Vermont, New York, and Pennsylvania south to Maryland and West Virginia (Fernald 1950, Gleason and Cronquist 1991, Crow and Helquist 2000) and is extirpated from New Brunswick (NatureServe 2008). *Polemonium vanbruntiae*'s natural habitat is characterized as minerotrophic wetlands in forest gap habitats with underlying calcareous basal till (D. Burbank *personal communication*, 2008). Soils are saturated, poorly drained, and apparently develop from accumulated organic material in depressions or deposits of alluvium from rivers (Sabourin and Paquette 1994). *Polemonium vanbruntiae* has a global conservation rank of G3 ("vulnerable to extirpation or extinction;" NatureServe 2008), and fewer than 100 populations occur in eastern North America (Vermont Nongame and Natural Heritage Program 2001).

Reproductive adults have an erect flowering stem ranging in height from 0.4 - 1.0 m. Flowers are campanulate, arranged in a compact terminal raceme, and each inflorescence produces an average of 15 flowers per plant with a range of 5-37 flowers (Deller 2002, Hill et al. 2008). Flowers are protandrous as the stamens become functionally mature and dehisce pollen prior to maturation of the pistil. *Polemonium vanbruntiae* is also herkogamous, as the unreceptive stigma is exerted beyond the stamens as the corolla opens (Gleason and Cronquist 1991). Autonomous selfing may be

limited due to both spatial separation of anthers and stigma (herkogamy) and temporal separation of male and female function (dichogamy). General assemblies of animal pollinators visit *P. vanbruntiae* flowers, including several species of bees, flies, butterflies and hummingbirds (Hill et al. 2008). Because *Polemonium vanbruntiae* reproduces clonally, geitonogamy may occur among ramets of the same genet. However, the degree of between-ramet geitonogamy has not been measured empirically. Upon fertilization, flowers mature into fruits, and the calyx surrounding the capsule becomes papery as it matures. Seeds are passively dispersed around the parent plant and may be dispersed long distances by winter winds and spring floodwaters (COSEWIC 2002). Seeds require a period of winter dormancy in order to germinate successfully (Brumback 1989).

3.3.2 Pollination experiments

We collected seeds from 70 plants in 2 Vermont populations, Forest Rd. 233 (FR) and Abbey Pond (AP), located in the Green Mountain National Forest in central Vermont, USA in September 2004. We collected ripened fruits from 30 plants from site FR (1424 seeds) and from 40 plants from site AP (6019 seeds). We kept a randomized mix of 10% of the seeds for the pollination experiments described here, and returned the remaining seeds to each respective population and passively dispersed the seeds around the maternal plant. Seeds were germinated in the lab in December 2004. Eleven plants produced flowers in March 2006 and these were used for hand-pollination treatments in the laboratory. Additionally, in June 2006, we conducted hand-pollinations in a natural

population located in Lordsland Preserve (LP) in Otsego County, New York. Precise GPS coordinates for site locations are not provided for conservation reasons.

To examine the effects of pollen source on seed production and early offspring traits, we haphazardly selected 118 treatment plants throughout the entire LP population in 2006. We randomly assigned individual flowers to one of four pollination treatments: (1) *geitonogamous-self* - bagged, emasculated, self-pollinated by hand with a mixture of within-plant self-pollen, (2) *autonomous-self* - bagged, no emasculation, no hand-pollination, (3) *intrasite-outcross* - bagged, emasculated, hand-pollinated with pollen from another flower on a flowering stem at least 8m away, and (5) *open-pollinated controls*. If stigmas of the treatment flowers were still receptive on the next visit, pollen was again applied. For plants grown in the laboratory in growth chambers, we performed all aforementioned pollen-crosses, with the exception of open-pollinated, but also included *intersite-outcross* hand-pollinations. Intersite crosses were only performed in the laboratory due to stipulations outlined in the Vermont Threatened and Endangered Species permit. We performed *intersite crosses* by collecting pollen from a single donor plant from either site AP or FR and delivered the outcross pollen to receptive stigmas of a plant originating from the other site, resulting in an AP × FR cross. We delivered pollen to all treatment flowers using a fine-tipped paintbrush. Pollination treatments were performed on all open female-phase flowers throughout the blooming period. After applying pollen, we marked the calyx with a specific color of indelible ink representing the pollen treatment. We later collected fruits, scored them as expanded or not, counted all seeds per fruit, and weighed all seeds in the laboratory.

3.3.3 Effect of pollen source on early offspring traits

To examine if pollen source affected offspring quality, after a 12-week stratification period at 4°C in a growth chamber (Percival Scientific Inc., Model I-37LXX), we germinated all seeds from both lab and field treatment plants. We randomized all seeds in germination mix in 4.5cm pots with 6 cells per pot arranged in a plastic tray. We assessed successful germination by emergence of the hypocotyl and calculated probability of germination. We measured the following variables of all surviving seedlings at 100 days after seedling germination: number of true leaves, leaf area (mm²), and seedling height (cm).

3.3.4 Data analysis

Prior to statistical analysis, we determined whether offspring measures differed significantly among laboratory and field plants using separate ANOVA models. We designated experimental location of plants (laboratory vs. field) as a fixed factor, and seeds/fruit and seed mass as separate response variables. We found no significant differences between our field and laboratory crosses (see Results for F-statistics and p-values). Therefore, we pooled all data prior to statistical analysis.

3.3.4.1 Differences in early offspring traits among pollination treatments

To determine whether seeds/fruit, seed mass and probability of germination differed significantly among different pollen crosses, we used separate ANCOVA models. For these models, we designated site as random factor, treatment as a fixed factor, parent plant height (cm) as the covariate, and seeds/fruit, seed mass and probability of germination as separate response variables in each model. If the models were significant, we used Tukey's HSD to detect differences among pollination treatments. We used parent height of the plants that were pollen receptors as the covariate to adjust for maternal effects (Becker et al. 2006). In order to avoid pseudoreplication, we calculated mean seed set, seed mass, and probability of germination for each pollination treatment within each plant. Seeds/fruit and seed mass were both natural log-transformed, and probability of germination was arcsine square-root transformed to improve normality and meet model assumptions.

3.3.4.2 Offspring performance comparison between outcross and selfed seedlings

A growth chamber malfunction severely reduced our sample sizes. Thus, we were only able to compare offspring performance between intersite-outcross and geitonogamous-self seedlings. However, although our offspring sample sizes were reduced, we were still able to compare later-offspring traits (# leaves, leaf area, height) of the seedlings that survived. Because offspring performance estimates may be correlated, we initially ran a MANOVA. We assigned treatment as a fixed factor, and offspring

height (cm), number of leaves, leaf area (mm^2), and a multiplicative offspring performance function ($M = \# \text{ leaves} \times \text{leaf area} \times \text{height}$) as response variables. We also performed univariate tests for all response variables following a significant MANOVA. We performed the following transformations for offspring performance data to meet ANOVA assumptions of homoscedascity and normality: square-root number of leaves, and natural log of leaf area, offspring height, multiplicative function, and seed mass.

3.3.4.3 Heterosis and relative performance of offspring

To estimate the relative performance of outcrossed and selfed offspring, we combined our hand-pollination treatments into 3 treatment classes: Self (geitonogamous-self treatment), Open-pollinated controls, and Outcross (intersite-outcross treatments). The degree of genetic diversity within populations is unknown, and, due to the clonal nature of *P. vanbruntiae*, intrasite pollination may represent a cross between ramets of the same genet or between closely related individuals. To be conservative, we used intersite-outcross offspring, rather than intrasite offspring, to represent our Outcross treatment. Additionally, we omitted autonomous selfing from the “self” treatment category, because autonomy is likely to be rare in natural populations given strong protandry and herkogamy within individual flowers, and autonomy results in very few seeds.

To determine the effect of pollen source on offspring quantity and quality, we compared early offspring traits among Open-pollinated, Outcross, and Self-offspring using separate ANOVA models. Seeds/fruit (natural log-transformed), seed mass (natural log-transformed), and probability of germination (arcsine square root-

transformed) were examined as response variables and hand-pollination treatment was the independent variable.

3.3.4.4 Relative performance

For each crosstype, we obtained an estimate of early offspring performance by comparing the mean performance of outcross and selfed progeny. We estimated relative performance for each early offspring trait (seeds/fruit, seed mass, and germination). We also calculated a cumulative measure of early offspring performance from the product of seed mass and probability of germination. The “relative performance of crosstypes” (RP) estimate of outcrossed and selfed offspring were modified from the methods of Ågren and Schemske (1993) as

Equation 3.1

$$RP_i = \frac{z_o - z_s}{z_{max}}$$

where z_o is the mean phenotype of outcrossed progeny, z_s is the mean performance of selfed progeny, and $z_{max} = z_o$ when $z_o \geq z_s$ and $z_{max} = z_s$ when $z_s \geq z_o$ (Ågren and Schemske 1993, Johnston and Schoen 1994). The index ranges from -1 to +1 and, following the tradition of Ågren and Schemske (1993), positive values indicate inbreeding depression. We did not use the traditional estimate of W for the relative performance indices because we did not follow offspring to flowering, and we therefore lack a complete estimate of offspring fitness (W) of different crosstypes.

Similarly, we used the relative performance index of intra— and intersite crosses as an estimate of outbreeding depression as

Equation 3.2

$$RP_o = \frac{Z_{intra} - Z_{inter}}{Z_{max}}$$

where z_{intra} is the mean performance of intrasite progeny, z_{inter} is the mean performance of intersite progeny, and $z_{max} = z_{intra}$ when $z_{intra} \geq z_{inter}$ and $z_{max} = z_{inter}$ when $z_{inter} \geq z_{intra}$. Positive values indicate outbreeding depression.

We used equation 3.3 to examine whether offspring resulting from crosses between isolated populations exhibited heterosis (i.e., hybrid vigor):

Equation 3.3

$$H = \frac{Z_{inter} - Z_{intra}}{Z_{intra}}$$

The value z_{intra} represents the mean performance of intrasite progeny and z_{inter} represents the mean performance of intersite progeny. Positive values indicate heterosis (Busch 2006).

We estimated RP_i , RP_o , and H for all early offspring traits, including seeds/fruit, seed mass, probability of germination, and cumulative-offspring measure. We generated 95% confidence intervals to test whether the RP_i , RP_o , and H indices deviated significantly from 0.

Our statistical analyses were carried out using JMP version 4.0.2 (SAS Institute 2000) and SPSS version 16.0 statistical software programs (SPSS 2001).

3.4 Results

3.4.1 Differences in early offspring traits among pollination treatments

Pollen source had a significant effect on early offspring traits. Plants receiving pollen from a distant site, as well as plants receiving within-population and within-plant pollen performed equally well in early life-history stages, whereas offspring of autonomously-selfed plants performed most poorly compared to all pollen treatments (**Figure 5**). Seed set for geitonogamously selfing plants was approximately 4 times higher than the autonomous treatment ($F_{4, 162} = 6.10$, $p = 0.0001$; **Figure 5A**). Probability of germination differed significantly among pollination treatments ($F_{4, 105} = 12.03$, $p < 0.0001$; **Figure 5B**), but seed mass was not different among hand-pollination treatments ($F_{4, 161} = 0.64$, $p = 0.64$). Seeds from intersite pollen crosses tended to have the highest probability of germination ($82.4 \pm 10.0\%$), but intersite germination was statistically equivalent to open-pollinated ($74.0 \pm 13.0\%$) and geitonogamous ($76.3 \pm 10.6\%$) seedlings. Autonomously-selfed seeds had the lowest probability of germination of 48.3%. Parent height in the ANCOVA models was not significant in any of the analyses and therefore results are presented without the covariate. Site was also not significant for any response variable. We found no differences in female reproductive fitness between field and laboratory pollen crosses (seeds/fruit: $F_{1, 160} = 3.11$, $p = 0.08$; seed mass: $F_{1, 160} = 2.26$, $p = 0.13$), and therefore pooled all data prior to statistical analysis.

3.4.2 Offspring performance comparison between outcross and selfed seedlings

Intersite-outcross seedlings performed significantly better than the geitonogamous-self seedlings in the number of leaves produced ($F_{1,7} = 17.03$, $p = .006$; **Figure 6A**), leaf area ($F_{1,7} = 18.83$, $p = .005$; **Figure 6B**) and seedling height ($F_{1,7} = 29.82$, $p = .002$; **Figure 6C**). As the effect of pollen source may be cumulative over the plant's life cycle (Lázaro and Traveset 2006), and allocation to any structure may result in trade-offs, e.g., fewer, larger leaves, we also calculated a multiplicative function of offspring performance ($M = \# \text{ leaves} \times \text{leaf area} \times \text{height}$), which was also significantly different between outcross and self progeny ($F_{1,7} = 11.73$, $p = 0.01$, **Figure 6D**).

3.4.3 Heterosis and relative performance of offspring

Seed set and seed mass of self-pollinated plants did not differ significantly from the open-pollinated and outcross plants (seed set: $F_{2,175} = 1.10$, $p = 0.34$; seed mass: $F_{2,170} = 0.55$, $p = 0.58$). However, outcross offspring had a significantly higher probability of germination than selfed offspring ($F_{2,101} = 3.22$, $p = 0.04$; **Table 3**).

Overall, outcrossed offspring performed relatively better than selfed offspring, indicated by positive RP_i values for seed mass, probability of germination, and the cumulative measure of offspring quality (**Figure 7A**). The RP_i was significantly greater than 0 for germination and cumulative offspring quality, but was not significant for seed mass (i.e., 95% confidence intervals overlapped 0). Selfed plants set significantly more seeds than outcrossed plants, as indicated by the negative RP_i value. Intrasite-outcross plants produced more and larger offspring than intersite-outcross treatment plants, as

indicated by the significant RP_o index for seeds/fruit and seed mass (**Figure 7B**). The RP_o index was significantly less than 0 for germination, implying that intersite offspring germinated at a higher rate than intrasite-outcross offspring. This trend is again observed as significant heterosis for intersite-outcross offspring germination (**Figure 7C**). The cumulative measure of offspring quality for heterosis was not significantly different from 0; thus, heterosis is only significant at the seedling germination stage.

3.5 Discussion

Our results indicate that while self-pollination may provide some reproductive assurance of seed set, the result is a reduction in relative performance of selfed offspring. Selfed seedlings had a significant reduction in relative performance for the germination stage and cumulative offspring quality, and outcross offspring exhibited significant heterosis for germination. Our findings corroborate studies examining the offspring quality of long-lived perennial plants with mixed mating systems existing within small populations (Herlihy and Eckert 2002, Mooney and McGraw 2007, Michaels et al. 2008). As in these studies, our experiment did not follow offspring to reproduction due to the multiple years it takes for long-lived perennial plants to flower. However, if selfed offspring suffer from a reduction in survival to reproductive maturity, there could be selection against self-fertilization in *P. vanbruntiae* populations (Eckert and Allen 1997, Morgan et al. 1997).

Seed production involves an interaction among multiple factors, including maternal, paternal, and offspring fitness, but seed germination is a more direct indicator

of offspring fitness (Mooney and McGraw 2007). Outcrossed progeny outperformed selfed offspring at the germination stage, which is an important life-history stage for establishment in natural populations. It is interesting that we detected any differences in relative performance (RP_i) in early offspring traits in *P. vanbruntiae*, because a reduction in relative performance of selfed progeny is often not detected until the growth / reproduction stage, whereas lower rates of inbreeding depression are commonly found at the seed and germination stage (Husband and Schemske 1996). One explanation for these findings is that plant populations that primarily self are generally more likely to exhibit decreased performance of selfed progeny for early offspring traits (Byers and Waller 1999). This may imply that population of *P. vanbruntiae* are primarily self-fertilizing, although this has not been tested empirically. Even though we were unable to measure later offspring life history traits, such as survivorship to flowering and fruit production, our results may imply that inbreeding depression in populations of *P. vanbruntiae* may be evident in seedling growth due to the significant differences in offspring performance between the intersite-outcross and geitonogamous-self crosses. However, more information is needed on the degree of inbreeding and outbreeding depression for adult survival and fitness to be able to definitively state that inbreeding depression poses a significant threat for populations of rare plant *P. vanbruntiae*. Our results must be interpreted carefully, as we assessed early offspring traits under growth chamber conditions, whereas the effects of inbreeding depression are often magnified under field conditions (Goodwillie et al. 2005, Waser 1993).

We detected reduced seed set when flowers were pollinated with distant, intersite-outcross pollen. However, the effects may be negligible as plants receiving outcross pollen from a distant site set less seed, but had a higher probability of germinating. Offspring that germinate and grow significantly faster are more vigorous and may outcompete neighboring plants for resources under natural conditions. Previous studies in other plant species have also found increased performance of first generation hybrids relative to parents, but hybrid fitness can decrease in later generations due to the breakup of co-adapted gene complexes (Hufford and Mazer 2003). Therefore, it is important to follow hybrid offspring to reproduction and perform additional crosses to test whether heterosis holds up through multiple generations. Additionally, outbreeding depression (RP_0) is expected to increase with the geographic or genetic distance between populations (Waser and Price 1994). Our crosses were between relatively proximal populations (< 10km); if we had crossed more distant populations, we may not have detected heterosis for our intersite crosses. The next step would be to determine the optimal crossing distance (i.e., the crossing distance that results in the highest fitness and offspring vigor) for *Polemonium vanbruntiae* (Waser and Price 1989).

It is worth noting that the open-pollinated, intrasite-outcross, and geitonogamous-self treatments did not differ within sites. These results may indicate that natural populations of *P. vanbruntiae* exhibit low genetic variation and little gene flow due to small populations that may have experienced population bottlenecks and genetic purging (Charlesworth and Charlesworth 1987). Additionally, the combination of simultaneously open flowers on a single plant and vegetative reproduction in *P. vanbruntiae* increases

the probability of geitonogamy if pollinators regularly visit multiple flowers on the same genetic individual, thus increasing the selfing rate (de Jong et al. 1992, Eckert 2000).

Our study provides evidence for differences in relative performance in early offspring traits of rare plant *P. vanbruntiae*, leading us to conclude that offspring performance is dependent on pollen source. Additionally, we detected a significant reduction in relative performance of selfed offspring for early life-history stages, which may indicate evidence of inbreeding depression. However, our study did not extend to adult survival and reproduction, and therefore we are unable to determine whether inbreeding depression poses significant fitness consequences for *P. vanbruntiae*. Because we detected heterosis for seed germination, artificially increasing gene flow among populations may serve as a means of increasing levels of genetic diversity within small, isolated populations. Genetically mixing plant populations often leads to substantial levels of heterosis in the survival and reproduction of interpopulation hybrid offspring (Levin 1984, van Treuren et al. 1993, Heschel and Paige 1995, Paland and Schmid 2003), which may be the case for *P. vanbruntiae*. These results should serve to inform conservation management strategies for remaining populations of this rare plant.

3.6 References

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Table 3: Average values of early offspring traits in *Polemonium vanbruntiae* among Open-pollinated, Outcross, and Self-pollination treatments (± 1 S.D.). Means with different letters denote significant differences in offspring quality among pollination treatments.

POLLINATION TREATMENT	SEEDS/FRUIT	SEED MASS (MG)	PROBABILITY OF GERMINATION*
Open-pollinated	7.79 \pm 4.56 ^a	1.03 \pm 0.24 ^a	0.739 \pm 0.06 ^a
Outcross	5.31 \pm 2.95 ^a	1.45 \pm 1.66 ^a	0.795 \pm 0.15 ^b
Self	8.33 \pm 4.86 ^a	1.22 \pm 1.12 ^a	0.754 \pm 0.05 ^a

* p < .05

3.7 Figure Legends

Figure 5: Comparisons of early offspring traits including seeds/fruit (A) and probability of germination (B) of *Polemonium vanbruntiae* among all pollination treatments. Bars sharing a letter do not differ significantly. N refers to sample size (# flowers) for each pollination treatment. Error bars represent the standard error of the mean (SEM).

Figure 6: Comparison of early offspring traits of *Polemonium vanbruntiae* seedlings resulting from intersite-outcross and geitonogamous-self pollination treatments: number of true leaves (A), leaf area (B), seedling height (C), and the multiplicative offspring performance function (D). The multiplicative function (M) = # leaves × leaf area × height. Error bars represent SEM.

Figure 7: Relative performance (RP) and heterosis (H) estimates of early offspring traits in rare plant *Polemonium vanbruntiae*. Following the tradition of Ågren and Schemske (1993), RP_i is an estimate for inbreeding depression (A), RP_o is an estimate for outbreeding depression (B), and H is an estimate of heterosis (C). The cumulative function is the product of seed mass and probability of germination. RP and H estimates are significant if the 95% confidence intervals do not overlap 0 and if RP and H values are greater than 0. Significant values are denoted by an *.

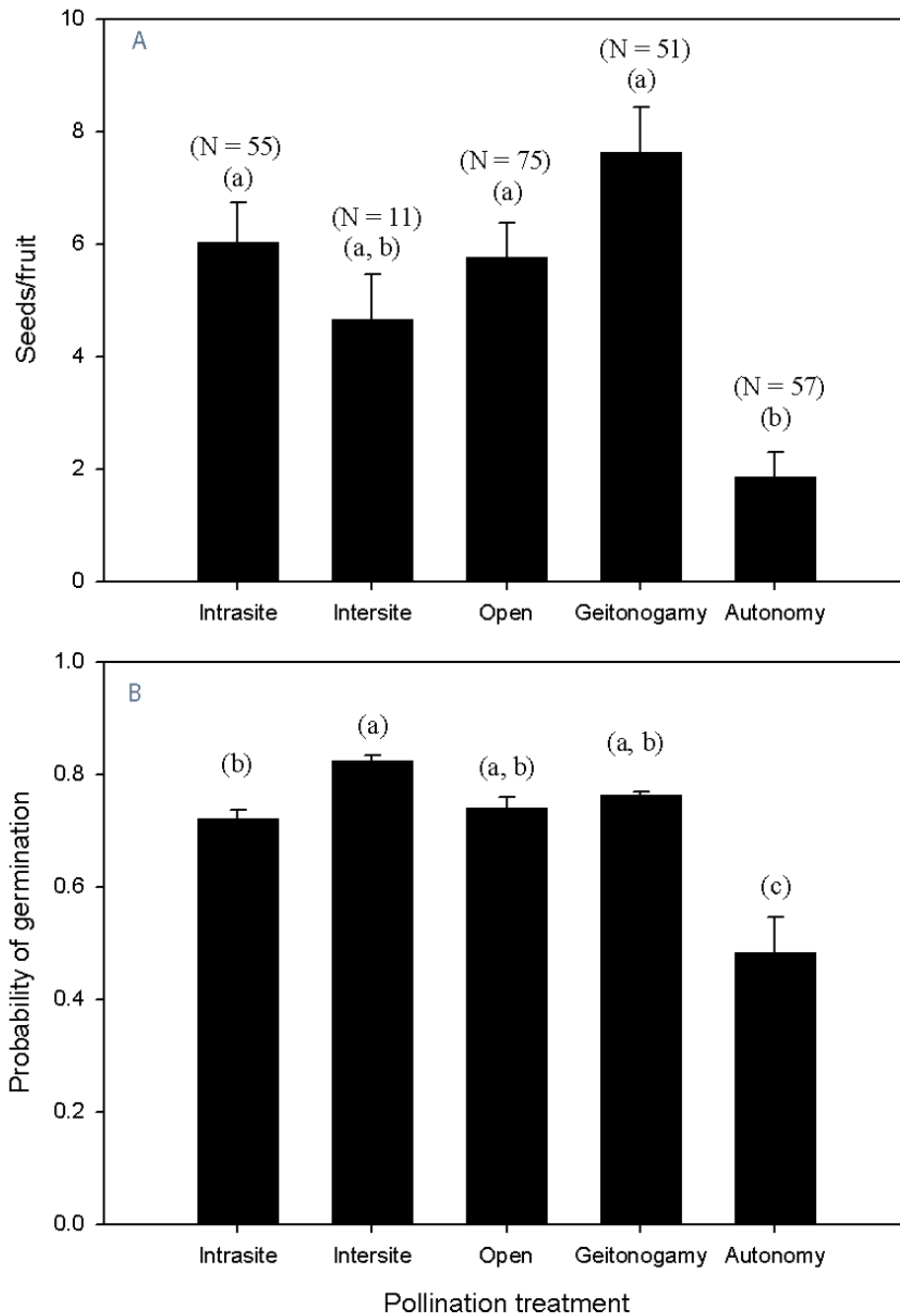
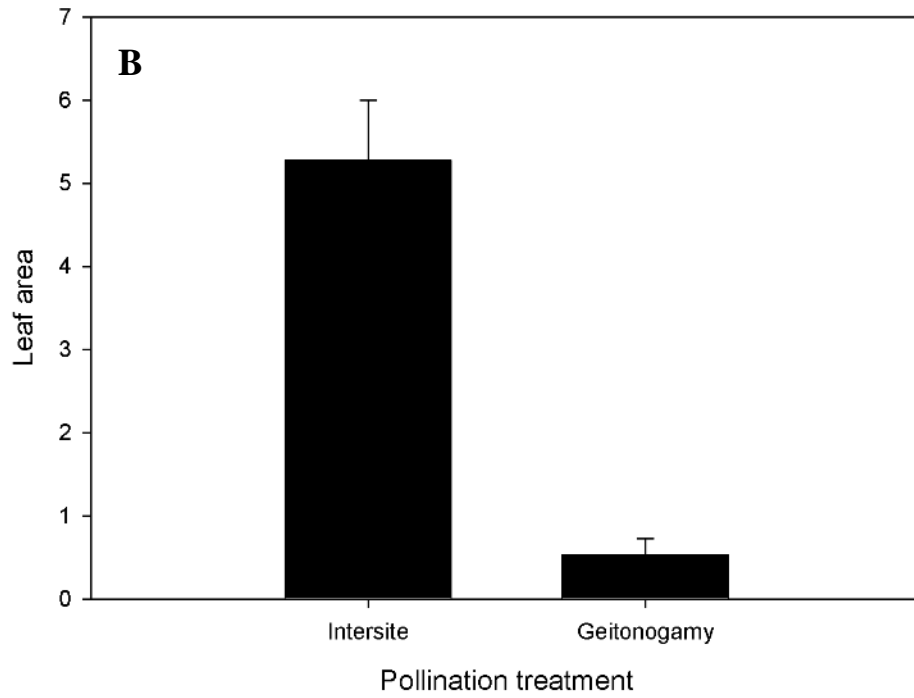
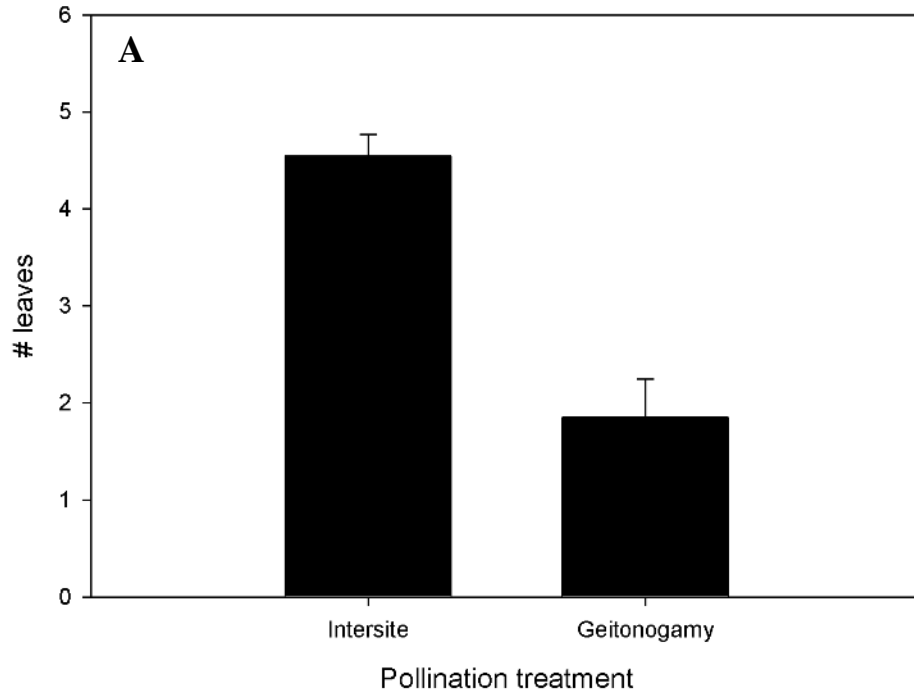


Figure 5: Comparisons of early offspring traits including seeds/fruit (A) and probability of germination (B) of *Polemonium vanbruntiae* among all pollination treatments. Bars sharing a letter do not differ significantly. N refers to sample size (# flowers) for each pollination treatment. Error bars represent the standard error of the mean (SEM).



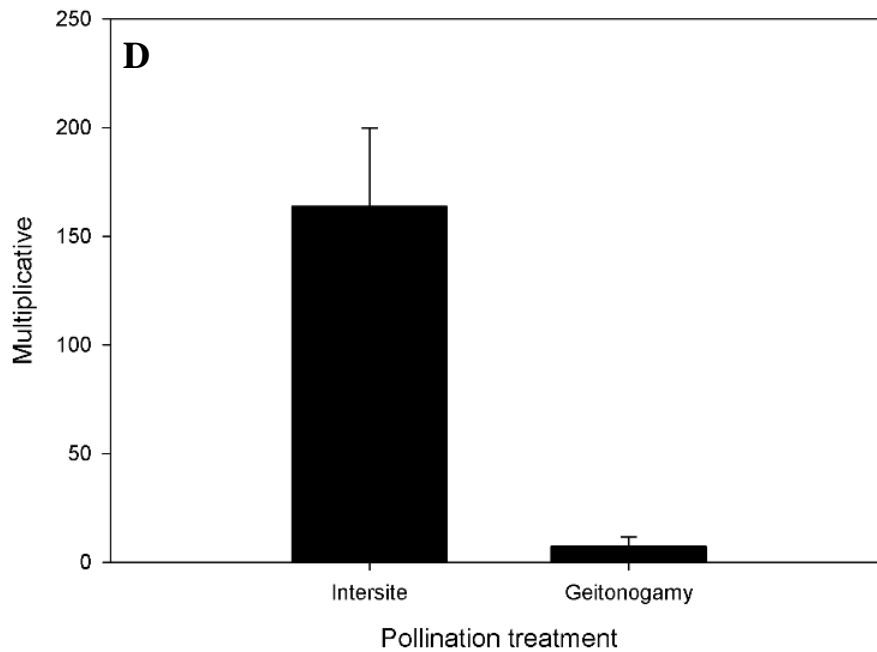
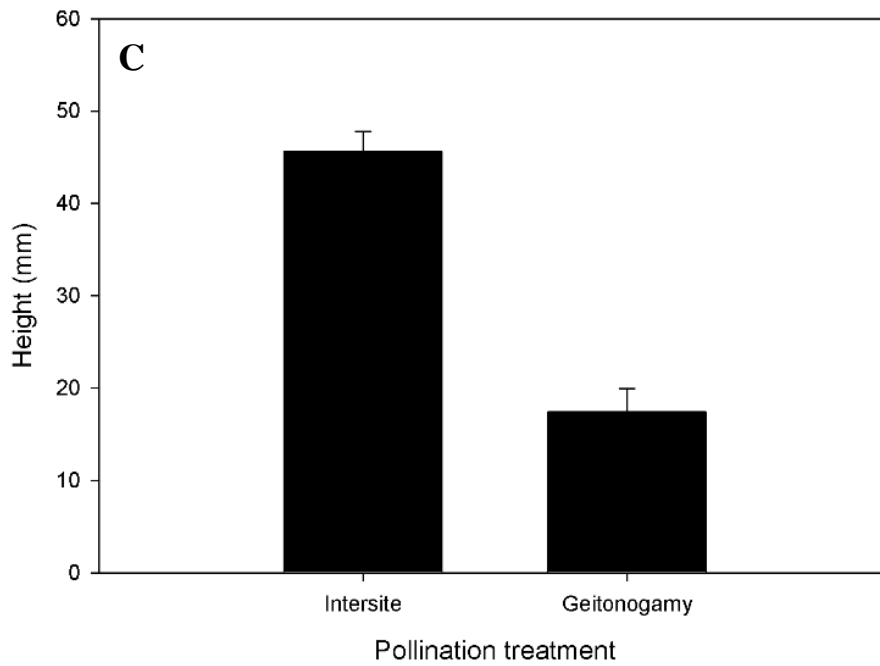


Figure 6: Comparison of early offspring traits of *Polemonium vanbruntiae* seedlings resulting from intersite-outcross and geitonogamous-self pollination treatments: number of true leaves (A), leaf area (B), seedling height (C), and the multiplicative offspring performance function (D). The multiplicative function (M) = # leaves \times leaf area \times height. Error bars represent SEM.

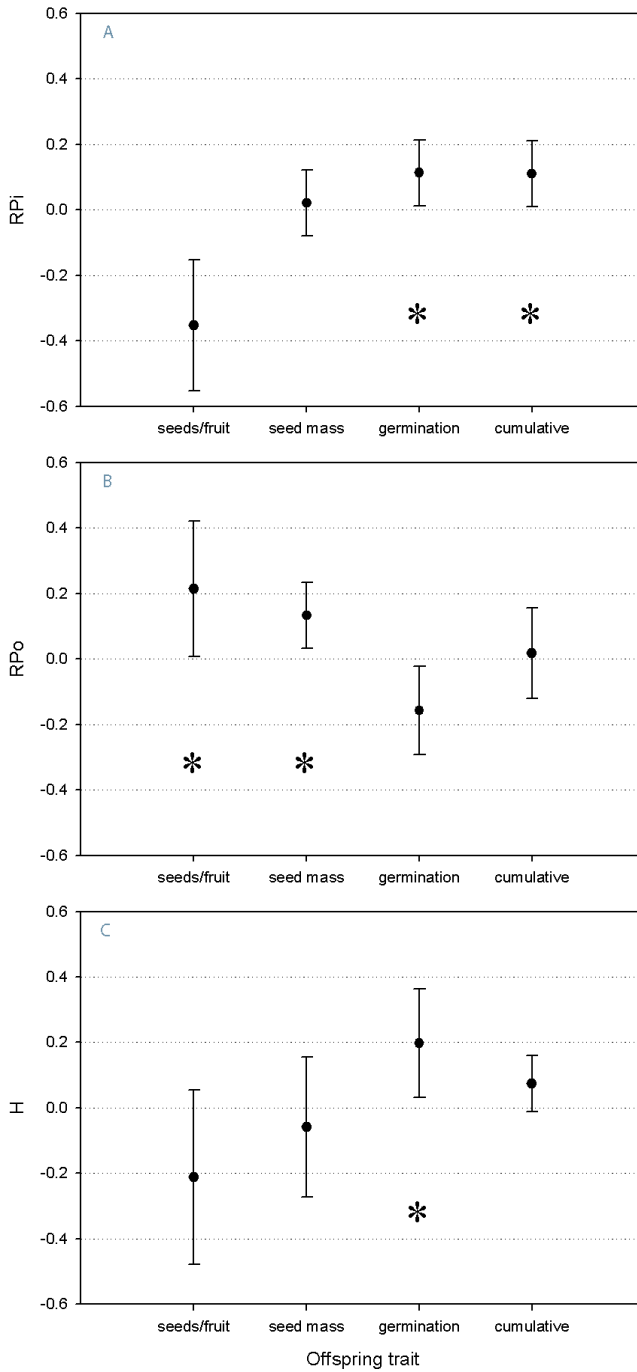


Figure 7: Relative performance (RP) and heterosis (H) estimates of early offspring traits in rare plant *Polemonium vanbruntiae*. Following the tradition of Ågren and Schemske (1993), RPi is an estimate for inbreeding depression (A), RPo is an estimate for outbreeding depression (B), and H is an estimate of heterosis (C). The cumulative function is the product of seed mass and probability of germination. RP and H estimates are significant if the 95% confidence intervals do not overlap 0 and if RP and H values are greater than 0. Significant values are denoted by an *.

**CHAPTER 4: THE EFFECT OF HABITAT TYPE AND WHITE-TAILED DEER
HERBIVORY ON LONG-TERM PERSISTENCE OF A
WETLAND ENDEMIC PLANT**

4.1 Abstract

For long-lived organisms such as perennial plants, it is often difficult to determine which environmental factors will have the largest effects on population dynamics. In this study, I used 4-years of demographic data on rare plant *Polemonium vanbruntiae* (Eastern Jacob's ladder, Polemoniaceae) and incorporated the effects of habitat type (wet meadow and forest seep) and white-tailed deer herbivory (presence/absence) into matrix population models to predict their effects on long-term population dynamics. Although differences in plant morphology, nutrient availability, and fertility revealed that ramets were smaller but more fertile in wet meadow sites compared to the forest seep sites, wet meadow sites are expected to increase only at a slightly faster rate than forest seep sites. White-tailed deer (*Odocoileus virginianus*) preferentially browsed vegetative adult and reproductive adult ramets over younger life history stages, and deer herbivory occurred exclusively in forest seep sites. Herbivory significantly increased the probability that small and large vegetative ramets will remain vegetative the following year. Herbivory also significantly reduced fertility of reproductive ramets due to the direct consumption of flowers, fruits, and seeds. Deer herbivory significantly decreased the predicted population growth rate under stochastic conditions. However, *P. vanbruntiae* ramets are expected to increase at a slow rate in the future as $\lambda > 1$ under both "browse" and "no

browse” scenarios. Yet, deer herbivory increased the extinction risk to a detectable level, although the probability of ramet extinction is still quite low (i.e., < 5%). Deer browsing shifted the elasticity values of vital rates, and changed the potential for younger life histories stages, such as seedlings, to change future population growth. Instead, survival and stasis of large vegetative ramets became the vital rates having the largest potential impact on future population growth. Overall, I found that the most important ecological factor affecting long-term population dynamics of *P. vanbruntiae* is white-tailed deer herbivory.

4.2 Introduction

Demographic studies are useful in understanding long-term population dynamics of threatened and endangered species. These analyses are especially informative when they are conducted in different habitat types where changes occur as a result of environmental stochasticity and forest canopy succession (Oostermeijer et al. 1996). Stochastic events are common in natural populations, but those that affect demographic parameters may be especially important in compromising the ability for a rare species to persist through time.

Many rare plants require canopy-opening events such as the creation of tree-gaps to avoid extinction (Eisto et al. 2000, Lennartsson and Oostermeijer 2001). The abandonment of agricultural land over the past few hundred years in eastern North America has led to a large increase in reforested areas, which has resulted in a drastically changing landscape for many eastern endemic plants (Singleton et al. 2001). Woody

vegetation inhibits sunlight from reaching the herbaceous layer, thus leading to a decrease in plant fitness, offspring recruitment, and survival (Gross 1980, de Jong and Klinkhamer 1988). Most herbaceous plants experience increased growth and reproduction in response to increased light levels (Collins and Pickett 1988, Quintana-Ascencio and Menges 2000, Neufeld and Young 2003). However, increased light levels may be partially offset by the negative effects of interspecific competition due to an increase in species diversity in open-canopy habitats (Hughes 1992). If rare plants occur in different habitat types, it is informative to understand the effect of habitat on long-term population growth. From a management perspective, it may be useful to create artificial disturbances (i.e., mowing, selective removal of trees and shrubs, controlled burns) in an attempt to thwart extinction in habitats where rare plants are not expected to persist.

Habitat type can also influence species interactions. For example, an increase in forested habitat in the Northeast coincides with increased abundance of white-tailed deer (*Odocoileus virginianus*). White-tailed deer are particularly notorious herbivores in part due to their increasing abundance and potential to modify natural vegetative communities (Côté et al. 2004). Many studies have shown that herbivores can have strong deleterious effects on plant vital rates, such as growth, survival, and reproduction (Crawley 1988, Louda 1989, Gange 1990, Doak 1992, Marquis 1992, Strauss and Zangrel 2002). Studies that have examined the effect of deer herbivory on population growth of perennial plants have found that deer herbivory has a measurable impact on long-term population dynamics, especially for small plant populations (Augustine and Frelich 1998, Rooney and Gross 2003, Knight 2004, McGraw and Furedi 2005).

Certain life history strategies, including the ability to produce clonal offspring, may provide the flexibility needed for plants to persist under stressful environmental conditions. Because sexual reproduction is costly and may not be feasible under low light levels or frequent herbivory, clonal plants have the ability to allocate resources to vegetative reproduction until conditions are more appropriate for sexual production of offspring. This strategy may allow clonal plants a degree of “buffering” against natural disturbances (Eriksson 1994).

Here, I examined the effect of habitat type (wet meadow and forest seep) and white-tailed deer herbivory on population persistence of a globally threatened plant, *Polemonium vanbruntiae* (Eastern Jacob’s ladder). *Polemonium vanbruntiae* is a clonal plant endemic to eastern North America in wetland regions throughout the Appalachian Mountains. The species is listed as threatened or endangered in each state and province it occurs. Encroachment of woody vegetation and white-tailed deer herbivory has been cited as two potential threats to *P. vanbruntiae* population persistence (Deller 2002). Because *P. vanbruntiae* is clonal, it is important to describe the different levels of organization of clonal plants. First, the genet is the inclusive genetic individual originating from one zygote, and the ramet is the functional, yet potentially independent, part of the genet (Eriksson 1994). In non-clonal species, the ramet and genet are equivalent. Here, I followed the demography of individual *P. vanbruntiae* ramets, rather than genets, to parameterize transition matrix models to project future population dynamics.

It is difficult to obtain demographic data for clonal plants because clones may fragment, move and intermingle over time, thus making it difficult to follow the demographic fate of genetic individuals (de Kroon and van Groenendael 1997, Cain and Damman 1997). Because of the difficulties involved in following the fates of genets, few studies have addressed the demography of genets, but have rather been performed on the ramet-level (Harper 1977, Hartnett and Bazzaz 1985, de Steven 1989, Falinska 1995, Damman and Cain 1998, Fair et al. 1999, Jongejans and de Kroon 2005). Therefore, the dynamics of clonal plants have been followed at the ramet, clonal fragment (i.e., a fragment of physically connected ramets), and genet level (Eriksson 1993, Pederson and Tuomi 1995, Cain and Damman 1997).

Describing ramet behavior is informative as the clone itself can be described as a population of individuals (Corradini et al. 2002). Although ramet demography may not accurately predict growth of the population as a whole, knowledge of ramet persistence and fecundity will ultimately inform studies of clonal plants. The matrix population modeling technique used here adequately describes a population of ramets, and the relative success of the population can be determined by its growth rate, λ (Caswell 1985). However, whether a population is described as a population of ramets or genets has an influence on how the model should be interpreted.

The first step in any demographic analysis is to build a life-cycle graph, which is then used to construct a matrix population model (Caswell 2001). The matrix population model, or transition matrix, represents the demographic data that describe the life cycle of a population. The contribution that an average individual belonging to size or stage class

j makes during a time interval (t to $t + 1$) to another class is expressed as coefficient a_{ij} of matrix \mathbf{A} whose number of rows and columns is equal to the number of life history classes chosen. In general, a matrix population model describes the dynamics of a population comprised of life-history classes (characterized by age, size, or stage classes) that best characterize the organism's life cycle. Age-structured matrices are often referred to as Leslie matrices, after P.H. Leslie (1945) who first established their importance as ecological models. Leslie matrices paved the way for stage- and size-structured models, which were introduced years later by L.P. Lefkovitch (1965), and are known as Lefkovitch matrices. Here, I utilize Lefkovitch matrices (stage- and size-structured), as they best describe life-history transitions of plant and are most often used for plant demography studies (Caswell 2001).

The main objectives of this study were the following: (1) to describe the life cycle and life history transitions of *P. vanbruntiae* ramets, (2) to utilize matrix population modeling to predict future population growth at the ramet level, (3) to determine whether habitat type or white-tailed deer herbivory significantly affected long-term population dynamics, (4) to project probability of ramet extinction under each habitat and deer browsing scenario, and (5) to evaluate the importance of each life history stage transition using sensitivity and elasticity analysis.

4.3 Materials and Methods

4.3.1 Study species

Polemonium vanbruntiae Britton (Eastern Jacob's ladder) is a globally threatened clonal perennial plant in the Phlox family (Polemoniaceae). *Polemonium vanbruntiae* is endemic to wetland regions in southern Québec, eastern Maine, Vermont, New York, and Pennsylvania south to Maryland and West Virginia (Fernald 1950, Gleason and Cronquist 1991, Crow and Helquist 2000) and is extirpated from New Brunswick (NatureServe 2008). The species has a global conservation rank of G3 ("vulnerable to extirpation or extinction;" NatureServe 2008), and fewer than 100 populations occur in eastern North America (Vermont Nongame and Natural Heritage Program 2001).

Polemonium vanbruntiae reproduces both sexually and vegetatively. The demographic analysis performed here focuses on ramet behavior and sexual reproduction only as genets are difficult to empirically measure in the field. The root structure of *Polemonium vanbruntiae* consists of a thick rhizome with an extensive network of rootlets with fine root hairs extending from the main rhizome. Ramets connected by a single rhizome form a clonal fragment, which consist of ramets representing various life history stages. However, it is unknown whether physical connections of ramets disintegrate over time, so a *P. vanbruntiae* clonal fragment may not necessarily represent a true genetic individual (i.e., genet). The shallow-rooted nature of *P. vanbruntiae* allows plants to grow in rocky calcareous basal till soils and on top of fallen, decomposing logs.

Reproductive ramets have an erect flowering stem with flowers arranged in a compact terminal raceme. Each inflorescence produces a range of 5-37 flowers per plant

with an average of 15 flowers per plant (Hill et al. 2008). Flowering within inflorescences is basipetalous. The flowers are protandrous, as the anthers dehisce pollen for 1-2 days before the stigma becomes receptive. Upon fertilization, flowers mature into fruits comprised of a capsule with three locules containing 1-10 seeds per locule (Thompson 1991). The capsule is surrounded by the calyx, which becomes papery as it matures. *P. vanbruntiae* flowering stalks can reach heights of >100cm, and occasional uprooting has occurred due to strong winds and seasonal flooding (Hill Bermingham, *pers. obs.*). Seeds are passively dispersed around the parent plant and may be dispersed long distances by winter winds and spring floodwaters (Sabourin 2002). Seeds may either remain viable in the seed bank or germinate the following spring.

4.3.2 Study sites

Polemonium vanbruntiae's natural habitat is characterized as minerotrophic wetlands where soils are saturated, poorly drained, and apparently develop from accumulated organic material in depressions or deposits of alluvium from rivers (Sabourin and Paquette 1994). Nine *P. vanbruntiae* sites occurring within or adjacent to the Green Mountain National Forest in central Vermont, USA were chosen for this demographic study. The sites represent two habitat types (i.e., forest seep and wet meadow) with differing habitat characteristics and associated plant communities. Four sites represent the wet meadow habitat and 5 represent the forest seep habitat (**Table 4**). The "wet meadow" sites have a relatively open-canopy and high occurrence of wetland grasses, sedges, and herbaceous plants. Conversely, the "forest seep" sites have a partially-open to

completely closed canopy with high leaf litter and a shade-tolerant herbaceous layer with a low frequency of grasses. All sites occur in elevations between 390 and 485m. Precise coordinates are withheld due to conservation concerns.

4.3.3 Demographic analysis

To characterize ramet behavior and assess transition probabilities in differing habitat types, I permanently tagged 2025 ramets (954 ramets at the forest seep sites and 1071 ramets in the wet meadow sites) during the 2004 and 2005 field seasons. I tagged the base of each ramet with a numbered aluminum tag, and inserted a numbered flag to ease the relocation of demographic ramets. Individual ramets throughout the entire population were haphazardly selected to gain a representative sample of ramets throughout the entire site in a variety of microhabitats.

A time-step of one year was utilized for data collection, as this is the common time-step included in stage-based matrix population models for other long-lived perennials (Caswell 1988, Menges 1990, Gotelli and Ellison 2002). I collected annual data on the vital rates (survivorship, growth, and fertility) for all tagged plants during three censuses per year. The spring census was conducted in May and June during initial leaf emergence. At this time, I relocated all tagged ramets and recorded survival. Then, in June and July during flowering, I collected growth data including height, number of leaves, and longest leaf length and width (cm). In late August and early September, I collected all fruits and seeds from tagged flowering ramets and brought the fruits into the lab for seed counting and weighing to score fertility. During each annual census, I also

recorded evidence of white-tailed deer herbivory, evidenced as a straight cut to the stem (Knight 2004). In most cases, deer tracks or nearby scat provided supplementary evidence that browsing was caused by deer.

4.3.3.1 Experimental analysis of seed, seedling, and yearling vital rates

Seed bank survival— I collected 15 total soil cores from the forest seep sites and 14 soil cores from the wet meadow sites in July 2004 to preliminarily test for a *Polemonium vanbruntiae* seed bank. At the time of soil collection, seed germination had already taken place in the field, and current-year seeds had not yet dispersed. Thus, the densities of the seed banks were at their lowest and positive seed germination reflected a persistent seed bank. I stratified the soil cores in a humidified growth chamber at 5-7°C for 4 months. I then placed the samples under fluorescent lights with an 14h:light and 10h:dark photoperiod, and watered the cores regularly and counted and identified the emerging seedlings every second day.

After identification of a viable seed bank from the soil cores in 2004, I setup additional field experiments to estimate seed bank survival of the seed class in the matrix models. I placed 6 replicate seed cages at each site in September 2005 following methods described in McGraw and Furedi (2005). I constructed seed cages with PVC pipe (approximately 20cm in length and 10cm in diameter). Hardware cloth (i.e., mesh screening) was affixed to the bottom of the cage in order to contain the soil and seeds. Hardware cloth was also fastened to the top of the cage to prevent the loss of experimental seeds without preventing seed germination. I filled each cage with soil

collected from nearby but not within the *P. vanbruntiae* sites to avoid collecting existing seeds that may be already present in the seed bank. Cages were buried at randomly selected locations within each site.

I randomly collected 3 cages from each site in May 2006 and brought them to the lab to identify viable seeds. Seed viability was determined using germination trials supplemented with a 90% crystalline gibberellic acid (GA₃) solution to stimulate seed germination. I made a solution of 100 ml of 0.1% GA₃, and added 0.111 g (to account for the 90% purity) to 2 ml of 95% ethyl alcohol (Keeley and Fotheringham 1998). I thoroughly mixed the solution and then added 98 ml distilled water. The GA₃ solution was covered with aluminum foil and kept in dark conditions at 4°C in the lab. I kept the seed cages in humidified growth chamber at 21°C under fluorescent lights with an 14h:light and 10h:dark photoperiod. I watered the seeds within the seed cages with the GA₃ solution to promote germination and counted and identified all emerging seedlings every 2 days. Because seeds require an 8-month stratification period, the number of viable seeds obtained from the cages removed in 2006 was used to estimate the proportion of seeds remaining viable in the seed bank from September 2005 to May 2006. I repeated this procedure in May 2007.

Seed-seedling-yearling transition— In 2004 and 2005, I planted 1450 *P. vanbruntiae* seeds in seed grids in both wet meadow and forest seep sites. I collected seeds from flowering tagged ramets at each site and then planted a random sample of those seeds in the seed grids. One hundred seeds per 10×10 cm grid were planted at the edge of the

existing populations. At site NBF (forest seep site), only 50 seeds were available at the entire site, and so the grid was planted with 50 seeds in 2005. Therefore, I planted 800 seeds in 8 seed grids at the wet meadow habitats and 650 seeds in 7 seed grids at the forest seep sites. Control grids were placed adjacent to the experimental grids to control for background seed germination in the experimental plots. I subtracted the number of germinants in control plots from number of germinants in the experimental seed grids to control for background seed germination. By quantifying seedling emergence and seedling-to-yearling transitions in the seed addition plots over 3 years, I parameterized vital rates for the younger plant stages in the transition matrices.

4.3.4 Soil chemistry analysis

In order to determine whether habitat types differed in soil composition, I collected soil cores for soil analysis at all 9 *P. vanbruntiae* sites. Samples were collected in late July 2005. I collected 10 soil cores from each site at randomly chosen intervals along each of three (3) parallel 10m transects. I used a hand trowel to carve out a 5cm diameter and 2cm depth soil core, ensuring that all cores were taken from an area at the same diameter and depth. I placed the samples in clean, dry, plastic container and labeled with site name and transect location and immediately returned the samples to the lab. The soil cores were analyzed by the University of Vermont Agricultural and Environmental Testing Laboratory for conductivity, pH, nutrients, and soil chemistry.

The soil cores were dried overnight at 55°C in a large oven in the lab. After drying, coarse fragments were removed with a 2 mm sieve. Then, 4mL of the soil was

shaken for 15 minutes with 20mL of modified-Morgan's solution (1.25 M ammonium acetate at pH 4.8). Calcium, K, Mg, Zn, and Al were measured on an inductively coupled plasma (ICP) instrument, which is an analytical instrument capable of accurately and simultaneously determining many soil elements. Phosphorous was analyzed using visible spectroscopy. Soil pH was measured in 10mL of a 0.01M CaCl solution combined with 5mL of soil. Organic matter was determined by obtaining a dry, weighed sample of soil, and then heating the sample at 370°C until all the soil organic matter had burned off. After drying, the sample was reweighed, and percent organic matter was calculated from the weight loss.

4.3.5 Statistical analysis

Morphological differences between habitats— To examine whether habitat type affected plant morphology, I compared vegetative and reproductive traits of each life history stage (excluding the seed stage) in each habitat using parametric and non- parametric statistical models.

(1) *Seedling and yearling morphology*— I did not regularly measure leaf area and height of seedlings and yearlings, because these measurements would have been destructive due to their small size and delicate structure. Therefore, I only compared number of leaves for these 2 life history stages. Number of leaves did not meet ANOVA assumptions of normality and homoscedascity even after transformation, so I used a Wilcoxon rank sums nonparametric test to compare number of leaves of seedlings and

yearlings in each habitat. I designated number of leaves as the response variable and habitat as the predictor variable. I ran separate tests for seedling and yearling plants.

(2) *Vegetative adult morphology*— To assess plant size of vegetative rosettes, I used the product of number of leaves and average leaf length (cm) as a proxy for vegetative plant size, as has been done for a closely related species, *P. viscosum* (Galen 1993). Before analysis, I examined normality of the data, and natural-log transformed plant size to meet model assumptions. Transformed data were tested for homogeneity of variance with Levene's test, and variances among treatment groups of response variables were equal. To test whether vegetative plant traits differed significantly between wet meadow and forest seep habitats, I ran a multivariate analysis of variance (MANOVA identity response design) for plant height and number of leaves for reproductive adult plants to control for correlations among vegetative plant structures. Following a significant MANOVA, I ran individual ANOVAs on each response variable separately.

(3) *Reproductive adult vegetative morphology*— Reproductive ramets have a different growth structure than vegetative ramets. Reproductive ramets form a tall, erect flower scape with alternatively arranged leaves extending from the base of the stem to the base of the terminal raceme. Therefore, I used plant height (cm) and number of leaves separately as the best estimate of plant size for reproductive ramets. I excluded from the analysis plants that were browsed by white-tailed deer or had a broken flowering stem. In the MANOVA model, I designated number of leaves and height (both natural-log transformed) as response variables and habitat as the fixed-factor predictor variable.

Following a significant MANOVA, I ran individual ANOVAs on each response variable separately.

(4) Reproductive adult flowering morphology— I also examined whether flowering morphology differed between the wet meadow and forest seep habitats by comparing number of flowers, percent fruit set, seed set, and seed mass (g) for reproductive adults. Before analysis, I examined normality of the data. Percent fruit set was arcsine square-root transformed, and the remaining variables except for number of flowers were natural log-transformed to meet model assumptions. All transformed data were tested for homogeneity of variance with Levene's test, and variances among treatment groups of response variables were equal, excluding number of flowers. Therefore, I used a Wilcoxon rank sums nonparametric test to examine whether habitat type affected number of flowers. To control for correlations among fruit set and seed set, I ran a MANOVA with percent fruit set and seed set as response variables and habitat as the predictor variable. Finally, I used ANOVA to examine the relationship between seed mass (g) and habitat type.

Using ANOVA, I also examined whether survival, growth, and fertility rates differed between habitat type and also between ramets that were browsed by white-tailed deer and those that were not browsed.

Lastly, I used logistic regression to analyze whether ramet height (cm), white-tailed deer herbivory (0,1), and life history stage (1-6) influenced *P. vanbruntiae* ramet survival (0,1) in year $t + 1$.

Soil composition analysis— I used ANOVA to determine whether there were differences in soil characteristics between the habitat types. To meet normality assumptions, I natural log-transformed the following variables: % Ca, % Mg, Fe, Mn, pH, and ppm K. The other variables met normality assumptions. All data were tested for homogeneity of variance with Levene’s test prior to statistical analysis. In the ANOVA model, I designated habitat as the predictor variable and soil characteristic as the response variable.

4.3.6 Model parameterization

In the field, I sampled all *P. vanbruntiae* ramets within a total of 26 randomly placed 0.5m² grids at each demographic site in order to parameterize the population vector (n_0) for model simulations. The population vector n_0 equals to the initial number of individuals in each life history stage in the population, and the sum of the vector equals the total population size (Morris et al. 1999). I sampled a total of fourteen 0.5m² grids at the 5 forest seep sites and twelve 0.5m² grids at the 4 wet meadow sites. I counted the number of individuals represented by each life-history stage within each 0.5m² grid. To parameterize n_0 , I converted # of plants per m² to number of individuals in each stage class by multiplying the average densities by the total population area (Morris and Doak 2002).

I constructed 4 types of matrices for the 3 annual transitions of the demographic study (2004-2005, 2005-2006, 2006-2007), thus yielding a total of 11 transition matrices (i.e., the “browse” matrix contained only 2 annual transitions). The habitat type

transition matrices contained pooled the data on ramets from the 5 forest seep sites and the 4 wet meadow sites. Additionally, I pooled plants that had been browsed by deer into a “browse” matrix, and those that had not been browsed by deer into a “no browse” matrix. The “no browse” matrix consisted of plants from both forest and meadow sites, but the “browse” matrices consisted of plants only from forest sites (i.e., sites AP, FR, NBF, and DR), as I detected no browsing of meadow ramets. Due to lack of data to parameterize the “browse” matrix, I used survival of seeds, seedlings and yearlings from the original forest seep matrices. In order to keep these rates consistent between “browse” and “no browse” matrices, I used the same estimates of survival of seeds, seedlings, and yearlings for both “browse and “no browse” matrices. The lack of data for younger life history stages for the “browse” matrix was due to the fact that all browsed plants were vegetative and reproductive adults, as no seedlings or yearlings were browsed during the study. However, I was able to directly estimate the survival and growth rates of small vegetative ramets, large vegetative ramets, and reproductive ramets separately for both the browse and no browse matrices. I only used only the 2005-2006 and 2006-2007 transition matrices to parameterize the browse matrix due to lack of data for the 1st annual transition. The no browse matrix included all 3 annual transition matrices.

Estimation of vital rates— In any demographic study, the vital rates (i.e., survival, growth, and fertility) are the basic biological components of the model. These vital rates are used to calculate the matrix elements that compose the transition matrix models

(Morris and Doak 2002). The calculation of individual matrix elements is described in the following section.

I use capital letters throughout this paper to refer to matrix elements, whereas lower case letters refer to vital rates. s_j represents the survival rate for stage class j , which is the expected proportion of individuals in a certain stage class at time t that are still alive at time $t + 1$. g_{ij} refers to growth rate, or the probability an individual in stage class j at time t makes the transition to stage class i at time $t+1$ given survival. f_j is the fertility rate for stage class j . In this analysis, fertility is the average number of offspring (i.e., seed set) of reproductive ramets (**Table 5**).

Calculation of matrix elements from the vital rates— In stage-based matrix models, P_{ij} represents the product of 2 vital rates: s_j , the probability that an individual in stage class j at time t survives to time $t + 1$, and g_{ij} , the probability that a survivor from stage j transitions to class i (Morris and Doak 2002). F_{ij} , the reproduction term in the model, combines both fertility (i.e, seed production) and survival of offspring (Morris and Doak 2002). P_j refers to persistence, which can be represented by stasis, regression, or growth (Caswell 2001). The model presented in **Table 6** outlines the calculation of matrix elements. For more detail in matrix element calculations, see **Appendix I**. Additionally, **Appendix II** includes all 11 transition matrices.

4.3.7 Matrix population model simulations

I performed matrix model simulations in MATLAB v.7.5. I analyzed each population matrix for each transition interval (2004-2005, 2005-2006, 2006-2007) to calculate the following: the deterministic (λ) and stochastic growth rates (λ_s), future population density under stochastic conditions, the stable stage distribution (SSD), the reproductive value for each stage class, the cumulative probability of extinction, and the sensitivity and elasticity values for all vital rates. I performed all analyses and simulations for all 4 types of transition model (i.e., forest seep, wet meadow, browse and no browse matrices).

I. Estimation of the deterministic growth rate (λ) — I first used the transition matrices to project future population size and structure in a deterministic (i.e., constant) environment. The transition matrix condenses the contribution of each stage class at one census to all stage classes at the next census, which is the underlying mechanism by which matrix population models can project the number of individuals in each stage class as an estimate of future population growth (Morris and Doak 2002).

Let \mathbf{A} represent the transition matrix containing matrix elements a_{ij} , which define life history stage transitions from stage j to stage i . Vector $\mathbf{n}(t)$ is a column vector containing the total number of individuals in the population in each life history stage at time t (Caswell 2001). Matrix \mathbf{A} is multiplied by vector $\mathbf{n}(t)$ in order to project population growth by a single time step (i.e., $\mathbf{n}(t + 1)$):

Equation 4.1

$$n(t + 1) = A(t) \times n(t)$$

Equation 4.1 is iterated to solve for the dominant eigenvalue, λ , which represents the deterministic population growth rate. In the simplest deterministic model with only one matrix, over time the population eventually converges to an asymptote, grows at a fixed growth rate (λ) and has a constant proportion of individuals in each life-history stage (Caswell 2001). Analysis of population growth rate is as follows: $\lambda > 1$ implies an increasing population, $\lambda = 1$ implies a stable population, and $\lambda < 1$ implies a decreasing population.

Additionally, the matrix was solved for its unique right and left eigenvectors, which provide useful information about the distributions and values of individuals comprising the population. The right eigenvector (\mathbf{w}) is the stable stage distribution (SSD), which contains the proportion of individuals in each stage class as the population reaches the asymptotic growth rate. The left eigenvector (\mathbf{v}) contains the reproductive values of each stage class, which is informative in deciphering the relative contributions of each stage class to future population growth, taking into account number of offspring produced and offspring survival to reproduction (Caswell 2001, Morris and Doak 2002). I performed the deterministic analysis for all 4 matrix types (i.e., forest seep, wet meadow, browse, and no browse matrices).

The effect of sampling variation— It is important to calculate the variation accounting for sampling error, which causes the variation in a vital rate to overestimate the variability due to true environmental variation (Morris and Doak 2002). Variation in binomial data when individuals have 2 fates is best modeled with the beta distribution when random variables are confined from 0 to 1 (Morris and Doak 2002). Kendall (1998) developed a specific approach using the beta distribution and maximum likelihood methods to estimate sampling variation separate from environmental variation. Kendall's method also provides corrections for vital rates that can be affected by unequal sample sizes and sampling variation (Morris and Doak 2002).

In Kendall's simulation, variation is modeled with a beta distribution where the values are confined to an interval from 0 to 1. However, *P. vanbruntiae* has transition growth rates for which there are more than 2 possible outcomes, for which a multinomial distribution would be best suited to describe the natural distribution of outcomes. Yet, there are problems associated with including multinomial vital rates in a stochastic simulation, as it is not amenable to Kendall's method to correct for low sample sizes (Morris and Doak 2002). Therefore, the best solution is to convert multinomial growth rates to binomial rates. To do this, I designated the probability that a surviving individual in class j will be in class i or any larger stage class (i.e., $g_{\geq i,j}$) in order to classify growth rates as a binary outcome having only two possible results. I subsequently used Kendall's simulation to estimate sampling variation for both growth and survival rates. Then, I reran the deterministic analysis with the corrected vital rates to estimate λ for both

the average forest seep and wet meadow matrix, in order to compare matrices constructed with the raw data and corrected rates.

II. Future population density under stochastic conditions — Once I had examined the transition matrices in a constant environment, I was interested in incorporating the effects of environmental variation to see how this affected population dynamics of *P.*

vanbruntiae. To do this, I simulated population growth of a structured population in a stochastic environment with the assumption that the environmental conditions were “independently and identically distributed” (iid). This assumption asserts that the environmental conditions are aperiodic and uncorrelated (Morris and Doak 2002). Each matrix was chosen in equal frequencies in the simulation under the iid assumption that the probability of choosing a matrix does not change over time.

The simulation begins with population vector n_0 equal to the initial number of individuals in each class in the population. I extrapolated numbers of seedlings, small vegetatives, large vegetatives, and reproductive ramets directly from life history stage analysis from the 0.5m² grids at each demographic site. Because I did not have direct estimates of seeds or yearlings, I estimated the number of individuals representing these 2 life history stages from the deterministic stable stage distributions (SSD). See **Appendix III** for more information on the calculation of population vector n_0 for all 4 matrix types. The simulation ran for 50 years and 50,000 independent realizations.

III. Estimating the stochastic log growth rate (λ_s) — It is most realistic to determine a population's growth rate in a variable environment rather than a constant one. In these more realistic stochastic models, population dynamics are simulated by sampling one of each possible matrices over each time step where each matrix has a constant probability of sampling (Morris and Doak 2002). A first-order Markov chain describes environmental variability. The result of the stochastic simulations is a stationary distribution of population structures to which all initial populations converge (Caswell 2001). In addition, estimating the log population growth rate over a long sequence of years yields the best projection of long-term population growth (Caswell 2001). One common way to project long-term population growth is to link environmental stochasticity to the matrix elements, which are functions of the vital rates (Morris and Doak 2002).

To do this, I used a simulation to estimate stochastic log growth rate (λ_s). Each matrix was drawn at random with equal probability for each time interval in order to calculate $\mathbf{n}(t+1)$ from $\mathbf{n}(t)$ and projected for 50,000 time intervals. I set the number of years of simulated population growth at a high level in order to gain a reasonably accurate λ_s (Caswell 2001). Additionally, the simulation used the total population density in successive years to calculate the arithmetic mean and variance of all simulated population growth increments to generate an estimate of $\log \lambda_s$. The approximate normal distribution also allows an estimation of confidence intervals (Morris and Doak 2002).

IV. Calculating probability of extinction— Calculating population growth under stochastic conditions allows the calculation of risk of population extinction (Caswell 2001). However, it is mathematically impossible for a simulated population to ever reach true 0, so it is necessary to define a quasi-extinction threshold (Ginzburg et al. 1982, Morris and Doak 2002). To measure extinction risk, I ran a simulation using the cumulative distribution function (CDF), which estimates the probability that the population hits the quasi-extinction threshold at any given point from the present time to the future date specified in the simulation.

I set the quasi-extinction threshold to 50 ramets and the probability of extinction in 50 years over 5000 iterations. I ran the simulation in 2 different ways. The first simulation included all 6 life-history stages, and the quasi-extinction threshold was set at 50 total ramets (including seeds in the seed bank, seedlings, and yearlings). The second simulation was run for only small and large vegetative adults and reproductive ramets. The intention of running the second simulation was to predict whether population would reach the extinction threshold of 50 plants representing the larger, more conspicuous life history stages. I ran this simulation because seeds in the seed bank, seedlings and yearlings are more difficult to census in the field, and previous count-based efforts have been focused on estimating number of *P. vanbruntiae* plants in larger life history stages. The starting population density (n_0 vectors) for the matrices was the same as in **Appendix III**.

V. Calculating sensitivities and elasticities of vital rates— I used the vital rate means to calculate deterministic sensitivities and elasticities of λ to vital rates. Sensitivity and elasticity analysis estimates the relative contribution of each vital rate and life history transition to changing future population growth (Morris and Doak 2002). I used deterministic models to estimate sensitivity and elasticity values, because they are simpler and more straightforward to calculate and are valid when compared to sensitivity and elasticity values for stochastic models (Caswell 2001).

Caswell (2001) introduced an elegant method of calculating the sensitivity (S_{ij}) of λ to changes in matrix elements a_{ij} from the dominant left and right eigenvectors (v_i and w_j , respectively) of the transition matrix:

Equation 4.2

$$S_{ij} = \frac{\partial \lambda}{\partial a_{ij}} = \frac{v_i w_j}{\sum_{i=1}^s v_i w_i}$$

where s is the number of stage classes.

Equation 4.2 was used to calculate the derivatives of matrix elements, not for vital rates, which are the true units of the transition matrix. In order to estimate the sensitivity values for vital rates rather than matrix elements, the chain rule for differentiation needs to be employed (Caswell 2001). The chain rule states that if both Y and Z are functions of X , then

Equation 4.3

$$\frac{\partial Z}{\partial X} = \frac{\partial Z}{\partial Y} \frac{\partial Y}{\partial X}$$

In order to make actual comparisons between the sensitivity values of vital rates, it is necessary to scale the sensitivity values to reflect true proportional differences.

Elasticity analysis is the standard way to rescale sensitivity values to reflect the proportional change in λ resulting from a proportional change in a vital rate (Caswell et al. 1984, de Kroon et al. 1986). Therefore, vital rate elasticities measure the proportional contribution to future population growth (Silvertown et al. 1993, Horvitz and Schemske 1995, de Kroon et al. 2000). Elasticity analysis is also useful because the analysis allows a prediction of the response of λ to minor modifications in the vital rates (Morris and Doak 2002).

In order to estimate sensitivities and elasticities of λ to *P. vanbruntiae* vital rates, I defined the transition matrices symbolically with equations used to estimate each matrix element from the underlying vital rates (see **Table 6**). I then used these symbolic functions to find the derivatives to solve for equ. 4.2 and transformed sensitivity values to reflect proportional changes, thus also solving for elasticities of each vital rate.

One problem associated with elasticity analysis is that the analysis tends to identify vital rates with higher means as being the most important (Morris and Doak 2002). In order to examine this issue with *P. vanbruntiae* transition matrices, I examined

the elasticity of both survival and mortality ($1 - \text{survival}$) rates in order to determine if there are differences between these analyses.

In order to estimate the uncertainty of the sensitivity and elasticity analysis, I used a simulation to analyze a range of vital rate values, reflected by the mean value and bounded by their minimum and maximum values. This analysis provides a method to account for variation and uncertainty in each rate using parametric bootstrap to generate confidence limits on the elasticity values (Morris and Doak 2002). I used the symbolic definition of the transition matrix to run 500 replicate randomly chosen matrices for the analysis. This analysis also calculated percentage of variation in λ explained by each vital rate (i.e., r^2 between λ and each vital rate).

4.4 Results

4.4.1 Life cycle analysis of *Polemonium vanbruntiae*

I examined the life cycle of individual ramets of *Polemonium vanbruntiae*, specifically focused on sexual reproduction and ramet behavior (**Figure 8**). A persistent **seed bank** exists in *P. vanbruntiae* populations, although seed bank survival is quite low in both habitats. In the meadow sites, 1% of seeds remained viable in the soil from year 1 to year 2, and 1.6% of seeds remained viable in the soil from year 2-3. Seed bank survival is similar in the forest sites, where 0.8% of seeds remained viable in the first year, and 2% of seeds survived in the seed bank in year 2. The remaining seeds either died (75% and 73.3%, forest and meadow, respectively) or became seedlings.

Seeds germinated and became **seedlings** during May and June. Seedlings characteristically have cotyledons and 1-3 leaves. Seed germination rate in the field

averaged 24% for forest sites and 25% for meadow sites. All seedlings that survived transitioned to the yearling stage in the following year.

Yearlings are defined as seedlings that survived the winter. Yearlings generally remained small with ~3 leaves, but they visually differed morphologically from seedling as they lacked cotyledons. Of the seedlings that survived the first winter in the forest sites, an average of 48% advanced to the yearling stage, whereas 70% of seedlings matured to the yearling stage in the meadow sites. Yearlings either died or became small or large vegetative adults. Twelve percent of yearlings that survived transitioned to small vegetative adults, and 84% skipped the small stage and transitioned directly to the large vegetative stage.

Thirty-three percent of **small vegetative ramets** died at the forest sites, compared to a 52% mortality rate at the meadow sites. For ramets that survived in the meadow sites, an average of 21% remained small, and 26% percent transitioned to the large vegetative stage. Less than 1% transitioned directly from the small vegetative stage to the reproductive adult stage at the meadow sites, but 3% flowered in the following year at the forest sites. The vast majority (41%) of small ramets at the forest sites remained small, but an average of 23% grew to the large vegetative stage.

An average of 44% of **large vegetative adults** died in the meadow sites, whereas the mortality rate was 35% in the forest seep sites. The majority of large vegetative ramets remained large the following year (30% and 33% in the meadow and forest sites, respectively). Fewer large vegetative ramets transitioned to the reproductive adult stages at the meadow sites (7%), compared to 18% at the forest sites. Regression to

the small vegetative stage was not uncommon in either habitat (19% and 12% regression in the meadow and forest, respectively).

Reproductive adult mortality was high as an average of 67% flowering ramets died in year $t+1$ in the meadow sites, and 55% died at the forest seep sites. *Polemonium vanbruntiae* ramets are largely semelparous as 88% and 60% of flowering ramets flowered once and died in the wet meadow and forest seep habitats, respectively. In the wet meadow sites, 10% of flowering ramets flowered twice and died, and only 2% flowered three times. In the forest seep sites, 36% of flowering ramets flowered again in year 2, and 4% of flowering ramets were observed flowering 3 years in a row. I observed no ramets that flowered 4 years in a row. Reproductive adult regression to a vegetative stage class was more common in the forest (23%) than in the meadow (16%).

Life history stage significantly affected survival in year $t + 1$ ($\chi^2 = 130.6$, $p < 0.0001$, $df = 5$). Reproductive ramets suffered the highest mortality rates when compared to small and large vegetative ramets.

All vital rates were statistically equivalent between the 2 habitat types (**Figure 9A**). The wet meadow sites tended to have higher fertility when compared to the forest seep sites, although this trend was only marginally significant (**Figure 9C**; $F_{1,5} = 5.52$, $p = 0.08$).

Deer preferentially browsed adult ramets. At the forest sites, deer browsed 15% of small vegetative ramets, 17% of large vegetative ramets, and 16% of reproductive adults. However, browsing in year 1 did not significantly influence survival in year 2 ($\chi^2 = 0.87$, $p = 0.35$, $df = 1$).

The probability of small vegetative survival and large vegetative stasis was significantly higher for ramets that had been browsed (s_4 : $F_{1,5} = 43.9$, $p = 0.003$; $g_{5,5}$: $F_{1,5} = 6.86$, $p = 0.05$; **Figure 9B**). Yet, unbrowsed ramets had a higher probability of regressing from a large to a small vegetative ramet ($g_{4,5}$: $F_{1,5} = 7.75$, $p = 0.05$).

Fertility was also higher for plants that had not been browsed by white-tailed deer (f_6 : $F_{1,5} = 528.01$, $p < 0.0001$). Deer most often removed most to all of reproductive tissues (i.e., flowers, fruits, and seeds). Therefore, browsed plants suffered a decrease in fertility (**Figure 9C**), which translated into a 3-fold decrease in contributions to the seed bank and contributions to the seedling class was 14-times less compared to non-browsed plants.

4.4.2 Morphological differences between habitat types

Ramets from the forest seep and wet meadow habitats exhibited significantly different vegetative and reproductive morphology for certain life-history stages. Number of leaves for seedlings occurring in the forest and meadow habitats did not differ ($\chi^2 = 0.27$, $p = 0.60$, d.f. = 1), but yearling leaf number was different in the 2 habitat types ($\chi^2 = 14.3$, $p < 0.0001$, d.f. = 1). Yearlings at the meadow sites had significantly more leaves than those at the forest sites (**Table 7**). Small vegetative ramets did not differ in overall size between the 2 habitats ($F_{1, 292} = 0.3$, $p = 0.87$), but large vegetative ramets were significantly larger in the forest sites ($F_{1, 373} = 15.2$, $p < 0.0001$). The MANOVA for reproductive adult vegetative traits was significant ($F_{2, 372} = 15.95$, $p < 0.0001$). Yet, number of leaves completely drove the significant trend ($F_{1, 374} = 27.88$, $p < 0.0001$), as plant height was not different between the 2 habitat types ($F_{1, 375} = 0.007$, $p = 0.93$).

Flowering stalks begin developing during mid-May and elongate until mid-June when flowers begin to bloom. Reproductive adults reach an average height of 81cm with 10 scape leaves. Flowering ramets produce an average of 17 flowers, and 63% of flowers develop into viable fruits, producing an average of 105 seeds per ramet. Seeds are passively dispersed around the parent plant beginning in early September. Seeds are light (mean = 0.9 mg) and wind and summer floods may be possible mechanisms of dispersal (Sabourin 2002).

Number of flowers did not differ between sites ($\chi^2 = 1.82$, $p = 0.17$, $df = 1$), although significant differences were detected for overall plant fitness ($F_{2,90} = 6.14$, $p = 0.003$). Both percent fruit set and seed set was significant higher in the meadow sites (% fruit set: $F_{1,96} = 8.2$, $p = 0.005$; seed set: $F_{1,99} = 4.61$, $p = 0.03$). Seed mass tended to be higher in the forest sites, although this trend was only marginally significant ($F_{1,96} = 3.54$, $p = 0.06$). Increased seed mass may be due to the trade-off between number of seeds set and seed mass. As *P. vanbruntiae* produces more seeds, as in the meadow sites, seed mass decreases (Hill et al. 2008).

Soil analysis of wet meadow and forest seep habitats— In both the wet meadow and forest seep sites, available P (ppm) was low compared to average nutrient levels in Vermont soils (Jokela et al. 2004). Conversely, available K was in the medium range for the forest seep sites (51-100 ppm), and in the optimum range for the wet meadow sites (101-130 ppm). The cation exchange capacity (CEC) measures the amount of positively charged ions (i.e., Ca⁺, Mg⁺, K⁺) that the soil can retain, and CEC values for *P.*

vanbruntiae sites range from 4.8 to 16.2 meq/100g. The low conductivity levels indicate general nutrient deficient conditions for *P. vanbruntiae* sites under natural conditions. Demographic sites contained a wide range of levels for percent organic matter, and the wet meadow site BBC had the highest percent organic matter (see **Appendix IV**). Both forest and meadow sites had a pH < 7.0, indicating an acidic environment. Levels of percent calcium and iron were high in both habitats, but did not vary significantly between forest seep and wet meadow sites (% Ca: $F_{1,8} = 2.28$, $p = 0.18$; Fe: $F_{1,8} = 2.16$, $p = 0.19$). High levels of magnesium and zinc characterize both forest and meadow sites, but percent Mg tended to be higher in forest seep sites, although this trend was only marginally significant (**Figure 10A**; $F_{1,8} = 4.76$, $p = 0.06$). Manganese (Mn) was significantly higher in the wet meadow sites (**Figure 10B**; $F_{1,8} = 6.0$, $p = 0.04$), and meadow sites BBC and BBD had by far the greatest concentration of Mn.

4.4.3 Model simulation results

I. Estimation of the deterministic growth rate (λ) — When future population size and population structure was projected in a deterministic (i.e., constant) environment for each habitat type, λ values were slightly higher in the wet meadow sites when compared to the forest seep sites, but $\lambda > 1$ in each habitat type (**Table 8**). λ ranged from 0.87 to 1.95 for forest seep sites and 1.40 to 2.12 for wet meadow sites over the 3 annual transitions. The 2005-2006 transition was the lowest for the forest seep sites, and the 2006-2007 transition was the lowest for the wet meadow sites. The deterministic analysis implies that the

forest sites increased in 2 of the annual transitions and declined in 1 year, whereas meadow sites increased in all 3 annual transitions.

Future population growth in a constant environment for the browse matrix yielded 1 year when the population increased (2005-2006; $\lambda = 1.16$) and 1 year when the population decreased (2006-2007; $\lambda = 0.67$). However, the average browse matrix predicted an increase of *P. vanbruntiae* ramets over time ($\lambda = 1.08$; **Table 8**). The no browse matrix had an expected future population growth rate nearly twice the browse matrix ($\lambda = 2.05$), and all annual transitions yielded λ values significantly greater than 1.

The dominant right eigenvector (\mathbf{w}), which represents the stable stage distribution (SSD), designates the proportion of individuals in each life history stage, and the values in the vector sum to 1. The SSD for all 4 transition models were similar. Seedlings made up the majority of plants, followed by yearlings and large vegetative plants (**Table 8**). The fewest individuals were represented as seeds in the seed bank, small vegetative plants, and reproductive adults. The dominant left eigenvector (\mathbf{v}) represents the reproductive value of each life history stage. Clearly, reproductive adults have the greatest contribution to future population growth, as this is the only life history stage in the ramet-based model that creates sexually produced offspring.

The effect of sampling variation—To test the effect of environmental variation on the future population growth rate, I compared the λ values computed from the raw vital rate estimates to the λ values computed from the maximum likelihood corrected mean estimates. In general, the raw mean estimate in growth and survival rates agree well with

the maximum likelihood corrected mean (MLE) estimates from Kendall's method (for raw and corrected estimates, see **Appendix V**). Additionally, the computation of deterministic analysis of λ for mean matrices for both forest and meadow sites also yielded similar results. The average wet meadow matrix with the raw estimates yielded a $\lambda = 1.69$, whereas $\lambda = 1.71$ with the corrected mean values. The forest seep matrix with the raw data had a λ value of 1.67, and $\lambda = 1.65$ for the corrected matrix. Nevertheless, the large 95% confidence intervals for the MLE mean and corrected variance estimates reinforce the importance of large sample sizes and collecting long-term data for demographic analysis.

II. Future population density under stochastic conditions — Insofar, the results presented have only examined future population growth of *P. vanbruntiae* ramets in a constant environment. Under stochastic conditions that are aperiodic and uncorrelated (“independently and identically distributed” or “iid”), population density of *P. vanbruntiae* ramets is expected to increase over time in both forest and meadow sites. The meadow sites started with an initial population density of 92,840 ramets and it is projected to increase to a median value of 1.3222e+17 in 50 years (**Figure 11**). The median value is less than the mean value presented in Figure 4, but, due to the lognormal distribution of population density, the median is more informative than the mean value. The projected median number of ramets in the forest sites, beginning with an initial population density of 11,033 ramets is 4.2664e+14. Additionally, both browse and no browse matrices exhibited increasing population density of *P. vanbruntiae* ramets.

Browsed ramets exhibited a slow increase from 7,810 initial ramets to a median of 29,651 ramets in 50 years. The no browse ramets are expected to increase at a much faster rate, from 9132 ramets to $2.7434e+19$ ramets in 50 years. Final population density for all matrices was lognormally distributed, which is a common characteristic for density-independent growth of structured populations in a stochastic environment (Tuljapurkar and Orzack 1980). These values indicate vigorous growth of *P. vanbruntiae* ramets in the near future at both forest seep and wet meadow sites, and under white-tailed deer herbivory.

III. Estimating the stochastic log growth rate (λ_s) — *Polemonium vanbruntiae* ramets in both habitat types are expected to increase under variable environmental conditions in the future (**Figure 12A**). The forest sites have a stochastic population growth rate (λ_s) of 1.57, or a log growth rate of 0.4503 ramets/ramet/year (95% C.I.: 0.4442, 0.4564). Ramets in the meadow sites are also expected to increase in the future at a rate of 0.5081 ramets/ramet/year (95% C.I.: 0.5054, 0.5107), which translates into a stochastic growth rate of 1.66. Both values are far greater than 1, implying that numbers of *P. vanbruntiae* ramets are expected to increase in the future in both habitat types. It is important to note here that the population viability analysis is for ramets, rather than genets, and therefore the analysis presented does not incorporate the entire clonal individual, but can only predict future ramet expansion.

I found similar results for both browse and no browse matrices, although the no browse matrix had significantly higher growth rate ($\lambda_s = 1.99$) than the browse matrix (λ_s

= 1.02; **Figure 12B**). Browsed ramets are only expected to increase at a log growth rate of 0.019 ramets/year (95% C.I.: 0.015, 0.023), but ramets that are not browsed are expected to increase at a rate of 0.693 ramets/year (95% C.I.: 0.687, 0.698).

IV. Calculating probability of extinction — All matrices had a low risk of declining to 50 ramets in 50 years time. The wet meadow, forest seep, and no browse matrix has no detectable risk of quasi-extinction (set at 50 ramets) for both types simulations (i.e., including all 6 life history stages and only including the 3 larger life history stage classes). The browse matrix was the only matrix type that had any quantifiable risk of quasi-extinction. When only the 3 larger stage classes were included in the model, browsed ramets had a 3.1% risk of extinction (**Figure 13**), whereas when all 6 life history stages were evaluated, the risk of extinction declined slightly to 1.1%. Risk of extinction for browsed ramets began to increase at around 12 years, and continued to consistently increase to the maximum future time set at 50 years.

V. Sensitivities and elasticities of vital rates

Comparison of sensitivity and elasticity rates— The mean sensitivity and elasticity rates showed the same trends in highlighting the same vital rates as having the largest influence on future population growth (**Figure 14**). However, the sensitivity values were much larger than the elasticity values. In order to make actual comparisons between the sensitivity values of vital rates, it was necessary to scale the sensitivity values to

elasticities in order to reflect true proportional differences. Elasticity analysis is a better reflection of the proportional change in λ resulting from a proportional change in a vital rate.

Comparison of elasticity values of survival and mortality rates— I examined whether the elasticity of survival and mortality rates gave different results. The importance of the majority of the vital rates did not change when I switched the values to represent survival rather than mortality (**Figure 15**). However, the importance of reproductive adult mortality (s_6) and reproductive adult stasis (g_{66}) was not evident until I switched the elasticity analysis from survival to mortality, but these values were not significantly different. The negative value for g_{11} is simply an error resulting from the simulation-based estimation of very small true values (Morris and Doak 2002). The remaining discussion of elasticity values for forest and meadow sites, and browse and no browse matrices is for survival rates, rather than mortality rates.

Forest and meadow elasticities— The vital rates with the highest mean elasticity values in both the forest seep and wet meadow habitats are survival of seedlings (s_2) and survival of large vegetative adults (s_5) (**Figure 16**). Survival of seedlings (s_2) has a high r^2 value in both the forest and meadow sites, indicating that this vital rate has the potential to change future population growth in both habitats. However, changing survival of large vegetative adults will only have a large impact on future population growth in the meadow sites ($r^2 = 0.36$ for meadow; $r^2 = 0.03$ for forest). Other rates with high mean

elasticity rates in both habitats include survival of yearlings (s_3) and the seedling-to-yearling transition (g_{32}). However, these rates all have low r^2 values (r^2 ranges from 0 to 0.0014). Fertility of reproductive adults (f_6) has a high mean elasticity and a high r^2 value for the meadow sites, but not for the forest sites. Conversely, germination of overwintered seeds had a high elasticity and r^2 value for forest sites, but not meadow sites. The transition from large vegetative-to-reproductive adult is important for both sites, which is indicated by a high mean elasticity value and a high r^2 value.

Browse and no browse elasticities — There were large differences in elasticity values between the “browse” and “no browse” matrices. For the browse model, survival and stasis of large vegetative ramets had the highest combined mean elasticity rates and the highest correlation coefficients ($r^2 = 0.63$ and 0.15 , respectively) of all life cycle transitions (**Figure 17**). These vital rates combined to represent the large vegetative adult self-loop in **Figure 18A**. Other vital rate with a high mean elasticity rate was the large vegetative-to-reproductive adult transition, although correlation coefficient value was comparatively small for this rate ($r^2 = 0.05$). Although survival of reproductive ramets had a relatively high mean elasticity, stasis of reproductive adults (g_{66}) had a low elasticity rate of 0.05 . The reproductive adult self loop represents the product of reproductive adult survival and stasis, and overall, the correlation coefficients was very small for this transition ($r^2 = 0.02$).

The life history transitions with the largest impact on future population growth at no browse sites were different than the browse sites. Although the transition from

large vegetative-to-reproductive adult and reproductive adult survival and stasis had high mean elasticity rates as in the browse sites, the seedling-to-yearling transition moved up in importance for no-browse sites (**Figure 17**). The seedling-to-yearling transition contains the probability of seedling survival, which had the second-highest correlation coefficient in the no-browse sites ($r^2 = 0.15$). Growth of large vegetative ramets to reproductive adults had the highest correlation with future population growth ($r^2 = 0.73$), highlighting the importance of this life history transition in sites not experiencing deer herbivory (**Figure 18B**). The no browse matrix results were similar to the forest seep and wet meadow elasticity analysis (see **Figure 16**).

4.5 Discussion

The demographic analysis indicates that *Polemonium vanbruntiae* ramets are predicted to increase in the future in both habitat types and under white-tailed deer herbivory pressure. There were differences in plant morphology and the nutrient profile between the forest seep and wet meadow habitats, but these differences did not translate into large differences in vital rates or long-term population dynamics. However, the population growth rate decreases sharply and extinction risk increases significantly when ramets are browsed by deer. Elasticity analysis revealed that the importance of certain vital rates shifted as a result of habitat type and presence or absence of deer herbivory.

4.5.1 The effect of deer herbivory on long-term population dynamics

Deer browsing significantly reduced the stochastic population growth rate for *P. vanbruntiae*. However, λ_s was still greater than 1, indicating an increasing population of ramets, but deer browsing decreases the population growth rate dangerously close to experiencing a future decline. Vegetative and reproductive *P. vanbruntiae* ramets were more likely to suffer from deer browsing than younger ramets, which agrees with studies showing a positive relationship between plant size and herbivory risk (Strauss 1988, Hulme 1994, Ehrlén 1995, Alonso and Herrera 1996, Piqueras 1999). The vital rates of unbrowsed plants followed similar trends to those estimated for the wet meadow and forest seep habitats. However, plants that were browsed suffered a sharp decrease in fertility. The reproductive value of flowering ramets decreased 15-fold when compared to the reproductive value of unbrowsed plants, most likely from both the loss of offspring to deer eating flowers and fruits, and from plants spending more of their lives in vegetative stage classes (Knight 2004). Herbivory may cause a shift in allocation of resources from the production of tall flowering ramets, which are conspicuous and browsed at high frequencies, to a shift to clonal growth. An allocation of energy into the production of flowers and fruits under intense herbivore pressure would be wasted at the expense of producing clonal recruits if flowering ramets are browsed. This would also explain the observed trend that wet meadow sites had higher rates of sexual reproduction, as all browsed ramets occurred in forest seep habitats.

Plants that were browsed also experienced a significant shift in the survival and growth of vegetative adult ramets. Browsed ramets had higher survival of small vegetative ramets, plus a higher probability of large vegetative adults remaining large

vegetative adults in year 2. Small and large vegetative ramets, through physiological integration with the genet, may avoid high mortality by drawing on parental reserves for sustenance (Huenneke 1987). This shift in life history strategy may be a method for ramets that suffer high levels of herbivory to allocate more resources to vegetative reproduction than to sexual reproduction. Therefore, small and large vegetative ramets may have a higher probability of being produced by vegetative reproduction, rather than sexual reproduction. Indeed, it has been shown that vegetative propagules often have a higher probability of survival than sexually produced offspring (Lamont 1988, de Kroon and van Groenendael 1997, Hoffmann 1998), which may be especially important under stressful conditions such as herbivory.

White-tailed deer herbivory had a large effect on the future population growth rate of *P. vanbruntiae* ramets, but, even under herbivore pressure, *P. vanbruntiae* ramets are expected to increase in the future. Deer have been noted as keystone species that can incur large direct and indirect effects on plant communities (Rooney and Waller 2003, Côté et al. 2004). Deer herbivory was also shown to be a serious threat to declining populations of perennial herbs *Trillium grandiflorum* (Knight 2004) and *Panax quinquefolia* (McGraw and Furedi 2005). Studies have shown that empirically excluding herbivores from herbaceous plant populations significantly improved plant reproduction, growth, and opportunities for expansion (Bevill et al. 1999, Opperman and Merlander 2000). Deer herbivory is, in fact, a serious environmental pressure experienced by *P. vanbruntiae* populations, as browsing decreased the future population growth rate 2-fold and increased the probability of extinction to detectable levels.

In the no browse matrix, population growth was most sensitive to survival of seedlings and large vegetative ramets transitioning to the reproductive stage. Deer browsing shifted the elasticities of *P. vanbruntiae* vital rates from an emphasis on younger life history stages and sexual reproduction to an emphasis on the survival and growth of large vegetative ramets. Therefore, in *P. vanbruntiae* sites experiencing recurring white-tailed deer herbivory, protection of large vegetative ramets would result in the largest increase in future population growth. This management recommendation should be followed by a note of caution, because elasticity estimates are meant to simulate how small changes in a vital rate would affect future population expansion (Knight 2004). However, conservation managers are often interested in enforcing large changes in the growth rate of a populations of threatened and endangered species, which may not be a practical following the results of elasticity analysis (Mills et al. 1999, de Kroon et al. 2000, Bessinger 2002, Morris and Doak 2002). Fortunately, *P. vanbruntiae* ramets are still expected to increase under herbivore pressure. *Polemonium vanbruntiae* can also reproduce vegetatively, and, thus, populations have an additional safeguard against extinction.

Given the importance of vegetative survival and growth, clonal propagation may release *P. vanbruntiae* from the negative impact of deer herbivory by providing a risk-spreading strategy within the clone. For example, when ramets are browsed within a clone, spreading the risk of herbivore browsing minimizes the risk of genet extinction by spreading the risk of mortality among ramets (Cook 1985, Eriksson and Jerling 1990, Piqueras 1999). In general, clonal plant population dynamics change very little to various

disturbance regimes, and, therefore, have the ability to “buffer” variation in local environmental conditions (Eriksson 1994). Resource allocation and risk spreading among physically connected ramets may buffer genet population growth differently than was observed in this ramet-level investigation. Therefore, the combination of the potential for vegetative reproduction and a slowly increasing ramet growth under herbivore pressure indicates future persistence of existing *P. vanbruntiae* populations.

4.5.2 The influence of habitat type

Habitat type significantly influenced the morphology of *P. vanbruntiae* ramets. Overall, vegetative and reproductive ramets were larger in the forest seep habitat. Even though I did not directly test the mechanism driving morphological differences in forest and meadow habitats, preliminary evidence points to interspecific competition as a potential driver of these overall patterns. The associated plant communities differ greatly between meadow and forest sites, as forest sites have a significantly lower frequency of wetland grasses. Although *P. vanbruntiae* ramets existing at the forest sites may receive less sunlight resources due to the high degree of canopy cover from woody trees and shrubs, these ramets are under less competitive stress. Conversely, the wet meadow sites contain a high frequency of grasses, including the invasive reed canarygrass (*Phalaris arundinacea*). Reed canarygrass is an official “pest species” in the U.S. due to its broad habitat tolerance, phenotypic plasticity, and competitive dominance (Comes 1971, Marten 1985). Preliminary research has shown that reed canarygrass decreases *P. vanbruntiae* leaf number, leaf area, above-ground biomass, and below-ground biomass

(measured indirectly as basal area), thus demonstrating its potential to reduce the growth of *P. vanbruntiae* (Hill Birmingham, *unpublished data*). However, it remains unknown whether all grasses occurring at the wet meadow sites are as competitively aggressive as reed canarygrass. Nonetheless, *P. vanbruntiae* ramets occurring at forest sites may be larger as a result of existing in a less competitive environment.

Both forest seep and wet meadow habitats were acidic and generally contained low to moderate levels of nutrients. In temperate wetlands, seasonal flooding may cause nutrient input (Baldwin and Mitchell 2000, Keddy 2000), therefore maintaining moderate levels of nutrients in *P. vanbruntiae* sites. Both habitats are characterized as having high levels of calcium and iron, and wet meadow sites were high in potassium. High K at the meadow sites may also reflect the high occurrence of grasses, which have an increased potential to forage for available K due to fibrous root systems (Jokela et al. 2004). In wet meadow sites, *P. vanbruntiae* often co-occurs with reed canarygrass. Many studies have shown increased nutrient pools in topsoil in response to invasion (e.g. Musil 1993, Scott et al. 2001, Duda et al. 2003, Vanderhoeven et al. 2005, Chapuis-Lardy et al. 2006) because invasive species are more efficient at uptaking nutrients, which are returned to the site in litterfall (Dassonville et al. 2008). The large amount of above-ground biomass at wet meadow sites, especially site BBC, may also explain the high percent organic matter due to high rates of decaying plant material, especially for the productive reed canarygrass.

An increase in resource allocation to sexual reproduction in the wet meadow sites may also be a result of interspecific competition with reed canarygrass (Gardner and

Mangel 1999, van Kleunen et al. 2001). Allocating resources to sexual reproduction is a means of dispersal, and may be a mechanism to escape invaded habitats (Gardner and Mangel 1999, Prati and Schmid 2000, Sakai 1995), and for colonize new sites (Tamm 1972, Eriksson 1997, Verburg et al. 2000). In addition, population growth rates of herbaceous plants occurring in forested habitats often rely more heavily on growth, including clonal reproduction, and survival, while plants of other habitats rely more on seed production (Bierzychudek 1982, Silvertown et l. 1993, Silvertown et al. 1996).

Similarity in the vital rates of *P. vanbruntiae* ramets in the forest seep and wet meadow habitats is reflected in the remarkably similar population dynamics between the 2 habitats. Even though *P. vanbruntiae* ramets were smaller in meadow sites, ramets are expected to increase at a faster rate in the meadow compared to forest sites. Deterministic estimates of λ in both habitats consistently overestimated the population growth rate when compared to population growth simulated in a more realistic, stochastic environment (Tuljapurkar 1982, Menges 1992, Benton and Grant 1996). Nonetheless, ramets are expected to increase in both ramet number and ramet density in the future under stochastic conditions in both habitat types, and there is no clear risk of *P. vanbruntiae* ramet extinction in either habitat.

Elasticity analysis revealed that seedling survival and growth were the vital rates contributing most to future population growth in both forest and meadow habitats, highlighting the importance of offspring recruitment and survival in *P. vanbruntiae* populations. In other clonal species, seedling recruitment has also been found to be important for population growth (de Kroon et al. 1987, Kéry et al. 2000). This may be

due to the fact that *P. vanbruntiae* seeds lack a method of long-distance dispersal, can remain dormant in the seed bank, and seedlings frequently occur in nature. Conversely, seeds in the seed bank had little to no effect on population dynamics and future population growth. The seed bank may only be important if a natural disaster kills all above ground plants at a particular site in which the seed bank or recruitment from other sites would be the only method of persistence. Taken together, these factors suggest that seed production is of importance in natural populations of *P. vanbruntiae*, as has been found in other clonal herbs (e.g., de Kroon et al. 1987, Kéry et al. 2000, Berg 2002). Therefore, I conclude that *P. vanbruntiae*'s mixed mating strategy, including clonal reproduction and maximizing seed production and seedling recruitment under certain habitat conditions, has significance for the growth and long-term persistence of *P. vanbruntiae* populations.

Survival of yearlings (s_3) and the seedling-to-yearling transition (g_{32}) both had high elasticity rates and high average values, indicating a significant contribution to future population growth. However, elasticity analysis must be carefully interpreted, as the vital rates that have the highest elasticities are not always the most important for population growth (de Kroon et al. 2000). Because elasticity analysis tends to identify vital rates with higher means as being the most important (Morris and Doak 2002), the high elasticity values for these rates may be artifacts of the analysis. In addition, both s_3 and g_{32} had very low correlation coefficients, which brings the actual importance of these rates to changing future population growth into question. Therefore, I am not confident that yearling vital rates have great importance to future *P. vanbruntiae* population

growth, and I do not expect that parameterizing all 4 transition model types with yearling data from the meadow sites significantly affected the results of this study.

Survival and growth of large vegetative ramets at the forest and meadow sites also contributed significantly to future population growth. Vegetative ramets play a major role in resource acquisition, which is beneficial to other ramets of the genet. Genets with physically connected ramets may shunt resources to flowering ramets within the clone while vegetative ramets gather resources from the local environment (Araki and Ohara 2008). Within the clone, *P. vanbruntiae* ramets may also transition between flowering and vegetative phases as a method of switching between resource acquisition (vegetative) and sexual reproduction (flowering) in order to accomplish multiple years of the production of flowers, fruits, and seeds within a single ramet. Yet, Damman and Cain (1998) found that flower production was positively correlated with clonal reproduction in the clonal herb *Asarum canadense*, so factors that affect sexual reproduction in *A. canadense* also affected rates of vegetative reproduction.

The balance of sexual and clonal modes of reproduction may create an evolutionary predicament, as there is an inherent trade-off in partitioning each method of reproduction within a genet (Abrahamson 1980). If the population is growing rapidly, seedling development is slow, and seedling survival is low, increases in clonal reproduction will be favored at the expense of sexual reproduction (Caswell 1985). This is not the case for the *P. vanbruntiae* sites examined in this demographic analysis, as reproductive adults produce a range of 50-200 seeds per year, of which 7 to 40% germinate the following season. Seedling survival is variable, but survival rates are

generally high, especially in the wet meadow habitats. Also, for clonal plants, the production of new ramets via clonal growth at the expense of new sexually-produced genets tends to be favored when genet survival is high, which translates into a large gain in the reproductive value by growth (i.e., production of new ramets) to the next stage (Caswell 1985). Because the demographic model presented is ramet-based, rather than genet-based, I am unable to draw valid conclusions regarding genet survival and long-term dynamics. Nonetheless, ramet survival was relatively high in both habitat types and *P. vanbruntiae* ramets are expected to increase both in number and in density in the future.

4.5.3 Do the results of this study change the status of *Polemonium vanbruntiae* as a rare species?

Damman and Cain (1998) discovered that numbers of ramets of the herbaceous perennial *Asarum canadense* are expected to decrease slowly over time, whereas the number of genets are expected to increase in the future. The demographic analysis of *P. vanbruntiae* presented here found the opposite result at the ramet-level (i.e., ramets are expected to increase over time). However, the importance of the Damman and Cain (1998) study is to highlight that the fates of ramets and genets do not necessarily follow the same trajectory. However, most studies assume that demographic patterns of genets may be inferred from that of ramets (Silvertown et al. 1993), but this clearly is a faulty inference.

At this time, I am unable to conclude that increases in genet number are an inevitable consequence of predicted increases *P. vanbruntiae* ramets. In addition, the demographic analysis only provided a 3-year glimpse into the long-term patterns of *P.*

vanbruntiae populations, whereas population viability analyses are best informed with 5+ years of data (Menges 2000). Also, I only analyzed 9 sites within a restricted area of *P. vanbruntiae*'s entire geographic range. Therefore, the demographic study is narrow in scope both temporally and spatially and cannot be used to infer that threatened species *Polemonium vanbruntiae* should no longer continue to be considered a rare species of conservation concern.

Polemonium vanbruntiae occurs in habitats that are vulnerable to human and natural disturbance. For example, 4 of the 9 demographic sites in this analysis occur immediately adjacent to roads. Road graders were been found to increase extinction risk in plant populations (de Kroon et al. 1987, Kiviniemi 2002), and roads and disturbed areas may provide corridors and suitable habitats for invasive species. Populations of rare endemic plants that occur along the roadside may be more susceptible to species invasion (Hobbs 2001). This is especially true for wetland invasives reed canarygrass (*Phalaris arundinacea*) and purple loosestrife (*Lythrum salicaria*), in which roads, particularly drainage ditches, act as dispersal corridors (Apfelbaum and Sams 1987, Wilcox 1989). Additionally, although *P. vanbruntiae* is a wetland species, flooding may cause the permanent loss of populations and suitable habitat. In fact, heavy rains caused river flooding, which is the suspected cause of extinction for one Vermont subpopulation of *P. vanbruntiae* and one population in Pennsylvania (Deller 2002). In addition, 1 demographic site occurs immediately adjacent to a beaver pond, so flooding may be an inevitable consequence for this site.

Polemonium vanbruntiae is a globally threatened species with a G3 status, which indicates that this species has a restricted range and relatively few populations globally (NatureServe 2008). Where *P. vanbruntiae* occurs, populations are small in area and form a dense aggregation of ramets, which are expected to increase over time. However, if anthropogenic or natural forces destroy these habitats, it may limit *P. vanbruntiae*'s ability to persist over time due to unavailability of suitable habitat. Even if suitable habitats occur in space, *P. vanbruntiae* seeds are passively dispersed which may lead to dispersal limitation to new sites. All in all, protection and conservation attention must still be afforded to *P. vanbruntiae* in order to ensure future persistence of this rare species.

4.6 References

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Table 4: Associated plant community for each *Polemonium vanbruntiae* demographic site located in the Green Mountain National Forest of central Vermont. Most woody vegetation occurs interspersed with *P. vanbruntiae* in the forest seep sites (A) and on the periphery of the wet meadow *P. vanbruntiae* sites (B).

(A) Forest seep sites

POPULATION	ASSOCIATED PLANT SPECIES	
	Woody vegetation	Herbaceous layer
Abbey Pond (AP)	<i>Acer rubrum</i> <i>Acer saccharum</i> <i>Alnus rugosa</i> <i>Fagus americana</i> <i>Picea rubens</i> <i>Viburnum alnifolium</i>	<i>Carex crinita</i> <i>Galium palustre</i> <i>Hydrocotyle americana</i> <i>Impatiens capensis</i> <i>Onoclea sensibilis</i> <i>Thalictrum pubescens</i> <i>Thelyptris palustris</i>
Blue Banks subpopulation E (BBE)	<i>Abies balsamea</i> <i>Acer rubrum</i> <i>Picea rubra</i>	<i>Arisaema triphyllum</i> <i>Equisetum sylvaticum</i> <i>Laportea canadensis</i> <i>Thalictrum pubescens</i>
Duclos Rd. (DR)	<i>Acer rubrum</i> <i>Acer saccharum</i> <i>Alnus incana</i> <i>Cornus</i> spp. <i>Rubus pubescens</i>	<i>Carex crinita</i> <i>Equisetum sylvaticum</i> <i>Galium palustre</i> <i>Geum rivale</i> <i>Impatiens capensis</i> <i>Onoclea sensibilis</i> <i>Symphyotrichum puniceum</i>
Forest Rd. 233 (FR)	<i>Acer rubrum</i> <i>Acer saccharum</i> <i>Betula alleghaniensis</i>	<i>Carex crinita</i> <i>Galium palustre</i> <i>Hydrocotyle americana</i> <i>Impatiens capensis</i> <i>Polygonum sagittatum</i> <i>Viola cucullata</i>
North Branch subpop. F (NBF)	<i>Abies balsamea</i> <i>Betula lenta</i> <i>Hamamelis virginiana</i>	<i>Caltha palustris</i> <i>Carex crinita</i> <i>Galium palustre</i> <i>Impatiens capensis</i> <i>Saxifraga pensylvanica</i> <i>Symphyotrichum puniceum</i> <i>Thalictrum pubescens</i> <i>Tiarella cordifolia</i>

(B) Wet meadow sites

POPULATION	Woody vegetation	Herbaceous layer
Blue Banks subpop. C (BBC)	<i>Abies balsamea</i> <i>Acer rubrum</i> <i>Betula papyifera</i> <i>Salix alba</i>	<i>Calamagrostis canadensis</i> <i>Carex crinita</i> <i>Equisetum sylvaticum</i> <i>Eupatorium maculatum</i> <i>Galium asprellum</i> <i>Galium palustre</i> <i>Hydrocotyle americana</i> <i>Impatiens capensis</i> <i>Onoclea sensibilis</i> <i>Phalaris arundinacea</i> <i>Polygonum sagittatum</i> <i>Sphagnum angustifolium</i>
Blue Banks subpop.D (BBD)	<i>Acer rubrum</i> <i>Amelanchier arborea</i> <i>Populus tremuloides</i>	<i>Calamagrostis canadensis</i> <i>Carex crinita</i> <i>Dryopteris intermedia</i> <i>Eupatorium maculatum</i> <i>Galium asprellum</i> <i>Impatiens capensis</i> <i>Onoclea sensibilis</i> <i>Osmunda claytoniana</i> <i>Osmunda cinnamomea</i> <i>Phalaris arundinacea</i> <i>Veratrum viride</i>
Camel's Hump State Forest (CH)	<i>Acer rubrum</i> <i>Betula papyifera</i> <i>Malus</i> spp. <i>Populus tremuloides</i> <i>Rubus pubescens</i> <i>Salix alba</i> <i>Spiraea latifolia</i>	<i>Asclepius syriaca</i> <i>Calamagrostis canadensis</i> <i>Carex crinita</i> <i>Clematis virginiana</i> <i>Dennstaedtia punctilobula</i> <i>Galium palustre</i> <i>Geum rivale</i> <i>Hydrocotyle americana</i> <i>Hypericum perforatum</i> <i>Onoclea sensibilis</i> <i>Prunella vulgaris</i> <i>Symphotrichum</i> spp. <i>Thalictrum pubescens</i> <i>Thelypteris palustris</i>
North Branch subpop. E (NBE)	<i>Abies balsamea</i> <i>Hamamelis virginiana</i> <i>Betula lenta</i>	<i>Calamagrostis canadensis</i> <i>Caltha palustris</i> <i>Carex crinita</i> <i>Chelone glabra</i> <i>Galium palustre</i> <i>Impatiens capensis</i> <i>Thalictrum pubescens</i> <i>Tiarella cordifolia</i> <i>Saxifraga pensylvanica</i> <i>Symphotrichum puniceum</i> <i>Veratrum viride</i>

Table 5: Description and abbreviations for *P. vanbruntiae* vital rates. Stages: 1 = seed; 2 = seedling; 3 = yearling; 4 = small vegetative; 5 = large vegetative; 6 = reproductive adult.

Vital rate abbreviations	Vital rate description
s_1	Seeds surviving the 8-month overwinter period in the soil
s_2	Seedling survival
s_3	Yearling survival
s_4	Small vegetative adult survival
s_5	Large vegetative adult survival
s_6	Reproductive adult survival
g_{11}	Seeds that do not germinate and remain viable in the seed bank
g_{21}	Proportion of seeds that survived the winter in the soil and germinated
g_{32}	Seedling-to-yearling transition given winter seedling survival
g_{43}	Yearling-to-small vegetative transition given winter yearling survival
g_{53}	Yearling-to-large vegetative transition given winter yearling survival, skipping the small vegetative stage
g_{44}	Survival and stasis of small vegetative adults
g_{54}	Growth from a small to large vegetative adult
g_{64}	Growth of a small vegetative to a reproductive adult, skipping the large vegetative stage
g_{45}	Regression of large vegetative ramets to the small vegetative stage
g_{55}	Survival and stasis of large vegetative adults
g_{65}	Growth of large vegetative to reproductive adults
g_{46}	Regression of reproductive adults to small vegetative ramets
g_{56}	Regression of reproductive to large vegetative ramets
g_{66}	Survival and stasis of reproductive adults
f_6	Seed set of reproductive adults

Table 6: Matrix population model parameters and equations for the calculation of matrix elements from the underlying survival (s_j), growth (g_{ij}), and fertility (f_j) rates. The subscripts represent placement of the matrix element in the matrix, represented by row, column (i.e., $F_{1,6}$ is the fecundity value in row 1, column 6).

	Seed	Seedling	Yearling	Small vegetative	Large vegetative	Reproductive adult
Seed	$\mathbf{P}_{1,1} = s_1 \times (1 - g_{21}) \times g_{11}$	0	0	0	0	$\mathbf{F}_{1,6} = f_6 \times s_1 \times (1 - g_{21}) \times g_{11}$
Seedling	$\mathbf{P}_{2,1} = s_2 \times g_{21} \times (1 - g_{11})$	0	0	0	0	$\mathbf{F}_{2,6} = f_6 \times s_2 \times g_{21} \times (1 - g_{11})$
Yearling	0	$\mathbf{P}_{3,2} = s_2 \times g_{32}$	0	0	0	0
Small vegetative	0	0	$\mathbf{P}_{4,3} = s_3 \times g_{43}$	$\mathbf{P}_{4,4} = s_4 \times g_{44}$	$\mathbf{P}_{4,5} = s_5 \times g_{45}$	$\mathbf{P}_{4,6} = s_6 \times g_{46}$
Large vegetative	0	0	$\mathbf{P}_{5,3} = s_3 \times g_{53}$	$\mathbf{P}_{5,4} = s_4 \times g_{54}$	$\mathbf{P}_{5,5} = s_5 \times g_{55}$	$\mathbf{P}_{5,6} = s_6 \times g_{56}$
Reproductive adult	0	0	0	$\mathbf{P}_{6,4} = s_4 \times g_{64}$	$\mathbf{P}_{6,5} = s_5 \times g_{65}$	$\mathbf{P}_{6,6} = s_6 \times g_{66}$

Table 7: Vegetative morphology of *Polemonium vanbruntiae* ramets in forest seep and wet meadow habitat types. Plant size for vegetative ramets was calculated as the product of average leaf length (cm) and number of leaves. Asterisks represent significant differences between habitat types. Values represent mean \pm 1 S.D.

		VEGETATIVE CHARACTER		
LIFE HISTORY STAGE	HABITAT TYPE	No. leaves	Plant size	Height (cm)
Seedling	<i>Forest seep</i>	3 \pm 0.7	N/A	N/A
	<i>Wet meadow</i>	3.1 \pm 1.1	N/A	N/A
Yearling	<i>Forest seep</i>	2.6 \pm 1.0**	N/A	N/A
	<i>Wet meadow</i>	3.2 \pm 0.5**	N/A	N/A
Small vegetative	<i>Forest seep</i>	3.8 \pm 2.0*	78.2 \pm 57.1	19.1 \pm 7.2
	<i>Wet meadow</i>	3.3 \pm 1.7*	71.3 \pm 50.1	19.6 \pm 6.7
Large vegetative	<i>Forest seep</i>	5.9 \pm 2.7**	237.2 \pm 137.5**	39.2 \pm 6.9
	<i>Wet meadow</i>	4.7 \pm 2.3**	188.7 \pm 111.6**	39.9 \pm 7.7
Reproductive	<i>Forest seep</i>	10.9 \pm 3.3**	N/A	80.4 \pm 11.3
	<i>Wet meadow</i>	9.4 \pm 2.8**	N/A	82.1 \pm 17.8

* $p < 0.05$

** $p < 0.0001$

Table 8: The deterministic population growth rate (λ), stable stage distribution (w), and reproductive values (v) for the average of 3 annual transition matrices for both habitat types and “browse” and “no browse” sites. The stable stage distribution and reproductive value vectors are arranged so that each value corresponds to life history stages in the following order: seed, seedling, yearling, small vegetative, large vegetative, and reproductive adult.

MATRIX TYPE	λ	w	v
Forest	1.67	0.00 0.65 0.18 0.03 0.12 0.02	1.00 7.46 28.00 18.63 53.23 379.93
Meadow	1.69	0.00 0.56 0.24 0.04 0.15 0.01	1.00 7.58 17.67 9.77 34.32 668.75
Browse	1.08	0.00 0.34 0.23 0.07 0.29 0.07	1.00 5.01 7.66 20.46 6.99 35.59
No browse	2.05	0.00 0.63 0.22 0.03 0.11 0.01	1.00 9.14 25.80 20.52 60.23 540.69

4.7 Figure Legends

Figure 8: Life cycle graph for the sexual reproductive cycle of *Polemonium vanbruntiae* ramets.

Figure 9: Survival and growth rates for each habitat type (A) and under 2 white-tailed deer herbivory scenarios (B). Asterisks in (B) represent significant differences between browse and no browse matrices. Fertility rates in (C) represent each habitat type and each white-tailed deer browsing scenario. Errors bars represent the S.E.M.

Figure 10: Soil characteristics of wet meadow and forest seep habitats, including percent calcium and magnesium (A) and available iron and manganese (B). Asterisks denote significant differences between habitat types.

Figure 11: Prediction of population density of *P. vanbruntiae* ramets in 50 years for wet meadow sites. Note that the x-axis is on a scale of 10^{19} .

Figure 12: Estimation of stochastic population growth rate (λ_s) for different *P. vanbruntiae* habitat types (A) and under two white-tailed deer browsing scenarios (B). Note the differences in scale in y-axes. The error bars represent 95% confidence intervals.

Figure 13: Probability of extinction in 50 years for *P. vanbruntiae* ramets experiencing white-tailed deer herbivory. The lines represent the cumulative probability of quasi-extinction to 50 ramets from 10 simulations including only the small vegetative, large vegetative, and reproductive adult life history stages.

Figure 14: Comparison of mean elasticity and sensitivity rates for wet meadow sites.

Figure 15: A comparison of mean elasticity values of survival and mortality rates in the no browse matrices. Error bars represent the 95% confidence interval.

Figure 16: Mean elasticities of vital rates for the forest seep and wet meadow habitats with 95% confidence intervals. Asterisks (*) denote elasticity values with high correlation coefficients (r^2) in the forest seep sites, and + denotes high r^2 values of elasticity values in the meadow sites.

Figure 17: Mean elasticities of vital rates for the browse and no browse sites with 95% confidence intervals. Asterisks (*) denote elasticity values with high correlation coefficients (r^2) in the browse sites, and + denotes high r^2 values of elasticity values in the no browse sites.

Figure 18: Elasticities for life cycle transitions of ramets in the browse (A) and no browse (B) matrices. Bold values represent the most important transitions.

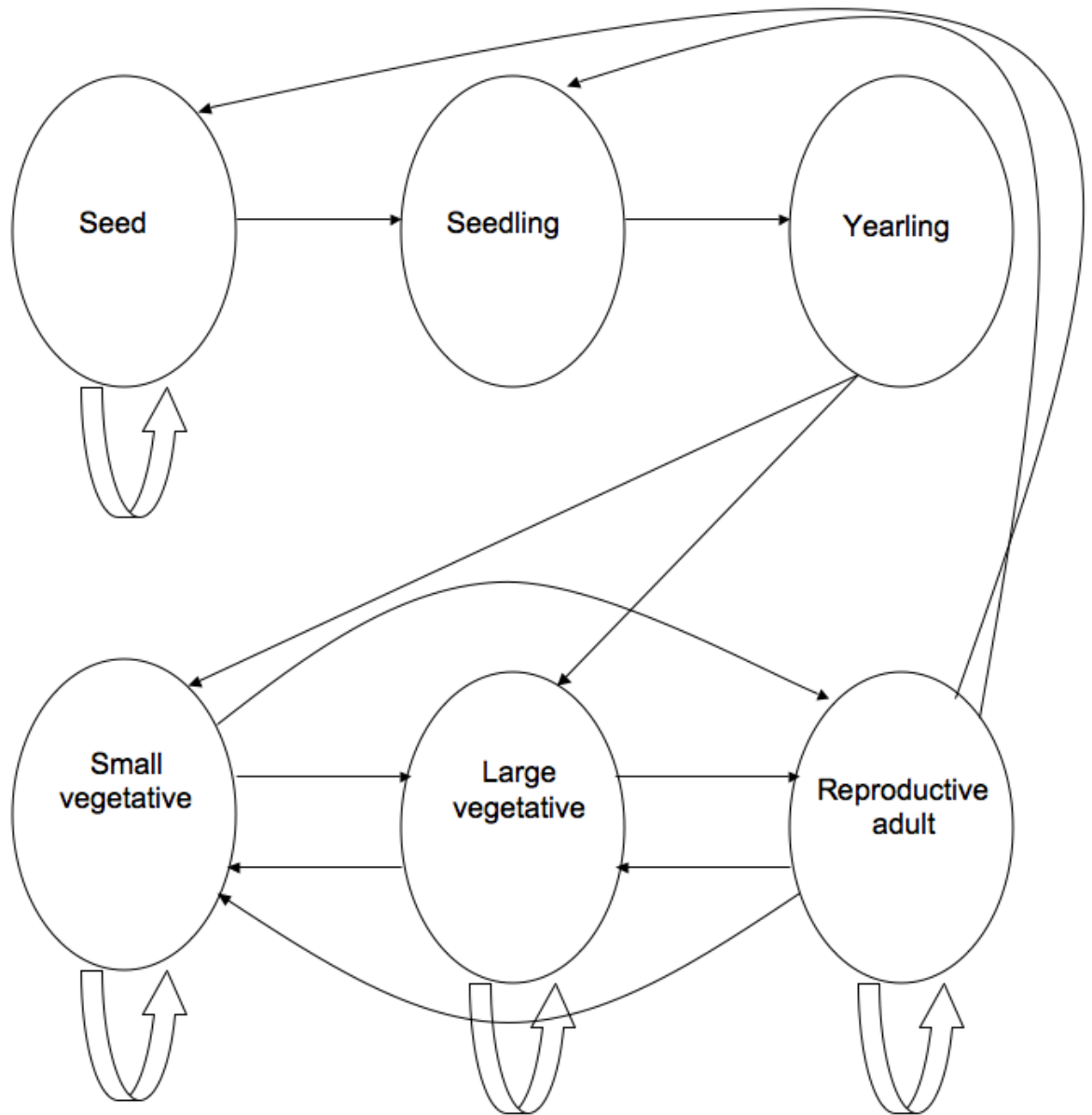
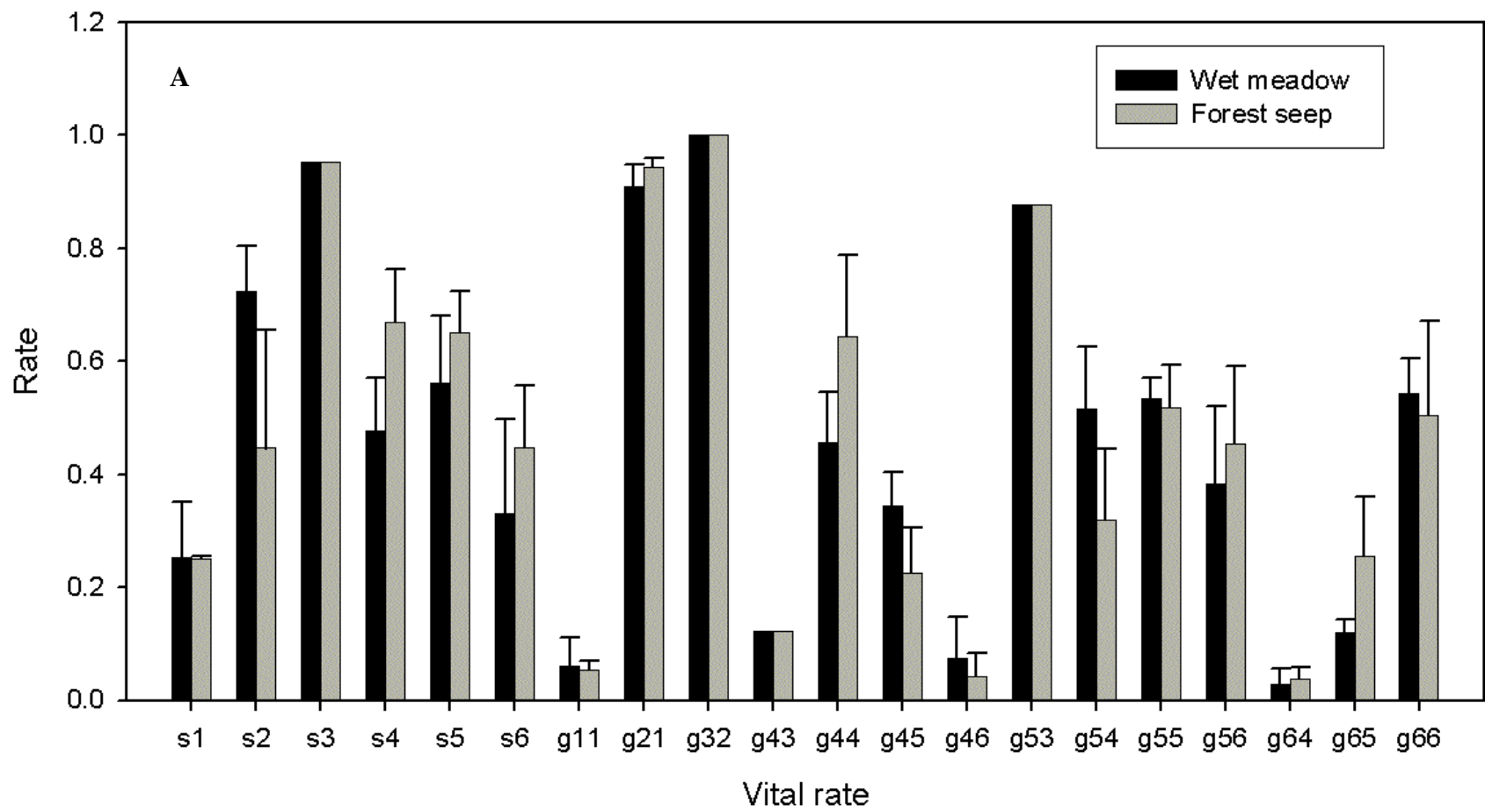
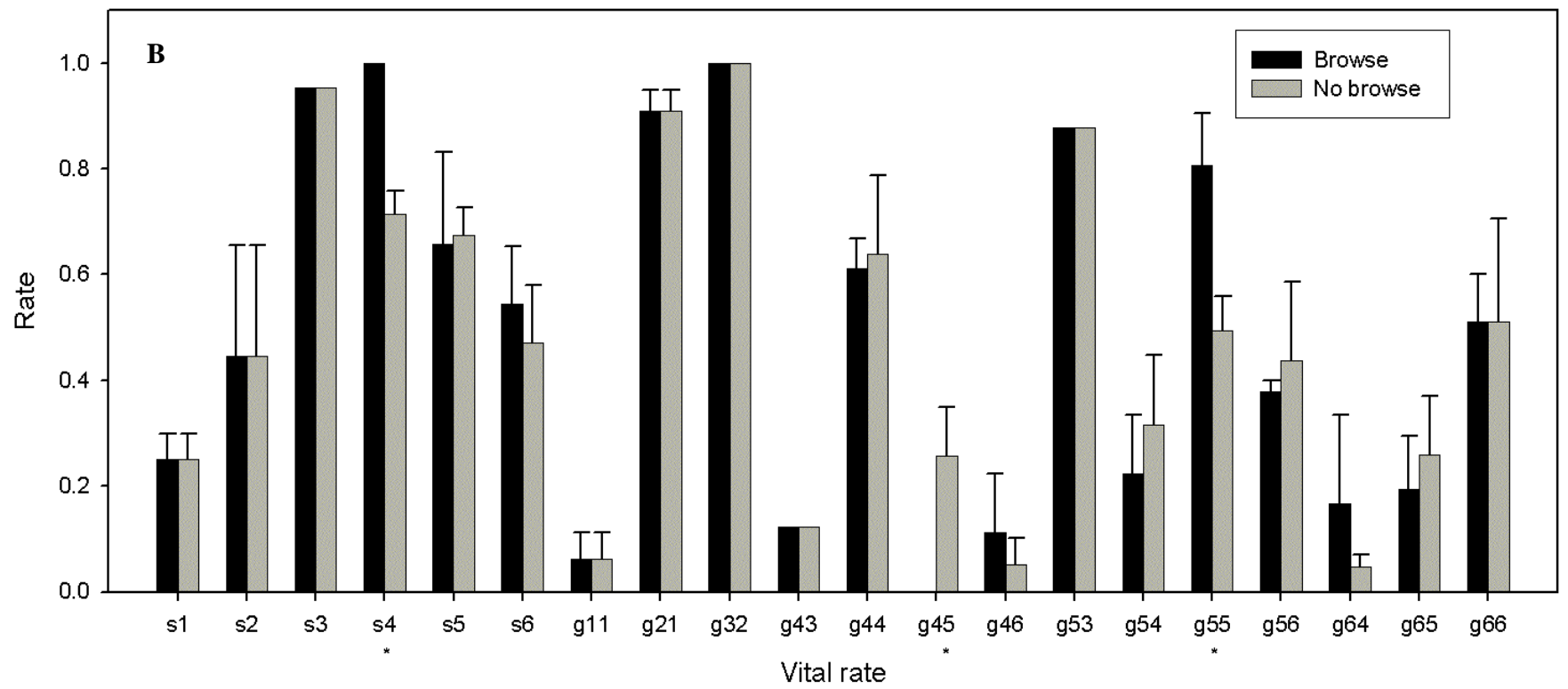


Figure 8: Life cycle graph for the sexual reproductive cycle of *Polemonium vanbruntiae* ramets.





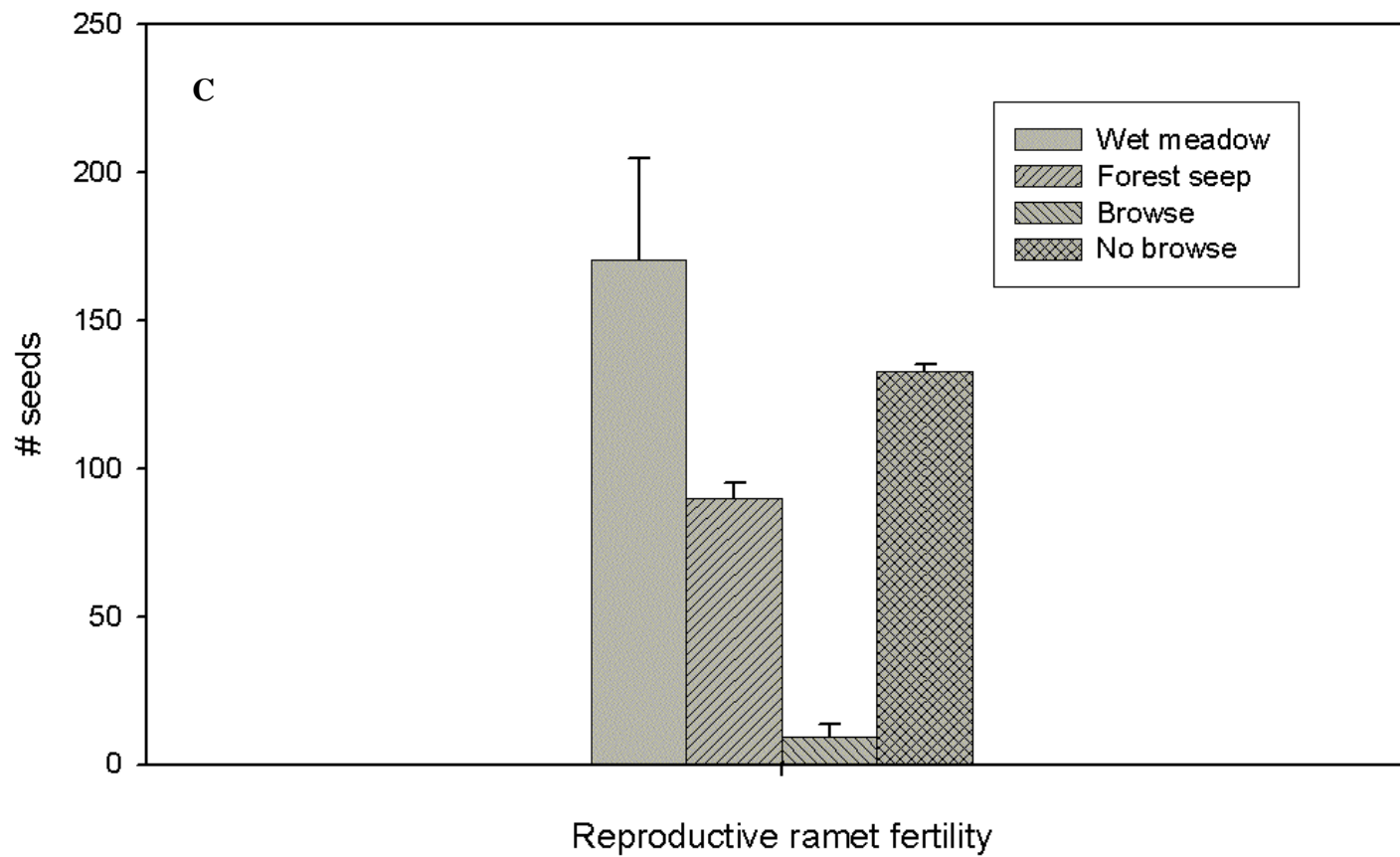


Figure 9: Survival and growth rates for each habitat type (A) and under 2 white-tailed deer herbivory scenarios (B). Asterisks in (B) represent significant differences between browse and no browse matrices. Fertility rates in (C) represent each habitat type and each white-tailed deer browsing scenario. Errors bars represent the S.E.M.

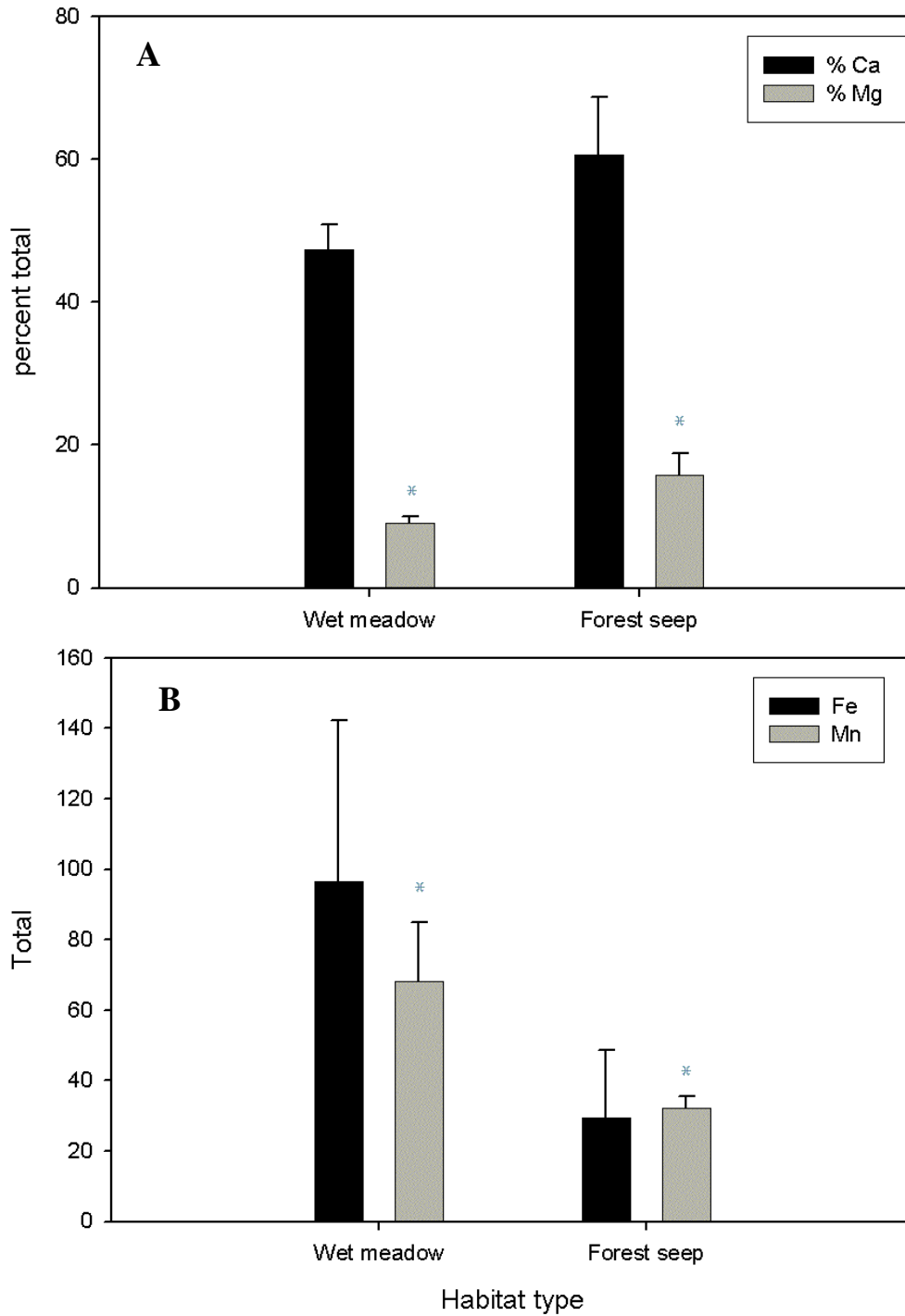


Figure 10: Soil characteristics of wet meadow and forest seep habitats, including percent calcium and magnesium (A) and available iron and manganese (B). Asterisks denote significant differences between habitat types.

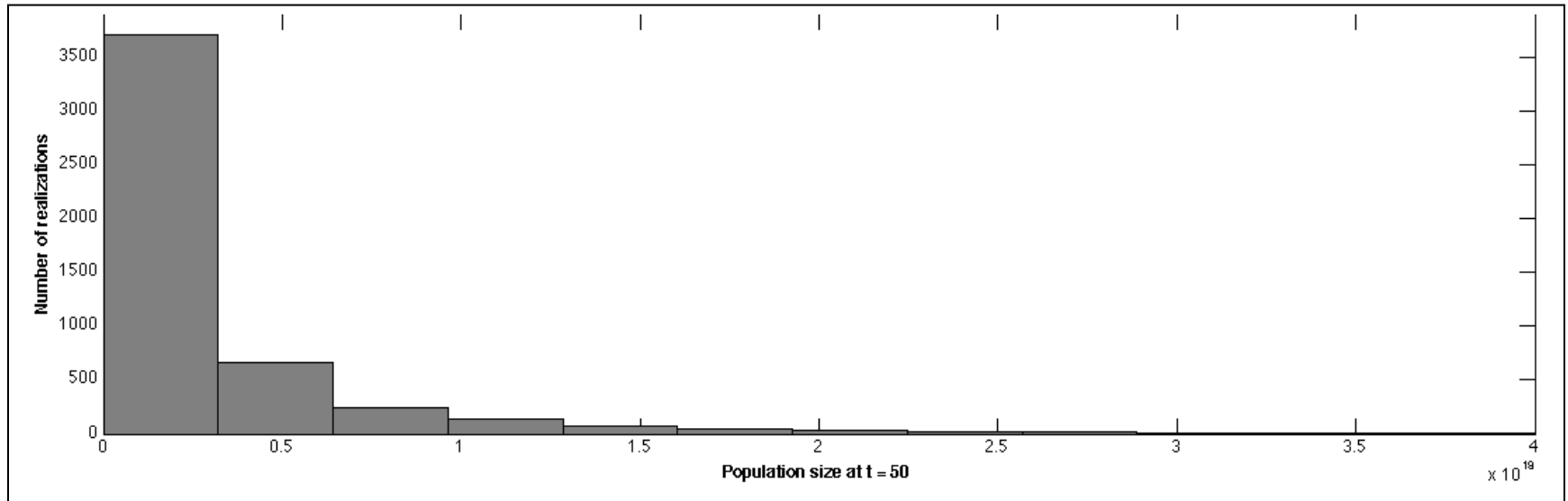
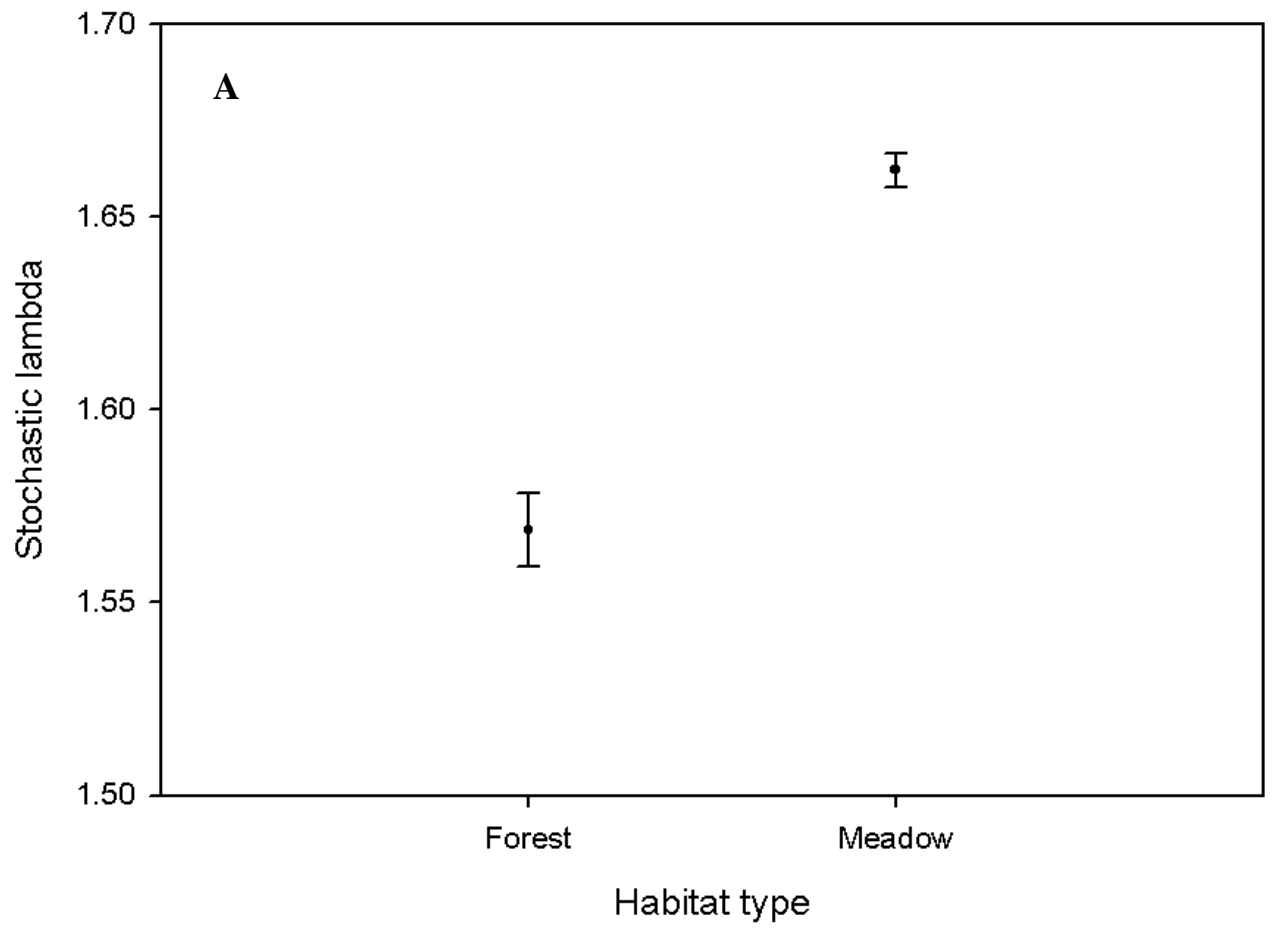


Figure 11: Prediction of population density of *P. vanbruntiae* ramets in 50 years for wet meadow sites. Note that the x-axis is on a scale of 10^{19} .



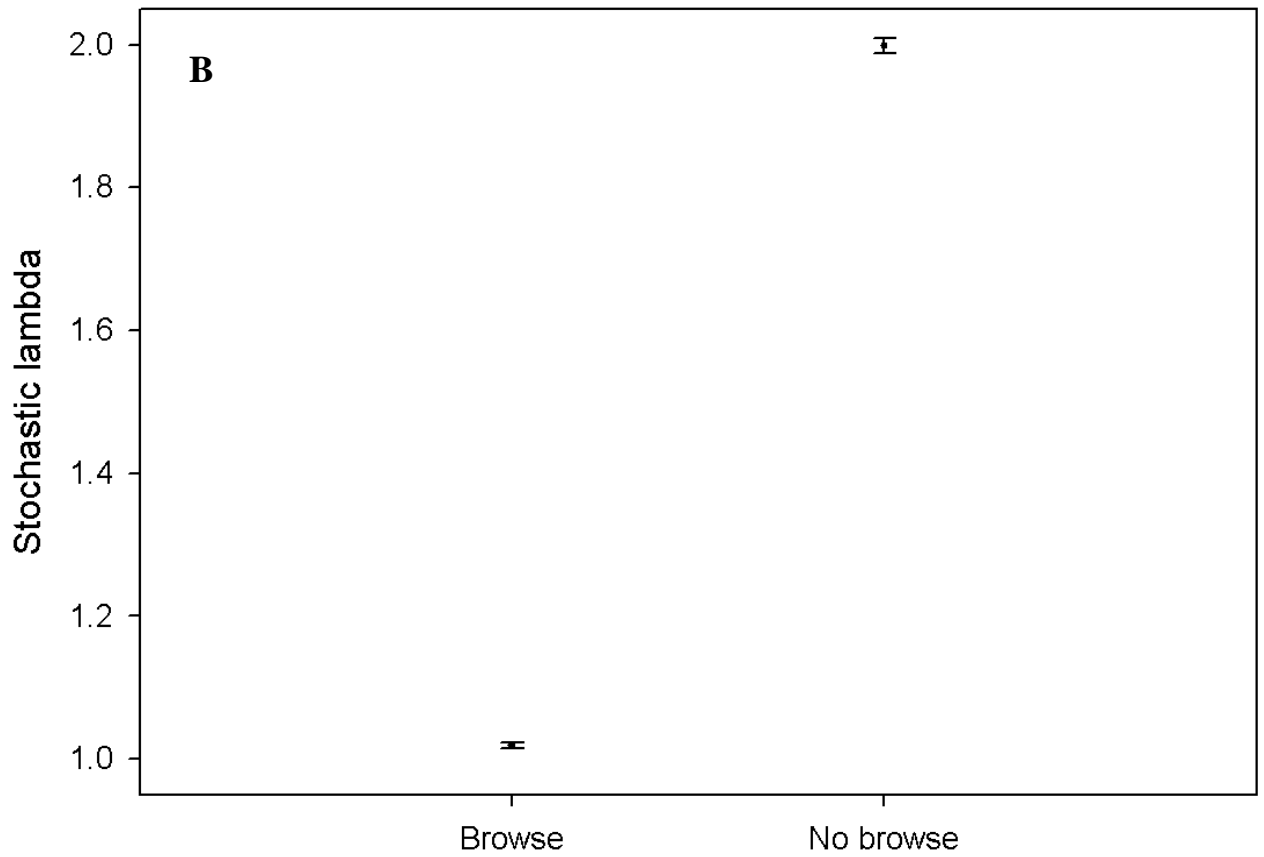


Figure 12: Estimation of stochastic population growth rate (λ_s) for different *P. vanbruntiae* habitat types (A) and under two white-tailed deer browsing scenarios (B). Note the differences in scale in y-axes. The error bars represent 95% confidence intervals.

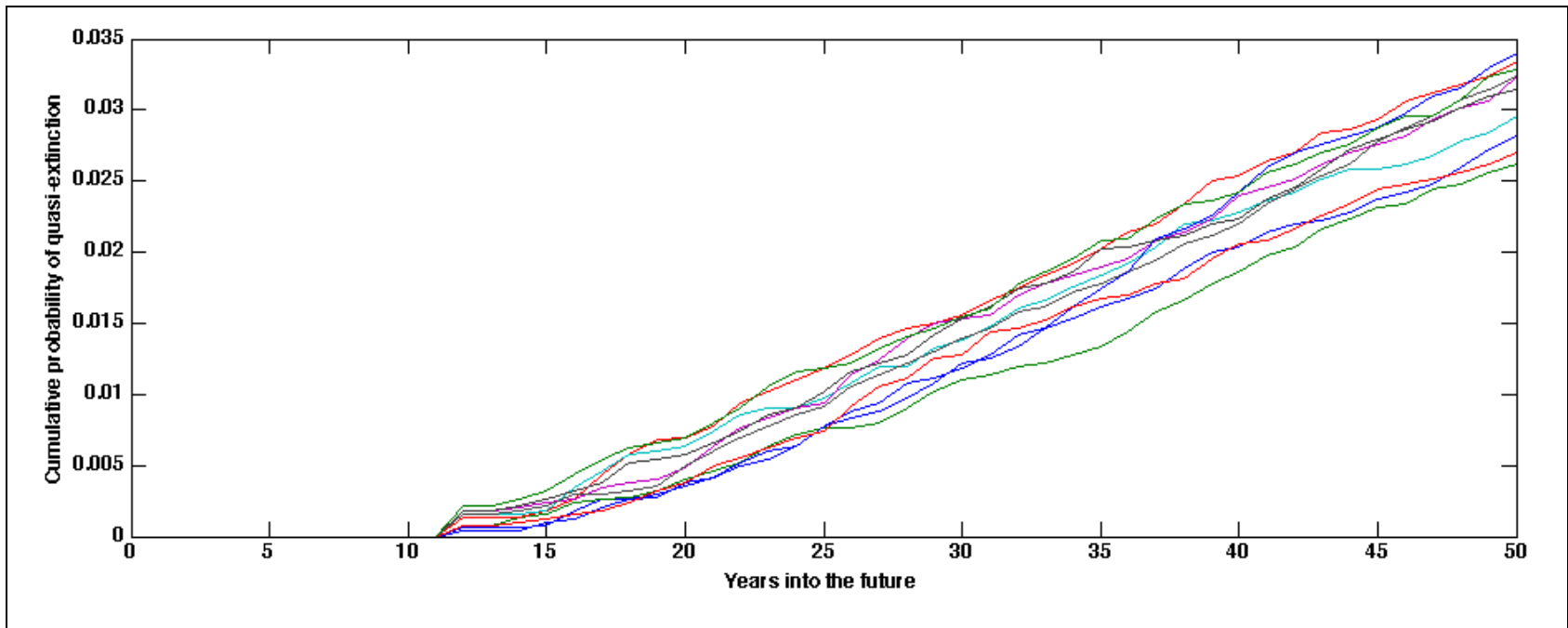


Figure 13: Probability of extinction in 50 years for *P. vanbruntiae* ramets experiencing white-tailed deer herbivory. The lines represent the cumulative probability of quasi-extinction to 50 ramets from 10 simulations including only the small vegetative, large vegetative, and reproductive adult life history stages.

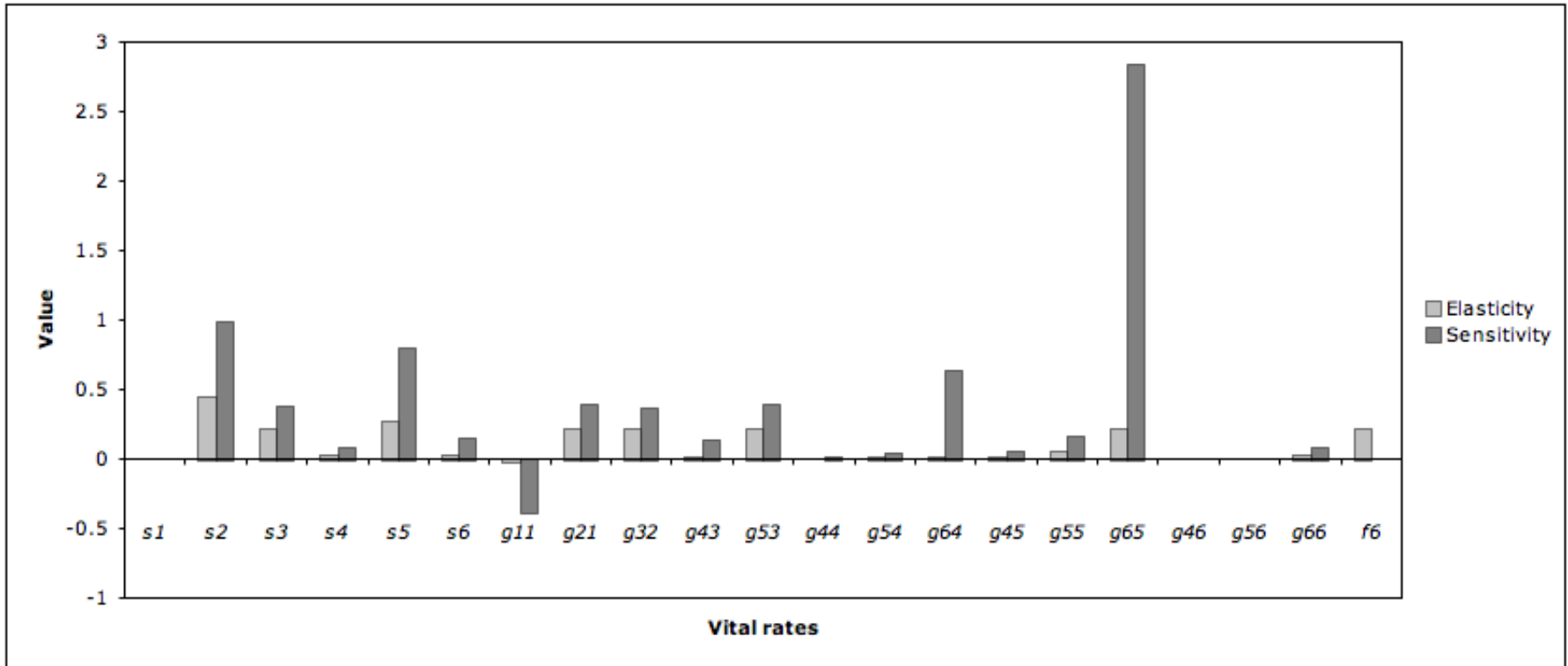


Figure 14: Comparison of mean elasticity and sensitivity rates for wet meadow sites.

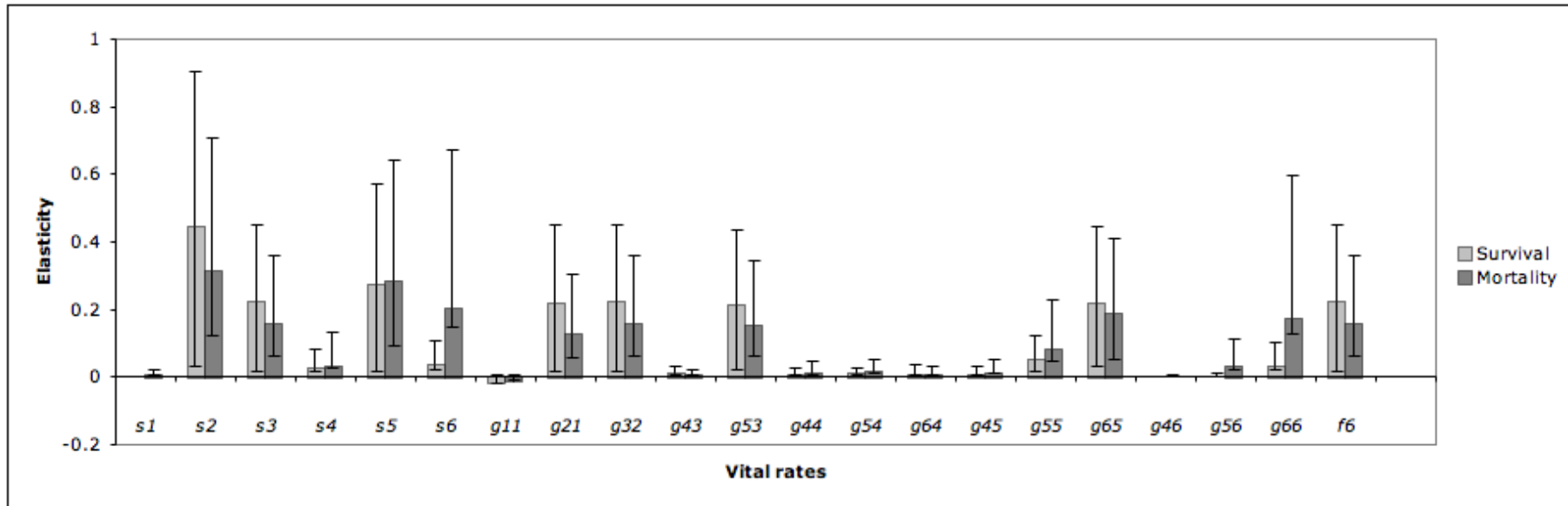


Figure 15: A comparison of mean elasticity values of survival and mortality rates in the no browse matrices. Error bars represent the 95% confidence interval.

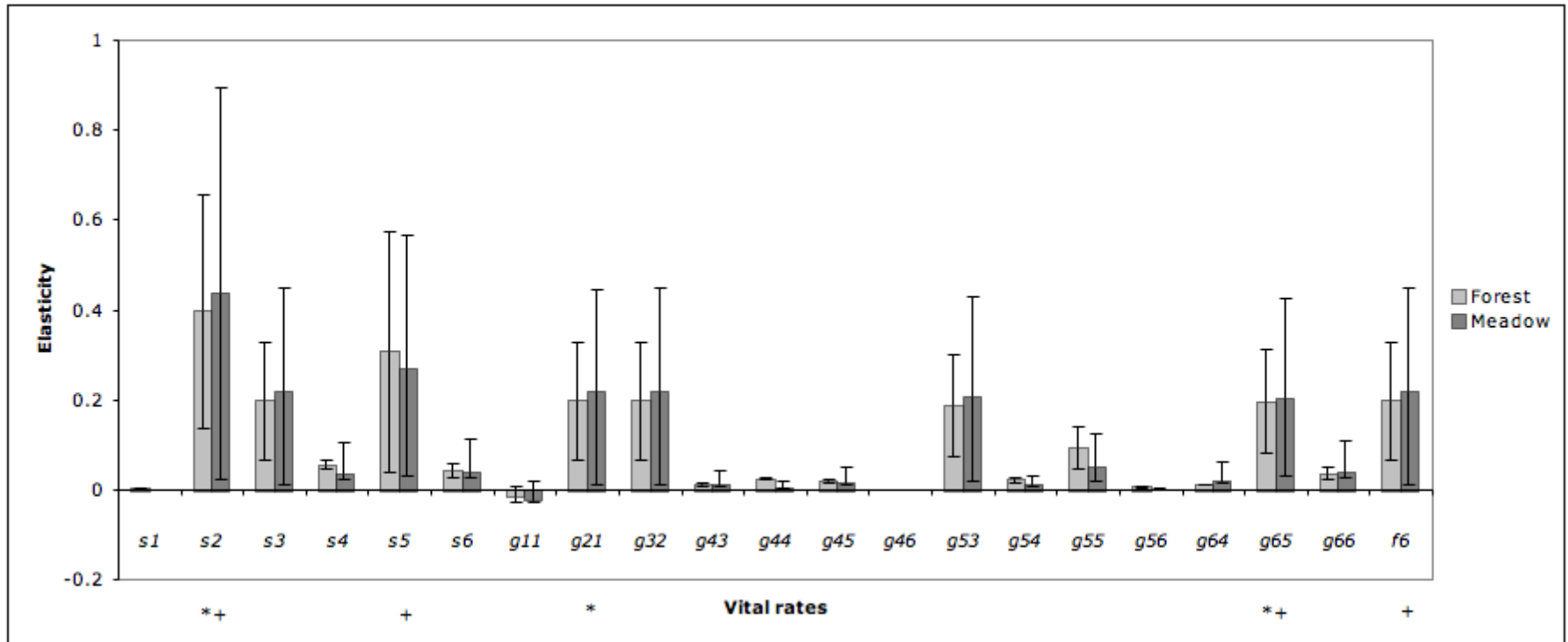


Figure 16: Mean elasticities of vital rates for the forest seep and wet meadow habitats with 95% confidence intervals. Asterisks (*) denote elasticity values with high correlation coefficients (r^2) in the forest seep sites, and + denotes high r^2 values of elasticity values in the meadow sites.

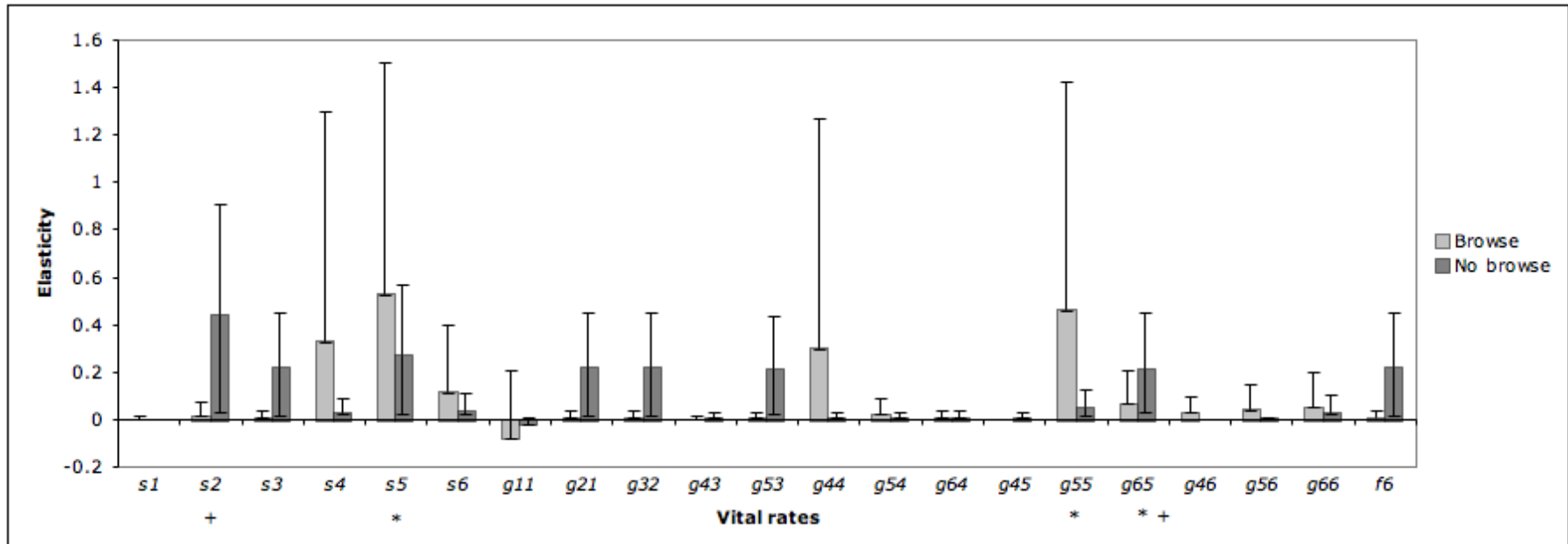
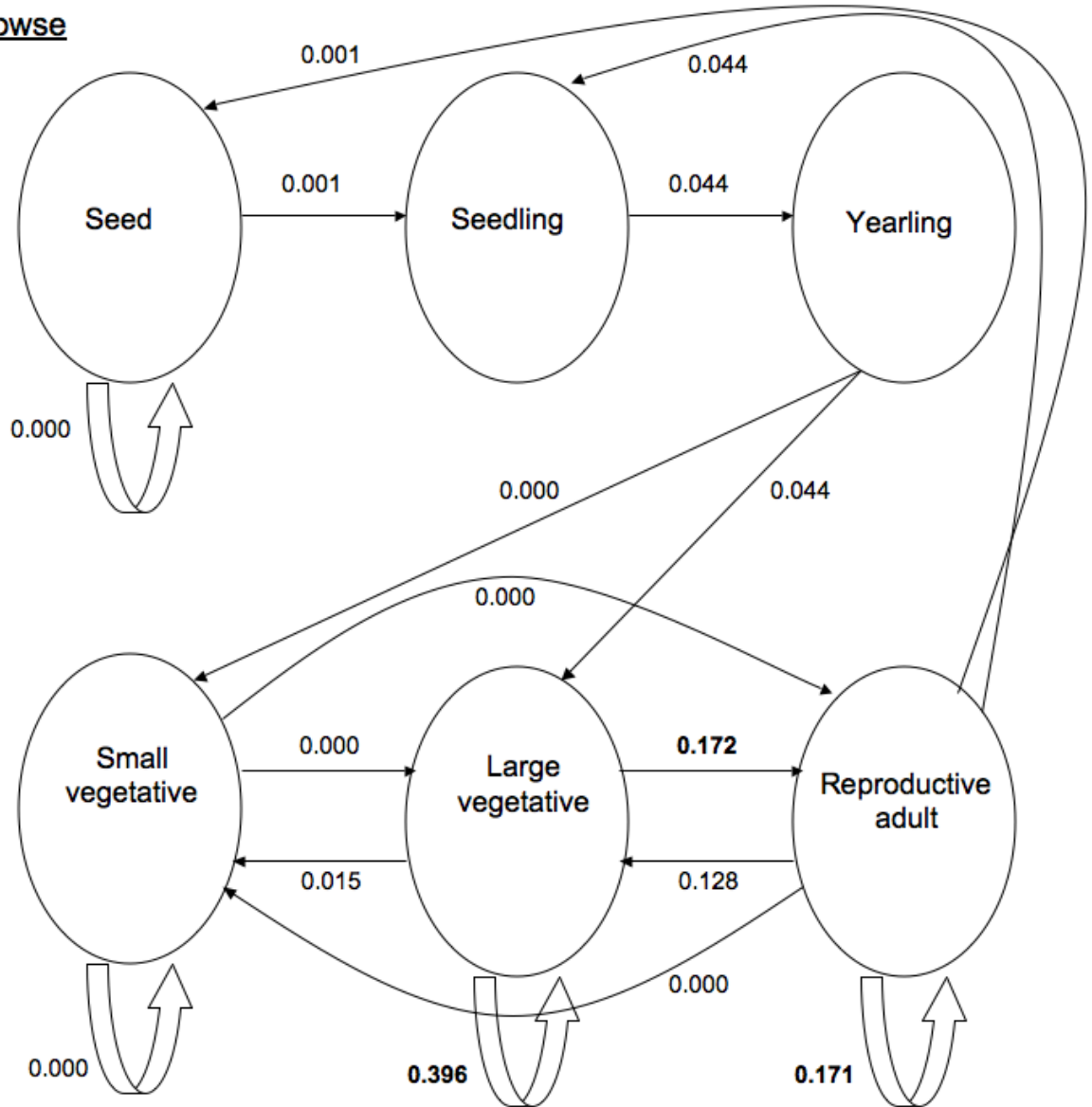


Figure 17: Mean elasticities of vital rates for the browse and no browse sites with 95% confidence intervals. Asterisks (*) denote elasticity values with high correlation coefficients (r^2) in the browse sites, and + denotes high r^2 values of elasticity values in the no browse sites.

A

Browse



B

No browse

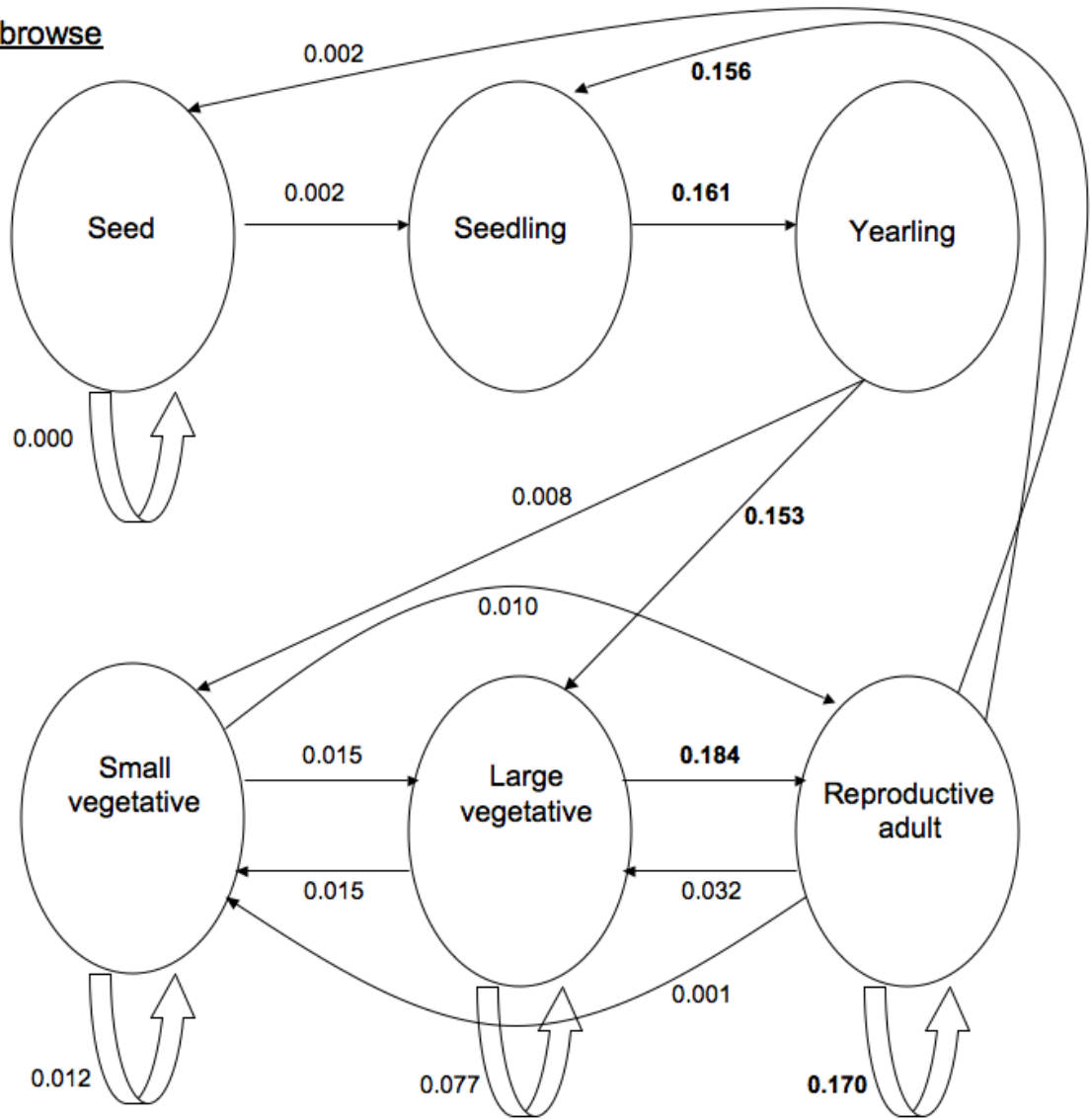


Figure 18: Elasticities for life cycle transitions of ramets in the browse (A) and no browse (B) matrices. Bold values represent the most important transitions.

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APPENDIX I

Matrix model parameterization

The following section gives more detailed information on matrix model parameterization (see **Table 6**). Reproductive adults can contribute to the seed bank ($F_{1,6}$) or directly to seedling production ($F_{2,6}$). To calculate the fecundity matrix element $F_{1,6}$ for contributions to the seed bank, I multiplied the mean number of seeds/fruit by the mean number of fruits to estimate seed production (f_6). I then multiplied this value by the probability of seed surviving the overwinter period (s_1) by the probability that a seed will not germinate ($1 - g_{21}$) but will remain viable in the seed bank (g_{11}). For each individual habitat type (i.e., wet meadow and forest seep), I used each population's individual germination rate from the seed grids to represent each habitat. Seed bank survival was assessed from seed cage experiments.

To calculate the fecundity matrix element $F_{2,6}$ for reproductive adult contribution to seedlings, I multiplied annual seed production by the probability of seedling survival (s_2) by the probability of germinating (g_{21}) and not remaining in the seed bank ($1 - g_{11}$). Seed germination and survival were assessed from the seed grids on a site-by-site basis.

For matrix element ($P_{1,1}$), seed bank survival, I multiplied the probability that the seed survives the winter in the soil (s_1) by the probability that a seed does not germinate ($1 - g_{21}$) by the probability that the seed will remain viable in the seed bank (g_{11}). A seed in the seed bank will become a seedling at the next census ($P_{2,1}$) if the seed

survives the winter in the soil (s_1), germinates the following spring (g_{21}), and does not remain in the seed bank ($1 - g_{11}$).

Because a seedling cannot remain a seedling for more than 1 year, there are only 2 fates: death or progression to the yearling stage ($P_{3,2}$). Therefore, $P_{3,2}$ is the number of seedlings that survive (s_2), and those that survive always advance to the yearling stage (i.e., $g_{32} = 1$). Seedling survival and seedling-to-yearling transitions were directly assessed from seed grids on a site-by-site basis.

Yearling survival (s_3) and yearling-to-vegetative adult transition probabilities (g_{43} and g_{53}) in both the forest and meadow matrices were estimated from probabilities from meadow sites in year 2006-2007, which were the only sites and year (year 3) that survived yearlings were observed making the transition to vegetative plants. All censused yearlings transitioned into a vegetative stage (i.e., no yearlings skipped the vegetative adult stage and directly transitioned to the reproductive adult stage). Because a yearling cannot remain a yearling in year $t + 1$, it has 3 fates: death or progression to one of the 2 vegetative stages. I estimate matrix element $P_{4,3}$, yearling-to-small vegetative transition, by multiplying the probability of yearling survival (s_3) by the probability that the yearling grows to the small vegetative stage (g_{43}). The yearling-to-large vegetative transition ($P_{5,3}$) is the probability of yearling survival (s_3) multiplied by the probability that the yearling grows to the large vegetative stage (g_{53}).

If a small vegetative ramet survives, it can remain small in year $t + 1$ ($P_{4,4}$), transition to the large vegetative stage ($P_{5,4}$), or transition into a reproductive ramet ($P_{6,4}$). A large vegetative ramet can regress to the small stage class ($P_{4,5}$), remain large ($P_{5,5}$), or

become a flowering ramet ($P_{6,5}$). Additionally, reproductive ramets can regress to either the small or large vegetative stage class ($P_{4,6}$ and $P_{5,6}$, respectively), or remain flowering in year 2 ($P_{6,6}$).

APPENDIX II

Transition probabilities for all matrix types: forest seep (A), wet meadow (B), no browse (C), and browse (D). Browse matrices only have 2 annual transitions (2005-2006, 2006-2007). Column must sum to (1 – mortality) for the given stage class represented in the column, except for column # 6 where only rows 4-6 sum to (1 – mortality).

(A) Forest seep transition matrices

2004-2005						
	Seed	Seedling	Yearling	Small vegetative	Large vegetative	Reproductive
Seed	0.00	0	0	0	0	0.02
Seedling	0.24	0	0	0	0	80.43
Yearling	0	0.38	0	0	0	0
Small vegetative	0	0	0.12	0.29	0.06	0
Large vegetative	0	0	0.84	0.46	0.36	0.09
Reproductive	0	0	0	0.06	0.34	0.40
<i>1-mortality</i>	0.24	0.38	0.96	0.81	0.76	0.49

2005-2006						
	Seed	Seedling	Yearling	Small vegetative	Large vegetative	Reproductive
Seed	0.00	0	0	0	0	0.13
Seedling	0.20	0	0	0	0	59.81
Yearling	0	0.12	0	0	0	0
Small vegetative	0	0	0.12	0.41	0.12	0
Large vegetative	0	0	0.84	0.08	0.34	0.14
Reproductive	0	0	0	0	0.05	0.11
<i>1-mortality</i>	0.20	0.12	0.96	0.49	0.51	0.25

2006-2007						
	Seed	Seedling	Yearling	Small vegetative	Large vegetative	Reproductive
Seed	0.00	0	0	0	0	0.06
Seedling	0.23	0	0	0	0	76.87
Yearling	0	0.84	0	0	0	0
Small vegetative	0	0	0.12	0.53	0.24	0.08
Large vegetative	0	0	0.84	0.16	0.28	0.38
Reproductive	0	0	0	0.03	0.15	0.15
<i>1-mortality</i>	0.23	0.84	0.96	0.72	0.67	0.61

(B) Wet meadow transition matrices

2004-2005						
	Seed	Seedling	Yearling	Small vegetative	Large vegetative	Reproductive
Seed	0.00	0	0	0	0	0.05
Seedling	0.41	0	0	0	0	204.07
Yearling	0	0.77	0	0	0	0
Small vegetative	0	0	0.12	0.31	0.22	0.03
Large vegetative	0	0	0.84	0.31	0.44	0.02
Reproductive	0	0	0	0	0.11	0.10
<i>1-mortality</i>	0.41	0.77	0.96	0.62	0.77	0.15

2005-2006						
	Seed	Seedling	Yearling	Small vegetative	Large vegetative	Reproductive
Seed	0.00	0	0	0	0	0.05
Seedling	0.07	0	0	0	0	96.20
Yearling	0	0.83	0	0	0	0
Small vegetative	0	0	0.12	0.18	0.11	0
Large vegetative	0	0	0.84	0.10	0.21	0.09
Reproductive	0	0	0	0.03	0.05	0.08
<i>1-mortality</i>	0.07	0.83	0.96	0.31	0.37	0.17

2006-2007						
	Seed	Seedling	Yearling	Small vegetative	Large vegetative	Reproductive
Seed	0.00	0	0	0	0	0.27
Seedling	0.18	0	0	0	0	106.02
Yearling	0	0.57	0	0	0	0
Small vegetative	0	0	0.12	0.14	0.25	0
Large vegetative	0	0	0.84	0.36	0.25	0.33
Reproductive	0	0	0	0	0.04	0.33
<i>1-mortality</i>	0.18	0.57	0.96	0.50	0.54	0.67

(C) No browse transition matrices

2004-2005						
	Seed	Seedling	Yearling	Small vegetative	Large vegetative	Reproductive
Seed	0.00	0	0	0	0	0.03
Seedling	0.24	0	0	0	0	115.62
Yearling	0	0.77	0	0	0	0
Small vegetative	0	0	0.12	0.29	0.06	0
Large vegetative	0	0	0.84	0.46	0.36	0.09
Reproductive	0	0	0	0.06	0.36	0.40
<i>1-mortality</i>	0.24	0.77	0.96	0.81	0.78	0.49

2005-2006						
	Seed	Seedling	Yearling	Small vegetative	Large vegetative	Reproductive
Seed	0.00	0	0	0	0	0.21
Seedling	0.20	0	0	0	0	96.50
Yearling	0	0.83	0	0	0	0
Small vegetative	0	0	0.12	0.58	0.17	0
Large vegetative	0	0	0.84	0.09	0.36	0.12
Reproductive	0	0	0	0	0.05	0.16
<i>1-mortality</i>	0.20	0.83	0.96	0.67	0.58	0.28

2006-2007						
	Seed	Seedling	Yearling	Small vegetative	Large vegetative	Reproductive
Seed	0.00	0	0	0	0	0.09
Seedling	0.23	0	0	0	0	107.85
Yearling	0	0.57	0	0	0	0
Small vegetative	0	0	0.12	0.47	0.27	0.10
Large vegetative	0	0	0.84	0.16	0.27	0.45
Reproductive	0	0	0	0.05	0.14	0.10
<i>1-mortality</i>	0.23	0.57	0.96	0.67	0.67	0.65

(D) Browse transition matrices

2005-2006						
	Seed	Seedling	Yearling	Small vegetative	Large vegetative	Reproductive
Seed	0.00	0	0	0	0	0
Seedling	0.20	0	0	0	0	0
Yearling	0	0.83	0	0	0	0
Small vegetative	0	0	0.12	0.67	0	0
Large vegetative	0	0	0.84	0.33	0.35	0.15
Reproductive	0	0	0	0	0.18	0.23
<i>1-mortality</i>	0.20	0.83	0.96	1.00	0.53	0.38

2006-2007						
	Seed	Seedling	Yearling	Small vegetative	Large vegetative	Reproductive
Seed	0.00	0	0	0	0	0.01
Seedling	0.23	0	0	0	0	9.76
Yearling	0	0.57	0	0	0	0
Small vegetative	0	0	0.12	0.50	0	0.17
Large vegetative	0	0	0.84	0	0.33	0.17
Reproductive	0	0	0	0.50	0.11	0.17
<i>1-mortality</i>	0.23	0.57	0.96	1.00	0.44	0.51

APPENDIX III

Calculation of population vectors for model simulations:

The forest sites had a total of 9566 ramets, and 1% of these ramets were seeds and 18% were yearlings according to the stable stage distribution results. The meadow sites had a total of 74470 ramets, and 1% of ramets were seeds and 24% were yearlings according to the SSD. Therefore, for the forest sites $n_0 = [96, 1166, 1721, 3002, 4501, 897]$, and for the meadow sites $n_0 = [745, 21877, 17872, 13274, 21812, 17507]$.

For the browse matrices, I used the sum of the total ramets from site FR to parameterize the n_0 vector, because FR contained the majority of the browsed plants (i.e., 30 out of 40). Because site FR is a forest seep site, I used the proportions from the forest SSD to estimate the number of seeds and yearlings. Therefore, the total population density was 6860, and n_0 for the browse matrix was $[69, 267, 1235, 1871, 4098, 624]$.

In the no browse matrix, sites AP and FR had the majority of ramets that were not browsed, so I used the combined sum of total ramets from site AP and FR to parameterize n_0 for the no browse model. Sites AP and FR are also both forest seep sites, so again I used the SSD for forest to estimate seeds and yearlings for the n_0 vector. Therefore, the total population density for the no browse matrix was 7913, and n_0 was $[79, 715, 1424, 2081, 4309, 808]$.

APPENDIX IV

Soil composition for all *P. vanbruntiae* demographic wet meadow and forest seep sites. Site abbreviations can be found in Table 4.

Site	Habitat type	% Ca	% K	% Mg*	Na	Fe	B	Mn*	Cu	Zn	S
AP	Forest seep	56.2	1.3	13.6	14	9.1	0.4	38.9	0.2	8.2	45
DR	Forest seep	70.5	0.8	19.1	10	3.9	0.5	28.5	0.2	1.5	43.8
FR	Forest seep	39.7	1.3	8	11	86.9	0.7	37	0.3	6.8	23
NBF	Forest seep	75.8	2	22.2	9	17.7	0.4	23.7	0.2	1.3	18.3
	<i>Forest average</i>	6.55	1.35	15.73	11	29.4	0.5	32.03	0.23	4.45	32.53
BBC	Wet meadow	41	2.3	6.4	24	183	1.1	128.4	0.2	3.9	47.1
BBD	Wet meadow	59.8	1.3	9.7	10	27	0.4	75	0.2	6.9	32.4
BBE	Wet meadow	50.2	1.2	8.4	9	14.5	0.3	39.1	0.2	8.8	30.3
CHSF	Wet meadow	43.9	2	9.8	11	27.4	0.4	38.5	0.2	3.2	28.2
NBE	Wet meadow	41.5	1.5	11.2	21	231.1	1.4	60.4	0.2	3.2	28.7
	<i>Meadow average</i>	47.28	1.66	9.1	15	96.62	0.72	68.28	0.2	5.2	33.34

* refers to significant difference between habitat type. pH was too low to estimate the cation exchange capacity (CEC) at sites CHSF, FR, and NBE. CEC units are expressed as milliequivalents per 100 grams (meq/100g) of soil. Conductivity units refer to milliSiemens per cm.

Site	Habitat type	pH	ppm P	ppm K	ppm Mg	ppm Al	ppm Ca	ppm Zn	CEC (meq/100g)	% organic matter	Conductivity (mS/cm)
AP	Forest seep	6	2.4	111	351	23	2420	8.2	15.3	31	0.058
DR	Forest seep	6.5	2.2	57	411	29	2525	1.5	16.2	16.4	0.08
FR	Forest seep	5.4	1.5	38	73	73	606	6.8	N/A	6.3	0.052
NBF	Forest seep	6.3	1.8	37	129	25	734	1.3	4.8	5.3	0.127
	<i>Forest average</i>	6.05	1.98	60.75	241	37.5	1571.25	4.45	12.1	14.75	0.0793
BBC	Wet meadow	5.8	1.9	196	165	40	1773	3.9	10.7	48.7	0.058
BBD	Wet meadow	5.9	1.4	59	138	65	1425	6.9	8.4	12.5	0.111
BBE	Wet meadow	5.7	1.5	64	139	77	1381	8.8	8.2	18.1	0.074
CHSF	Wet meadow	5.5	1.1	105	158	84	1177	3.2	N/A	16.4	0.061
NBE	Wet meadow	5.5	2	78	175	55	1080	3.2	N/A	19.3	0.089
	<i>Meadow average</i>	5.68	1.58	100.4	155	64.2	1367.2	5.2	9.1	23	0.0786

APPENDIX V

Raw and corrected mean and variance vital rate estimate with 95% confidence intervals.

Vital rate	Habitat	MEAN RATE		VARIANCE	
		Raw estimate	MLE mean (95% C.I.)	Raw estimate	Corrected variance (95% C.I.)
s_1	Meadow	0.2523	0.2518 (0.0843, 0.6115)	0.0285	0.0261 (0.0054, 0.2144)
	Forest	0.2499	0.2548 (0.2072, 0.2994)	0.0001	0.0003 (0.0003, 0.0081)
s_2	Meadow	0.7251	0.7374 (0.4401, 0.8860)	0.0188	0.0114 (0.0003, 0.1652)
	Forest	0.4458	0.4520 (0.1230, 0.8365)	0.1328	0.1066 (0.0291, 0.2916)
s_3	Meadow	0.9535	0.9574 (0.8603, 0.9851)	0	0 (0.0003, 0.0237)
	Forest	0.9535	0.9574 (0.8603, 0.9851)	0	0 (0.0003, 0.0237)
s_4	Meadow	0.4763	0.4381 (0.2112, 0.7473)	0.0262	0.0165 (0.0003, 0.1898)
	Forest	0.6695	0.6621 (0.3578, 0.8662)	0.0259	0.0177 (0.0003, 0.1889)
s_5	Meadow	0.5626	0.5035 (0.2518, 0.8137)	0.0422	0.0150 (0.0003, 0.2075)
	Forest	0.6513	0.6442 (0.3895, 0.8325)	0.0162	0.0126 (0.0006, 0.1544)
s_6	Meadow	0.33	0.2875 (0.0873, 0.7314)	0.0852	0.0366 (0.0003, 0.2595)
	Forest	0.4480	0.4193 (0.1854, 0.7364)	0.0357	0.0225 (0.0006, 0.2024)
$g_{2,1}$	Meadow	0.939	0.9277 (0.5223, 0.9901)	0.0075	0.0051 (0, 0.2643)
	Forest	0.963	0.9505 (0.7500, 0.9890)	0.0017	0.0003 (0, 0.1090)
$g_{3,2}$	Meadow	1.0	0.999 (0.5650, 0.999)	0	0.0012 (0, 0.3000)
	Forest	1.0	0.999 (0.54, 0.999)	0	0.0012 (0, 0.3000)
$g_{\geq 4,3}$	Meadow	0.5	0.4995 (0.4054, 0.5937)	0	0.0006 (0.0006, 0.0297)
	Forest	1.0	0.999 (0.54, 0.999)	0	0.0012 (0, 0.3000)
$g_{\geq 5,4}$	Meadow	0.2718	0.2449 (0.1339, 0.4609)	0.0059	0.0003 (0.0003, 0.0916)
	Forest	0.1784	0.1805 (0.0576, 0.5075)	0.0159	0.0108 (0.0003, 0.1868)
$g_{6,5}$	Meadow	0.1209	0.1230 (0.0328, 0.3608)	0.0014	0.0003 (0, 0.1144)
	Forest	0.2558	0.2558 (0.0784, 0.6343)	0.0324	0.0270 (0.0021, 0.2270)

APPENDIX V continued...

Raw and corrected mean and variance estimates—

Growth rates $g_{\geq 4,3}$ and $g_{\geq 5,4}$ represent the binomial representation yearling to either small or large vegetative growth and small vegetative to either large vegetative or reproductive growth. Yearling survival (s_3) and yearling-to-vegetative transitions ($g_{\geq 4,3}$) have 0 variance due to only 1 year of available data. The seedling-to-yearling transition has 0 variance because all surviving seedling automatically transition to the yearling stage (i.e., $g_{3,2} = 1$).