Mating strategies and pollen limitation in a globally threatened perennial Polemonium vanbruntiae

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1. 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 Matthes, 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, personal communication), and P. caeruleum’s mating system has been characterized as 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. Materials and methods

2.1. 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 roadides, at elevations generally above 330 m in the northeastern US. 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; Fig. 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 (Deller, 2002), although the degree of clonal diversity in natural populations has yet to be assessed.

2.2. Study sites

In 2004, we conducted pollen limitation studies in three 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 Vermont population in Camel’s Hump State Forest (CHSF). The farthest sites...
total effective population size by calculating the number of reproductive adults per m² quadrat averaged over the five quadrats per site, and multiplying the mean by the total population area (m²).

2.3. Pollen limitation experiments

We tested whether seed production of Polemonium vanbruntiae was pollen limited during the summers of 2004 and 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 centimeter. Flowers of supplemental treatment plants received outcross pollen collected from a single donor plant 1–5 m 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 10 m 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 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 nine control plants and seven 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 (mg). We kept 10% of the seeds in a vial and delivered pollen to the receptive stigmas of the treatment flowers using a fine-tipped paintbrush. Flowers of supplemental treatment 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.

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**Fig. 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.

(CHSF and AP) are 29 km apart, and the closest sites (BB and FR) are 6 km apart. Precise GPS coordinates are not provided for conservation reasons, but site information can be obtained from the Vermont Nongame and Natural Heritage Program. In 2006, self-compatibility experiments were conducted at 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 1 m² 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 Mover. 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 two transects and counted every reproductive adult. For sites CHSF, AP, and BB, we estimated...
2.4. 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 10 m away from the recipient plant; and (4) open-pollinated controls. After 100 days and number of leaves (100 days) as our response variables.

2.5. 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-min 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.

2.6. 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 (mg) using separate analysis of variance (ANOVA) models with our pollination treatments as a fixed effect, site as a random factor, and a treatment × site interaction term. Before analysis, we examined normality of the data. Seed set and seed mass (mg) 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 60 days (natural log-transformed), plant height (cm) at 100 days and number of leaves (100 days) 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, number of seeds as the covariate, site and treatment as random and fixed effects, respectively, and number of seeds × site and number of seeds × 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).

3. Results

Reproductive *P. vanbruntiae* adults have an erect flowering stem ranging in height from 0.4 to 1.0 m. The leaves are alternate, pinnately compound, and consist of 15–21 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 basipetallous. The flowers are protandrous, and the anthers dehisce pollen for 1–2 days before the stigma becomes receptive.

3.1. Pollen limitation experiments

Supplemental outcross pollen did not significantly increase female reproductive success of *P. vanbruntiae* in any of the populations in any year (Fig. 2). Open-pollinated control plants set an average (±1 SD) of 169.87 ± 79.6 seeds and plants supplemented with outcross pollen set 164.06 ± 84.3 seeds.
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 ± 10.9% for control plants and 89.1 ± 10.6% for supplemental outcross plants ($F_{1,85} = 0.49, p = 0.49$). Seed mass averaged 0.874 ± 0.13 mg for control plants and 0.895 ± 0.15 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: $F_{2,85} = 1.1, p = 0.32$; 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 × 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: $F_{1,27} = 0.40, p = 0.54$; seed mass: $F_{1,27} = 0.03, p = 0.86$).

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 (Fig. 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 × 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 SD) of outcrossed seeds was 23.7 ± 0.18% compared to 23.8 ± 0.23% 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.

### 3.2. Self-compatibility experiments

Geitonogamously self-fertilized plants at CHSF in 2005 produced an average (±1 SD) of 86.79 ± 66.5 seeds per plant with an average seed mass of 0.83 ± 0.27 mg. Fifty-three-percent (±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 (Fig. 4). 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 ($F_{3,45} = 5.03, p = 0.03$), and open-pollinated controls ($F_{1,64} = 5.36, p = 0.02$). Seed mass (mg) did not differ significantly among selfing treatments ($F_{3,156} = 0.12, p = 0.95$). At site LP, autonomously-selfed flowers set the lowest average number of seeds (±1 SD) 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.

### 3.3. 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 ($F_{1,36} = 5.35, p = 0.03$). 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-min 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.

### 4. 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

### Table 1 – Among-population differences in female reproductive success

<table>
<thead>
<tr>
<th>Site</th>
<th>Population size</th>
<th>Plant height (cm)</th>
<th>No. flowers</th>
<th>Fruit set (%)</th>
<th>Seeds/plant</th>
<th>Seeds/fruit</th>
<th>Seed mass (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP</td>
<td>155</td>
<td>76.58 ± 1.9</td>
<td>15.9 ± 1.4</td>
<td>90.7 ± 1.7</td>
<td>158.94 ± 10.02</td>
<td>10.16 ± 0.62</td>
<td>0.968 ± 0.02</td>
</tr>
<tr>
<td>BB</td>
<td>225</td>
<td>84.73 ± 2.3</td>
<td>21.3 ± 1.2</td>
<td>89.9 ± 1.7</td>
<td>210.63 ± 10.53</td>
<td>10.85 ± 0.67</td>
<td>0.845 ± 0.02</td>
</tr>
<tr>
<td>FR</td>
<td>75</td>
<td>79.81 ± 1.8</td>
<td>13.2 ± 1.3</td>
<td>82.7 ± 2.6</td>
<td>47.99 ± 11.29</td>
<td>6.05 ± 0.88</td>
<td>1.01 ± 0.04</td>
</tr>
<tr>
<td>CHSF</td>
<td>205</td>
<td>90.57 ± 2.2</td>
<td>16.7 ± 1.8</td>
<td>66.5 ± 5.1</td>
<td>119.59 ± 17.17</td>
<td>10.02 ± 0.37</td>
<td>0.948 ± 0.16</td>
</tr>
</tbody>
</table>

The values represent the mean of each fitness estimate ± 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.
P. viscosum assures for the strongly pollen limited though the ability to self-fertilize would provide reproductive excludes both self-compatible and self-incompatible species. Al-
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-compatible 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 from 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

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**Table 2 – ANCOVA table results**

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>Sums of squares</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of seeds</td>
<td>1</td>
<td>9.0e–8</td>
<td>6.13</td>
<td>0.02</td>
</tr>
<tr>
<td>Site</td>
<td>2</td>
<td>6.2e–9</td>
<td>0.20</td>
<td>0.82</td>
</tr>
<tr>
<td>Treatment</td>
<td>1</td>
<td>1.0e–8</td>
<td>0.72</td>
<td>0.40</td>
</tr>
<tr>
<td>Site × no. of seeds</td>
<td>2</td>
<td>1.0e–7</td>
<td>3.31</td>
<td>0.04</td>
</tr>
<tr>
<td>Treatment × no. of seeds</td>
<td>1</td>
<td>2.0e–8</td>
<td>1.51</td>
<td>0.22</td>
</tr>
<tr>
<td>Error</td>
<td>84</td>
<td>1.3e–6</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

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.
Fig. 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.

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; Eckhart, 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; Hererra 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 the 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.

5. Conclusion

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 and found that self-incompatibility rates appear to be relatively low for P. vanbruntiae. However, reproductive assurance provided by autonomous selfing 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.

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