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Reproductive Effort and Output in Atypical Morphotypes of Mountain Purple Pitcher Plant, *Sarracenia purpurea* var. *montana* Schnell & Determan

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Abstract

In 2022, an unusual population of the carnivorous Mountain Purple Pitcher Plant, *Sarracenia purpurea* var. *montana*, was observed in western North Carolina. This population included some individuals exhibiting floral characteristics not typically observed in other *Sarracenia,* including duplicate petal and sepal whorls and an upward floral orientation. Atypical floral traits can lead to negative consequences, including increased energy expenditure to floral tissue, deterrence of pollinators, and susceptibility to pollen damage. The objective of this study was to investigate relationships between these flower features and reproductive success. To achieve this, we selected flowers from 73 plant clumps and observed them over a nine-week period during the summer of 2023. We identified the number of flowers per clump, floral orientation, and number of petal and sepal whorls. We collected anthers to quantify pollen viability and gathered mature flowers in early fall to count seeds. X^2 tests revealed significant associations between sepal and petal characteristics. Wilcoxon tests highlighted that clumps with duplicate sepal morphologies yielded more flowers per clump. Among the floral components, flowers with duplicate petals had more viable and total pollen production but less inviable pollen. Atypical pedicel orientation was associated with more viable, inviable, and total pollen production. Furthermore, duplicate petals were associated with fewer mature seeds, while duplicate sepals were associated with fewer immature seeds. The co-occurrence of duplicate petals and sepals in individual flowers could suggest shared genetic factors controlling these two floral characteristics. Additionally, pollen production could be influenced by resource allocation going towards duplicate petal whorls or influenced by flower orientation and associated environmental damage. Lastly, the arrangement of flower whorls might influence pollinator interactions, potentially enhancing attraction or affecting the accessibility of the ovary, thereby influencing fertilization. Future research will explore genetic markers that could be responsible for variations in flower orientation and whorl duplication.

1. Introduction

Atypical floral morphologies can come with a suite of reproductive and population-level consequences. Angiosperm reproductive effort includes the production of flowers and the subsequent production of pollen and ovules within those flowers. The energy that goes into successful fertilization of ovules within the ovary can be followed by the production of seeds, reproductive output. Many characteristics affect reproductive success and influence the potential for seed germination. For instance, extra or enlarged floral structures are energetically costly (Sargent et al., 2007; Huang et al., 2022), and morphologies that stray from normal flower shape may deter specialized pollinators (Wang et al. 2017) or attract herbivores and florivores (Huang et al., 2022). Atypical floral architecture may also cause damage to pivotal organs and structures; for instance, pollen could be exposed to rain (Tadey et al. 2001) or increased ultraviolet light, affecting reproductive effort.

Here we describe flowers of the Mountain Purple Pitcher Plant, *Sarracenia purpurea* var. *montana* Schnell & Determan, a perennial, carnivorous plant restricted to *Sphagnum* bogs, fens, and seepage swamps in the northeastern United States and Canada (Landis et al., 2011). Typical *S. purpurea* var. *montana* flowers extend higher than the pitcher leaves, have three bracts, five sepals, and five maroon-red petals (Ne'eman et al., 2006). The pedicel of *S. purpurea* var. *montana* var *montana* is directed downward, giving flowers a pendulous orientation and holding the umbrella-shaped style upside down. Petals hanging between the stylar lobes act as a curtain that pollinators must pass through (Figure 1) to obtain pollen and nectar (Guo et al., 2020). The umbrella-shaped style provides a stage for insects to collect pollen and nectar and a location for pollen retention and pollen tube transmission. The style retains dehisced pollen grains from the flower while stigma papillae facing the top and outside of the pollination chamber capture pollen grains as pollinators enter, encouraging cross pollination (Guo et al., 2020). Pollinators include bees of the genus *Bombus* as the principal pollinator and small solitary bees of Halicitidae and Megachilidae as other effective pollinators (Guo et al., 2020).

In 2022, a population of *S. purpurea* var. *montana*, co-occurring with Mountain Sweet Pitcher Plants (*Sarracenia jonesii*) and putative hybrid individuals, was observed in Transylvania County, North Carolina (hereafter referred to as Silicon Valley, SV). Many individual *S. purpurea* var. *montana* flowers exhibited atypical morphological features not normally seen in other *S. purpurea* var. *montana* populations (Figure 2). The unusual features found in this location include upward and intermediate flower pedicels and duplicate petal and sepal whorls. Preliminary data from 34 flowers in late summer 2022 showed that 56% exhibited upward flower orientation and 29% had intermediately-positioned flowers, while 65% of flowers exhibited two or more petal whorls. A more recent survey in summer 2023 censused the frequencies of multiple sepal whorls, multiple petal whorls, and pedicle orientation for all clumps (Table 1).

Figure 1. A Bumble Bee (*Bombus* sp.) entering through the curtain-like petals of a typical *S. purpurea* var. *montana* flower. Photo by Gabi Parker.

S. purpurea var. *montana* has been declared a Federal Species of Concern by the U.S. Fish and Wildlife Service as a result of habitat loss due to agriculture, fragmentation, alterations in hydrology, fire suppression, susceptibility to pollutants, and illegal or over-collection (Furches et al. 2013). While the southeastern United States has more wetland coverage than any other region, its rate of wetland loss is also the highest (Furches et al., 2013). General concerns in sites across western North Carolina include hybridization of *S. purpurea* var. *montana* and *S. jonseii*, loss of genetic diversity, and poaching (Koopman & Carstens, 2010). Site-specific concerns at SV include the increased possibility of poaching due to their striking flower whorls and the potential for low reproductive success from the atypical morphologies being selected against in the environment.

Since plant fitness could be influenced by floral morphology, it is important to understand these imperiled plants' success in terms of reproductive effort and reproductive output. While the flowers are self-compatible, inbreeding might result in fewer seeds, lower seed mass, decreased germination rate, and survival (Guo et al., 2020), thus, facilitating sexual reproduction is an important factor affecting seed quantity and quality. However, atypical flower morphology may negatively impact pollinators' access to flowers, resulting in limitations to outbreeding. Since changing orientation in *S. purpurea* var. *montana* would result in the stylar umbrella turning sideways or upside down, this could impair pollinator behavior and landing (Wang et al. 2014). Studies on pollen limitation in *Sophora microphylla* (Fabaceae) resulted in lower seed quantity and quality (Hildesheim et al., 2016). Additionally, carnivorous plants derive most of their resources from the mineral nutrients of arthropods captured in their modified leaves (Ne'eman et al., 2006), so it is worth noting floral structure could potentially be secondary in importance compared to leaf structure. Understanding where plants allocate energy resources such as pollen production is important.

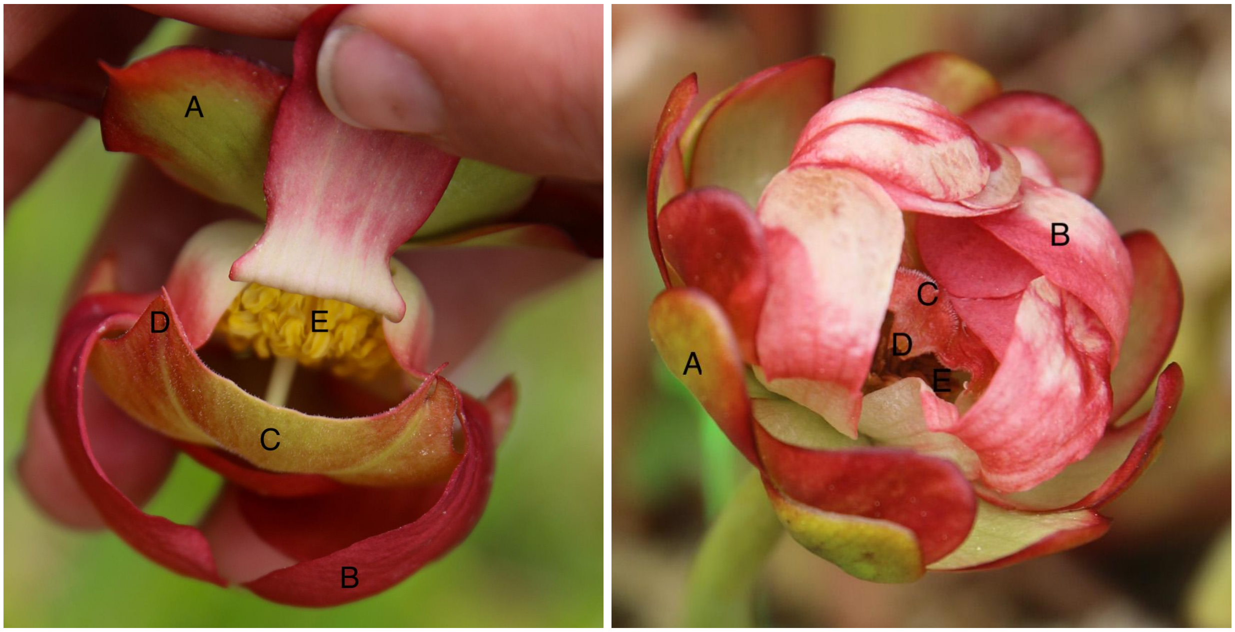

Figure 2. Parts of a typical (left) and atypical (right) *Sarracenia* flower. A, Sepals; B, Petals; C, Stylar umbrella; D, Stigma lobe; E, Anthers. The typical flower on the left hangs downwards with one whorl of sepals and petals while the atypical flower on the right faces upward with multiple petal and sepal whorls. Photo by Gabi Parker.

Table 1. Frequency of duplicate petal and sepal whorls, and pedicel orientations in *S. purpurea* var. *montana* flowers at site SV.

Floral Morphotype	**Frequency (Percent)**
Petals whorls	
One (Typical)	85.94
Two	12.50
Three	1.56
Sepal Whorls	
One (Typical)	69.53
Two	9.38
Three	11.72
Four	9.38
Pedicel Orientation	
Down (Typical)	34.38
Intermediate	58.59
Up	7.03

The recent finding of atypical *Sarracenia* at SV raises novel questions about how flower morphology could affect the reproduction of these imperiled plants. The aim of this study was to compare flower morphology with reproductive effort and output in atypical plants to determine potential correlations. Our hypotheses were that 1) *S.purpurea* var *montana* plants with duplicate sepal and petal whorls would produce significantly less flowers per clump, 2) *S.purpurea* var *montana* plants with atypical pedicel orientations would produce significantly less viable pollen per anther, 3) flowers with atypical orientation would yield a significantly lower amount of mature seeds than those flowers with typical orientation resulting from less viable pollen, and 4) flowers with duplicate petal and sepal whorls would yield a decreased production of seeds.

2. Methods

Flowers were observed over a nine week flowering period from April to July 2023 at site SV, a bog in Transylvania County, North Carolina. This bog is located in a temperate rainforest in the southeastern Appalachian Mountains with an elevation range between 900 and 1,100 meters. A total of 73 rosette-forming clumps at least 0.5 m apart were identified and permanently tagged. Within each clump, three flowers were chosen at random and labeled for weekly monitoring. In clumps with many atypical morphologies, additional flowers (up to 5 total) were tagged.

2.2 Floral monitoring

Clumps were monitored weekly for total number of flowers, orientation of the flowers, number of petal and sepal whorls (Table 1), and the floral stage of each flower (Table 2). One anther per flower was collected weekly until a week after the flower reached stage six (Table 2). Mature flower heads were collected from each flower over a two week period in September and October 2023.

Table 2. Floral stages based on phenology of reproductive effort.

Floral Stage	Anther Attachment
0	Flower unopened
1	Flowers open with all anthers attached; no pollen evident
2	Flowers open with all anthers attached; pollen evident
3	25% of anthers have fallen
4	50% of anthers have fallen
5	75% of anthers have fallen
6	100% of anthers have fallen

2.3 Pollen counts

Each anther was preserved in a 2 mL microcentrifuge tube of Carnoy's solution (Carnoy’s Fluid Fixation 2023). Once the anthers were ready for processing, the Carnoy’s solution was pipetted out, and 100 µL of modified Alexander stain (Rathod et al. 2018) was added. Modified Alexander Stain identifies metabolically active pollen by staining protoplasm and pollen wall cellulose (Rathod et al. 2018). The samples were macerated with a micropestle to expose pollen from the anther, vortexed to homogenize the pollen grains in the solution, then stored until ready for counting. To begin pollen counting, the microcentrifuge tube was vortexed again to homogenize the sample. Using a pipette, 10 µL of the sample was placed on a hemocytometer; viable pollen was counted on the nine square grid, containing 0.9 µL of the sample, using a compound microscope at 400X total magnification. Viable pollen grains stained dark purple to magenta-red, and inviable pollen stained yellow or blue-green, if at all (Rathod et al. 2018).

2.4 Seed counts

Flower heads were stored in open, brown paper bags at room temperature to prevent mold. Fruits were split, and seeds were gently extracted from the locules using forceps, placing them on a black sheet of paper for contrast. Mature and undeveloped seeds were counted with a dissecting microscope at 10X - 40X magnification.

2.5 Data analyses

Co-occurrence of petal, sepal, and pedicel morphologies were evaluated using a X^2 Test for Independence in R (R Core Team 2023). Pollen and seed counts were compared between plants with typical and atypical morphologies in a nonparametric Wilcoxon Rank-Sum Test in R (R Core Team 2023). A Spearman correlation analysis was performed to observe a relationship between reproductive effort and output (R Core Team 2023).

3. Results

3.1 Morphology

The X^2 Test for Independence showed a significant co-occurrence of duplicate petal and sepal whorls ($\chi^2 = 35.1$, $p < 0.05$). However, no relationship was found between pedicel orientation and petal morphology ($\chi^2 = 1.2513$, $p = 0.26$) or between pedicel and sepal morphology ($\chi^2 = 3.7603$, $p = 0.05$).

3.2 Reproductive Effort

The Wilcoxon rank-sum test with continuity correction revealed significantly greater flower production per clump in the group with duplicate sepals ($W = 199.5$, $p = 0.03$) and nearly significant differences in flower production between pedicel morphologies ($W = 123.5$, $p = 0.05$; Figure 3). In contrast, there were no significant differences in flower production between typical or atypical petal groups ($W = 121$, $p = 0.09$).

When assessing the impact of sepals on viable pollen, inviable pollen, and total pollen throughout the season, all tests yielded non-significant differences (viable W =

12974, p = 0.91; inviable W = 13241, p = 0.68; total W = 1317, p = 0.74). On the other hand, petal morphology had a significant influence on all three variables in pollen production, with duplicate petals associated with increased viable and total pollen production (viable W = 4948.5, p < 0.005; total W = 5059, p <0.005). Typical petals were associated with more inviable pollen (W = 5883.5, p = 0.0002). Additionally, flowers with atypical pedicel morphologies had significantly more viable (W = 10388, p = 0.046), inviable (W = 9666, p = 0.004), and total pollen (W = 10310, p = 0.04; Table 4 and 5).

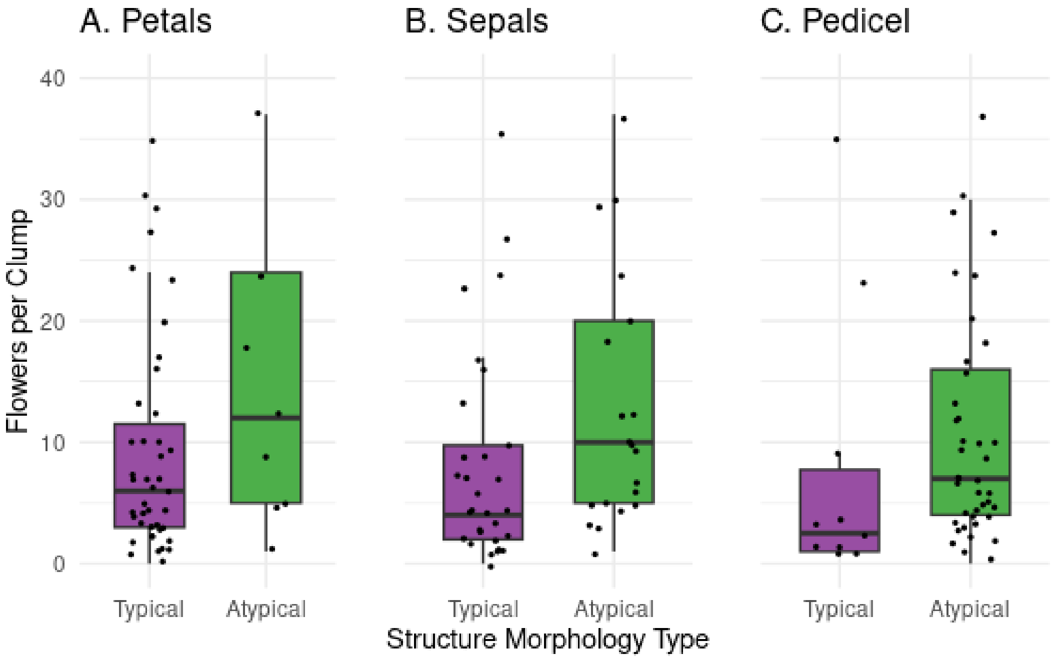

Figure 3. Flowers produced per clump, a measure of reproductive effort, in flowers with typical and atypical petals, sepals, and pedicel orientation. Maximum counts from summer 2023 were used.

3.3 Reproductive Output

In analyses of total seeds, there were no significant differences between plants with typical and atypical floral morphologies (petals W = 1099.5 p = 0.0444; sepals W = 1904, p = 0.0613; pedicel W = 2110, p = 0.0941). Typical petal whorls resulted in marginally higher mature seed counts (W = 1102.5, p = 0.05) but not immature seeds (W = 1025, p = 0.0945). Sepal morphology was not related to mature seed counts (W = 1879, p = 0.0834), but more immature seeds were produced in flowers with typical sepal whorls (W = 1934.5, p = 0.0163). Pedicel orientation was not significantly associated with differences in any reproductive output variables (mature W = 2100, p = 0.1045; immature W = 1949.5, p = 0.1821; Table 6 and 7). A Spearman correlation analysis showed a significant correlation between pollen production and seed production throughout the season (Figure 8; Spearman= -0.446, p<0.005, r^2= 0.199).

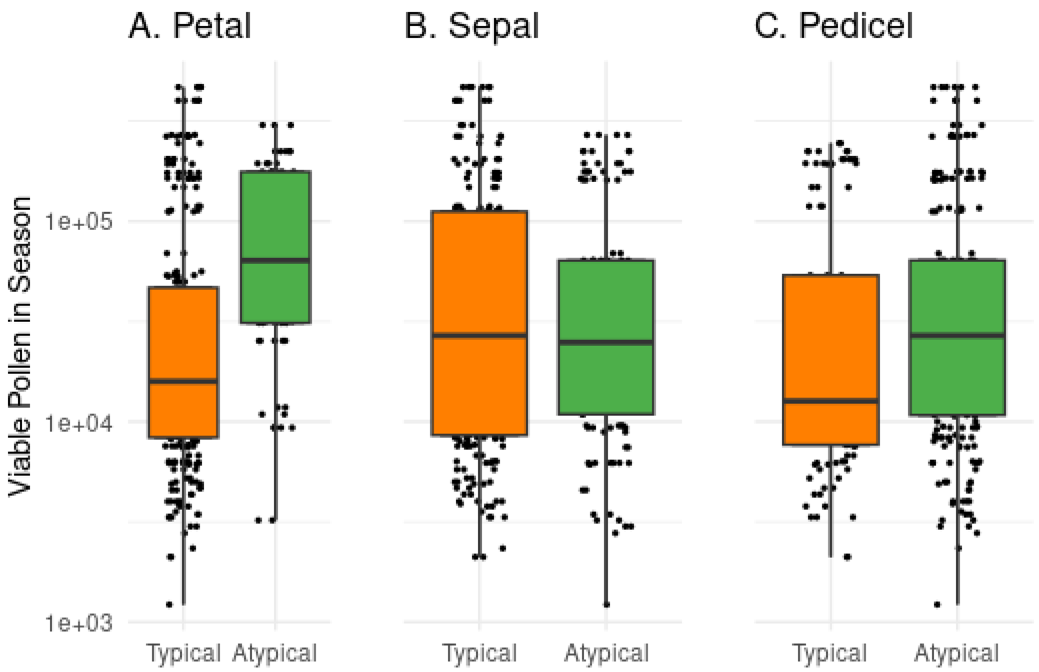

Figure 4. Pollen viability, a measure of reproductive effort, in relation to typical and atypical morphologies in petals, sepals, and pedicels. Total counts were taken from one anther per week over a nine week period in summer of 2023 at SV. The y-axis is log-scaled.

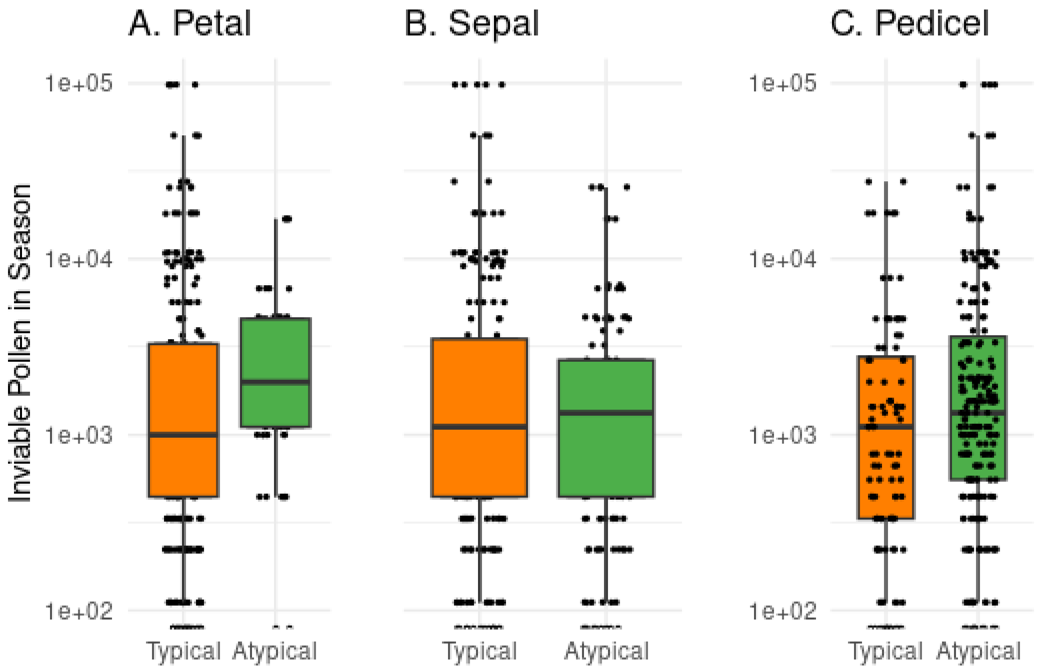

Figure 5. Inviable pollen grains per anther comparing petals, sepals, and pedicel between typical and atypical morphologies. Total counts were taken from one anther per week over a nine week period in summer of 2023 at SV. The y-axis is log-scaled.

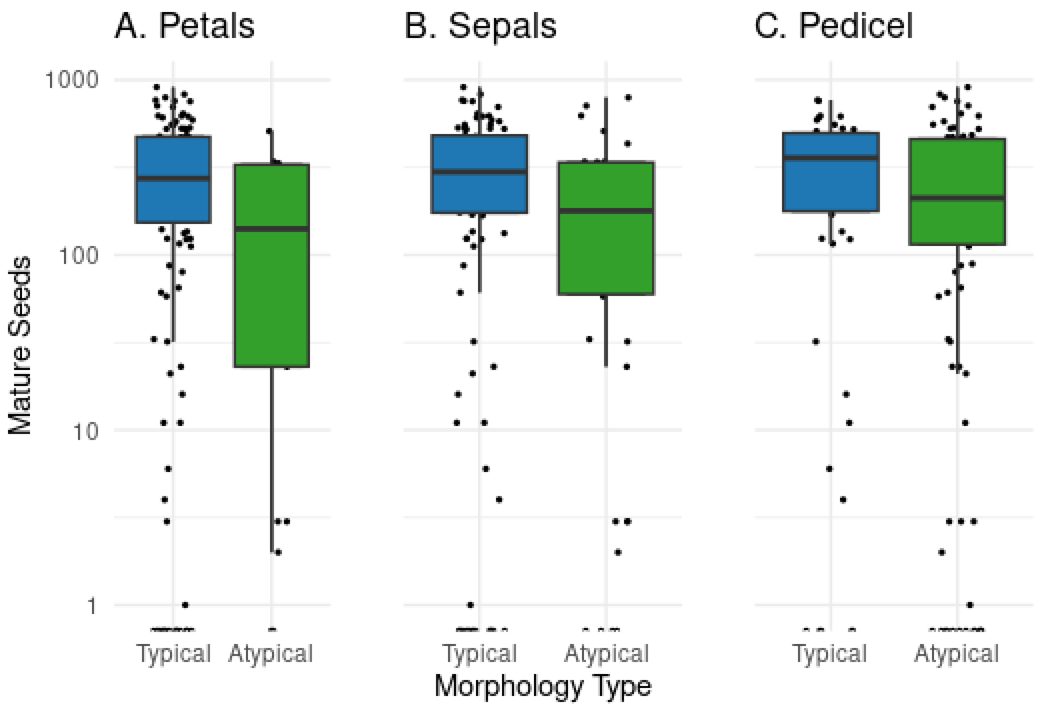

Figure 6. Mature seeds, a measure of reproductive output, by petal, sepal, and pedicel morphotypes. Seeds were counted from mature flowers at the end of the flowering season in 2023. The y-axis is logged to scale.

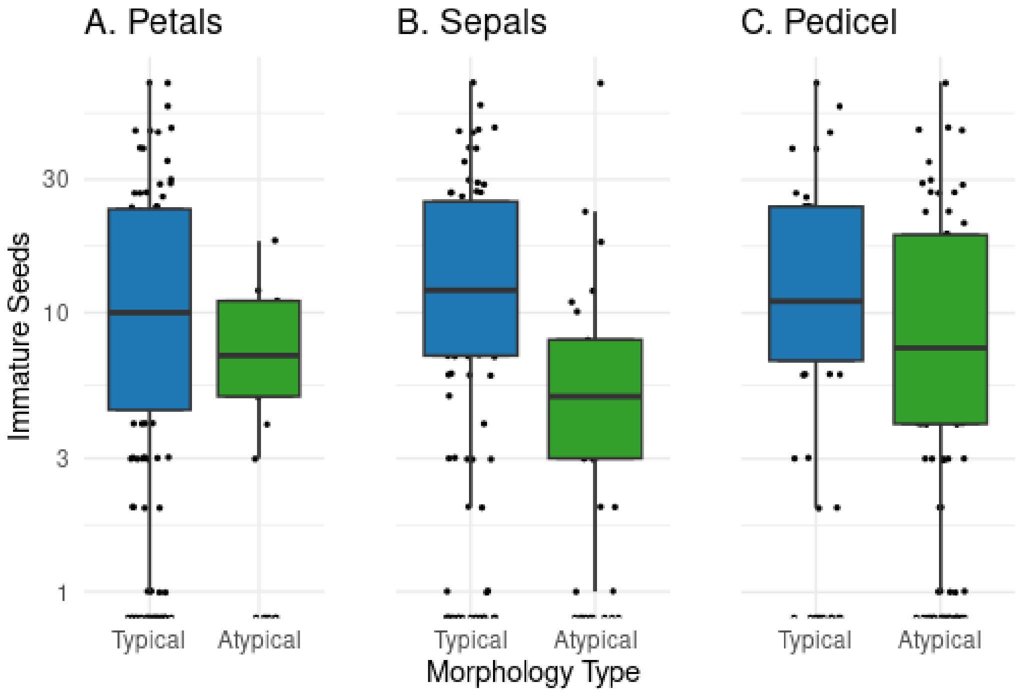

Figure 7. Immature seeds by petal, sepal, and pedicel morphotype. Seed output was counted from mature flower fruits at the end of flowering season at SV. The y-axis is logged to scale.

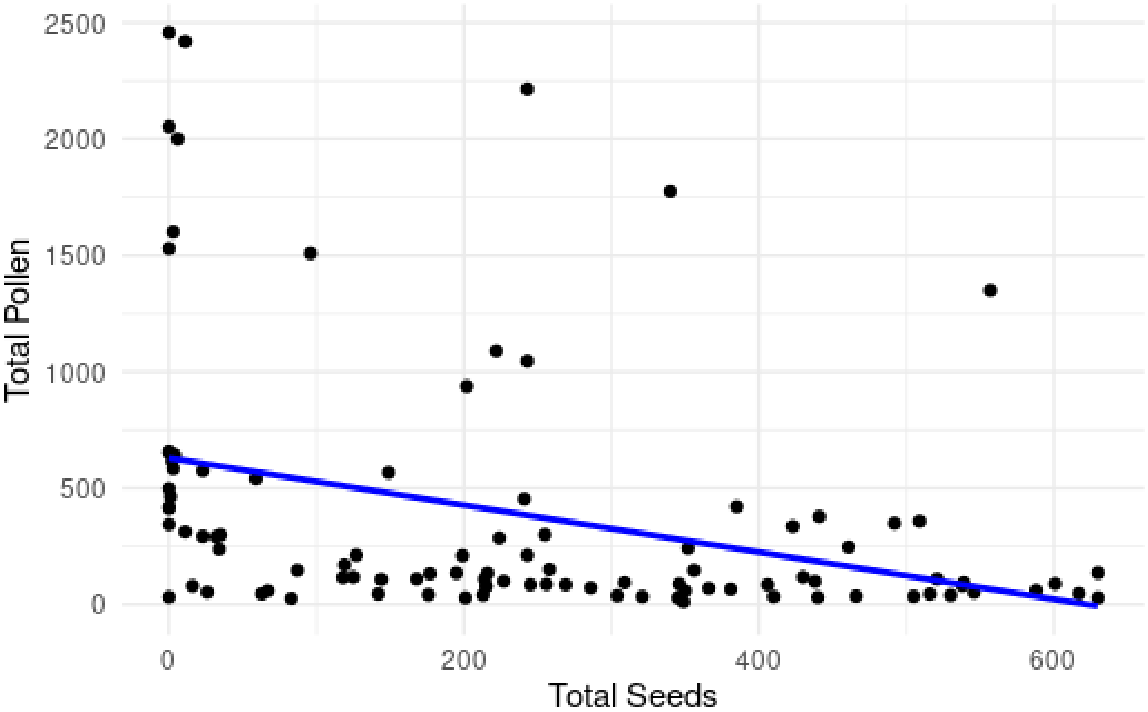

Figure 8. A Spearman correlation analysis shows the relationship between pollen production and seeds produced (r^2= 0.199). As seed production increases, total pollen decreases.

4. Discussion

We tested four hypotheses concerning the role of reproductive strategies of *S. purpurea* var. *montana* between typical flowers and the novel flowers exhibiting duplicate floral petal and sepal whorls, as well as upward pendation of the flower. We predicted that atypical morphologies would be associated with lower reproductive effort in terms of flower and pollen production and reproductive output in terms of total seed production. We found mixed support for these hypotheses. Although there was an association between petal and sepal morphologies on the same flower, they did not always co-occur, indicating that these are two distinct traits rather than traits that have shared genetic control. Further, pedicel orientation appears to be independent of these variables.

We found that duplicate sepal whorls were associated with greater flower production, contrary to our prediction. We hypothesized that greater investment into sepals would occur at the cost of flower production. However, flower production is primarily influenced by resource allocation, while sepals are photosynthetic and are able to supplement critical energy to the plant (Guo & Halston 2020). Contrary to what we thought, the addition of duplicate sepal whorls could provide extra resources to the clump, which could be stored to produce more flowers the following year. Multiple studies with *Sarracenia* suggest flower production is linked to energy reserves. For example, Hale et al. (2020) found *Sarracenia* clumps with more invertebrate prey in their pitchers were more likely to flower the following year. This suggests that first, pitcher fluid communities benefit host pitcher plants with increased reproductive effort and second, the extra energy is stored over winter and utilized the following year. Additionally, Primack and Hall (1990) demonstrated plants that produce fruit in a given year experience a decrease in leaf area and a lower probability (5% -16%) of flowering in the following year. This suggests that there is a cost associated with reproduction, and that investment into flowers is tightly linked to the energy budget. Next steps include the continued monitoring of these clumps to track flower production and energy storage.

In this study, an intermediate or up pendation of the flower resulted in more inviable pollen; our original hypothesis suggested an atypical pendation would be associated with less viable pollen. Conversely, up or intermediate pedicels were also associated with more viable and total pollen, overall. Up or intermediate orientations of the pedicel expose pollen to potential environmental damage, and it is this factor that could increase the amount of non-viable pollen per anther. Pollen contains genetic information that must be transmitted to the stigma via sexual reproduction, with pollinators and flower structure being important vectors in the fertilization process. The typical downward orientation of pedicels protect the pollen from rain and UV light due to the curtain-like petals and sepals. The increase of all pollen categories could be due to the fact that flowers with atypical pedicels had more pollen overall than regular pedicled flowers, increasing the volume for viable and inviable pollen grains. In a study with *Tristerix corymbosus*, an Andean perennial with multiple flowers per individual, changes in pedicel orientation did not cause changes in pollen loads when exposed to rain (Tadey et al. 2001). Interestingly, duplicate petal whorls also were associated with more total and viable pollen, while flowers with typical petal whorls produced more inviable pollen, contrary to what we predicted in terms of allocating resources to both petals and pollen.

The correlation between pollen and seed production suggests that reproductive output is related to reproductive effort, but not in the way we initially hypothesized. We found that more pollen was related to lower seed production, contrary to our belief that increased pollen would yield an increased seed set. Therefore, our hypothesis that atypical pedicel orientations would lead to lower mature seed counts as a result of pedicel affecting viable pollen was not supported. A study by Hildesheim et al. (2019) failed to detect an interaction between pollen load and seed count in the model testing effects on seed mass. It could be *S. purpurea* var. *montana* is an income breeder, relying heavily on current prey resources for reproductive success (Ne'eman & Ne'eman 2016).

Our results supported our hypothesis as they suggest that flowers with typical petal whorls yielded more seeds while flowers with typical sepal whorls had more immature seeds. Generally, the mechanical match between plants and pollinators is important and stronger in specialized systems. For example, the Dwarf Rattlesnake Plantain (*Goodyera repens*), a North American orchid, has a flower in which the sepals and petals form a narrow tube, preventing the flowers from opening wide. Little room is available for insects to penetrate, but the rigid proboscis of the bumblebee is well suited to the attachment of the viscidium to the adhering pollinia (Brzosko et al. 2023). This showcases how a flower's morphology can influence the specialization of pollinators and their ability to successfully pollinate. While we could have assumed duplicate petal whorls would attract more pollinators, leading to increased fertilization, duplicate petals could have made it difficult to enter the flower, since petals act as a curtain to the reward center. The deterrence from the duplicate petals may have given co-occurring flowers with typical petals increased visitation by pollinators and fertilization. Additionally, other factors contribute to decreased seed production such as seed predation, resource limitations, allocation to prior reproduction, and genetic factors such as inbreeding depression and self-incompatibility (Emilie et al. 2007). Conversely, flowers with the single sepal whorl produced more immature seeds. Duplicate sepal whorls could have resulted in more floral visitors and outbreeding since sepals do not block entry to the anthers.

4.2 Future Recommendations

The next steps are to investigate the reasons for the abnormal floral morphologies, such as genetic or developmental mutations affecting floral whorls and orientation. In a study by Sun et al. (2019), a spontaneous upward-pedicel mutant named *upward-pedicel (up)* was found in cucumber. Most cucumber inbred lines possess downward growth of pedicels with downward-facing or horizontal-facing female flowers. In this mutation, a 5-bp deletion resulted in a frameshift mutation and earlier translation termination, causing the altered orientation. Vandenbussche et al. (2004) explored the genetic mechanisms underlying flower development in *Petunia hybrida*, specifically focusing on the role of duplicated B-class *MADS-box* genes, *PhDEF and PhGLO*, which exhibit functional redundancy in specifying petal and stamen identity (Vandenbussche et al. 2004). It would be informative to know the roles of these loci in *S. purpurea* var. *montana* floral architecture.

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