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Analyzing Reproductive Effort, Reproductive Output, and Pitcher Morphology Variation to Monitor *Sarracenia purpurea* var. *montana* Populations in Southern Appalachia

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Abstract

Sarracenia purpurea var. *montana* is a carnivorous pitcher plant endemic to the southern Appalachian region of North Carolina, South Carolina, and Georgia. It is a federal species of concern under review with the U.S. Fish and Wildlife Service. This study focuses on a population of *S. purpurea* var. *montana*, located in Transylvania County, North Carolina, that exhibits unique flower morphology. Typically, *S. purpurea* flowers are pendulous and oriented downward with one sepal whorl, one petal whorl, and a smooth style. In this population, some individuals have intermediate and upward-oriented flowers, multiple sepal whorls, multiple petal whorls, and frilled styles. We hypothesize that this atypical flower morphology might be associated with differences in fitness, including reproductive effort (pollen production) and reproductive output (seed production). To test this, we monitored the population throughout the 2023 reproductive season and compared our findings to data collected in previous years at three other *S. purpurea* var. *montana* sites in western North Carolina. We collected

anthers and recorded floral stage and orientation weekly, measured the morphological characteristics of the pitchers, and counted seeds once the ovaries had matured. Analyses show that the reproductive effort, reproductive output, and pitcher morphology of the site's plants are comparable to those at other sites in the region. Future research includes exploring the genetic and developmental mechanisms underlying this population's unique morphology. Comparing morphological and reproductive traits among populations documents the natural variation that exists within *S. purpurea* var. *montana*. Such comparisons can inform conservation efforts by identifying populations that may be of particular interest to preserve.

Introduction

Sarracenia purpurea L., the purple pitcher plant, is a species of carnivorous plant distributed across eastern regions of the United States and most of Canada (U.S. Forest Service, n.d.). *Sarracenia purpurea* is the widest-ranging species in the *Sarracenia* genus with several known subspecies and varieties across North America (Juniper et al., 1989). It is listed as a federal species of concern by the U.S. Fish and Wildlife Service and is categorized as endangered in Georgia, Illinois, and Michigan. *Sarracenia purpurea* is a rhizomatous plant whose evergreen leaves are morphologically adapted to capture invertebrates (Slack, 1985; Schnell, 1976). They grow in peat bogs and pine stands and exhibit distinct pitcher morphology. Pitchers are decumbent with an average length of around 45 cm (Schnell, 1976). While other pitchers may rely on trapping prey using hood shape, *Sarracenia purpurea* pitchers are specialized in shape and orientation to capture rainwater that can be used to capture prey (Slack, 1985). Mature *S. purpurea* leaves also contain inquline communities (Miller and Kneitel, 2005).

Sarracenia purpurea has two main subspecies: *S. purpurea* L. subsp. *venosa* (Rafinesque) Fernald and S. *purpurea* subsp. *purpurea* L. These subspecies are separated by their geographic distribution and morphological distinctions (Ellison et al., 2004). *S. purpurea* subsp. *venosa* is found in the southeastern region of the United States, while subsp. *purpurea* is found in the northeastern United States and Canada. The pitchers and flowers of these subspecies are morphologically distinct on the basis of pitcher aperture, flower appearance, pitcher surface morphology, and pitcher height-to-aperture ratio (Schnell, 1976; Ellison et al., 2004). Morphology of these subspecies is correlated with differences in precipitation and temperature.

While *S. purpurea* is the widest-ranging species in the *Sarracenia* genus, a variant of the *S. purpurea* subsp. *venosa*, *S. purpurea* L. var. *montana* Schnell & Determan, is only found in the southern Appalachian region in western North Carolina, northern South Carolina, and northern Georgia (ECOS, n.d.). S. *purpurea* var. *montana* is listed as a Federal Species of Concern, as determined by the US Fish and Wildlife

Service (Schnell, 2000). Conservation and maintenance of small populations is vital for maintaining genetic diversity that exists in this region.

Flowers of *S. purpurea* var. *montana* are characterized by downward-oriented blooms that hang pendulously from the stem (Fig. 1) (Schnell, 1976). Typical flowers have one whorl of five sepals at the base of the bloom that extend beyond the bloom itself. There is one whorl of five petals, as well, that hang down and drape around the style like curtains. The star-shaped style is attached to the base of the bloom, acting like a “floor” that catches pollen falling from the anthers. At each tip of the style is the stigma. The stigma is pollinated when an insect visitor brushes against it with pollen it has carried over from other flowers. Flowers in the clade *Sarraceniaceae* can self-pollinate, and previous studies find that self-pollination results in lower quality and quantity of offspring (Sheridan et al., 2000). However, the shape of the style discourages self-pollination, preventing high rates of self-pollination (Schnell, 1976). The anthers hang from the base of the bloom underneath the whorl of petals, facing down so pollen is free to fall from them. They surround the flower’s ovary, on which the style hangs. The pollen itself also has a distinct morphology with six to ten colpi (Oswald et al., 2011).

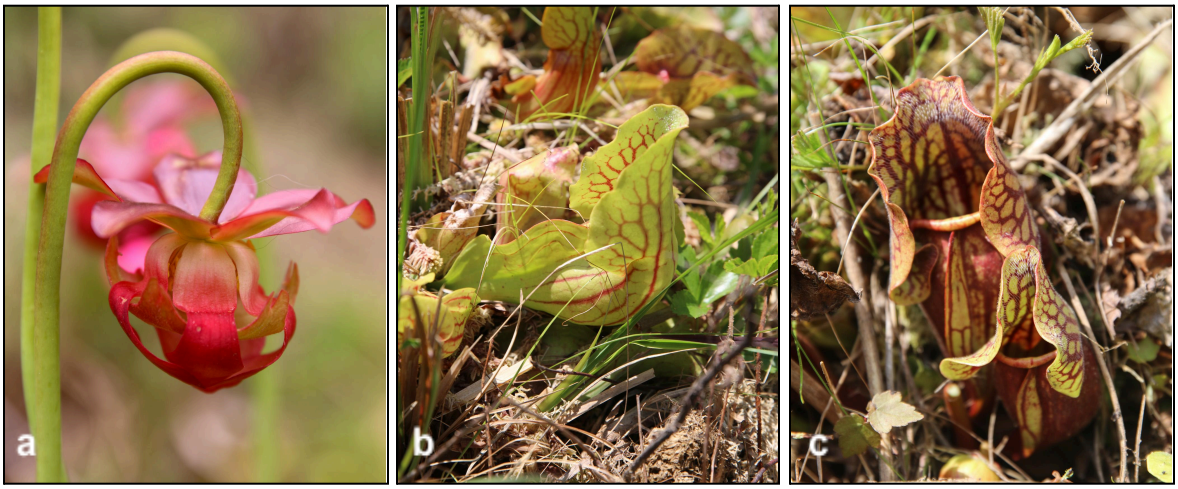

Figure 1. Example of typical floral morphology of *Sarracenia purpurea* var. *montana* (a), an example of typical pitcher morphology of *Sarracenia purpurea* var. *montana* (b, c). Photo credits to Gabi Parker.

At one site in western North Carolina, referred to as SV, several individuals displayed atypical floral morphology (Fig. 2). Instead of the bloom being oriented downward, some flowers were intermediate or upwardly oriented. Additionally, some of the upwardly oriented flowers lacked a style and perhaps ovaries, so these flowers may have been unable to produce seeds. It is important to note that flowers naturally assume an intermediate or upward position after pollination and fertilization (Schnell, 1976). However, the flowers observed with the aforementioned characteristics had only

recently flowered. Some flowers in the population had two or three sepal whorls as opposed to the typical single whorl. Some individuals, particularly those from a specific rosette, had frilled styles rather than smooth ones. Others in the population had abnormally oriented stems. Instead of straight, vertical stems, many bent in several directions before terminating in a flower. Occasionally, the bends were so dramatic that the flower grew in an arc, pushing back into the pitcher crown.

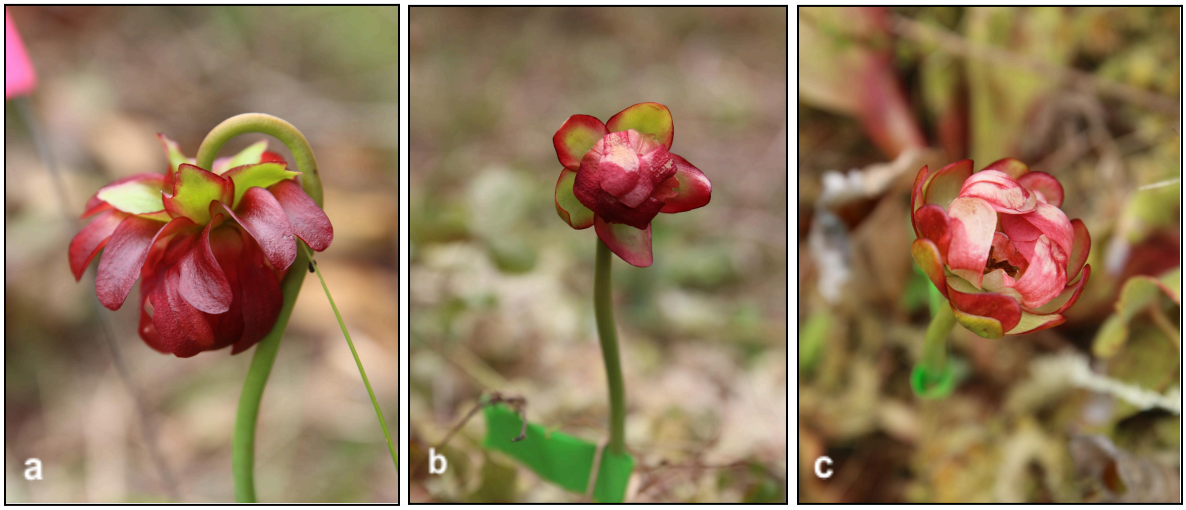

Figure 2. Examples of atypical floral morphology observed at site SV. Downward floral orientation with multiple sepal whorls (a), intermediate floral orientation (b), and upward floral orientation with multiple sepal whorls (c). Photo credits to Gabi Parker.

Overall, the population's atypical floral morphology led us to wonder if site SV is an outlier in pitcher morphology as well as reproductive effort and output. Reproductive effort refers to the total proportion of energy, over a biologically meaningful time interval, that an organism devotes to reproduction (Hirshfield and Tinkle, 1975). Plants' reproductive effort is multifaceted and should encompass all structures that are considered "reproductive" (Thompson and Stewart, 1981). For our purposes and limitations, we used viable pollen production to measure reproductive effort. Reproductive output also encompasses a myriad of measurements, but seed production over a given time is one frequently used measure (Wenk and Falster, 2015). Atypical floral morphology itself may affect reproductive effort and output. Further, if the pitchers at site SV also have abnormal morphology, this could affect prey capture and thus reproductive effort. Indeed, Ne'eman et al. (2006) found that *S. purpurea* var. *montana* resource availability was a key predictor of reproductive output. We predicted that due to atypical floral morphologies, site SV would have lower reproductive effort and output than the other compared sites. Comparisons of reproductive effort and output between *S. purpurea* var. *montana* sites may aid in a better understanding of how carnivorous plants allocate their resources, as well as in conservation efforts. Lower reproductive

productivity may indicate more resource allocation to the production of additional plant tissue.

Materials and Methods

During the months of May to July of 2023, we monitored and collected samples from an isolated population of *Sarracenia purpurea* var. *montana* in western North Carolina. In May, we assessed the bog's population and marked individual pitcher plant clumps. *S. purpurea* var. *montana* is a rhizomatous plant, so individual rosettes that are close to one another may be clones. U.S. Fish and Wildlife Services defines rosettes as being distinct from one another if they are separated by a distance of 0.5 m or greater (Murdock, 1990). Following this convention, we identified 70 individual clumps and marked each with a metal tag. We selected at least three flowers from each clump and labeled them with the letters A, B, or C. To capture the variation in floral morphology exhibited by this population, additional flowers with especially atypical morphology were marked and included in the study, with some clumps having as many as five flowers marked. Each week, we returned to the site to collect anthers and document the total number of flowers in each clump. We recorded floral orientation, stage of floral maturity; number of sepal whorls, number of petal whorls, and the total number of flowers in the clump. In this study, we also used data gathered from other *Sarracenia purpurea* var. *montana* sites in previous years. Our methods of data collection were modeled after theirs.

2.1 Morphological Pitcher Measurements

Morphological measurements of one pitcher from each of the 70 clumps at SV were taken in August of 2023. A vernier caliper and digital protractor were used to measure pitcher front height, pitcher posterior height, maximum hood width, hood height, hood angle, aperture width, perpendicular width aperture, and rib width (Fig. 3). The tallest flower from each clump was identified, and stalk measurements were recorded.

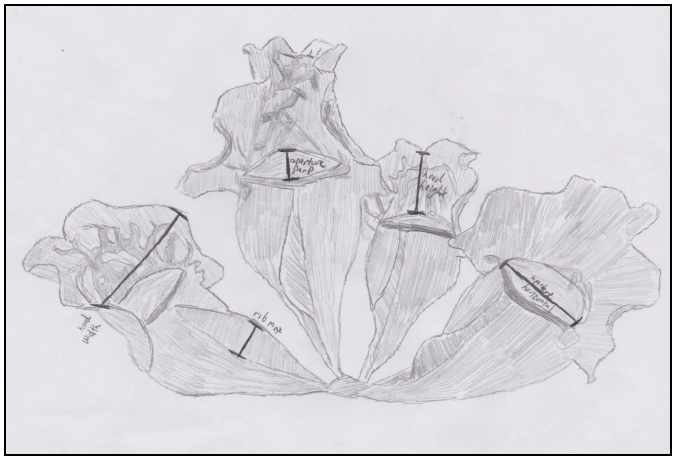

Figure 3. Morphological measurements obtained from each selected pitcher. Drawing credits to Todd Brasseur.

2.2 Pollen Collection

Among the four sites CM, MB, RL, and SV, 655 total anthers were collected and counted. We used viable pollen production as a measure of the reproductive effort of *S. purpurea* var. *montana*. While reproductive effort is best measured by assessing all reproductive structures (Wenk and Falster, 2015), pollen is a discrete measurement that can easily be utilized for site comparisons. On a weekly basis, we collected anthers and scored each flower for floral maturity. The flowers were assigned a status of 0-6 based on the approximate percentage of anthers present and the presence or absence of visible pollen.

Table 1. Criteria for distinguishing pollen stage.

Floral Stage	Characteristics
0	Unopened
1	All anthers attached; no pollen evident
2	All anthers attached; pollen evident
3	25% of anthers have fallen
4	50% of anthers have fallen
5	75% of anthers have fallen
6	All anthers have fallen/no anthers attached

We collected one anther per flower using forceps and placed it in a microcentrifuge tube. In the lab, Carnoy's preservative solution [one part glacial acetic acid and three parts 95% or 100% ethanol] was added to each anther and left for at least 24 h.

2.3 Pollen Counting

After the anthers sat for at least 24h in the Carnoy's solution, we used a pipette to remove excess solution from the tube. The tubes were left open for 24h to allow any remaining Carnoy's solution to evaporate. Anthers were resuspended in 100 µL of modified Alexander's Stain [9.5% ethanol, 0.1% malachite green, 0.5% acid fuchsin, 0.05% orange G, 4% glacial acetic acid, 25% glycerol] and allowed to sit for at least 30 min. Samples were then macerated using a micro pestle to release pollen grains from the anther.

To count the amount of pollen in each sample, the anther was re-macerated and agitated using a vortexer for at least 30s. After pipetting 10µL of the sample onto a hemocytometer with Neubauer rulings, viable and inviable pollen was counted on the nine-square grid, containing 0.9µL of the sample. All nine squares were counted. This was done using an Olympus CH30 compound microscope at 400X magnification.

We calculated the total anther pollen, P_T, as

$$P_T = \frac{P_S \cdot V_T}{V_S}$$

where P_S is the pollen counted in our sample, V_T is the total volume of the full suspension (100µl), and V_T is the sample volume (0.9µl).

2.4 Seed Collection

In September and early October of 2023, mature ovaries were gathered from all marked flowers at the site. We placed a paper bag over the flower, pinched the opening closed around the stem, and cut the flower off at the base. The mature ovaries were stored in open bags at room temperature in the lab.

2.5 Seed Counting

Each flower was removed from the collection bags and the outer layer of petals and sepals were removed. We scraped all of the seeds and ovules off the ovary and

seeds were placed in a petri dish and counted. An Olympus SZ51 Stereo Zoom microscope was used to distinguish seeds from ovules, as well as observe their viability. We distinguished between mature seeds, aborted seeds, and undeveloped ovules. Only the mature seeds and aborted seeds were counted. After counting, the seeds were put into accession at the North Carolina Botanical Garden.

2.6 Data Analysis

All data analyses were performed in RStudio (R Core Team 2023).

Pitcher Morphology:

To compare pitcher morphology between sites on the basis of eight standardized morphological measurements, a principal component analysis was performed. Measures were examined for loading positively and negatively on PC1 and PC2 to deduce the variance captured by each principal component. Variation in pitcher morphology by site was visualized in a violin plot based on PC1 scores for each site. ANOVA with post-hoc Tukey HSD quantified which sites exhibited statistically significant variation in pitcher morphology from one another.

Pollen Counts:

To determine if SV's pollen production varied from that of other sites, we used a Scheirer-Ray-Hare test to assess the effects of site location and floral stage on pollen production. A Kruskall-Wallis test was used to determine whether the sites differed in pollen production within each stage. A post hoc Dunn test with Bonferroni correction was run on groups with significant interactions.

Seed Counts:

A Shapiro-Wilk test was first performed to check for the normality of the distribution. A Scheirer-Ray-Hare test was used to compare between site, year (1 vs. 2), and the relationship between site and year. A post hoc Dunn test with a Bonferroni correction was also performed to pool all years and compare the sites, with year 2 of CM excluded as it was an outlier.

Results

3.1 Pitcher Morphology

Principal component analyses indicate that PC1 accounted for 36.8% of variation among pitchers with all measures loading positively on this component, while PC2 accounted for 15.5% of variation among pitchers (Fig. 4). PC1 indicated variation in pitcher size while PC2 represented variation in pitcher shape. Further, it was found that taller pitchers exhibited smaller apertures and hoods, along with sharper hood angles. Looking specifically at the morphological variation captured by PC1, pitcher morphology varied significantly by site ($F_{3, 96}$=3.533, p=0.018).

Pitcher morphology at SV did not vary from morphology at all of the other sites (TukeyHSD *post hoc* test, p ≥ 0.05; Fig. 5). However, pitcher morphology at MB was distinct from pitcher morphology at CM and RL (TukeyHSD *post hoc* test, p ≤ 0.05). This indicates that pitchers at SV exhibit an intermediate morphology between that of CM and RL.

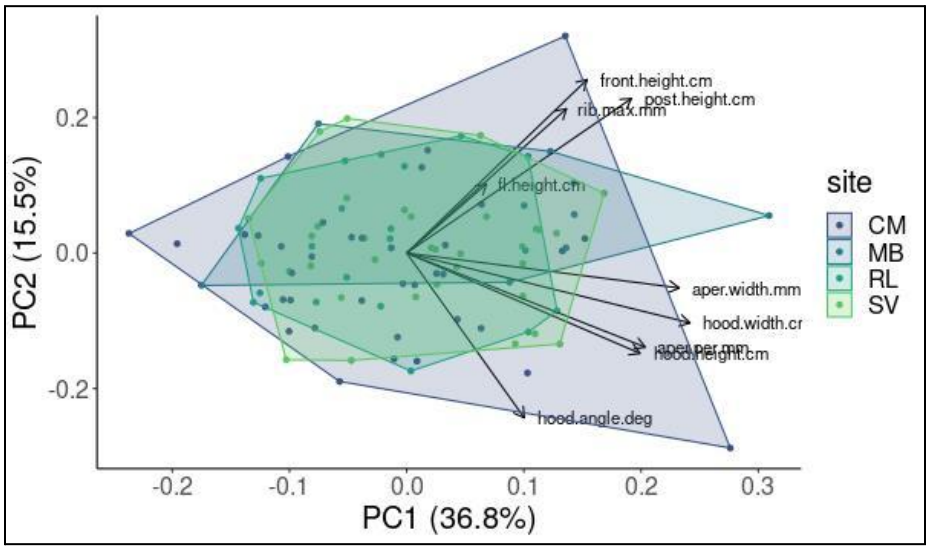

Figure 4. Principal component analysis (PCA) of pitcher morphology measurements by site.

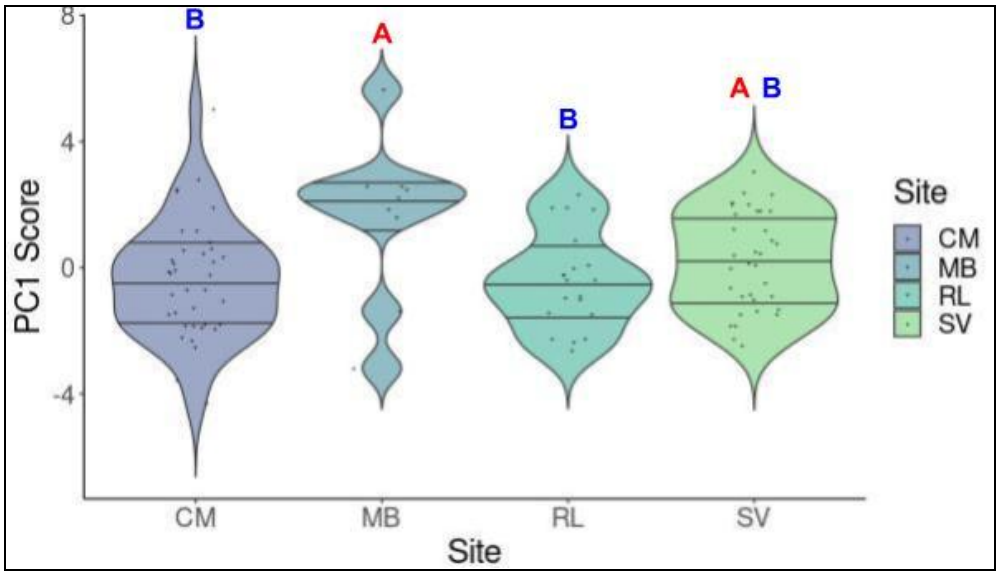

Figure 5. Violin plots of PC1 scores compared between sites.Sites with different letters (**A** and **B**) differed significantly.

3.2 Reproductive Effort

Pollen counts ranged from 0 to 367,666 pollen grains per anther. Through the pollen counting process, we discovered that some of the pollen had abnormal morphology, with some pollen grains lacking colpi entirely. After comparing the four sites using a Scheirer-Ray-Hare test, we found that overall, viable pollen production was significantly affected by site ($H=30.837$, $df=3$, $p < 0.0001$), by floral stage ($H=39.144$, $df=4$, $p < 0.0001$), and by the interaction between site and floral stage ($H=30.093$, $df=12$, $p=0.0027$; Fig 6).

While the results of the Schierer-Ray-Hare tests show significance, not every floral stage had significant results. At floral stages 1, 3, and 4, there were no significant differences found. In other words, pollen production did not differ across sites at these floral maturity stages. The only pollen stages with significant results were stages 2 and 5. For floral stages 1, 3, and 4, there was no significant difference between them. At floral stage 2, significant differences were found between CM, RL, and SV ($p>0.05$). Site CM had the lowest pollen count at stage 2 and RL had the highest pollen count. At floral stage 5, significant differences were found between sites CM and MB ($Z=-2.640$, $p=0.0497$) with stage 5 flowers at site CM having lower viable pollen counts than site MB.

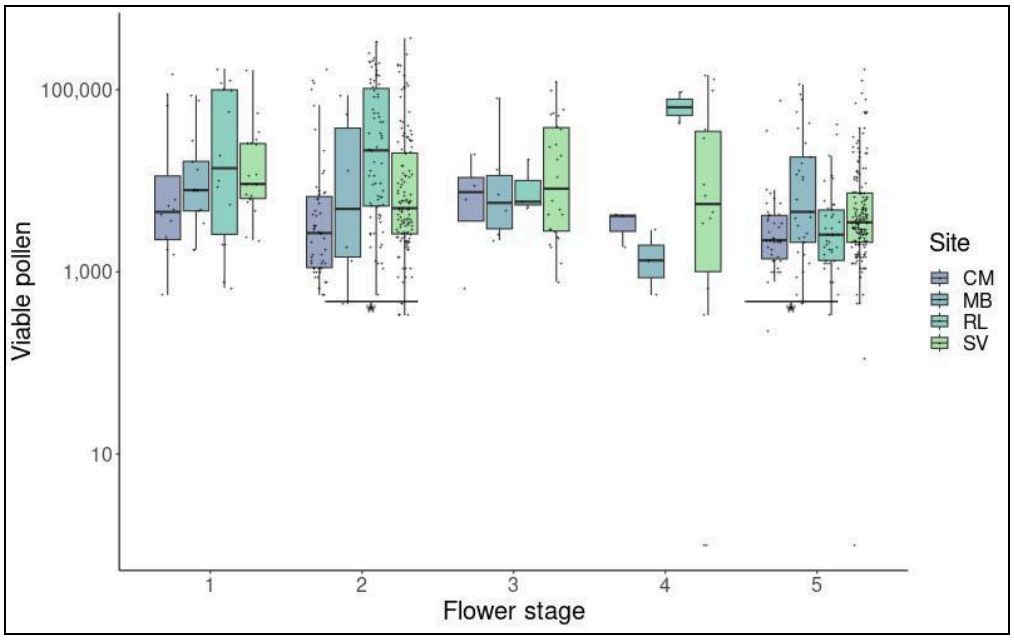

Figure 6. Box plot depicting the production of viable pollen per anther at each site by the stage. Stage correlates to the percent of anthers that are still attached to the flower. * indicates a significant difference

3.3 Reproductive Output

A Scheirer Ray-Hare test showed mature seed production was significantly affected by site ($H=64.564$, $df=3$, $p= 0.00000$) and by the interaction between site and year ($H=26.659$, $df=3$, $p=0.000007$). Mature seed production was not shown to be significantly affected by year ($H=2.693$, $df=1$, $p=0.100814$; Fig. 7). A post-hoc Dunn test with a Bonferroni correction was also run. After pooling all years and excluding year 2 CM, MB and RL exhibited significant differences in reproductive output, with RL producing more mature seeds ($p=0.007$). Additional significant differences were found between sites SV and RL for both years ($p=0.040$).

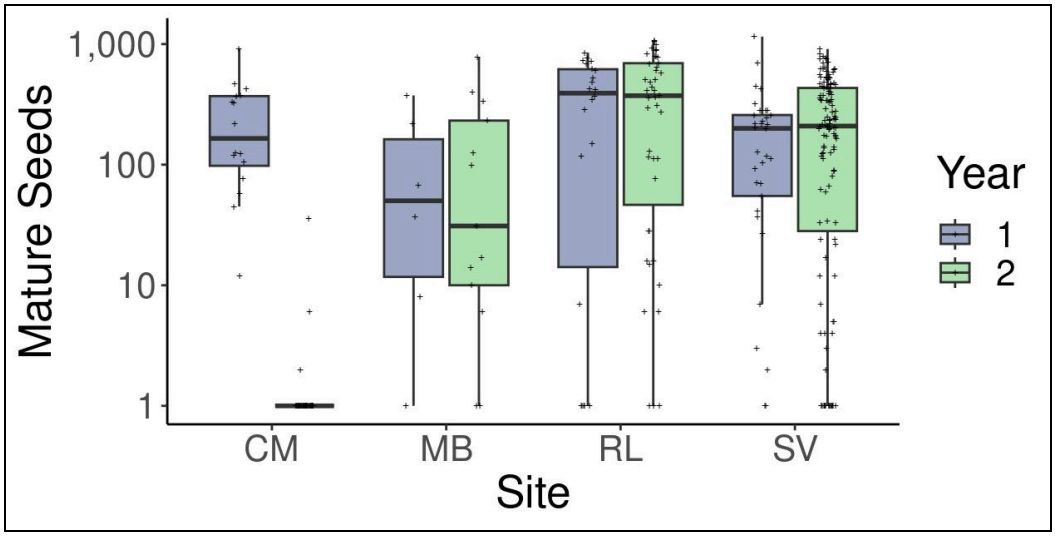

Figure 7. A comparison of two years of seed production of *S. purpurea* var. *montana* among all four sites.

Discussion

Pitcher morphology, reproductive effort, and reproductive output at site SV were overall consistent with other sites. Because SV had consistent measures with other sites, it was disproven that resource allocation was affected by the production of additional plant tissue. In this population, our data do not suggest a negative consequence of atypical floral morphology in terms of pollen and seed production. The pitcher morphology of plants at SV was also similar to other sites. Reproductive effort, measured by pollen production, was not significantly lower in SV than in the other sites. Another factor linked to reproductive effort in carnivorous plants is prey capture (Ne'eman et al., 2006). Because pitcher morphology at site SV does not differ from pitcher morphology at other sites, it can be assumed that prey capture abilities of plants at SV are not compromised. The reproductive output, measured by the number of seeds produced, yielded similar results. Significant differences were found between sites MB and RL ($p<0.05$). Additional significant differences were found between the output for both years between SV and RL, but this slight difference appears to be due to abnormally high success rates in RL, as opposed to a lack of output in SV.

The reason why the flowers differed morphologically from the typical phenotype is still unknown. Although the phenotypic differences in the SV population have not been described before in *Sarracenia purpurea* var. *montana*, populations of other vascular plants have displayed abnormal floral morphology. For example, fascination is a phenomenon associated with the elongation of the apical meristem caused by a hormonal imbalance, genetic mutation, or a viral, bacterial, or fungal infection (Voyle, 2015). While this is not what is being observed in the SV population, it shows

documented phenotypic differences as a result of some infection, genetic change, or hormone imbalance.

Another possible explanation for the abnormal floral morphology is inherited epigenetic mutations. Because plants are sessile, they have evolved highly adaptable epigenetic capabilities (Voyle, 2015). Epigenetic changes can be passed to offspring produced asexually, and if conditions are consistently stressful, epigenetic changes can be passed down to offspring produced sexually (Brukhin and Albertini, 2021; Kumari et al., 2022). Because *Sarracenia purpurea* var. *montana* reproduces both sexually through seeds and asexually through rhizomal cloning, both scenarios are possible (Schnell, 1976).

Conclusion

Sarracenia purpurea var. *montana* is a Federal Species of Concern (Schnell, 2000). Understanding variations in life histories and population dynamics among populations will help better inform regional conservation efforts. Future work includes genotyping plants at SV to determine if atypical floral morphology is of genetic and/or epigenetic origin.

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References

Brukhin, V., & Albertini, E. (2021). Epigenetic modifications in plant development and reproduction. Epigenomics, 5(4). https://doi.org/10.3390/epigenomes5040025

Ellison, A. M., Buckley, H. L., Miller, T. E., & Gotelli, N. J. (2004). Morphological variation in *Sarracenia purpurea* (Sarraceniaceae): Geographic, environmental, and taxonomic correlates. American Journal of Botany, 91(11), 1930-1935. https://doi.org/10.3732/ajb.91.11.1930

Environmental Conservation Online System (ECOS) (n.d.). Mountain Purple Pitcherplant (*Sarracenia purpurea* var*. montana*). U.S. Fish and Wildlife Service. [Accessed 2024 April 11] https://ecos.fws.gov/ecp/species/9940

Hirshfield M.F. and Tinkle D.W. 1975. Natural selection and the evolution of reproductive effort (life history/energy budgets/fitness/reproductive value). Proceedings of the Natural Academy of Sciences of the United States of America. 72(6): 2227-2231.

Juniper, B. E., Robins, R. J., & Joel, D. M. (1989). The Carnivorous Plants. Academic Press.

Kumari, P., Khan, S., Wani, I., Gupta, R., Verma, S., Alam, P., & Alaklabi, A. (2022). Unravelling the role of epigenetic modifications in development and reproduction of angiosperms: a critical appraisal. Frontiers in Genetics, 13. https://doi.org/10.3389/fgene.2022.819941

Miller, T., & Kneitel, J. (2005). Inquiline communities in pitcher plants as a prototypical metacommunity. Metacommunities: Spatial Dynamics and Ecological Communities. 122-145.

Murdock, N. (1990). Mountain Sweet Pitcher Plant Recovery Plan. Atlanta, Georgia: U.S. Fish and Wildlife Service Southeast Region. Available from: https://ecos.fws.gov/docs/recovery_plan/Mountain%20sweet%20pitcher%20plant%20RP.pdf

Ne'eman, G., Ne'eman, R., & Ellison, A. M. (2006). Limits to reproductive success of *Sarracenia purpurea* (Sarraceniaceae). American Journal of Botany, 93(11), 1660-1666. https://doi.org/10.3732/ajb.93.11.1660.

Oswald, W. W., Doughtry, E. D., . Ne'eman, G., . Ne'eman, R., & Ellison, A. M. (2011). Pollen morphology and its relationship to taxonomy of the genus *Sarracenia* (Sarraceniaceae). BioOne, 113(955), 235-251. https://doi.org/10.3119/0035-4902-113.955.235

Pikaard, C. S., & Scheid, O. M. (2014). Epigenetic regulation in plants. Cold Spring Harbor Perspectives in Biology, 6(12). https://doi.org/10.1101/cshperspect.a019315

R Core Team (2023). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Schnell, D. (1976). Carnivorous Plants of the United States and Canada (1st ed.). Blair.

Schnell, D. (2000). The IUCN Red List of Threatened Species: *Sarracenia purpurea* var. *montana*. IUCN Red List of Threatened Species. [Accessed: 2024 April 11]. https://www.iucnredlist.org/species/40116/10314744

Sheridan, P. M., & Karowe, D. N. (2000). Inbreeding, outbreeding, and heterosis in the yellow pitcher plant, *Sarracenia flava* (Sarraceniaceae), in Virginia. American Journal of Botany, 87(11), 1628-1633. https://doi.org/10.2307/2656739

Slack, A. (1985). Carnivorous Plants. The MIT Press.

Thompson, K., & Stewart, A. J. (1981). The measurement and meaning of reproductive effort in plants. The American Naturalist, 117(2), 205-211. https://doi.org/10.1086/283700

U.S Forest Service (n.d.). Purple Pitcherplant, Saddle Flower (*Sarracenia purpurea L.*). U.S. Forest Service. https://www.fs.usda.gov/wildflowers/plant-of-the-week/sarracenia_purpurae.shtml

Voyle, G. (2015). Plants with abnormal growths: The interesting phenomenon of plant fasciation. MSU Extension. [Accessed 2024 April 11]. https://www.canr.msu.edu/news/plants_with_abnormal_growths_the_interesting_phenomenon_of_plant_fasciation

Wenk E.H. and Falster D.S. (2015). Quantifying and understanding reproductive allocation schedules in plants. Ecology and Evolution. 5(23): 5521-5538.