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Potential for Hybridization between *Sarracenia* Species in Sympatry

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Abstract

Sarracenia jonesii (mountain sweet pitcher plant) is a Federally Threatened species, and *Sarracenia purpurea* var. *montana* (mountain purple pitcher plant) is a Federal Species of Concern. Due to habitat loss, *S. jonesii* is occasionally outplanted into sites where *S. purpurea* var. *montana* occurs. However, anecdotal evidence and grey literature from breeders suggest that species distinctions within this genus are maintained primarily through allopatry, and that plants in sympatry hybridize readily. Observations of phenotypic hybrids at sites where these species co-occur, and genetic confirmation of hybrid parentage, led us to ask about the potential for, and realization of, interspecific breeding. In summer 2019, we monitored *S. jonesii* and *S. purpurea* var. *montana* at two western North Carolina field sites where they are sympatric. Parental species and their phenotypic hybrids were visited weekly during the flowering season to assess plant phenological stages and to collect anthers for pollen viability analysis; ovaries were collected after floral senescence to analyze seed production and viability. Seed collections were repeated in summer 2020. Results revealed significant overlap in flower production and stigma receptivity, and showed that pollen from both species maintained high viability even after anthers were shed. Parental species and their hybrids all produced large numbers of seeds, and seed production varied significantly by site but not by species. These data demonstrate the potential for hybridization between two species of conservation concern under field conditions, and suggest that interventions such as floral bagging should be undertaken to restrict gene flow across these permeable species boundaries. Future investigations will compare parental species' seed production in sympatry vs. allopatry, and will discern parental versus hybrid status of the seed generation.

1. Introduction

Hybridization is a process of evolution that can occur when pre- and postzygotic barriers between populations are weak or absent[1]. Hybridization is a natural process that can lead to speciation, extensive introgression, or depressed F_1 fitness such that hybrids have little effect on parental species; however, it can raise conservation concerns when it is the result of human interference or when one or both parental species are threatened or endangered[2]. This appears to be the case for the hybridization between the pitcher plant species *Sarracenia purpurea* L. var. *montana* D.E. Schnell & Determann (mountain purple pitcher plant) and *Sarracenia jonesii* Wherry (mountain sweet pitcher plant).

Sarracenia, in the Sarraceniaceae family, is a genus of 11 herbaceous, carnivorous pitcher plants native to North America. While most species occur in the southeastern coastal plains, some members of the genus occur in the southern Appalachian Mountains, and one species (*S. purpurea*) has a range that extends north to Canada[2]. This study focuses on two species located in the bogs and fens of the southern Appalachian Mountains: *S. jonesii* and *S. purpurea* var. *montana*. *Sarracenia purpurea* var. *montana* and *S. jonesii* can be phenotypically differentiated by the morphology of the pitchers. While *S. jonesii* has tall, slender pitchers with a height of 21-73 cm[3], *S. purpurea* var. *montana* has short, wide pitchers with a height up to 45 cm[4]. Both of these species reproduce sexually from seeds and asexually from rhizomes[3]. Hybrids of these two species, which are typically phenotypically intermediate between the two parent species, are found in the field sites where this study was conducted.

There has been a lot of interest in *Sarracenia* pitcher plants from cultivators and researchers alike due to their carnivorous nature, unique morphology of hollow pitchers derived from leaf tissue, and their potential for hybridization[2]. However, attention has recently been focused on the taxonomy of this genus, and away from hybridization. Hybridization becomes a topic of concern when it affects the gene pools of parent species, especially if the species involved are threatened or endangered. Of the 11 species *Sarracenia*, three are Federally Endangered, including *S. oreophila* (Kearney) Wherry, *S. alabamensis* Case & R. B. Case, and *S. jonesii*[2]. *Sarracenia* populations are threatened by altered habitats due to a lack of fire maintenance or hydrologic changes, habitat destruction, and overcollection of plants[5]. *Sarracenia oreophila* and *S. alabamensis* are found completely apart from other *Sarracenia* species, making hybridization and potential genetic swamping highly unlikely. However, *S. jonesii* can be found in sites also containing *S. purpurea* var. *montana*, which can hybridize when in sympatry[2]. Formal research on hybridization of *Sarracenia* species under field conditions is lacking[2].

The purpose of this study was to determine the potential for hybridization between *S. purpurea* var. *montana* and the endangered *S. jonesii* in three western North Carolina mountain bogs where these species co-occur. Assessments were made using flower phenology data, pollen counts, pollen viability analyses, and seed counts from the two species and their hybrids. Based on breeder accounts of hybridization potential among all possible pairs of *Sarracenia* species[2], we predicted that there would be a high potential for hybridization between these two species in sites where they co-occur. This study could provide valuable insight concerning the conservation of *S. jonesii* in sites where its gene pool and thus evolutionary potential could be negatively affected by hybridization with *S. purpurea* var. *montana*.

2. Methods

2.1 Study Sites

Three sites were identified in western North Carolina that have both *Sarracenia purpurea* var. *montana* and *Sarracenia jonesii*. For the sake of protecting the threatened species within these habitats, sites will be referred to as CM (Transylvania County), MB (Henderson County), and SF (Transylvania County). At all three sites, *S. purpurea* var. *montana* and *S. jonesii* are the dominant *Sarracenia* species, with SF also containing some *S. flava* individuals. At CM, *S. purpurea* var. *montana* was the dominant *Sarracenia* species, while *S. jonesii* was the dominant *Sarracenia* species at MB and SF.

2.2 Field Sampling: Flowers

Weekly flower sampling took place at the three study sites from May through June 2019. At each of the three sites, clumps of flowering *S. jonesii* and *S. purpurea* var. *montana* were identified and tagged. Clumps were defined as rosettes of the same species that were clustered, likely as a result of asexual rhizomatous reproduction.

At each visit to the three sites, all tagged flowers were observed, and the condition and number of anthers in each flower was recorded. Each tagged flower was also placed into one of seven categories based on its phenological state (Table 1). At least one anther from each flower was sampled weekly, then immersed in Carnoy's solution (60% ethanol, 30% chloroform, 10% acetic acid, 1g ferric chloride) until analysis[6].

2.3 Pollen Counts and Viability Analyses

Pollen from field-collected anthers was analyzed from August 2019 to December 2020. One anther per sample was removed from Carnoy's solution and resuspended in 100 µL of modified Alexander's stain[7]. The modified Alexander's stain allowed the viable pollen to appear purple, while inviable pollen appeared nearly colorless or a light tan (Figure 1). The solution was macerated and mixed until it was homogenous, and then vortexed. Ten µL of the solution was pipetted onto a hemocytometer and observed under a compound microscope at 200X total magnification. The pollen was counted in the 9 largest squares (0.9 µL) of a hemocytometer, and viable and inviable counts were recorded. Hemocytometer counts were extrapolated to total sample volume. A total of 491 anthers were used for pollen counts: 232 from *S. jonesii*, 205 from *S. purpurea* var. *montana*, and 54 from hybrids.

2.4 Seed Collection & Counting

Seeds were collected from tagged flowers in September 2019 and again in October 2020. During seed collection, tagged flowers were cut on the pedicel below the ovary, then stored in an unbleached, open paper bag at room temperature until analysis. For each flower, the total number of seeds was counted under a dissecting microscope, and the counts were recorded. Since numbers of inviable seeds from a preliminary analysis was negligible (N = 14), seed viability was not tested over the larger sample set. After seeds were counted, they were repatriated to their respective sites.

2.5 Statistical Analyses

All statistical analyses were performed using R[8]. Analysis of variance (ANOVA) was performed to determine if pollen production or viability varied by site, species, floral stage, or their interactions. ANOVA was also performed to determine if seed production varied by site, species, year, or their interactions. Additionally, Pearson's Chi-Squared tests were performed for each taxon (parental and hybrids) at each site to examine associations among pollen production, viability, and floral stage.

Table 1. Floral stage based on pollen release and number of anthers attached.

Stage	Flower Status
0	Flower not open
1	Flower open, no pollen visible
2	Flower open, pollen visible
3	~75% of anthers attached
4	~50% of anthers attached
5	~25% of anthers attached
6	No anthers attached

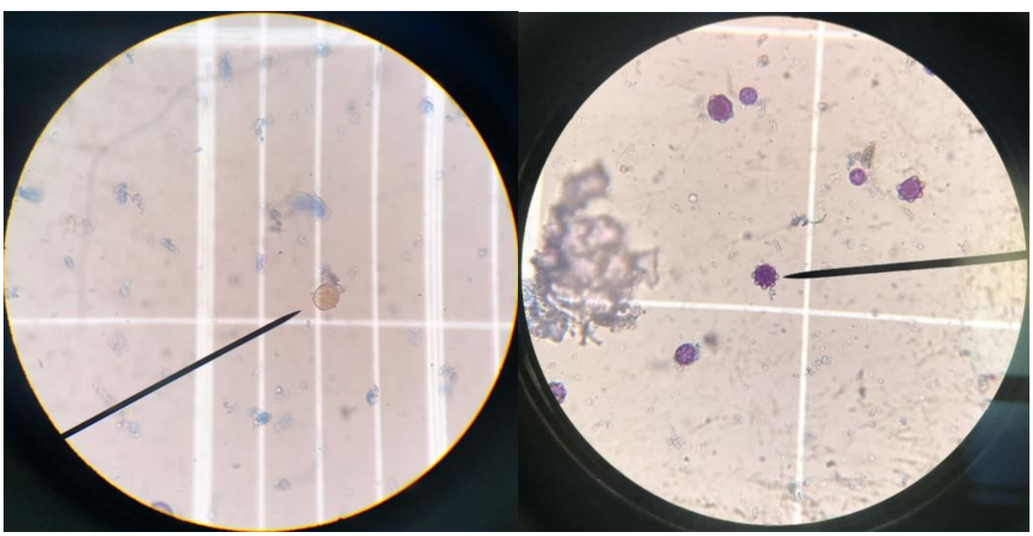

Figure 1. An inviable *Sarracenia* sp. pollen grain (left) and viable *Sarracenia* sp. pollen grains (right), stained with modified Alexander's stain[7].

3. Results

Pollen production per anther varied significantly by time ($F_{1,479} = 27.07$, $P = 2.906*10^{-7}$), floral stage ($F_{1,479} = 4.36$, $P = 0.0373$), and *Sarracenia* species ($F_{2,479} = 8.56$, $P = 0.0002$). Pollen production was greatest at Floral Stage 1 for both *S. purpurea* var. *montana* and *S. jonesii*, with a general downward trend at all sites (Figure 2). Pollen viability was high for both *S. purpurea* var. *montana* and *S. jonesii* in Floral Stages 1-6 at all sites (Figure 3). Viability was significantly affected by floral stage ($F_{1,471} = 26.99$, $P = 3.05*10^{-7}$) and species x stage interactions ($F_{2,471} = 3.47$, $P = 0.0319$). Additionally, seed production varied significantly by site (CM vs. MB; $F_{1,63} = 5.54$, $P = 0.0217$) and site x species interactions ($F_{1,63} = 11.92$, $P = 0.0010$); but not by species ($F_{2,41} = 2.567$, $P = 0.0891$), year ($F_{1,38} = 3.12$, $P = 0.0855$), or species x year interactions ($F_{2,38} = 0.206$, $P = 0.8148$). In general, *S. jonesii* seeds per flower was higher at MB than at CM, and the opposite trend was seen for *S. purpurea* var. *montana* (Figure 4). While *S. purpurea-jonesii* hybrids produced seeds, they made fewer per flower than the parental species. Floral phenology among *S. purpurea* var. *montana*, *S. jonesii*, and hybrids were also broadly similar at all three sites, with significant overlap (Figure 5).

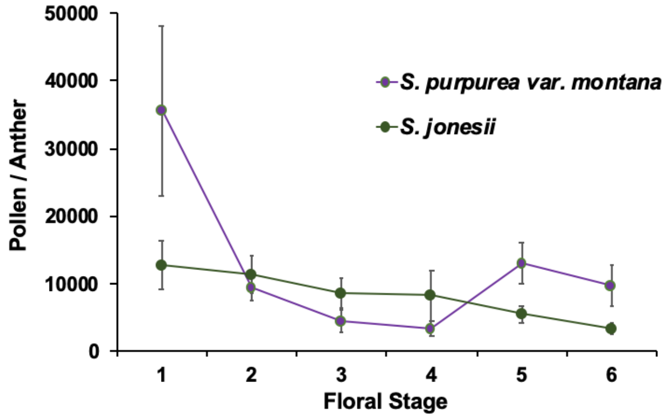

Figure 2. Mean (+/- 1 S.E.) pollen grains/anther of *S. jonesii* and *S. purpurea* var. *montana* over the six floral stages.

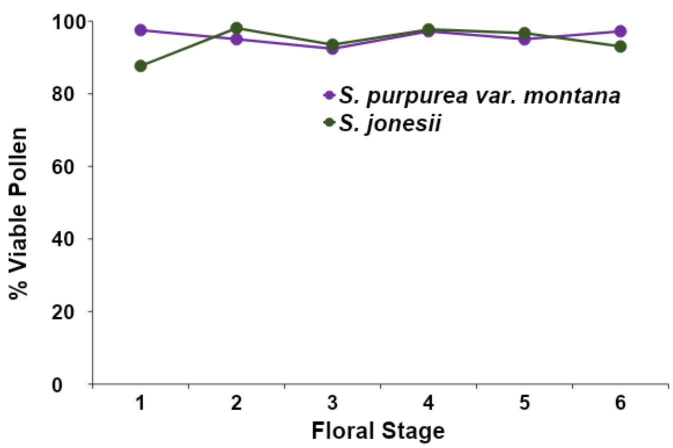

Figure 3. Percent viable pollen for *S. purpurea* var. *montana* and *S. jonesii* over the six floral stages.

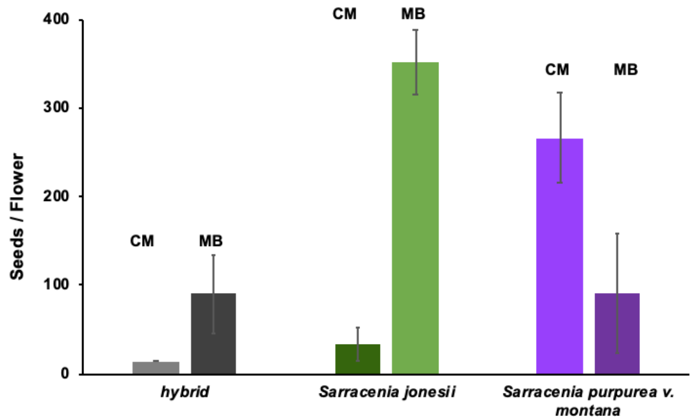

Figure 4. Seed production per flower by site (CM vs. MB) and *Sarracenia* taxon.

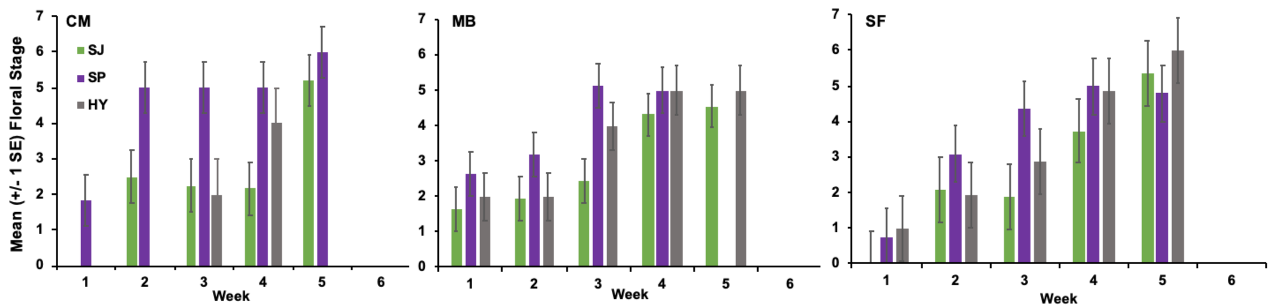

Figure 5. Flower phenology of *S. jonesii*, *S. purpurea* var. *montana*, and hybrids over the six-week flowering period, from May 16 to June 13, 2019.

4. Discussion

Hybridization can occur when pre- and postzygotic barriers between species are weak or absent[1]. The presence of hybrid taxa, which are themselves fertile, though producing fewer seeds than parentals, indicate an absence of strong pre- and postzygotic barriers maintaining species integrity in these sites. Since these species co-occur in these sites, there is no spatial barrier separating them; in fact, *Sarracenia* congeners were sometimes located right next to each other in the sites monitored for this study. Additionally, previous studies from breeders have provided evidence that *Sarracenia* species readily hybridize when in sympatry[2]. Our study suggests a lack of temporal barriers preventing hybridization between these two species; there was phenological overlap in pollen production and viability between *S. jonesii* and *S. purpurea* var. *montana*, creating a long window for interspecific hybridization. Field observations of floral visitors also support this, as potential pollinators were observed to visit multiple flowers of both *Sarracenia* species in sequence. These results indicate that there is an absence of prezygotic barriers between *S. jonesii* and *S. purpurea* var. *montana*.

Typically, hybridization is not considered a problem when it is naturally occurring and does not involve endangered species; hybridization is a natural process that can contribute to the evolution of species[2]. However, conservation concerns arise in cases of hybridization among endangered species that may threaten the genetic integrity of the parental species. Due to the Federally Endangered status of *S. jonesii* and the status of *S. purpurea* var. *montana* as a Federal Species of Concern, hybridization between these species should be prevented when possible. Therefore, since species distinctions may not be maintained when in sympatry, conservation efforts should avoid outplanting different *Sarracenia* species in the same habitat. Alternatively, in sites where species co-occur, breeding between species should be controlled via floral removal.

Ongoing work related to this project includes monitoring phenological overlap to determine the degree of interannual variation, repeating formal observations of floral visitors to determine their potential role in interspecific hybridization, and genetic analyses to determine hybridization indices. Additionally, we would like to implement a more formal method to test pollen transfer in order to further determine the role of floral visitors in hybridization. More research should be done on the effects of hybridization between *S. jonesii* and *S. purpurea* var. *montana*, and the potential for genetic swamping where these species co-occur. If there is high fitness among hybrids, there could potentially be detrimental effects to the parental species due to competition. This research, as well as future studies, may aid in advising conservation efforts of these rare carnivorous plants.

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6. Resources

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