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Helping conservationists easily identify *Sarracenia purpurea* var. *montana, S. jonesii*, and their hybrids in the field

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Abstract

Hybridization between rare or endangered species poses complicated issues for land managers. Hybridization, and resulting introgression, can blur the boundaries between species and pose challenges for maintaining species identity. However, gene flow into populations of rare or endangered species can increase genetic diversity, may facilitate adaptation to changing environments, and may result in increased persistence of threatened populations. Regardless of whether managers want to prevent or facilitate hybridization, it is important for them to recognize which plants are hybrids. The goal of this research was to develop a quick and reliable method that managers could use to identify hybrid pitcher plants in the field. Morphology of *Sarracenia purpurea* var. *montana* Schnell and Determann (mountain purple pitcher plant), *S. jonesii* Wherry (Jones' pitcher plant), and their putative hybrids were compared at two sites in Western North Carolina using principal components analysis on eight pitcher measurements and found two major clusters that differed significantly in PC1. One cluster was distinguished by tall, narrow pitchers with hoods angled tightly over pitcher apertures, whereas the other cluster was distinguished by shorter, broader pitchers with hoods angled away from the pitcher aperture. These clusters mostly, but not entirely, corresponded to our *a priori* identification of individual *S. purpurea* var. *montana* and *S. jonesii* plants. Two plants we identified as putative hybrids were intermediate in their PC1 scores. Although the genetic identity of these plants remains to be confirmed, these morphological measurements provide a promising method of identifying *S. purpurea* var. *montana* x *S. jonesii* hybrids.

1. Introduction

Hybridization is common in plants but when this phenomenon happens in rare or threatened species it raises issues that conservation managers must address. Often when plants hybridize, they form genetic and sometimes phenotypic intermediates (Arnold 1992). This can cause confusion when managers are trying to identify species. Further, distinguishing hybrids from parental species could become more difficult when species hybridize over multiple generations. Early generations' often have morphological features that might be intermediate between the two parental species, whereas later generations can show features more similar to the parental forms (Goulet et al. 2017). Therefore, conservationists need to recognize the varying phenotypes when observing F1 and subsequent specimens. Hybridization can cause confusion for conservation managers in the field, but this phenomenon can also slow down research when researching rare or threatened plants.

Hybridization in rare or threatened plant species is of special concern because there have not been many studies that address this issue, and this could impede research that conservationists are trying to conduct. One reason why conservationists need to be mindful of the effects of hybridization in rare plant species is because hybridization has a direct impact on taxonomy and management decisions. Taxonomy and systematics are important to conservation biology because they provide a baseline for conservation managers to make decisions (Rieserberg 1991). However, early taxonomic methodologies are not precise when identifying and differentiating first-generation, intermediate, and bona fide hybrid species (Rieserberg 1991). Considering the aforementioned hinderance, conservationists could utilize

current methods for identifying hybrids phenotypically and genetically to aid in their research and ensure that rare species of plants can be studied effectively.

There are many examples of how the dangers of hybridization can affect the fitness of a plant species. Loss of genetic identity through introgression is a common occurrence (Allendorf et al. 2010). Introgression occurs when two species hybridize and one or both species are nearly replaced by hybrids when hybrids have equal or higher fitness than one or both parent species (e.g., Pecos pupfish, Kodric-Brown and Rosenfield 2004). Such introgression can leave native species and their habitats vulnerable to invasion by non-natives (Schierenbeck and Ellstrand 2008). When invasive plants utilize the resources that native plants need, this creates a downfall in the fitness of native plants and makes it harder for them to reestablish a healthy population. Another example of the dangers of hybridization is if alleles are introduced from outside of the rare plant's community. This could lead to loss of local adaptation for rare or threatened species. One way in which outside alleles can cause loss of local adaptation for rare plants is if genes from cultivated crops travel to wild populations. This could have ecological consequences because these genes reduce fitness advantages of the wild plants (Vilà et al. 2000). Hybridization could also become a problem if native species hybridize with aggressive taxa and their hybrids compete with the native species. If the native species is outsourced by the hybridized individuals, then this will allow the exotic taxa to spread (Vilà et al. 2000, Schierenbeck and Ellstrand. 2008).

Declining populations can experience this phenomenon more intensely because allele frequencies undergo large and unpredictable fluctuations due to drift (Ellstrand and Elam 1993). Whenever a small population is experiencing unstable genomes of hybrid origin influenced by natural selection and genetic drift, this accumulates gene and chromosomal differences. When plant populations go through these gene and chromosomal differences, then new reproductive isolating mechanisms are created which increases genetic isolation of a new race or species (Rodionov et al. 2019).

Although the dangers of hybridization are evident, there are ways in which hybridization is beneficial. For example, hybridization can introduce new genetic variation via gene flow. In smaller populations, inbreeding depression could reduce fitness by reducing selection and maintaining the deleterious recessive gene instead of selection eliminating it (Ellstrand and Elam, 1993). Gene flow between rare plants and more abundant sympatric species can help maintain the abundance of rare species because it introduces alleles that may facilitate adaptation to changing conditions, keeping the species' population thriving. Another example of beneficial hybridization is in *Ranunculus reptans* L. In this species, interpopulation outbreeding positively affected the measure of fitness, and fitness superiority was maintained in the second offspring generation (Willi et al. 2007). The small populations benefited more from interpopulation outbreeding. Overall, the researchers saw that the benefits of interpopulation outbreeding outweigh potential drawbacks that happen to populations that experience inbreeding (Willi et al. 2007). This study shows how fitness was bolstered when using interpopulation outbreeding, which can assist in future studies when trying to get rare taxa to adapt to changing environments and increase genetic diversity. When focusing on the importance of maintaining fitness in rare plant taxa, hybridization can have its benefits, but it can also cause issues for conservation managers in the field.

When conservation managers are studying hybrids in the field, confusion can arise. Recognizing hybrids morphologically, one would assume that individuals will represent intermediate characteristics of their parental individuals. This is not always the case because hybrids can show a myriad of their parent's phenotype. F1 generations and beyond that contain most of their parent's genes can also possess phenotypes that are indistinguishable from their parents. Conservation managers need to distinguish these characteristics because if a population still has a healthy number of parental individuals, then removal of the hybrids could be accomplished much easier (Allendorf et al 2001). Conservationists could identify hybrids using genetic markers, such as PCR applications, but these are more useful in ex situ situations and would not be applicable in the field. In order for managers to easily identify hybrids and their parents in the field, a composite metric needs to be administered. This composite metric will assist researchers in the field to easily identify certain rare taxa of plants by measuring physical characteristics that can easily identify hybrids.

The species that this project focused on were the pitcher plants *Sarracenia purpurea* var. *montana* (mountain variety purple pitcher plant), *Sarracenia jonesii* (mountain sweet pitcher plant) and their hybrids. *S. purpurea* var. *montana* and *S. jonesii* are native to the bogs of the Southern Appalachian Mountains. The two species of *Sarracenia* are of concern to conservationists because the fragile environment these plants inhabit is becoming limited, which is depleting their populations (Karberg et al. 2010). *S. jonesii* is federally listed as Endangered and is being actively managed through daylighting efforts, captive breeding, and reintroductions. These two species are known to hybridize (Muñoz 2015), which will have important implications for reintroduction and management. Therefore, it is crucial for conservation managers to recognize the hybrids from the parents and to be able to monitor localities for hybridization.

The morphology of these carnivorous plants is an important characteristic to study in order to recognize hybridization between the two species. In this study, we measure eight morphological characteristics of the two parent

species and their putative hybrids and ran PCA tests to identify whether morphological differences in the three plant groups exist. We develop a composite metric that uses two of the most distinct morphological features of the plants to distinguish the groups and we propose that conservation managers use this metric to accurately and quickly identify hybrids in the field.

2. Methods

2.1 Study Site

Field work was performed at two sites in North Carolina where *S. purpurea* var. *montana* and *S. jonesii* co-occur. These sites are not precisely identified in order to protect the sensitive populations. The Transylvania County site has an abundance of *S. purpurea*, with a few *S. jonesii* and putative hybrids. The Henderson County site has mainly *S. jonesii* with a few *S. purpurea* and putative hybrids.

2.2 Field Work

Sampling was conducted at the Transylvania County site on July 6, July 10, and October 3, 2020. Sampling was conducted at the Henderson County site on July 23 and October 3, 2020. Clumps of rosettes were located along a transect and marked using flags. A single pitcher was measured at each clump; pitchers were selected that had no overwinter browning, were fully open, and held water, suggesting they were fully developed. Field sampling involved counting and measuring tallest flower stalks, measuring front and posterior heights of the pitchers, measuring width, height, and angles of the hoods, measuring aperture perpendicular and width, and measuring rib width (Figure 1). A 3–4-centimeter length (< 5 mm width) of the rib was cut from each specimen and stored in a tube with desiccant, transported on ice, then stored at 80°C for genetic species identification in. separate study (results not recorded here). All of these procedures were conducted on the largest pitcher of the clump. The same procedures were used for *S. jonesii* and the hybrids at Cedar Mountain Preserve and McClure's Bog.

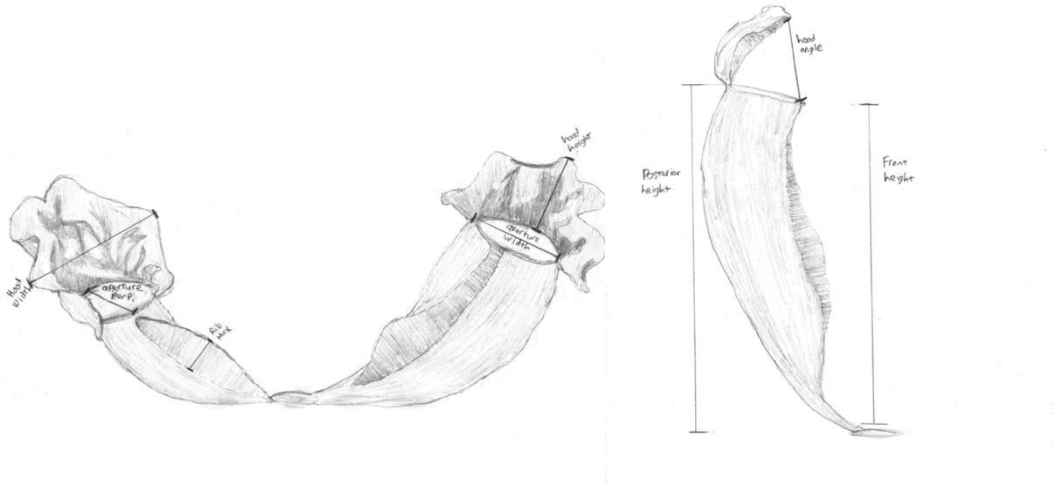

Figure 1. Morphological features that were measured and analyzed.

2.3 Data Analyses

Principal components analysis was used to analyze the morphological features for *S. purpurea*, *S. jonesii*, and the hybrids. The PC1 and PC2 data were compared between the taxa using ANOVA and Tukey HSD tests. One *S. purpurea* was excluded from PC1 and PC2 because its values for PC1 were outliers.

3. Results

3.1 Principal Components Analysis

The plant's morphological traits formed two major clusters that differed significantly in PC1 (Figure 2). One cluster was distinguished by tall, narrow pitchers with hoods angled tightly over pitcher apertures, whereas the other cluster was distinguished by shorter, broader pitchers with hoods angled away from the pitcher aperture. These clusters mostly, but not entirely, corresponded to our *a priori* identification of individual *S. purpurea* var. *montana* and *S. jonesii* plants. Two plants we identified as putative hybrids were intermediate in PC1 score.

The morphometric analyses showed that the pitcher front and posterior heights had strong positive loading values, whereas the hood angle and width had strong negative loading values for PC1 (Table 1). Posterior width and height, hood angle, rib max and hood width all corresponded with component 1. Aperture perpendicular and width and hood height corresponded with PC2. All three plant groups differed significantly in PC1 ($F_{2,62} = 257.38$, $p<0.0001$, Tukey HSD *post hoc* test = 0.05), but there were no significant differences among groups in PC2 ($F_{2,62} = 2.63$, $p = 0.08$; Figure 3).

Although the three plant groups were clearly different in morphology, it is impractical to measure eight traits and perform PCA quickly in the field. To explore whether more simplified metrics could be used to quickly identify hybrids from the two parent species, we selected the morphological traits that loaded most heavily, either positively or negatively, on PC1 and combined them into three separate metrics. We then evaluated whether these metrics could distinguish the three plant groups using ANOVA.

Table 1. Loading values of the plant morphological traits in PC1 and PC2.

Morphological trait	PC1	PC2
Pitcher Front Height	0.407	0.294
Pitcher Posterior Height	0.405	0.297
Hood Height	~0	0.623
Hood Angle	- 0.410	- 0.147
Hood Width	- 0.440	0.150
Aperture Perpendicular	- 0.229	0.507
Aperture Width	- 0.355	0.366
Maximum Rib Width	- 0.357	~0

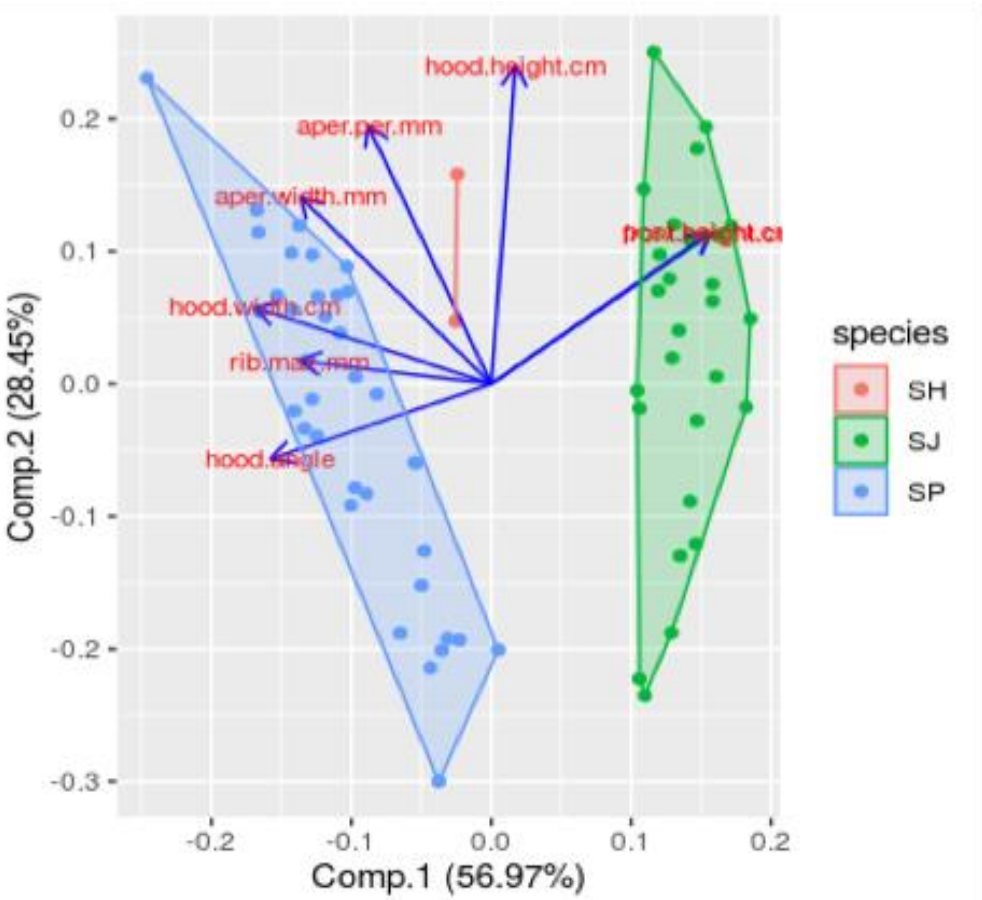

Figure 2. Morphological measurements for *S. purpurea*, *S. jonesii*, and their hybrids and their placement in principle components 1 and 2.

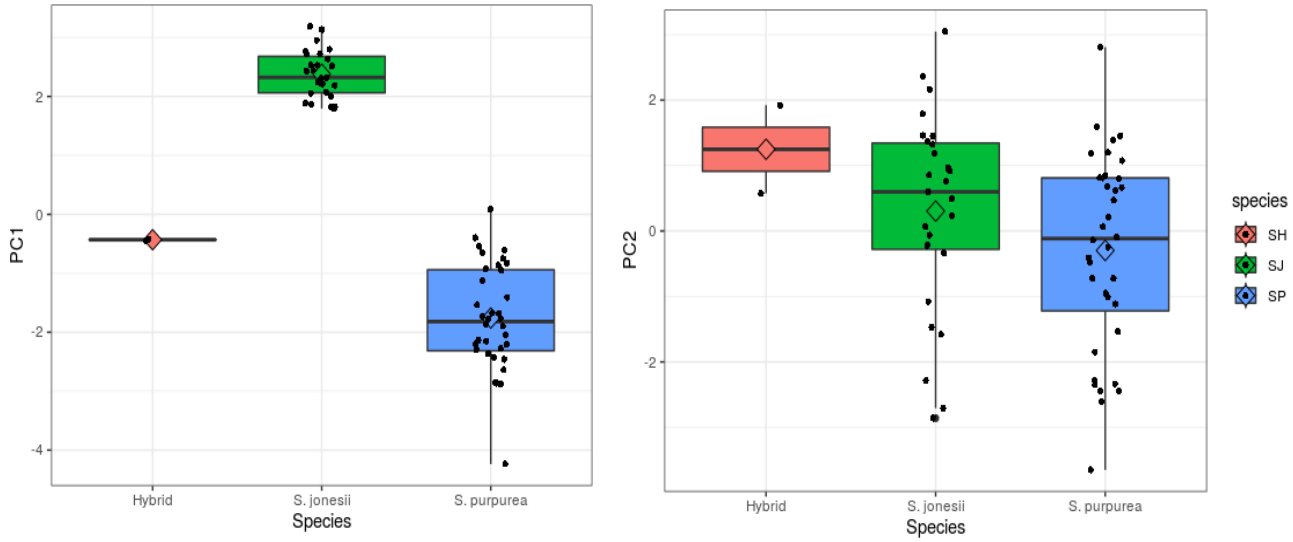

Figure 3. Data from the three composite measurements of each plant group in PC1 and PC2.

3.2. Composite Metrics

We developed and tested three simplified metrics for field identification of the three plant groups. Metric 1 uses pitcher front height and hood width in the formula: log(front height) / hood width. For Metric 1, all three taxa differed from one another ($F_{2,63}$ =322.52, p<0.0001, Tukey HSD *post hoc* test p<0.05). Metric 2 uses hood width and hood angle in the formula: log(hood width / hood angle). The three taxa did not differ in Metric 2 ($F_{2,63}$ =201.92, p<0.0001, Tukey HSD *S. jonesii*-hybrid p>0.05). Metric 3 uses front height and hood angle in the formula: log(front height / hood angle). The three taxa did not differ in Metric 3 ($F_{2,63}$ =167.97, p<0.0001, Tukey HSD *S. purpurea*-hybrid p>0.05; Figure 4).

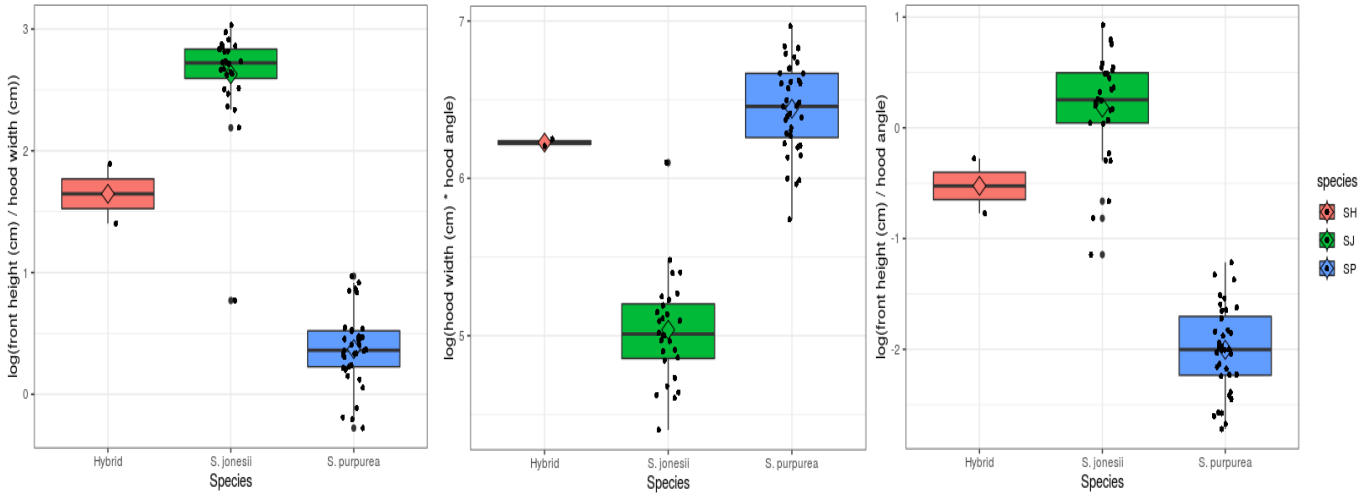

Figure 4. Comparison of a) Metric 1, b) Metric 2, and c) Metric 3 between the three plant groups. Only Metric 1 [log(pitcher height / hood width)] was significantly different between the three groups when outliers were removed.

Because there were few putative hybrids in the 2020 data, we tested the validity of Metric 1 for distinguishing the three plant groups using a data set collected in 2018 from a site with more hybrids (Morgan 2018). Metric 1 distinguished these plants well (Figure 5). We established threshold ranges for Metric 1 that could be used for field identification of plant groups (Table 2).

Table 2. Quantiles for Metric 1: log(pitcher height / hood width) and proposed thresholds for distinguishing plant identification groups.

Plant group	25th Quantile	50th Quantile	75th Quantile	Threshold range
S. purpurea	0.109	0.254	0.347	< 0.5
S. jonesii	2.60	2.73	2.96	> 2.0
Hybrid	1.07	1.27	1.54	1.0-1.75

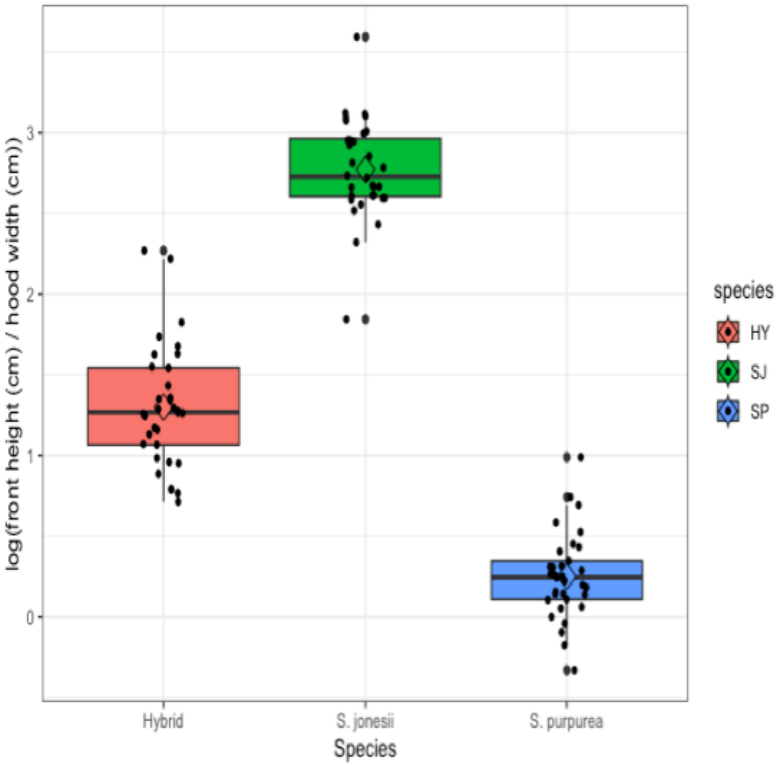

Figure 5. Metric 1 measurements from log(pitcher front height/ hood width) applied to 2018 data.

4. Discussion

Identifying different species of plants that have similar phenotypes can cause problems in the field. However, if the plant's features can be easily measured and recognized, then scientists can perform their tasks with more ease and enhance the efficacy of their studies. The principal components analyses test verified that the three plant groups showed morphological differences. We used a PCA test because it reduced the dimensionality of our dataset and made it easier to interpret while decreasing information loss (Jolliffe and Cadina 2016). This test focused on distinct morphological features and placed each plant group into different clusters in the principal components.

The posterior height of the pitcher in *S. jonesii* was the most distinct morphological trait for that species. The hood width, rib width, and hood angle were the most distinct morphological traits for *S. purpurea.* The morphological traits that showed the most intermediate features were the aperture width, aperture perpendicular, and the hood height, which all three plant groups displayed similarly. The phenotype features that distinguished the three plant groups significantly were placed in PC1 and the physical characteristics that did not distinguish the three taxa were placed in PC2.

PCA analyses is a very effective tool to accurately measure the morphological features of the three taxa, but it is not pragmatic for making quick identification in the field. In order for measurements to be easier for managers to apply, we developed and validated three metrics that each focused on two morphological traits. Out of the three metrics, we found that Metric 1 showed that all three taxa differed the most, thus better distinguishing between the different species and hybrids. The 2020 data had few putative hybrids, so we decided to use data from 2018 that had more hybrids at a different site. With this data, we could validate Metric 1 in the hybrid plant group to be more effective by analyzing more specimens from the 2018 site. Since Metric 1 best distinguished the three taxa, we believe that this would be the best tool for conservationists to use in the field for easy identification.

The next step is to confirm genetic identity of plants we putatively identified as hybrids and maintain their genotypes. Even though phenotypical identification can be a reliable way to distinguish the three plant groups, genetics is a guaranteed way of knowing what species the three plant groups belong to. By identifying a plant's genetics, plant conservationist geneticists can design experiments that can halt the loss of rare plant biodiversity.

One way to protect a rare plant's genetics is by safeguarding genetic diversity. Safeguarding genetic diversity in rare plant species involves measuring how genetic diversity is distributed across the landscape and then coming up with a strategy to conserve the taxa that will maximize the total amount of genetic diversity that needs to be protected. This conservation method should be conducted in situ in the species' natural habitat. If in situ methods are not applicable, then ex situ methods should be applied by using conservation seed banks or maintaining the endangered plants in botanical gardens (Edwards 2017).

To understand genetic variations when in situ experiments are conducted, conservationists need to use neutral genetic markers. Neutral genetic markers in plants such as mating systems, seed dispersal and pollination, and geographic distribution interact to affect neutral genetic variation. In small populations, pollinators/seed dispersers travel short distances, and this ensures that genetic variation can be maintained. The sites in which the *S. purpurea*, *S. jonesii* and their hybrids grow are protected, so safeguarding their genetic diversity could be advantageous (Edwards 2017). Examining the genotypes of *Sarracenia purpurea*, *S. jonesii* and their hybrids is crucial for proper identification, and conservation managers should rely on this method, in addition to recognizing their phenotypes in order to accomplish the main goal, which is to continually educate ourselves and further protect this rare species of pitcher plant.

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6. Works Cited

1. Allendorf FW, Leary RF, Spruell P, Wenburg JK. 2001. The problems with hybrids: setting conservation guidelines. Trends in Ecology and Evolution. 16 (11): 613-622.
2. Arnold ML. 1992. Natural hybridization as an evolutionary process. Annual Review of Ecology and Systematics. 23: 237-261.
3. Cross AT, Krueger TA, Gonella PM, Robinson AS, Flieschmann AS. 2020. Global Ecology and Conservation. 24: 1-30.
4. Edwards, CE. 2017. Strengthening the link between international conservation policy and plant conservation genetics to achieve more effective plant conservation. Annals of the Missouri Botanical Garden. 102(2): 397-407.
5. Ellstrand NC. 1992. Gene flow by pollen: implications for plant conservation genetics. Oikos.63(1): 77-86.
6. Ellstrand NC, Elam DR. 1993. Population genetic consequences of small population size: implications for plant conservation. Annual Review of Ecology and Systematics. 24: 217-242.
7. Gaume L, Bazile V, Huguin M, Bonhomme V. 2016. Different pitcher shapes and trapping syndromes explain resource partitioning in *Nepenthes* species. Ecology and Evolution. 6(5):1378-1392.
8. Goulet BE, Roda F, Hopkins R. 2017. Hybridization in plants: old ideas, new techniques. Plant Physiology. 173:65-78.
9. Horner JD. 2014. Phenology and pollinator-prey conflict in the carnivorous plant, *Sarracenia alata.* The American Midland Naturalist. 171:153-156.
10. Hovick SM, Campbell LG, Snow AA, Whitney KD. 2012. Hybridization alters early life-history traits and increases plant colonization success in a novel region. The American Naturalist. 179(2).
11. Iritani R, Noriyuki S. 2020. Reproductive interference hampers species coexistence despite conspecific sperm precedence. Ecology and Evolution. 11: 1957-1969.

12. Jennings DT, Cutler B, Connery B. 2008. Spiders (Arachida:Araneae) associated with seed heads of *Sarracenia purpurea* (Sarraceniaceae) at Acadia National Park, Maine. Northeastern Naturalist. 15(4): 523-540.
13. Jollife IT, Cadina J. 2016. Principle componenet analysis: a review and recent developments. Philosophical Transactions: Physical and Engineering Sciences. 374(20150202): 1-16.
15. Karberg JM, Gale MR. 2010. Exploration of meaningful subspecies definitions of the carnivorous Northern Pitcher Plant (*Sarracenia purpurea)* throughout its geographic distribution. Conservation Genetics. 11: 2369-2378.
15. Kodric-Brown A, Rosenfield JA. 2004. Populations of pecos pupfish (*Cyprinodon pecosensis*) differ in their susceptibility to hybridization with sheepshead minnow (*C. variegatus*). Behavioral Ecology and Sociobiology. 56:116-123.
16. Levin DA, Francisco-Ortega J, Jansen RK. 1996. Hybridization and the extinction of rare plant species. Conservation Biology. 10(1): 10-16.
17. Montserrat V, Weber E, Antonio CMD. 2000. Conservation implications of invasion by plant hybridization. Biological Invasions. 2: 207-217.
18. Morgan GW. 2018. *Sarracenia purpurea* var. *montana* and *Sarracenia jonesii*: A study of effects of hybridization [theses]. [Asheville (NC)]: University of North Carolina at Asheville.
19. Muñoz C. 2015. Characterization of genetic diversity in demes of Mountain Purple Pitcher Plants (*Sarracenia purpurea* L. var. *montana*) from Western North Carolina [thesis]. [Asheville (NC)]: University of North Carolina at Asheville.
20. Rhymer JM, Simberloff D. 1996. Extinction by hybridization and introgression. Annual Review of Ecology and Systematics. 27: 83-109.
21. Rieserberg LH. 1991. Genetics and conservation of rare plants. Faulk DA, Holsinger KE, editors. Hybridization in rare plants: insights from case studies in *Cercocarpus* and *Helianthus.* New York, Oxford: Oxford University Press. P. 158-178.
22. Rodionov AV, Amosova AV, Belyakov EA, Zhurbenko PM, Mikhailova YV, Punina EO, Shneyer VS, Loskutov IG, Muravenko AV. 2019. Genetic consequences of interspecific hybridization, its role in speciation and phenotypic diversity of plants. Russian Journal of Genetics. 55: 278-294.
23. Schierenbeck KA, Ellstrand NC. 2008. Hybridization and the evolution of invasiveness in plants and other organisms. Biological Invasions. 11: 1093.
24. Vilà M, Weber E, Antonio CMD. 2000. Conservation implications of invasion by plant hybridization. Biological Invasions. 2: 207-217.
25. Wang L, Zhou Q. 2016. Surface hydrophobicity of slippery zones in the pitchers of two *Nepenthes* species and a hybrid. Scientific Reports. 6:19907: 1-11.
26. Willi Y, Kleunen MV, Dietrich S, Fischer M. 2007. Genetic rescue persists beyond first-generation outbreeding in small populations of a rare plant. Proceedings of the Royal Society B. 274 (1623): 2357-2364.