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Sarracenia purpurea var. *montana* and *Sarracenia jonesii*: A Study of Effects of Hybridization

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Abstract

Sarracenia purpurea var. *montana* and *Sarracenia jonesii* are two species of pitcher plants that are known to hybridize if in sympatry. The parent pitcher plants vary greatly in morphology and how they obtain nutrients, and these differences may cause the hybrid pitcher to be less suited for their environment. In this study, several morphological measurements of each type of pitcher are taken and analyzed to determine morphological differences within species using principle components analysis. Phytotelma communities were quantified in *S. purpurea* var. *montana* and the hybrids to determine trends in each of these pitcher's endosymbiont communities. In completion of these measurement, it was found that *S. purpurea* var *montana*, *S. jonesii*, and their hybrids are distinct morphologically. Species richness and organism abundance were correlated with increased water volume in the pitcher and with the first principle component describing morphology. Size of the pitcher's aperture was not found to significantly affect species richness or abundance.

1. Introduction

Hybridization regularly occurs in nature and can have positive, negative, or neutral effects on offspring and parental fitness (Fridman 2015). Hybridization occurs in up to 40% of plant families, and genetic research has shown that new plant species have arisen through hybridization (Hopper 2018). These data suggest hybrids can be successful; however, hybridization can have detrimental effects as well (Fridman 2015). For example, hybrids may have poor fitness when they have complex interactions with organisms such as prey and mutualists, including pollinators (Whitney et al 2010). Pollinator-plant interactions have been studied in domestication of crops, giving insight into effects of hybridization in nature. Chen et al. (2018) have shown that domestication often alters the timing of flowering, as well as floral coloration, and structural phenotypes compared to wildtype lineage. This is important because organisms that evolved to interact with plants with certain features may not recognize the plant and not interact as usual. Hybrid offspring may have reproductive advantages to their descendent species. Beddows (2018), found that, of species that hybridize, 97% are perennial. Many of these species are also known to reproduce vegetatively, which Beddows (2018) suggests may be the reason why hybrids are successful given that many times they are not as fecund as their parent species.

For pitcher plants, pollination is not the only important relationship with other organisms that may be disrupted by hybridization. Pitcher plants also interact with arthropod communities (Bradshaw 1983). Pitcher plants of the genus *Sarracenia* are unique because they are not found in fertile soil, but rather soils that with low nutrient availability, such as bogs (Heard 1998). Like other carnivorous plant species, most pitcher plants, including *S. jonesii*, attract invertebrate prey and derive their nutritional requirements from the prey captured in their modified pitcher-like leaves (Heard 1998).

According to Bradshaw (1983), many insects and microorganisms naturally inhabit the pitcher and have a symbiotic relationship with the pitchers. *S. purpurea* collects water that is colonized by a large community of invertebrates

(Adlassnig et al. 2010, Jaslow 2015), and this community releases the nutrients that are used by the plant (Butler et al. 2008). *Sarracenia jonesii* acquires nutrients by attracting and digesting prey using enzymes rather than pitcher communities (Beikmohamadi 2018).

When *S. purpurea* var. *montana* and *S. jonesii* hybridize, the resulting hybrid may not succeed at either type of feeding. Indeed, Jaslow (2015) found that while both *S. purpurea* var. *montana* and hybrid species harbored the same species, hybrids had fewer total organisms. One hypothesis is that the hybrid water is not attractive to colonists, such as midges and mosquitoes. However, Jaslow (2015) conducted a water swap study to determine whether colonizing invertebrates avoid hybrids due to characteristics of the pitcher water or due to characteristics of the pitchers, themselves, and found water source did not influence invertebrate communities. Instead, the *S. purpurea var. montana* pitchers had more organisms, regardless of the type of water they contained.

Prior study has described the pitcher plants *S. purpurea* var. *montana*, *S. jonesii*, and the hybrid as distinct phenotypically, though this observation was not quantified (Beikmohamadi 2018). According to Beikmohamadi, *S. purpurea* is low-lying and wide, whereas *S. jonesii* is tall and thin in comparison (2018). Beikmohamadi did quantify fluid volumes of *S. purpurea* var. *montana* and the hybrid and found that *S. purpurea* var. *montana* did not contain more fluid than hybrids (2018). This finding indicates that even though there is no significant difference in volume of fluid accumulated in a pitcher, the morphological hybrids are still visually distinct and has an intermediate shape compared to *S. purpurea* var. *montana* and *S. jonesii*.

Although differences in morphology have been described qualitatively, they have not been quantified, and their association with invertebrate community composition is not known. As described earlier, Jaslow (2015) found fewer symbionts living within hybrids than within *S. purpurea* var. *montana* (*S. jonesii* was not quantified because it does not contain water), and his water swap study suggested that factors of the pitchers, such as morphology, strongly influence colonization. However, differences in the communities may not be consistent over time, as Beikmohamadi studied the same population in 2017 and found that there was not a difference between hybrids and *S. purpurea* var. *montana* with respect to pitcher inhabitant communities.

The purpose of this study was to survey several aspects of *Sarracenia* pitcher plants to better understand the conflicting results of Beikmohamadi (2018) and Jaslow (2015) regarding *S. purpurea* var. *montana*, hybrids, and symbionts in their pitchers. This study also attempts to quantify shape of pitchers as described by Beikmohamadi (2018) by taking numerous morphological measurements of *S. purpurea* var. *montana*, *S. jonesii*, and hybrids to determine whether the hybrid morphometrically is intermediate between its parent species. Differences in shape could explain why hybrids have fewer symbionts, if colonizing invertebrates use pitcher shape as a cue for colonization. Alternatively, aperture area could determine how many organisms and which taxa colonize pitchers, if pitchers with larger openings capture or attract more organisms. Therefore, we will compare whether our measurements of the communities vary as a function of overall morphology or aperture area.

2. Methods

2.1. Study Site

S. purpurea var. *montana* and *S. jonesii* were studied at an artificial lake in Brevard, North Carolina. The plants are located within 2 meters of the lake. There is a dense population of *Sarracenia* at this site consisting primarily of *Sarracenia jonesii* and *S. purpurea* var. *montana*, as well as a few individuals of *S. flava* and *S. leucophylla*. There is genetic evidence of hybridization of all the *Sarracenia* at this site (unpublished data, Rhode Ward), and hybridization between *S. jonesii* and *S. purpurea* appears to be most common.

2.2 Field Sampling

The sampling schedule consisted of four visits over three months in 2018. The first measurements were taken 25 May. The second measurements were taken 7 and 20 June, and the third measurements were taken on 17 July. Rosettes initially were marked in 2014 and have been sampled annually. One pitcher on each rosette was sampled on each date. On 25 May, a pitcher without browned edges or apparent freeze damage and with young, tender leaf tissue was selected for sampling. On 25 May and 7 June, we also selected a pitcher for the next sampling date, by marking an unopened pitcher that would open soon (estimated at most one week). Pitchers were selected this way to control for pitcher age and development.

On each visit there were several measurements taken for morphometric quantification (Fig.1). The measurements were taken using calipers and cloth sewing tapes. The sewing tape was used to measure the anterior height of the pitcher by measuring from the base of the rosette to the aperture of the pitcher. The posterior height was determined by measuring the base of the rosette to where the hood of the pitcher began. The hood height was measured with the cloth sewing tape as well by measuring the distance from where the hood connects to the pitcher to the tip of the hood. The width of the hood was also observed by measuring the maximum width of the pitcher hood using the cloth sewing tape as well. Two aperture measurements were obtained with calipers. The horizontal measurement was taken by measuring the widest portion of the sides of the aperture. The perpendicular measurement was taken by measuring the inside of the aperture from the base of the hood to the other side of the aperture. The calipers were also used to measure the widest portion of the pitcher rib.

Plastic pipettes were used to determine the fluid volume present in the pitcher as well as the maximum volume of each pitcher. The volume of fluid present in the pitchers was removed and placed in tubes for later pitcher community studies. The pipette was then used to add tap water to maximum capacity and this volume was recorded as well. The tap water was removed after measurement.

2.3. Micro- and Macro-Organisms

Dissecting and compound microscopes were utilized to quantify the community within each pitcher fluid sample. Micro-organisms (flagellates, ciliates, copepods, rotifers) were quantified by placing four separate 50 μL drops on a glass slide and observing this under the compound microscope at a magnification of 100X. If rotifers and copepods were present in the first 200 μL, two additional slides were prepared in the same manner for a total of 600 μL. All rotifers and copepods were counted. Ciliate and flagellate organisms were scored as present or absent.

Macro-organisms (midges, mites, mosquitoes) were counted using a dissecting scope at a magnification range of 7X-45X. The entire sample was placed in a Petri dish, and larval midges (*Metriocnemus knabi)*, larval mosquitoes (*Wyeomyia smithii),* and mites were enumerated.

2.4 Data Analysis

We conducted principal component analysis (PCA) using the Vegan package in R (R Development Core Team 2018) to determine morphological differences among pitcher species. Principal components 1 and 2 were then compared between taxa using analysis of variance followed by Tukey's HSD *post hoc* test, where appropriate.

Animal abundance, species richness, and Shannon's diversity index (H) were compared between taxa using analysis of variance and Tukey's HSD tests. Initial analyses included taxon, month, and fluid volume as independent variables. Month did not significantly affect communities in any analysis, but fluid volume affected all community variables and was retained in all analyses. Regression was used to determine whether organism abundance of species richness varied significantly with PC1 or aperture area, which was calculated as aperture horizontal x aperture perpendicular x 0.25 x.

3. Results

3.1. Principal Components Analysis

The first three principal components of this analysis explained 90 percent of the variance of the morphological traits (Table 1). In principal component one (PC1), the front height and posterior height of the pitchers loaded positively (Table 2). The hood width and perpendicular aperture measurements of the pitcher loaded negatively. In principal component two (PC2), all measurements loaded positively, except for hood width, which did not show significant variation along PC2. This suggests that PC2 captures overall variation in pitcher size, whereas PC1 captures allometric differences in shape.

There was a significant difference in PC1 between taxa ($F_{2,92}$=105.45, p=< 2.2e-16; Fig. 2). All three taxa differed significantly, with the hybrid intermediate (Tukey HSD *post hoc* test, p≤ 0.05, Fig 3a). The three taxa also differed in PC2 ($F_{2,92}$=10.53, p=7.609e-05). There was a strong

Table 1. Standard deviations and proportion of variance in morphological measurements explained by each of the seven principal components.

	Comp 1	Comp 2	Comp 3	Comp 4	Comp 5	Comp 6	Comp 7
SD	1.95	1.36	0.81	0.59	0.47	0.37	0.09
Proportion of Variance	0.54	0.26	0.09	0.05	0.03	0.02	0.00
Cumulative Proportion	0.54	0.81	0.90	0.95	0.98	1.00	1.00

Table 2. Loadings of each of the seven morphological measurements on the principal components.

	Comp.1	Comp.2	Comp.3	Comp.4	Comp.5	Comp.6	Comp.7
front.height.cm	0.322	0.557	0.173	0.133		0.122	0.721
post.height.cm	0.322	0.553	0.194	0.140		0.248	-0.687
hood.height.cm	-0.311	0.474	-0.120	-0.732	-0.324	-0.154	
hood.width.cm	-0.477		-0.220		0.188	0.825	
aper.per.mm	-0.416	0.326		0.190	0.691	-0.443	
aper.width.mm	-0.435	0.194		0.613	-0.614	-0.141	
rib.max.mm	-0.327	-0.112	0.928	-0.117			

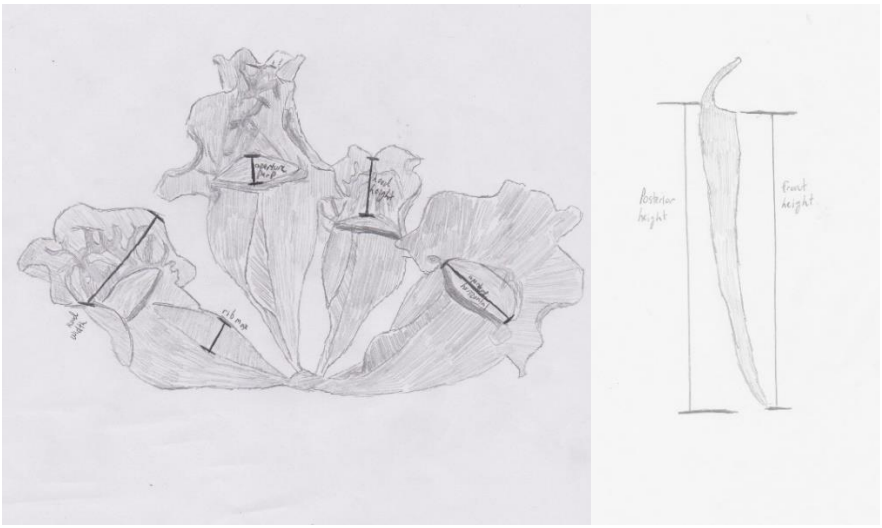

Figure 1. Depiction of morphological measurements of (a) hood, aperture, rib and (b) posterior and front height of pitchers

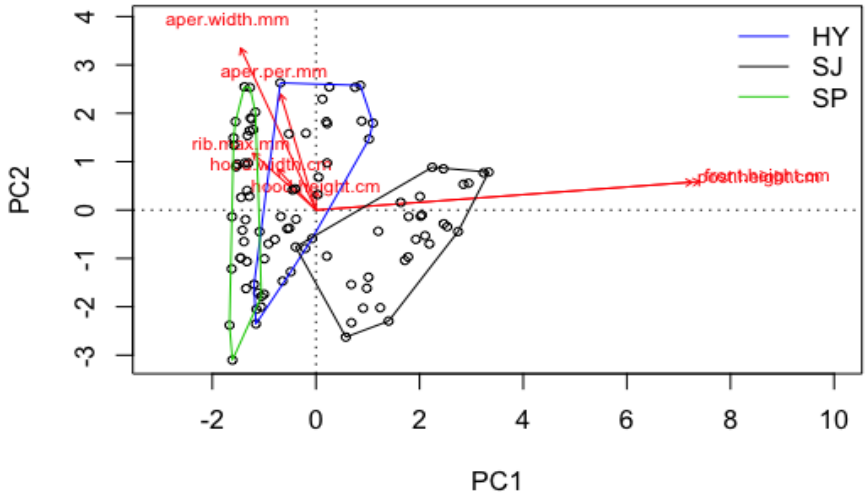

Figure 2. Biplot from principal components analysis showing values of individuals plants with respect to PC1 and PC2, as well as loadings of the seven morphological measurements. Loadings and values for individual rosettes are scaled to the same axes. HY= hybrid, SJ = *S. jonesii*, SP = *S. purpurea* var. *montana*. Front height and posterior height loaded very similarly for both PCs, and are superimposed.

The difference between *S. jonesii* and *S. purpurea* and *S. jonesii* and the hybrid, however there was no significant difference in PC2 between *S. purpurea* and the hybrid (Tukey HSD *post hoc* test, $p \leq 0.05$, Fig 3b). We also ran an analysis of covariance to see if there was a difference between the taxa in the relationship between the two PCs. We used PC2 as an independent variable to see whether the allometric variation captured by PC1 scales differently with the overall size of the pitcher. The relationship between PC1 and PC2 differed among taxa (PC1xPC2 interaction: $F_{2,89}$=45.54, p=2.39e-14, species: $F_{2,89}$=513.94, p=2.20e-16, PC2: $F_{1,89}$=0, p=1.00).

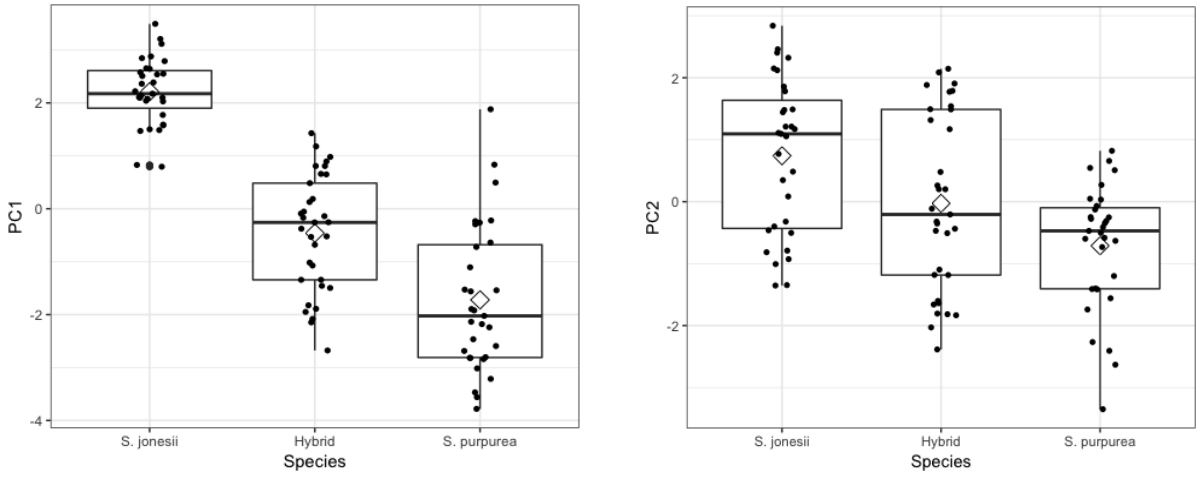

Figure 3. Interspecific differences among *S. jonesii*, hybrid, and *S. purpurea* var. *montana* in (a) PC1 and (b) PC2 scores.

3.2. Community Analysis

Communities were compared between *S. purpurea* var. *montana* and hybrids only. The total number of organisms increased with volume and was greater in *S. purpurea* var. *montana* (anova; taxon: $F_{1,37}$=17.57, p=0.0002; log(fluid): $F_{1,37}$=6.47, p=0.015; Fig 4a). Species richness also increased with volume and was greater in *S. purpurea* var. *montana* (anova; taxon: $F_{1,36}$=20.71, p<0.0001; log(fluid): $F_{1,36}$=10.21, p=0.003; Fig. 4b). Likewise, Shannon's H increased with volume and was greater in *S. purpurea* var. *montana* (anova; taxon: $F_{1,36}$=24.61, p<0.0001; log(fluid): $F_{1,36}$=9.25, p=0.004; Fig. 4c).

There was no effect of aperture area on organism abundance (linear regression: $F_{1,37}$=2.01, p=0.164, R^2 = 0.05; Fig. 5a). Similarly, there was no effect of aperture area on species richness (linear regression: $F_{1,36}$=0.51, p=0.48, R^2 = 0.01; Fig. 5b). In contrast, abundance (linear regression: $F_{1,36}$=6.68, p=0.014, R^2 = 0.16; Fig. 6a) and richness both decreased with increasing values of PC1 (linear regression: $F_{1,35}$=4.04, p=0.052, R^2 = 0.10; Fig. 6b).

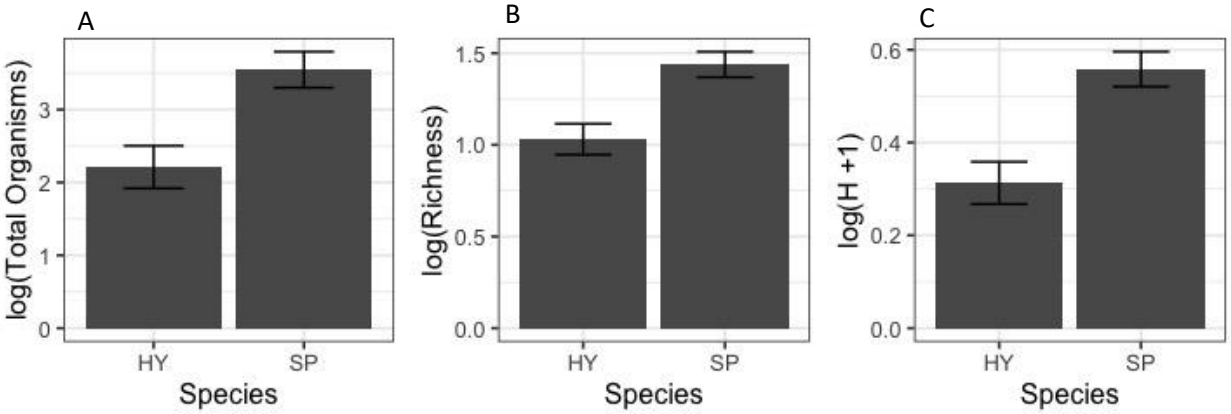

Figure 4. Mean ± SE (a) total organisms, (b) species richness, and (c) Shannon's H for hybrids (HY) and *S. purpurea* var. *montana* (SP). All dependent variables were transformed as log(y+1) to achieve normality.

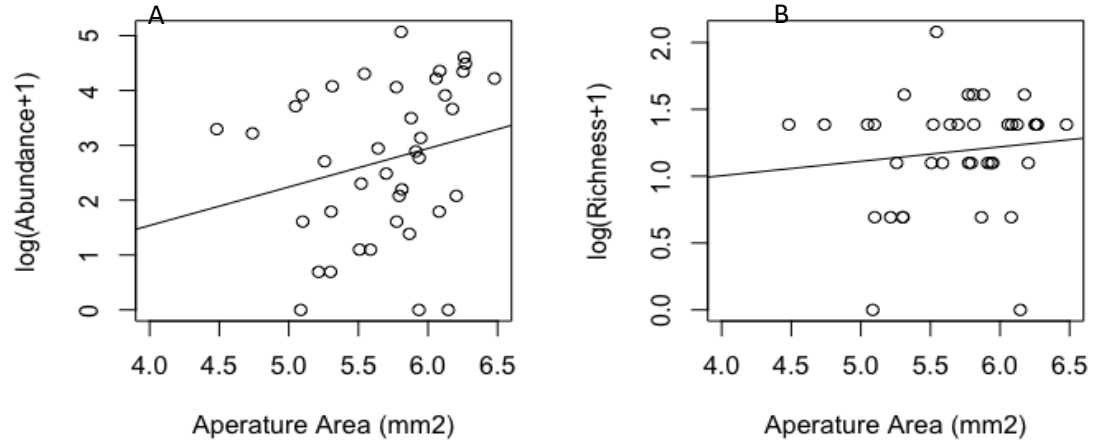

Figure 5. Relationship between aperture area and (a) organism abundance and (b) species richness. Abundance and richness were transformed as log(y+1).

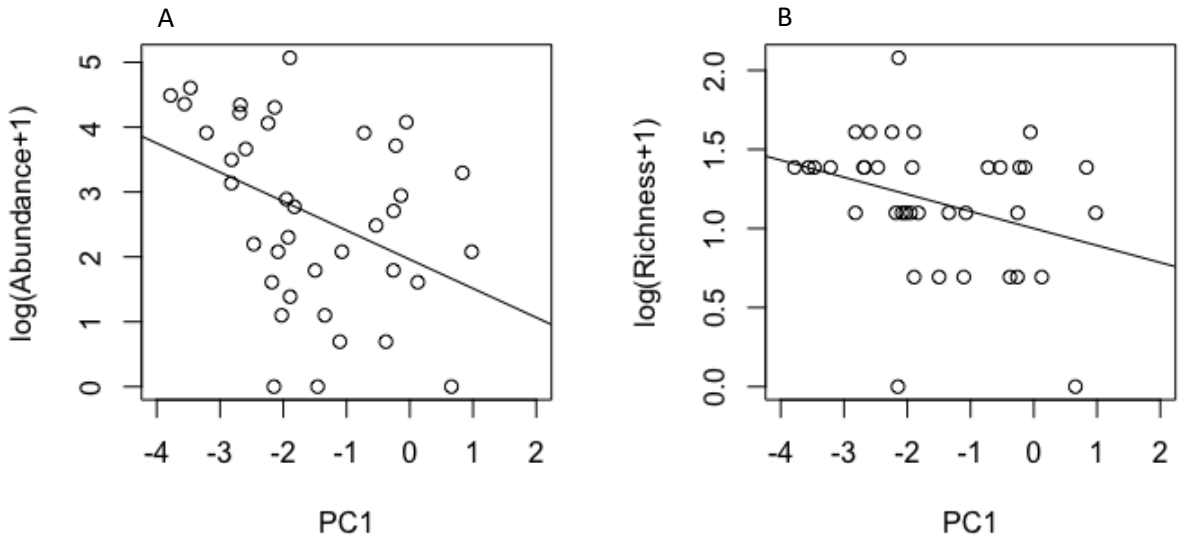

Figure 6. Relationship between PC1 and (a) organism abundance and (b) species richness. Abundance and richness were transformed as log(y+1).

5. Discussion

Interspecific differences appear to be displayed in PC1, verifying anecdotal evidence provided by Beikmohamadi (2018). Intraspecific differences appear to be displayed in PC2. As shown in PC1, the hybrid pitcher is in fact morphologically intermediate between *S. purpurea* and *S. jonesii*, verifying our hypothesis. Principal component analysis shows that a taller pitcher has a decreased aperture area and a shorter pitcher has an increased aperture area. Principal component analysis verifies anecdotes of pitcher size showing taller height and smaller aperture in *S. jonesii* and short and large aperture in *S. purpurea*, respectively. Hybrids, which were identified based on their intermediate morphology, were indeed intermediate with respect to PC1 and PC2. Analysis showing a strong difference between *S. jonesii* and *S. purpurea* and hybrid verifies visual differences.

The community analysis showed that as the volume of fluid found in the pitcher increased, the number of organisms in the pitcher increased as well. Prior studies did not find this to be the case, as they found that organism abundance was not linked to fluid volume (Beikmohamadi 2018, Jaslow 2015). This finding makes sense because an organism would probably be more likely to reproduce in pitchers that contained more fluid than low volume or empty pitchers. Significant differences on pitcher volume to richness showed that as volume increased so did richness. This once again could show that the more volume of fluid present in a pitcher allows for more species of organisms to colonize a pitcher before a maximum capacity is reached. The hybrid pitcher had lower total organisms and species richness as compared to *S. purpurea*. This further verifies our hypothesis that the hybrid is intermediate and possibly less effective at attracting symbionts. Our result of decreased hybrid species richness is supported by similar findings from Jaslow (2015) but not in total organisms.

Finally, regression analysis of aperture area versus richness and aperture area versus abundance had an interesting finding. The fact that neither regressions were statistically significant shows increased area did not affect species richness or abundance. One would hypothesis a larger target aperture would mean more organisms or species richness; however, this was not the case. Furthermore, finding both abundance and species richness were significantly different among PC1 was an important finding. This suggests that organisms may be evolved to recognize an overall shape of pitcher, rather than just an aperture area. The low-lying wide pitchers of *S. purpurea* var. *montana*, which were found to host more taxa and total organisms than the hybrid, appear to be the overall shape that is most preferred.

These findings bring forward more questions to be answered in the future. Although it appears morphology is key to colonization, what is it about the overall shape? Is a certain morphology a cue to a more habitable environment? Is

the low-lying pitcher less likely to dry up killing the symbionts? These are all questions to be answered as to why certain shapes of pitchers are preferred.

6. Acknowledgements

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