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Effects of Hybridization on Phytotelma communities in *Sarracenia purpurea* and *Sarracenia jonesii*

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Abstract

Sarracenia purpurea var. *montana*, the mountain variety purple pitcher plant, inhabits rare mountain bogs and readily hybridizes with the endangered mountain sweet pitcher plant, *Sarracenia jonesii.* As carnivorous plants, these two species rely on trapped prey for nutrients, but do so in very different ways, and hybrids are assumed to be less effective at capturing prey. As a result, hybridization between these two species is thought to be detrimental to offspring. This study examined hybridization effects specifically in the phytotelma communities on two levels, the mirco and macro invertebrate communities. Fluid samples containing invertebrates and micro communities were collected from randomly selected plants from a study site in Transylvania county, North Carolina. The micro community was quantified by first counting one 50 µl drop, if any organisms were seen then we would count six more 50 µl drops. The macro community was quantified by directly counting mosquito larvae, midge larvae and mites. In addition to looking individually at the communities, patterns in micro communities were compared with those from macrocommunities. It was shown that there was no relationship between fluid volume and species richness. It was also shown that there was a successional relationship between macro communities and micro communities being present. There was a significant effect of pitcher age on species richness in the micro-invertebrate community. The successional relationship in the macro and micro communities can be explained by differences in colonization. Individual organism differences can be due a multitude of factors such as pitcher age and morphology.

1. Introduction

Sarracenia purpurea var. *montana*, the mountain variety purple pitcher plant, occupies mountain bogs and hybridizes with the mountain sweet pitcher plant, *Sarracenia jonesii.* Both of these plant species inhabit areas that have limited nitrogen and therefore rely on trapped prey to obtain nutrients[1]. However, these species do so in very different ways. *Sarracenia purpurea* has short, broad pitchers that are able to collect water and attract a diverse group of aquatic organisms. This group, which is referred to as a phytotelma community, is commonly made up of mosquitos, midges, and mites, which make up the macro-community; and rotifers, protozoans, paramecium and copepods, which make up the micro-community [2]. These organisms feed on and break down insects inside of the plant while the plant itself absorbs nitrogen and other nutrients released by the symbiont organisms[3]. In comparison, *Sarracenia jonesii* is taller, thinner, and does not hold water and therefore does not host a phytotelma community. Instead, its own enzymes break down trapped prey.

Since both of these species rely on symbionts in different ways, both pitchers are thought to differ in a wide range of traits including the amount of digestive enzyme produced by the plant and the secondary compounds used to attract prey and symbionts. In addition, pitcher shape differs quite drastically between species, affecting the ability to collect rainwater. Collectively, these trait differences suggest these two species occupy different foraging niches. These two species hybridize in some parts of Western North Carolina and hybridization may have important consequences for these species. Hybrids appear to be morphologically intermediate between the two species and may also be

biochemically intermediate. It has been observed in the past that phytotelma communities differ in the macro scale between the hybrids and parent species; the hybrids have a smaller community in large invertebrates. However, it is just as important to look at the differences on micro scales.

2. Methods

2.1 Field Sampling

Pitcher fluid samples were collected from 74 *S. purpurea* var. *montana* and 47 *S. purpurea* var. *montana* x *S. jonesii* hybrid plants at a site in Transylvania County in the summer of 2016. Twenty-three rosettes of each species were sampled in June, July and twice in August and samples were taken from the same rosettes each month.

Samples were collected by directly pipetting the fluid from the pitchers into 50 mL centrifuge tubes. We recorded the volume of the fluid that was removed from the pitchers ('fluid volume'). The pitcher volume was determined by measuring the amount of deionized water required to fill the pitcher ('pitcher volume'). Deionized water was then removed and discarded.

We used a beading system to mark pitchers; we placed a monofilament necklace with one, two or three beads (June, July and August, respectively) around the base of the pitcher to be sampled. In June, we sampled and marked a young pitcher that had not overwintered, as indicated by the lack of freeze damage to the leaf tissue. We then identified and marked for July sampling an unopened pitcher. In July, we similarly marked for August sampling an unopened pitcher. This method ensured that the communities we sampled were approximately the same age – less than one month old ("young" pitchers). In August, we also sampled an older pitcher ("old" pitchers), matched for size and apparent age to the June pitcher. This pitcher was marked with a four-bead necklace and presumably contained a community at least 2 months old.

2.2 Measuring Communities

The samples were stored in a cooler and transported to the lab in order to be quantified. The smaller, "micro-invertebrates" were counted by first placing four separate 50 µl drops on to a microscope slide and looking for any activity under a compound scope at 10x magnification. If any activity was found, we then looked at two more slides of four 50 µl drops for a total of 600 µl. We counted directly total rotifers, copepods, copepod nauplii nematodes, and planarians in all 600 µl. We recorded presence/absence of smaller and more abundant organisms such small flagellates and paramecium.

After the micro-invertebrates were quantified, the samples were filtered, through 10um Nitex (Bolt Cloth-Nitex 10 µm 40" W), to separate the smaller organisms from the larger, "macro-organisms". Macro-organisms were stored in 50% ethanol in 15mL tubes. These samples were then quantified under a dissecting scope by directly counting the organisms in the sample. We quantified mosquito larvae, midge larvae and mites. The remaining fluid was stored at 4˚C for another study.

2.3 Data Analysis

S. purpurea var. *montana* and *S. jonesii* pitchers differ in shape. Therefore, we first analyzed whether fluid volume or pitcher volume differed between the two species using a *t*-test. Then, we evaluated and whether fluid volume was correlated with the number of macro- and micro-organisms present using a generalized linear mixed effect model (glmm) with log(abundance+1) as the dependent variable and log(fluid volume + 1) as the fixed variable and pitcher month/age as the random variable. There were four levels of pitcher month/age: June, July, August young, and August old. The effect of fluid volume on species richness similarly was evaluated with a glmm with richness as the fixed effect and pitcher month/age as the random effect. Mixed effect models were evaluated using the lme function in the nlme package of the R v. 3.4.3[4].

Anecdotal observations from previous seasons suggested that micro-invertebrates more often are found in older pitchers than in younger ones and that there may be a negative correlation between the abundance of micro- and macro-invertebrates. To evaluate this, we first calculated the abundance and species richness of micro- and macro-invertebrate communities, separately. Then we conducted four glmms with pitcher month/age and species as independent variables and each of these calculations as dependent variables. Rosette identity as included as a random variable. Models were evaluated as above. Finally, we conducted a χ^2 test of independence to determine whether there

was a negative association between the presence/absence of macro-invertebrates and the presence/absence of micro-invertebrates.

3. Results

S. purpurea var. *montana* collected slightly more pitcher fluid (4.04 ∓ 0.49 ml) than the hybrids (3.46 ∓ 0.95 ml), but this difference was not significant (t=1.90, df=103, p=0.06; Fig. 1) There was no relationship between fluid volume and species richness (slope=0.01, $F_{1,103} = 3.61$, p=0.06) or number of micro-invertebrates (slope = -0.11, $F_{1,50} = 1.76$, p = 0.19; Fig. 2A); however, the number of macro-invertebrates increased with fluid volume (slope = 0.311, $F_{1,50} = 30.57$, p < 0.001; Fig. 2B).

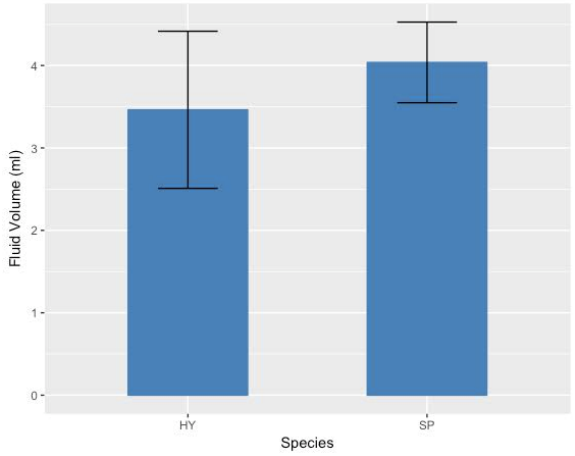


Fig 1. Mean ± SE log fluid volume for hybrids and *S. purpurea* var. *montana* pitchers sampled in June, July, and August.

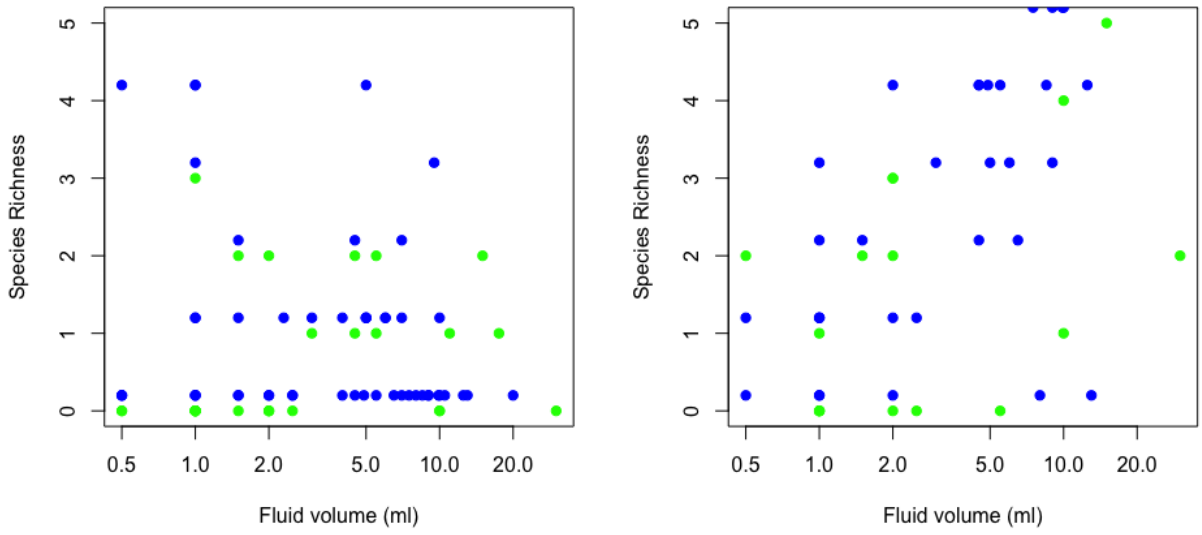
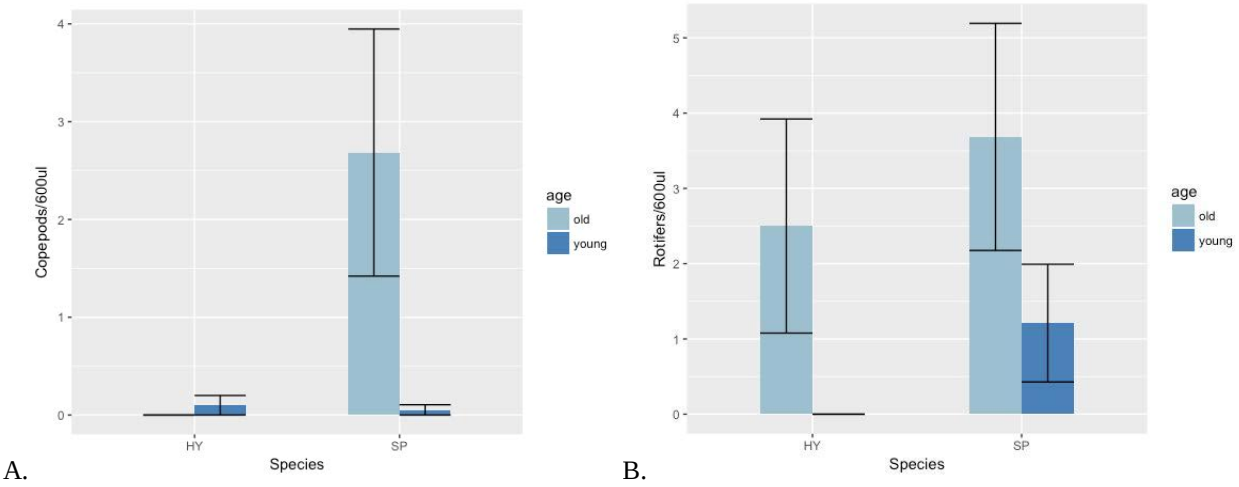


Fig 2. The relationship between fluid volume and A) macro-invertebrate and B) micro-invertebrate species richness in hybrid (blue) and *S. purpurea* var. *montana* (green) plants collected in summer of 2016 in Transylvania County.

Micro-invertebrate communities

Micro-invertebrates sampled were rotifers, copepods, copepod nauplii, paramecium, amoebas, small flagellates and nematodes. There was an effect of pitcher age, but not species, on copepod abundance (age: $F_{1,4}$=22.11, p=0.009; species: $F_{1,4}$=0, p=1; Fig. 3A); no copepods were found in older hybrid pitchers. There was not an effect of pitcher age or species on rotifer abundance (age: $F_{1,11}$=0.36, p=0.56; species: $F_{1,11}$=0.0032, p=0.96) (Fig. 3B). Total macro-inverterbrate abundance was higher in June and July than in both young and old pitchers sampled in August, but there was not a consistent difference between hybrids and *S. purpurea* var. *montana* (pitcher month/age: $F_{2,49}$ = 17.88, p = 0.022; species: $F_{1,49}$ = 4.03, p = 0.23; Fig. 4). Similarly, macro-invertebrate species richness was higher in June and July than in both young and old pitchers sampled in August, but there was no difference between hybrids and *S. purpurea* var. *montana* (pitcher month/age: $F_{2,49}$ = 11.25, p = 0.0001; species: $F_{1,49}$ = 0.22, p = 0.64; Fig. 5).



A. B.

Fig 3. Mean ± SE log abundance of A) copepods and B) rotifers in 600 µl pitcher fluid of young and old, hybrid and *S. purpurea* var. *montana* pitchers. Young pitchers (HY: N=?, SP: N=?) include all young pitchers from August. Old pitchers (HY: N=?, SP: N=?) include old pitchers from August.

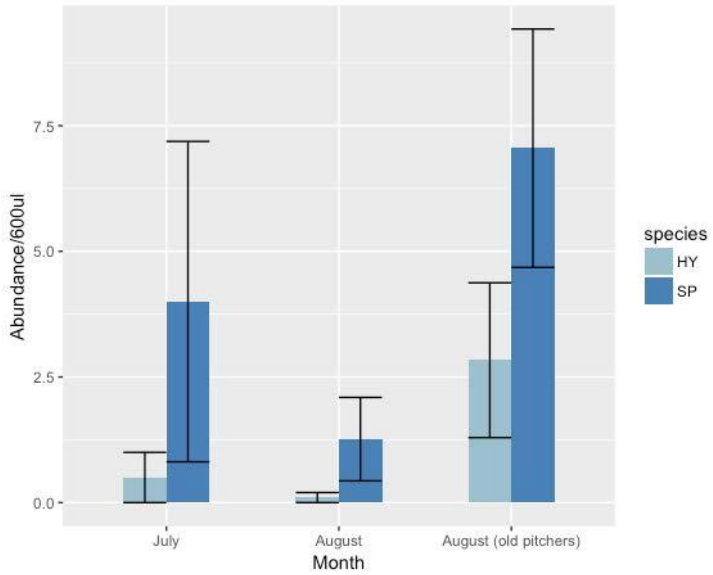


Fig. 4. Mean ∓ SE log abundance of all micro-invertebrates, combined, for hybrids and *S. purpurea* var. *montana* sampled in July, and August of 2016. Micro-invertebrates were not sampled in June.

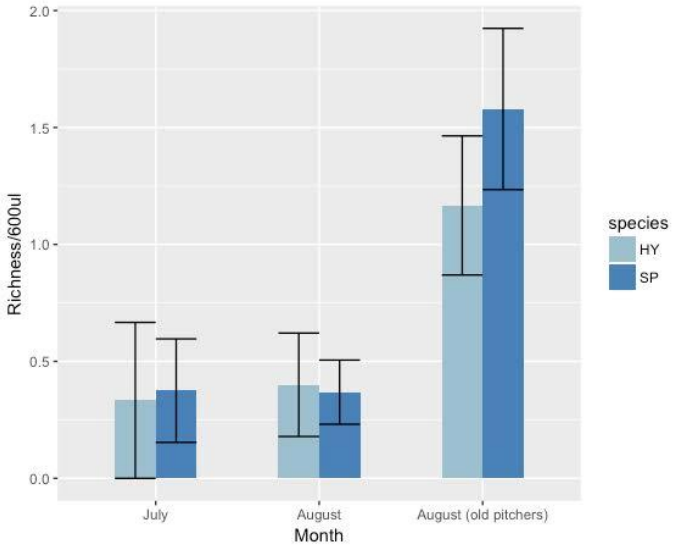


Fig. 5. Mean ∓ SE log micro-invertebrate species richness for hybrids and *S. purpurea* var. *montana* sampled in July, and August of 2016. Micro-invertebrates were not sampled in June.

3.1 Macro-Invertebrate Communities

Macro-invertebrates sampled were pitcher plant mosquito (*Wyeomyia smithii*), pitcher plant midge (*Metriocnemus knabi*), and mites. We recognized 12 different morphospecies of mite, but did not identify them taxonomically. Total macro-inverterbrate abundance was higher in June and July than in both young and old pitchers sampled in August, but there was not a consistent difference between hybrids and *S. purpurea* var. *montana* (pitcher month/age: $F_{3,21}$ = 17.88, $p < 0.0001$; species: $F_{1,26}$ = 4.03, p = 0.055; Fig. 6). Similarly, macro-invertebrate species richness was higher in June and July than in both young and old pitchers sampled in August, but there was no consistent difference between hybrids and *S. purpurea* var. *montana* (pitcher month/age: $F_{3,21}$ = 12.95, p = 0.0001; species: $F_{1,26}$ = 4.03, p = 0.18; Fig. 7).

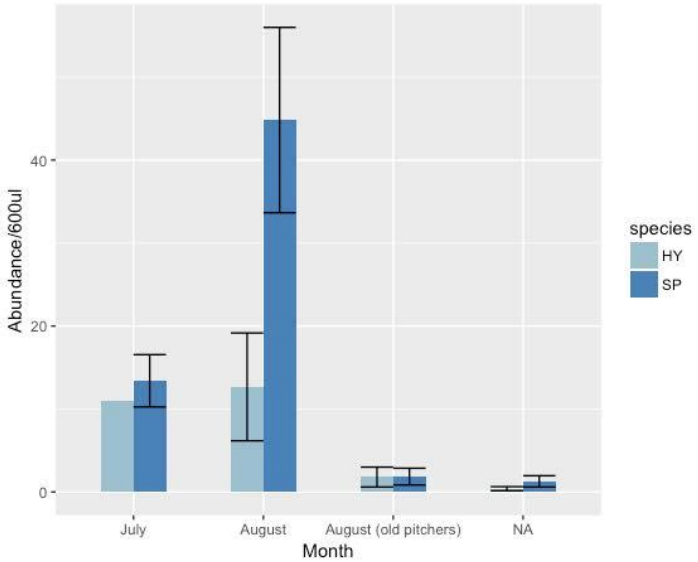


Fig. 6. Mean ∓ SE log abundance of all macro-invertebrates, combined, for hybrids and *S. purpurea* var. *montana* sampled in June, July, and August of 2016.

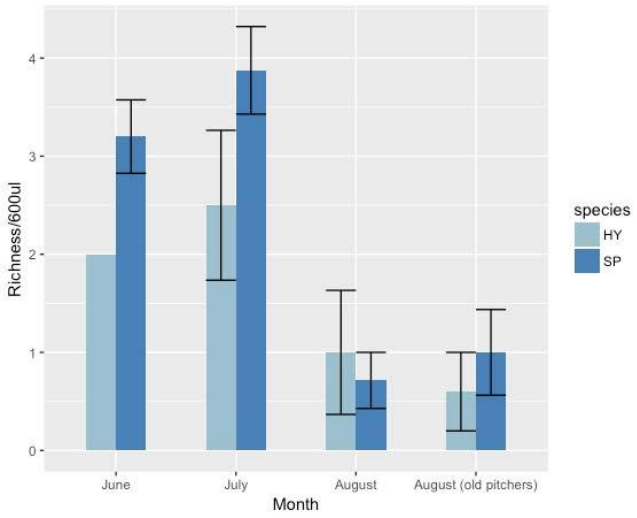


Fig. 7. Mean ∓ SE log macro-invertebrate species richness for hybrids and *S. purpurea* var. *montana* sampled in June, July, and August of 2016.

There was a trend of macro-organisms being more abundant during the earlier months (June and July) rather than the older months and micro-organisms being more abundant in old August pitchers rather than in any young pitchers. Indeed, micro-invertebrates were less likely to occur in pitchers that contained macro-invertebrates, and vice versa ($\chi^2_1 = 17.77$, $p < 0.0001$).

4. Discussion

Differences in communities could be due to multiple reasons, one of them being the volume of the phytotelma within the pitchers. If there is larger pitcher volume, it could be assumed that there is a higher chance for diversity in the phytotelma. However, we found that there was no difference in the fluid volume between the two species. Also, the amount of fluid volume did not affect the number of organisms in the pitchers.

Rotifers were much more abundant in older pitchers than younger ones. We also did not find any rotifers in young hybrid pitchers. In general, not much was found in young pitchers since they have been open for a shorter amount of time open. When pitchers are open for a shorter amount of time then they are unable to catch as much pretty as those open for a longer time. Copepods showed an opposite effect of many of the results in this paper, they were not found in old hybrid pitchers. Copepods were usually found in samples that were small in volume and were very debris heavy. An explanation for this is that the *S. purpurea* are closer to the ground and therefore it is easier for them to be flooded with water and debris. This flooding can then help copepods get in as well as help sustain them since the debris can provide food material. Hybrid's shape do not allow for easy flooding since they are taller and not as close to the ground.

Qualitatively, macro-organisms were more abundant in early season, young pitchers, whereas some micro-organisms were more abundant in later season and old pitchers. A possible explanation for this is because of differences in colonization rates between macro- and micro-organisms. Many of the macro-organisms are able to fly from pitcher to pitcher and readily colonize newly opened pitchers, whereas micro-organisms must walk or be carried in by other organisms. For example, mites may travel on the legs of the mosquitos or midges. So the micro-organisms being in the pitchers is dependent on if the macro-organisms are in the pitchers. Even if they arrive with macro-organisms, they may not be detectable until they are able to reproduce to density high enough to be observed.

Another explanation could be seasonal effects on organism prevalence. For example, mosquitoes and midges use pitchers as egg and larval habitats. If these organisms colonize early, the larvae may emerge and leave pitchers just as micro-organisms are becoming more abundant. Finally, the interaction between the two groups of organisms may be more direct. Mosquito and midge larvae and mites may feed on rotifers, copepods, and unicellular organisms.[2] If so, they these micro-organisms may be present, but kept in low density, until the mosquitoes and midges emerge and leave pitchers.

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