University of North Carolina Asheville
Undergraduate Research Journal
Asheville, North Carolina
April 2015

Characterization of Genetic Diversity in Demes of Mountain Purple Pitcher Plants (*Sarracenia purpurea* L. var. *montana*) from Western North Carolina

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Abstract

Purple pitcher plant (*Sarracenia purpurea* L.) is a carnivorous species found throughout the eastern United States and throughout much of Canada. In western North Carolina, *S. purpurea* var. *montana* (mountain purple pitcher plant) exhibits a much more limited distribution, inhabiting only in isolated montane *Sphagnum* bogs and fens, which serve as ecosystem islands. Population sizes of mountain pitcher plants are fairly small, ranging from as few as 2 up to approximately 300 individuals. In addition, many bogs are spatially isolated, with *S. purpurea* var. *montana* plants up to 21 km from the next known population. We characterized the genetic structure of three *S. purpurea* var. *montana* populations in Western North Carolina (DF, MB, and SF) using 4 microsatellite loci. DNA was extracted from leaf tissue using Qiagen© Plant DNeasy kits, and DNA was amplified at the polymorphic *Sarr005*, *Sarr009*, *Sarr029*, and *Sarr042* microsatellite loci. PCR products were separated via agarose gel electrophoresis and visualized to quantify band sizes. Results showed relatively high numbers of alleles, and private alleles were found in all populations. Heterozygosity was depressed at all sites, most genetic variation was found within sites, and genetic and geographic distances were related. These data are consistent with our hypotheses of reduced gene flow with increasing distance between the populations and inbreeding within populations. Results of this project will help the United States Fish and Wildlife Service determine *S. purpurea* var. *montana*'s suitability as a candidate species for conservation and preservation programs.

1. Introduction

Purple pitcher plant (*Sarracenia purpurea* L.) is a carnivorous plant species that is widespread throughout the eastern United States and throughout much of Canada[1, 4]. Members of the *Sarracenia* genus collect water in their pitcher capsules[2], which aids in capturing arthropod prey and extracting their nutrients[3]. The distribution of *S. purpurea* var. *montana* is patchy in Western North Carolina, restricted to isolated *Sphagnum* seep bogs, poor fens, and seepage swamps[4, 5, 6]. These habitats might serve as ecosystem islands for the species. Population sizes range from as few as 2 up to approximately 300 plants[7]. In addition, some bogs are extremely isolated, over 21 km from the nearest known population. This separation raises the questions of how these sites were initially colonized, and whether gene flow currently exists among them.

Gene flow is likely to be quite low in *S. purpurea* var. *montana* because of the short traveled distances of its pollinators and its seed dispersal strategies. Bumblebees (*Bombus* spp.)[8] and sarcophagid flies (*Sarcophaga sarraceniae*)[8, 9], also known as flesh flies, have been found to be the primarily pollinators of *S. purpurea*[10]. Bumblebees typically travel no more than 600-1,700 m to forage, as shorter trips are safer and more energy-efficient[11, 12, 13, 14]. Similarly, flesh flies display a foraging range of 1 to 4 km[9]. Like most herbaceous plants of wetlands, woodlands, and fields of North America[15], seed dispersal in *S. purpurea* occurs over short distances; moreover, seedlings cluster around the maternal plant, and seed mortality is believed to be high[6]. Seeds are thought

to be primarily water-dispersed[4] and exhibit limited movement between isolated bogs[6]. Therefore, the presence of *S. purpurea* throughout the coastal plain for Eastern North America can be explained by retreating glaciers or by rare, long-distance transport circumstances[6].

Complicating our understanding of historic gene flow and colonization are accounts of plants being moved among sites. Anecdotal evidence indicates that at least one population examined in this study was planted from the North Carolina Botanical Garden accession, which might not be *S. purpurea* var. *montana*. Restoration efforts at another site have included importation of at least one *Sarracenia* species, so the history of *S. purpurea* var. *montana* there is unknown. We expected patterns of gene flow across populations to reflect this complicated history of natural migration between geographically proximate sites and transplantation.

In this study, we characterized the structure of genetic diversity in three *S. purpurea* var. *montana* demes in Western North Carolina using four microsatellite loci[16]. It was hypothesized that small and isolated populations would exhibit reduced gene flow and heterozygosity as a result of little pollen or seed movement. We also expected that populations would show little similarity among one another, due to non-local origins or post-isolation evolution. This study is important because understanding aspects that limit reproductive success such as pollination and seed dispersal strategies could provide help in modeling migration dynamics for this carnivorous species. This study is key in identifying *S. purpurea* var. *montana* populations for conservation and preservation efforts by the U.S. Fish and Wildlife Service.

2. Materials and Methods

2.1 Sample Collection and DNA Extraction

During fall 2014, thirty-five leaf tissue samples of *S. purpurea* were collected from three Western North Carolina populations: DF and SF (Transylvania County, NC), and MB (Henderson County, NC). DF and SF were 5.7 km away from each other. DF and SF were 16 km and 21 km, respectively, from MB. Each site drains to a different stream, so seed movement among sites is unlikely.

For populations containing more than 20 individuals, we sampled 20 plants evenly distributed across the population. A transect was established either through or around the population, depending on the plants' spatial distribution. Twenty evenly distributed rosettes along, and at varied distances perpendicular to, the transect were sampled. Tissue from the fullest, non-senescing pitcher in the selected rosette was sampled. For populations with 20 or fewer individuals, tissue was collected at every rosette using the same criteria.

A leaf tissue sample of approximately 2 cm x 0.5 cm was cut from the rib on the back of the pitcher, stored on ice, then transferred to a -80ºC freezer. Leaf tissue samples were subjected to total DNA extraction using the Qiagen© DNeasy Plant Mini kits (Valencia, CA), and DNA concentrations were quantified utilizing a Nanodrop ND-200 Spectrophotometer (Nanodrop©, Wilmington, DE). Ideal DNA concentrations were approximately 10 ng/µL.

2.2 PCR Set-Up for Allelic Markers Determination

Four microsatellite primer pairs (*Sarr005*, *Sarr009*, *Sarr029*, and *Sarr042*)[16] were ordered from Eurofins MGW Operon (Huntsville, AL). Microsatellite DNA amplification reactions contained 1X PCR Buffer A (containing 15 mM $MgCl_2$), 2.2 mM dNTP mix, 1.8 mM forward and reverse primer, 2 mM $MgCl_2$, 5 U *Taq* DNA polymerase, and 20-300 ng of total genomic DNA. PCR was performed using a T100 thermal cycler (BIO-RAD, Hercules, CA) with the following procedure: 30 cycles at 94°C for 4 minutes, 94°C for 1 minute, 56°C for 1 minute, 72°C for 1 minute, and a final extension at 72°C for 4 minutes.

2.3 Electrophoresis of PCR Products

One hundred forty PCR products were analyzed by size separation along with a 100 bp DNA Step Ladder (Promega, Madison, WI) on a 2% agarose gel, stained with 4 µL of ethidium bromide, at 90 V for approximately 1.5 hours . Bands were visualized using UV light, and gels were photographed using the KODAK® Gel Logic 100 Imaging System at an f-stop of 5 (Fig. 1). Carestream© MI Software (Rochester, NY) was used to edit images and quantify band sizes. Band sizes were recorded and compiled into a Microsoft Excel spreadsheet.

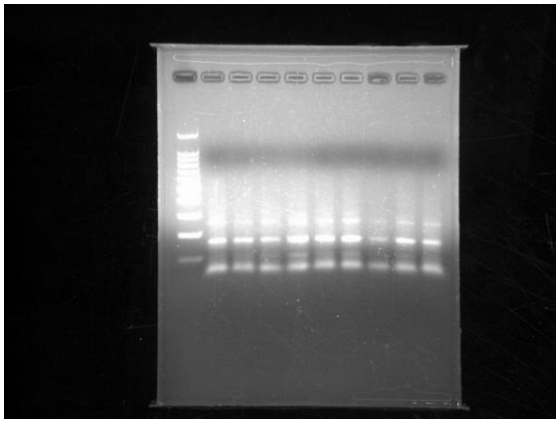

Figure 1. PCR products using primer *Sarr042* on a 2% agarose gel. From left to right: Promega 100 bp DNA Step Ladder, T8SP01, SF08, T10SP01, T16SP02, SF04, SF06, T10SP02, T12HY01, T6HY01.

2.4 Statistical Analysis

The GenAlEx 6.5 add-in for Microsoft Excel 2013© (Microsoft Corporation, Redmond, WA) was utilized to run descriptive analyses for genetic patterns within and among three *S. purpurea* populations (N = 35). For each population, the number of different alleles and private alleles was estimated, as were expected and observed levels of heterozygosity. F_{ST} values were used to conduct an analysis of molecular variance (AMOVA), comparing the relative amounts of genetic variation within and among individuals and populations (9999 iterations). The genetic distance between populations was determined using a pairwise population matrix of Nei's genetic identity[17].

3. Results

3.1 Allelic Patterns Across Populations

Population SF exhibited the greatest number of different alleles and private alleles (Fig. 2). Population DF showed the second highest number of different alleles as well as the second highest number of private alleles (Fig. 2). In contrast, population MB exhibited the lowest number of alleles and the lowest number of private alleles (Fig. 2). Nei's Genetic Identity[17] values showed that SF and DF were the most related populations (Table 1). The AMOVA test showed that most genetic variation was found within populations and little was distributed among populations (Fig. 3). In addition, all individuals from DF and MB were homozygous at the four microsatellite loci; individuals from SF were the only ones that exhibited heterozygosity (Fig. 4).

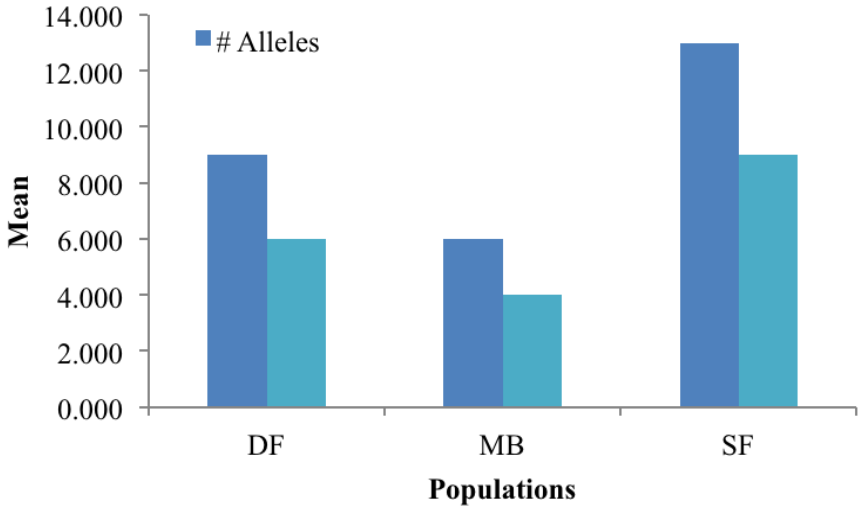

Figure 2. Number of alleles and private alleles in three populations of *Sarracenia purpurea* var *montana*.

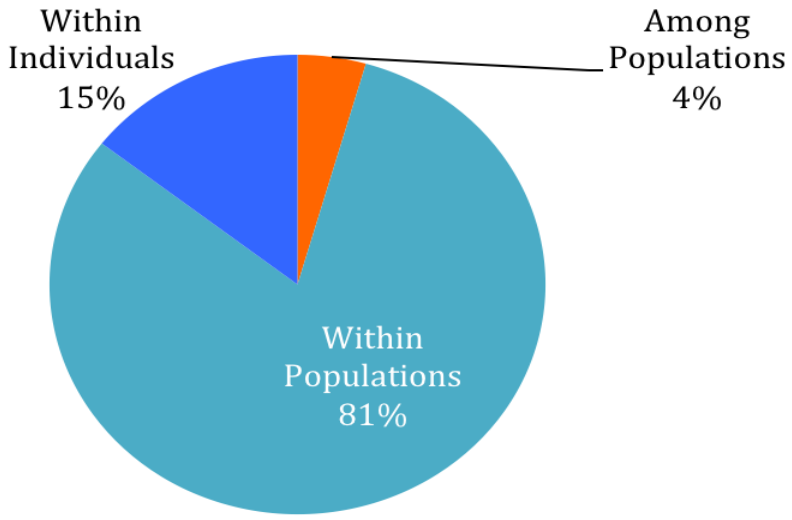

Figure 3. Results of AMOVA for three *Sarracenia purpurea* var *montana* populations.

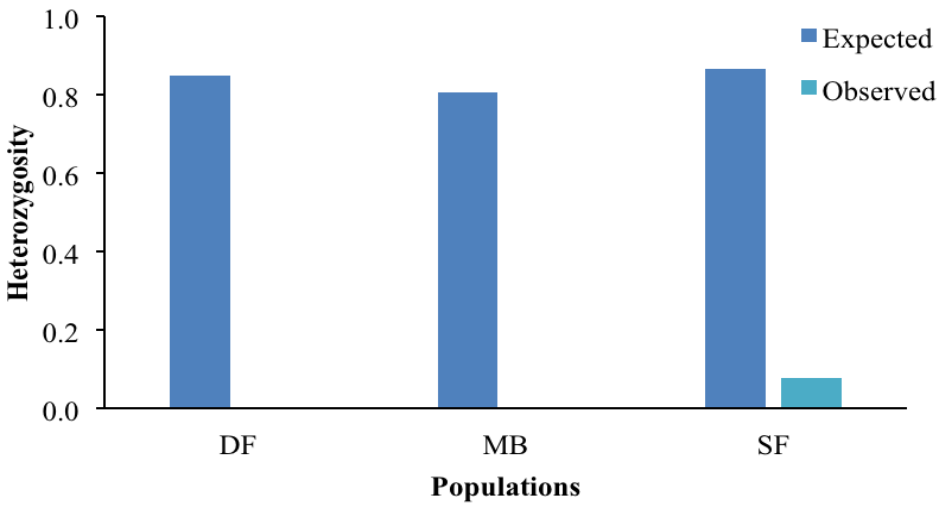

Figure 4. Observed and expected heterozygosity for three *Sarracenia purpurea* var *montana* populations.

Table 1. Pairwise population matrix of Nei's genetic identity for three *Sarracenia purpurea* var *montana* populations

	DF	**MB**	**SF**
DF	1		
MB	0.069	1	
SF	0.179	0.103	1

4. Discussion

Reduced allelic diversity in MB could be due to a combination of small sample size (8 plants; population size = 20) and its isolation from other populations. In contrast, SF shows the highest number of different alleles, perhaps due to its larger sample and population size (20 plants; population size > 50). SF also displays the highest number of private alleles (N = 19), which could also be attributed to its large population size. The presence of private alleles in all populations indicates a lack of panmixis.

The AMOVA results suggest that most genetic variation was found within populations and the least was observed among populations. This indicates that interpopulation gene flow is low. Depressed heterozygosity suggests that populations might be inbred or subject to genetic drift.

Nei's genetic distance results are aligned with populations' geographic distributions. DF is 5.7 km away from SF, while MB is 21 km away from SF and almost 16 km from DF. The genetic similarity between DF and SF suggests that SF was not planted from the North Carolina Botanical Garden accession as previously believed, or at least that if it were planted, this population has then experienced gene flow between it and nearby natural populations.

The results from this study are crucial for *S. purpurea* conservation and preservation efforts in Western North Carolina. Although the risk of extinction of *S. purpurea* is small[3], the rapid increase in nitrogen deposition, bog fragmentation by deforestation, clearing for development, wetland-loss, and anthropogenic forces such as illegal collection or overcollection can lead to habitat destruction and decrease population growth of purple pitcher plants[3, 18, 19, 20, 21, 22]. Future studies should focus on a more comprehensive examination of *S. purpurea* plants from this region, analysis of additional microsatellite loci, and tracking seed and pollen movement.

5. Acknowledgements

The author wishes to express his great appreciation to Elise Powell, Zack Mackenzie, and Dr. Sarah Seaton for collecting leaf tissue samples for this study, and Dr. Mara Alexander from the U.S. Fish & Wildlife Service, Asheville Field Office, for providing *S. purpurea* locations and funding for this work. Jessey Neder assisted with DNA extraction and quantification process.

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