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Development and characterization of microsatellite markers in *Sarracenia L.* (pitcher plant) species

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Abstract

Sarracenia species (pitcher plants) are carnivorous plants which obtain a portion of their nutrients from insects captured in the pitchers. *Sarracenia* species naturally hybridize with each other, and hybrid swarms have been identified. A number of the taxa within the genus are considered endangered. In order to facilitate evolutionary, ecological and conservation genetic analyses within the genus, we developed 25 microsatellite loci which show variability either within species or between species. Three *S. purpurea* populations were examined with 10 primer sets which showed within population variability.

Keywords

Sarracenia; Carnivorous plants; Microsatellite loci; Hybridization; Genetic diversity

Carnivorous plants fascinate both scientists (Darwin 1875) and the general public (Corman 1960). *Sarracenia* (pitcher plants) typically grow in highly acidic, nutrient poor soils that are water saturated for at least part of the year, such as bogs and similar wetlands. They obtain a portion of their nutrients from prey captured in their pitchers, highly modified tubular leaves; however, the morphologies of these pitchers vary greatly across the genus and are diagnostic of different *Sarracenia* species. *Sarracenia* species may digest their prey directly with secreted proteases, phosphatases, nucleases (Hepburn et al. 1920; Gallie and Chang 1997); however, some species (*S. purpurea* and *S. rosea*) host complex food webs of bacteria, protozoa, and arthropods that mineralize the prey and release nutrients that are taken up by the plant (Ellison et al. 2003; Gotelli and Ellison 2006).

The North American genus *Sarracenia* consists of 14–16 perennial herbaceous taxa, most of which are restricted to wetlands of the coastal plain of the southeastern US. The more wide-ranging *S. purpurea* is found in *Sphagnum* bogs throughout the coastal plain of eastern North America and in Canada east of the Rocky Mountains (Buckley et al. 2003). Several southern *Sarracenia* species are listed as threatened endangered species at either the federal or state levels as a result of habitat loss, changes to their habitat in response to lack of fire, or over collection. The number and rank of *Sarracenia* taxa recognized varies among

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taxonomic treatments. Weakley (2006) recognizes 16 taxa labeled as species, subspecies, or varieties. Bayer, Hufford, and Soltis (1996) used chloroplast *rbcL* and nuclear *rRNA* ITS sequences to examine phylogenetic relationships within the family, but, phylogenetic positions within the genus *Sarracenia* were not well resolved. There was weak support for *S. purpurea* and *S. leucophylla* as a group, and moderate support of *S. minor*, *S. flava*, and *S. psittacina* as a group. Naczi et al. (1999) named a new species, *S. rosea*, by separating *S. purpurea* subsp. *venosa* var. *burkii* and elevating it to species status. This new species was supported by further morphological work (Ellison et al. 2004).

Sarracenia species freely hybridize. Hybrid swarms occur in southern Georgia and in the Gulf Coast regions of Florida and Alabama (Bell and Case 1956; McDaniel 1971), and occasionally in the Atlantic coastal plain (Godt, personal communication). Natural F1 hybrids between 21 species pairs have been documented (Slack 1980). The extent to which naturally occurring hybrid swarms contain other than F1 individuals is unclear. The factors and processes that maintain individual *Sarracenia* species as species are unknown, if, in fact, such factors and processes exist. Given the rampant hybridization within the genus, it is reasonable to question the taxonomic level and nature of speciation among the identified taxa.

Thus, there are multiple evolutionary and ecological genetic questions, as well as studies of genetic diversity within endangered taxa, all of which could be addressed in *Sarracenia* with the aid of appropriate molecular markers.

The chromosome number is constant within the genus at $N = 26$ (Bell and Case 1956). We estimated the nuclear DNA amounts of *S. purpurea* and *S. psitticina* by nuclear flow cytometry, using rice as a standard with 1.01 pg per 2C and 430 Mbp per 1C. This gave estimates of 8.64 pg per 2C and 3,581 Mbp per 1C for *S. purpurea*, and the very similar values of 8.69 pg per 2C and 3,602 Mbp per 1C for *S. psitticina*. Thus, these have about 25% more nuclear DNA than maize. Genomes of this size typically have many repetitive sequences and transposable elements and could be polyploid.

Genomic DNA was isolated from greenhouse-grown plants of *S. purpurea* and of *S. psitticina* using slight modifications of the protocol of Peterson et al. (2000). The samples were homogenized by grinding to a powder in liquid nitrogen and then continuing the grind with liquid buffer; afterwards the samples were filtered through nylon mesh; unlike the original protocol, cheese cloth was not used, and the additional homogenizations in a blendor and a polytron were not performed. We made genomic fosmid libraries of both in pCC2FOS (Epicentre) using the kit supplied by the manufacturer. From these libraries we sequenced the ends of inserts in randomly picked clones; we obtained 262 reads for 170,000 bp of *S. purpurea* and 270 reads for 167,000 bp of *S. psitticina* sequence. The results were analyzed for potential microsatellite loci using the program *msatcommander* (Faircloth 2008). We identified 58 potential microsatellite locus primer pairs. To test these, we used four plants from each of the ten taxa shown in Table 1, selected from the collection maintained by the Atlanta Botanical Garden. The plants were grown from wild collected seeds that were surface sown and cold stratified at 4.4°C for 4 weeks on a mixture of moist milled *Sphagnum*, peat moss and sand. The seeds were then covered and head started under grow lights (17 h light/day) for about 4 months before they were moved to the conservation greenhouse. For each primer pair, we varied the annealing temperature (T_a) to optimize the fragment amplification. Standard PCR conditions included 4 min at 94°C, 1 min at the T_a shown in Table 2, and 1 min extension at 72°C, with a final 4 min 72°C finishing extension. Of the primer pairs, 16 failed to amplify anything from the 40 plants, while 17 amplified a constant banding pattern across all 40.

The results from the remaining 25 primer pairs are shown in Table 1. A number of loci showed good variability in fragment size between plants of the same species, making them useful for within species analyses. Others were variable in fragment size between species, including one locus which only amplified a fragment from *S. purpurea*, and another which only amplified a fragment from *S. psitticina*; this category may prove useful both for studies of species hybridization and in studies of endangered taxa.

We identified 10 primer pairs which showed variability within *S. purpurea* to characterize population-level variation; for this, we examined 84 individuals across three populations. The data was analyzed using the Excel add-in GenAlEx (Peakall and Smouse 2006) with the results shown in Table 2. Most of the primers tested gave more than one segregating band, consistent with the idea that *S. purpurea* is polyploid. Most of the loci were significantly out of Hardy-Weinberg equilibrium.

These are the first microsatellites of which we are aware identified for *Sarracenia* species. They should prove useful in answering a variety of questions with this genus.

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Table 1

Results with 25 *Sarracenia* microsatellites across species

Name	Primers	T_a	Comment
Sarr002	F: 5′ TGTGTGACAAGACTAAGCTCC R: 5′ AATTCTACTGGGGCGTGGG	52°	*S. flava* and *S. leucophylla*
Sarr005	F: 5′ CCCACGAGAAGAATTACCCG R: 5′ TGCCAAATCAACCCATTCACC	56°	Variable within all species
Sarr006	F: 5′ TGGCTGCCTGTCTGCATAG R: 5′ AGTGCCTTCCCCTTACGAC	54°	*S. purpurea* and *S. rosea*
Sarr007	F: 5′ CTGTGGAGCAAGCAACGTC R: 5′ CTGCCGAATCTCCCTCTCC	56°	Variable within some species, *S. purpurea* and *S. rosea* differ from others
Sarr009	F: 5′ TGCCCCAAATAAATGCCCG R: 5′ CCATTTGGCATGGTGGCAG	48°	Variable within all species
Sarr010	F: 5′ GTGGTGGCTGAGATTTGGAG R: 5′ AATCAAAACCCAACACGGC	52°	*S. purpurea* and *S. rosea*
Sarr014	F: 5′ CCGCGAATACCCCAAATCG R: 5′ TCGTGCTTGATTTCGGTCG	50°/60°	Variable within all species
Sarr015	F: 5′ CTTTCCCACGGCTCAAAGG R: 5′ CAGGGCTCAGGTCAGTCG	48	Variable within species except *S. purpurea* and *S. rosea*
Sarr020	F: 5′ GTAAAGGATTACCAAGTTTTACTGC R: 5′ TGGAGGAAACATGGCTGGG	52°	Variable within most, not *S. alata*, *S. minor*, *S. rosea*
Sarr023	F: 5′ ACCAGTAGCAAAACAAAGCC R: 5′ GCTTCATCTTTCCTCTCCCC	56°	Variable within *S. oreophila*; *S. minor* and *S. psitticina* unique
Sarr027	F: 5′ ACTGCGAGAGTTTGGGCTG R: 5′ TCGGTTTGATTTTAACGGACAAC	56°	Variable within *S. alata*, *S. minor*, *S. oreophila*, *S. rubra*
Sarr028	F: 5′ CGGCGGCTAATTCCAACTG R: 5′ ACTCGCGTCCCGTTATCTC	52°	Variable within most, not *S. flava*, *S. leucophylla*, *S. purpurea*, *S. rosea*
Sarr029	F: 5′ AAGCTCCTCGATTGGACCG R: 5′ TTGCCAAACACTCCCTTGG	56°	*S. purpurea* specific
Sarr032	F: 5′ GCGCACTTACCACGATCAC R: 5′ CAGCTGAAGCATCCAGGTC	56°	Absent in *S. flava*, *S. minor*, *S. oreophila*, *S. psitticina*
Sarr035	F: 5′ AAGTCCAGCCGTAGTTGGG R: 5′ AGCAATGCAAAACGTAAAACAC	56°	Absent in *S. jonesii*, *S. leucophylla*
Sarr040	F: 5′ TCTGAAGCGGATCAGGACG R: 5′ CGGTTGACCACAGATTGCC	48°	Variable within most, not *S. flava*, *S. jonesii*, *S. purpurea*, *S. rosea*
Sarr042	F: 5′ CGAACCCTAGTTCATCCAATACC R: 5′ ACTTTGAGCTTTACGGTGGC	54°	Absent in *S. jonesii*
Sarr045	F: 5′ GTTGAACGAAAACGGTGCC R: 5′ TGTGACCAAAAGGGAGTCG	52°	Variable in *S. purpurea* and *S. rosea*

Name	Primers	T_a	Comment
Sarr050	F: 5′ GAGGACTAAGGGATGCCCG R: 5′ TCACAAAGTCAAAGCAAGGAAAC	52°	Variable within *S. minor*, *S. oreophila*, *S. purpurea*, *S. rosea*, *S. rubra*
Sarr052	F: 5′ TTTCCAAACACGGGCAAGG R: 5′ CGACATCACCAAAGGGGTC	48°	*S. flava* and *S. minor* differ from others
Sarr053	F: 5′ CAGCTTTTGCAATACACTGGAC R: 5′ ATTGGGCAAGAGGGACACC	56°	Variable within all species
Sarr055	F: 5′ ACGTTGGCCATAGCATTCAAG R: 5′ CGGCTTGTGGCAGTTGTAG	52°	Variable within *S. psitticina*
Sarr056	F: 5′ TGACGACCACCATCGTTGC R: 5′ TCGTATTGTTCGATTTGGTTTGC	56°	Variable within species except not *S. alata*, *S. minor*
Sarr058	F: 5′ TGGTTTGGTGCTGGTATGC R: 5′ CATGATACTCCTCACGCACC	56°	Variable within species except not *S. leucophylla*
Sarr060	F: 5′ GTTCTCTCCTCTCTGGCCG R: 5′ TCCAGAGGTGTGTTGAGAGG	56°	*S. psitticina* specific

S. alata		***S. flava***		***S. jonesii***		***S. leucophylla***		***S. minor***	
No. frag.	**Size range**	**No. frag.**	**Size range**	**No. frag.**	**Size range**	**No. frag.**	**Size range**	**No. frag.**	**Size range**
2	220–240	1	240	2	220–240	1	240	2	220–240
4	120–550	3	200–375	4	190–450	5	190–450	3	125–300
1	220	1	220	1	220	1	220	1	220
2	166–250	2	166–250	2	166–250	2	166–250	2	166–250
7	170–300	4	210–240	7	175–260	8	160–270	7	150–270
1	135	1	135	1	135	1	135	1	135
6	125–250	8	110–250	6	150–230	9	150–350	7	160–250
4	160–280	3	155–250	4	160–280	2	240–250	2	240–250
1	225	2	225–227	2	225–227	2	223–225	1	225
1	170	1	170	1	170	1	170	1	165
2	150–160	1	162	1	155	1	165	2	162–165
4	225–245	1	245	3	225–245	1	238	3	235–245
0		0		0		0		0	
1	225	0		0	220	1	225	0	
1	185	1	185	0		0		1	185
2	170–190	1	190	1	190	2	170–190	2	185–190
1	168	1	166	0		1	166	1	168
1	150	1	150	1	150	1	150	1	150

Conserv Genet Resour. Author manuscript; available in PMC 2011 December 1.

S. alata		***S. flava***		***S. jonesii***		***S. leucophylla***		***S. minor***	
No. frag.	**Size range**	**No. frag.**	**Size range**	**No. frag.**	**Size range**	**No. frag.**	**Size range**	**No. frag.**	**Size range**
1	230	1	230	1	230	1	230	2	215–230
1	195	1	210	1	195	1	195	1	200
4	165–200	6	165–195	4	160–190	6	155–200	4	160–185
1	233	1	233	1	233	1	233	1	233
1	345	5	270–345	2	300–345	2	300–345	1	345
2	225–260	3	180–260	3	225–260	1	255	4	180–260
0		0		0		0		0	
S. oreophila		*S. psitticina*		*S. purpurea*		*S. rosea*		*S. rubra*	
No. frag.	Size range	No. frag.	Size range	No. frag.	Size range	No. frag.	Size range	No. frag.	Size range
2	220–240	2	220–240	2	220–240	2	220–240	2	220–240
3	225–475	2	220–375	9	120–450	3	150–300	6	120–450
1	220	1	220	1	230	1	230	1	220
3	166–250	2	166–250	2	166–175	2	166–175	2	166–250
6	160–250	7	155–300	5	190–290	6	190–250	8	150–250
1	135	1	135	1	162	1	162	1	135
8	150–290	8	160–300	12	140–300	10	180–400	10	180–400
2	220–250	3	210–250	1	240	1	240	4	150–240
2	223–225	1	225	3	150–155	3	150–155	3	223–227
2	170–175	1	180	1	170	1	170	1	170
2	155–162	1	162	1	162	1	162	2	155–162
2	225–238	2	238–242	1	238	1	245	3	225–238
0		0		1	204	0		0	
0		0		1	202	1	202	1	220
1	185	1	185	1	185	1	185	1	185
2	170–190	3	165–190	1	190	1	190	2	170–190
1	168	1	166	1	168	1	168	1	168
1	150	1	150	0		0		1	150
5	210–240	1	230	3	210–240	3	220–245	2	230–240
1	195	1	195	1	195	1	195	1	195
4	165–195	4	165–190	5	170–205	5	170–205	3	170–195

S. alata		*S. flava*		*S. jonesii*		*S. leucophylla*		*S. minor*	
No. frag.	**Size range**	**No. frag.**	**Size range**	**No. frag.**	**Size range**	**No. frag.**	**Size range**	**No. frag.**	**Size range**
1	233	2	160–233	1	233	1	233	1	233
2	340–345	3	300–400	3	340–400	3	345–395	2	340–345
1	255	2	225–260	2	220–255	2	220–255	3	250–300
0		1	151	0		0		0	

No. Frag. is the number of different sized amplified fragments observed among the individuals screened for a species. Sarr014 worked better with T_a at 50° in some plant extracts and 60° in others

Table 2

Results with *Sarracenia* microsatellites within three *S. purpurea* populations

Primer	L	Population 1				Population 2				Population 3			
		Na	Ho	He	H.W.	Na	Ho	He	H.W.	Na	Ho	He	H.W.
Sarr005	a	3	0.04	0.32	<0.001	2	0.67	0.44	<0.01	3	0.20	0.24	<0.001
Sarr005	b	2	0.92	0.50	<0.001	2	0.83	0.49	<0.001	3	0.37	0.47	<0.001
Sarr005	c	2	0.00	0.08	<0.001	1	0.00	0.00	mono.	2	0.97	0.50	<0.001
Sarr005	d	2	0.00	0.22	<0.001	1	0.00	0.00	mono.	2	0.00	0.06	<0.001
Sarr009	a	2	0.83	0.49	<0.001	1	0.00	0.00	mono.	1	0.00	0.00	mono.
Sarr009	b	3	0.79	0.54	<0.001	1	0.00	0.00	mono.	1	0.00	0.00	mono.
Sarr027	a	2	0.08	0.15	<0.05	2	0.00	0.32	<0.001	2	0.40	0.32	NS
Sarr027	b	1	0.00	0.00	mono.	1	0.00	0.00	mono.	2	0.00	0.06	<0.001
Sarr032	a	3	0.38	0.60	<0.001	2	0.00	0.50	<0.001	2	0.00	0.44	<0.001
Sarr032	b	2	0.00	0.08	<0.001	2	0.00	0.36	<0.001	2	0.00	0.18	<0.001
Sarr032	c	2	0.00	0.08	<0.001	2	0.00	0.18	<0.001	2	0.00	0.32	<0.001
Sarr032	d	1	0.00	0.00	mono.	2	0.00	0.39	<0.001	1	0.00	0.00	mono.
Sarr035	a	2	0.00	0.38	<0.001	1	0.00	0.00	mono.	2	0.50	0.47	NS
Sarr045	a	2	0.00	0.15	<0.001	2	0.00	0.36	<0.001	1	0.00	0.00	mono.
Sarr045	b	1	0.00	0.00	mono.	2	0.00	0.46	<0.001	1	0.00	0.00	mono.
Sarr053	a	1	0.00	0.00	mono.	1	0.00	0.00	mono.	2	0.63	0.49	NS
Sarr056	a	2	0.00	0.28	<0.001	2	0.00	0.28	<0.001	2	0.77	0.50	<0.01
Sarr056	b	1	0.00	0.00	mono.	1	0.00	0.00	mono.	2	0.87	0.50	<0.001
Sarr058	a	2	0.75	0.47	<0.01	2	0.50	0.38	NS	2	0.77	0.47	<0.001

L is locus, since most of the primer pairs had more than one band segregating. Na is the number of alleles. Ho and He are the observed and expected heterozygosity, H.W. is the significance level of a test for Hardy-Weinberg equilibrium