Genetics of Anthocyanin Deficiency in *Sarracenia* L.

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Abstract. *Sarracenia* L. is a genus of insectivorous plants confined to wetlands of the United States and Canada. Green mutants, lacking red pigmentation in the leaves, flowers, and growing point, have been found in most *Sarracenia* species. Controlled crosses were made using green mutants from *S. rubra* Walter ssp. *gulfensis* Schnell, *S. purpurea* L., *S. psittacina* Mich., and *S. leucophylla* Raf. Self-pollination of mutant green individuals in four different species resulted in green offspring, whereas reciprocal crosses with respective wild-types resulted in red offspring. Three of six self-pollinated heterozygous *S. rubra* ssp. *gulfensis* yielded offspring exhibiting a 3 red : 1 green ratio. Progeny from a testcross and two self-pollinated heterozygous plants of *S. purpurea* fit the expected ratios, whereas offspring from two *S. purpurea* crosses had significant deviations in field and laboratory sowing experiments. Offspring from testcrosses with *S. rubra* Walter ssp. *jonesii* (Wherry) Wherry met expected ratios under field conditions. Interspecific crosses between green individuals resulted in green offspring. These results suggest that anthocyanin pigmentation is controlled by two alleles at a single locus, with red dominant to green.

Sarracenia is a genus of insectivorous plant found in acid, moist savannas and seepage bogs of the southeastern United States and acid bogs and alkaline meadows of Canada and the northern United States. Leaves are modified into tubular or funnel-shaped structures that catch and digest insects by means of a pitcher or pitfall trap. Insects are thought to be attracted by color, nectar, and scent to the pitcher mouth where they lose their footing, fall, and drown in a pool of water in the depths of the pitcher. Bacterial and plant enzymes digest the insect, and the products are used for plant growth (Hepburn et al., 1927; Plummer and Jackson, 1963; Plummer and Kethley, 1964).

Eight species are recognized in the genus (Bell, 1949; McDaniel, 1966; McFarlane, 1908; Uphof, 1936) with flower color ranging from red to cream. Eaton (1822, 1833) described *S. heterophylla* Eaton as "a remarkably distinct species, but very rare," character-

ized by an overall yellow leaf and flower color. Wherry discussed the redesignation of *S. heterophylla* to a variety and finally to a form of *S. purpurea* ssp. *purpurea* Wherry (Wherry, 1933). Wherry (1933) also applied the term "anthocyanin-free mutation" to this entity and Bell (1949) recognized this mutation as a form. Schnell (1976) qualified *S. purpurea* ssp. *purpurea* f. *heterophylla* (Eaton) Fernald as completely lacking red pigment in any tissues. This same phenotype has been found in five different *Sarracenia* species at various locations over the past 50 years (Korolas, 1977; Robinson, 1981; Sheridan and Scholl, 1993b). These mutants occur singly or as a few individuals intermixed with normal wild-type plants in the southern United States (Sheridan and Scholl, 1993b, 1996) or more abundantly in northern bogs (Bell, 1949; Case, 1956; Griesbach, 1977; Sheridan and Scholl, 1993a). The anthocyanin-free phenotype has also been obtained in *S. purpurea* ssp. *venosa* Raf. through large-scale commercial propagation of wild seed, suggesting spontaneous mutation in wild populations (Bob Hanrahan, pers. comm.). Sheridan and Scholl (1996) proposed the term "green" for this mutation and "red" for the wild-type.

Sheridan (1994, 1996, 1997) also determined in preliminary studies that the green phenotype was recessive to the red, based on the phenotype of F_1 seedlings. The objective of this experiment was to determine the inheritance of the green phenotype and whether it was controlled by the same gene in all species.

Materials and Methods

Pollination, seed storage, seed stratification, and germination closely followed the methods of Sheridan (1997). Pollinations were performed in 1994 at Meadowview Biological

Research Station in Caroline County, Va., and in Chesterfield County, Va., during normal flowering periods, and in the Virginia Commonwealth Univ. (VCU) greenhouse. The 1989–91 crosses were performed in Chesterfield County using similar pollination methods.

In 1987, seed was obtained in Santa Rosa County, Fla., from a naturally pollinated mutant green *S. rubra* ssp. *gulfensis*. Seedlings were raised to maturity and wild-types used as presumed heterozygotes for self-pollinations. *Sarracenia rubra* ssp. *jonesii* seed was also collected in 1987 from a peat bog in Henderson County, N.C., and germinated and raised in Chesterfield County, Va. Wild-type seedlings raised to maturity were then used as putative heterozygotes. Wild-type plants of *Sarracenia purpurea* from a natural population containing the green mutation in Nova Scotia, Canada, were also used as putative heterozygotes.

Pots and trays with seed were placed under fluorescent lights in the VCU greenhouse after 1 month of stratification and kept moist. Seedling phenotype was scored from Jan. to July 1995. Progeny from testcrosses and self-pollinated heterozygotes were repotted and rescored after an adjustment period, during which new leaves developed, to ensure accuracy of classification.

A selection of 1995 crosses (1995-151I.1, 1995-151I.2, 1995-174A, 1995-174B, 1995-175) were used to determine whether natural selection under field conditions would alter expected ratios for testcrosses and self-pollinated heterozygotes. Seeds were sown in lots of 50 in a sunny, seepage ecotone at Vole Haven Research Tract, Caroline County, Va., on 8 Apr. 1996. Placement of seed was determined using a random number table. The soil surface was prepared by mechanically removing grass, sedges, and debris to expose the organic and mineral soil. Evening temperatures during April occasionally approached freezing and provided a stratification period of ≈ 1 month. Seedling phenotype was scored in Nov. 1997 to ensure complete germination and color development after two growing seasons.

Reds in one species were self-pollinated twice and greens in four species were self-pollinated 11 times. Greens were pollinated with reds three times and reds pollinated with greens five times. Green species were crossed with green species five times, an F_1 green interspecies cross was self-pollinated, and another F_1 interspecies cross was outcrossed with another green species. Two testcrosses each were performed on two species and six heterozygotes in one species, and three heterozygotes in another species were self-pollinated.

Results

Forty-two crosses yielded 14,671 seeds, with germination averaging 40% (Table 1). Number of seed per capsule ranged from 1 to 829 while capsule percent germination ranged from 3 to 84.

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Table 1. *Sarracenia* crosses made, seeds obtained, percent germination, and phenotype of seedlings.

Cross code	Parentage	Total no. seeds	Germination		Phenotype	
			No.	(%)	Red	Green
Greenhouse sown						
Red self-pollinated						
1994-149A	<i>S. psittacina</i>	165	9	5	9	0
1994-150A	<i>S. psittacina</i>	181	60	33	60	0
Green self-pollinated						
1989-122	<i>S. rubra</i> ssp. <i>gulfensis</i>	21	2	10	0	2
1990-123	<i>S. rubra</i> ssp. <i>gulfensis</i>	515	112	22	0	112
1990-124	<i>S. rubra</i> ssp. <i>gulfensis</i>	800	42	5	0	42
1991-118.1	<i>S. rubra</i> ssp. <i>gulfensis</i>	322	125	39	0	125
1991-118.2	<i>S. rubra</i> ssp. <i>gulfensis</i>	231	51	22	0	51
1991-118.3	<i>S. rubra</i> ssp. <i>gulfensis</i>	366	38	10	0	38
1994-145A	[<i>S. rubra</i> ssp. <i>jonesii</i> (green) x <i>S. psittacina</i> (green)]	69	2	3	0	2
1994-147A	<i>S. psittacina</i>	277	11	4	0	11
1994-148A	<i>S. psittacina</i>	175	17	10	0	17
1994-152	<i>S. purpurea</i> f. <i>heterophylla</i>	745	294	39	2	292
1994-156	<i>S. purpurea</i> f. <i>heterophylla</i> control	5	2	40	0	2
1994-160	<i>S. psittacina</i>	15	3	20	0	3
1994-163	<i>S. leucophylla</i>	699	200	29	0	200
Red x green						
1994-149B	<i>S. psittacina</i> x <i>S. psittacina</i>	307	20	7	20	0
1994-158A	<i>S. rubra</i> ssp. <i>gulfensis</i> x <i>S. rubra</i> ssp. <i>gulfensis</i>	579	231	40	231	0
1994-158B	<i>S. rubra</i> ssp. <i>gulfensis</i> x <i>S. rubra</i> ssp. <i>gulfensis</i>	503	311	62	311	0
1994-159A	<i>S. leucophylla</i> x <i>S. leucophylla</i>	829	622	75	622	0
1994-159B	<i>S. leucophylla</i> x <i>S. leucophylla</i>	562	344	61	344	0
Green x red						
1994-148B	<i>S. psittacina</i> x <i>S. psittacina</i>	297	37	13	37	0
1994-157A	<i>S. rubra</i> ssp. <i>gulfensis</i> x <i>S. rubra</i> ssp. <i>gulfensis</i>	305	234	77	234	0
1994-157B	<i>S. rubra</i> ssp. <i>gulfensis</i> x <i>S. rubra</i> ssp. <i>gulfensis</i>	291	212	73	212	0
Green x green						
1994-161	<i>S. psittacina</i> x <i>S. leucophylla</i>	4	1	25	0	1
1994-162	<i>S. rubra</i> ssp. <i>jonesii</i> x <i>S. leucophylla</i>	170	63	37	0	63
1994-164A	<i>S. rubra</i> ssp. <i>gulfensis</i> x <i>S. leucophylla</i>	442	331	75	0	331
1994-164B	<i>S. rubra</i> ssp. <i>gulfensis</i> x <i>S. leucophylla</i>	508	428	84	1	427
1994-165	[<i>S. purpurea</i> f. <i>heterophylla</i> x <i>S. psittacina</i>] x <i>S. leucophylla</i>	18	3	17	0	3
1994-166	<i>S. purpurea</i> f. <i>heterophylla</i> x <i>S. leucophylla</i>	174	8	5	0	8
Self-pollinated heterozygotes						
1994-143B	<i>S. rubra</i> ssp. <i>gulfensis</i> (F ₁)	186	65	35	41	24
1994-143C	<i>S. rubra</i> ssp. <i>gulfensis</i> (F ₁)	340	210	62	144	66
1994-143D	<i>S. rubra</i> ssp. <i>gulfensis</i> (F ₁)	449	256	57	178	78
1994-143E	<i>S. rubra</i> ssp. <i>gulfensis</i> (F ₁)	249	124	50	89	35
1994-143F	<i>S. rubra</i> ssp. <i>gulfensis</i> (F ₁)	274	145	53	113	32
1994-143G	<i>S. rubra</i> ssp. <i>gulfensis</i> (F ₁)	512	180	35	129	51
Testcross						
1994-153	<i>S. purpurea</i> f. <i>heterophylla</i> green x <i>S. purpurea</i> (F ₁) red	829	497	60	248	249
1994-154	<i>S. purpurea</i> (F ₁) red x <i>S. purpurea</i> f. <i>heterophylla</i> green	715	411	58	176	235
Field sown						
Testcross						
1995-151I.1	<i>S. rubra</i> ssp. <i>jonesii</i> (F ₁) x <i>S. rubra</i> ssp. <i>jonesii</i> (green)	223	32	14	18	14
1995-151I.2	<i>S. rubra</i> ssp. <i>jonesii</i> (F ₁) x <i>S. rubra</i> ssp. <i>jonesii</i> (green)	195	33	17	15	18
Self-pollinated heterozygotes						
1995-174A	<i>S. purpurea</i> (F ₁)	210	22	11	15	7
1995-174B	<i>S. purpurea</i> (F ₁)	486	54	11	33	21
1995-175	<i>S. purpurea</i> (F ₁)	428	33	8	26	7
Total		14671	5875	40	3308	2567

Self-pollination of green plants and green interspecies crosses resulted in green offspring with the exception of crosses 1994-152 and 1994-164B, where <1% of the progeny were red. Self-pollination of red plants resulted in red offspring, and reciprocal crosses of red and green plants resulted in red offspring.

Self-pollination of heterozygotes and testcrosses resulted in red and green offspring. A Chi-Square goodness of fit test of the testcrosses fit a 1:1 ratio for red and green in *S. purpurea* and *S. rubra* ssp. *jonesii* under field and laboratory conditions, with the exception of cross 1995-154 (Table 2). Progeny from three of the six self-pollinated heterozygous *S. rubra* ssp. *gulfensis* (1994-143 series) fit a 3:1 ratio and offspring from two of the three self-pollinated heterozygous *S. purpurea* fit the expected 3:1 ratio in the field experiment (Table 2).

Discussion

Inheritance of the pigmentation previously reported by Sheridan (1994, 1997) was confirmed in reciprocal crosses between red and green plants in *Sarracenia leucophylla*, *S. psittacina* and *S. rubra* ssp. *gulfensis*. No green or intermediate-colored offspring were observed in F_1 plants.

Self-pollinated red and green plants were true breeding with the exception of the self-pollinated Nova Scotia *S. purpurea* f. *heterophylla* (1994-152) where two red seedlings were obtained. The interspecific cross 1994-164B between *S. rubra* ssp. *gulfensis* (green) x *S. leucophylla* (green) also resulted in a red seedling. The most likely causes for these aberrant red seedlings could have been pollen or seed contamination or reversion of the green allele (Sheridan, 1997). A relatively high spontaneous mutation rate of 1 out of 20,000 seeds has been observed (Bob Hanrahan, pers. comm.), suggesting that this locus may be unstable. This possibility merits further investigation.

In all of the species tested the green phenotype was inherited as a single recessive gene. When green *S. leucophylla* was crossed with green individuals of the other species, all offspring were green, signifying that the lesion was in the same gene.

Some self-pollinated heterozygous *S. rubra* ssp. *gulfensis* progeny did not fit the expected ratio of three reds to one green as predicted. In crosses 1994-143B, 1994-143C, and 1994-143D, the expected ratio was not observed while in crosses 1994-143E, 1994-143F, and 1994-143G, the expected ratio was observed.

Progeny from the testcross of *S. purpurea* 1994-154 also failed to fit a 1 red : 1 green ratio because of a second wave of germinations. Normally, we disposed of seed bearing soil after recording phenotype and repotting, but tray 1994-154 was retained because a few seedlings were in poor health and could not be scored for color. Plants from a second wave of germinations were largely greens resulting in rejection of a 1:1 color ratio.

Fortunately, field experiments were also conducted over a 2-year period with *S.*

Table 2. Testcross and self-pollinated heterozygote progeny segregation in *Sarracenia*.

Cross code	Parentage	Phenotype		Expected ratio ^a	χ^2
		Red	Green		
Greenhouse sown					
Self-pollinated heterozygotes					
1994-143B	<i>S. rubra</i> ssp. <i>gulfensis</i> (F ₁)	41	24	3:1	5.3 ^o
1994-143C	<i>S. rubra</i> ssp. <i>gulfensis</i> (F ₁)	144	66	3:1	5.0 ^o
1994-143D	<i>S. rubra</i> ssp. <i>gulfensis</i> (F ₁)	178	78	3:1	4.1 ^o
1994-143E	<i>S. rubra</i> ssp. <i>gulfensis</i> (F ₁)	89	35	3:1	0.7 ^{ns}
1994-143F	<i>S. rubra</i> ssp. <i>gulfensis</i> (F ₁)	113	32	3:1	0.6 ^{ns}
1994-143G	<i>S. rubra</i> ssp. <i>gulfensis</i> (F ₁)	129	51	3:1	1.1 ^{ns}
Testcross					
1994-153	<i>S. purpurea</i> f. <i>heterophylla</i> (green) x <i>S. purpurea</i> (F ₁)	248	249	1:1	0.0 ^{ns}
1994-154	<i>S. purpurea</i> (F ₁) x <i>S. purpurea</i> f. <i>heterophylla</i> (green)	176	235	1:1	8.5 ^o
Field sown					
Testcross					
1995-1511.1	<i>S. rubra</i> ssp. <i>jonesii</i> (F ₁) x <i>S. rubra</i> ssp. <i>jonesii</i> (green)	18	14	1:1	0.5 ^{ns}
1995-1511.2	<i>S. rubra</i> ssp. <i>jonesii</i> (F ₁) x <i>S. rubra</i> ssp. <i>jonesii</i> (green)	15	18	1:1	0.3 ^{ns}
Self-pollinated heterozygotes					
1995-174A	<i>S. purpurea</i> (F ₁)	15	7	3:1	0.6 ^{ns}
1995-174B	<i>S. purpurea</i> (F ₁)	33	21	3:1	5.5 ^o
1995-175	<i>S. purpurea</i> (F ₁)	26	7	3:1	0.3 ^{ns}

^aExpected ratio based on a single gene model.

^{ns}: Nonsignificant or significant at $P < 0.05$.

purpurea, which we think address any problems with delayed germination. Self-pollination of heterozygous *S. purpurea* (1995-174 and 1995-175) from the same location as 1994-154 resulted in progeny from two of the three crosses fitting expected ratios. Offspring from *S. purpurea* cross 1994-174B failed to fit expected ratios. Field experiments with testcross progeny of *S. rubra* ssp. *jonesii* fit expected ratios.

Sheridan (1997) discussed the potential for heterozygous individuals occurring in the wild. At the time of writing no heterozygotes had actually been found, although their existence was suspected. This presumption was confirmed by red and green offspring from heterozygous parents in testcrosses 1994-153, 1994-154, and in self-pollination of 1995-174A, 1995-174B, and 1995-175. The plants involved in these crosses were obtained from a large population of wild-types and green mutants growing together in Nova Scotia. Green mutants and wild-types originally were crossed to test dominance of wild-type in the F_1 generation. Observation of greens in the progeny and a subsequent Chi-Square test led to the conclusion that these wild-type parents were heterozygous. Thus heterozygous individuals were present in at least one population, which is not surprising considering the large number of mutants found at this site. The green phenotype, however, is rarely seen in southern pitcher plant populations, although it can be quite abundant in the northern bogs of *S. purpurea* (Bell, 1949). Very few green *S. leucophylla* plants have been found in two adjoining parcels in Baldwin County, Ala. (Sheridan and Scholl, 1996), and several green *S. psittacina* were found among abundant wild-types over a 30-km stretch of highway in Gulf County, Fla. Green *S. rubra* ssp. *jonesii* formerly occurred in the North Carolina Blue Ridge, but was extirpated, possibly by collec-

tors (Ron Determan, pers. comm.). The paucity of greens in the southern range of the genus may indicate that greens are spontaneously occurring and only recently established, with no heterozygotes present or a low green allele frequency. Another possibility is a selection pressure against the green phenotype and/or some advantage possessed by heterozygous or homozygous reds. The extirpation of the green phenotype of *S. rubra* ssp. *jonesii* from its bog may offer the opportunity to test various hypotheses under field conditions at that site.

Color, nectar, and scent are the presumed means by which *Sarracenia* attract prey for capture (Juniper et al., 1989). The discovery of green mutants lacking reddish-purple color and elucidation of the inheritance of this trait may now allow the role of color in attracting prey to be more rigorously investigated. If reddish-purple color significantly increases the capture of prey, and captured prey increase fitness, then red phenotypes should have a selective advantage over greens. The presence of green mutants in wild-type populations raises questions as to the role of color in the fitness of *Sarracenia*, and indicates that other factors may play a role in competition (if any) between these two morphs.

Literature Cited

- Bell, C.R. 1949. A cytotoxic study of the *Sarraceniaceae* of North America. J. Elisha Mitchell Sci. Soc. 65:137-166.
- Case, F. 1956. Some Michigan records for *Sarracenia purpurea* forma *heterophylla*. Rhodora 58:203-207.
- Eaton, A. 1822. Manual of botany for the northern and middle states of America. 3rd ed. Webster and Skinners, Albany, N.Y.
- Eaton, A. 1833. Manual of botany for North America. 6th ed. Oliver Steele, Albany, N.Y.
- Griesbach, R. 1977. News and views. Carnivorous Plant Nwsl. 6(3):47.

- Hepburn, J.S., F.M. Jones, and E.Q. St. John. 1927. The biochemistry of the American pitcher plants: Biochemical studies of the North American Sarraceniaceae. Trans. Wagner Free Inst. of Sci. Philadelphia 11:1-95.
- Juniper, B.E., R.J. Robins, and D.M. Joel. 1989. The carnivorous plants. Academic, New York.
- Korolas, J. 1977. News and views. Carnivorous Plant Nwsl. 6(2):33.
- McDaniel, S.T. 1966. A taxonomic revision of *Sarracenia* (Sarraceniaceae). PhD Diss., Florida State Univ.
- McFarlane, J.M. 1908. Sarraceniaceae, p. 1-39. In: A. Engler (ed.). Das Pflanzenreich 3Y Heft (IV. 110). Engelmann, Leipzig, Germany.
- Plummer, G.L. and T.H. Jackson. 1963. Bacterial activities within the sarcophagus of the insectivorous plant *Sarracenia flava*. Amer. Midland Naturalist 69:462-469.
- Plummer, G.L. and J.B. Kethley. 1964. Foliar absorption of amino-acids, peptides and other nutrients by the pitcher-plant *Sarracenia flava*. Bot. Gaz. 125:245-260.
- Robinson, J.T. 1981. *Sarracenia purpurea* L. forma *heterophylla* (Eaton) Fernald: New to Connecticut. Rhodora 83:156-157.
- Schnell, D. 1976. Carnivorous plants of the United States and Canada. John F. Blair, Winston-Salem, N.C.
- Sheridan, P. 1994. Genetics of aberrant *Sarracenia* leaf and flower color. Virginia J. Sci. 45(2):71.
- Sheridan, P. 1996. Biochemistry and genetics of *Sarracenia* anthocyanins and flavonoids. MS Thesis, Virginia Commonwealth Univ.
- Sheridan, P. 1997. Genetics of *Sarracenia* leaf and flower color. Carnivorous Plant Nwsl. 26(2):51-64.
- Sheridan, P. and B. Scholl. 1993a. *Sarracenia purpurea* ssp. *purpurea* f. *heterophylla* (Eaton) Fernald in Nova Scotia. Carnivorous Plant Nwsl. 22(4):106-107.
- Sheridan, P. and B. Scholl. 1993b. Noteworthy *Sarracenia* collections. Carnivorous Plant Nwsl. 22(3):58-61.
- Sheridan, P. and B. Scholl. 1996. Noteworthy *Sarracenia* collections II. Carnivorous Plant Nwsl. 25(1):19-23.
- Uphof, J.C.T. 1936. Sarraceniaceae, p. 1-24. In: A. Engler and H. Harms (eds.). Die naturlichen Pflanzenfamilien, 2nd ed. 17b. Engelmann, Leipzig, Germany.
- Wherry, E. 1933. The geographic relations of *Sarracenia purpurea*. Bartonia 15:1-8.