Genetics of *Sarracenia* leaf and flower color

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Keywords: genetics: pigmentation-genetics: *Sarracenia.*

Abstract

Sarracenia is a genus of insectivorous plants confined to wetlands of eastern U.S. and Canada. Eight species are generally recognized with flower and leaf color ranging from yellow to red. Fertile hybrids occur in the wild under disturbed conditions and can be artificially produced in the greenhouse. Thus genetic barriers between species are weak. Normally when crosses occur or are induced between species or between different color types the progeny exhibit a blending of parental phenotypes called incomplete or partial dominance. In most species all-green mutants have been found which lack any red pigment in leaves, flowers or growth point. Controlled crosses were performed on all-green mutants from S. *purpurea* and two subspecies of the S. *rubra* complex. Self pollinated all-green plants result in all-green offspring and self pollinated wild-type red plants result in red offspring. Crosses between red and all-green plants produce wild-type colored red progeny. These results suggest that the red alleles are "dominant" to the "recessive" all green mutant alleles in the three independent all-green variants tested. Since partial dominance is the usual genetic pattern in the genus, dominant/recessive characteristics are an unusual phenomenon.

Figure 1: A pink flowered hybrid in cultivation. This specimen was collected by Fred Case and is the cross S. *rubra* subsp. *wherry) x S. alata.*

Introduction

The Sarraceniaceae (American pitcher plants) is a family of insectivorous pitcher plants restricted to wet, sunny, generally acid, nutrient poor habitats of the southeastern United States, Canada, northern California, southern Oregon, Venezuela, British Guiana (Lloyd, 1942), and Brazil (Maguire, 1978). The family contains a total of three genera: *Darlingtonia, Heliamphora* and *Sarracenia. Darlingtonia is* found in coastal swamps, moist mountain meadows and serpentine creeks of northern California and southern Oregon. *Heliamphora* occurs in savannas and peat bogs of the sandstone table mountains of Venezuela, Brazil, and British Guiana. *Sarracenia is* restricted to acid, moist savannas and seepage bogs of the southeastern United States and acid bogs and alkaline meadows of Canada and the northern U.S.

American pitcher plants are herbaceous, rhizomatous plants which have leaves and stems modified into tubular or funnel shaped structures. These modified leaves catch and digest insects by means of a pitcher or pitfall trap. Presumably insects are attracted by color, scent and nectar to the pitcher mouth although experiments testing this hypothesis need to be done. Insects then lose their footing and fall into a pool of water in the pitcher. Escape is prevented by smooth waxy walls, downward pointing hairs and a stupefying or narcotic agent in the pitcher liquor (Hepburn *et al.,* 1927; Mody *et al.,* 1976). Bacterial and plant enzymes then digest the insect and the by-products are used by the plant for growth (Hepburn *et al.,* 1927; Plummer & Jackson, 1963; Plummer & Kethley, 1964). It is believed that the trapping of insects evolved in order to compensate for the lack of nutrients in pitcher plant habitats (Romeo *et al.,* 1977).

The evolution of the three genera is poorly understood due to the lack of any fossils. Albert *et al.* (1992) suggest an evolutionary relationship among the three genera based on similarities in the plastic *rubisco L* gene.

Botanical treatments (McFarlane, 1908; Uphof, 1936; Bell, 1949; McDaniel, 1966) of the genus *Sarracenia* have led to a general acceptance of eight species: *Sarracenia alata, S. flava, S. Ieucophylla, S. minor, S. oreophila, S. psittacina, S. purpurea,* and S. *rubra. Sarracenia purpurea* has been split into the two subspecies *venosa* and *purpurea. Sarracenia purpurea* subsp. *venosa* contains a recently described variety named *burkii* (Schnell, 1993) while S. *purpurea* subsp. *purpurea* has a form lacking purple or red pigment called forma *heterophylla.* Some taxonomists advocate splitting S. *rubra* into as many as three species with two subspecies (Case & Case, 1974, 1976), five species (McDaniel, 1986), one species with five subspecies (Schnell, 1977, 1979b) or just one species (Bell, 1949). The taxonomy of Schnell (1977, 1979b) will be followed in this paper.

Known species flower colors are red, pink, yellow and cream (Table 1). *Sarracenia alata, S. flava, S. minor* and S. *oreophila* have yellow flowers with S. *alata* variants producing cream flowers. S. *Ieucophylla, S. psittacina, S. purpurea* and S. *rubra* have red flowers with variants in all four species producing yellow flowers. S. *purpurea* subsp. *venosa* var. *burkii* has pink to cream flowers. Leaf shapes range from upright to decumbent. Upright species are S. *alata,*

S. flava, S. Ieucophylla, S. minor, S. oreophila and S. *rubra*. Decumbent species are *S. psittacina* and S. *purpurea.*

Table 1: Species in the genus *Sarracenia* are normally either red, pink or yellow to cream-yellow flowered. Flowers of normally red flowered species can be yellow, pink flowered species can be cream, and yellow and cream-yellow flowered species can be cream.

Taxon	Normal Flower Color	Variant Flower Color
S. Ieucophylla	Red	Yellow
S. psittacina	Red	Yellow
S. purpurea subsp. *purpurea*	Red	Yellow
S. rubra complex	Red	Yellow
S. pupurea subsp. *venosa* var. *burkii*	Pink	Cream
S. alata	Yellow/Cream	Cream
S. flava	Yellow/Cream	Cream
S. minor	Yellow/Cream	Cream
S. oreophila	Yellow/Cream	Cream

Wild-type *Sarracenia* plants contain some purple or red pigment in either the growth point, leaves, flowers or a combination of the three. Normally, species leaf color can be either red, yellow, purple, red striped and splotched. Striped or splotched individuals possess a yellow background with varying intensities of pigmentation. Yellow leaved individuals maintain pigment in the growth point which is brilliant reddish-purple. Leaf and flower color variation have been extensively discussed in the literature (Masters, 1881; McFarlane, 1908; Bell, 1949; Case, 1956; McDaniel, 1966; Schnell, 1978b, 1979a, 1993).

Flower color, leaf color, leaf shape and leaf number are both genetically and environmentally controlled (Bell, 1949; Mandossian, 1966a; Schnell, 1978b). As an example I have observed that red-flowered species growing in shaded habitats will produce flowers that are still red but not as *intense* as those growing in full sun. Yellow-flowered species maintain yellow in the shade but the color may not be as vibrant. Low light levels may result in reduction of pitchers to flattened leaves. Soil pH can effect the number and size of leaves but has no effect on color. The effect of environment is most pronounced in pigment production in the leaves. Individuals with a genetic predisposition to be red show these characteristics best in full sun. Root disturbance or shading can result in reduction in quantity and distribution of red pigment.

Offspring of crosses between *Sarracenia* species or varieties normally exhibit blending of the parental characteristics (Russell, 1919) called incomplete or partial dominance. As an example, crosses between red- and cream-flowered species typically produce hybrids with pink flowers (Figure 1). Species can be easily crossed and the resulting hybrids can be back-crossed with the parents without deleterious effect on offspring fertility. Natural hybrids are known between almost all species in the genus (Bell, 1952; Bell & Case, 1956). Hecht (1949) reported a

reduced chromosome number of n=12 for all species in the genus while Bell (1949) identified one more chromosome and arrived at n=13 which is now the accepted figure.

The species distinction in plants allows for hybrids to occasionally occur between taxonomically recognized species (Jones & Luchsinger, 1986). In *Sarracenia*, barriers between species interbreeding are not dictated by a difference in chromosome numbers. Rather, species integrity is maintained by a combination of different flowering times, flower color and habitat preferences (Bell, 1949).

Two recurring unusual variant forms are found, however, in the genus *Sarracenia.* The first involves the total lack of red or purple pigment in leaves, flowers and growth point (hereafter called "green") and the second is normally red-flowered species that are yellow-flowered. The green variant of S. *purpurea* subsp. *purpurea* was recognized as early as 1822 by Eaton (Eaton, 1822, 1833) and is known as f. *heterophylla.* Both variants have been found in a number of species at a variety of locations over the past fifty years (Robinson, 1981; Sheridan & Scholl, 1993a, 1993b; Shomin, 1993). Green or yellow-flowered variants occur singly or as a few individuals intermixed with normal wild-type plants in the field (Case, 1956; Sheridan & Scholl, 1993a, 1993b).

Scholl (1994) and Baumgartl (1993) report individuals of *Heliamphora* which lack purple or red pigment in the leaves and are analogous to the green variant. Unfortunately research can not be pursued in this genus at this time due to insufficient plant material and breeding problems. Yellow-flowered variants in *Sarracenia* are also limited in number and require several years to reach maturity and for these reasons this study focused specifically on the genetics of red and green leaf color in *Sarracenia.*

In preliminary, casual work with *Sarracenia,* Bill Scholl and I (1993b) observed that when red or green plants were self-pollinated the offspring were true-breeding red or green. When red and green plants were reciprocally crossed the offspring from each parent appeared to be a mix of red and green plants. The purpose of this study was to perform controlled matings between green plants of *S. purpurea, S. rubra* subsp. *gulfensis* and S. *rubra* subsp. *jonesii* and red plants of the same or similar subspecies in order to determine the validity of our casual observations and to begin elucidation of the genetic interactions between wildtype red and green alleles.

Materials and Methods

Plants of *Sarracenia* were brought from winter storage in Caroline County, Virginia and placed in the Virginia Commonwealth University (VCU) greenhouse from 17 March-24 March (1993). Plants in need of repotting or those collected bare root from a research bog were planted in a 50/50 mixture of Canadian peat moss and sandy soil collected on the Reedy Creek drainage in Caroline County. Potted plants were then assigned a clone number and placed in a 2 mil (0.05 mm) thick plastic lined bed measuring 90 x 240 cm (3 x 8 ft) with a southern exposure in the

Additional plant specimens remained in the research bog and were allowed to grow under natural conditions and flowering times. Their treatment of flower covering and pollination was the same as plants maintained in the greenhouse as discussed below.

As flowers emerged they were covered securely with tobacco netting before flower maturity and pollen deposition to prevent possible insect pollination. In most cases there were extra flowers on a clone involved in a cross which served as controls. Most of these flowers were covered in a similar manner but a few were left uncovered to see if any pollinator activity might occur in the greenhouse.

As soon as pollen was observed in the flower, self and cross pollinations were performed. Pollinations were done by first removing netting from the flower and then dipping a clean toothpick in vegetable oil. Pollen was scraped from the umbrella with the oiled toothpick and placed on all stigma tips of the pollinated flower. After each pollination the toothpick was discarded and a clean toothpick obtained for additional crosses. Toothpicks were only dipped once in vegetable oil and at no time were redipped after contacting pollen. After a cross was done a plastic label with an alphanumeric identifier was attached to the flower designating the cross.

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The number of seeds per capsule was then determined by first separating and discarding capsular debris. Seeds were counted and then returned to the refrigerator in film vials.

After all seeds had been counted and cleaned, ten seeds each were placed in ten labeled 5.5 coin sub. 24 Universal Brown Kraft envelopes for a total of one hundred seeds for each capsule. Seeds from capsules of the same type of cross were not mixed. In this manner every capsule from a cross was allotted a test of one hundred seeds. In cases where a capsule produced less than one hundred seeds all seeds were sown. Two inch square plastic pots were filled to within 1.3 cm (0.5 in) of the top with a premoistened 50/50 sand and peat mixture and placed in plastic lined trays measuring 46 x 2 x 85 cm (18 x 11 x 2 in). Seeds were then sown in pots by tapping the contents of a single labeled envelope into a single pot and placing identifying labels in each pot. Trays were placed in a 4°C refrigerator and the seeds were allowed to stratify (a period of cool, moist conditions is required for germination). Two repetitions were done in this manner.

The first was in stratification from 27 November 1993—24 January 1994 and the second from 28 December 1993 4 February 1994 for a total stratification period of 59 and 39 days respectively.

At the end of the stratification period seed trays were brought into the greenhouse and placed under continuous illumination by 1.3 m (4 ft) cool white fluorescent lamps. Trays were placed on a metal stand with four trays per bank of five fluorescent lights. The total assembly contained four vertical sets of trays with fluorescent lights placed 13 cm (5 in) from the soil surface.

Pots were kept at constant moisture levels by maintaining 2.5 cm (1 in) of deionized water in the trays. High and low air temperature in the growth area was monitored on a daily basis with a minimum/maximum thermometer.

Initial germination of seedlings was noted and development observed. On 22 March 1994 the numbers of red and green seedlings per pot were noted and the percent germinations determined by dividing the numbers of seedlings by total numbers of seeds sown.

The above procedure was followed for the third replicate and for selected repeated crosses performed in 1994 with the following exceptions.

1. Netting over flowers was not removed from 1994 crosses until harvest time in mid-August.

2. All seeds of a cross were dusted with the fungicide Captan and were sown at the same time in fiberglass "Permanest" trays measuring 22 x 30 cm (8.5 x 12 in). Soil moisture was maintained by removing a row of soil on the edge of the tray and irrigating via this "drainage ditch".

3. 1994 crosses were harvested on 13 August 1994, stratified starting 8 November 1994 (2 November 1994 for replicate 3) and placed under lights 23 December 1994. Assessment of seedling phenotype and repotting of seedlings was done from 26 June-2 July 1995.

Results

10,202 seeds were produced as a result of twenty-two crosses with germination averaging 23% between the three replicates in 1993 crosses (Table 2a). 4042 seeds were produced in four crosses in the 1994 repetition of selected crosses with germination averaging 35% (Table 2b). One viable seed was produced by a control flower in 1994 (#156) while no seeds were produced by other controls. No germination occurred with crosses involving plants #71 and #116. Seed was produced by plant #32B and #35A even though they were not intentionally pollinated.

Reds in two species were self-pollinated once with one repetition and greens in one species and two subspecies were self-pollinated six times with possibly two additional unintentional

self-pollinations and one repetition. Greens were pollinated by reds six times and reds pollinated with greens four times (one repetition of each). Self-pollinations of green plants resulted in green seedlings with one exception in 1994 (cross #114A) where one red seedling was produced. Self-pollinations of red plants resulted in red seedlings with one exception in the third replicate in 1993 where three greens were produced (cross #113A). Reciprocal crosses of red and green plants resulted in red seedlings (Table 3). Greenhouse temperatures averaged 30°C with highs reaching 51°C and lows to 15°C.

Discussion

Overall germination rate and the difference in percent germination between replicates and the repetition may have several explanations. Mandossian (1966b) found optimal germination of S. *purpurea* occurred at 28°C but inhibition and/or death of seed/seedling occurred at 33°C. Replicate 1 seeds were placed on the lowest tiers of the light racks while replicate 2 was on the top tiers. It is possible that heat from the fluorescent light ballasts along with warm days in the greenhouse may have elevated temperatures in the upper tiers to lethal levels. Greenhouse highs of 51°C were recorded and this would have been lethal if sustained. These high temperatures, however, were not sustained or the normal environment. Other factors to consider are stratification and water mold. Mandossian (1966b) got germination rates averaging 59% in constant light at 28°C between one, two and three month prechills. Thus stratification time in this experiment was probably not a factor in germination rate. Rather the most likely culprit was an outbreak of water mold on the seeds as they were in stratification. I have since gone to one month prechills with dusting the seeds in Captan and this seems to control the deleterious effects of water mold on seeds. In addition, initial drying of seeds in Caroline County in film vials resulted in fungus attacks on the drying seeds.

The lack of germination in #71 and #116 was likely due to two reasons. Plant #71 was attacked by a fungal infection soon after pollination and most of the plant was destroyed in the attack and the flower stalk withered. Seeds were not fully developed. Plant #116 was in the research bog and the seedpod was attacked at maturity by a seed eating larva. Apparently seeds that appeared undamaged may have been incapacitated by the larva in some manner.

It is interesting to contrast my preliminary work (Sheridan & Scholl, 1993b) in which reciprocal crosses produced both red and green offspring and the results of this study where only one phenotype (red) was produced by this cross. This difference may be explained by the manner in which the preliminary work was done. Although flowers were covered to prevent accidental pollination, the assistant employed in performing the pollinations may have contaminated the crosses by tainting the oil with pollen or any one of the previously mentioned scenarios could have occurred. In any case the carefully controlled crosses of this experiment did not fully support my earlier observations of mixed red and green progeny in reciprocal crosses with this genus.

Various growers have postulated that green forms in S. *purpurea* arise by a gradual progression from red forms to lighter colored intermediates to the green form indicating polygenic inheritance or codominance. In polygenic inheritance an additive effect is seen on the phenotype through the cumulative effect of a number of genes (Klug & Cummings, 1991). In S. *flava* leaf color variants range from red to yellow with various intermediate color variants. This range of color in S. *flava* could indicate an additive effect of color genes in this species. Unfortunately no formal experiments have been conducted in any *Sarracenia* species to actually test whether partial dominance or polygenic inheritance is actually occurring although it is indicated by field observations.

The results of this experiment, however, do not support polygenic inheritance or partial dominance (blending) between green and red *Sarracenia* varieties. Rather a dominant/recessive genetic basis is indicated. Red is dominant to a recessive green since only red was seen in reciprocal crosses and red self-pollinations, not an intermediate color. Green was only seen in self pollination of green plants (exceptions are discussed below). When only one of two parental phenotypes is observed in crosses, the trait is said to be dominant to the washed or hidden trait, which is said to be recessive. Since all reciprocal crosses of red and greens were red, I conclude that the red allele is dominant to the green allele. Thus dominant/recessive characteristics represent an unusual genetic behavior in the genus *Sarracenia*.

Different crosses have been made by *Sarracenia* growers between greens in different species and in every case the result has been green offspring. One interpretation of these results is that the occurrence of greens in different species is caused by mutations effecting the same gene. If crosses between greens in different species had resulted in reds then different genetic events might be suspected to account for the occurrence of green plants in different species. Since greens are produced, mutation of the same gene is probably sporadically occurring throughout the genus.

The production of viable seeds by plants #32B, #35A and #156 may support Mandossian's assertion (1965) that a certain amount of self-pollination may occur in *Sarracenia.* It is also possible that the retrieval of pollen from flowers #32B and #35A for other crosses may have inadvertently resulted in some self pollination even though the stigmas were not touched. Plant #156 was in the research bog in Caroline County and was not handled at all after covering. I suggest that the limited amount of self pollination observed by Mandossian and myself is due to a small amount of airborne pollen landing on stigma tips.

The occurrence of a few green seedlings in the selfing of a red (third replicate in cross #113A) and a red seedling in a selfed green (cross #114A in 1994 repetition) may have several explanations. These two plants were growing intertwined at the same research bog in Caroline County, Virginia as control #156. There are several possible explanations for these outcomes.

1. Pollen was blown from one plant and landed on the stigma tips of another managing to circumvent the netting in both cases. This would explain the occurrence of a red in a green but not a green in a red self-pollination under a dominant red/recessive green situation.

2. Green reverted (mutated) back to red and a red mutated to a green.

3. Seeds jumped inadvertently during watering or other handling. *Sarracenia* seeds are very hydrophobic and extreme care must be exercised in the watering process to avoid seeds jumping during contact with falling water droplets and subsequent contamination of other pots.

4. Although I like to say my bags are insect-proof I have observed that small ants are able to penetrate small gaps at the tied stalks in bagged flowers and harvest nectar from the base of the ovaries in outside pollinations. Although these are by no means the main pollinator it is possible that they could transfer a small amount of pollen to a stigma tip in their foragings.

5. A combination of all of the above could occur.

To reduce or eliminate the possibility of accidental selling, crossing or seed contamination during stratification or germination I would make the following suggestions to other workers.

1. An effective and efficient system of flower emasculation needs to be developed to perform large scale crosses. Mandossian (1965) commented on how time consuming and damaging to the flowers this process can be. I experimented with this process on a flower prior to petal descent but pollen grains were still being dislodged and I considered the process potentially more contaminating than a careful cross.

2. Perform crosses between physically different species. This would tend to eliminate an obvious self pollination but we should keep in mind that the genetics of this group is being sorted out and a cross potentially may look like one of the parents.

3. Immediately after sowing trays should be covered with a transparent protective covering. I am now using glass plates or plastic domes to cover a particular tray. Covers should remain on through stratification and initial germination until repotting of seedlings. Obviously, the time of year has to be considered in this to avoid cooking the seedlings and lids can be vented to reduce heat buildup.

This experiment demonstrated that the green trait can be transmitted through the seeds in self pollination of green plants but does not answer how it arises in the wild. Conservation work at the Atlanta Botanical Garden by Ron Determan has helped to shed some light on how green forms are occurring in the wild. Two green seedlings of S. *rubra* were isolated out of four capsules collected along a creek in western Georgia. The remainder of the seedlings were all wildtype. All seed came from a local, vigorous colony of wild type plants where only S. *rubra* now grows.

In addition, Bob Hanrahan (1994) reports that he has isolated green seedlings of S. *purpurea* subsp. *venosa* from wild seed on several different occasions.

At least two scenarios could explain how Mssrs. Determan and Hanrahan obtained green plants from populations of wild-type reds. Either a premitotic mutation occurred or there was a preexisting mutation present in the population. A premitotic mutation from red to green could result in a flower bud with varying degrees of heterozygosity in the ovules. The seed yield of a plant of S. *rubra* used in this experiment (#23B) from the same region of Georgia that Ron Determan obtained his seed was 154 seeds. Since this experiment yielded a 23% germination rate, a capsule of 154 seeds would germinate 35 seeds (154 seeds x 23%=35 seeds). The proportion of recessive greens expected from self pollinating a heterozygous individual would then be nine seeds (35 seeds x 25%=9 seeds). Given that some outcrossing will probably take place in the field a yield of two green seedlings from a heterozygous plant is within the realm of possibility. If the plant was only 1/4 heterozygous due to a premitotic mutation two green seedlings is entirely realistic.

The second possibility is that a preexisting green individual was in the area at one time or that heterozygotes are present. Sheridan & Scholl (1993b) and Troup & McDaniel (1980) have extensively explored the area prior to Determan's work and no green plants were observed. Also, pitcher plant pollen is carried locally (within one mile) by *Bombus* species (Schnell, 1983) which would preclude long distance pollination by a distant green form. It is unlikely then that a green plant is in the immediate area. However, the chance that a heterozygous individual is present is much more likely. When Mr. Determan harvested seed he may have retrieved only one or the only capsule from a heterozygous plant. Using the same arithmetic as above, two green seedlings could be obtained from a heterozygous individual. Green may have a low frequency in the population and thus is rarely seen.

Schnell (1978a) concluded leaf color variation in *S. flava* was non-adaptive while Bell (1949) suggested that the reproductive success of the green S. *purpurea* subsp. *purpurea* f. *heterophylla* might have some selective advantage. If green is occurring at a low frequency what are the selection pressures (if any) for or against the allele? Further genetic experiments as well as studies of prey capture effectiveness of red and green variants are warranted. Studies of pollination and resultant seed set of red and green variants in natural conditions are being studied at this time.

It is worth commenting on the direction of change in yellow flower color variants even though these plants were not examined in this study. Flower color changes from red to yellow but not the reverse. No variants in a yellow-flowered species have been found which spontaneously become red-flowered. Schnell (1978b) found that red-flowered species have red pigment on a yellow background. This could indicate that the development of yellow-flowered individuals from redflowered species could be due to the loss or suppression of a gene controlling red pigment production in flowers. The all-green mutant forms studied in this

experiment may be experiencing mutation of the same gene yet earlier in the metabolic pathway which controls leaf, flower and growth point color.

Acknowledgements

I would like to thank the Biology Department at Virginia Commonwealth University with particular appreciation to Drs. Carolyn Conway and Robert Fisher in the undergraduate research program and to Dr. William Eggleston for his careful review, supervision and advice on this project. This work was largely supported by a grant from the V.C.U. Undergraduate Research Grant Program. (DES)

Table 2a:
Seed Produced and Percent Germination in 1993 Crosses

Cross #	Seed Yield	Rep. 1	Rep. 2	Rep. 3	Type of Cross
10	116	28/100	2/17	-------	green selfed
22A	164	49/100	18/64	-------	red x green
23A	378	54/100	23/100	42/88	red x green
23B	155	46/100	16/55	-------	red selfed
29A	269	38/100	9/100	0/9	green selfed
30.1	288	34/100	10/100	-------	green selfed
30.2	78	23/100	-------	-------	green selfed
32A	597	37/100	19/100	79/307	green x red
32B	76	35/100	-------	-------	green selfed
35A	63	20/100	-------	-------	green selfed?
37.1	354	37/100	38/100	31/64	green x red
37.2	462	43/100	20/100	38/172	green x red
71A	390	0/100	0/100	0/100	red x green
71B	409	0/100	0/100	0/119	red x green
113A	1540	14/100	5/100	224/1250	red selfed
113B	1641	21/100	6/100	165/1351	red x green
114A	945	31/100	14/100	212/655	green selfed
114B	1586	25/100	9/100	573/1296	green x red
115B	335	54/100	39/100	3/45	red x green
116A	86	0/100	-------	-------	green selfed
116B	22	0/100	-------	-------	green x red
117	248	37/100	14/100	-------	green x red
Total	10,202	626/2200	242/1536	1367/5456	
Average	464	29%	16%	25%	

Table 3
Outcome of Self and Cross Pollinations

3A. S. rubra subsp. *gulfensis* (green)

Cross #	Type of Cross	Seedling Phenotype
10	green selfed	green
117	green x red	red

3B. S. rubra subsp. jonesii (green)

Cross #	Type of Cross	Seedling Phenotype
22A	red x green	red
23A	red x green	red
23B	red selfed	red
29A	green selfed	green
30.1	green selfed	green
30.2	green selfed	green
32A	green x red	red
32B	green selfed?	green
35A	green selfed?	green
37.1	green x red	red
37.2	green x red	red
115B	red x green	red

3C. S. *purpurea subsp. purpurea* f. *heterophylla* (green)

Cross #	Type of Cross	Seedling Phenotype
113A	red selfed	red (3rd rep 1993 had three green seedlings)
113B	red x green	red
114A	green selfed	green (1994 one red seedling)
114B	green x red	red
156	control	green

Appendix: Identity of crosses

Cross #	Identity
10	*S. rubra* subsp. *gulfensis* (green) —Selfed
22A	*S. rubra* (red-Taylor Co., GA) x S. *rubra* subsp. *jonesii* (green)
23A	*S. rubra* (red-Taylor Co., GA) x S. *rubra* subsp. *jonesii* (green)
23B	*S. rubra* (red-Taylor Co., GA)—selfed
29A	*S. rubra* subsp. *jonesii* (green)—selfed
30.1	*S. rubra* subsp. *jonesii* (green)—selfed
30.2	*S. rubra* subsp. *jonesii* (green)—selfed
32A	*S. rubra* subsp. *jonesii* (green) x *S. rubra* (red-Taylor Co., GA)
32B	*S. rubra* subsp. *jonesii* (green)—selfed
35A	*S. rubra* subsp. *jonesii* (green)—selfed
37.1	*S. rubra* subsp. *jonesii* (green) xS. *rubra* subsp. *jonesii* (red-Etowah, NC)
37.2	*S. rubra* subsp. *jonesii* (green) xS. *rubra* subsp. *jonesii* (red-Etowah, NC)
71A	*S. rubra* subsp. *jonesii* (red-Etowah, NC) x S. *rubra* subsp. *jonesii* (green)
71B	*S. rubra* subsp. *jonesii* (red-Etowah, NC) —selfed
113A	*S. purpurea* (red-Reynolds Pond, Del.) —selfed
113B	*S. purpurea* (red-Reynolds Pond, Del.) x S. *purpurea* forma *heterophylla* (green)
113D	*S. purpurea* (red-Reynolds Pond, Del.) x S. *purpurea* forma *heterophylla* (green)
114A	*S. purpurea* forma *heterophylla* (green) —selfed
114B	*S. purpurea* forma *heterophylla* (green) x S. *purpurea* (red-Reynolds Pond, Del.)
115B	*S. rubra* subsp. *jonesii* (red-Greenville Co., S.C..) xS. *rubra* subsp. *jonesii*
116A	*S. rubra* subsp. *jonesii* (green)—selfed
116B	*S. rubra* subsp. *jonesii* (green) x S. *rubra* subsp. *jonesii* (red-Greenville Co.,SC)
117	*S. rubra subsp. gulfensis* (green) x S. *rubra subsp. gulfensis* (red-Blue Ridge Rd.)
156	*S. purpurea* forma *heterophylla* (green)—control

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