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INTERSPECIFIC AND INTRASPECIFIC VARIATION IN SEED SIZE AND GERMINATION REQUIREMENTS OF *SARRACENIA* (SARRACENIACEAE)[1]

AARON M. ELLISON

Department of Biological Sciences, Mount Holyoke College, South Hadley, Massachusetts 01075-6418 USA

Seed size and germination requirements of eight (of nine) *Sarracenia* species, and 13 populations of *S. purpurea* were studied. All species except for *S. purpurea* are restricted to the southeastern United States, whereas *S. purpurea* ranges across Canada, southward along the eastern United States into Maryland and Virginia (*S. purpurea* ssp. *purpurea*), and from New Jersey southward into northern Florida and the coast of the Gulf of Mexico (*S. purpurea* ssp. *venosa*). I tested the hypotheses that dormancy-breaking requirements vary predictably among species across a latitudinal gradient. I also sought to determine whether seed size and germination requirements were useful characters for resolving systematic and phylogenetic questions within this genus. Seed size varied significantly among species, but variability in seed size within *S. purpurea* exceeded the variability in seed size observed across all eight species studied. Seeds of all species are morphophysiologically dormant upon dispersal. Length of required cool, moist pretreatment varied among species, and germination in higher latitude populations is enhanced with longer pretreatment. In contrast, variability in germination requirements of subspecies, varieties, and populations of the geographically wide-ranging *S. purpurea* was not related clearly to geographic location (latitude or elevation). Germination requirements do not map onto a proposed phylogeny of *Sarracenia*, but observed differences in germination requirements of *S. purpurea* ssp. *venosa* var. *burkii* relative to other populations of *S. purpurea* support the recent proposal to elevate this variety to species status.

Key words: dormancy; germination; latitude; morphophysiological dormancy; *Sarracenia*; Sarraceniaceae; seed size.

Seed traits, including seed size, dormancy, germination, and dispersal, are central components of plant life histories (Harper, 1977; Fenner, 1983; Thompson, 1987), and their importance to plant fitness and are widely appreciated (e.g., Leon, 1985; Venable, 1985, 1989; Foster, 1986; Lord, Westoby, and Leishman, 1995; Venable et al., 1998; Higgins and Richardson, 1999). Comparative analyses of seed size (e.g., Foster, 1986; Mazer, 1989; Leishman, Westoby, and Jurado, 1995; Lord, Westoby, and Leishman, 1995; Venable et al., 1998) and seed germination (e.g., Garwood, 1983, 1989; Leon, 1985) have been especially useful in illustrating not only the broad range of seed traits that occur within and among taxa, but also the environmental and phylogenetic constraints that limit variability in characters. Seed traits, including seed size, length of dormancy, germination requirements, and germination rate, often vary in parallel and are thought to comprise co-evolved complexes or life history strategies for many species (e.g., Harper, 1977; Foster, 1986; Venable and Brown, 1988; Evans and Cabin, 1995; Rees and Westoby, 1997). These co-evolved complexes may be correlated with relatedness among species (Mazer, 1989). Alternatively, locally strong environmental forces could decouple seed traits from phylogeny (Leishman, Westoby, and Jurado, 1995; Lord, Westoby, and Leishman, 1995). In this study, I examine seed size, germination, and dormancy and their covariation across a latitudinal gradient among a set of closely related species in the genus *Sarracenia.* An explicit goal of this study is to test whether seed traits are useful taxonomic characters for distinguishing among these species, or whether local environmental conditions that change predictably across a latitudinal gradient have resulted in geographic patterns among seed traits that are independent of an existing phylogeny of *Sarracenia* species.

Germination biology of carnivorous plants, including *Sarracenia* (the pitcher plant), has received little attention from botanists or plant ecologists (Baskin and Baskin, 1998), although carnivorous plant collectors and enthusiasts have a rich lore of suggestions and recipes for germination of these plants (on-line Carnivorous Plant Archive at http://randomaccess.unm.edu/www/cp/cparchive.html). The lack of studies on the germination of carnivorous plants is in striking contrast to the more extensive literature on their morphological adaptations for carnivory (e.g., Darwin, 1875; Arber, 1941; Juniper, Robins, and Joel, 1989) and the community of invertebrates that live within the pitcher-shaped leaves of *Sarracenia*, *Darlingtonia* (both Sarraceniaceae), and *Nepenthes* (Nepenthaceae) (reviews in Higley, 1885; Bradshaw, 1983; Fish, 1983). Within the genus *Sarracenia*, the germination requirements of only one species, *S. purpurea*, have been carefully studied (Mandossian, 1966; Gotsch and Ellison, 1998). *Sarracenia purpurea* is the most widespread species in the genus, occurring throughout Canada and the eastern United States. The remaining species in the genus occur only in the southeastern United States, and there is no a priori reason to suspect that their germination requirements are identical to those of *S. purpurea.*

Relying on Mandossian's (1966) study, Baskin and Baskin (1998) suggested that all *Sarracenia* species have nondeep

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morphophysiological dormancy (MPD), in which the embryos are underdeveloped at dehiscence and the radicle, once developed, is prevented from emerging because of physiological inhibition (definition after Nikolaeva, 1977; Baskin and Baskin, 1998). Gotsch and Ellison's (1998) observations on embryo development at time of dehiscence and germination requirements confirmed that *S. purpurea* seeds have nondeep MPD, but data from other species in this genus are unavailable. Several authors have observed dramatic increases in population size of southern *Sarracenia* species following fires (McDaniel, 1971; Weiss, 1980; Barker and Williamson, 1988; Brewer, 1999), suggesting that some southern species could require fire for germination. Even if all *Sarracenia* species have nondeep MPD, length of dormancy could be shorter among species growing in the southeastern United States relative to *S. purpurea* at the northern limits of its range. Both increases in mean temperature and decreases in number of subfreezing days could select for shorter dormancy periods among southern species (Baskin and Baskin, 1998). To test these hypotheses, I examined the germination requirements of eight of the nine species of *Sarracenia* and of both subspecies (and three of the four subspecific varieties) of *S. purpurea.*

Seed characteristics, including seed size and germination requirements, could also be helpful in resolving systematic and phylogenetic questions within this genus. Taxonomy of *Sarracenia* is based principally on the morphology of the carnivorous, pitcher-shaped leaves (Macfarlane, 1908; McDaniel, 1971; Schnell and Krider, 1976; Case and Case, 1976; Naczi et al., 1999). The Sarraceniaceae are clearly a monophyletic group (Albert, Williams, and Chase, 1992), but recent attempts to resolve interspecific relationships using molecular techniques have provided little resolution among the species of *Sarracenia* (Bayer, Hufford, and Soltis, 1996), although isozyme analysis (Godt and Hamrick, 1996, 1998a, b) has tended to support subspecific-level distinctions proposed by earlier authors (Case and Case, 1976; Schnell, 1979) on morphological grounds. McDaniel (1971) asserted that seed size was a diagnostic characteristic and could be used to distinguish among species, but presented insufficient data to test this hypothesis. I therefore also collected data on seed size, and examined interspecific relationships in seed size and germination requirements in the context of prior systematic work in *Sarracenia.*

STUDY SPECIES AND COLLECTION INFORMATION

I studied germination in eight of the nine species of *Sarracenia*: *Sarracenia alata* (Wood) Wood, *S. flava* L., *S. jonesii* Wherry, *S. leucophylla* Raf., *S. psittacina* Michx., *S. rubra* Walt., *S. minor* Walt., and *S. purpurea* L. (nomenclature follows Case and Case, 1976; Schnell, 1979; Godfrey and Wooten, 1981). Within *S. purpurea,* I studied germination of 11 populations of *S. purpurea* ssp. *purpurea* (Raf.) Wherry, one of *S. purpurea* ssp. *venosa* var. *venosa* (Raf.) Fernald, and one of *S. purpurea* ssp. *venosa* var. *burkii* Schnell. Seeds of the federally endangered *S. oreophila* (Kearney) Wherry were unavailable. All but *S. purpurea* ssp. *purpurea* are found only in the southeastern United States, where they occur in wet pine savannas, bogs, or pineland seepage slopes (Godfrey and Wooten, 1981). *Sarracenia alata* occurs in southwestern Alabama, southern Mississippi, and Louisiana and extends into eastern Texas. *Sarracenia leucophylla* and *S. psittacina* both occur in southern Georgia, northern Forida, southern Alabama, and southeastern Mississippi. *Sarracenia minor* and *S. rubra* occur along the southeastern coastal plain, from southern North Carolina to the central peninsula of Florida. *Sarracenia flava* occurs sympatrically with *S. minor* and *S. rubra,* but extends farther northwards into southeastern Virginia. The federally endangered species *Sarracenia jonesii* was separated from the *S. rubra* complex by Case and Case (1976) and is known from only ten populations in North and South Carolina (Godt and Hamrick, 1996). Its identity as a distinct species is supported by isozyme studies (Godt and Hamrick, 1996). *Sarracenia purpurea* is a common and widespread bog species that ranges from Newfoundland westward across Canada to Manitoba, southward along the eastern United States to Florida and Mississippi, as well as into Ohio, Illinois, Iowa, and northern Indiana.

Schnell (1979) divided *S. purpurea* into two subspecies, ssp. *purpurea* and ssp. *venosa* (Raf.) Wherry. *Sarracenia purpurea* ssp. *purpurea,* the ''northern'' pitcher plant, grows as far south as Maryland, whereas ssp. *venosa,* the ''southern'' pitcher plant, occurs from Maryland (possibly extending north into southern New Jersey) southward. These two subspecies of *S. purpurea* are sympatric in southern Maryland and northern Virginia (Wherry, 1933), although recent field observations on the degree of sympatry are lacking. Schnell later subdivided ssp. *venosa* into three varieties: var. *venosa* that occurs on the Atlantic coastal plain, var. *montana* Schnell & Determann that occurs in the southern Appalachian Mountains of Georgia and the Carolinas, and var. *burkii* that occurs on the Gulf coastal plain from the Florida panhandle westward into Louisiana (Schnell, 1993; Schnell and Determann, 1997). Gleason and Cronquist (1991) rejected the subspecific and varietal designations of Schnell (1979), and recognized only two varieties of *S. purpurea,* the ''northern'' pitcher plant, *S. purpurea* var. *purpurea* and the ''southern'' pitcher plant, *S. purpurea* var. *venosa.* Most recently, isozyme analysis (Godt and Hamrick, 1998b) has supported the subspecific designations proposed by Schnell (1979), and morphological analysis has been used to further elevate *S. purpurea* ssp. *venosa* var. *burkii* to the species level—*S. rosea* Naczi, Case & Case (Naczi et al., 1999). To add insult to nomenclatural injury, Reveal (1993) pointed out that the correct name for the northern pitcher plant should be *S. purpurea* var. *terrae-novae* de la Pylaie and that the correct name for var. *venosa* actually should be var. *purpurea*! To avoid confusion with common usage, I follow current convention here and refer to the northern pitcher plant as *S. purpurea* ssp. *purpurea* and the southern pitcher plant as *S. purpurea* ssp. *venosa,* and refer to the varieties of ssp. *venosa* where necessary.

I used wild-collected seeds for all species except *S. jonesii* and *S. rubra,* for which I obtained outcrossed seeds from cultivated plants. Seeds of *Sarracenia flava* were collected from a bog in Miramar Beach, Walton County, Florida, USA in July 1997. Seeds of *Sarracenia minor* were collected from a small bog in Jedborg, Dorchester County, South Carolina, USA in September 1997. Seeds of *S. leucophylla* were collected from another bog in Walton County, Florida in September 1997. Seeds of *S. alata* and *S. psittacina* were collected from a bog in Stone County, Mississippi, USA in November 1997. Seeds of *S. rubra* and *S. jonesii* were collected in September 1998 from outcrossed-plants grown by David Mellard in Atlanta, Georgia, USA.

For intraspecific comparisons within the widespread *S. purpurea,* I studied germination of seeds from 13 populations (Ta-

TABLE 1. Geographic information on the studied populations of *Sarracenia purpurea*. Latitude and longitude are given in decimal degrees, and elevation is given in metres above sea level. Populations are ordered by absolute latitude (North to South). Apparent latitude is calculated as latitude + 1° for every 100 m of elevation.

Variety	Population	Latitude	Longitude	Elevation	Apparent latitude
Sarracenia purpurea ssp. *purpurea*	Scott's Corner, MN	46.61	92.43	329	49.90
	Molly Bog, VT	44.50	72.64	250	47.00
	Snake Mountain Bog, VT	44.06	73.27	313	47.19
	North Springfield Bog, VT	43.23	72.50	158	44.81
	Sadawga Lake Bog, VT	42.78	72.88	166	44.44
	Rowe Bog, MA	42.71	72.88	490	47.61
	High Ledges Bog, MA	42.62	72.72	412	46.74
	Hawley Bog, MA	42.58	72.89	543	48.01
	Kampoosa Bog, MA	42.29	72.64	256	44.85
	Granby Bog, MA	42.27	72.49	89	43.16
	Calico Ridge Bog, NJ	39.69	74.51	11	39.80
Sarracenia purpurea ssp. *venosa* var. *venosa*	Arden Bog, MD	39.06	76.59	9	39.15
Sarracenia purpurea ssp. *venosa* var. *burkii*	Appalachicola Nat. Forest, FL	30.08	84.95	11	30.19

ble 1). Wild-collected seeds of *S. purpurea* were obtained between September and November 1998. Latitude and longitude of each population were recorded using GPS. Elevation of each site (metres above sea level) was determined from USGS topographic maps.

MATERIALS AND METHODS

For each species and for each population of *S. purpurea,* an entire fruit was collected from each of ten plants. For this population-level study, all seeds from each site were thoroughly mixed to minimize effects of a single parental plant on germination. For all experiments, seeds of each species or population were chosen at random from that species' or population's mixed collection. All species were tested for dormancy-breaking requirements and germination in the year they were collected.

Seed size (length, width) was determined for 200 seeds per species or population using an ocular micrometer in a Willd dissecting scope at 50× magnification. Twenty seeds per species and population were also weighed (±0.01 mg) to determine length–mass relationships.

Seeds of each species and population were tested for morphological dormancy (sensu Baskin and Baskin, 1998) by attempting to germinate freshly collected seeds. Under a laminar-flow hood, ten seeds were surface-sterilized (10% bleach solution, followed by a triple rinse with distilled water), then placed in sterile 9 cm diameter petri dishes between two sheets of sterilized filter paper and moistened with 6 mL of sterile distilled water. Petri dishes were placed on a warm (20°C) bench in high light (PAR [Photosynthetic Active Radiation] > 1000 $\mu mol \cdot m^{-2} \cdot sec^{-1}$), long-day (14 h) conditions in the Mount Holyoke College greenhouse. Seeds were checked daily for 60 d for germination (radicle emergence); 2 mL of distilled water were added to each dish every 2 d to prevent desiccation. Ten additional seeds of each species and population were dissected to examine the state of the embryo upon dispersal. Dissected embryos were stained with tetrazolium to determine viability (Lakon, 1949). The remaining seeds were stored dry at 4°C prior to using them in the experiments involving stratification (cold and moist pretreatment) or scarification.

These seeds were then divided randomly into five germination treatments: (1) no pretreatment other than cold, dry storage (dry treatment); (2) cold, dry storage plus scarification in 1 mol/L H_2SO_4 for 60 sec followed by a triple rinse in distilled water (scarification treatment); (3) seeds moist-chilled at 4°C for 2 wk (short stratification treatment); (4) seeds moist-chilled at 4°C for 4 wk (intermediate stratification treatment); (5) seeds moist-chilled at 4°C for 5 wk (long stratification treatment). The dry and stratification treatments tested for the need for a period of additional embryo development, whereas the scarification treatment tested for the impermeability of the seed coat. Cold, moist stratification also is a common, dormancy-breaking treatment for seeds with MPD (Baskin and Baskin, 1998). Because no germination occurred in the scarification treatment in 1997, this treatment was eliminated for the species from which seeds were collected and studied in fall 1998 (*S. jonesii, S. rubra,* all populations of *S. purpurea*).

For stratification, seeds were (1) surface-sterilized for 60 sec in a 10% bleach solution; (2) triple rinsed in distilled water; (3) placed in sterile 9 cm-diameter petri dishes between two sheets of sterilized filter paper; (4) moistened with 6 mL of sterile distilled water; (5) placed in a dark refrigerator at 4°C. All plating was done aseptically under a laminar-flow hood to minimize possibility of fungal or mold growth. For each treatment × species or population combination, I used five replicate plates of ten seeds. Petri dishes were placed on greenhouse benches in full sun (maximum PAR 1500 $\mu mol \cdot m^{-2} \cdot sec^{-1}$). Because germination experiments were conducted in the winter, supplemental lighting (sodium flood lights) was used to maintain a day length of 14 h. Greenhouse temperature (20°C) and relative humidity (70%) were maintained by computer controllers. Temperatures within the petri dishes were not monitored, although they probably exceeded ambient temperature. Once they were in the greenhouse, I added 2 mL of distilled water to each plate every 48 h to prevent desiccation. Plates were examined every 2 d for 34 d for evidence of radicle emergence.

Statistical analysis—Data were analyzed using ANOVA and regression procedures in Systat version 8.0 (SPSS Inc., Chicago, Illinois, USA). For interspecific comparisons, I used the Hawley Bog population of *S. purpurea* ssp. *purpurea* (chosen at random from among the northern populations) and the Appalachicola (Florida) population of *S. purpurea* ssp. *venosa.* Although Hawley Bog is not at the northern limit of the range of *S. purpurea,* its high elevation gives it an apparent latitude well north of its location in northwestern Massachusetts (Table 1). The Appalachicola population is assuredly a population of *S. purpurea* ssp. *venosa,* whereas the Arden Bog (Maryland) population likely is *S. purpurea* ssp. *venosa* (personal observation), but is within the zone of sympatry of the two subspecies of *S. purpurea* (Wherry, 1933). All 13 populations of *S. purpurea* were used for the intraspecific comparisons.

Results are presented as means ± 1 SE throughout. Comparisons were made both among and within species (or populations) using a nested ANOVA design, for which the factors were species (or populations) and treatment nested within species (or populations). I compared time (days) to first observed germinant, time to 50% germination (50% of the actual number of seeds that germinated in a given species or population × treatment combination), total number of seeds germinated after 34 d, time to maximum germination, and germination rate (germinants per day, calculated as maximum number of germinants / [days to maximum germination − days to initial germination]) across species or populations (main effect in the ANOVA), and among treatments within species or populations (nested effect in the ANOVA). All data were examined to ensure that they conformed to assumptions

TABLE 2. Northern range limits, optimal pretreatment (weeks of cold, moist stratification; $N = 5$ replicates of ten seeds each), seed size (length in mm; $N = 200$ seeds), and ranks (based on Tukey's LSD post hoc test for multiple comparisons among means) for the studied *Sarracenia* species.

Species	Northern range limit	Optimal pre treatment (wk)	Mean seed size (SE)	Ranks				
				Seed size	Time to onset	Time to 50% germ.	Time to max. germ.	Rate
S. purpurea ssp. *purpurea*	Newfoundland	4–6	1.94 (0.013)	3	1	1	1	1
S. purpurea ssp. *venosa*	Maryland/Virginia	4-6	1.88 (0.009)	4	4	4	3	3
S. flava	Virginia	4	2.23 (0.021)	1	3	3	3	3
S. minor	N. Carolina	4	1.66 (0.007)	5	3	3	3	3
S. rubra ssp. *rubra*	N. Carolina	4	1.76 (0.035)	5	3	3	2	3
S. jonesii	N. Carolina	4	1.73 (0.064)	5	3	3	2	3
S. leucophylla	southern Georgia	2–4	2.19 (0.015)	1	3	3	3	2
S. psittacina	southern Georgia	2	1.69 (0.013)	5	2	2	2	3
S. alata	southern Alabama	2	1.99 (0.015)	2	1	2	2	3

of parametric ANOVA. Only germination rate required transformation (to their natural logarithm) prior to analysis.

RESULTS

Seed viability—Embryos of all *Sarracenia* species were linear, filled one-third to one-half of the mature seed, were creamy white in color and had obvious cotyledons and a hypocotyl. Differentiated plumules were not apparent. Endosperm filled the volume of the seed not taken up by the embryo. Of the dissected seeds, >90% had live embryos, as indicated by positive (red) tetrazolium staining. Freshly collected seeds of all eight species and all 13 populations of *S. purpurea* failed to germinate within 60 d, however. Thus, although embryos were not completely developed at dehiscence, they failed to develop immediately under summer conditions. Thus, these species were not simply morphologically dormant (sensu Baskin and Baskin, 1998).

Interspecific variability in seed size—*Sarracenia* species produced very small, obovate, light brown seeds that ranged from 1.2 to 2.7 mm in length (Table 2), and 0.8–1.8 mm in width. Five seed-size groups (based on seed length) were apparent, and were distinguished using Fisher's Least Significant Difference post-hoc test for multiple comparisons among means. The largest seed group consisted of *S. flava* and *S. leucophylla* and the smallest seed group consisted of *S. jonesii*, *S. rubra*, *S. minor*, and *S. psittacina*. Seeds of *S. alata* and the two subspecies of *S. purpurea* were intermediate in size, but were all statistically dissimilar in length (Table 2). Seed length and width were weakly but significantly correlated ($r = 0.24$, $P < 1 \times 10^{-15}$). Seed length and width were significant predictors of seed mass (mass in g $= 0.0004 \times$ length $\times$ width $- 0.003$) and this regression explained 56% of the observed variance in seed mass.

Intraspecific variability in seed size—Across the populations of *Sarracenia purpurea*, seeds ranged from 1.1 to 2.8 mm in length ($X = 2.09$, SE $= 0.004$) and 0.6–1.7 mm in width ($X = 1.18$, SE $= 0.002$; Table 3). Seed size varied significantly among populations ($F_{12, 3689} = 184.7$, $P < 0.0001$; Table 3). Although intersite differences in seed size were observed, this variability did not show any relationship either with absolute latitude ($r^2 = 0.13$, $P = 0.23$) or with latitude corrected for elevation (100 m elevation $\approx 1°$ latitude) ($r^2 = 0.04$, $P = 0.50$). Variability in seed size within *S. purpurea* exceeded the variability in seed size observed across all eight species studied.

Interspecific variability in germination—Germination trajectories of all species in all treatments are illustrated in Fig. 1. Except for a small number (<4 per species) of seeds of *S. alata*, *S. jonesii* and *S. rubra*, no seeds germinated in either the dry or scarification treatments, and so subsequent analysis was restricted to the three moist-cold (stratification) treat-

TABLE 3. Optimal pretreatment (week of cold, moist stratification), seed size (length in mm), and ranks (based on Tukey's LSD post hoc test for multiple comparisons among means) for the studied populations of *Sarracenia purpurea*.

Population	Optimal pre-treatment (wk)	Mean seed size (SE)	Ranks				
			Seed size	Time to onset	Time to 50% germ.	Time to max. germ.	Rate
Scott's Corner, MN	4–6	1.93 (0.013)	6	2	2	2	2
Molly Bog, VT	4–6	2.38 (0.012)	1	2	1	1	1
Snake Mountain Bog, VT	4	2.28 (0.011)	2	2	2	2	2
North Springfield Bog, VT	6	2.21 (0.016)	3	2	1	1	1
Sadawga Lake Bog, VT	4	2.14 (0.012)	4	1	1	1	1
Rowe Bog, MA	4	2.04 (0.009)	5	2	2	1	1
High Ledges Bog, MA	4–6	1.95 (0.011)	6	2	2	2	2
Hawley Bog, MA	4–6	1.94 (0.013)	6	1	1	1	1
Kampoosa Bog, MA	4	1.87 (0.014)	7	2	1	1	2
Granby Bog, MA	2–4	2.19 (0.012)	3	2	1	1	1
Calico Ridge Bog, NJ	2–4	2.12 (0.009)	4	2	2	1	1
Arden Bog, MD	4	2.06 (0.010)	5	1	1	1	1
Appalachicola Nat. Forest, FL	4–6	1.88 (0.009)	7	3	3	3	3

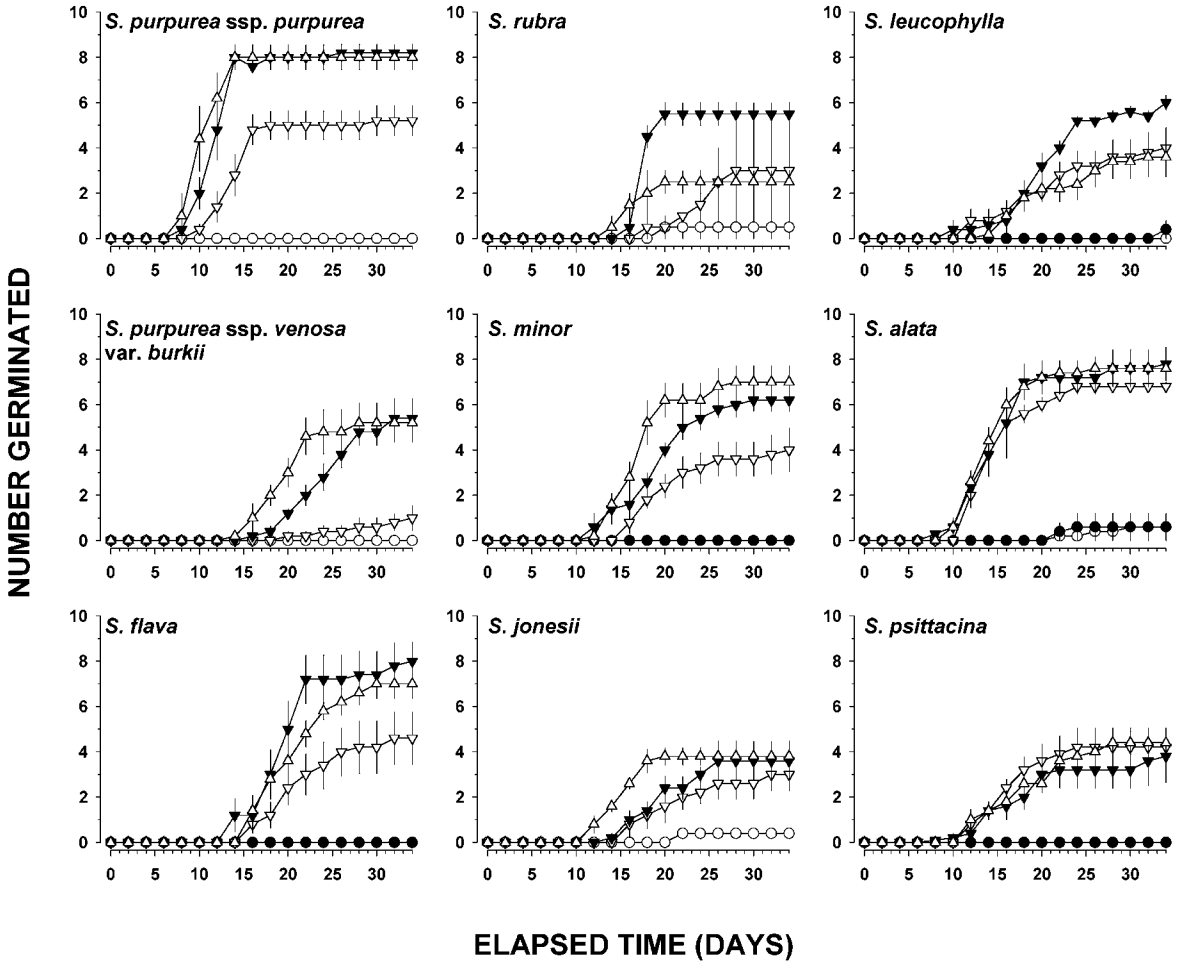

Fig. 1. Germination trajectories for eight *Sarracenia* species and two subspecies of *S. purpurea.* Values shown are mean number germinated with 1 SE indicated ($N = 5$ plates of ten seeds each per species × treatment combination). ○ dry treatment; ● scarification only; ▽ short stratification; ▼ intermediate stratification; △ long stratification.

ments. Analysis of variance indicated significant differences among species (main effect) in: time to onset of germination ($F_{8,97} = 24.1$, $P < 0.001$); time to 50% germination ($F_{8,97} = 22.1$, $P < 0.001$); maximum number of seeds germinated during the experiment ($F_{8,97} = 12.5$, $P < 0.001$); time to maximum germination ($F_{8,97} = 9.3$, $P < 0.001$); and germination rate ($F_{8,97} = 7.9$, $P < 0.001$).

Similarly, ANOVA indicated significant differences among treatments within species (nested effect) in: time to onset of germination ($F_{18,97} = 3.961$, $P < 0.001$); time to 50% germination ($F_{18,97} = 2.1$, $P = 0.013$); maximum number of seeds germinated during the experiment ($F_{18,97} = 3.7$, $P < 0.001$); and germination rate ($F_{18,97} = 2.1$, $P = 0.013$). Differences were not found among treatments within species in time to maximum germination ($F_{18,97} = 1.4$, $P = 0.420$).

Subsequent pairwise comparisons (Fisher's Least Significant Difference test) among treatments within species illustrated that these significant effects were due to strong effects of treatment on a few species. Onset of germination was significantly accelerated by increasing stratification from 2 to 4 wk in *S. minor*, *S. jonesii*, and both subspecies of *S. purpurea.* Onset of germination showed further acceleration with additional stratification (6 wk) only in *S. jonesii* and both subspecies of *S. purpurea.* In contrast, onset of germination was significantly slowed with increasing stratification in *S. leucophylla.* Time to 50% germination was accelerated by increasing stratification from 2 to 4 wk in *S. jonesii*, *S. rubra*, and *S. purpurea* ssp. *venosa.* No further effect on time to 50% germination was found by increasing stratification time to 6 wk. Total numbers of germinated seedlings of *S. flava*, *S. minor*, and both subspecies of *S. purpurea* increased significantly as stratification time increased from 2 to 4 wk, but no additional enhancement was observed by increasing stratification time to 6 wk. Maximum number of *S. leucophylla* seedlings occurred with 4 wk stratification and was lowest in the 6-wk stratification treatment. Germination rate increased significantly between 2- and 4-wk stratification time only in *S. rubra* and both subspecies of *S. purpurea*, and only *S. purpurea* ssp. *purpurea* showed a further increase in germination rate with the 6-wk stratification period. Seed size was not correlated with germination requirements ($P = 0.79$, Spearman correlation test on ranked seed size group vs. ranked optimal pretreatment time [columns 3 and 5 of Table 2]).

Intraspecific variability in germination—Germination trajectories of all populations of *S. purpurea* are illustrated in Fig. 2 (that of *S. purpurea* ssp. *purpurea* var. *burkii* is in Fig. 1). Analysis of variance indicated significant differences

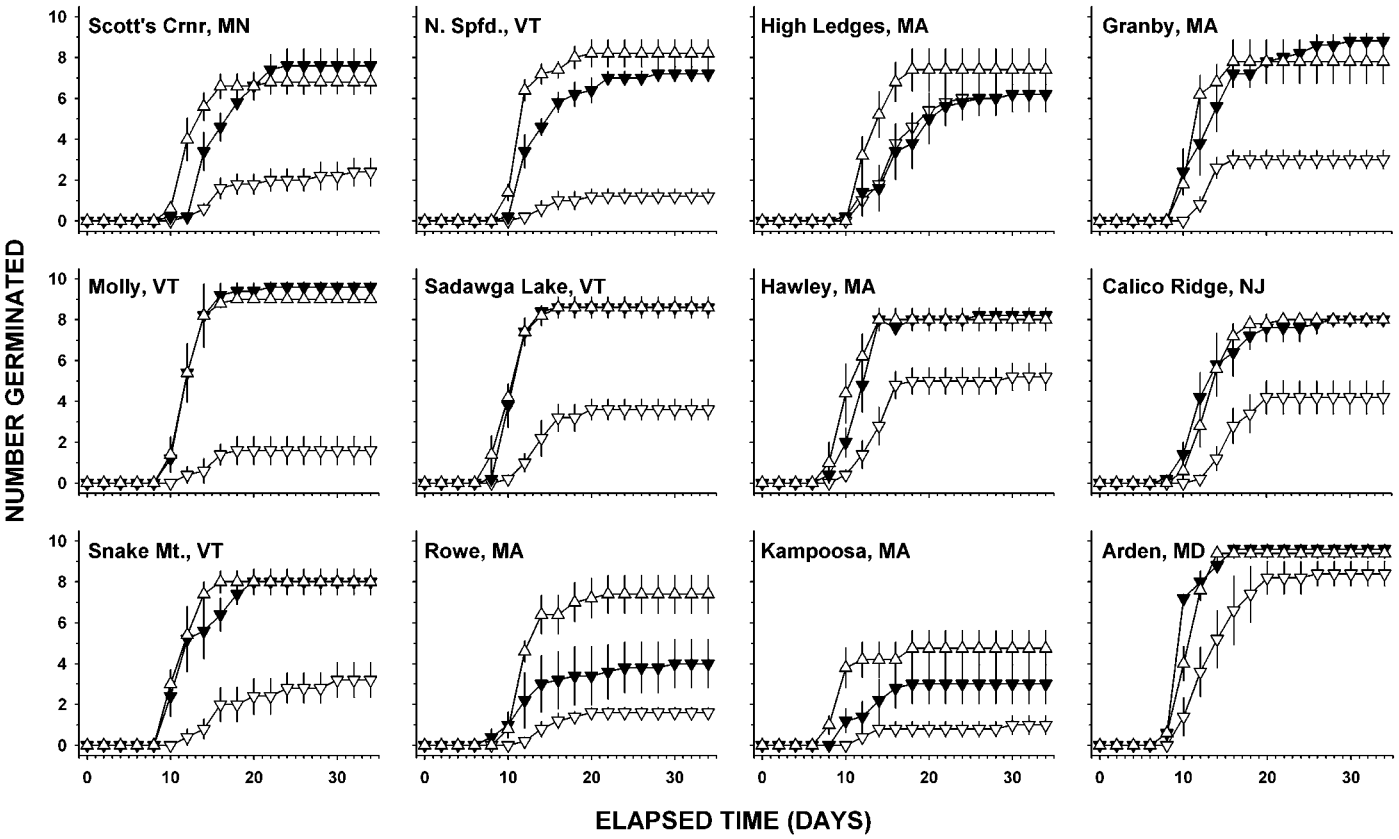

Fig. 2. Germination trajectories for the twelve populations of *Sarracenia purpurea.* Values shown are mean number germinated with 1 SE indicated (N = 5 plates of ten each per population × treatment combination). Trajectories for *S. purpurea* ssp. *venosa* var. *burkii* are in Fig. 1. ▽ short stratification; ▼ intermediate stratification; △ long stratification.

among populations (main effect) in: time to onset of germination ($F_{12,26} = 17.7$, $P < 0.001$); time to 50% germination ($F_{12,26} = 22.7$, $P < 0.001$); maximum number of seeds germinated during the experiment ($F_{12,26} = 19.0$, $P < 0.001$); time to maximum germination ($F_{12,26} = 8.6$, $P < 0.001$); and germination rate ($F_{12,26} = 4.6$, $P < 0.001$). Similarly, ANOVA indicated significant differences among treatments within populations (nested effect) in: time to onset of germination ($F_{26,147} = 4.4$, $P < 0.001$); time to 50% germination ($F_{26,147} = 3.6$, $P < 0.001$); maximum number of seeds germinated during the experiment ($F_{26,147} = 16.9$, $P < 0.001$); time to maximum germination ($F_{26,147} = 3.1$, $P < 0.001$); and germination rate ($F_{26,147} = 3.2$, $P < 0.001$).

Subsequent pairwise comparisons among treatments within populations illustrated that these significant effects were due to strong effects of treatment across all of the populations. In all populations of *S. purpurea* (Fig. 2), onset of germination and time to 50% germination were significantly accelerated by increasing stratification from 2 to 4 wk. Onset of germination showed further acceleration with additional stratification (to 6 wk) in populations from Minnesota, Rowe, Hawley, and Granby. Time to 50% germination also was accelerated further with a 6-wk stratification in populations from Minnesota, High Ledges, and North Springfield. Total numbers of germinated seedlings rose as stratification time increased significantly from 2 to 4 wk in all populations except High Ledges and Arden, and additional enhancement was observed by increasing stratification time to 6 wk in the North Springfield and Rowe populations. Germination rates increased significantly between 2- and 4-wk stratification time in all populations except for Granby and Calico Ridge, and showed a further increase in germination rate with the 6-wk stratification period in the Molly, North Springfield, High Ledges, Hawley, Kampoosa, and Arden populations. Seed size was not correlated with germination requirements ($P = 0.91$, Spearman correlation test on ranked seed size group vs. ranked optimal pretreatment time [columns 2 and 4 of Table 3]), or with any of the other measures of germination success ($P > 0.25$, Spearmean correlation test on ranked seed size group vs. ranks of germination metrics [columns 4–8 of Table 3]).

DISCUSSION

Interspecific patterns—Response to pretreatment varies among species, and the varied responses appear to be related to the northern-range limits of each species. Those species that grow only in the deep south, *S. alata, S. psittacina,* and *S. leucophylla,* show no response to pretreatment beyond 2 wk of cold, moist stratification. In fact, *S. leucophylla* shows a negative response (longer onset of germination and lowered germination success) to 6-wk pretreatment. The five species that grow along the southeastern coastal plain and extend northward into the Carolinas and Virginia, *S. flava, S. minor, S. rubra, S. jonesii,* and *S. purpurea* ssp. *venosa,* all show positive germination responses (earlier onset of germination, increased germination rate, increased number of germinants)

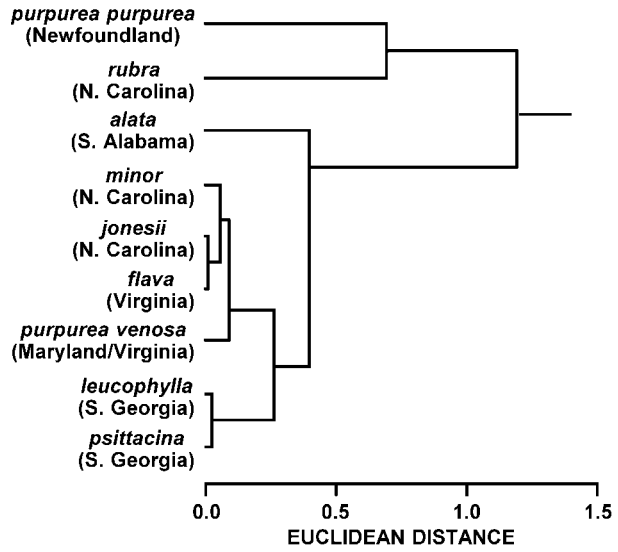

Fig. 3. Cluster diagram of the eight *Sarracenia* species and two subspecies of *S. purpurea.* Northern range limits are given in parentheses. Tree was computed from nearest neighbor clustering using Euclidean distance between species based on four germination characteristics: time to onset of germination, time to 50% germination, time to maximum germination, and germination rate. Values were standardized in SD units prior to clustering.

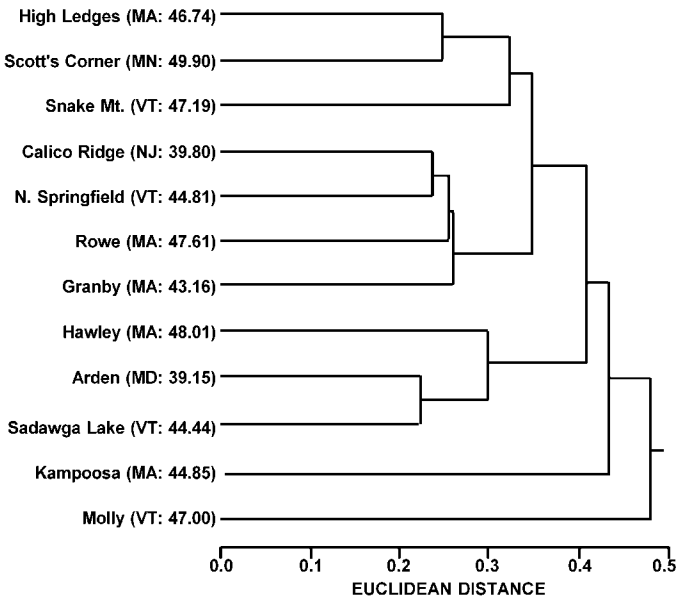

Fig. 4. Cluster diagram of 12 populations of *Sarracenia purpurea* (*S. purpurea* ssp. *venosa* var. *burkii* excluded), with apparent latitude given. Tree was computed from nearest neighbor clustering using Euclidean distance between species based on four germination characteristics: time to onset of germination, time to 50% germination, time to maximum germination, and germination rate. Values were standardized in SD units prior to clustering. Note difference in scale from Fig. 3.

to 4-wk pretreatment relative to 2-wk pretreatment, but no further responses when subject to 6-wk pretreatment. The northernmost species, *S. purpurea* ssp. *purpurea,* shows positive germination responses to 6-wk pretreatment relative to 4 and 2 wk pretreatments. Analysis of these germination properties together with seed size (all ranked, Table 2) using Kendall's coefficient of concordance shows that all these properties change in parallel (Kendall's $W = 0.196$, $P = 0.116$ with 5 df). Thus, the species with the longest optimal pretreatment time (*S. purpurea* ssp. *purpurea*) has relatively large seeds and the fastest time to onset of germination and germination rate. Species with small seeds and intermediate pretreatment requirements have intermediate germination rates, and those with short pretreatment requirements have the slowest germination rates and small to intermediate-sized seeds. None of the species respond to scarification only, suggesting that the germination of these species is not dependent on fire. Fire appears to increase population growth of pitcher plants only by removing potential competitors (Weiss, 1980; Barker and Williamson, 1988; Brewer, 1999).

Cluster analysis of the eight species and two subspecies of *S. purpurea* based on their average germination rates (time to onset, time to 50% germination, time to maximum germination, germination rate) illustrate strong geographic groupings (Fig. 3). The northern pitcher plant, *S. purpurea* ssp. *purpurea,* and the coastal plain species, *S. rubra,* clearly separate out from the rest of the species. The coastal plain species with broad ranges, *S. minor, S. jonesii, S. flava,* and *S. purpurea* ssp. *venosa,* form a second cluster. The three southern species occur in two clusters, one formed by *S. leucophylla* and *S. psittacina* and the other by *S. alata* alone. Addition of seed size data into the cluster analysis (tree not shown) maintains the tree topology, but the positions of *S. flava* and *S. psittacina* are interchanged.

Intraspecific patterns—Seed size and germination responses to the duration of cool, moist stratification pretreatment varied among populations of *S. purpurea,* but the variability is not related clearly to the geographic location (latitude or elevation) of each population (Table 3, Fig. 2). Although germination responses and seed size changed concordantly *among* different species of *Sarracenia* (see above), similar correlated changes are not observed among populations within this single species (Kendall's $W = 0.603$, $P < 0.001$ with 5 df). There is no relationship between optimal pretreatment time, seed size, time to onset of germination, or germination rate in this widespread species. Lack of concordance among seed traits in populations of *S. purpurea* and absence of a relationship between geography and seed traits within this species suggest that gene flow among populations of *S. purpurea* could be maintaining high variance in these traits. Dispersal dynamics have not been studied for any species of *Sarracenia,* but their predictable occurrence in widely spaced habitats (isolated bogs) suggests that long-distance seed dispersal is not uncommon (cf. Clark et al., 1998, 1999).

Cluster analysis of 12 of the 13 populations of *S. purpurea* based on their average germination rates (time to onset, time to 50% germination, time to maximum germination, germination rate) illustrate the geographic heterogeneity in germination characteristics within this species (Fig. 4). The Appalachicola population of *S. purpurea* ssp. *venosa* (var. *burkii*) was not included in this cluster, as preliminary analysis indicated that it was a strong outlier in all parameters, and its inclusion in the cluster analysis resulted in virtually no discrimination among the other 12 populations. This cluster analysis, together with the data in Table 3, do not suggest that the Maryland population, identified on the basis of field characteristics as *S. purpurea* ssp. *venosa* var. *venosa,* is distinguishable from *S. purpurea* ssp. *purpurea* on the basis of seed size and germination characteristics.

Relationship of germination characteristics to Sarracenia systematics—Seed size alone is not a useful character for distinguishing among species of *Sarracenia* (contra McDaniel, 1971). Within-species variability in seed size overlaps and exceeds between-species variability in seed size, and among species, seeds of several species are similarly sized (Tables 2 and 3).

At the species level, the groupings of *Sarracenia* based on germination characteristics (Fig. 3) differ from a cladistic analysis of these species based on ITS-1 and ITS-2 sequences (Bayer, Hufford, and Soltis, 1996). Their phylogenetic analysis suggested *S. alata* as the sister group of a large subclade composed of the other *Sarracenia* species. Within this subclade, they identified two monophyletic groups: one consisting of *S. purpurea* (probably ssp. *purpurea*) and *S. leucophylla,* and the other consisting of *S. flava, S. minor,* and *S. psittacina*. *Sarracenia rubra* was not assignable to either of these groups (Bayer, Hufford, and Soltis [1996] included *S. jonesii* within *S. rubra*). Support for the existence of the two monophyletic groups, however, was not strong (bootstrap value of 36% for the *S. purpurea–S. leucophylla* group and 70% for the *S. flava–S. minor–S. psittacina* group; Bayer, Hufford, and Soltis, 1996). Lack of congruence between germination characteristics and the molecular phylogeny of these species is consistent with the hypothesis that as *Sarracenia* species migrated north following the deglaciation at the end of the Pleistocence, their dormancy requirements evolved in response to the increasingly long winters associated with higher latitudes. The observed decoupling of germination ecology and seed traits from *Sarracenia* phylogeny also suggests that the species had diversified prior to deglaciation.

In contrast, the intraspecific groupings of populations of *S. purpurea* support current hypotheses concerning the relationships of the subspecies and varieties. In both the interspecific analysis (Fig. 3) and the intraspecific analysis (Fig. 4), *S. purpurea* ssp. *venosa* var. *burkii* is clearly separate and distinct from the other populations of *S. purpurea.* Naczi et al. (1999) proposed assigning this variety to a new species, *S. rosea,* based on flower color, scape height, and leaf morphology. Godt and Hamrick (1998b), using isozymes, clearly identified this variety as distinct from other varieties and subspecies of *S. purpurea.* On the basis of germination properties, *S. purpurea* ssp. *venosa* var. *burkii* is more closely allied with other southern coastal plain species than it is to *S. purpurea* ssp. *purpurea* or to the more northern *S. purpurea* ssp. *venosa* var. *venosa.* Thus, these studies on morphology, isozymes, and germination all point to a clearly distinctive entity of *S. purpurea* along the Gulf Coast of the United States. The nesting of the Arden Bog population of *S. purpurea* ssp. *venosa* var. *venosa* firmly within more northerly populations of *S. purpurea* ssp. *purpurea* implies either that the distinction between these two subspecies is not useful (as suggested by Gleason and Cronquist, 1991), that this population is actually *S. purpurea* ssp. *purpurea,* or that extensive hybridization between the subspecies has occurred in this putative zone of sympatry. Isozyme studies (Godt and Hamrick, 1998b) suggest that *S. purpurea* ssp. *venosa* var. *venosa* is difficult to distinguish from *S. purpurea* ssp. *purpurea.* Further field work, common garden experiments, and genetic analysis on *S. purpurea* ssp. *purpurea* and ssp. *venosa* var. *venosa* are required to clarify their range limits and relationships.

These data illustrate that a comparative analysis of seed germination, in groups for which some phylogenetic information exists, can be used to test hypotheses about the relative strength of environmental factors and phylogeny on the evolution of seed traits. The results presented here support earlier studies that pointed to strong environmental control on seed characters (e.g., Leishman, Westoby, and Jurado, 1995; Lord, Westoby, and Leishman, 1995; Baskin and Baskin, 1998). Although seed characters show some degree of covariance among species, this covariance appears to be related more to geography than to phylogeny. Additional comparative studies on phylogenetically well-known groups with broad geographical distributions are needed to determine the relative importance of local environment and phylogenetic history on early life histories of plants.

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