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Isozyme Variation in *Sisyrinchium sarmentosum* (Iridaceae)

Abstract

Sisyrinchium sarmentosum is a rare, duodecaploid plant endemic to the Mt. Adams and Mt. Hood areas of Washington and Oregon. Isozyme variation in six Washington populations was assessed to infer genetic variation in this species. Five of the six sampled populations were monomorphic for the sixteen enzymes surveyed, although at one putative locus one of these populations was monomorphic for an enzyme band pattern not seen in any other population. The sixth population had low levels of variation in two enzymes. This low level of variation has often been observed in very rare species and high polyploids. Apparently, the species has recently gone through a bottleneck, perhaps during its recent origin as a species.

Introduction

Ideally, management practices designed to preserve rare plant biodiversity are based in part on an understanding of the population genetics of the rare species (Falk and Holsinger 1991). For practical reasons, such practices are usually based on the generalization that narrowly endemic species usually exhibit low genetic variation (Hamrick et al. 1991). However, individual endemic species vary widely in their genetic diversity (Karron 1991, Gitzendanner and Soltis 2000) and in the degree to which that variation is partitioned among populations (Hamrick et al. 1991). This study uses isozyme electrophoresis to characterize the genetic variation in the narrowly endemic, high polyploid plant, *Sisyrinchium sarmentosum* Suksdorf ex Greene, and to address some management-related questions.

Sisyrinchium sarmentosum, Pale Blue-eyed Grass, is endemic to a small area of south-central Washington and northern Oregon, in the vicinity of Mt. Adams and Mt. Hood (Henderson 1976, John Gamon pers. com.). Two thirds of the known populations occur in the Gifford Pinchot National Forest of Washington (Andrea Raven pers. com.). Its habitat includes mesic to wet meadows and forest openings at elevations of 480 to 1220 m (Gamon and Raven, pers. com). *Sisyrinchium sarmentosum* is characterized by glaucous foliage and pale blue, rather small flowers with tepals that are not emarginate (Henderson 1976). It reproduces vegetatively, spreading by rhizomes at least on a scale of many centimeters (Raven pers. com.). It also sets seed, and is highly self-compatible (Henderson 1976).

Sisyrinchium sarmentosum is a duodecaploid (12X) thought to have originated within the *S. idahoense* E. P. Bicknell complex (Henderson 1976). *Sisyrinchium idahoense* is widely distributed from British Columbia south to northern California, east to the Rocky Mountains. All Pacific Northwest *Sisyrinchium* species are tetraploid to duodecaploid (Henderson 1976). *Sisyrinchium idahoense* is usually octoploid (8X), but duodecaploid populations are known from central Oregon (Henderson 1976). No duodecaploid species or populations of *Sisyrinchium* are known to be sympatric with *S. sarmentosum,* but we are not aware of chromosome counts for *S. idahoense* from the range of *S. sarmentosum.* Artificial hybridization experiments indicate that many Pacific Northwest species are interfertile, and artificial crosses between *S. sarmentosum* and duodecaploid individuals of *S. idahoense* produce seed (Henderson 1976). Pollination among *Sisyrinchium* species results in seed set only when the pollen chromosome number equals or exceeds that of the ovule (Henderson 1976).

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Management-related issues led to this study. First, censusing of *Sisyrinchium sarmentosum* is complicated by the rhizomatous growth form of these plants. The inconspicuous shoots (ramets) can be counted, but many of them may belong to the same genetic individual (genet). Therefore, censusing genets is impossible, and the genetic effect of a reduction in the population of ramets is not obvious. Second, the observation that cattle eat and trample the flowering and fruiting stems of *S. sarmentosum,* significantly reducing seed set (Raven pers. com.), led to questions about the role of seed production in *S. sarmentosum* populations. Seed production affects both demographics and genetic diversity. Two demographic effects of seeds are obvious; they are the only method for producing new genetic individuals (genets), and seeds, rather than rhizomes, are the units of long-distance dispersal. The genetic effect of seed production in this vegetatively spreading, self-compatible, potentially long-lived, perennial plant depends on its genetic diversity, its breeding system, and the rate of seedling establishment. None of these factors is known for *S. sarmentosum,* but isozyme analysis can often shed light on the first two. An additional concern is the potential for outbreeding depression (Barnett and Kohn 1991) or blurring of species boundaries due to hybridization between *S. sarmentosum* and sympatric *S. idahoense.* Therefore, we evaluated the hypothesis that hybridization has occurred in the one *S. sarmentosum* population observed to be morphologically diverse.

Methods

This study used plants from the six populations of *S. sarmentosum* in the Gifford Pinchot National Forest that could be relocated and were large enough for sampling (Table 1). Leaves were collected in 1997 and transported on ice to the National Forest Genetic Electrophoresis Laboratory (NFGEL). Based on foliage color, the Little White Salmon River samples were believed to include *S. idahoense, S. sarmentosum,* and their hybrids. A tentative identification was recorded for each sample from that population.

Sample Preparation

Samples were prepared using standard NFGEL procedures (Anonymous 1995). A leaf sample approximately 8-11 cm long was ground in a cold mortar with 400 microliters of a Tris buffer pH 7.5 (Gottlieb 1981). The resulting slurry was transferred to wells in a microtiter plate and stored at −70 °C. In preparation for electrophoresis, slurry was thawed and absorbed onto 3 mm wide wicks prepared from Whatman 3MM chromatography paper.

TABLE 1. *Sisyrinchium sarmentosum* populations used in this study. All populations were located in Skamania County, Washington, in the Mt. Adams Ranger District of the Gifford Pinchot National Forest. "Outside" and "inside" refers to the location relative to the grazing exclosure at Cave Creek. Except as noted, the most important grazing animals were cattle. FS Rd. = Forest Service Road.

Population	Location	Latitude/ Longitude	Collection Date	Grazing Status	Sample Size
Cave Creek inside	north of FS RD 8631, ca. 0.5 mile south of the jct. of Rds. 8631 & 8620	45.9333°N 121.6437°W	2 July 1998	light for three years	35
Cave Creek outside	north of FS RD 8631, ca. 0.5 mile south of the jct. of Rds. 8631 & 8620	45.9333°N 121.6437°W	30 June 1998	Intense	35
Cayuse Meadows	ca. 400 feet north of Cayuse Meadow and west of Meadow Creek	46.1076°N 121.7892°W	24 July 1998	light (big game)	35
Little White Salmon River	small meadow south of FS Rd. 18, 0.25 mile south of jct. of Rds. 18 and 18.068	45.8317°N 121.6645°W	19 July 1998	light	35
Peterson Prairie	the northwest section of Peterson Prairie	45.9769°N 121.6645°W	9 July 1998	none until fall	35
South Prairie	north end of the prairie, ca. 0.6 mile south of junction of FS Rds. 860 & 8620.130	45.9188°N 121.7061°W	10 July 1998	light to moderate	35

Electrophoresis

Methods of electrophoresis followed the general methodology of Conkle et al. (1982) except that most enzyme stains are somewhat modified (Anonymous 1995). A lithium borate electrode buffer (pH 8.3) was used with a Tris citrate gel buffer (pH 8.3) (Conkle et al. 1982) to resolve the enzymes alcohol dehydrogenase (ADH), fluorescent esterase (FEST), leucine aminopeptidase (LAP), malic enzyme (ME), phosphoglucomutase (PGM), and phosphoglucose isomerase (PGI). A sodium borate electrode buffer (pH 8.0) was used with a Tris citrate gel buffer (pH 8.8) (Conkle et al. 1982) to resolve glutamate-oxaloacetate transaminase (GOT), glucose-6-phosphate dehydrogenase (G6PDH), triosephosphate isomerase (TPI), and uridine diphosphoglucose pyrophosphorylase (UGPP). A morpholine citrate electrode and gel buffer (pH 6.1) (Conkle et al. 1982) was used to resolve diaphorase (DIA), isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), and phosphogluconate dehydrogenase (6PGD). All enzymes were resolved on 11% starch gels. Stain recipes for enzymes follow Anonymous (1995) except that GOT was stained using the recipe from Wendel and Weeden (1989). Two people independently scored each gel. When they disagreed, a third person resolved the conflict. For quality control, 10% of the individuals were run and scored twice.

Data Analysis

Sisyrinchium sarmentosum is duodecaploid (Henderson 1976). Therefore, in the most simple case, a single band may represent the activity of enzymes coded by six loci on 12 chromosomes. We treated each enzyme as if it were encoded by one putative locus, except that we treated PGI and TPI as encoded by two putative loci (actually two putative sets of homologous loci) each (Table 2). Therefore, we analyzed these data as if they consisted of sixteen isozymes (16 putative loci) (Table 2), referred to in the text and tables as sixteen enzymes. Gels were scored for banding pattern differences and band presence/absence. In this type of analysis, an enzyme banding pattern is considered a single phenotypic trait at which variation can be assessed.

Samples from the Little White Salmon River were analyzed as a single population of *S. sarmentosum*.

Phenotypic diversity measures were calculated from both band presence/absence and multi-band patterns (Table 3). For presence/absence data,

TABLE 2. Enzyme band pattern frequencies for *Sisyrinchium sarmentosum* populations. "Band Patterns" are schematic diagrams of bands observed for each pattern; 1 = presence of a band; 0 = absence of a band seen in other patterns of that enzyme.

Enzyme	Pattern	Cave Creek outside	Cave Creek inside	Cayuse Meadows	Peterson Prairie	Little White Salmon River	South Prairie	Band Pattern
ADH	A	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1111
DIA	A	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	11
FEST	A	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	11
GOT-1	A	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1
G6PDH	A	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1
IDH	A	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1
LAP	A	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1
MDH	A	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1
ME	A	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1
6PGD	A	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	11
PGI-1	A	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1
PGI-2	A	1.0000	1.0000	1.0000	1.0000	1.0000	0.9091	110
	B	0.0000	0.0000	0.0000	0.0000	0.0000	0.0909	111
PGM	A	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	11
TPI-1	A	1.0000	1.0000	0.0000	1.0000	1.0000	1.0000	01
	B	0.0000	0.0000	1.0000	0.0000	0.0000	0.0000	11
TPI-2	A	1.0000	1.0000	1.0000	1.0000	1.0000	0.9118	1110
	B	0.0000	0.0000	0.0000	0.0000	0.0000	0.0882	1111
UGPP	A	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	11

TABLE 3. Genetic variation in *Sisyrinchium sarmentosum* populations, revealed by isozymes treated as sixteen putative loci. N = mean sample size/enzyme. %P = percent polymorphic putative loci; see methods for definition. P.I. = polymorphic index. S.W. = Shannon-Weaver diversity index. s.d. = standard deviation. "Outside" and "inside" refers to the location relative to the grazing exclosure at Cave Creek.

Population	N	# bands	# unique bands	% P	patterns/ locus	P.I	S.W.	% S.W. among populations
TOTAL	200	30	—	18.75	1.1875 (0.403)	0.3480	0.0387	0.1070
Cave Creek inside	35	27	0	0.00	1.0000	0.0000	0.0000	
Cave Creek outside	35	27	0	0.00	1.0000	0.0000	0.0000	
Cayuse Meadows	35	27	1	0.00	1.0000	0.0000	0.0000	
L. White Salmon River	26	27	0	0.00	1.0000	0.0000	0.0000	
Peterson Prairie	35	27	0	0.00	1.0000	0.0000	0.0000	
South Prairie	34	29	2	12.50	1.1250	0.3261	0.0377	
Mean/population	33	27	0.5	2.08	1.02	0.0544	0.0064	
s.d.	(3.6)	(0.82)	(0.84)	(5.1)	(0.051)	(0.133)	(0.015)	

phenotypic diversity was measured by a polymorphic index (PI), based on the frequency of occurrence of each band. For multi-band patterns, phenotypic diversity measures include: (1) the number of bands found in each plot, (2) percent of stains that yield more than one band pattern, (3) the average number of band patterns per stain in each plot, and (4) Shannon-Weaver Diversity Index values (Shannon and Weaver 1949). The Shannon-Weaver Diversity Index uses the frequency of each band pattern in each plot. The larger the Shannon-Weaver Index, the more diverse the plot. The distribution of the total variation within and among plots was determined by partitioning the total Shannon-Weaver Diversity Index. Band pattern frequencies and the Shannon-Weaver Diversity Index were calculated using Popgene (Yeh et al. 1997).

Isoelectric Focusing

Enzyme banding patterns in the Little White Salmon River populations were analyzed with isoelectric focusing (Acquaah 1992, Westermeier 1997). Methods of isoelectric focusing follow HyPure procedures (Anonymous 1994-1998). Tissue samples varied from 0.12 to 0.51 gram, prepared with proportional amounts of loading buffer. Polyacrylamide gels were stained for the enzymes acid phosphatase (ACP), diaphorase (DIA), esterase (EST), malate dehydrogenase (MDH), peroxidase (PER), and phosphohexose isomerase (PHI). Both buffers FS5480 (pH 3-10) and FS5080 (pH 4-5) were used to resolve ACP, EST, MDH, and PER. Buffer VG1110 (pH 3-7) was used for DIA and PHI. Buffer VG1080 (pH 3-10) was used for DIA, EST, MDH, PER, and PGM. Further information on buffer composition is available in Anonymous (1994-1998).

Results

Two adjustments were made to the data set before final analysis. One individual from South Prairie was removed from the analysis because it had unique patterns at 15 of the 16 enzymes sampled; it was presumed to be of a graminoid species rather than *S. sarmentosum*. Samples from the Little White Salmon River population were all treated as *S. sarmentosum* because their enzyme band patterns were all identical.

Although most isozyme patterns were simple and invariant, some enzymes exhibited multiple bands in all individuals (Table 2). Because of lack of variation in this species, band segregation could not be used to identify specific alleles and loci. Therefore, we treated the band pattern as the unit of analysis. However, each gel stained for PGI or TPI exhibited two regions of activity, which we considered to represent isozymes from different subcellular compartments (Gottlieb 1982). Therefore, we divided the PGI and TPI bands into two units. We refer to these units as putative loci.

Five of the six *S. sarmentosum* populations tested using starch-gel electrophoresis were monomorphic for all 16 enzymes examined (Table 2). One of these (Cayuse Meadow) was monomorphic

TABLE 4. Genetic variation in *Sisyrinchium* populations, revealed by isozymes, and in a rare *Iris*. N = mean sample size/enzyme. Pops = number of populations in the study. Enzymes = the number of different stains used. Loci* = the number of different regions on the gel treated as if they were loci. %P Enz. = percent of enzyme stains that revealed polymorphisms. %P = percent polymorphic putative loci or enzymes treated as loci; see methods for definition. Patterns/locus = patterns per putative locus. S.W. = Shannon-Weaver diversity index. Data for *S. montanum* and *S. septentrionale* reanalyzed as band patterns by the authors, with all high polyploid populations treated as *S. montanum*.

Taxon	N	Pops	Enzymes	Loci*	%P Enz.	%P	Patterns/ locus	S.W.	Source
S. montanum	36	5	13	24	61	54	1.9583	0.2661	Pavek 1989
S. sarmentosum	200	6	14	16	14	19	1.1875	0.0387	this study
S. septentrionale	6	1	13	24	46	21	1.2500	0.1417	Pavek 1989
Iris lacustris	229	9	10	22	0	0	1.000	0.0000	Simonich & Morgan 1994

for a TPI1 band pattern found in no other sampled population (Table 2). One population (South Prairie) was polymorphic at two enzymes, PGI2 and TPI2. Band patterns obtained were simple and consisted of a single band in 43% of the patterns. An additional 37% of the patterns consisted of two bands each, 10% were made up of three bands, and 10% were made up of four bands (Table 2). The Cayuse Meadows population had one unique band, and the South Prairie population had two. *Sisyrinchium sarmentosum* had very low levels of enzyme variation (Table 3). The levels of variation observed in this species was lower than that observed previously in *Sisyrinchium* species (Table 4).

In the Little White Salmon population, isoelectric focusing revealed approximately 186 bands, counting bands on each enzyme/buffer combination separately. Eleven (6.5%) of these bands were polymorphic (Table 5). No band differentiated the putative parental samples or the putative hybrids.

Discussion

A genetic interpretation of *S. sarmentosum* isozyme band pattern data would be preferred over a phenotypic analysis, because a genetic analysis provides information about population genetics and results can be compared directly with compilations of plant isozyme genetics (Hamrick and

TABLE 5. Polymorphic bands revealed by isoelectric focusing in the Little White Salmon population of *Sisyrinchium*. Sampled individuals are numbered. 1 = band presence; 0 = band absence. * = same band revealed on buffers VG1080 and FS5080. See Methods for enzyme abbreviations.

		S. idahoense											Putative hybrids												*S. sarm.*		
Enz.	Buffer	1	2	3	4	5	6	7	8	9	10	11	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3
DIA	1080	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
EST	1080	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1
EST	1080	0	0	1	0	0	1	1	1	0	0	0	0	0	1	1	1	0	0	1	0	0	0	0	0	0	0
EST	5080	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	1	1	0	0	1	1	1	0	1	1
EST	*	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	1
MDH	5080	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
PER	5080	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
PER	5480	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0
PER	5480	1	1	0	1	1	1	0	1	1	1	1	0	1	0	0	0	0	0	1	0	1	1	0	0	0	0
PHI	1080	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PHI	1110	1	1	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Godt 1990). Two factors made genetic interpretation of *S. sarmentosum* enzyme band patterns impossible. First, almost no bands segregated within or among populations, and therefore the number of loci and their relationships to specific alleles were difficult to judge. Second, uncommon alleles may escape detection because *S. sarmentosum* is duodecaploid (12X) (Henderson 1976). For example, the most obvious interpretation of a pattern consisting of a single band is that the genotype producing it is AA AA AA AA AA AA. However, the true genotype might be AA AA AA AA 00 00 if gene silencing occurs (Crawford 1990). In addition, the one visible band may result from the genotype AA AA AA AA AA AB if dosage regulation of gene expression occurs and limits the amount of enzyme produced from all these loci jointly. The enzymes produced by the B allele would be so rare compared to the enzymes produced by the A allele that they would probably not show up on the gel (AB heterodimers could be produced because of interlocus interactions, but these would still occur at a small frequency relative to AA homodimers, and therefore likely be invisible). If the plant is autopolyploid, an allele may be shared among all the homologous loci, and the plant might have a genotype such as AA AA AA AB BB (Soltis and Rieseberg 1986). If a two- or three-band pattern shows up occasionally, one must wonder how many copies of the alternative allele must be present, and/or how many null (silenced) alleles must be present, before the enzyme produced by that alternative allele can be observed. Phenotypic analysis provides a way of using isozyme data, such as that in *S. sarmentosum,* which cannot be interpreted genetically (e.g. Chung et al. 1991).

Enzyme variation in *S. sarmentosum* (as detected in starch gel electrophoresis) was extremely limited. Comparison with genetic analyses of plant isozyme data (Hamrick and Godt 1990) is instructive, if certain trends are kept in mind. First, the percent polymorphic enzymes is often higher than the percent polymorphic loci, because a single enzyme stain may reveal both variable and monomorphic loci. Second, the number of patterns reported per enzyme (or per putative locus) is often somewhat greater than the number of alleles per locus, because every combination of two alleles can produce a different pattern. Also, patterns are not independent. The addition of one allele for a dimeric enzyme will produce two additional bands. Third, the Shannon-Weaver diversity index may overestimate differences, because patterns produced by homozygotes are considered completely different from patterns produced by heterozygotes.

Sisyrinchium sarmentosum appears to have much less enzyme variation seen in the average narrowly endemic taxon (Hamrick and Godt 1990, Gitzendanner and Soltis 2000). For example, *S. sarmentosum* had 19% polymorphic enzymes, compared with the average of 26% polymorphic loci for endemic species, and 1.19 patterns per enzyme, compared to 1.80 alleles per locus in endemic species (Hamrick and Godt 1990). The variation in *S. sarmentosum* is also lower than that reported for other *Sisyrinchium* species of the Pacific Northwest, despite much smaller sample sizes in those other studies (Table 4), and the variation in those species was considered low (Pavek 1989). Like the monomorphic *Iris lacustris* Nutt. (Simonich and Morgan 1994), *Sisyrinchium sarmentosum* has characteristics (self-compatibility, restricted range, and moist habitat) associated with extremely low isozyme variation (Cole and Biesboer 1992).

Why are enzyme patterns in *S. sarmentosum* populations so nearly invariant? There are several possibilities. First, a high polyploid taxon that arose once would include a very limited sample of the genetic variation present in the parental species because it includes only the variation present in the one (or two) individual(s) that were parent(s) of the first high polyploid individual (Crawford 1990). Second, low population sizes for several generations would reduce variation, even if the population was initially variable. *Sisyrinchium sarmentosum* has had small populations and a very limited range since it was collected by Suksdorf (Gamon pers. com.). Third, low variation could characterize a species that reproduces entirely vegetatively. However, vegetatively reproducing taxa are often highly variable (Ellstrand and Roose 1987), and *S. sarmentosum* appears to reproduce sexually. It produces abundant seed when grazing does not prevent seed set (Raven pers. com.). The seed is probably produced sexually, because *S. sarmentosum* fails to produce seed in certain artificial crosses (Henderson 1976). A fourth hypothesis is that the observed low variation results from many generations of selfing. Selfing produces a preponderance of homozygous individuals,

but whether selfing populations are genetically variable or not depends on the variability of founding individuals and whether the population has gone through a prolonged period of low size. Some largely selfing populations are highly variable (Knapp and Rice 1996). We favor the hypothesis that duodecaploid *S. sarmentosum* arose once (or rarely) from an ancestor with lower chromosome number.

Sometimes isozyme analysis can determine if a species is an autopolyploid or allopolyploid, because allopolyploids usually exhibit fixed heterozygosity at many loci (Crawford 1990). *Sisyrinchium sarmentosum* has consistent multiple-band patterns for several enzymes, which may represent fixed heterozygosity at homologous loci. However, that does not help determine the origin of polyploidy in this species. The ancestor of *S. sarmentosum* was probably polyploid itself. We are not aware of information about the isozyme variation in *S. idahoense,* but duplicated loci were inferred for tetraploid *S. septentrionale* E. P. Bicknell (Pavek 1989). Duodecaploid *Sisyrinchium sarmentosum* may be an autopolyploid derived from an allopolyploid ancestor, but isozyme analysis of *Sisyrinchium sarmentosum* alone cannot eliminate other hypotheses.

The variation revealed by isozyme electrophoresis is not always representative of variation in physiology and other traits (Barrett and Kohn 1991, Hamrick et al. 1991), but there are several reasons to think *S. sarmentosum* has little genetic variation of any kind. The species shows less floral variation than most other Pacific Northwest species of *Sisyrinchium* (Henderson 1976). Morphological variation observed during recent field work is limited; single plants with white or twelve-tepaled flowers were observed in the Cave Creek population (Raven pers. com.), and foliage color varied only in the Little White Salmon River population. In addition, all populations occupy similar, open, wet to somewhat dry habitats within a very limited range. The apparent lack of genetic variation in *S. sarmentosum* does not imply that the species suffers inbreeding depression, because invariant plants may lack harmful genetic variants (Allard et al. 1986, Husband and Schemske 1997).

Variation in foliage color and tepal shape suggested that *S. idahoense, S. sarmentosum,* and their hybrids might have been collected at the Little White Salmon River site. That hypothesis was not supported by the results of this study. The plants all had the same enzyme bands as revealed by starch gel electrophoresis (Table 2). Isoelectric focusing of enzymes known to be highly variable and to exist in multiple forms revealed limited variation among individuals in this population but no consistent differences among the samples labeled as different species. If the sample identifications are correct, this uniformity of enzyme variation among species suggests that *S. sarmentosum* is the recently derived autopolyploid descendent of the widespread *S. idahoense.* However, the observed variation in foliage color may represent intraspecific variation in *S. sarmentosum.* Other *Sisyrinchium* species in the Pacific Northwest are known to exhibit variation in foliage color (Henderson 1976). This point could be clarified by additional research that included samples from single-species stands of *S. idahoense* and voucher specimens with flowers pressed in the field, to preserve the floral features necessary to confirm species identification (Henderson 1976).

Although the Shannon-Weaver diversity index indicates that populations of *Sisyrinchium sarmentosum* are little differentiated, gene flow among populations may be low (Table 3). All individuals in the Cayuse Meadow population had a band at TPI1 that was observed in no other population (Table 2). This situation could be stable only if gene flow between Cayuse Meadow and any other sampled population were very low.

Because *S. sarmentosum* had such low enzyme pattern variation and most populations were monomorphic, we cannot use enzymes to answer certain management-related questions. Enzyme patterns cannot delimit genets (clones), and therefore censusing genets remains problematic. Enzyme patterns cannot reveal the relative importance of sexual and vegetative reproduction in this species, and therefore decisions about protecting seed set cannot be made on the basis of enzyme patterns. The differentiation between the Cayuse Meadow population and the others hints at limited gene flow, suggesting that population establishment through long-distance seed dispersal events (which requires seed production) is important in the patterning of genetic diversity in this species. Perhaps different molecular techniques, such as isoelectric focusing, RAPDs, or ISSRs, can generate enough variation to address these questions more effectively.

The lack of observed variation suggests that few restrictions need be placed on programs to increase the *S. sarmentosum* population through transplants. Moving plants (seeds or rhizomes) among populations may be undesirable because it might lead to loss of rare genetic variations now restricted to single populations. However, as long as gene flow is as limited as it appears, establishment of new populations at sites not now occupied by *S. sarmentosum* need be restrained only by the pragmatic test that populations can be established only where the plants can grow.

This study suggests that preserving enzyme (and genetic) diversity in *S. sarmentosum* requires preserving many populations because some, like Cayuse Meadow, contain unique alleles. Also, a large population should be maintained at South Prairie, the only population known to be variable. However, this study also suggests that management practices directed toward demographics, rather than genetics, can be effective in long-term preservation of *S. sarmentosum* biodiversity. Unless populations are extremely small (<50), demographic changes are more important than loss of genetic variability for long-term population survival (Menges 1991, Schemske et al. 1994). This is especially true in a species such as *S. sarmentosum* that has little genetic variability.

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