



Sisyrinchium sarmentosum
Population and Taxonomic
Status
2014

Prepared for
U.S. Fish and Wildlife Service

Prepared by
Joseph Arnett
August 7, 2014



**Monitoring *Sisyrinchium sarmentosum*
(pale blue-eyed grass)**

Including Taxonomic Review

Award # F12AP00782

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Prepared for:

U.S. Fish and Wildlife Service
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Region 1
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by

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Acknowledgments

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Introduction

A petition was submitted to the U.S. Fish and Wildlife Service (FWS) on July 24, 2007 to list *Sisyrinchium sarmentosum* under the Endangered Species Act, prompting the FWS to initiate a status review of the species. This current report, and a previous Washington Natural Heritage Program (WNHP) report (Arnett 2012) are both in support of that FWS status review. Arnett (2012) summarized the status of all Washington populations through 2011; this report incorporates inventory and monitoring conducted in 2012 and 2013, efforts to develop an appropriate monitoring methodology, and a summary of the status of ongoing genetic analysis. This work was done in collaboration with the Rare Care program from the University of Washington and Interagency Sensitive and Special Status Species Program (ISSSSP) of the U.S. Forest Service and Bureau of Land Management.

Evaluating the distribution and abundance of a species, and from this, conservation status, cannot appropriately be done until the taxonomy of the species is resolved. In this case the relationship between *Sisyrinchium sarmentosum* and *S. idahoense* remains unclear. Photographs of examples of these two species are presented in figures 2 and 3. As reported in Arnett (2012), we conferred with Anita Cholewa at the University of Minnesota and confirmed that the populations of *Sisyrinchium* from the Naches District of the Wenatchee National Forest, previously reported as *S. sarmentosum*, were best considered to be *S. idahoense*. Dr. Cholewa is co-author of the Flora of North America treatment of *Sisyrinchium* (Cholewa and Henderson 2002). The questions that remain pertain to plants at some of the sites that appear intermediate between *S. sarmentosum* and *S. idahoense*. We participated in collection of leaf material for molecular analyses and seeds for conducting a common garden experiment at the University of Washington.

Distribution of *Sisyrinchium sarmentosum* in Washington

Sisyrinchium sarmentosum is known from fifteen confirmed element occurrences in Washington (following standards established in NatureServe 2002). One of these, along Ice Caves Road, is a historical occurrence, last observed in 1971. The occurrences are listed in Table 1 and mapped in Figure 1.

Development of a *Sisyrinchium sarmentosum* monitoring protocol

A working group formed in late 2010 to develop a monitoring protocol for *S. sarmentosum*. Team members included Jim Alegria and Carol Apple (Region 6 Forest Service statisticians), Joe Arnett (WNHP rare plant botanist), Andrea Ruchty (South Zone botanist for the Gifford Pinchot National Forest), and Kelly VanNorman (Inventory Coordinator for ISSSSP). Phone conference calls were made in November and December 2010, and January through June 2011, and a draft monitoring protocol was developed by Jim Alegria in January 2011. This plan included a fairly precise method of recording the perimeters of each occurrence, permanently marking the vertices of each polygon, and then sampling within each polygon to determine

Table 1. Summary of *Sisyrinchium sarmentosum* element occurrences (EOs) in Washington. Highlighted rows indicate that plants appearing intermediate between *S. sarmentosum* and *S. idahoense* have been observed at this location.

| EO #* | EO Rank* | Owner | Location | Notes |
|-------|----------|---------------|-------------------------------------|---|
| 3 | H | Private | Ice Caves Road | A small population reported by D. Henderson in 1971. Attempts to relocate have been unsuccessful. |
| 8 | E | Private/ DNR | Trout Lake | Population estimates in the hundreds. Private land has not been surveyed. Wright and Bugner last observed in 2013. |
| 9 | AB | USFS-GPNF | South Prairie | A large complex occurrence, composed of 12 subpopulations. Ruchty, Arnett, and Gibble last monitored in 2012, when 6,103 plants were counted in the subpopulation west of Lost Creek. In 2006, 10,000 plants were estimated at South Prairie. |
| 10 | BC | USFS-GPNF | Little Mosquito Lake/Tillicum Creek | Many revisits since 1971. Raven reported it as declining. Gibble reported 42 stems in 2011 at Little Mosquito Lake; Scott reported 14 plants in 2012 and Arnett and Scott reported 1 plant in 2013 at Tillicum Creek. |
| 12 | CD | USFS-GPNF | Cave Creek | Large population, several different areas, apparent hybrids present. Ruchty, Scott, and Deshong last observed in 2012. Estimated total size over 10,000 plants. |
| 17 | | Private | Schoolhouse Meadow | Small population, grazed and previously plowed. Gamon last observed in 1986. |
| 18 | E | USFS-GPNF | Little White Salmon River | Miller observed hybrids in 2009. All plants were observed to be identical in isozyme banding. In 2000, 500-1,000 plants observed. |
| 19 | E | USFS-GPNF | Peterson Prairie | Large population, <i>S. idahoense</i> ; apparent hybrids present. Arnett et al. last observed in 2012. |
| 20 | E | USFS-GPNF | Cayuse Meadow | Plants appear more purple than usual. Isozyme analysis by Wilson et al. revealed banding pattern unique to this population. Scott and Raven reported 100+ plants in 2004. |
| 24 | | USFS/ Private | Bergen Road | Large population, In 2003 Raven reported 2,000 to 3,000 plants and that most plants present appear to be hybrids. |
| 25 | E | USFS-GPNF | Falls Creek horse camp | Large population, with lumpy distribution. Scott last observed in 2004, estimated 1,000 clumps. |
| 28 | E | USFS-GPNF | Lost Meadow | Small population. Ruchty reported 30 plants in 2004. |
| 31 | E | USFS-GPNF | Rd. 24 Spur Meadow | Ruchty reported one small clump in 2011; <i>S. idahoense</i> is also present. |
| 32 | E | USFS-GPNF | East of North Butte | Hobbs and Hobbs reported 17 plants in 2012; Arnett revisited in 2013. |
| 33 | E | USFS-GPNF | Lost Creek Spring | Saul et al. reported 1 plant in 2012. |

*In NatureServe methodology, an Element Occurrence (EO) is roughly analogous to a population, a clustering of individuals defined by specified distances between them. EO Rank is an indicator of condition: A is the highest quality, D is the lowest quality, and E indicates that the EO is documented, but its condition has not been ranked.

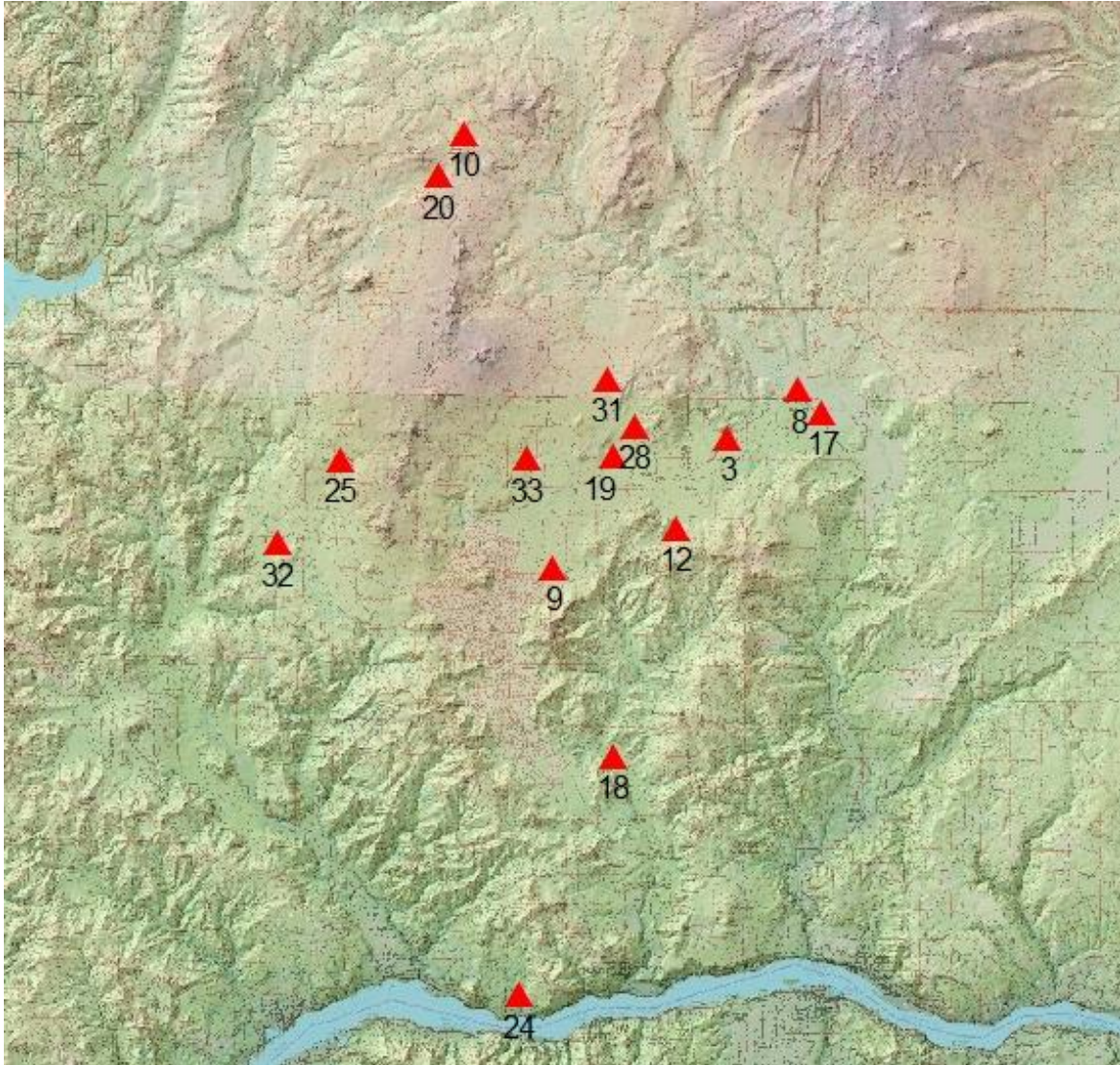


Figure 1. Overview of the distribution of *Sisyrrinchium sarmentosum* Element Occurrences (EOs) in Washington. Numbers below each triangle are EO numbers, listed in Table 1.

Sisyrrinchium densities within each. The protocol was quite labor intensive, and it assumed that polygon boundaries would not change appreciably from year to year. The method, as drafted, would require using metal detectors to relocate rebar markers, and survey grade GPS equipment, possibly done by engineering staff rather than field botanists. In the summer of 2011, efforts were made to implement the plan at a few selected sites. WNHP staff participated in a visit to Peterson Prairie to test the method, but it proved to be impractical in the field; the species is cryptic under most conditions, and often extremely patchy in distribution. Defining static



Figure 2. *Sisyrinchium sarmentosum* from South Prairie.



Figure 3. *Sisyrinchium idahoense* from Boistfort, Lewis County, WA.

polygon boundaries was not practicable, and the patchiness within population boundaries would have necessitated an extremely large number of plots to provide statistically valid population estimates. *Sisyrinchium* plants are extremely difficult to see when not flowering, which is transitory, and the flowers open and close in response to sunlight during the course of a day. An additional complication is that *S. sarmentosum* and *S. idahoense* cannot readily be distinguished from each other unless they are flowering. The difficulties in 2011 were compounded by the extremely wet year and lingering snowpack. In the process of attempting to monitor *S. sarmentosum*, additional or “satellite” occurrences were found in several areas, indicating that the distribution of the species was not as well-known as had been assumed. In general, the monitoring methodology developed by the working group was not practical. An alternative approach, to record GPS positions of clusters of plants, has usually been followed. Within each cluster a count is made of *Sisyrinchium sarmentosum* plants.

In order to get a more complete inventory of potential habitat for *S. sarmentosum*, and to focus searches for additional populations, a habitat model was developed in 2012 by Richard Helliwell, a U.S. Forest Service modeler, using MaxEnt software (Helliwell 2012). The WNHP was not part of this effort, but U.S. Forest Service staff and Rare Care volunteers did field verification of selected plots in 2012.

In 2012 and 2013, monitoring generally used GPS points to record the locations of clusters of plants, and complete counts of plants were made rather than sampling. These counts, identified with accurate locations, provide a precise record of distribution and abundance, and comprise a record that could be used in the future to monitor trend. As an example, Figure 4 shows an array of points collected at the large population of *S. sarmentosum* at Lost Creek Meadow in 2012. This array of points is recorded as a polygon in the WNHP Biotics database; point specific data is maintained in other WNHP paper and electronic files. The Element Occurrences and Source Features of *Sisyrinchium sarmentosum* in the WNHP Biotics database are included in Appendix A. The point data have been used to generate Element Occurrences following standard Natural Heritage Methodology (NatureServe 2002).

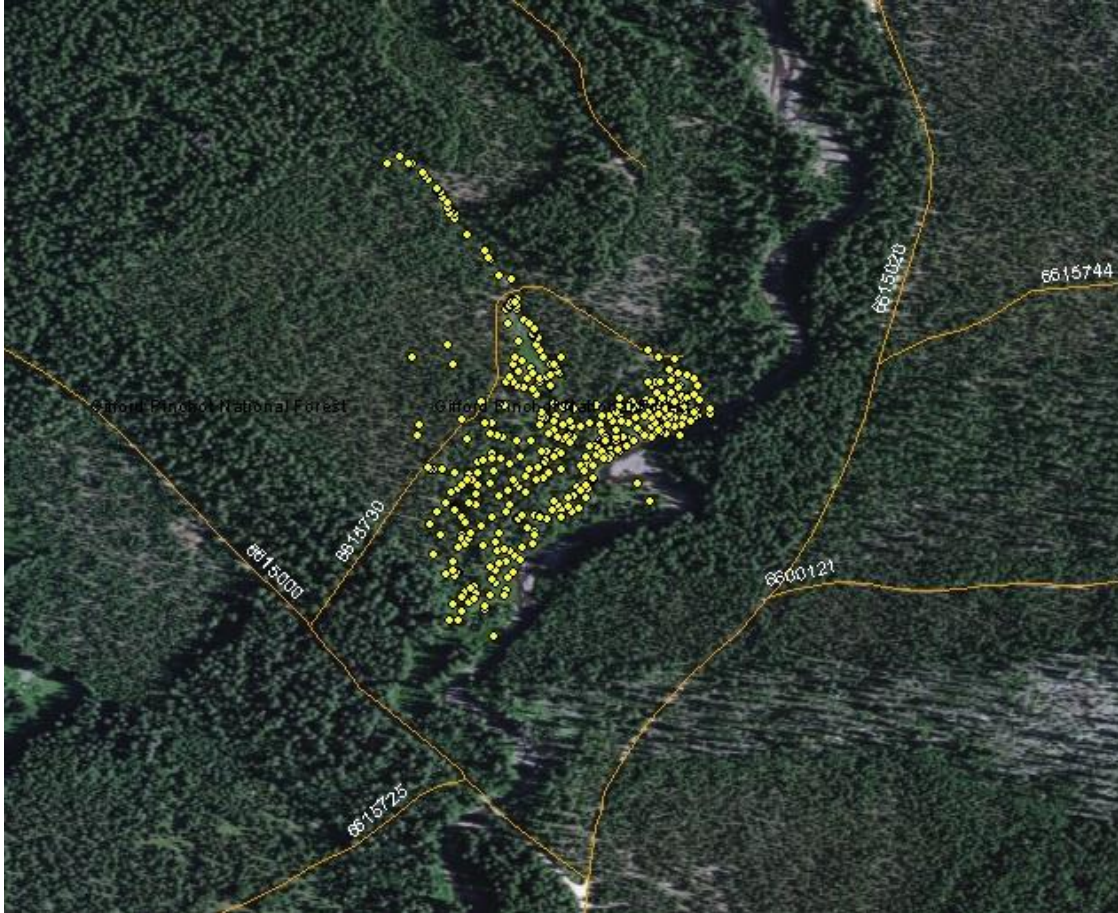


Figure 4. Array of clusters of *Sisyrrinchium sarmentosum* at Lost Creek Meadow, 2012.

Taxonomy of *Sisyrrinchium* in Washington

“*Sisyrrinchium* is a complex polyploidy taxon in which the species are not always easily distinguished.”

Cholewa and Henderson 2002

Hybridization in *Sisyrrinchium*

Cholewa and Henderson (2002) report that ploidy number appears to be a strong barrier to hybridization. Cholewa (pers. com.) has not seen hybrids in the field, though she and Henderson made crosses in the lab. *S. sarmentosum* is $n=48$, and most *S. idahoense* is $n=32$. However, *S. idahoense* is extremely variable, and Groberg et al. (2010) reported *S. idahoense* var. *idahoense* in western Oregon as $n=48$; The FNA (Cholewa and Henderson 2002) also reports that *S. idahoense* var. *idahoense* can be $n=48$ as well as $n=32$.

Raven (2003) reported on Henderson’s work (1972, 1976) performing hand pollinations in the greenhouse showing hybrids between *S. sarmentosum* and *S. idahoense*. Field observations also suggest hybridizations in nature, based on flower color. Apparent hybrids were observed at Cave

Creek, Little White Salmon, Peterson Prairie, and Bergen Road. Apparent hybrids at Little White Salmon flowered earlier than typical *S. sarmentosum*.

DeWoody and Hipkins (2006) performed isozyme and Random Amplified Polymorphic DNA (RAPD) analyses of Washington plants from one population of *S. sarmentosum*, two populations of *S. idahoense*, and two populations of unknown *Sisyrinchium* plants from sites where *S. sarmentosum* is known to occur. They also analyzed one population of *S. sarmentosum* from Oregon. They asserted that hybridization can only be considered to be present if differentiation among populations of different species is greater than between any two populations of the same species. Their isozyme analyses (referring to functional enzyme systems, and thus conservative) did not indicate that this was the case with these two *Sisyrinchium* species; one *S. sarmentosum* population (South Prairie) appeared to be more similar to a *S. idahoense* population (from north of the range of *S. sarmentosum*) than it was to another *S. sarmentosum* population. Consequently, their isozyme analyses could not substantiate that hybridization was present between *S. sarmentosum* and *S. idahoense*.

Their RAPD analyses (including the entire genome and thus less conservative than the functional enzyme systems assayed in isozyme analyses) revealed more variation among the populations and was successful in identifying a distinct marker for *S. sarmentosum*; however, a distinct marker was not discovered for *S. idahoense*. They concluded that DNA data was also insufficient to identify hybridization between *S. sarmentosum* and *S. idahoense*.

The ISSSSP, in discussions of this question, initiated a genetic study with four purposes: 1) to verify that *S. sarmentosum* is a distinct species, 2) to determine whether hybridization is occurring in nature between *S. sarmentosum* and *S. idahoense*, 3) to determine whether hybridization, if it is occurring, poses a threat to the genetic integrity of *S. sarmentosum*, especially in sites where both species are present, and 4) to characterize the genetic differences and similarities among *S. sarmentosum* populations, to determine whether this information might be considered in conservation planning for the species. A broader purpose of the genetic study is also to contribute to understanding genetic relationships within *Sisyrinchium*, placing *S. sarmentosum* in a broader taxonomic context. The genetic study was with the guidance of Andy Bower, U.S. Forest Service geneticist, with support from Valerie Hipkin at the National Forest Genetics Laboratory (NFGEN) in Placerville, California.

Material was collected from several outliers, as well as the core populations, and from areas where color variation suggested that hybridization had occurred. Locations of collection sites are presented in Table 2. These specimens were sent as fresh material to NFGEN, where DNA was successfully extracted. The most detailed analysis conducted was complete sequencing of the chloroplast genome; this analysis was not able to discriminate between *S. sarmentosum* and *S. idahoense*; rather, the chloroplast variation observed was better explained by geography. This analysis showed greater genetic similarity within a geographically isolated population, irrespective of flower color, than between separated examples of flowers identified as *S. sarmentosum*.

Cronn (2013) makes the point, however, that species are not defined by genetic variation, because species sampled close to the time of their divergence may still share a large part of their

genetic variation. I am interpreting this to mean that while plants we identify as *S. sarmentosum* and *S. idahoense* may yet be best regarded as distinct taxonomic entities, the genetic analyses conducted thus far do not support this distinction.

Table 2. Locations of *Sisyrinchium* collections for molecular analysis.

| Species | Site | Samples | Area | State | Notes |
|-----------------------|-----------------------------|----------|--|-------|--|
| <i>S. idahoense</i> | Camas Prairie | 25 | Willamette NF, Sweet Home RD | OR | |
| <i>S. idahoense</i> | Frog Creek Tributary | 20 | Mt Hood NF, Hood River District | OR | |
| <i>S. idahoense</i> | Camas Prairie | 20 | Mt Hood NF, Barlow RD | OR | |
| <i>S. idahoense</i> | North Fork, Rock Creek | 20 | Mt Hood NF, Barlow RD | OR | |
| <i>S. idahoense</i> | Rock Creek | 21 | Siuslaw NF | OR | |
| <i>S. idahoense</i> | Lava Lake | 24 | Deschutes NF | OR | |
| <i>S. idahoense</i> | Peterson Prairie | 24 | Gifford Pinchot NF | WA | hybrids present |
| <i>S. idahoense</i> | Forbes Point | 20 | Forbes Point, Whidbey Is., Island County | WA | |
| <i>S. idahoense</i> | Lozier Preserve | 20 | Lozier Prairie Preserve, Lewis County | WA | |
| <i>S. idahoense</i> | Conboy | 10 | Conboy NWR, Klickitat County | WA | |
| <i>S. idahoense</i> | Boistfort | 20 | Lewis County | WA | |
| <i>S. idahoense</i> | Lost Meadow | 25 | Gifford Pinchot NF | WA | |
| <i>S. idahoense</i> | Andy Creek | 14 | Okanogan-Wenatchee NF | WA | |
| <i>S. idahoense</i> | Iron Stone Mt | 10 | | WA | |
| <i>S. sarmentosum</i> | Clackamas Lake, North Shore | 20 | Mt Hood NF, Zig Zag District | OR | hybrids present; DNA at NFGEL from 2005 collection |
| <i>S. sarmentosum</i> | Little Crater Meadow | 30 | Mt. Hood NF | OR | |
| <i>S. sarmentosum</i> | Lost Meadow | 30 | Gifford Pinchot NF | WA | hybrids present |
| <i>S. sarmentosum</i> | Peterson Prairie | 24 | Gifford Pinchot NF | WA | hybrids present |
| <i>S. sarmentosum</i> | Falls Creek Horse Camp | 15 (14?) | Gifford Pinchot NF | WA | DNA already at NFGEL from 2005 collection |
| <i>S. sarmentosum</i> | South Prairie | 30 | Gifford Pinchot NF | WA | |
| suspected hybrid | Falls Creek Horse Camp | 9 (10?) | Gifford Pinchot NF | WA | |

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Appendix A: *Sisyrinchium sarmentosum* Element Occurrences in Washington, revised July 2014

In NatureServe methodology, which WNHP uses in assessing conservation status, an Element Occurrence (EO) is roughly analogous to a population, a clustering of individuals defined by specified distances between them. An EO Number is assigned to each EO within a species; an EO ID is a unique identification number that explicitly identifies each EO in the WNHP database. An EO may include several points, lines, or polygons; each is given a unique Shape ID and Source Feature ID; the former is no longer used in Biotics but is included here in reference to some Source Features to cross reference with earlier reports. EO Rank is an indicator of condition: A is the highest quality, D is the lowest quality, and E indicates that the EO is documented, but its condition has not been ranked.

| EO # | EO ID | Shape ID | Source Feature ID | EO Rank | owner | Location | notes (may pertain to the whole EO) |
|------|-------|----------|-------------------|---------|-----------|----------------------|--|
| 9 | 2781 | 14694 | 1764 | AB | USFS-GPNF | South Prairie | Large population, 24,000-27,000 plants (Raven). DeWoody & Hipkins and Wilson et al. used in analyses; Wilson found plants here dimorphic for two enzymes. most similar to Peterson Prairie. 8 or 9 distinct patches. 31Aug1893, 27June1981, 18June1984, 16July1985, 17July1990, 24July1997, 25June2003. Detailed monitoring by WNHP, RareCare, and FS on 13July2012 documented 231 clumps with count of 6,103 plants. NRIS includes report from 9July2003. New point in South Prairie EO. Susan Saul et al RareCare observation 14July2012 |
| 9 | 2781 | 14695 | 1765 | AB | USFS-GPNF | South Prairie | |
| 9 | 2781 | 14696 | 1757 | AB | USFS-GPNF | South Prairie | |
| 9 | 2781 | 14697 | 1758 | AB | USFS-GPNF | South Prairie | |
| 9 | 2781 | 14698 | 1759 | AB | USFS-GPNF | South Prairie | |
| 9 | 2781 | 14699 | 1760 | AB | USFS-GPNF | South Prairie | |
| 9 | 2781 | 14700 | 1761 | AB | USFS-GPNF | South Prairie | |
| 9 | 2781 | 14701 | 1762 | AB | USFS-GPNF | South Prairie | |
| 9 | 2781 | 14702 | 1763 | AB | USFS-GPNF | South Prairie | |
| 9 | 2781 | 27806 | 14079 | AB | USFS-GPNF | South Prairie | |
| 9 | 2781 | 27807 | 14080 | AB | USFS-GPNF | South Prairie | |
| 9 | 2781 | 27808 | 14081 | AB | USFS-GPNF | South Prairie | |
| 9 | 2781 | 27814 | 14082 | AB | USFS-GPNF | South Prairie | |
| 9 | 2781 | | 19789 | AB | USFS-GPNF | South Prairie | |
| 10 | 2783 | 2773 | 9668 | BC | USFS-GPNF | Little Mosquito Lake | 5Aug1971, 6Aug1981, 20Aug1984, 22July1985, 18July 1990, 25 plants 24Aug2001, 100-150 plants 17July2003; Raven visit in 2000, reported it as declining. Gibble reported 3 visits in 2011. The southern point, Site 1 in Gibble 2011 report. |
| 10 | 2783 | 27820 | 14083 | BC | USFS-GPNF | Little Mosquito Lake | The northern point, Site 2 in Gibble 2011 report |
| 10 | 2783 | | 19519 | E | USFS-GPNF | Tillicum Creek | J. Scott visit 31July2012; J. Arnett and J. Scott revisit to site on 18July2013. |
| 12 | 4142 | 14689 | 1754 | CD | USFS-GPNF | Cave Creek | 7,500 to 10,000 in 2000. Raven visit in 2000, apparent hybrids present. Wilson et al included in isozyme analysis. 16July1985, 19July1990, 5July1995, 28June1997. NRIS report form 4July2011. Report in NRIS labeled Coyote #304818 19July2011, 2 observations. |
| 12 | 4142 | 14690 | 1755 | CD | USFS-GPNF | Cave Creek | |

| | | | | | | | |
|----|------|-------|-------|---|---------------|------------------------------|---|
| 18 | 756 | 2789 | 9842 | E | USFS-GPNF | Little White Salmon River | Rare Care revisit unsuccessful in 2009. Raven visit in 2000, apparent hybrids appear to flower much earlier than typical SISA. Most plants present appear to be hybrids. DeWoody & Hipkins and Wilson et al. used in analyses, distinct from South and Peterson Prairies. All plants identical in isozyme banding. 1June1988, 18July1990, 16Aug1995. NRIS report for 1June2005. |
| 19 | 4910 | 7122 | 11130 | E | USFS-GPNF | Peterson Prairie | At least 200. Raven: 1,000 plants, apparent hybrids present, 1/4 to 1/3 of plants examined. DeWoody & Hipkins and Wilson et al. used in analyses. Appears to have SIID. Site visit 31July2012 by J. Arnett, FS and RareCare. Did not do any counts. NRIS reports from 12July2011. |
| 19 | 4910 | 7123 | 11131 | E | USFS-GPNF | Peterson Prairie | |
| 20 | 4908 | 2771 | 9667 | E | USFS-GPNF | Cayuse Meadow | Plants appear more purple than usual. Raven had not relocated. Isozyme analysis by Wilson et al revealed banding pattern unique to this population. 20July1988, 18July1990, 17June2004 |
| 24 | 6303 | 19190 | 3909 | | USFS/ private | Bergen Road | 2,000 to 3,000. Raven: most plants present appear to be hybrids. 8June1998 |
| 25 | 7212 | 27785 | 14069 | E | USFS-GPNF | Falls Creek horse camp | 1,000+ clumps. Lumpy distribution. 18Aug1999, 20July2004. Paper map includes more refined polygons than currently in biotics, recommend revising to correspond to the better map. |
| 25 | 7212 | 27786 | 14070 | E | USFS-GPNF | Falls Creek horse camp | |
| 25 | 7212 | 27787 | 14071 | E | USFS-GPNF | Falls Creek horse camp | |
| 25 | | | | E | | Falls Creek horse camp | Several additional patches just to south of currently mapped polygons, Area 1 and Area 7? First noted by John Scott, visited by Arnett, Ruchty and Ling on 16July2013. |
| 32 | 8748 | | | E | USFS-GPNF | East of North Butte | New site by Janka and Michael Hobbs, RareCare, 15July2012, 3.5 mi SW of Falls Creek horse camp. Revisit by Arnett in 2013. |
| 28 | 7889 | 32096 | 16522 | E | USFS-GPNF | Lost Meadow | 30 plants. NRIS reports from 30June2004 and 18July2011 |
| 31 | 8605 | 35802 | 18564 | E | USFS-GPNF | Rd. 24 Spur Meadow | Ruchty observation 2011. |
| 32 | 8748 | | 19399 | E | USFS-GPNF | SW of Falls Creek horse camp | Arnett observation in 2013, fruits only, ID needs confirmation. |
| 32 | 8748 | 36406 | 19788 | E | USFS-GPNF | SW of Falls Creek horse camp | Hobbs and Hobbs obbserver in 2012. |
| 33 | 8887 | 36942 | 19795 | E | USFS-GPNF | Lost Creek Spring | New site observed by Susan Saul et al. 2012. |

Appendix B

Cronn, Richard. 2013. *Sisyrinchium sarmentosum* vs. *S. idahoense*, complete chloroplast genome screening summary. Preliminary summary of genetic work at the National Forest Genetic Electrophoresis Laboratory, April 29, 2013.

SISYRINCHIUM SARMENTOSUM VS. S. IDAHOENSE

COMPLETE CHLOROPLAST GENOME SCREENING SUMMARY

Chloroplast genome information

Genome size: 145,000 bp (estimated from genome assembly)

Surveyed region: 103,254 bp (invariant IR regions excluded)

Data set: 9,809,130 nucleotides

Average sequencing depth per genome: TBD

Sample information

Individuals screened:

59 *S. idahoense* morphotypes

28 *S. sarmentosum* morphotypes

4 *S. bellum*

5 *S. x hybrid/unknkowns*

Variation detected: Single nucleotide polymorphisms (SNPs)

250 total variants, when including *S. bellum*; 35 total haplotypes

83 variants for *S. idahoense/S.sarmentosum*; 31 total haplotypes

Variation detected: Insertions/deletions (indels)

TBD

The big question: Can morphotypes be discriminated using SNPs?

- *S. bellum* and *S. idahoense/S.sarmentosum* are easily discriminated and can be identified by a number of fixed sites.
- *S. idahoense* and *S. sarmentosum* cannot be discriminated using chloroplast genome SNPs. Results based on SNP variation indicates that the two species are segregating for the same chloroplast variation. This is illustrated on the circular distance phenogram (Fig. 1). While the labels are messed up (sheez!), you can still see that blue (SSID) and red (SISA) are not unique to a single cluster or branch. A more detailed example of this is provided in the rectangular phenogram, where a large number of SSID (N=5) and SISA (N=13) from the Gifford Pinchot NF share the same haplotype (highlighted in grey, part I; Fig. 2). This can also be seen in SSID (5) and SISA (3) from Oregon (highlighted, part III; Fig 4).

The big question, part II: Can species be discriminated using Indels?

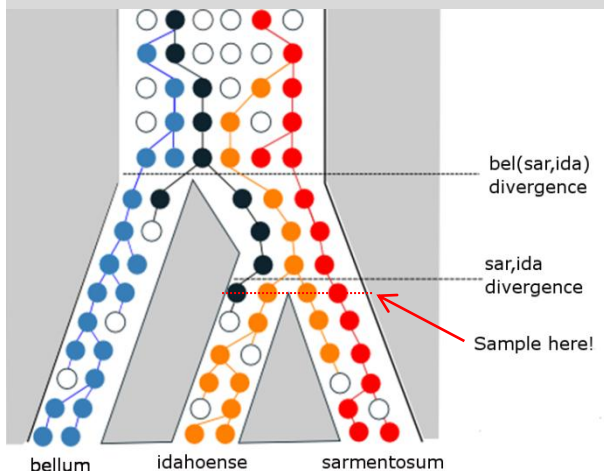
- We're evaluating the dataset to determine whether indels are diagnostic for different morphotypes. Given the high degree of spatial differentiation observed in these sequences (see below), we don't expect indels to be informative.

How much of the variation we see is due to geographic isolation?

- A lot. We'll calculate this soon, but it's evident that populations are frequently composed of single haplotypes, or a small number of haplotypes that derive from a recent common ancestor. Great examples of this are provided by haplotypes in the Washington populations (Whidbey Island, Conboy NWR, "Peterson", North Fork Rock Creek).
- One of the most interesting patterns is illustrated by the grey highlighted haplotypes on panel I, Fig. 2. The SIID and SISA from Lost Meadow all share the same haplotype. In this specific case, it's probably reasonable to conclude that the haplotypes are shared through introgression.

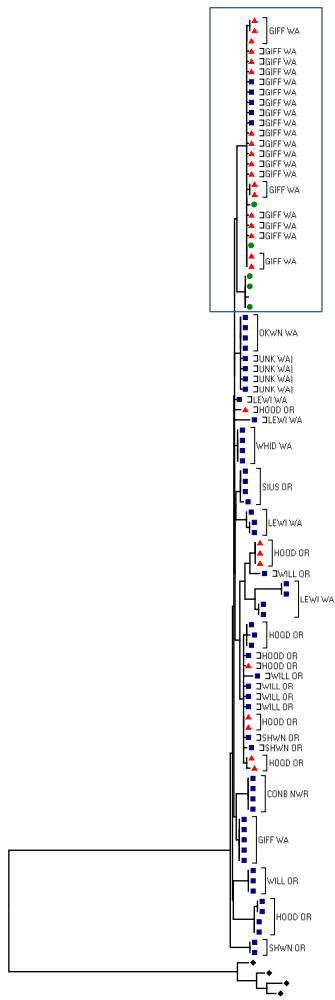
Does chloroplast variation support the distinction between species?

- No. Chloroplast variation appears better explained by geography than 'species'. We will formally test this, but the results are pretty evident, even without a statistical test.
- That said, consider my comments in an earlier email – species aren't defined by genetic or genomic variation. The biggest reason for this is that 'gene trees' often fail to accurately depict 'species trees' because of the lag time involved in sorting genetic variation. This is illustrated in the image



below. If you sample two species close to the time of their divergence, it takes time for their shared genetic variation to 'sort' into descendent lineages.

- This same pattern can be seen in many closely related species, such as closely related pines, or even Gorillas/Chimps/Humans.



Panel I

- *S. idahoense*
- ▲ *S. sarmentosum*
- *S. x hybrid*
- ◆ *S. bellum*

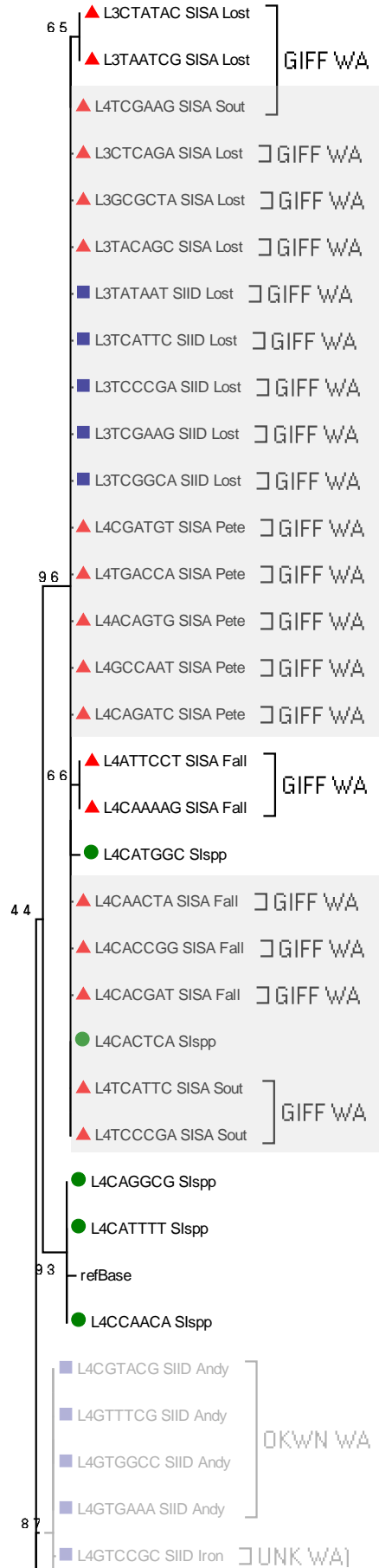


Fig. 2. Rectangular phenogram

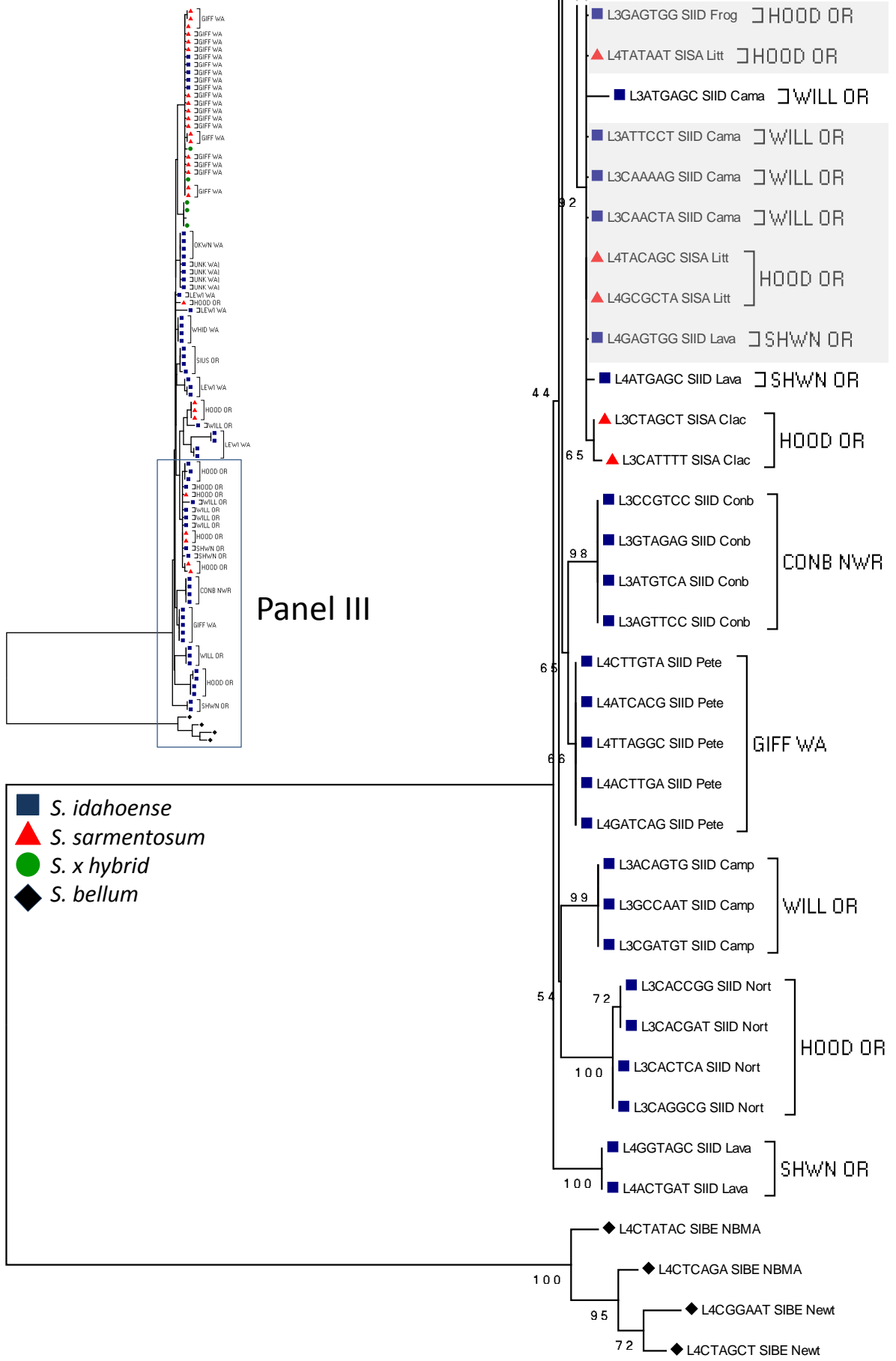


Fig. 4