



**Willamette Valley Prairie
Restoration: The Native
Seed Network and *Sidalcea***

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AN ABSTRACT OF THE FINAL REPORT OF

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Abstract Approved:

My internship was conducted with the Native Seed Network program within the Institute for Applied Ecology, a non-profit organization located in Corvallis, whose focus is education, applied research and active restoration and of native ecosystems. My internship goals were to increase my experience in applied restoration ecology and conservation biology with the emphasis on native plant communities in particular the endangered prairie communities of the Willamette Valley. These goals were met through my tenure with the Native Seed Network's native seed collection and increase program during the 2007 field season. This work provided me strong experience with a process necessary for genetically conservative restoration and provided the NSN with a willing intern to complete their goals for 2007. Additional work I accomplished included a morphometric analysis of the native Willamette Valley prairie forb *Sidalcea campestris*

which served as one constituent towards resolving the general ambiguity of this focal species for prairie restoration. Included in this analysis is a presentation and my interpretation of an initial molecular phylogenetic analysis on a selection of *S. campestris* accessions by Liston and deFeniks (unpublished) that may aid in the future resolution of *S. campestris* and other congeners in the Willamette Valley. The purpose of the morphological research was not mastery of population genetics or statistics but an exercise in concentration and achievement towards a more integrated and advanced application of taxonomic work oriented towards preserving biological diversity. This entire report completes the written requirement for the Professional Science Masters degree in Applied Systematic through the Botany and Plant Pathology Department at Oregon State University.

A FINAL REPORT

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APPROVAL:

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I understand that my final report will become part of the permanent collection of the Oregon State University Professional Science Master's Program. My signature below authorizes release of my final report to any reader upon request.

Joshua K. Lambert, Author

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List of Abbreviations:

PSM	Professional Science Masters
NSN	Native Seed Network
USFWS	United States Fish and Wildlife Service
DEB	Diversity Enhancement Blocks
PMC	Plant Materials Center
AOSCA	Association of Official Seed Certifying Agencies
PVG	Pre-Varietal Germplasm
NMS	Nonmetric Multidimensional Scaling
MRPP	Multi-Response Permutation Procedure
ITS	Inter-Transcribed Spacer (region)
nrDNA	Nuclear Deoxyribonucleic Acid
GIS	Geographic Information System
SICA	<i>Sidalcea campestris</i> Greene
SICU	<i>Sicalcea cusickii</i> Piper
SINE	<i>Sidalcea nelsoniana</i> Piper
SIVI	<i>Sidalcea malvaeflora</i> (D.C.) A. Gray ex Benth. ssp. <i>virgata</i> (Howell) C.L. Hitchc.

Chapter 1: The Native Seed Network and the Willamette Valley

ABSTRACT: The following report summarizes my YR 2007 work as an intern with the Native Seed Network (NSN) program at the Institute for Applied Ecology (IAE) in 2007. Included is an overview of the program components of the NSN and goals which outline my responsibilities as an intern. Further discussion follows some of the major technical and ecological challenges involved with restoring at-risk native plant populations and the translocation of native species during restoration and seed increase programs.

Introduction

The following report serves as a review of my educational priorities as a graduate student in applied plant systematics at Oregon State University's Botany and Plant Pathology Department, and as the deliverable for the internship that is the foundation of the Professional Science Masters program (PSM). In chapter 1, I strive to outline the concepts that have enriched my understanding of conservation biology within the framework of restoration ecology, as it applies to the Willamette Valley, a hotspot of applied restoration and conservation. I also outline the foundation and goals of the Institute for Applied Ecology's Native Seed Network which serves as a guideline to my experience as an intern in field season 2007. Overall the PSM program has allowed me to refine my theoretical knowledge of conservation biology and restoration ecology concepts, plant taxonomy, along with applied conservation work. Chapter 1 and the remaining two chapters will cover the following topics with Willamette Valley prairie community restoration as the unifying theme:

- ⇒ History and Status of Willamette Valley prairie and grassland ecosystems: the prevailing focus for conservation and restoration in the Willamette Valley
- ⇒ The Institute for Applied Ecology (IAE): one organization working toward the conservation and restoration of native prairie ecosystems
- ⇒ Conceptual challenges for restoring native ecosystems: literature review on pertinent topics associated with the management and restoration of genetic diversity in prairie ecosystems
- ⇒ IEA's Native Seed Network (NSN): focusing on the linking of seed suppliers and restoration practitioners at the local and national levels
- ⇒ *Sidalcea campestris*: an observational study applying modern systematics and taxonomy in resolving restoration based issues with a local native species
- ⇒ The Native Seed Network: an overview of their national online database

Willamette Valley Prairies

Upon entering the northern flats of the Willamette Valley in the fall of 1826, explorer David Douglas noted in his journal a wide open landscape extensively blackened by recent fire (Douglas, 1959). During the fifteen trip through the Willamette Valley his crew found barely enough fodder for his horses to forage and practically no game in sight (Douglas, 1959). This charred landscape is attributed to the thorough and systematic work of the native Kalapuya people and is a remarkable testament to the forces that shaped the vegetation of the Willamette Valley bottomlands and surrounding uplands prior to European settlement (Boyd, 1986; Towle, 1982). Evidence of fire scars among valley trees suggest that fire had been used frequently at least as far back as 1647 (Sprague and Hansen, 1946). With the exception of the gallery forests surrounding flood plains, frequent application of fire excluded dense tracts of trees in the bottomlands of the valley and the surrounding upland hills resulting in vast swaths of grassland prairie with a host of fire adapted plant and animal species (Towle, 1982; Johannessen et al. 1971; Habeck, 1961). To a lesser extent, bottomland wetlands and perched water tables from thick clay soils created wet prairie ecosystems with seasonally inundated soils that resulted in a unique yet heterogeneous vegetation composition.

Following the settlement of Europeans beginning in the early-mid 1800's, fire was increasingly suppressed leading to a noticeable shift towards the reintroduction of woody shrubs and trees from the uplands surrounding the valley (Towle, 1982; Johannessen et al. 1971; Habeck, 1961). Along with the exclusion of fire, European cultivation of open grassland introduced many new plant species to Oregon. Sixty years after European settlement a survey of the 106 known species of grass in the valley, 55 were determined to be introduced (Nelson, 1919). A published account of forb and shrub species does not exist from this time but Nelson's (1919) account suggests that many such non-natives were likely established throughout the valley at this point in history.

By the 1890's, agriculture in the surrounding upland prairies and oak savannah was mostly abandoned for the more fertile bottomlands and left to absorb the encroaching forest (Towle, 1982). As European settler progress continued into the 1900's the bottomland prairies and

gallery forests of the major rivers were increasingly tilled and removed, respectively, for agriculture and increasing infrastructure development (Towle, 1982).

Currently, only 10% of the extensive prairie and gallery forest of the pre-European Willamette Valley remain (Titus et al. 1996). Wet prairies of the valley bottomlands have seen the largest decline. Land conversion to agriculture and development and flood control dams and stream channelization projects, resulting in a vastly different hydrologic regime in the Willamette Valley (Seddell and Froggatt, 1984), have reduced wet prairie ecosystems to less than 1% of their original extent (Titus et al. 1996). The remaining wet prairie remnants have been faced with encroachment by exotic and native woody species for so long that no example (or original record) of the original native species or compositions of these systems exists. Upland or Willamette Valley foothill prairie and oak savannah ecosystems have decreased to less than 13% of their original extent (Titus et al. 1996). Much of this ecosystem has been altered for agricultural purposes or converted to non-native pastures or grassy woodlands for domestic livestock grazing. A majority of sites that have not been actively managed to exclude woody perennial species are not dominant components of prairie communities have reverted or are in the process of returning to closed-canopy woodland or mixed conifer and deciduous forest due to decades of fire suppression (Titus et al. 1996).

Conservation Measures

Willamette Valley conservation efforts to maintain and restore some level (not necessarily pre-European) of structure, function and self sustainability of upland and wet prairies in the Willamette Valley is a result of evident societal value in biodiversity (Kaye et al. 1997; USFWS 2006). At its most evident, this value has been manifested in regulatory documents in the State and Federal Endangered Species Act to protect and restore species and subspecies (population viability) listed as threatened or endangered within these ecosystems. More local efforts have seen inclusion of a Plant Conservation Biology program within the Oregon State Department of Agriculture, and continuity of the Oregon Natural Heritage Program. Finally, local nonprofit organizations have taken up the goal of putting

conservation biology concepts to work in an applied conservation framework (i.e. The Institute for Applied Ecology and The Berry Botanical Gardens).

Currently, twenty-three species of rare native plants occur in the Willamette Valley (ORNHIC 2007). Many of these species are endemic to the Willamette Valley. Five prairie species currently reside on the State and/or Federal endangered list (*Erigeron decumbens* var. *decumbens*, *Lomatium bradshawii*, *Eucephalus vialis*, *Delphinium leucophaeum*, and *Delphinium pavonaceum*) and four are listed as State and/or Federally threatened (*Castilleja levisecta*, *Lupinus sulphureus* ssp. *kincaidii*, *Sidalcea nelsoniana*, *Sericocarpus rigidus*,) (ORNHIC 2007). Many more native taxa that are important components to prairie ecosystems have experienced negative anthropogenic impact (Titus et al. 1996; Wilson 1998) raising concerns about their future viability in the face of continued habitat alteration and loss.

Further considerations for conservation are the fauna of Willamette Valley prairies. Species that are currently most dependent on these plant communities are birds and invertebrates however, most have not been well documented or studied (Wilson, 1998). One well studied case of species dependence on upland prairies is the Federal and State protected Fender's Blue butterfly (*Plebejus icarioides fenderi*). This species is endemic to the Willamette Valley and dependent upon many prairie plant species; in particular the endemic Kincaid's lupine (*Lupinus sulphureus* ssp. *kincaidii*) which serves as the obligate egg and larval host for the Fender's Blue butterfly (Wilson et al. 1997). The listing of the Fender's Blue butterfly in 2000 has served as an integral tool for providing protection and funding for research, restoration and management of upland prairie systems.

Due to the fragmented and degraded (defined as reduced native component) condition of remaining upland and wet prairie ecosystems in the Willamette Valley (Wilson, 1998), ecological restoration poses many challenges. Past restoration efforts in the Willamette Valley have shown that upland prairie remnants react differently to restoration treatments (Schultz, 2001), cautioning managers against applying generic treatments across multiple

sites. Heterogeneous soil nitrogen levels in addition to weed seed, biomass, and diversity (Schultz, 2001) across the Willamette Valley are just a few factors that may result in different levels of success to treatments.

Wilson (1998) outlined four issues deemed crucial for future conservation of Willamette Valley wet prairies which provide a proper surrogate for upland prairies as well: The need for 1) protection from further habitat destruction (conversion); 2) effective management tools, such as prescribed burning or mowing, to prevent natural succession of protected prairie sites to shrub lands and forests; 3) restoration techniques developed and tested to promote the establishment of key native plant species and to reduce the abundance of aggressive pest plants in protected wetland prairies; 4) expanded scientific studies (and monitoring) to provide the basic biological information necessary for effective conservation. All four of these measures have been enacted to various degrees in many public and private prairie reserves within the Willamette Valley and surrounding foothills.

The Native Seed Network

The Institute for Applied Ecology (IAE) is a non-profit organization whose broad focus is applied research and active restoration of rare and endangered Oregon plant species and Pacific Northwest regional plant communities with an emphasis on grassland ecosystems. The Native Seed Network (NSN), is a program of the Institute for Applied Ecology with which I completed my internship. The mission of the NSN is to facilitate and support a genetically conservative approach to provide native seed for the restoration of native plant communities. This mission is founded on the substantial evidence that plants exhibit high degrees of genetic adaptation to their local environment (Endler 1986; Linhart and Grant 1996). This principle leads to the preferred use of native plant materials from local sources with similar environmental conditions in order to maximize the conservation of biological diversity and maintain the adaptive capability of plant populations, communities, and ecosystems.

The NSN is essentially two separate but integrated entities: 1) a national online networking effort to connect the demand of land managers seeking to use native and locally sourced genetic material with resource suppliers of native seed, many of which agree to grow seed in a certifiable manner; 2) a supplier of locally sourced seed for Willamette Valley prairie plant communities and an active research and restoration based organization. The former exists currently as a searchable and updatable (for suppliers) online database (Chapter 3). The latter is part of a long term (thru 2015) Cooperative Agreement with the U.S. Fish and Wildlife Service to develop a supply of ecologically appropriate genetically diverse native plant materials, particularly forbs, for restoration of Willamette Valley wetland and upland prairie ecosystems (Fiegener et al. 2006). These efforts are aimed at supporting historically known native plant species that are necessary for the federally listed Fender's Blue butterfly (*Plebejus icarioides fenderi*). These species include obligate species of *Lupinus* for the larval phase of the butterfly's life cycle, many generalist nectar species (Table 1) that support the adult phase as well as structural components, such as bunchgrasses (e.g. *Festuca roemerii* and *Danthonia californica*), that are naturally dominant components of upland prairie associations.

In 2006, the USFWS published a recovery outline for *Lupinus sulphureus* ssp. *kincaidii*, requiring a Willamette Valley ecoregion (Omernick 1987) wide restoration effort aimed at meta-population development with genetic viability as a major concern (USFWS 2006). This effort outlines plans to place ten meta-populations or recovery zones each with a combined total of 5000m² *Lupinus sulphureus* ssp. *kincaidii* cover. Their guidelines for creating prairie habitat for the lupine require a high quality index (ratio of native to non-native cover), an effort that presumably will strive to recover the characteristics and diversity of native Willamette Valley upland prairie habitat. The project goal is a habitat increase or recovery of at minimum 50,000m² or 12.5 acres of quality upland prairie habitat throughout the Willamette Valley and surrounding areas. Based on an ecoregion wide recovery effort such as this, there is no doubt need for increased production along with development of genetically conservative methods for providing the base germplasm for upland prairie habitat

restoration. The IAE’s Native Seed Network is a partner in this greater challenge to reestablish the prairie ecosystems that support Fender’s Blue Butterfly.

Table 1. Wet and Upland Prairie Species of Focus for Seed Increase

* species currently undergoing seed increase as of YR 2006, 2007 (grow out phase)

☒ currently in common garden research program

ω species currently wild collected and stored

<i>Allium amplexans</i> ω	<i>Lomatium nudicaule</i> ω
<i>Asclepias speciosa</i> ω	<i>Lupinus polyphyllus</i> * ☒
<i>Calochortus tolmiei</i> ω	<i>Microseris laciniata</i>
<i>Camassia leichtlinii</i> ω	<i>Potentilla gracilis</i> *
<i>Camassia quamash</i> ω	<i>Prunella vulgaris</i> var. <i>lanceolata</i> ☒
<i>Carex densa</i> *	<i>Ranunculus occidentalis</i> ω
<i>Carex unilateralis</i> *	<i>Ranunculus orthorhynchus</i> *
<i>Danthonia californica</i> *	<i>Saxifraga oregana</i> * ☒
<i>Deschampsia caespitosa</i>	<i>Sidalcea campestris</i> * ☒
<i>Epilobium densiflorum</i> * ☒	<i>S. malvaeflora</i> ssp. <i>virgata</i> ω
<i>Eriophyllum lanatum</i> ☒	<i>Sisyrinchium idahoense</i>
<i>Festuca roemerii</i> *	<i>Symphotrichum hallii</i> (syn. <i>Aster hallii</i>) * ω
<i>Grindelia integrifolia</i> *	<i>Wyethia angustifolia</i> ☒
<i>Juncus tenuis</i> *	
<i>Lotus unifoliolatus</i> (syn. <i>Lotus purshianus</i>) *	

NSN Seed Increase Program: History

The goals and methods of NSN’s Willamette Valley seed increase program were developed in part with the Natural Resource Conservation Service (NRCS), the US Fish and Wildlife Service (USFWS), and input from numerous other knowledgeable individual contributors. By 2006, this group developed a list of target species that have been desired but not available in significant quantities for restoration, in particular local restoration projects by the USFWS and the NRCS’s Wetland Re-enhancement Project (WRP). Table 1, provides a list of focus species for collection and increase that were the result of this meeting. Most of these species are currently either in the collected or seed increase phase of the program. The NSN’s seed increase program comprises a few major steps: tracking of wild seed populations, collection, seed processing (cleaning), development of seed transfer guidelines, research to refine seed transfer zones, initiation of grow-out with local seed producers and the applied steps for certification of seed output as “sourced seed”. In 2006, a total of 403 individual collections

were made from 5 graminoids and 13 forbs, from an average of 20 collection sites per species, covering 7 counties. The year 2007, the season I worked on the NSN seed crew, culminated in a collection of approximately 14 forbs with numerous collection sites per species. The following methods outline the major components of NSN's seed increase program and summarize part of the full time work I performed as an intern during the season of 2007 with the NSN field crew. Appendix A provides a daily detailed account of my internship.

Program Methods

The first step involves wild seed collection. This entails the groundwork of locating (often via vehicle) and documenting multiple wild populations of each focus species. Populations ranged from highway to country roadsides with anywhere from 10 to 1000's of individuals of a particular taxa (often multiple prairie species) to public or private land and preserves often with greater than 1000 individuals. After acquiring owner permission in cases in which populations are located on private or public land, population information is gathered and areas are continually monitored for collection. Locations of species populations are carefully documented and mapped using GPS units and often a voucher specimen is collected when a new population is found. The protocol used by the NSN allows for collection of up to 50% of the seed on any particular inflorescence or population whichever is deemed most appropriate on site. Often much less than 50% is collected. Following collection, the NSN workers decide upon which populations will be combined, if appropriate, in accordance to seed transfer zone (see below) protocols developed and researched before, during or after collection.

Once seeds have been grouped according to seed transfer zone, seeds are transferred to the cooperating USDA Plant Materials Center (PMC) where they are prepared. Preparation may involve cold stratification and/or scarification for select species following mechanical removal of extraneous non-seed materials (exocarp, vegetation, weed seeds etc.) for the purposes of uniformity. Following preparation, seed is cold stored until delivery to local seed producers. Once delivered, producers are responsible for planting, growing and

harvesting seed according to the NSN outlay for seed transfer zones, however NSN workers maintain contact with farmers and monitor results over the growing season. Multiple farms are contracted for seed increase. Following harvest, seed is transferred back to the PMC for cleaning.

Some species, in particular perennial herbaceous species, have posed challenges for efficient propagation via seed (e.g., *Carex* species). Often these are propagated by vegetative material or by seed in a greenhouse and then out-planted to restoration sites via young plugs. Other perennials, such as *Camassia*, may not produce seed following germination for many years and thus can not be increased in a financially viable way. Species such as these, if available in appropriate amounts, can be hand seeded directly to restoration sites with wild collected seed.

Seed Certification

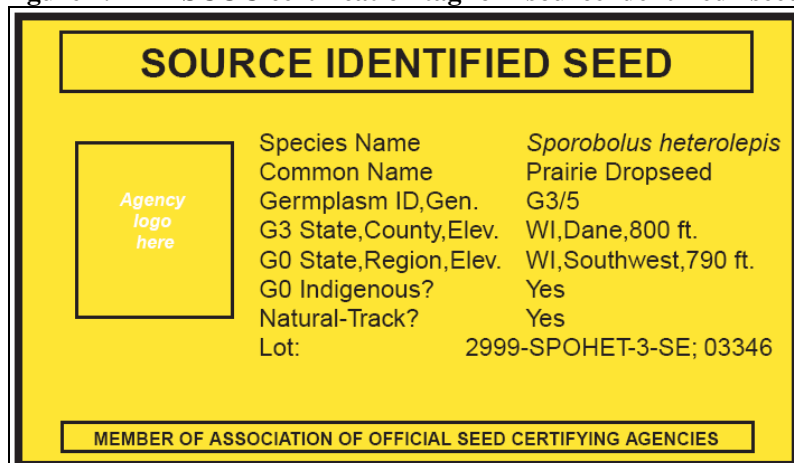
The NSN has placed an emphasis on providing certified “source identified” seed from its wild sourced seed increase operations. For this process the NSN utilizes the Association of Official Seed Certifying Agencies (AOSCA) certification procedures which provide third-party verification of source, genetic identity, and genetic purity of wild collected field or nursery grown plant germplasm materials. As an alternative to formal variety release which often covers highly manipulated and controlled plant cultivars, AOSCA Pre-Variety Germplasm (PVG) categories (below) provide certification for lower level seed production not aimed at typical varietal cultivation. The PVG program offer a parallel progression of stages for certification designated as:

- (1) Source Identified Class (unevaluated germplasm identified only as to species and location of the wild growing parents)
- (2) Selected Class (germplasm showing promise of desirable traits, having been selected either within or as a common site comparison among accessions or populations of the same species)

(3) Tested Class (germplasm for which progeny testing has proven desirable traits to be heritable)

For the practical purposes of ecological restoration, “source identified” class certification is appropriate for the NSN. Such source identified germplasm originating from a wild (native, naturalized, or feral) stand is assigned a genetic status of either “manipulated-track” or “natural-track”. The natural track or “yellow tag” (example, Figure 1), for which the NSN adheres, is reserved for germplasm accessions that are an unrestricted representation of the intact wild plant population on the original site. To achieve this status, genetic manipulation must be purposefully avoided when such accessions are increased in field or nursery production. Accessions that are purposefully or inadvertently hybridized with other accessions or selected for distinctive traits within the population (whether on the original site or in succeeding field or nursery generations) are routed to the “manipulated-track”. As stated by the AOSCA, utilization of the above three PVG categories is applicable when a) identification and propagation of species and/or ecotypes at various stages of evaluation are needed for timely (often immediate) restoration of specific geographic areas, b) market potential is limited beyond specific geographic areas, and/or c) accommodating consumer special plant material demands.

Figure 1. An ASCOC certification tag for “source identified” seed:



In general, AOSCA seed certification agencies (acting as a third-party) require seed collectors/producers to follow established requirements, procedures, and standards to assure germplasm identity and purity for the seed consumer. Certification procedures include:

- Pre-collection application filed before harvest
- Proper permitting and/or permission for collecting on public and private lands
- Site identification log filled out during and after harvest
- Verification of the collection site and identification and evaluation of plant and seed samples before, during, and/or post harvest
- Tagging of the seed lot after compliance with applicable requirements and standards; seed purity and viability analysis may be required
- Germplasm accessions acquired within established protocols of recognized public or private agencies are normally eligible (with appropriate data on file in lieu of the above procedures) to enter the certification process as planting stock

Willamette Valley Prairie Restoration Challenges

Prairie communities that are the focus of many restoration efforts in the Willamette Valley are considered some of the most rare and endangered ecosystems in the world (Noss et al. 1995). Challenges for restoration and conservation efforts include land conversion resulting in a loss of original prairie habitat and increasing disconnection and proximity (fragmentation) of remaining sites. There exist few high quality examples remaining (Wilson et al. 1997) as reference sites. Much of the remaining habitat is undergoing transition towards a higher ratio of nonnative and woody species to native herbaceous perennial species (Hammond and Wilson 1993), defined here as degradation. Along with larger sites of remnant habitat there exists many smaller residual or refuge populations found along roadsides or along the unconverted edges of farm and forest. Whether they are recently colonized populations or remnants of larger original populations is likely different for each

site but all are important in recovery efforts that follow in the footsteps of many conservation programs whose focus is to conserve existing levels of genetic variation in rare or threatened populations (Simberloff 1988). The following is an overview of some of the challenges that land managers and conservation biologists are facing in order to preserve or even increase viable, self sustaining prairie communities in the Willamette Valley as a lasting component of Oregon's heritage.

Fragmentation

Habitat fragmentation can be viewed as both the level of reduction in the area covered by a habitat type or population, and a change in habitat or population configuration to smaller and more isolated patches (Harris 1984; Wilcove et al 1986; Saunders et al. 1993). While many view the latter component as fragmentation in the literal sense, both typically occur in tandem (Noss et al. 2006). Lord and Norton (1990) define fragmentation as, "the disruption of continuity" in ecosystems. This definition provides insight into the processes that are important for maintaining an ecosystem, community or population. At the landscape or ecosystem level most plant communities are heterogeneous, with a patchy appearance. This can be attributed to the heterogeneity of past or current disturbance events, the natural heterogeneity of the substrate conditions (soil, elevation, hydrology), and stochastic propagation dispersal. With increased isolation of fragmented patches, natural disturbance regimes and gene flow can be disrupted or stopped. Over time these processes may lead to a landscape different from which species have adapted over evolutionary time (Noss and Cooperider 1994).

It is important to appreciate the different spatial scales of fragmentation. While the effects of landscape or geographic fragmentation are relatively readily seen, fragmentation can also occur in a subpopulation or at the individual plant level in what Lord and Norton (1990) deem structural fragmentation. Using short-tussock grassland in New Zealand as an example, Lord and Norton (1990) suggested that fine scale fragmentation (individual plants), such as with increasing presence of nonnative species among native grass and herb patches, disrupted

connectivity and ultimately ecosystem function. Lennartson (2002), showed quantitatively that while habitat area had an effect on the viability curves (fitness) of the herb *Gentianella*, the largest impact on fitness came from local, site/population level fragmentation from shrub invasion which interrupted plant-pollinator interactions and resulted in increased levels of inbreeding due to increased selfing rates. This structural fragmentation is especially of concern in larger habitat preserves which have a large area protected from fragmentation but may be becoming increasingly disconnected and less functional by increased exotic presence (degraded) from the inside (Meurk et al. 1989).

In naturally occurring small populations immigration is known to be a necessary component for preventing inbreeding depression or local extirpation (Harris, 1984) and forms the theoretical basis for metapopulation dynamics (Levins 1970) and the source-sink concept (Pullium 1988). In populations that have been disconnected or fragmented, a reduction of immigration and corresponding gene flow can be a real threat to the existence of these populations.

Inbreeding and Drift

Inbreeding depression is well documented in wild populations and is known to increase in populations isolated by fragmentation (Keller and Waller 2002). A population completely isolated from a historically connected metapopulation or larger population results in a smaller effective population (true number of breeding individuals), and lower survival probability (Fahrig and Merriam 1985), and can lead to a genetic bottleneck: a reduced representation of the entire genetic diversity of a population due to reduction to a fewer number of individuals (Barrett and Kohn, 1991). In these small populations (e.g., <100 individuals), gene frequencies can undergo large fluctuation in few generations, leading to loss of rare alleles, commonly known as random genetic drift. In populations that are small and isolated from one another, genetic drift can have a dominant influence on population genetics in the form of reduced polymorphism or increased homozygosity of allele loci (Graur and Li 1991). One result of increased homozygosity is an increased genetic load, or greater expression of deleterious recessive alleles, resulting in reduced fitness expressed as lower success rates

during stages of the reproductive cycle (Charlesworth and Charlesworth 1987), and reduced genetic variation due to selection against these individuals. For example, Menges (1991a) showed that reduced rate and reliability of seed germination was greater for smaller versus larger populations of the prairie associated *Silene regia*. The accumulated results of drift and inbreeding depression in association with stochastic disturbance events make small populations particularly prone to local extinction (Barrett and Kohn, 1991).

Many plants however are not prone to the same genetic effects of demographic fluctuation due to a diversity of breeding systems (e.g. autogamy vs. heterogamy) and high levels of polyploidy in plants. Predominantly autogamous (selfing) or apomictic (clonal) species may have naturally low genetic variation and may not respond negatively in isolated populations whereas inbreeding depression is often strongly associated with outcrossing plants in these situations. In general, inbreeding depression usually occurs in outcrossing plants, is less severe in species that are partially self-fertilizing, and may be absent altogether in species that are highly selfing (Wright 1977).

Rescuing Populations

One of the goals of many conservation programs is to maintain existing levels of genetic variation in species that are rare or threatened (Simberloff 1988). Some have even called for the need to increase adaptive ability (genetic diversity) within and among populations in a rapidly changing climate (Rice and Emory 2003). McKay et al. (2005) have recommended that one practical strategy for restoring isolated but historically connected populations is “coarse selective tuning”, where a restored population is planted with a composition of genotypes from climatically local populations which are often within close geographic proximity to the planting site. Since we generally lack an understanding of which particular traits confer adaptation to particular environments, collection efforts aimed at simply sampling genetic variation across major ecological gradients within the species’ range may help to preserve the adaptive potential (McKay & Latta 2002) of species at risk of inbreeding depression or below minimum viable population levels. A population with higher levels of heritable genetic variation has a better chance of evolving towards the new optimum in a

changing environment, because it has a greater number of genotypes with the correct phenotype for selection (Gilpin and Soulé 1986). This approach can be substantially improved by sampling within the framework of historical gene flow, where clines are likely to contain a great deal of allelic variation (McKay & Latta 2002).

Along with other criteria such as ecological role, the biological distinctiveness of a group can be an important factor when making conservation priorities (Soltis and Gitzendannern 1999). Andreasen (2005) demonstrated that phylogenetic analysis of taxonomic groups may also provide such information on the levels of distinctiveness and evolutionary potential (measured by relative amount of genetic variation) that could provide managers information on which species lineages may have a higher diversity value for protection and propagation.

Outbreeding Depression

It is well known that certain species suffering from inbreeding depression and isolation often respond to outcrossing with increased vigor or heterosis. Such crossing is often important for sustaining the flow of heritable genetic variation to allow adaptation and evolutionary change during changes in environmental conditions (Gilpin and Soulé 1986). However, the field of plant ecological genetics has provided overwhelming support that many native plant populations are locally adapted over a variety of spatial scales (Endler 1986; Linhart and Grant 1996). In isolated subpopulations, a consequence of fragmentation, small effective population size, limited gene dispersal and inbreeding may favor restricted recombination and facilitate the evolution towards locally adapted or co-adapted gene complexes (Levin 1978; Templeton 1986). These processes may be even more likely to occur with isolated populations at the extremes of a species geographic or ecological range. With decisions to reestablish gene flow between such subpopulations, such as in a seed increase program, mixing between infraspecies without similar allelic combinations can result in progeny less fit for a specific locality or even lethal disruption and dilution of epistasis (Hufford and Mazer 2003) within the local gene pool known as outbreeding depression (Templeton 1986). Brown and Knapp (2007) demonstrated that the origins of plant material introduced at a site may be interlinked by local adaptive processes and that the ultimate success of the restoration

may depend critically on recognition of this evolutionary history. At the most extreme, germplasm augmentation may result in population extinction if natural selection does not purge maladapted progeny, but more benign risks such as loss of genetic variation crucial for the future adaptive ability of a species, and persistence of locally adapted traits over many generations especially under highly competitive conditions (Rice and Knapp 2007) is worthy of concern.

Many factors including the magnitude and scale of local selection pressures and a species' mating system influences genetic differentiation between subpopulations. This information provides some level of predictability as to whether outbreeding depression poses a major risk during germplasm mixing (Hufford and Mazer 2003). While outbreeding depression is far less documented than heterosis, it has been shown that it may follow after a few generations of heterosis leading some workers to possibly dismiss the effects (Barrett and Kohn 1991).

But in a discipline such as restoration ecology the balance of risk is in constant flux with management objectives. In general, management may be grounded in pragmatic actions in order to apply efforts with often a large number of unknown genetic parameters within a community or ecosystem. Often the question arises as to whether we risk preserving a locally adapted genotype for the sake of genetic diversity at the expense of saving a population that may not have long term viability due to one factor or another. Do we risk diluting through hybridization and introgression the very genetic diversity restoration is striving to preserve (Hufford and Mazer 2003)? When manipulating the genetic constitution of at-risk populations, it might be possible to strike a balance between an acceptable level of genetic load and the capacity for further adaptive ability (Rice and Emory 2003). Often, information on taxa such as breeding systems and polyploidy (Severns and Liston, in press), and simple experiments (see below for common gardens and seed transfer zones) can provide information about how best to approach translocations without detailed molecular information about a population.

Seed Transfer Zones

Guidelines to help alleviate the paucity of genetic information regarding local populations slated for augmentation (due to financial or other limitations) have been presented by many conservation biologists (McKay et al. 2005). The overarching priority is to source germplasm as geographically and/or environmentally local as possible to the restoration site with careful efforts made to match germplasm for both abiotic and biotic factors, such as elevation, soil characteristics, climatic regime, pathogens and predators (McKay et al. 2005). One such conservative pathway with seed increase programs is using seed transfer zones during seed increase to separate (prevent gene flow and seed mixing) and preserve the genetic integrity of a species with a known genetically based geographic/environmental gradient. The development of seed transfer zones was first defined in recognition of strong regional differences in life-history traits of commercially important species of conifers (Buck et al. 1970; Kitzmiller 1990). This concept has been adopted by some restoration ecology and genetics frameworks in efforts to maximize the viability of genotypes introduced in restoration and to mitigate depressing effects on the receiving gene pool (Hufford and Mazer 2003).

Another important consideration is that seed transfer zones incorporate life-history characteristics of the focal species (Hufford and Mazer 2003). Specifically, the mating system of species will determine patterns of gene flow and levels of within and among population differentiation (Hamrick and Godt 1996). Highly outcrossing taxa are less likely to represent genetic differentiation because of the homogenizing effect of gene flow but highly inbreeding taxa are more likely to form ecotypes and/or epitypes because of their greater isolation and independent evolution (Hufford and Mazer 2003; Rice and Knapp 2007). Consequently, seed transfer zones should be larger for outcrossing species given that populations linked by longer distance pollen dispersal are less likely to exhibit outbreeding depression if they hybridize (Hufford and Mazer 2003). As a final consideration, once a seed zone is delineated, collections must be made from a large enough number of individuals to represent population variation adequately and to avoid severe genetic bottlenecks (Hufford and Mazer 2003).

Common Gardens

Common gardens as well as the more complicated and costly reciprocal transplant procedures inform seed transfer zone decisions by separating environmental-based variation from actual genetic based variation in focus taxa (McKay and Latta 2002). Common gardens specifically control for confounding effects from competition and limited resources in order to visualize the least hindered phenotypical representation of a species of interest. Species chosen for NSN's common garden experiment (Table 1) are sourced from multiple collection sites throughout the Willamette Valley. Although some ecologists have suggested that the Willamette Valley below 1000ft is a single seed zone for some native species (limited native prairie taxa), they have also recognized there can be different ecotypes for xeric and riparian sites (Johnson 2006, unpublished). Many of the species in the NSN's seed increase program grow in both wet and dry upland prairie sites. Considering the effects of disconnected gene flow between many prairie remnants and the diverse range of life history and breeding traits of prairie species, common garden studies that may ultimately suggest and support diverse seed transfer zones in the valley appear to be pertinent to a conservative approach for seed increase programs in the Willamette Valley.

Diversity Enhancement Block Designs

The NSN is currently investigating morphological variation of many native species (Table 1) that are designated as focus species within the seed increase program. This investigation includes common garden research, morphometric analysis and to a lesser extent molecular analysis to delineate and further refine the use of seed transfer zones. Until this data can be procured, they have applied a novel design, the Diversity Enhancement Block (DEB) (Fiegener et al. 2006). This design delineates individual taxa plantings into separate but adjacent blocks. These blocks are based on informed but preliminary Willamette Valley regions that maintain pollen flow but are separately harvestable if phenology should differ between blocks. This increases the amount of diversity harvested within seed mixes without creating potential inbreeding conditions (Fieneger et al. 2006).

Seed Increase Management

During agronomic seed increase operations of wild sourced seed, taxa are subject to much different conditions than in the wild. The longer a plant is removed from its native environment, the more it is subjected to artificial selection, whether intentional or not. To reduce the potential for unconscious selection during seed increase, it is believed to be important to harvest from the entire planted population as often as possible and in as few generations as possible (McKay et al. 2005). Additionally, it has been recommended to conduct seed-increase operations as close as possible to the source of germplasm (in an environmental distance sense) (McKay et al. 2005) to mitigate as many abnormal selective pressures as possible. These considerations are appropriated and verified with the AOSCA seed certification program which the NSN uses for its native seed increase. Included in the seed certification tag in Figure 1, is a generation number “G3/5”, which indicates the filial generation of seed from the original wild planting over of the acceptable number of generations, in this example the third generation out of five acceptable generations designated for this particular species.

Conclusion

Conservation biology is a crisis oriented science, faced with the difficult challenge of understanding and predicting the long-term dynamics of complex systems (Soulé 1985). Conservation and management of remaining Willamette Valley prairie communities is faced with implementing conservation under a daunting paucity of information on such factors as in-depth information of life history traits of individual taxa, the genetic variation of individual populations and the minimum viable population (Menges 1991b) required to prevent extinction. Such management issues are compounded by additional themes including local competition pressures from non-natives and aggressive natives, lack of wild seed sources for restoration and unique site characteristics excluding the use of exact methods from past restoration efforts at other sites. Finally, preserving genetic variation while preventing population collapse due to the loss of connectivity, poses serious ethical concerns. Ultimately, managers must attempt to be conservative and adaptive and practical in their application while closely monitoring biological responses to measure success and to find

broader patterns that may provide insight to future management. Along with the majority of unavoidable risks that land manager's face with translocation and seed increase projects, the absence of such efforts may be more detrimental to the future of at-risk native plant communities.

Sidalcea campestris

Certain native prairie species of interest have shown variation throughout the Willamette Valley ecoregion raising concern as to whether such phenological variation is genetically based (epitypes) and require separate seed transfer zones or subsequent exclusion from collection programs. Common garden experiments help to address this issue but morphometric analysis can also provide a more cost efficient level of regional comparison, especially when paired with modern molecular phylogenetic methods.

One such species of concern is *Sidalcea campestris* Greene (meadow checker mallow). This species has been seen to show morphological variation in individual populations (Chapter 2: Table 5) and there is now evidence of potential gene flow between sympatric congeners (Chapter 2). However, it is currently unknown as to whether this variation is just due to high infraspecific variation (well known within *Sidalcea*), or if it is due to evolutionary divergence or possibly hybridization. Further, testing to see if the variation is only on a local basis or possibly clinal has not yet been formally addressed. Chapter 2 provides the results of molecular phylogenetic analysis using nrDNA ITS polymorphisms and a morphometric analysis on fifteen selected morphological traits. This analysis represents a further component of my PSM internship program.

Summary of Side Projects at the NSN

During my field assistantship (internship) with the NSN, I completed a few small projects involving taxonomy of native prairie species of focus for the NSN collection program. Below is a quick description of the two projects.

Grindelia

Grindelia integrifolia (Willamette Valley gumplant), like many gumplants is known to hybridize with congeners (Chambers 1998; Lane 1993). It is also a native component of Willamette Valley wetland prairies. One particular variant known to occur throughout the valley is a hybrid between the non-native *G. nana* and *G. integrifolia*, which appears to have intermediate traits between the two taxa. Since only pure *G. integrifolia* is desired for collection by the NSN, I was asked to do a rapid morphological assessment of a few samples from collection sites around the valley to assess where pure stands of *G. integrifolia* were located. Pure *G. integrifolia* populations ostensibly exist in the Willamette Valley but the rampant hybridization makes collection difficult since these populations are not well known. Based on literature review and in depth discussion with Dr. Kenton L. Chambers (Oregon State University), I compared 13 morphological traits (Tables 2, 3) from 26 vouchers from NSN collection sites in Lane, Benton, Polk and Linn County. Appendix C provides the data accumulated from this rapid and untested morphological assessment of pure *Grindelia integrifolia* verses the hybrid known as *G. integrifolia* ' *G. nana* var. *nana* (Chambers 1998). The result is that only a few populations appear to represent the true characteristics of Willamette Valley gumplant, which supports earlier claims that hybridization has created a large swarm of intermediates through out the valley (Chambers 1998).

Table 2: *Grindelia integrifolia* and *G. nana* trait comparison chart

Height:		
	GRIN	1.5 – 8dm
	GRNA	1.0 – 8dm
Leaf length; width; petiole morphology:		
	GRIN	40cm long; 4cm wide, lower lvs becoming sessile, occ. clasping
	GRNA	15cm long; 3cm wide, lower lvs barely clasping
Leaf shape:		
	GRIN	Lanceolate?
	GRNA	Linear to oblanceolate?
Pubescence:		
	GRIN	Glabrate or more often villous (glandular)
	GRNA	Glabrous
Ray flower length; width:		
	GRIN	10 – 35mm; 8 – 12mm
	GRNA	12 – 25mm; 5 – 15mm
Head:		
	GRIN	1 – 3cm wide; hemispheric
	GRNA	up to 1.5 cm wide; radiate
Phyllaries:		
	GRIN	Loose to spreading, not regularly reflexed green tips
	GRNA	Regularly reflexed green tips

Note: The above table was compiled as a description of the most typical traits of the pure species, in which intergradation of the two may can then be assessed.

Table 3: Raw Data From Rapid Morphological Assessment of *Grindelia* from NSN Collection Sites

County	Population	Voucher #	Height (cm)	Largest cauline leaf (below infl.)			
				Leaf length (cm)	Leaf width at widest point (cm)	Leaf shape	Clasping
Polk	Basket slough (Smithfield Rd.)	1	48.1	8.9	1.7	linear - lanceolate	yes
Polk	Basket slough (Smithfield Rd.)	2	49.5	9.5	1.2	linear	yes
Lane	Helt	3	55.0	7.5	1.8	oblolanceolate	no
Lane	Helt	4	35.5	6.1	1.1	oblolanceolate	no
Lane	Camas Swale	5	72.0	42.0	1.9	oblolanceolate	no
Lane	Camas Swale	6	58.0	12.2	1.7	oblolanceolate	yes
Lane	Vinci (WEW)	7	32.5	5.6	0.9	oblolanceolate	no
Lane	Vinci (WEW)	8	37.0	10.7	1.3	oblolanceolate	no
Lane	Greenhill (WEW)	9	44.0	6.8	1.1	oblolanceolate	no
Lane	Greenhill (WEW)	10	46.0	12.5	1.2	oblolanceolate	yes
Lane	Oxbow East (WEW)	11	43.0	10.0	0.9	oblolanceolate	yes
Lane	Balboa (WEW)	12	37.0	10.5	1.4	oblolanceolate	yes
Lane	Spores	13	54.0	14.2	2.2*	oblolanceolate	yes
Linn	Belts Drive	14	36.5	6.5	1.5	oblolanceolate	yes
Linn	Belts Drive	15	50.0	6.0	1.2	oblolanceolate	yes
Linn	Richardson Gap Rd	16	35.0	6.0	1.2	oblolanceolate	yes
Benton	Jackson Frazier	17	83.0	15.0	2.5	oblolanceolate	yes
Benton	Jackson Creek	18	56.0	9.5	2.2	lanceolate	yes
Benton	Timberhill Athletic Club	19	67.0	13.0	1.8	oblolanceolate	yes
Benton	Timberhill Athletic Club	20	48.0	7.0	1.2	oblolanceolate	yes
Benton	Bald Hill	21	62.0	6.4	1.3	lanceolate	yes
Benton	Philomath Priarie	22	57.0	12.5	2.1	oblolanceolate	yes
Polk	Jebousek	23	54.5	6.0	0.9	lanceolate	yes
Lane	Helt	24	67.0	6.9	1.1	lanceolate	yes
Polk	Basket slough (Area 1)	25	44.0	9.4	1.2	lanceolate	yes
Polk	Hwy 22 x Fort Hill	26	76.0	8.0	2.0	lanceolate	yes

Table 3 continued

V#	Largest upper infl leaf			Pubescence	Phyllaries	Ray flowers		Head width (cm)
	Leaf length (cm)	Leaf shape	Clasping	(1 to 4)*	(1 to 4)*	Length (mm)	Count	
1	3.4	lanceolate	yes	3	3	9	21	1.1
2	3.2	lanceolate	yes	3	3	12	24	2.0
3	3.6	linear - lanceolate	yes	2	2	10	22	1.9
4	3.2	linear - lanceolate	yes	1	3	9	22	1.5
5	5.5	lanceolate	no	1	4	10	15	1.3
6	2.5	lanceolate	yes	1	3	9	19	1.5
7	1.0	linear	yes	1	2	8	13	0.8
8	1.0	lanceolate	yes	1	2	7	20	1.4
9	1.0	linear	yes	1	2	8	15	0.9
10	1.3	lanceolate	yes	1	2	8	12	1.1
11	1.6	lanceolate	yes	1	2	6	9	0.7
12	1.5	lanceolate	yes	1	3	7	19	1.7
13	2.8*	lanceolate	yes	2	1	8	21	1.2
14	2.6	linear	yes	2	1	7	20	1.0
15	2.0	lanceolate	yes	1	3	10	18	1.2
16	3.4	linear	yes	2	1	9	17	1.0
17	4.0	lanceolate	yes	4	1	11	23	1.6
18	4.5	lanceolate	yes	4	1	10	24	1.6
19	4.4	lanceolate	yes	4	2	11	21	1.2
20	3.1	lanceolate	yes	4	3	10	20	1.5
21	3.0	lanceolate	yes	3	2	11	23	1.6
22	3.0	linear	yes	4	3	9	23	1.3
23	2.3	lanceolate	yes	4	1	13	15	1.2
24	2.2	lanceolate	yes	2	3	9	17	1.4
25	3.0	lanceolate	yes	4	4	9	20	1.5
26	3.7	lanceolate	yes	4	2	7	16	0.1

*** Pubescence**

- 1 - essentially glabrous
- 2 - glabrous with ciliate leaf margins
- 3 - short villous
- 4 - long villous

*** Phyllaries**

- 1 - loose to spreading
- 2 - spreading to occ. reflexed
- 3 - occ. spreading to regularly reflexed
- 4 - regularly reflexed to squarrose

Camas

The second project consisted of a literature review and personal field study to aid in field identification of the post-anthesis characteristics of Willamette Valley *Camassia* taxa. This project was spurred on by the difficulty of field identification during seed collection in 2007 (post anthesis) since both species are often sympatric and hybridization does appear to occur between the two species in certain regions of the valley (Liston, personal communication). Table 4, is the culmination of this effort. My ultimate recommendation is that collection of either species should only occur in pockets where the two species are not closely sympatric. Dr. Aaron Liston (Director of OSU herbarium) suggested that these distinctions may be more noticeable north and south (Eugene and Salem respectively) of Benton County and that these characteristics have possibly blended together in Benton County due to increased hybridization between *Camassia* taxa. Considerable caution should be taken regarding geographic location of seed source when reseeding with collection materials.

Table 4: Field ID of Willamette Valley Camas Species Post-Anthesis

	<i>Camassia leichtlinii</i>	<i>Camassia quamash</i> var. <i>maxima</i>
Post-Anthesis		
Tepals	Withering tepals spreading separately not covering the ovary	Withering tepals mostly twisting around ovary; eventually breaking apart during fruiting
Bulbs	Mostly single, 1.5-3 cm thick and 2-4cm long	Bulbs ovoid, 1-2.5cm thick and 2-4cm long
Lv and scape ratio	Lvs slightly longer than scape	Scape considerably longer than lvs
Fruiting Stage		
Flower bract	Smaller or sometimes equal to pedicel in fruit	Mostly longer or sometimes equal to pedicel in fruit
Pedicel length	Typically longer; 10-40mm	Typically shorter; (5) 10-20 (30)mm
Pedicel shape	Ascending to incurved, erect in fruit	Ascending in fruit

Chapter 2 – Morphometric and Phylogenetic Analysis of *Sidalcea campestris*

Abstract

Chapter 2 details the observational research I accomplished regarding the native Willamette Valley perennial *Sidalcea campestris*. Morphometric analysis on fifteen morphological traits using ordination techniques was applied to 39 populations of *S. campestris* (n~2 per site). Additionally the results of a molecular phylogenetic analysis based on ITS sequence polymorphisms are presented. Methods involved detailed measurements of pressed specimens and a few field measurements, with multivariate ordination analysis used to search for morphological trends. Multi-response permutation procedures were used to test similarity between artificial groups developed especially for this analysis. Phylogenetic extraction and sequencing followed Whittall et al. (2000) and Liston and deFeniks (unpublished) completed the initial phylogenetic analysis using maximum parsimony. Morphometric results were statistically weak for both morphs although both suggested a difference between the central and east combined regions and the north and south combined regions of the Willamette Valley with a zone of high variation in between (Benton Co. and southern Polk Co.) that may represent a mixing zone or evidence towards a clearer picture of the historical radiation of *S. campestris*. Morphological traits that support these trends include north and south populations having larger and more branched inflorescences and shorter aerial stems than the central and east populations. Other traits, such as floral characters were not found to be strongly correlated in the analysis. Strong support for widespread variants that have been cited in past reports was not found. Molecular phylogenetic results showed normal sequence types of *S. campestris* throughout the valley but the south and to a small extent the east region of the valley is experiencing gene flow and potential hybridization, in particular with the sympatric *S. malvaeflora* ssp. *virgata*. The most significant result was evidence of gene flow between *S. campestris* and the tetraploid *S. malvaeflora* ssp. *virgata* named later in this paper as *S. species A*. I propose that there is potential for successful backcrossing between *S. campestris* and the tetraploid *S. species A*, resulting in a pentaploid variant that could be explained by the data from the current molecular phylogenetic data in this paper. Based on

these results we have a new perspective for assessing variation between these two species and I have recommended maintenance of the NSN's current seed transfer zones and exclusions with a further refinement (if not already considered) to exclude the southern halves of Benton and Linn counties from collection. Finally I propose more avenues of research to clarify the current evolutionary trajectory of *S. campestris*.

Introduction

The following is an observational study using sterile and vegetative morphological characteristics of the Willamette Valley endemic, *Sidalcea campestris* to test for phenotypic evidence of geographically isolated variant populations that could warrant exclusion or partitioning of germplasm into separate seed transfer zones (see below) for NSN's seed increase program. This was carried out using morphometric analysis of traits that might exhibit geographically isolated, locally adaptive alleles within the fragmented populations of *S. campestris* throughout the Willamette Valley. In addition, phylogenetic analysis on subsamples from the Native Seed Network's (NSN) Willamette Valley *S. campestris* populations is included to see if there are molecular trends that might suggest delineation as well.

Distribution

Sidalcea campestris Greene is one of four perennial *Sidalcea* species native to the Willamette valley. This endemic species historic range throughout the valley has likely remained approximately the same although its population density and cover have been reduced in congruence with the loss of upper dry prairie and wet prairie ecosystems where it has historically flourished. *S. campestris* occurs on both wetland and upland prairie, and along roadsides (Halse and Glad 1986) or unused pastoral type land that often retain some structural aspect of prairie ecosystem substrate. *S. campestris* is currently present in every county in the Willamette Valley ecoregion (ORNHIC 2007). Roadside or pastoral populations are typically found in agricultural or rural settings (personal observation). The subsequent management (e.g. regular mowing, herbicides) of non-managed populations such

as roadsides have allowed *S. campestris* and other native prairie species to survive in populations often consisting of anywhere from 30 to 1000, but on average 150 plants (NSN, unpublished data) however, due to rhizomatous growth the number of genetically distinct individuals is unknown. Roadside and pastoral populations may provide critical sources of genetic variability left over from historically connected populations. Since among population genetic diversity is typically much higher than individual population variability, more subpopulations may provide the adaptive potential for future whole population viability (Scribner et al. 2006).

Status

The Oregon Department of Agriculture lists *S. campestris* as a “candidate” species (ORNHIC 2007). This listing includes species which are rare but currently secure, as well as taxa whose habitat or numbers are declining but are still too common to be proposed as threatened or endangered (ORNHIC 2007). The latter is likely the case for listing *S. campestris* since it is not considered rare but its habitat is currently in jeopardy due to habitat loss and degradation from land conversion and habitat degradation (Noss 1995). The Oregon Natural Heritage Institute Program, using the ranking method developed in part by the Nature Conservancy, has given *S. campestris* a state and global rank of S4 and G4, respectively, defining taxa at this level as common and apparently secure but with existing long term concern for the species viability.

Habit/taxonomy

According to Hitchcock (1957), *Sidalcea* is a taxonomically challenging genus due to relatively little modification of floral structures among species while its other characters, such as leaves, pubescence and inflorescence congestion, often vary formidably. In context of the Willamette Valley, *S. campestris* is distinguished relatively easily from other sympatric congeners by its often taller erect stature and off-white to pale pink petals that are typically larger than other Willamette Valley *Sidalcea* species. To a less reliable extent *S. campestris* is adorned with conspicuously hirsute herbage with simple (or bifurcate) hairs on

the lower portions of the stem (Hitchcock 1957). Below ground, *S. campestris* exhibits varying forms of rootstock from heavy taproots to short, thick rootstocks likely depending upon local substrate conditions, both of which can eventually produce woody rhizomes. In addition to these phenotypic characters, *S. campestris* is one of only two hexaploid *Sidalcea* species found in Oregon and the only hexaploid *Sidalcea* in the Willamette Valley. Beyond the allopatry, *S. hirtipes* differs morphologically from *S. campestris* in that it typically has heavier well developed rhizomes, longer more hirsute and non-stellate stem pubescence, and slightly accrescent and longer calyx with more hirsute pubescence (Hitchcock 1957).

Breeding system

S. campestris exhibits a gynodioecious gender arrangement found in some *Sidalcea* species (Roush 1931), consisting of genets with either hermaphrodite flowers or male-sterile flowers that are functionally female. The male-sterile flowers still retain the typical staminal column that surrounds hermaphrodite styles but the male-sterile column shows varying degrees of stamen development and all anthers are functionally sterile. These different flower types are often referred to as morphs or sexual morphs. Hermaphrodite flowers are protanderous (delayed stamen maturation), which promotes outcrossing in hermaphrodites although *S. campestris* is self compatible (Hitchcock 1957).

Variants in the Valley

The underlying emphasis of this paper stems from phenotypically unusual *S. campestris* populations observed in different parts of the Willamette Valley, evidence of divergence that could be genetically based. The following three variants of *S. campestris*, including the typical form, have been recognized by local plant ecologists (Alverson (unpublished); Gisler 2003). There also exists speculation about the origin of *S. campestris* and possible hybridization between *S. campestris* and *S. malvaeflora* ssp. *virgata* (Whittall et al. 2000) that may result in possible clinal variation or isolated variant populations. With the notice of individual variant populations it has come to the attention of local ecologists that perhaps a

more broad scale assessment of *S. campestris*, which is currently collected for seed increase by the NSN, is in order.

Table 5. Three potentially isolated variants existing in the Willamette valley

0	<u>Typical form</u> : found throughout WV. Standard <i>S. campestris</i> traits
1	<u>Early flowering form</u> : found east of Willamette River, Linn County
2	<u>Small flowering/late blooming form</u> : found east of Lebanon
3	<u>Pink Flowered and pubescent calyx form</u> : Polk Co. and Grande Ronde region

During a 2006 assessment of potential seed transfer zones for *S. campestris*, (SICA workgroup assessment seed transfer meeting), it was stated that a wild collection program for the purpose of seed increase for prairie restoration should consider the above phenotypic variants along with any other substantial variation found throughout the Willamette Valley. If it was found that there were perceptible clinal phenotypes a consensus followed that some sort of strategy of seed exclusion from the common typical *S. campestris* form should occur. The best seed transfer strategy was unclear due to the fact that the extent of the geographic variation is unknown. Based on NSN developed seed transfer zone protocol and the paucity of information on *S. campestris* demography and taxonomy, it was concluded that further decisions should be based on more information including: 1) a common garden study of *S. campestris* from throughout the valley; 2) taxonomic research; 3) ploidy investigation; 4) and isozyme study. In the accordance with this meeting, the Institute of Applied Ecology's Native Seed Network program initiated: 1) a common garden study of *S. campestris*; 2) a collection of morph pairs from all NSN collection sites around the valley for the purpose of morphometric analysis; 3) and a phylogenetic analysis of a subset of those morphs collected from NSN collection sites. The objective of this paper is to provide the results from the morphometric study of selected floral and vegetative characteristics of *S. campestris* and the preliminary molecular phylogenetic analysis results prepared by Liston and deFeniks (unpublished) to evaluate phenotypic and genotypic clinal trends within NSN collection populations. This paper specifically tested support for or against the future separation of discrete geographical populations of *S. campestris* into separate seed transfer zones.

Methods

Collection of *S. campestris* occurred between June and July of 2006, with the goal of collecting one hermaphrodite and one male sterile morph (although occasional duplicates were collected) that was representative of the population. The final collection resulted in 48 hermaphrodites and 25 females collected from 39 different sites throughout the majority of the Willamette Valley ecoregion comprising seven different counties from as far north as Washington County to as far south as Lane County (Figure 2).

Morphological Methods

Fifteen morphological and phenotypic metrics (Table 6) were chosen in order to reveal possible statistically significant phenetic variation between *S. campestris* field collections. The wide geographic range of field collections within the Willamette valley was an attempt to cover the range of *S. campestris* populations that could be undergoing locally selective pressures. Four morphological traits were initially measured in the field on live plants however only petal color and style length were retained from the field measurements with all remaining measurements taken in the lab.

Flower traits were measured using electric calipers with the aid of a dissecting microscope at approximately 20-30x, as were the congestion metrics from the inflorescence. Floral metrics were primarily taken from the terminal, or most well developed, branch of the inflorescence. The terminal branch of the inflorescence was included for the inflorescence branch count metric.

Predefined categories of a few nondiscrete traits aided in the relatively quick identification of their optically difficult-to-quantify differences. These categories included calyx pubescence type, flower color, sepal anthocyanin content, and glaucousness. Petal color and sepal anthocyanin content classes were arbitrarily chosen based on observed differences in the field. Calyx pubescence was derived from Hitchcock (1957). I added a sixth class of hair type

which I could not classify using the existing classes. All calyx pubescence classes are as follows:

Calyx Pubescence Classes

- 1) subglabrous, occasional short hairs
- 2) uniform finely appressed stellate hairs
- 3) densely and coarsely stellate hairs
- 4) bristly hirsute with forked to several rayed stiff hairs as much as 1.5-2 mm long
- 5) long lax hairs (presumably no appressed stellate hairs)
- 6) dense, short appressed stellate hairs mixed with sparser long, 1-2 rayed hairs < 2mm

Temporal variables involving three phenologically based frequency measurements of bud, mature (full anthesis) and post-anthesis (withered) flowers were used to develop a ratio to track phenology among valley populations. Since these measures were taken over a period of two to three months, this may not be a very precise metric because of the bias towards inflorescences measured earlier in the season vs. later in the season.

Occasionally, specimen specific metrics were unable to be taken due to missing material from pressed specimens. Morphometric methods of analysis are unable to deal with missing data in a nonarbitrary way (Rohlf, 1990) therefore missing data were artificially replaced with the mean of all collected metric data for a given morph-type. This most often occurred with aerial stems. The optical estimation of glaucousness was taken using the dissecting scope on pressed specimens. My experience with this metric dictates that this trait should be taken in the field on living specimens to prevent a false negative measurement due to rubbing of pressed samples and change in optical noticability due to drying of plant material.

Table 6. Morphological traits used in morphometric analysis

Morphological Trait	Units	Further Description
A. Flower		
Petal color	Categorical (1-4)^	1) Off-white to 4) Dark pink
Petal length	mm (0.01)*	-
Petal width	mm (0.01)*	-
Calyx length	mm (0.01)*	-
Sepal width	mm (0.01)*	-
Calyx pubescence	Categorical (1-5)*	See above
Calyx anthocyanin content	Categorical (1-3)*	1) no anthocyanin 2) noticeable anthocyanin 3) Strong anthocyanin
Style length	mm (0.01)^	-

Pedicel length	mm (0.01)*	-
B. Inflorescence		
Length from flower 1 to flower 2	mm (0.1)*	<i>First measurement of inflorescence congestion</i>
Length from flower 2 to flower 3	mm (0.1)*	<i>Second measurement of inflorescence congestion</i>
Height of inflorescence	cm (0.1)*	-
Number of branches	Numeric*	<i>Includes terminal branch</i>
Number of buds	Numeric*	<i>For one selected branch</i>
Number of mature flowers	Numeric*	<i>For one selected branch</i>
Number of fertilized flowers	Numeric*	<i>For one selected branch</i>
Percent Done (Phenology)	Numeric percentage*	<i>Done/(done+open+bud)</i>
C. Aerial Stem		
Height of aerial stem	cm (0.1)*	-
<i>Presence of glaucousness</i>	<i>Presence or absence*</i>	-

Measurement taken: In-lab/preserved specimen: *, In-field/living specimen: ^

Ratios

The use of ratios, multiplicative or additive combinations of continuous variables, appears useful in morphometric analysis for analyzing dimensionally complicated traits. This is useful, for example, on flowers with high degrees of morphologically complicated perianth structures (Ballard and Wujek, 1994). However, there are difficulties in interpreting statistical significance of non-dimensional variables following multivariate analysis (Atchley et al, 1976; Atchley, 1978). Due to these complications and the fact that *S. campestris* does not have any dimensionally complex vegetative or floral characters (the exception being basal leaves but these are currently not considered reliable metrics) I decided to exclude ratios from this analysis. Further, during the preliminary stages of analysis, the addition of a *petal length / petal width* ratio showed weak correlation in explaining variation in ordination space.

Data Omissions

Data omitted includes a few collection site specimens that were lost before morphometric analysis could be performed (See Appendix G for list of all sites). In particular, and most regrettably, the following three sites used in the phylogenetic analysis were omitted:

Panther Creek
Linn Richardson Subpop. 1
Sublimity Prairie

Phylogenetic Methods

Nineteen *S. campestris* accessions representing a subsample of the NSN collection sites used in the current morphometric analysis were examined for internal transcribed spacer region (ITS) DNA sequence variation. ITS regions are highly repeated sequences within nuclear ribosomal DNA (nrDNA), that are widely used in plant systematics due to their small size (<700 bp in angiosperms), highly conserved flanking regions (18S, 5.8S, 26S), and high copy number (Baldwin et al. 1995). ITS sequences can be treated as a single locus in phylogenetic reconstruction because the multiple copies of the gene tend to be identical (due to rapid concerted evolution via unequal crossing-over and gene conversion). The ITS has proven useful in the analysis of species relationships at many taxonomic levels (Baldwin et al. 1995). Methods for extraction, amplification, and sequencing follow the methods of Whittall et al. (2000).

Phylogenetic analysis of *S. campestris* was performed by Liston and deFeniks (unpublished) using maximum parsimony (MP), a character-based tree estimation method which uses a matrix of discrete phylogenetic characters to infer one or more optimal phylogenetic trees (Lipscomb 1998). MP operates by evaluating candidate phylogenetic trees according to an explicit optimality criterion; the tree with the shortest length is taken as the best estimate of the phylogenetic relationships (Lipscomb 1998). Liston and deFeniks (unpublished) analysis treated all polymorphic DNA positions as unique characters coding each polymorphism with the following prescription:

A or G =R	C or T = Y
A or C = M	A or T = W
G or T =K	C or G = S

Since PAUP (Swofford 1998) treats DNA polymorphisms as missing data, this coding allowed their inclusion and thus better resolution of trees.

Included with the subsample of NSN collection sites were *Sidalcea* accessions from Andreasen and Baldwin (2003) and Whittall et al. (2000). Species used from the former

include putative hybrids and multiple clones from four reputed clades (“glaucescens, malviflora, asprella, and oregana”) of *Sidalcea* (Andreason and Baldwin 2003) from throughout California and Oregon. Accessions of *S. campestris*, *S. nelsoniana*, *S. hirtipes*, *S. malvaeflora* ssp. *virgata* (including two ploidy levels) and *S. robusta*, all originating from western Oregon with the exception of *S. robusta* from California, were used from the latter paper.

Analysis

Data Handling and Transformation

The female flowers of almost all temperate gynodioecious species are smaller in relative size than those of hermaphrodites (Darwin, 1877; Baker, 1948; Delph, 1996). Bias caused by this condition was controlled by performing analyses on each gender separately.

A log transformation was applied to male-sterile morphs to reduce bias associated with a small sample size, to increase uniformity of data and provide a stronger resolution of dimensionality in ordination space. Due to the analysis’ inability to use negative numbers, variables that contained values of zero could not be transformed. Similarly, categorical variables were not log transformed. The variables log transformed for male-sterile morphs included:

HT1	(aerial stem height)
HT2	(inflorescence height)
INF1	(measure of inflorescence congestion)
INF2	(measure of inflorescence congestion)
PETALL	(petal length)
PETALW	(petal width)
BRANCH	(number of inflorescence branches)
CALYXL	(calyx length)
PEDL	(pedicel length)
SEPALW	(sepal width)
STYLEL	(style length)

Nonmetric Multidimensional Scaling (NMS)

Multivariate statistical techniques are often used to ordinate morphometric data so that biological parameters underlying morphological relationships among individuals or groups may be more readily discovered (Albrecht, 1979). Nonmetric multidimensional scaling (NMS) is an ordination method well suited to data that are non-normal or on arbitrary, discontinuous or otherwise questionable scales (McCune et al. 2002). NMS seeks to minimize departures from *stress* by arranging treatment units in species space such that similar units are closer together and dissimilar units are farther apart (Kruskal 1964; McCune et al. 2002). *Stress* is measured as the difference between an ideal configuration or full model (using one dimension for each variable or species) and the proposed configuration or reduced model (a lower dimensional, computer-generated solution). Low stress indicates that the reduced model with fewer dimensions adequately approximates the full model. For ecological data, a measure of acceptable stress levels is: <5 = excellent representation with no prospect of misinterpretation; 5-10 = a good ordination with no real risk of drawing false inferences; 10-20 = still corresponds to a usable picture although values at the upper end suggest a potential to mislead and users should avoid reading into plot details (Clark, 1993).

Fifteen *S. campestris* traits were ordinated, with hermaphrodites (n = 48) and females (n = 25) in separate matrices, using NMS based on Sørensen's distance measures (Kruskal 1964; McCune and Mefford 1997). Matrices were arranged by individual plants (rows) and morphological traits (columns) with the following three grouping overlays appended to the second matrices: soil type, county and an artificially derived Willamette valley region (Figure 2). The primary reason for utilizing grouping overlays is to clarify the distribution of sample units in ordination space. Many ecological study designs sample species multiple times from each population or replicate treatments which serves as a visually distinct grouping variable when viewed in ordination space. However, this study relied on collection of only one (rarely two) morphs from any given population resulting in a need for synthetic grouping categories or overlays. Due to the rather homogenous physical conditions (precipitation, elevation) of the Willamette Valley, grouping sample units by county, Willamette valley region, and soil type were the only obvious choices for observing possible

clinal trends. While a global positioning based coordinate system would have been ideal, the field collection of these coordinates was not strict, often used multiple coordinate systems, and many data were missing. The Willamette valley regional grouping variable (Table 7), which delineates naturally clustered NSN collection sites throughout the Willamette valley (Figure 2), does not infer ecological boundaries. It does however attempt to group collections in such a way as to isolate certain variants believed to exist. County was used for this purpose as well. A soil overlay was derived using free online based GIS soil layers from each county and subsequently reduced to generic soil constituents.

Table 7. Categorical variables (overlays) applied to NMS matrices

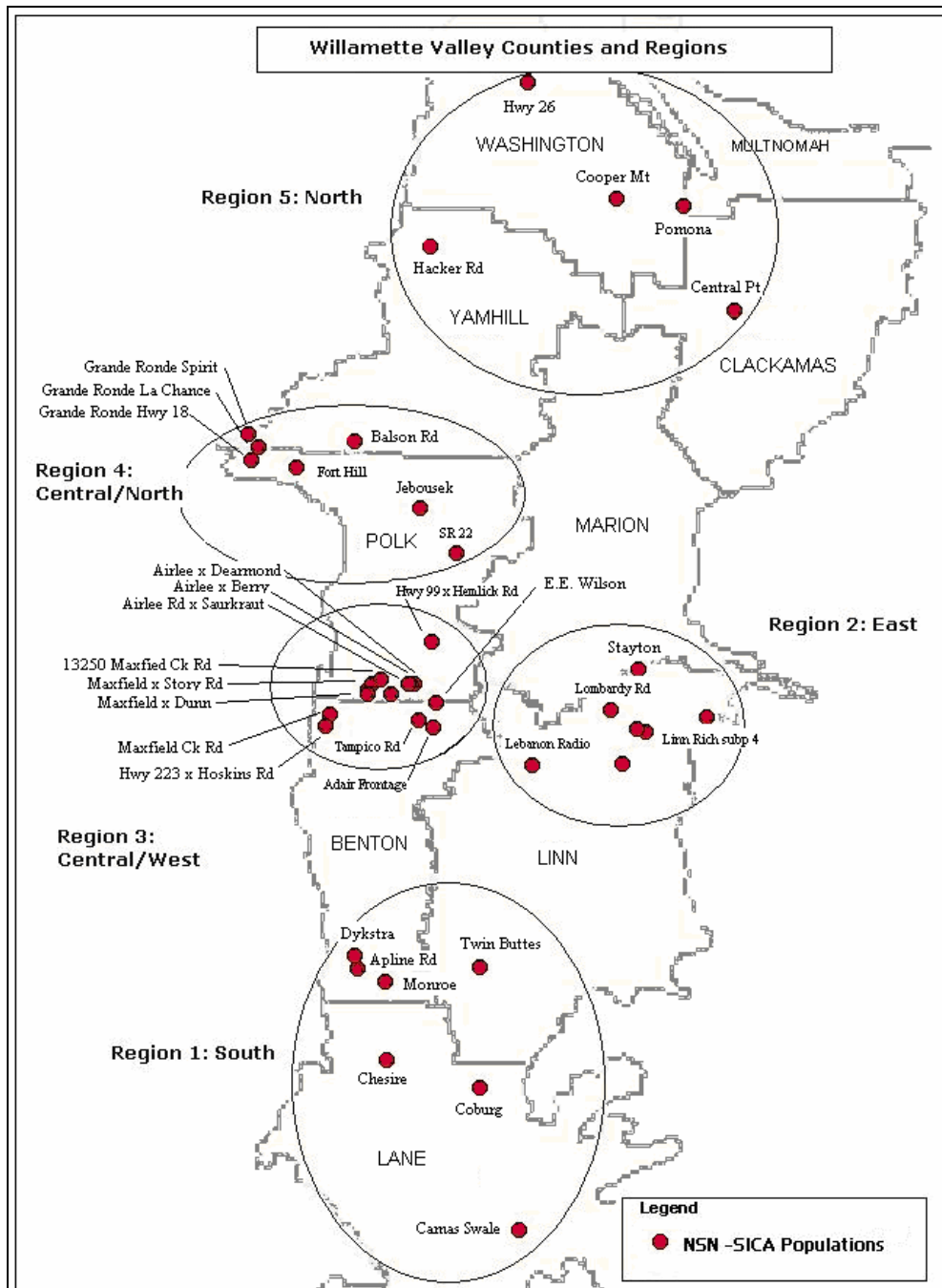
County	W.V. Region (Figure 2)	Soil
Benton	South	Silty Clay Loam
Polk	East	Silt Loam
Yamhill	Central/west	Gravelly Silt Loam
Washington	Central/north	Gravelly Loam
Clackamas	North	Unknown/Urban
Lane		
Linn		

Multi Response Permutation Procedure (MRPP)

Further analysis of artificial groups (overlays) was carried out by multi-response permutation procedures (MRPP). This multivariate algorithm provides the statistic T , as a measure of between group proximity in ordination space, which is a result of similarity within groups. The distance between groups is considered greater as T becomes larger in the negative direction, which infers that groups are more dissimilar and therefore farther apart in ordination space. A larger positive value of T infers more similarity within groups and less distance between groups. MRPP also provides the statistic A , which measures an effect size: the level of within-group heterogeneity. A value of 1 denotes complete within-group heterogeneity (all components of the group are different) and signals a strong within-group effect on T , the measure of dissimilarity between groups. A value of 0 denotes perfect homogeneity (all components of the group are the same) within groups and likewise a weak or absent affect on group dissimilarity (T). MRPP provides *p-values* based on pair-wise

comparisons between groups. MRPP does not correct for multiple comparison errors known to inflate p-values therefore a Bonferroni correction ($p\text{-value} * n \text{ groups}$) was applied to final estimates. Since MRPP cannot test between groups with less than 2 components, Washington and Clackamas County (with only one sample each) were left out of the analysis during MRPP testing for county similarity.

Figure 2. Willamette valley region overlays and NSN *S. campestris* population locations



Results

Hermaphrodite morphs

NMS ordination of *S. campestris* hermaphrodites resulted in a three dimensional solution with a final stress of 8.89 following 96 iterations and a final instability of <0.0001. The cumulative proportion of variation accounted for by all axes was 0.934 (Table 8). The resulting ordination, with three geographical and ecological overlays (Table 7), resulted in no distinct, separate clusters of individual plants in ordination space. However, the following MRPP analysis provided some remarkable signals with the Willamette valley and county overlays.

Table 8. Proportion of variation (R^2) in *S. campestris* male-sterile and hermaphrodite morphological variables accounted for by NMS ordination

<u>Hermaphrodite</u>	<u>Axis</u>	<u>Incremental R^2</u>	<u>Cumulative R^2</u>
	1	.339	.339
	2	.441	.780
	3	.154	.934
<u>Male Sterile</u>			
	1	.305	.305
	2	.504	.808

After subjecting p-values to Bonferonii adjustments to reduce multiple comparison errors there were no statistically distinct groups in the MRPP pair-wise group analysis however, the initial analysis did provide some suggestive statistically significant results that are treated here as trends worth discussing. Further, while MRPP *T* statistics suggest there is dissimilarity between some groups, all *A* statistics were close to zero values suggesting very little within group dissimilarity (i.e., unique groups). This may or may not be within an acceptable range for taxonomic data however.

MRPP analysis of Willamette valley groups provided suggestive evidence that there may be some between group dissimilarity between east and south groups and between south and

central/west groups (Table 9). Figure 2, shows that east groups consist of northern Linn County populations (east of the Willamette River); south populations consist of southern Benton, Linn and all Lane County populations; and central/west are all northern Benton and southern Polk County populations. The NMS output for hermaphrodites (Figure 3), shows east and south groups slightly overlapping in the middle of the plot. Central/west and south groups are seen to overlap quite a bit more than east and south groups in the present two dimensions (Figures 3,4,5) but a 3-dimensional view rotation (not shown) of the points in space corroborated that while there is overlap, south populations are just slightly nested in a corner of the central/west cluster. The suggested differences between Willamette Valley groups give the impression of a possible north south cline but only between approximately Benton/Linn and Lane counties. This gradient was not corroborated by MRPP using county grouping variables. Additionally, soil overlay resulted in no geographically discernable patterns.

Table 9: MRPP pair-wise comparisons for *S. campestris* hermaphrodite groups. Bonferroni corrected p-value in parenthesis. Alpha = 0.05

	T	A	p-value
Willamette valley region			
East vs. South	-2.36	0.056	0.027 (0.135)
South vs. Central/West	-1.73	0.024	0.063 (0.315)

Morphological Traits

Traits with strong correlation to the ordination axes govern the spatial trends in NMS ordination. The measure of a given trait's correlation, Pearsons product-moment correlation (r), can be seen as rays emanating from the center (word) of ordination space with larger values of a particular variable representing stronger correlation with a particular axis. This allows us to interpret morphological trends among groups if groups are well delineated in ordination space. The difference between a weak and strong Pearsons correlation is relative to the type of data that is being explored. The range I used to exclude weakly correlated traits in the analysis was $r > 0.500$, with $r > 0.800$ suggestive of a strong correlation.

Two traits that showed strong correlation (with axis 2) were the aerial stem height (HT1) and inflorescence height (HT2) (Table 10). One remarkable aspect of this trait was the inverse relationship between HT1 and HT2. As height of the aerial stem increased the height of the inflorescence decreased along this gradient and vice versa. The east Willamette Valley region shows the greatest trend (clustering) towards generally having taller inflorescence heights and therefore shorter aerial heights. The central/north region to a lesser extent shows this trend with two strong outliers in the opposite direction. Finally, the south region shows an opposite trend with shorter inflorescence heights and therefore longer aerial stems with the north region mimicking this trend to a lesser extent. The number of inflorescence branches (BRANCH) correlated in a similar manner with inflorescence (HT2), which seems rather intuitive and possibly an artifact of plants with longer inflorescences likely having a greater number of inflorescence branches. Correspondingly, the east and central/north Willamette Valley regions aligned predominately with a greater number of inflorescence branches while the south and north regions had less branching per inflorescence.

Table 10. Pearson product moment correlations for *S. campestris* hermaphrodite morphological variables (> .500) with ordination axes (N= 48)

Axis:	1	2	3
	r	r	r
HT1	.470	-.856	-.013
HT2	-.558	.813	-.056
INF1	-.742	-.115	.518
INF2	-.729	-.038	.315
BRANCH	-.240	.771	-.127
PERCDONE	-.239	.092	-.937

Figure 3. Hermaphrodite populations labeled in ordination space using the Willamette valley region overlay (trait $r > .500$)

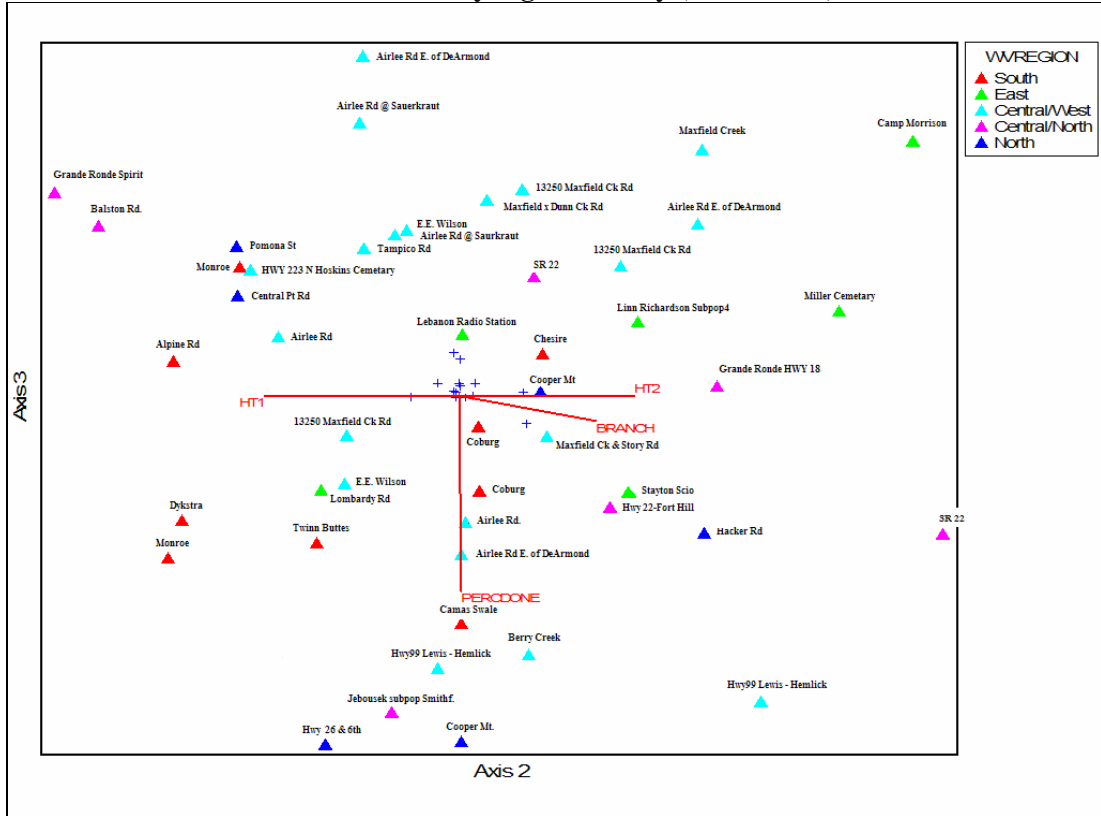
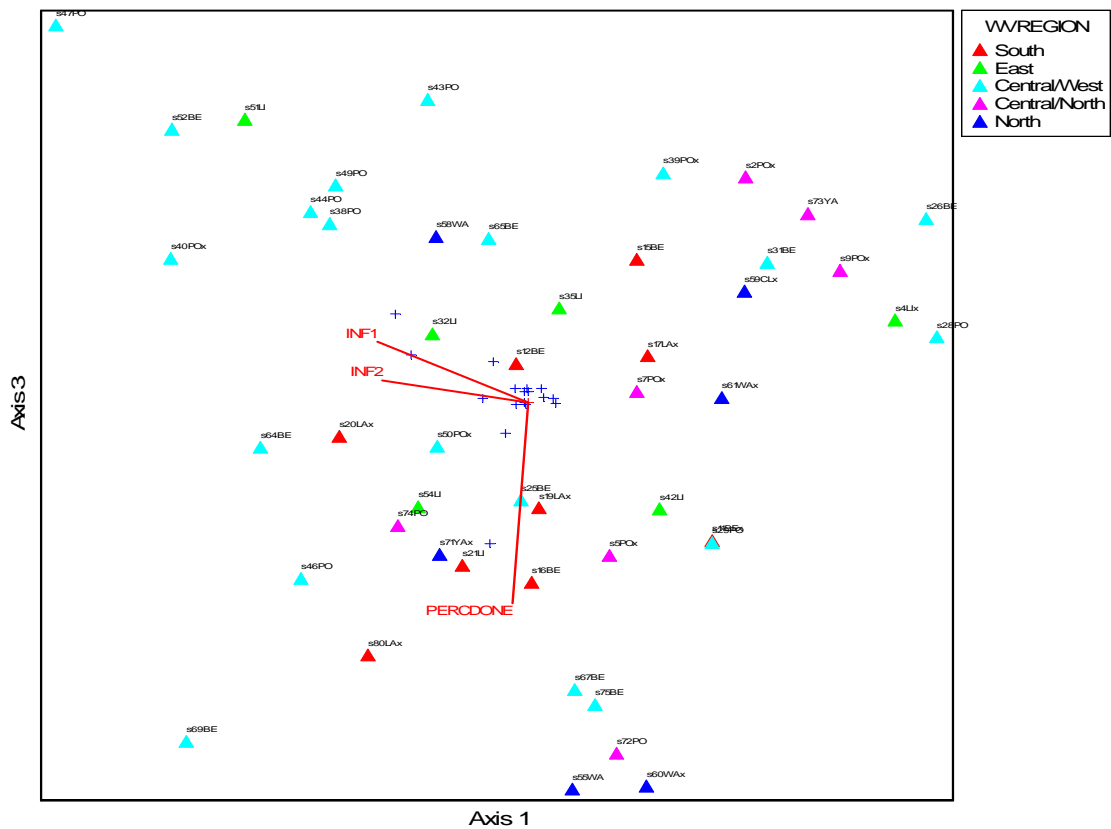


Figure 4. Hermaphrodites in ordination space using the Willamette valley overlay (trait $r > .500$)



The metric of inflorescence congestion: the distance between the first and second lowest flower on the inflorescence (INF1) and the second and third (INF2), exhibited strong to moderate correlation with axis 1 and 3 (Table 10). The north and central/north Willamette Valley groups showed a trend towards lesser inflorescence congestion.

The final metric, percentage of post anthesis flowers (PERCDONE), or measure of current phenology at time of sampling, showed the strongest correlation with axis 3. The south Willamette Valley group and Lane County showed a slight trend towards higher percentage

of individual plants in ordination space. The MRPP analysis provided one remarkable signal with the county overlay. However, with such a large final stress and weak data structure possibly due to a small sample size (n=25) with likely high variance, our graphical interpretation should be taken as speculative and with caution (see Methods).

Table 11: MRPP pair-wise comparisons for *S. campestris* male-sterile groups. Bonferroni corrected p-value in parenthesis. Alpha = 0.05

	T	A	p-value
County			
Benton vs. Lane	-2.80	0.18	0.017 (0.085)

As seen with hermaphrodites, MRPP analysis did not show statistically significant results for male sterile morph groups following correction for multiple comparisons. MRPP analysis suggested dissimilarity between Benton and Lane Counties (Table 11), which can be seen readily in ordination space (Figures 6 and 7). This suggested difference between central and southern counties emulates the suggested difference between the central/west and south Willamette Valley hermaphrodite groups.

Figure 6. Male-steriles in ordination space using the Willamette valley region overlay

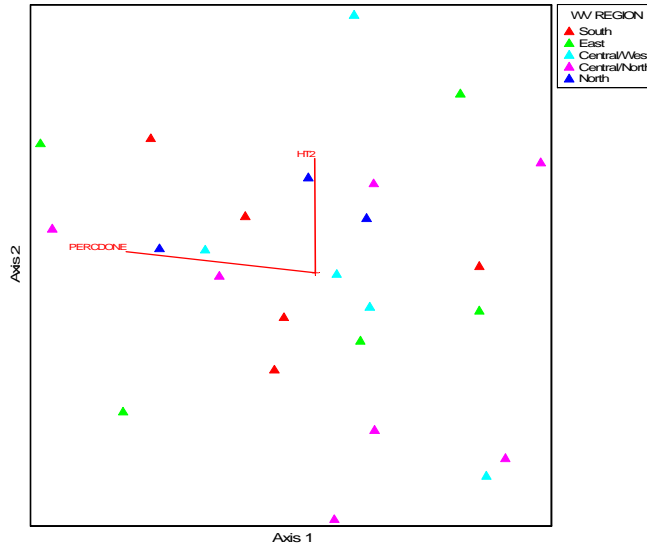
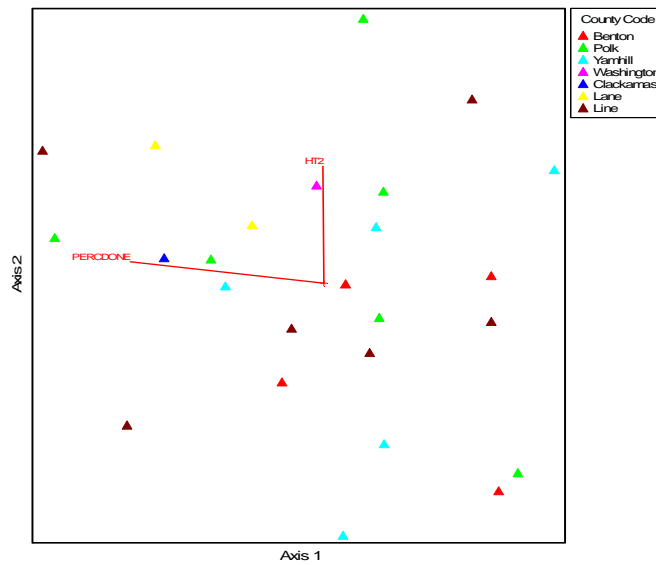


Figure 7. Male-steriles in ordination space using the Oregon County overlay



Morphological Traits

The trait that showed the strongest correlation, with axis 1 (Table 12), was the percent done ratio (PERCDONE). In this case, the southern and northern most groups appear to have a greater percentage of post-anthesis flowers. The second strongest trait was inflorescence height (HT1), which was inversely correlated with aerial stem height (HT2) similar to hermaphrodite results (although HT2 was not found to be very strongly correlated with an axis). Similar to hermaphrodites, inflorescence branch number (BRANCH, not shown) was also correlated along the same axis as inflorescence height but not as strongly as with hermaphrodites. Figure 6, shows that the north Willamette Valley region clustered towards taller inflorescences, supported by Washington County (Figure 7), having overall taller inflorescence heights and correspondingly shorter aerial heights and more inflorescence branches.

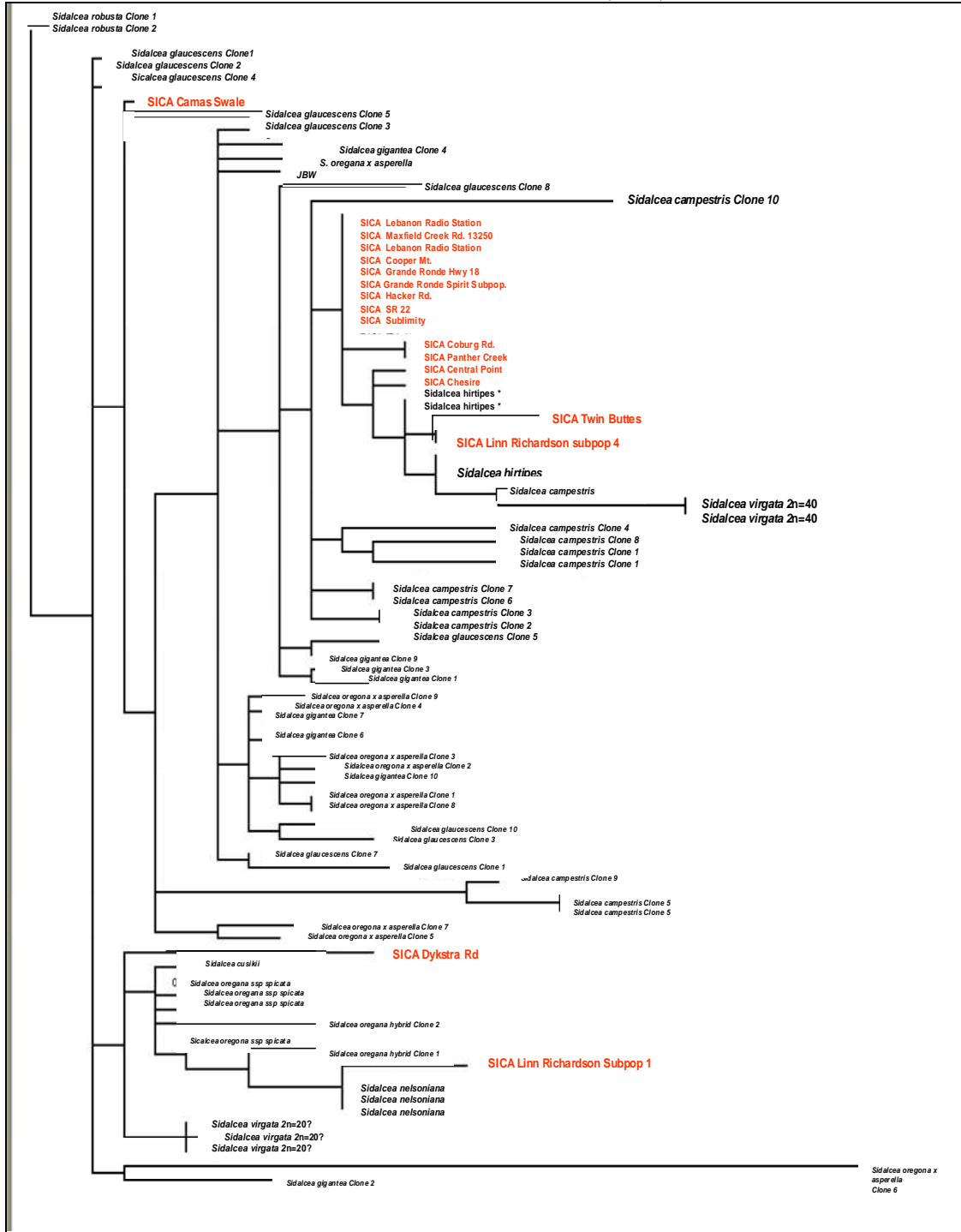
Table 12. Pearson Correlations of *S. campestris* male-sterile morphological variables (> .425) with ordination axes (N= 25)

<u>Axis:</u>	<u>1</u>	<u>2</u>
	<u>r</u>	<u>r</u>
HT2	-.078	.805
BRANCH	.058	.576
PERCDONE	-.943	.347

Molecular Phylogenetic Results

Of the 18 NSN accessions (Table 13) 15 resolved in a single "*S. campestris*" clade (Figure 8 and 10) that also contains previously published ITS sequences of *S. campestris*, as well as *S. hirtipes* and the tetraploid *S. malvaeflora* ssp. *virgata* variant. Within this clade, 10 NSN accessions have identical sequences, two accessions share another sequence, and another two accessions have a third set of unique sequences. It is noteworthy that the NSN accessions do not match the previously published *S. campestris* sequences.

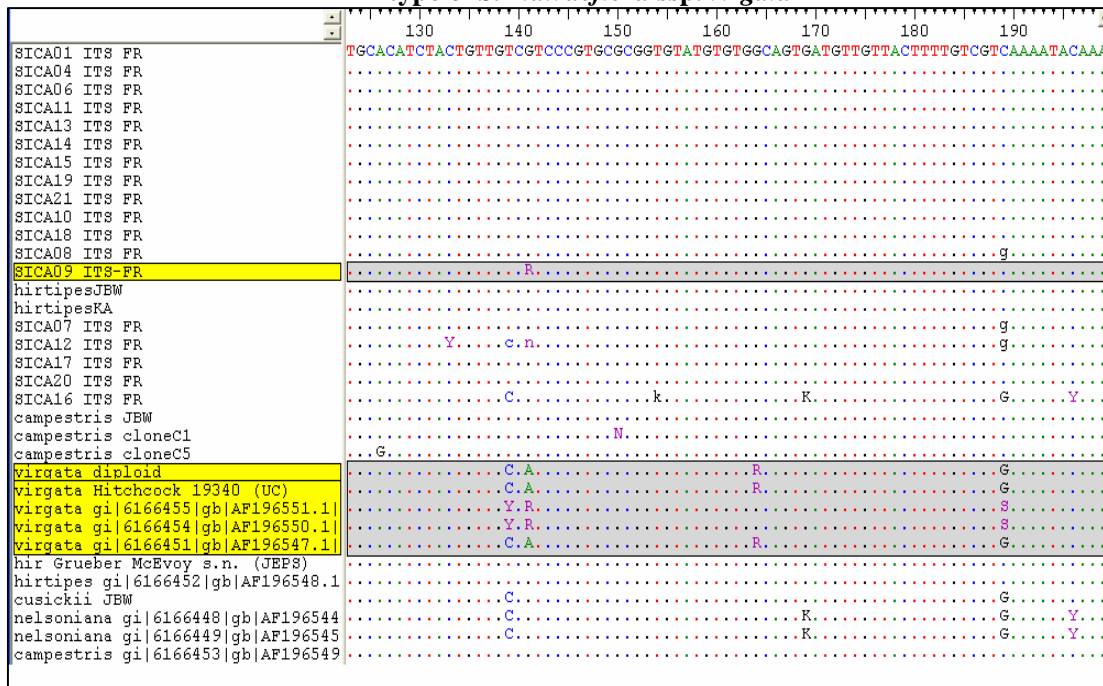
Figure 8: Molecular phylogenetic results based on ITS polymorphisms of NSN *Sidalcea campestris* accessions (red), *Sidalcea* congeners and outgroups from Whittal et al. (2001), and Andreason and Baldwin (2003)



The three accessions outside of the *S. campestris* clade were considered outliers. One outlier, Linn Richardson subpopulation 1, resolved with three *Sidalcea nelsoniana* accessions. Steven Gisler's thesis (2003) on interspecific compatibility of the four Willamette Valley congeners found almost complete sexual incompatibility between *S. campestris* and *S. nelsoniana* due to post-mating barriers which aids in suggesting that this individual is very likely *S. nelsoniana* and not a hybrid between *S. nelsoniana* and *S. campestris*. The remaining two outliers, Dykestra Rd. and Camas Swale, may be of hybrid origin, but this requires further verification.

Of most importance was the ITS polymorphism at base pair (bp) 141 (Figure 9). This polymorphism is unique to one of two known *S. malvaeflora* ssp. *virgata* tetraploid variant DNA types (Liston, personal communication) but was found in the Chesire (Lane County) *S. campestris* accession. The difference between the two *S. malvaeflora* ssp. *virgata* tetraploid variant DNA types are represented in Figure 8 and Figure 9. One of which places in the main "*S. campestris*" clade and is known for an Adenine at bp 141 (Figure 9), and one in the lower section of the tree, known for the polymorphism of Adenine or Guanine at bp 141 (Figure 9). The evidence of the shared polymorphism between *S. campestris* and the polymorphic variant of *S. malvaeflora* ssp. *virgata* provides direct evidence of gene flow between the two taxa which will be discussed further below.

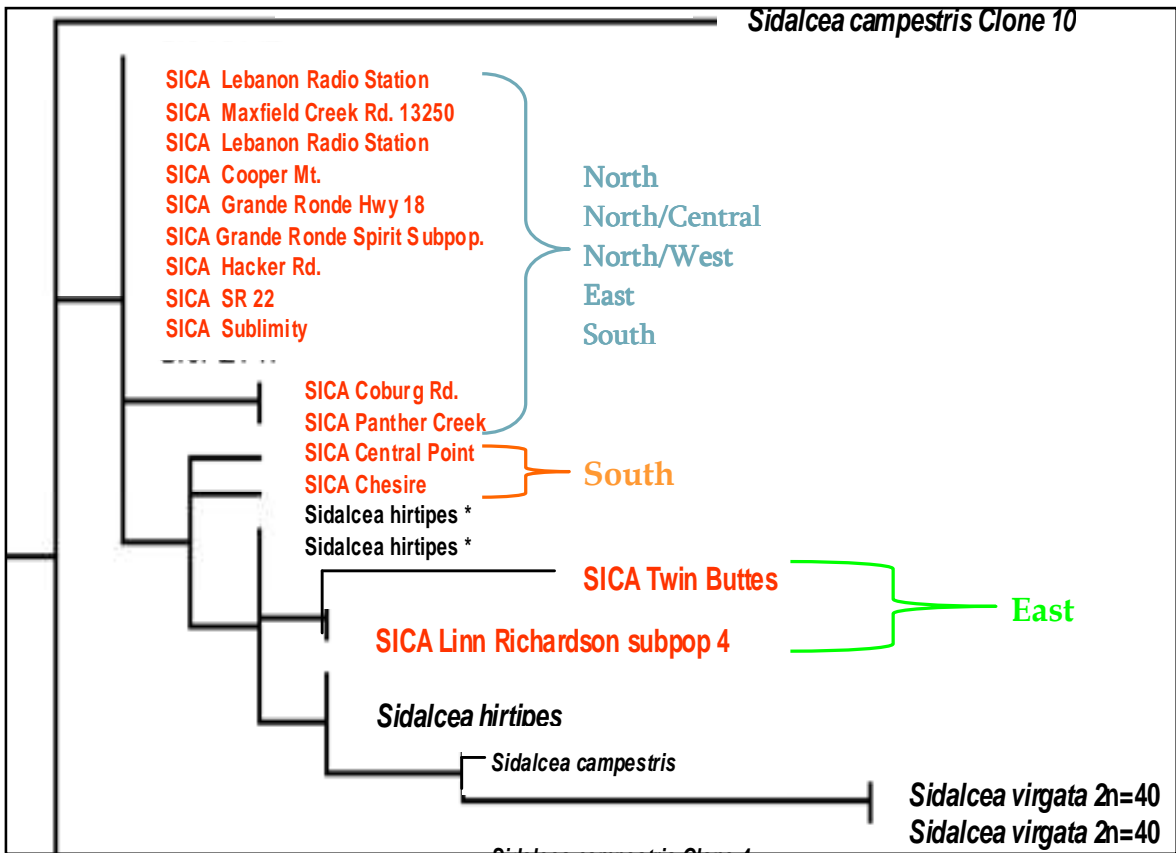
Figure 9: ITS region exhibiting shared polymorphisms between *S. campestris* and one sequence type of *S. malvaeflora* ssp. *virgata*



Geographic trends

Within the main “*S. campestris*” clade, there exist geographic patterns of interest. The first ten accessions represent the typical *S. campestris* ITS sequence and contain accessions representing populations from all Willamette Valley regions (Figure 10). The next sequence that deviates from the dominant sequence is composed exclusively of two south Willamette Valley regions one being the above mentioned Chesire population which shares the polymorphic condition at bp 141 with tetraploid *S. malvaeflora* ssp. *virgata* variant grouped at the far end of the “*S. campestris*” clade (Figure 8 and 10). Finally, the third unique ITS sequence is composed of populations east of the Willamette River, one which is grouped within the east Willamette Valley region (Linn Richardson subpop 1) and one which is grouped with the south Willamette Valley region (Twin Buttes). The outliers, or putative hybrids, are both from the south Willamette Valley region as well. One in particular (Dykstra Rd), is associated basally with the second tetraploid *S. malvaeflora* ssp. *virgata* variant sequence type (Figure 8).

Figure 10: Geographic patterns within the “*S. campestris*” clade



**Table 13: *S. campestris* populations and voucher numbers, F= male-sterile, H= hermaphrodite:
 *=Accessions used for molecular; Currently stored at IAE's herbarium**

Population		vouchers	Population		vouchers
Grand Ronde subpop. LaChance	1	SICA-JKL-06-001 (F)	13250 Maxfield Ck Rd	21	SICA-JKL-06-039 (H) SICA-JKL-06-040 (H) SICA-JKL-06-050 (H) (no F's)
Grand Ronde subpop. Spirit	2	SICA-JKL-06-002 (H) SICA-JKL-06-003 SICA-JKL-06-006 (F) SICA-JKL-06-008	Stayton-Scio Rd	22	SICA-JKL-06-041 (F) SICA-JKL-06-042 (H)
Grand Ronde subpop Hwy 18	3	SICA-JKL-06-007 (H) SICA-JKL-06-010 (F)	Airlee Rd E of DeArmond	23	SICA-JKL-06-044 (H) SICA-JKL-06-045 (H) SICA-JKL-06-046 (H) SICA-JKL-06-047 (H)
Linn-Richardson subpop. 4	4	SICA-JKL-06-004 (H)	Maxfield Creek x Dunn Forest Rd	24	SICA-JKL-06-048 (F) SICA-JKL-06-049 (H)
SR22	5	SICA-JKL-06-005 (H) SICA-JKL-06-009 (H)	Maxfield Creek	25	SICA-JKL-06-052 (H)
Dykstra	6	SICA-JKL-06-011 (H) SICA-JKL-06-014 (F),	Lombardy Rd.	26	SICA-JKL-06-053 (F) SICA-JKL-06-054 (H)
Alpine Rd	7	SICA-JKL-06-013 (F), SICA-JKL-06-12 (H)	HWY 26 & 6th Intersection	27	SICA-JKL-06-055 (H)
Monroe	8	SICA-JKL-06-015 (H) SICA-JKL-06-016 (H)	Central Point Rd	28	SICA-JKL-06-056 (F) SICA-JKL-06-059 (H)
Cheshire	9	SICA-JKL-06-017 (H), SICA-JKL-06-018 (F)	Pomona	29	SICA-JKL-06-057 (H) SICA-JKL-06-058 (H)
Coburg	10	SICA-JKL-06-019 (H) SICA-JKL-06-020 (H)	Cooper Mt.	30	SICA-JKL-06-060 (H) SICA-JKL-06-061 (H)
Twin Buttes (Steve G. recommended)	11	SICA-JKL-06-021 (H), SICA-JKL-06-022 (F)	Berry Creek (x with Airlee)	31	SICA-JKL-06-067 (H) SICA-JKL-06-068 (F)
Mt Richardson (Linn_Richardson subpop. 4???)	12	SICA-JKL-06-023 (F) SICA-JKL-06-024 (H)	Tampico Rd	32	SICA-JKL-06-065 (H) SICA-JKL-06-066 (F)
E.E. Wilson	13	SICA-JKL-06-025 (H), SICA-JKL-06-026 (F)	Story Road X Maxfield Creek Rd	33	SICA-JKL-06-063 (F) SICA-JKL-06-064 (H)
Adair Frontage	14	SICA-JKL-06-027 (H)	Hwy 99 - Lewisburg to Hemlick Pk. (Also 15 plants at Adair)	34	SICA-JKL-06-069 (H) SICA-JKL-06-075 (H)
Airlee Rd	15	SICA-JKL-06-028 (H), SICA-JKL-06-029 (H)	Hacker Rd	35	SICA-JKL-06-070 (F) SICA-JKL-06-071 (H)
Hwy 223 -- N of Hoskins Cemetery	16	6/12, SICA-JKL-06-031 (H)	Jebousek-sub population Smithfield Rd.	36	SICA-JKL-06-072 (H) SICA-JKL-06-077 (F)
Radio Station - Lebanon	17	SICA-JKL-06-032 (H) SICA-JKL-06-033 (F)	Balston Rd(Not Found)-data here are for new site called "Red Priairie Rd."	37	SICA-JKL_06-073 (H)
Miller Cemetary	18	SICA-JKL-06-034 (F) SICA-JKL-06-035 (H)	Hwy 22 - Fort Hill	38	SICA-JKL-06-074 (H) SICA-JKL-06-076 (F) SICA-JKL-06-078
Camp Morrison	19	SICA-JKL-06-036 (F) SICA-JKL-06-037 (INF not mature)SICA-JKL-06-051(H)	Camas Swale	39	SICA-JKL-06-079 (F) SICA-JKL-06-080 (H)
Airlee Rd @ Sauerkraut	20	SICA-JKL-06-038 (H) SICA-JKL-06-043 (H)			

Valley Wide Trends (summary)

In general, there are no statistically robust results to mandate delineation of NSN collection sites into individual seed transfer zones based on morphology alone however, there are some perceptible trends that when combined with molecular phylogenetic results may serve as means to renewed management considerations. First, the populations in the east near Lebanon and east of the Willamette River, noted as having two variant types based on phenology (Table 5), appear within a level of variation well within the range of other populations throughout the valley. As for darker petaled variants in the Grande Ronde region, they may not have been sampled well enough to register in this analysis as petal color was not a strong variable. It is likely these populations are not a large concern and are not widespread.

Based on hermaphrodite populations, the current analysis found similarities between the north and south Willamette Valley regions and similarities between the north/central and east Willamette Valley regions with a zone of mixing in the central/west region that appeared to encompass all the variation found in the other four regions. The primary trend among east and north/central Willamette Valley regions are towards taller inflorescences with greater branches and proportionally shorter aerial stems, while the south and north region exhibits shorter inflorescences with correspondingly taller aerial stems. It should be noted however, all regional groups have outliers diverging from these trends. The central/west region exhibits high variation without strong trends along any metric. Phylogenetic and ordination placement and alignment, respectively, of the two putative hybrid outliers (Dykstra Rd. and Camas Swale) show no close relationship.

Molecular phylogenetic results show a slightly different perspective and series of geographic patterns within Willamette Valley *Sidalcea* populations as compared to the morphological results. The evidence of gene flow, and possible hybridization between *S. campestris* and the tetraploid *S. malvaeflora* ssp. *virgata*, appears stronger in the south and east Willamette Valley regions with no evidence of gene flow in the central/west and northern regions based on this analysis. These complications will be discussed in the next section.

Discussion

Sampling Methods and Precision

It is possible that the artificial grouping variables used for this analysis were at a scale not fine enough to detect groups based on their selected parameters. Due to the type of analytical tools used, I was unable to test difference between individual populations which would have been ideal but would also have substantially increased multiple comparison error buffering. Thankfully, since grouping variables do not influence ordination output we are still able to view the overall spread of points to infer morphological deviance of NSN *Sidalcea* populations in the Willamette Valley ecoregion. One suggestion for possible future analyses is using UTM position of each collection site for a finer measure of latitudinal or longitudinal gradients in accordance with a larger sample per site to account for more within site variation. Another grouping variable suggested by Tom Kaye (personal discussion 2007), which was found to be useful for research regarding *Castilleja levisecta*, includes a measure of soil drainage at each site.

From Variant to Variation

It is evident that the selected morphological traits and overall sampling design from this analysis did not support discrete patterns of morphological variation among NSN *S. campestris* collection sites such as have been recently cited (Alverson 1996; Gisler 2003). What we can surmise from this analysis is that that these variants (Table 5) either 1) did not register in the analysis due to the current sampling design; or 2) were sampled but are within normal parameters of the overall variation within *S. campestris*. In contrast, phenology appeared to be more broadly strongly correlated along a latitudinal gradient (which is common with many species) with increased post-anthesis flowers in the central east and south valley. The phenology metric used in this analysis is ostensibly meant to represent relative bloom times throughout the valley. However if the east region, for example, did have an unusually early bloom but the south had considerably increased pollinator visitation and success, a phenology metric may not detect an early bloom in the east. The second metric, flower petal color, a trait witnessed to be darker in variant populations in the central/north

(Polk County) failed to even register as a strongly correlated trait in the analysis. If these variants were sampled properly, the current morphometric and phylogenetic analysis does not distinguish variants with early and later flowering populations on the east side of the Willamette River or pink flowered and heavily pubescent flowers in Polk County and the Grande Ronde region as unique. However, until more intense sampling (especially molecular) of the variant populations in Table 5 can be pursued I suggest they are too rare to be of importance in a collection program and can be simply excluded if they continue to produce these characteristic traits.

Regardless of the above results there were additional trends detected from this analysis. This analysis witnessed similarities of morphology at the farthest north and south range limits of *S. campestris* inflorescence and aerial stem heights divided by a central zone of opposite variation and a region with no strong trend. Ultimately, these phenotypic patterns could be variability common with a putatively recent radiation of *Sidalcea* (Andreasen and Baldwin 2001) and potentially all within the normal range of variation for *S. campestris*. Depending upon the point of radiation for this species these geographic trends may be informative for future research although this is beyond the scope of the current study. Most interestingly, evidence was quite unexpectedly found supporting gene flow between the sympatric and putatively rare tetraploid *S. malvaeflora* ssp. *virgata* variant which makes the current morphological results more intriguing when put into the perspective of congener interactions.

Phylogenetic analysis has begun to provide greater resolution of evolutionary relationships among Willamette Valley *Sidalcea* (Whittal et al. 2000; Andreasen and Baldwin 2001; Andreasen and Baldwin 2003a; Andreasen and Baldwin 2003b). Single nucleotide additivity polymorphisms (SNAPS) suggest that the hexaploid lineage represented by Willamette Valley *S. campestris* and Coastal Oregon and S.W. Washington *S. hirtipes* is likely of an allopolyploid origin (Whittal et al. 2000). In more modern evolutionary history, SNAPS point to increased support for hybridization between a hexaploid and the common diploid *S. malvaeflora* ssp. *virgata* as being the source of the rarely sampled tetraploid hybrid in the Willamette Valley which to date has been labeled as tetraploid *S. malvaeflora* ssp. *virgata*

(Whittal et al. 2000; Kruckeberg 1957). ITS polymorphisms from the current analysis show that in fact there has been some gene flow between *S. malvaeflora* ssp. *virgata* and *S. campestris*. This evidence and the fact that the hexaploid progenitor is likely sympatric with *S. malvaeflora* ssp. *virgata* (Whittal et al. 2000) implicates *S. campestris* almost without question as the hexaploid source of the putative tetraploid hybrid. Ultimately, Whittal et al. (2000) called for this putative hybrid to be recognized under a new taxonomic status (Whittal et al. 2000). To avoid confusion it (tetraploid *S. malvaeflora* ssp. *virgata*) will hereafter be referred to in this paper as *S. species A*.

Besides Kruckeberg's (1957) interspecific hybridization greenhouse studies of *Sidalcea*, Steven Gisler's thesis (2003) is the most thorough interspecies compatibility experiment among Willamette Valley *Sidalcea* species. Gisler's pairwise crosses with *S. nelsoniana* revealed an overall high level of interspecies compatibility between *S. malvaeflora* ssp. *virgata* and *S. cusickii* when pre-mating (temporal and geographic) barriers were removed. This experiment along with Kruckeberg (1957) show that infraspecies *Sidalcea* hybrids among different ploidy levels are in theory viable. More specific support for the potential hybridization between *S. malvaeflora* ssp. *virgata* and *S. campestris* noted in Whittal et al. (2000), comes from successful crosses between *S. campestris* and *S. species A*, (Gisler unpublished data) indicating incomplete crossing barriers among backcrosses of the hybrid to the putative hexaploid parent, *S. campestris*.

Pentaploids in the Valley

A cross between *S. campestris* and *S. malvaeflora* ssp. *virgata* is likely the cause of the tetraploid *S. species A* that is apparently successful at surviving in the valley. What has not been considered to this point is whether or not the variation in *S. campestris* is the result of backcrosses between *S. campestris* and *S. species A*, which might result in a variant pentaploid that is morphologically similar to *S. campestris* but with enough variation as to result in a rather erratic amount of morphological variation within the species.

Informal morphological comparisons of *S. malvaeflora* ssp. *virgata* ($2n=20$) and *S. species A* ($2N=40$) indicate that the tetraploid has much larger basal leaves, longer stems, more flowers

per inflorescence, a more whorled inflorescence structure and a northern Willamette Valley distribution (Gisler and Whittall, unpublished), although these characteristics were not found to be consistent among *S. species A* (Gisler, S. personal communication) and it has also been collected from Lane County (Hitchcock and Kruckeberg 1957).

Further, Kruckeberg (1957), found that crosses between *S. campestris* ($2n=60$) and *S. malvaeflora* ssp. *patula* ($2n=40$) resulted in a hybrid (ostensibly pentaploid) with 95% stainable pollen. This example and reported successful backcrosses between *S. campestris* and *S. species A* (Whittall and Gisler, unpublished), promote the possibility of pentaploid variants occurring naturally in the Willamette Valley. Although such pentaploid offspring would likely be sterile, the perennial and vegetative propagating (rhizomatous) nature of *Sidalcea* may confer the ability for persistence of such a variant. Chromosome doubling, for example, might eventually result in a viable hybrid form.

Ultimately, this conclusion is not meant to inject more complications and hair splitting into the management of *Sidalcea*, nor is it a call for naming rare variants of *Sidalcea* in the Willamette Valley, but is to provide additional insight into the congener interactions of a relatively new genus (Andreason and Baldwin 2001) still undergoing strong evolution selection and resolution into a more discreet species. Hybridization is a natural component of evolution and management considerations should of course attempt to preserve this potential.

Willamette Valley Congener Comparison

Based on the current morphometric findings I decided to compare traits from a small sample of Willamette Valley *Sidalcea* congeners to see if there were trends worthy of discussion. This analysis was carried out with the same methods from the earlier analysis but with a lesser number of traits and on a smaller sample size per taxa. Five samples from each morph came from the NSN *S. campestris* accessions and herbarium vouchers from Oregon State University Herbarium, Botany and Plant Pathology Department. The nature of dry herbarium material necessitated that only certain flower-based traits could be compared (Table 14).

Table 14: Morphological traits used in *Sidalcea* congener morphometric analysis

Morphological Trait	Unit of Measurement	Further Description
<i>Flower</i>		
Petal length	mm (0.01)*	-
Petal width	mm (0.01)*	-
Calyx length	mm (0.01)*	-
Sepal width	mm (0.01)*	-
Calyx pubescence	Categorical (1-5)*	See Above
Calyx anthocyanin content	Categorical (1-3)*	1) no anthocyanin 2) noticeable anthocyanin 3) Strong anthocyanin
Style length	mm (0.01)^	-
Pedicel length	mm (0.01)*	-

Results

Ordination and MRPP results show a strong overlap between *S. campestris* and *S. malvaeflora* ssp. *virgata* hermaphrodites with no difference between groups (p-value >0.9), a slight overlap between *S. campestris* and *S. cusickii* (p-value >0.9) and a well supported difference between *S. campestris* and *S. nelsoniana* groups (p-value 0.024) after Bonferroni corrections were applied. Male sterile groups showed a suggestive difference between *S. campestris* and *S. malvaeflora* ssp. *virgata* (p-value 0.048), a strong difference between *S. campestris* and *S. cusickii* (p-value 0.0084) and a suggestive difference between *S. campestris* and *S. nelsoniana* (p-value 0.051).

Table 15. Proportion of variation (R^2) in male-sterile and hermaphrodite *Sidalcea* morphological variables accounted for by NMS ordination

<u>Hermaphrodite</u>		
Axis	Incremental R^2	Cumulative R^2
1	.363	.363
2	.582	.945
<u>Male Sterile</u>		
1	.850	.850
2	.100	.950

Willamette Valley Hermaphrodites

Figure 11. Willamette Valley hermaphrodite *Sidalcea* species in ordination space

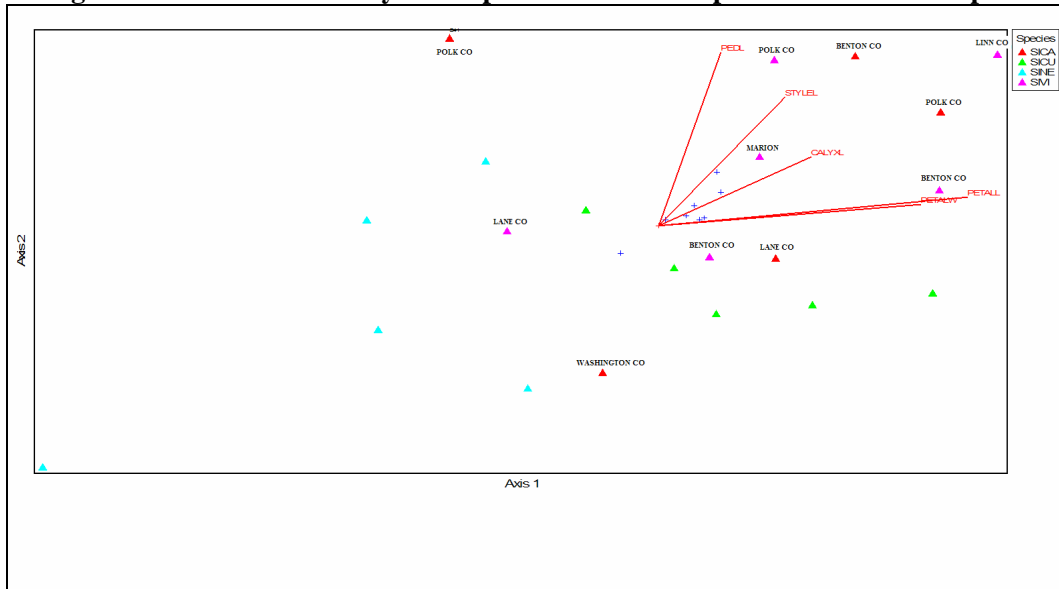


Table 16: MRPP pair-wise comparisons for hermaphrodite *Sidalcea*. Bonferroni corrected p-value in parenthesis. Alpha = 0.05

<i>Sidalcea</i> species	T	A	p-value
SICA vs. SICU	-0.63	0.019	0.24 (>0.90)
SICA vs. SINE	-3.31	0.17	0.0096 (0.058)
SICA vs. SIVI	0.52	-0.018	0.63 (>0.90)
SICU vs. SINE	-4.34	0.19	0.0035 (0.021)
SICU vs. SIVI	-1.013	0.037	0.15 (0.90)
SINE vs. SIVI	-4.53	0.22	0.0034 (0.02)

Table 17. Pearson product moment correlations of hermaphrodite *Sidalcea* morphological variables (> .500) with ordination axes

Axis:	1	2
	r	r
PETALL	-.909	-.803
PETALW	-.847	-.732
CALYXL	-.480	-.695
PEDL	-.030	-.699
STYLEL	-.304	-.741
SEPALL	.119	.534
CALYXH	-.266	-.610

Willamette Valley Male-Steriles

Figure 12. Willamette Valley male-sterile *Sidalcea* species in ordination space

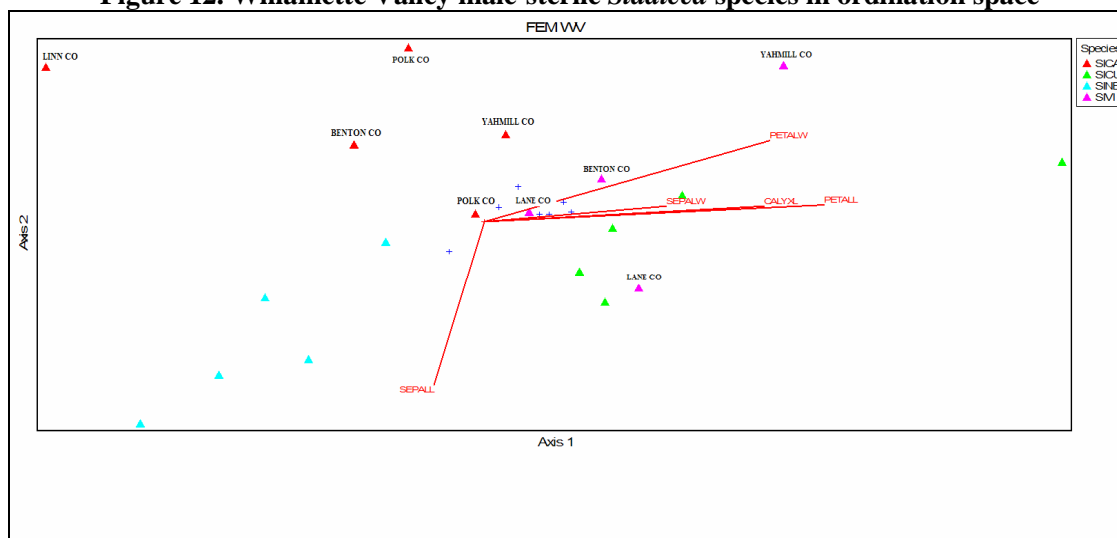


Table 18: MRPP pair-wise comparisons for male-sterile *Sidalcea*. Bonferroni corrected p-value in parenthesis. Alpha = 0.05

<i>Sidalcea</i> species	T	A	p-value
SICA vs. SICU	-4.75	0.15	0.0014 (0.0084)
SICA vs. SINE	-3.55	0.16	0.0085 (0.051)
SICA vs. SIVI	-2.96	0.11	0.0080 (0.048)
SICU vs. SINE	-5.40	0.33	0.0019 (0.011)
SICU vs. SIVE	0.54	-0.023	0.69 (>0.90)
SINE vs. SIVI	-4.60	0.34	0.0036 (0.022)

Table 19. Pearson product moment correlations of male-sterile *Sidalcea* morphological variables (> .500) with ordination axes (N=)

Axis:	1	2
	r	r
PETALL	-.979	.504
PETALW	-.871	.714
CALYXL	-.888	.221
SEPALW	-.712	-.413
SEPALL	.323	.736

Discussion

One of the more interesting aspects from these results is the extent of variability within each species morphology. *Sidalcea nelsoniana* and *S. cusickii* hermaphrodites show relatively tight grouping but *S. campestris* is quite varied (and *S. malvaeflora* ssp. *virgata* to a lesser degree). Such high variation of morphological traits may signal hybridization. For male-sterile morphs this variability appears more conservative for all four congeners. This analysis unlike the *S. campestris* analysis is driven by floral traits. However, these results when compared to relative sizes (Table 20) between congeners are shown to be within a normal range based on published accounts (Hitchcock and Cronquist 1973; Hitchcock 1957).

Table 20. Morphological trait ranges for Willamette Valley *Sidalcea* (Hitchcock and Cronquist 1973; Hitchcock 1957)

	<i>S. campestris</i>	<i>S. malvaeflora</i> ssp. <i>virgata</i>	<i>S. cusickii</i>	<i>S. nelsoniana</i>
Pedicel length mm	3-6	3-10 (15)	1-2 (5)	3 (2-10)
Calyx length mm	8-10	8-12	6-10	4-6
Petal length mm	12-25	15-30	10-18	5-15

Note these values are likely ranges between morphs since the above authors did not appear to separate morphs (Halse, R. personal communication)

Pedicel length drives the hermaphrodite group trends placing *S. cusickii* and *S. nelsoniana* at proportionally smaller sizes to the other species and petal length and width place *S. malvaeflora* ssp. *virgata* and *S. campestris* towards the larger end of the spectrum. Based on Table 20, there appears to be a significant amount of overlap between *S. campestris* and *S. malvaeflora* ssp. *virgata* floral traits. What we do not see are geographic trends separating different parts of the valley (based on county) towards different ends of a trait spectrum (Figure 11). However, county is not a very good geographical overlay as witnessed in the prior *S. campestris* analysis.

Petal length and width and calyx metrics drive male-sterile groups showing that *S. malvaeflora* ssp. *virgata* tends to have larger floral traits than *S. campestris*, which is supported by normal parameters (Table 20). Geographic patterns appear random which is similar to hermaphrodites (Figure 12).

Conclusions and Recommendations

In general, the morphological variation within *Sidalcea* species is so high (Roush 1931; Hitchcock and Kruckeberg 1957; Hill 1993) that morphometric analysis alone may not register extant hybrid swarms much less rare hybridization events, especially without large and thorough sampling protocols. However, including the same individuals in molecular phylogenetic analysis may assist future efforts to resolve the complex evolutionary history and current interactions of *Sidalcea* species. At this point there is mounting evidence of successful progeny resulting from a cross between *S. campestris* and *S. malvaeflora* ssp. *virgata* supported further by evidence of compatibility between the tetraploid entity *S. species A* and *S. campestris* through successful backcrosses between the two (Gisler unpublished data) and direct evidence of what could be a pentaploid offspring as seen with single accession of *S. campestris* (Chesire) in the south Willamette Valley. With this in mind we are provided a new perspective for assessing morphological variation in *S. campestris*, in particular where it is sympatric with *S. malvaeflora* ssp. *virgata*. It could be that some *S. campestris* variation may be resulting from backcrossing with *S. species A*. Unfortunately, the distribution of *S. species A* is not well documented and does not have consistent morphology as is to be expected with *Sidalcea*. Published vouchers come from the central/northern Valley (i.e. Monmouth, Polk County Hitchcock Voucher #19313) and South (Lorane, Lane Co., OR Hitchcock #19340). Since the distributions of *S. campestris* and *S. malvaeflora* ssp. *virgata* overlap looking for patterns of introgression based on sympatry alone may be of little help.

The morphometric trends from this analysis are not strongly supported but provide a source of information and comparison for the current common garden work on *S. campestris*. Without reasonable support from these analyses, strict seed transfer zones should be applied with caution as they may restrict gene flow in a dominantly outcrossing species that may already suffer from isolation among many subpopulations.

Currently the NSN has restricted *S. campestris* sampling from Lane County and separated the central/north and east region into two seed transfer zones with multiple DEBs within each

seed transfer zone (Tom Kaye, personal communication). I recommend that this be maintained and that the cut off from the south valley be located in the southern Benton and Linn County area as to restrict collecting from potential hybrid populations including Dykstra Rd. and Twin Buttes.

Future Research

Ultimately, more research is necessary to legitimize or refute the current papers concluding hypothesis of pentaploid hybrid populations or individuals in the Willamette Valley. More importantly, molecular phylogenetic and cytological sampling and analysis are recommended in order to learn more about the frequency and distribution of *S. species A*. The use of more loci is recommended to refine the accuracy of molecular phylogenetic testing for future genetic sampling of *Sidalcea*. Cytological sampling to assess ploidy levels can also be streamlined using techniques such as flow cytometry. Finally, morphological assessment of genetic based variation of *S. campestris* will eventually be fine tuned using the NSN common garden research which provides a better level of control for environmental variation over the current analysis.

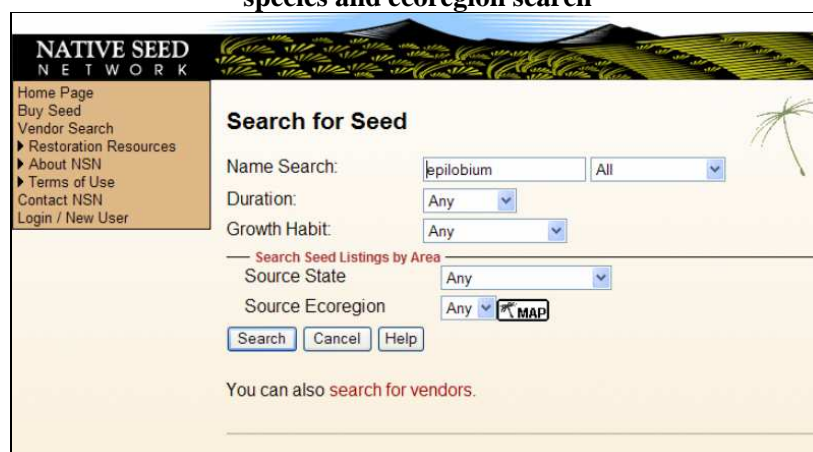
Chapter 3: The Native Seed Network Database

As discussed in Chapter 1, the Native Seed Network (NSN) is essentially two separate but integrated entities one of which is maintained as a national online networking and information effort that

- Creates awareness about the use of native plants in land management
- Supplies information about restoration genetics
- Offers recommendations and species lists for most eco-regions in the U.S.
- Provides a searchable data base of native seed vendors with detailed information about their stock, grow practices and certification
- Provides information on officially released plant materials such as cultivars

One way the NSN creates awareness about the use of native plants in land management is by providing up to date articles about successful uses of native plants in restoration. The site also posts information on the guidelines for native plant use in federal agencies as well as exemplary local information about the current uses, methods and concerns about genetically conservative restoration within the Northwest ecoregion and Willamette Valley with which the NSN is most intimate.

Figure 13. The Native Seed Network search engine showing options for species and ecoregion search



The screenshot shows the Native Seed Network search engine interface. The header features the logo "NATIVE SEED NETWORK" and a background image of a landscape with hills and a palm tree. A navigation menu on the left includes links for Home Page, Buy Seed, Vendor Search, Restoration Resources, About NSN, Terms of Use, Contact NSN, and Login / New User. The main search area is titled "Search for Seed" and contains the following fields and options:

- Name Search: Input field containing "epilobium" and a dropdown menu set to "All".
- Duration: A dropdown menu set to "Any".
- Growth Habit: A dropdown menu set to "Any".
- Search Seed Listings by Area: A section with two dropdown menus: "Source State" set to "Any" and "Source Ecoregion" set to "Any" with a "MAP" button next to it.
- Buttons: "Search", "Cancel", and "Help".


Below the search fields, there is a note: "You can also search for vendors." followed by a horizontal line.

Queries with the NSN database are intuitive making for very rich and cross-referenced searches. The search engine (Figure 13) provides many options for sourcing seed or getting information on a species or ecoregion. Choosing an ecoregion will provide a list of recommended species for that area followed by hyperlinks to vendors and species information.

Figure 14. Example of Native Seed Network database search based on species and ecoregion.

Plant Detail

denseflower willowherb - *Epilobium densiflorum*



Symbol	EPDE4
Common Name	denseflower willowherb
Scientific Name with Author	<i>Epilobium densiflorum</i> (Lindl.) Hoch & Raven
Family	Onagraceae
Growth Habit	Forb/herb
Duration	Annual
U.S. Nativity	Native to U.S.

[View NRCS Plant Profile for *Epilobium densiflorum*](#)
[Google Image Search for *Epilobium densiflorum*](#)

This seed is for sale in the marketplace:
Click on column header to sort by column

For Sale	Source State - County	Source Ecoregion	Release Name	Certification	Quantity Available	Price	Company
	OR	Willamette Valley			ask	ask	Pacific Northwest Natives
	OR	Willamette Valley			ask	ask	Native Seed Network
	OR - Marion	Willamette Valley			ask	\$50/lb	Heritage Seedlings Inc

synonyms	scientific_name
BODE	<i>Boisduvalia densiflora</i>
BODEP	<i>Boisduvalia densiflora</i> var. <i>pallescens</i>
BODES	<i>Boisduvalia densiflora</i> var. <i>salicina</i>
BOSA4	<i>Boisduvalia salicina</i>
OEDE6	<i>Oenothera densiflora</i>

Note: If this species has [releases](#), the list of releases will not include [synonyms](#)

Figure 14, provides an example of a search made based on a particular species and a desired ecoregion. This results in short life-history and habit information on the species of interest, synonyms of taxonomic nomenclature, and a table providing cursory information on vendors and hyper links to their specific information. Following an individual vendor link (Figure 15 and 16) provides more in-depth vendor contact and seed information such as source and certification information and pricing if available. The end result is a powerful resource that

can provide the desired value to satisfy demand for public and private land managers in search of genetically local seed while amplifying the market for locally sourced seed.

Figure 15. Example of choosing an individual vendor link from Figure 11 above; includes species availability and vendor contact information

NATIVE SEED NETWORK

- Home Page
- Buy Seed
- Vendor Search
- Restoration Resources
- About NSN
- Terms of Use
- Contact NSN
- Login / New User

Seed For Sale

elegant calicoflower - *Downingia elegans*

Symbol	DOEL
Certification	Uncertified
Source State	OR
Source Site	Linn/Benton County - Western OR
Source Ecoregion	3 - Willamette Valley
Date Available	2003-12-11
Quantity Available	ask
Price	ask
Last Updated	2006-01-14

Available From:

Name	Craig Edminster
Company	Pacific Northwest Natives
Address	1525 Laurel Heights Drive NW Albany, OR 97321
Primary Phone	541-928-8239
Secondary Phone	541-928-8239
Fax	541-924-8855
Email	Login to view email

[Seed search](#)

Figure 16. Example of choosing an individual vendor link from a species query

Vendor Contact

Name	Kimiora Ward
Company	Native Seed Network
Title	Restoration Ecologist
Address	563 SW Jefferson Ave Corvallis, OR 97333
Primary Phone	541-753-3099
Secondary Phone	541-602-0821
Web site	
Email	Login to view email
Ecosystem Specialization	Prairie
Region Specialization	Pacific Northwest
Services	Contract Growout Seed Collection Seed Cleaning Project Consulting Research
Products	Seed Seed Mixes Certified Seed
Percentage of Stock is Native Plant Materials	76-100%
Track Seed Collection Location?	yes

Seed listings for: Native Seed Network

Click on column header to sort by column

For Sale	Scientific Name	Common Name	Release Name	Source State - County	Ecoregion	Quantity Available	Price	Growth Habit
	Epilobium densiflorum	denseflower willowherb			Willamette Valley	ask	ask	Forb/herb

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Appendix A. Daily Internship Journal NSN J. Lambert; YR 2007

Week 1: June 18 - 22

Monday, June 18

Orientation: first day briefing was brief.

Common Garden:

- Species in experiment are *Prunella vulgaris*, *Lupinus polyphyllous*, *Saxifraga oregana*, *Epilobium densiflorum*, *Eriophyllum lanatum*, *Sidalcea campestris*.
- Checked flowering status on *Prunella* and *Epilobium*.

Scoping:

- Hwy 34 and Decker Rd: flagged *S. malvaeflora* ssp. *virgata*, took voucher specimens.
- Beazell Memorial Forest: Found access point for prairies, scoped first prairie, found unexpected and considerable *Achillea* and *S. malvaeflora* ssp. *virgata* (*Silene* spp.!) populations for future seed collections. Took voucher for each spp. Also notable were potential *Festuca idahoensis* (*roemeri*) and a possible *Grindelia integrifolia* population (~40 plants).

Discussions with Amy about extra internship projects included, researching non-well defined *Lupinus* spp *arbustus* and *albicaulis* and possibly others? (Herbarium research; writing up document on characteristics) as well as mapping future scoping sites.

Tuesday, June 19

Amy, Lisa and Me

Scoping:

- Camas Swale (HWY 99 north of Creswell): flagged ALLAMP and *Grindelia integrifolia*?
- SherKhan Rd: flagged CAMLEI no SIVI found. Note *Grindelia* is at this site (see notebook)
- Weiss Rd. (Lane Co.): Located, flagged and vouchered two moderately dense populations of SIVI.

Wednesday, June 20

Amy, Kimiora, Lisa and Me

Scoping:

- Mud Slough: Checked out WRP site and the effects of various seeding and treatments. Noted that coverage of some species such as *Dowlingia* was the result of seed bank rather than seeding mix. Silly pictures taken.
- Basket Slough Wildlife Reserve (Dusty Canadian Goose): flagged ERLA, ALLAMP
- Vouchered *Asclepias speciosa*, collected some *S. malvaeflora* ssp. *virgata* seed.
- Made contact with Aaron Liston about proposal and future meeting for the week of June 25th.

Thursday, June 21

Lisa and Me

Common Garden:

- Took measurements on *Prunella vulgaris* (flower size, color, physiognomy).
- Collected *Lupinus polyphyllus* inflorescences for later flower count
- Recorded flowering for *Epilobium densiflorum*

Friday, June 22

Lisa and Me

Scoping:

- -Beazell Memorial Forest. Scoped priority species (and nectar spp.) at all four prairies. High quality upland prairie, often very steep and enclosed by Douglas fir forest. Dominant grass included *Arhenatherum elatius* (Oatgrass), although sparse to moderately dense populations of *Festuca roemeri* were noticed. Also flagged route to access all four prairies. Largest *S. malvaeflora* ssp. *virgata* site known in Benton county. Stong populations of *Eriophyllum lanatum*, *Prunella vulgaris* and *Achillea millefolium*. Other possible future-interest upper-prairie species collected and identified included *Brodieae coronaria* and *Clarkia gracilis*, *C. amoena*.

Week 2: June 25 - 29

Monday, June 25

Lisa and Me

Common Garden:

- Finished measuring *Prunella vulgaris* flower characteristics
- Recorded flowering for *Epilobium densiflorum*

Collection:

- Northern WV population West of Dallas, OR. Roadside site near private property, site was mostly freshly mowed and most of remaining SIVI was still too immature for collection.

Tuesday, June 26

Jenn Cramer crew day:

- Assisted bagging immature inflorescences of largest Benton County *Lupinus sulphureus* var. *kincadii* population in Kings Valley (Private owner). Approximately 600-700 bags.

Wednesday, June 27

Ted, Lisa and Me

Collection:

- Ferguson Rd: SIVI south of Eugene: (6-10 plants)
- Found possible *Sidalcea nelsoniana* (SINE) site at corner of Oakhill and Greenbelt Rd. (approx. 80 plants)

Scoping:

- Found 20-30 SIVI plants at intersection of Cherokee Rd and Gimpel Hill Rd, no voucher taken

Thursday, June 28

Personal work day:

OSU herbarium:

- Studied WV *Camassia* species for post-anthesis worksheet (Appendix A) writeup for field ID of *Camassia* species. Specifically *Camassia leichtlinii* and *Camassia quamash* var. *maxima*.
- Discussed, with Aaron Liston, characteristics of WV *Lupinus* spp that are being considered for collection by NSN

Friday, June 29

Lisa and Me

Common Garden:

- Flowering date data for PRVU and EPDE

Collection:

- Timberline playground prairie: *Camas leichtlinii* (<1000 plants). Note: *Grindelia* not ready yet
- SIVI at Dunn property, not ready.
- Beaver and Decker Rd. (approx. 60 plants)

Scoping:

- Herbert Open Space: relatively small preserve surrounded by upland and wetland prairie components; spp. include: WYAN, DELPAV, FRAVIR, SICA, CAMLEI, LILCOL, *Eryngium petiolatum*, , LUPSUL, *Dowlingia* spp. Note: *Dowlingia yina* has stamens not exerted, *D. elegans* is the opposite. Collected CALTOL at this site.

Week 3: July 2 - 6

Monday, July 2

Ted and Me

Common Garden:

- LUPO seed maturity data and EPDE flowering data

Collection:

- Camas Swale (outside Creswell OR), Collected CAMLEI?

- Weiss Rd. SIVI collected, most not ready.
- Sher Khan: CAMLEI, large roadside pop. Collected far more than needed for this site.

Tuesday, July 3

Jenn Cramer Crew + myself

Collection/Scoping:

- Erickson property: Collected CAMLEI or CAMQUA. Noted and flagged ALLAMP and mapped ERLA populations
- Finley/Pigeon Butte: SIVI collection: lower prairie almost completely grazed. This is the first occasion where this has shown to be an issue. Upper prairie had substantial population. ERLA and ACMI at this site was flagged, CALTOL was noted in notebook.
- Allan and Allan property: Huge CAMQUA population collected, small fringe CAMLEI population along the tree line collected as well. Site is a premier location for ERIDEC and LOMBRA, found along drainage at the center of prairie site. Found and flagged new relatively large ALLAMP population

Spoke with Mike, one of the land owners. He is really interested in preserving the native prairie species diversity but is struggling to make some income of the property as well, hopefully in the form of a funding for his preservation efforts and possible protection status from a conservation organization. He mentioned his desire to increase the prairie into the adjacent field (West) to create a refuge for local turtles.

Wednesday, July 4

Amy, myself and Heather (volunteer)

Collection/Scoping:

- Sublimity Prairie: checked on whether or not ALLAMP was ready for collection. Seeds were black but many were still soft, decided it needed another week or so. Collected CAMLEI and CAMQUA. The difficulty of collecting when both species are present reared its head. Aaron Liston and Richard Halse are aware of hybridization of these species and it seemed apparent at times. More so at Kingston prairie where the species are more intermixed at times. I suggested and we decided not to collect CAMQUA when CAMLEI was around but to go ahead to collect CAMLEI when CAMQUA was around as CAMLEI seems less variable in size than the CAMQUA. Amy noted that there may just have to be a disclaimer with our seeds that both species may be present and also that some may not be used.
- Kingston Prairie (TNC land): Collected CAMQUA and CAMLEI. ALLAMP and ERLA were present. See above discussion about *Camassia* collection

Thursday, July 5

Amy, Lisa, myself

Collection/scoping:

- Philomath prairie (TNC land): scoped SIVI population which was very large and dense at times but essentially entirely grazed. We are wondering if the site may have had some SIVI that was just vegetative? Flagged and mapped ERLA and CALTOL populations in both prairies.
- Bellfountain and 53rd: SIVI not present
- Belts Drive (LINN Co.): large roadside CAMLEI population was collected. Noted WYAN, single but large *Sidalcea cusickii* (possibly *Sidalcea nelsoniana*). Saw large
- Wise property: small roadside prairie with SIVI and some CAMLEI, both collected.
- Noted a large ASLSPE population on same hwy that Wise property is on, Kimiora noted it has already been documented.

Friday, July 6

Lisa and Josh

Common Garden:

- Today me and Lisa categorized the EPDE populations pubescent type as this helps to determine which variety of the two known varieties each plant is and possibly the dominant at the site at which it was collected. We assigned two primary varieties with 3 intermediate types for the entire EPDE experiment. PRVU inflorescences were also counted using the quick technique of assuming most whorls are in

sixes with closer attention to tops and bottoms. There appears to be no real major trend with flower count based on cursory collection since most fall within 55 to 75 flwrs per inflorescence. Seed set was also begun today with withering calyxes of inflorescences as the determining site characteristic for seed set for a PRVU individual.

- Lupine inflorescence flower counts from the LUPO area were completed today, with an extreme sigh of relief.
- Day was finished with helping Matt Blakey Smith transplanted native spp plugs into larger containers for planting out at the Dunn property. This was done at the EPA green house.

Week 4, July 9 - 13

Monday, July 9

Lisa and Me

Common Garden:

- Lupine seed set and EPDE flowering data

Collection:

- Beazell Memorial Forest: Collected SIVI. First prairie SIVI was not ready and third and fourth prairies SIVI were not located but fifth and largest prairie was successfully sited and collected. ERLA is mostly at post anthesis but could wait another week or two to fully mature into hard achenes. ACHMIL was starting to show post anthesis in some individuals in the first thru second prairie.
- Hwy 223 and Mahogany dr.: SIVI collected
- Hwy 34 and Decker Rd: SIVI collected
- Dunn property: SIVI collected

Tuesday, July 10

Lisa and Me

Collection:

- Weiss Rd: SIVI still not ready
- Salt Creek Cutoff: CAMLEI, roadside population
- Robson Creek: CAMLEI roadside population

Listened to discussion about NSN participation at Florida conference for discussion about native seed usage.

Wednesday, July 11

Marsha, Lisa, Ted and Me

Portland Metro Public Property: Cooper Mtn. (two upland prairie restoration sites)

Collection:

- ALAM
- ERLA
- Additional Species for Portland Metro's restoration grow out program

Thursday, July 12

Marsha Lisa and Me

Portland Metro Public Property: Willamette Narrows

Collection:

- ALAM
- ERLA
- CAMQUA
- CAMLEI
- Additional Species for Portland Metro's restoration grow out program

Friday, July 13

Lisa and Me

Grow out:

- Pulled weedy *Potentilla recta* from grow out farm

Common Garden:

- EPDE flower data PRVU seed set data

Collection:

- Kingston Prairie: ALAM huge populations
- Sublimity Prairie: ALAM huge populations

Week 5: July 16 - 20

Monday, July 16

Me

Common Garden:

- PRVU flower counts and seed set data
- EPDE height width and flower data: note that earlier measurements of height taken by Amy were dramatically different this time around. Suggest taking height measurements a good three weeks after first flower.

Planning for week due to Amy being out of office

Tuesday, July 17

Lisa and Me

Collection:

- Weiss Rd.: SIVI
- Collins.: Site mowed, no ALAM present
- Barnswallow x Office Max: Camas not present

Meeting with Aaron:

Discussed the scope of the SICA project further and decided that I should adopt a two-prong analysis 1) SICA alone to see if morphological variation occurs through out the valley 2) comparison of morphological characteristics of other existing *Sidalcea* species in the valley to see if hybridization is occurring. This will require taking morphological measurements of herbarium specimens of other *Sidalcea* species. Aaron assured me that only a few herbarium specimens need morphometric data for SICA comparison. Note that certain traits will not be compared between species such as flower color if this was not preserved in herbarium species.

Wednesday, July 18

Laurel and Me

Collection:

- Allan and Allan: ERDE and ALAM

Grindelia Research:

- Talked with Rob about scope to refresh my mind
- Sent emails out to Paul Severens, Kenton Chambers
- Compiled Excel list of collection sites including past NSN collection sites, potentially viable Oregon Atlas sites and any other NSN database sites

Ted and Lisa

Common Garden:

- EPDE and PRVU flower and seed set data

Collection:

- Tampico Rd.: Could not find three flags, walked rd and still could not find ERLA or ALAM
- Starlight Rd.: No ALAM found
- Richardson Gap: No ERLA found
- Lebanon Radio Station: site mowed, ERLA only vegetative

Thursday, July 19

Lisa and Me

Scoping:

- Crowe Property: ERLA not flowering this year, both sites
- Balston Rd.: ERLA populations not ready at this time, maybe in about two weeks

Meeting with Matt Blakely Smith to discuss proper data entry and handling of SICA data into PCORD

Friday, July 20

Lisa, Ted and Josh

Common Garden:

- PRVU and EPDE flowering data

Collection/scoping:

- Erickson Property: ALAM
- Lupine Meadows: POGR collection based on Kimiora's suggestion

Week 6: July 23 - 27

Monday, July 23

Lisa, Ted and Me

Collection:

- Bald Hill: POGR, ERLA
- Finely Floodplain: POGR

Replant plugs at IAE greenhouse for Dunn WRP

Worked on EPDE pubescence scale and flowering/measurement data and programming

Tuesday, July 24

Lisa and Me

Collection:

- Rankin Tree Farm: POGR, ERLA
- HWY 226 x Thomas Creek: POGR, SICA
- Sublimity Prairie: POGR
- Honey Bear Rd: POGR

Replant plugs at IAE greenhouse for Dunn WRP

Wednesday, July 25

Lisa, Ted and ME

Collection:

- Spores private easement: (LANE Co.): POGR, checked SYHA phenology) one plant in full flower, the rest early budding stage. Only found two populations, one on each side of Rd.

Josh: research at OSU herbarium on *Grindelia* traits and discussion with Kenton Chambers

Thursday, July 26

Amy, Lisa and Me

Collection:

- Tupper x Turner Rd.: POGR and CAMLEI and SICA
- Hacker Rd.: SICA and ERLA and CAMLEI
- Mt. Richmond: ERLA

Friday, July 27

Scheduled day off

Week 7: July 30 – August 3

Monday, July 30

Lisa, Ted and Me

Collection:

- Basket Slough: ACMI; GRIN collection
- Balston Rd.: ERLA
- Fort Hill x HWY 22: nothing found
- Bestheldorf: nothing found

Common Garden:

- EPDE flowering data

Began collecting GRIN vouchers for *Grindelia* morphometric project as noted in red. Rob mentioned that Google Earth may be used for lat and long measurements instead of a GPS point taken at the site. I am also marking sites in a designated Oregon road atlas following the protocol of the SICA project.

Tuesday, July 31

Lisa, Amy and Me

Collection:

- Bezell: ACMI
- Pigeon Butte (Finley): ERLA, ACMI
- Bald Top (Finley): ACMI

Wednesday, August 1

Personal Day

GRIN collection: Lane Co. West Eugene Wetlands Complex and near Creswell

Thursday, August 2

Lisa and Me

Grindelia Collection, Linn County

Collection

- Rankin Tree Farm

Friday, August 3

Personal Day

GRIN collection: Benton County, Philomath

Week 8: August 8 - 10

Monday, August 6

Day Off

Tuesday, August 7

Lisa and Me

PORE pull at growout

Common Garden

- ERLA leaf characteristics
- SICA measurements need to be clarified

Wednesday, August 8

Personal Day

Group Meeting with NSN folk

GRIN collection: Polk County

GRIN collection is essentially finished at this point. A group meeting today refocused the *Grindelia* project down from a morphometric project to more of a crude estimation of the status (hybrid or not) of *Grindelia*'s at known NSN collection sites, therefore scoping for more sites will not be carried out unless there is reliable intelligence. A study of the collection (23 samples) will be carried out by me on my own time to elucidate the purity of NSN collection sites. The analysis is not urgent at this point and maybe put off until winter quarter or early Oct.

Thursday, August 9

Lisa and Me

PORE pull at growout (no PORE's found but we are uneasy with the PORE-like leaf characteristics we are seeing in the POGR that is currently flowering)

Common Garden:

- ERLA leaf measurements finished, discussed an additional categorical description of leaf shape to fully capture variation in ERLA leaves
- SICA glaucousness measured

Friday, August 10

Lisa Amy and Josh

Scoping:

- Ankeny WLP: *Asclepias* (none found)

Collection:

- Lake Park: LUPO seeds for Cornell research project regarding ploidy

SICA analysis with Kimiora

Week 9: August 13 - 17

Monday, August 13

Me

Scoping/Collection

- Helt: ACMI, SYHA, ASSP
- Applegate: 5% of SYHA flowering, ASSP not found
- E4 Ranch: LUPO not found
- Hwy 223 x Mahogany Ln: ERLA not found

Tuesday, August 14

Lisa, Amy and Josh

Scoping:

- Basket Slough/Butte (Area 1): *Symphiocarpum hallii* and GRIN collection
- Fort Hill x Hwy 22: SYHA and GRIN collection

Wednesday, August 15

Lisa, Ted and Josh

Collection:

For Jan at American Seed company

- Many species collected

Thursday, August 16

Day Off

Friday, August 17

Lisa and Josh

Seed Cleaning Training

Danthonia californica seed

Week 10: August 20 - 24

Monday, August 20

8-20

Lisa and Josh

Rain Day:

Mounted herbarium specimens

Tuesday, August 21

Lisa Amy and Josh

- EE Wilson: ASSP has pods, SYHA present near pond 2 in NW corner

Office work

Wednesday, August 22

Lisa, Josh

Collection/scoping

- Mc Farland Dr: ASSP, only 3 pods
- Diamond rd.: ASSP, lots of pods found
- Lupine meadows: found SYHA, ASSP no pods
- 228 x fisher: ASSP, lots of pods found
- Allen and Allen: SYHA found, collectable (collected ACMI too)
- Timberhill Athletic club: SYHA found, collectable
- Fitton Green: SYHA pop too small

Thursday, August 23

Lisa Josh

Collection/scoping

- Mt Richmond: Found SYHA and ASSP 10 pods
- Minto-brown park in Albany: found no SYHA
- Lebanon Airport: found SYHA
- Oak Creek: could not get in due to rusty lock

Friday, August 24

Lisa and Josh

Common Garden

- ERLA seed set
- Weed PORE at farm

Database entry and update of site collection forms

Week 11: August 27 - 31

Monday, August 27

Lisa and Me

Collection/Scoping:

- Diamond Hill Dr.: ASSP
- Oak Creek Rd. (Lane): SYHA (present)

Discussion with Carolyn Meinke about PCORD/NMS

Knee appointment at 3:45pm

Tuesday, August 28

Office Day

Working on loading SICA data into GIS format to visualize populations and to somehow develop geographical/ecological data to group populations further for analysis.

Wednesday, August 29

Lisa Amy and Me

Scoping:

- Portland

Thursday, August 30

Lisa Josh

Office Day:

POGR clean up,

Worked on GIS mapping of SICA's to include soil, elevation and spatial data into SICA analysis.

Friday, August 31

Lisa Josh

Scoping:

- Philomath Prairie: SYHA found

Continued GIS work

Week 12: September 3 - 7

Monday, September 3

Paid Holiday

Tuesday, September 4

Lisa Josh

Seed Cleaning at PMC

- *Lotus unifoliatus*

Wednesday - Friday, September 5 - 7

Seed collection with Plant material center folks at Mt. Rainier, Washington

Week 13: September 10 - 14

Monday, September 10

Seed Cleaning at PMC

- CALE, CAQU

Tuesday, September 11

Seed Cleaning at PMC

- SIVI, SICA

Wednesday, September 12

Seed Cleaning at PMC

- ALAM, POGR

Thursday, September 13

Seed Cleaning at PMC

- CATO, RAOC

Friday, September 14

Seed Cleaning at PMC

- ERLA

Week 14: September 17 - 21

Monday, September 17

Lisa and Josh

Scoping:

- Spores: SYHA still present
- Lebanon Radio Station: still present but site has been altered with Ash tree clearing
- Coburg Rd (Lane Co.): Two new populations of SYHA found on

Tuesday, September 18

Seed Cleaning at PMC

Wednesday, September 19

Seed Cleaning at PMC

Thursday, September 20

Seed Cleaning at PMC

Friday, September 21

Seed Cleaning at PMC

Week 15: September 24 - 28

Monday, September 24

Lisa and Josh and Kimiora

Pulled PORE from Keaneagy's grow out farm, collected SICA and POGR from same area.

Office work on SICA analysis

Tuesday, September 25

Meeting with Tom about SICA analysis

Wednesday, September 26

SICA analysis

Thursday, September 27

SICA analysis

Friday, September 28

SICA analysis

Week 16: October 1 - 4

Monday, October 1

Lisa and Josh

Scoping

- SYHA Spores and Lebanon

SICA analysis/paper writing

Tuesday, October 2

SICA analysis/paper writing

Wednesday, October 3

Lisa and Josh

Collection/scoping

- Allen and Allen: SYHA collected!
- Sublimity Prairie: SYHA collected!
- Kingston Prairie: SYHA collected!

SICA analysis/paper writing

Thursday, October 4

FINAL DAY

Scoping

- Bald Hill: SYHA

SICA paper