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Ecophysiology and Genetic Variation in Domestication of Shpaeralcea and Shepherdia Species for the Intermountain West

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ECOPHYSIOLOGY AND GENETIC VARIATION IN DOMESTICATION

OF *SPHAERALCEA* AND *SHEPHERDIA* SPECIES FOR

THE INTERMOUNTAIN WEST

by

Chalita Sriladda

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ABSTRACT

Ecophysiology and Genetic Variation in Domestication of *Sphaeralcea* and *Shepherdia*

Species for the Intermountain West

by

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Utah State University, 2011

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Low-water landscaping is an essential tool for water conservation in the arid

Intermountain West (IMW) for managing limited supplies and population-driven

increased demand. The IMW harbors a large number of drought-tolerant native species

that have potential for use in the low-water use landscape (LWL). However, many

species are not available in the nursery trade due to their morphological confusion and

establishment difficulty in the managed landscapes. The overall goal of this study is to

elucidate morphological, ecophysiological, and genetic distinctions within two IMW

native plant genera containing species with high urban low-water landscape potential.

For the first study, morphological and genetic variations among populations of

four putative *Sphaeralcea* species were evaluated using canonical variate analysis (CVA)

on the basis of morphological characteristics of their type specimens, and amplified

fragment length polymorphisms (AFLP). The putative *S. grossulariifolia* was not

significantly different morphologically and genetically from *S. coccinea*, similar to the relationship between *S. munroana* and *S. parvifolia*. The results in the Mantel's correlation tests suggest there are con-specific species due to isolation-by-distance within each composite group, and the putative *S. munroana* appeared to be an ecotype of *S. parvifolia*.

In the second study, environmental conditions, morphology, and AFLP genetic variations among populations of *Shepherdia rotundifolia* in its native habitats were evaluated. Environmental conditions in selected six populations varied among populations in terms of elevation, precipitation, temperature, relative light intensity (RLI), and soil properties. Leaf area, specific leaf area (SLA), and leaf trichome structure exhibited adaptive traits to shady environments as well as hot and dry summer and cold winter environments in its native habitats. The SLA was significantly correlated with RLI, soil organic matter, and potassium.

In the third study, interspecific hybrid *S. rotundifolia* x *argentea* was created to achieve the aesthetic quality of *S. rotundifolia* and wet soil tolerance of *S. argentea*. The hybrid was intermediate morphologically and genetically (AFLP) to its parents. Trichome structure and physiological responses in terms of diurnal stomatal conductance (g_s), photosynthetic light response curve, midday photosynthesis assimilation (P_n), and midday quantum efficiency (PhiPS2) of the hybrid were also more similar to *S. argentea* than to *S. rotundifolia*. The physiological responses of the hybrid compared to its parents may suggest tolerance to regularly watered conditions.

(96 pages)

PUBLIC ABSTRACT

Ecophysiology and Genetic Variation in Domestication of *Sphaeralcea* and *Shepherdia*

Species for the Intermountain West

Chalita Sriladda

Low-water landscaping (LWL) using native drought tolerant species is an

essential tool for water conservation in the arid Intermountain West (IMW) for managing

limited water supplies. However, many potential species have not been evaluated for

LWL. Some species are difficult to visually distinguish from each other, thus decreasing

confidence in products from native plant industry. Meanwhile, some species are difficult

to establish to urban landscape conditions. The overall goal of this study is to elucidate

morphological, ecophysiological, and genetic distinctions within two IMW native plant

genera containing species with high urban low-water landscape potential.

For the first study, a classification model based on morphological characteristics

of type specimens using canonical variate analysis (CVA) was successful in clarifying

morphological variation among four *Sphaeralcea* species. Genetic variation among

populations and species based on amplified fragment length polymorphisms (AFLPs)

revealed two pure types among four putative species. *Sphaeralcea munroana* and *S.

parvifolia* separated genetically from *S. coccinea* and *S. grossulariifolia*, and *S.

munroana* appeared to be an ecotype of *S. parvifolia*.

In the second study, environmental conditions in selected six populations of

Shepherdia rotundifolia varied among populations in terms of elevation, precipitation,

temperature, relative light intensity (RLI), and soil properties. AFLP genetic varied between high and low elevation populations. Leaf area, specific leaf area (SLA), and leaf trichome structure exhibited adaptive traits to shady environments as well as hot and dry summer and cold winter environments in its native habitats. The SLA was significantly correlated with RLI, soil organic matter, and potassium.

In the third study, interspecific hybrid *S. rotundifolia* x *argentea* was intermediate morphologically and genetically to its parents. Leaf trichome structure and physiological responses of the hybrid were more similar to riparian *S. argentea* than *S. rotundifolia*, suggesting tolerance to regular watered urban landscape conditions.

ACKNOWLEDGMENTS

CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Urban landscape management in arid and semiarid areas is complicated by concerns about water use efficiency of plants as well as their aesthetics. The Intermountain West (IMW) has a very limited water supply and increasing population growth, demand from which is straining the water supply. Approximately 50-70% of total municipal water consumption is applied to urban landscapes. The urban landscape is composed of largely turfgrass, which has high water need and thus drives urban irrigation. Consequently, urban water suppliers have targeted urban landscapes for conservation in order to reduce water demand.

Low-water landscaping is a key tool in water conservation. Drought adapted native plants are key fundamentals for low-water use landscapes (LWL). The IMW has many native woody and herbaceous species that are drought adapted with aesthetic qualities for LWL. Low-water use landscape not only has the potential to reduce water demand but also has potential to increase urban biodiversity. Urban biodiversity not only provides diverse aesthetic qualities, but also provides ecosystem services, such as sustenance for native birds and insect pollinators. Ultimately, LWL in the IMW diversified with native plants tells a compelling story about native systems in a high desert climate.

Many native plant species in Utah are adapted to harsh conditions and are ornamentally attractive, but have not been evaluated for use in managed landscape conditions. Some species are promoted for use in urban landscapes but difficult to distinguish due to genetic variation among accessions and species, therefore decreases

consumer confidence in the native plant industry. Clarification in terms of morphological and genetic variations among accessions and species may improve production and confidence in the native plant industry. Some species are grower-desirable to introduce to the native plant industry, but production has proved difficult to establish in LWL. It is important to understand how native plants adapt to environmental conditions in their native habitats before using them in urban landscapes, due to the difference between their natural habitats and landscape conditions.

The genus *Sphaeralcea* (Globemallow) produces attractive orange flowers, and has species, which are used in the urban landscape. *Sphaeralcea grossulariifolia* is promoted as a superior choice for urban landscapes. However, it is one of four common species in Utah, including *S. coccinea, S. grossulariifolia, S. munroana,* and *S. parvifolia*, that have confusing morphological characteristics. Difficulty in their accurate identification and therefore collection and propagation creates concerns for seed collectors and reduces consumer confidence in the native plant industry. The morphological confusion in identifying characteristics between species could be a result of either natural hybridization between them, and/or differences in geographical distribution among populations.

The genus *Shepherdia* (Buffaloberry) has attractive leaves and berries. *Shepherdia rotundifolia* occurs on hillsides and the bases of cliffs on very well drained rocky soils; it has an attractive crown shape, but is very sensitive to over-watering and difficult to establish in the urban landscape. Even though this species has potential for use in the landscape and has been sought by plant growers, it has not been studied enough to

understand and resolve its limits for landscape use; in particular, understanding how this species is adapted to the different ecological conditions in its natural habitats.

Although the two genera have potential for use in low-water use urban landscapes because they are drought tolerant and ornamentally attractive, there are not enough data to develop these species for domestication for use in low-water use landscapes. Understanding the morphological, ecophysiological, and genetic variations of *Sphaeralcea* and *Shepherdia* species may help to clarify factors that limit their successful introduction to low-water use landscaping. It also may help in selection of species or cultivars with genetically superior forms for use in low-water use urban landscapes. Genetic improvement by hybridization with other species that can adapt to urban landscape conditions may also accommodate the aesthetic characteristics and adaptability to low-water use landscapes in a hybrid.

CHAPTER 2

GOAL AND OBJECTIVES

The overall goal of this study is to elucidate morphological, ecophysiological, and genetic distinctions within two IMW native plant genera containing species with high urban landscape potential. The specific objectives are:

1. To clarify morphological and genetic distinctions among the four *Sphaeralcea* species, *S. coccinea, S. grossulariifolia, S. munroana*, and *S. parvifolia*, which may improve superior cultivar selections for use in low-water use landscapes.

2. To investigate variability in terms of environmental conditions, morphology, and genetics among populations of *Shepherdia rotundifolia* in its native habitats, which may help to understand mechanisms underlying sensitivity to irrigated LWL.

3. To compare genetic, morphological characteristics, and physiological responses of the interspecific hybrid between *S. rotundifolia* and *S. argentea* with its parents.

CHAPTER 3

MORPHOLOGICAL AND GENETIC VARIATION AMONG
SPHAERALCEA SPECIES[1,2]

ABSTRACT. The herbaceous perennial species in the genus *Sphaeralcea* have desirable

drought tolerance and aesthetics with potential for low-water use landscapes. However,

taxonomy of these species is ambiguous, which leads to decreased consumer confidence

in the native plant industry. The goal of this study was to test and clarify morphological

and genetic differentiation among four putative species. Morphological characteristics of

the type specimens were used as species references in canonical variate analysis to

generate a classification model, and assigned putative species names to USU herbarium

specimens to clarify morphological, and field specimens to clarify genetic variation

among species. Genotypic classifications were tested using Bayesian cluster analyses of

AFLP genotypes. The putative *S. grossulariifolia* was not significantly different

morphologically and genetically from *S. coccinea*, with similarities between *S. munroana*

and *S. parvifolia*. The composite group of *S. coccinea* and *S. grossulariifolia* was

distinguished morphologically and genetically from the *S. munroana* and *S. parvifolia*

composite group, with significant correlation between genotypic and morphological

characteristics in the overall samples. There was no correlation between geographical and

genetic distances when all putative species pooled together in the Mantel's correlation

tests. However, the correlation was significant when tested within each composite group,

suggesting they are con-specific species due to isolation-by-distance within each group.

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[1] Authors: Chalita Sriladda, Heidi Kratsch, Steven Larson, Roger Kjelgren
[2] Additional index words: *Sphaeralcea coccinea*, *Sphaeralcea grossulariifolia*, *Sphaeralcea munroana*, *Sphaeralcea parvifolia*, globemallow, AFLP

Morphological distance exhibited a correlation with geographical distance within the *S. parvifolia* and *S. munroana* composite group, suggesting morphological isolation by distance. Therefore, the putative *S. munroana* may only be an ecotype of *S. parvifolia*.

Globally, urbanization has dramatically increased, with nearly half of the world's population currently living in urban areas; it is expected that approximately three quarters of the world's population will be living in urban areas by the year 2025 (United Nations, 2010). Substantial increases in urban population lead to increased demand for water in commercial, industrial, and residential sectors (Foster and Beattie, 1979). This increased demand in arid and semiarid urban regions means facing the additional challenge of absolute water scarcity (Vorosmarty et al., 2000).

Demand for urban green spaces includes urban landscapes is increasing with population growth because those spaces provide aesthetic and mental health benefits and a positive association with the perceived general health of residents (Maas et al., 2006). Urban green spaces also mitigate a number of undesirable environmental effects such as urban heat islands (Deloya, 1993). Many urban green spaces require irrigation, particularly in arid and semi-arid areas. Thus, increasing irrigation for urban landscapes is causing an increased demand for efficient watering systems. Low-water landscaping describes landscapes full of plants that use substantially less water. Therefore, low-water landscaping is a key tool for conserving water in irrigated urban landscapes, particularly in arid and semiarid areas (Kjelgren et al., 2009).

Drought-tolerant plants, particularly native species promoting water conservation, are key elements for successful low-water landscapes because they require little maintenance, provide a natural look to the landscape (Cane and Kervin, 2009; Kjelgren et al., 2009; McKinney, 2002), and honor natural habitats. Native plant landscaping also supports local economies, facilitating sustainability in urban systems at multiple levels. However, lack of native plant availability, cost of native plant materials, inconsistent and unreliable demand, and lack of production knowledge has minimalized the native plant market (Peppin et al., 2010).

The U.S. Intermountain West (IMW) has an abundance of woody and herbaceous perennial native plants that are drought-tolerant and ornamentally attractive (Intermountain Native Plant Growers Association, 2011; Kratsch, 2011; Mee et al., 2003; Meyer et al., 2009). The genus *Sphaeralcea* (Malvaceae), Globemallow, is an annual or perennial herb or shrub wildflower, characterized by brilliant, largely orange, flowers. About 40 species of the genus are found throughout temperate North and South America (Holmgren et al., 2005). The genus *Sphaeralcea* includes 27 species in North America, from southern Canada through western United States to northern Mexico, with disjunct populations in temperate South America (Holmgren et al., 2005). Several of these species appear to have both aesthetic and drought-tolerance qualities that lend them well to low-water landscaping (Intermountain Native Plant Growers Association, 2011; Mee et al., 2003); however, they are ostensibly closely related and difficult to distinguish. Four promising low-water landscape species, *S. coccinea, S. grossulariifolia, S. munroana,* and *S. parvifolia* are commonly found in the IMW (Holmgren et al., 2005; Ring, 2005;

Ring and Cully, 2007; Ring et al., 2009), and they are particularly difficult to distinguish

from one another (Holmgren et al., 2005).

Polyploidy and hybridization have been reported as factors contributing to weak

morphological differences among species in the genus (La Duke and Northington, 1978).

Tate (2002) also suggested the possible importance of geographical isolation for

speciation. Analysis of nuclear ribosomal DNA internal transcribed spacer (ITS)

sequences showed that the North American *Sphaeralcea* species (*S. angustifolia* and *S.

wrightii*) cluster together, as do the two South American taxa (*S. crispa* and *S.

philippiana*) (Tate, 2002). Inter-gradation among species challenged early taxonomists in

their efforts to clearly identify *Sphaeralcea* species using only morphological

distinctions.

To varying degrees, these four species are being promoted by the nursery industry

(Intermountain Native Plant Growers Association, 2011), but it is difficult to determine

which species are actually being sold. Difficulty to determine species decreased

consumer confidence in these species. Leaf morphology was used as the first taxonomic

key to separate these species into two groups (Holmgren et al., 2005). Leaves shallowly

lobed putatively separate *S. munroana* and *S. parvifolia* from *S. grossulariifolia* and *S.

coccinea,* whose leaves are deeply lobed, cleft more than half way to the base, and in

many, cleft to the base. However, *S. munroana* is often difficult to distinguish from *S.

parvifolia*, while *S. grossulariifolia* can be easily confused with *S. coccinea* and some

specimens of *S. munroana* (Holmgren et al., 2005). Besides leaf morphology, the mature

fruiting carpel characteristics, density of leaf hairs, and hair ray orientation sometimes are

used as keys for taxonomists to identify specimens (Atwood and Welsh, 2002). However,

these characters are seldom-used in practical settings, and are not efficient for field use or for non-taxonomist collectors. The intergrading morphology of these species might be a result of genetic overlap, and makes selection for superior forms difficult.

In terms of the geological distribution of the four species (United States Department of Agriculture, 2010), *S. coccinea* is found extensively throughout the IMW, and *S. grossulariifolia* is found throughout the Great Basin area on the west side of the IMW. Distribution of *S. munroana* and *S. parvifolia* overlaps in Nevada, Utah, and Colorado, where *S. parvifolia* is distributed further south and *S. munroana* distributed further north. In Utah where the distributions of the four species overlap, geographical differences seem to play an important role in putative species distribution, particularly for *S. munroana* and *S. parvifolia*, which occur in the north and south, respectively (Shultz et al., 2010). The distribution has sometimes been used as a key for species identification because of difficulty due to morphological overlap among these species.

Difficulty in their accurate identification creates concerns for those collecting *Sphaeralcea* seeds for nursery production, and reduces consumer confidence in the native plant industry, with plants varying widely in morphology being sold as the same species. The goal for this study was to clarify morphological and genetic distinctions among the four *Sphaeralcea* species, *S. coccinea, S. grossulariifolia, S. munroana*, and *S. parvifolia*, which may improve superior cultivar selections for use in low-water landscapes.

Materials and Methods

Type specimens of the four *Sphaeralcea* species were used as references. We borrowed all available type specimens (holotype/isotype) representing the four species from herbaria across the U.S. and Great Britain. In summer 2008, we collected field specimens from 20 populations in Utah (Fig. 3-1). The field specimens were used for genetic and correlation analysis. In addition to the field specimens, existing voucher specimens from the Intermountain Herbarium at Utah State University (USU) were used to verify morphological variation within and among species. The voucher specimens from the USU Herbarium were also used to locate the field specimens.

MORPHOLOGY. Morphological variation within and among type specimens were evaluated by measuring ten morphological characteristics of fully mature leaves (3 leaves per plant) (Table 3-1). In order to observe all possible variation within and among the type specimens, we measured morphological characteristics of all type specimens even though the numbers representing each species were unequal. Morphological characteristics of the type specimens were subjected to analysis of variance (ANOVA) using PROC GLM in SAS software (SAS Institute, Cary, NC) to compare variation of each morphological character among species. The morphological characteristics were subjected to canonical variate analysis (CVA) in NTSYSpc 2.2N software (Exeter Software, Setauket, NY) to generate a model for the purpose of assigning field and existing herbarium specimens to one of four putative *Sphaeralcea* species groups.
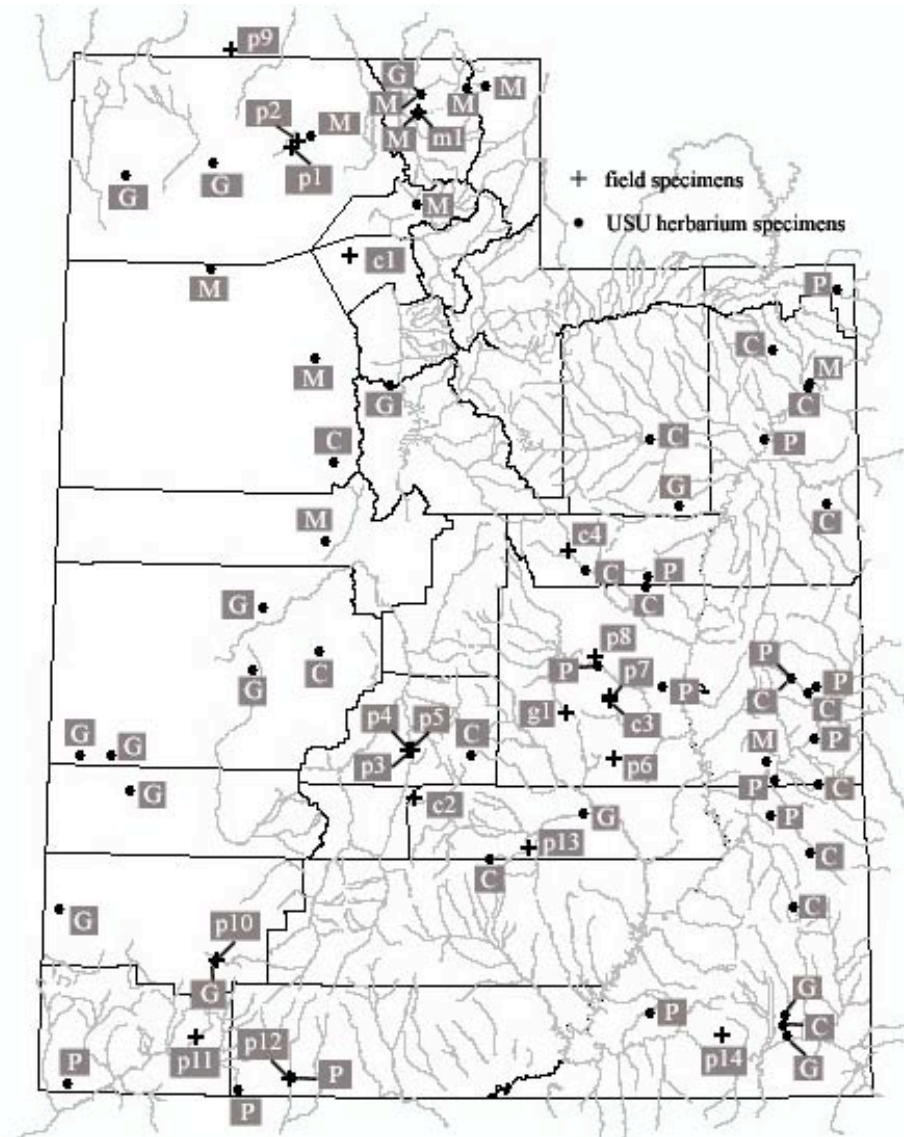
Fig. 3-1. Geographical distribution of *Sphaeralcea* species in Utah used in this study; letters were used to indicate species names; capital letters representing herbarium specimens (C = *S. coccinea*; G = *S. grossulariifolia*; M = *S. munroana*; P = *S. parvifolia*); lower-case letters representing field collected specimens (c = *S. coccinea*; g = *S. grossulariifolia*; m = *S. munroana*; p = *S. parvifolia*)

Table 3-1. Means (range) for morphological characteristics for type specimens among putative *Sphaeralcea* species, including *S. coccinea* (n = 8), *S. grossulariifolia* (n = 5), *S. munroana* (n = 3), and *S. parvifolia* (n = 17)

Character	*S. coccinea*	*S. grossulariifolia*	*S. munroana*	*S. parvifolia*
Petiole length (mm)[z]	10.63 (5.7 – 20.0)[a]	14.67 (7.0 – 17.7)[a]	8.72 (5.8 – 10.3)[a]	14.02 (8.5 – 25.3)[a]
Mid-lobe length (mm)	19.88 (8.0 – 35.7)[a]	25.53 (24.7 – 26.3)[a]	22.44 (19.7 – 26.7)[a]	20.55 (12.0 – 27.3)[a]
Mid-lobe width (mm)	3.31 (1.0 – 6.3)[b]	4.67 (4.3 – 5.7)[b]	3.72 (3.3 – 4.3)[b]	10.60 (7.0 – 15.7)[a]
Secondary-lobe length (mm)	13.08 (5.7 – 19.3)[a]	17.47 (16.0 – 21.0)[a]	14.33 (12.2 – 15.7)[a]	14.14 (8.0 – 18.3)[a]
Secondary-lobe width (mm)	2.56 (1.0 – 4.0)[b]	3.57 (3.0 – 4.0)[b]	2.78 (2.7 – 2.8)[b]	8.30 (4.3 – 12.3)[a]
Lobe-depth (%)	97.00 (80.0 – 100.0)[a]	83.00 (60.0 – 100.0)[a]	51.00 (30.0 – 70.0)[b]	22.00 (3.0 – 50.0)[c]
Max. number of flowers/ node	1.00 (1.0 – 1.0)[b]	5.20 (4.0 – 6.0)[a]	4.33 (4.0 – 5.0)[a]	5.59 (1.0 – 9.0)[a]
Pedicel length (mm)	3.08 (2.0 – 4.0)[a]	2.90 (1.7 – 3.7)[a]	2.89 (2.0 – 3.7)[a]	2.99 (1.7 – 5.0)[a]
Calyx length (mm)	6.67 (4.0 – 9.0)[a]	5.73 (4.7 – 6.7)[a]	5.89 (5.7 – 6.0)[a]	6.54 (5.2 – 8.0)[a]
Petal length (mm)	12.33 (7.0 – 19.0)[a]	11.73 (9.3 – 14.7)[a]	11.00 (10.0 – 11.7)[a]	11.65 (5.0 – 14.3)[a]

[z] Values within a row with different letters indicate statistical significance at α = 0.05. The type specimens (holotype and isotype) were on loan from Harvard University Herbarium, New York Botanical Garden Herbarium, Rancho Santa Ana Botanic Garden Herbarium (CA.), United States National Herbarium (Washington, D.C.), and Royal Botanical Garden, Kew Herbarium (England)

We selected 15 to 16 existing USU herbarium specimens of each species from different locations in Utah (Fig. 3-1) for morphological variation study. Similar to the type specimens, ten morphological characteristics were measured on fully mature leaves (3 leaves per plant). Morphological characteristics of the herbarium specimens were subjected to CVA in order to assign putative species names based on the classification model. Morphological variations within and among species were determined based on deviation of putative species names assigned to the USU herbarium specimens compared to names given by taxonomists.

GENETICS. Three voucher specimens were taken at each field population to represent morphological characters of the population. Similar to the type specimens, three fully mature leaves from each plant were used to measure ten morphological characteristics. Morphological characteristics of the field specimens were subjected to CVA in order to assign putative species names on the basis of the classification model of the type specimens. The assigned species names of the field specimens were later used for the genetic variation and correlation analysis.

Leaf samples (2-3 leaves per plant) collected from each field population (12 plants per population) were dried in 28-200 mesh silica gel (Fisher Scientific, Pittsburgh, PA). DNA was extracted with the Dneasy 96 Plant Kit (QAIGEN, Valencia, CA). Amplified fragment length polymorphisms (AFLPs) were assayed as described by Vos et al. (1995) with described modifications. The DNA samples were preamplified with EcoRI +1 / MseI +1 using A/C selective nucleotides. Selective amplification primers consisted of five EcoRI +3 / MseI +3 primer combinations using AAG/CAG, ACT/CAG, ACT/CTC, ACT/CAC, ACA/CTG selective nucleotides. The EcoRI selective

amplification primers included a fluorescent 6-FAM (6-carboxy fluorescein) label on

5' nucleotides. Selective amplification products were combined with GS600 LIZ internal

lane size standard and were fractionated using an ABI 3730 instrument with 50-cm

capillaries and sized between 50 and 600 bp with Genescan software (Applied

Biosystems, Foster City, CA). Although DNA molecules vary in length by increments of

1 bp, the relative mobility of bands is also affected by sequence composition. Thus, non-

homologous bands of the same length may not have the same relative mobility. Genescan

trace files for each individual were visually analyzed for the presence or absence of DNA

bands in bins that were at least 0.3 bp or more apart using Genographer software

(available free at http://hordeum.oscs.montana.edu/genographer/ or directly from the

author, Tom Blake, at blake@hordeum.oscs.montana.edu). Bayesian clustering of

individual plants without a priori assignment of individuals to hierarchical groups was

used to determine genetic structure and to test for possible admixture between taxa,

which might otherwise confound phylogenetic analysis, using Structure v2.1 (Pritchard et

al., 2000). Three analyses were used of each model with 100,000 iterations and 10,000

burn-in or 200,000 iterations and 20,000 burn-in with the dominant-allele, admixture

model of Structure v2.2 Falush et al., 2007; Pritchard et al., 2000).

CORRELATIONS. Morphological, genetic, and geographical distance matrices of

the field specimens were used for correlation tests. Fifteen field populations were used

for the correlation tests. The Euclidean distance matrix of morphology and NEI-72

distance matrix of genetics were computed using NTSYSpc 2.2N software (Exeter

Software, Setauket, NY). Geographic distance matrix was computed in Geographic

Distance Matrix Generator v1.2.3 (Center for Biodiversity and Conservation, the

American Museum of Natural History, New York, NY). Mantel's correlation tests (Mantel and Valand, 1970) were performed with 999 permutations in the NTSYSpc 2.2N software (Exeter Software, Setauket, NY). We also constructed neighbor-joining clustering trees, in order to compare similarity in classification between distance matrices of morphology and genetics of the field collected specimens using NTSYSpc 2.2N software (Exeter Software, Setauket, NY).

Results and Discussion

MORPHOLOGY. Morphological variation among the type specimens was mostly described by leaf lobing. Four out of ten morphological characters were significantly different among species in ANOVA, including lobe-depth, mid-lobe width, secondary-lobe width, and number of flowers per node (Table 3-1). Lobe-depth of more than 80% of the leaf of *S. coccinea* and *S. grossulariifolia* type specimens was significantly different and putatively separated from *S. munroana* and *S. parvifolia*. Shallow leaf lobing are associated with wide mid-lobe width and secondary-lobe width separated *S. parvifolia* from the other species. Having only one flower at a node separated *S. coccinea* from the other species. According to CVA of the type specimens, the first component accounted for 92% of the morphological variation among species (Table 3-2). This suggests the classification model on the basis of the ten morphological characters of the type specimens was powerful enough to use as a species reference. Lobe-depth contributed most to the variation, followed by mid-lobe width and secondary-lobe width, respectively.

Table 3-2. Principal component in canonical variate analysis of morphological

characters for type specimens of *S. coccinea* (n = 8), *S. grossulariifolia* (n = 5), *S. munroana* (n = 3), and *S. parvifolia* (n = 17)

Morphological Traits	Component 1	Component 2	Component 3
Petiole length (mm)	0.6093	-0.1236	0.5630
Mid-lobe length (mm)	-1.2335	-0.3611	-0.9685
Mid-lobe width (mm)	1.1507	-1.6038	0.8345
Secondary-lobe length (mm)	0.2947	1.4056	0.8925
Secondary-lobe width (mm)	0.6142	-0.3122	0.1624
Lobe-depth (mm)	-2.5134	-1.2082	1.1876
Number of flower per node	0.3356	1.0642	0.0845
Pedicle length (mm)	0.4761	-0.1044	0.0343
Calyx length (mm)	0.4654	-0.1782	-0.3516
Petal length (mm)	-0.5647	-0.1586	-0.0607
Variation (%)	92.3100	6.1600	1.5300

Morphological characteristics of *S. grossulariifolia* were similar to *S. coccinea,* as 75% of *S. grossulariifolia* USU herbarium specimens were assigned to the type group of *S. coccinea* (Table 3-3; Fig. 3-2). Meanwhile, only 6% of the *S. coccinea* USU herbarium specimens fell into the type group of *S. grossulariifolia*. Morphological characteristics of *S. munroana* were very similar to *S. parvifolia,* as all of the USU herbarium specimens of

Table 3-3. Percentage of numbers of existing USU herbarium specimens of the four putative *Sphaeralcea* species assigned to its type group based on the classification model generated from morphological characteristics of the type specimens in the canonical variate analysis

Herbarium specimens	Type specimens			
	S. coccinea (n=8)	*S. grossulariifolia* (n=5)	*S. munroana* (n=3)	*S. parvifolia* (n=17)
S. coccinea (n=16)	93.75% (15)	6.25% (1)	-	-
S. grossulariifolia (n=16)	75.00% (12)	12.50% (2)	12.50% (2)	-
S. munroana (n=15)	-	-	-	100.00% (15)
S. parvifolia (n=16)	-	-	-	100.00% (16)
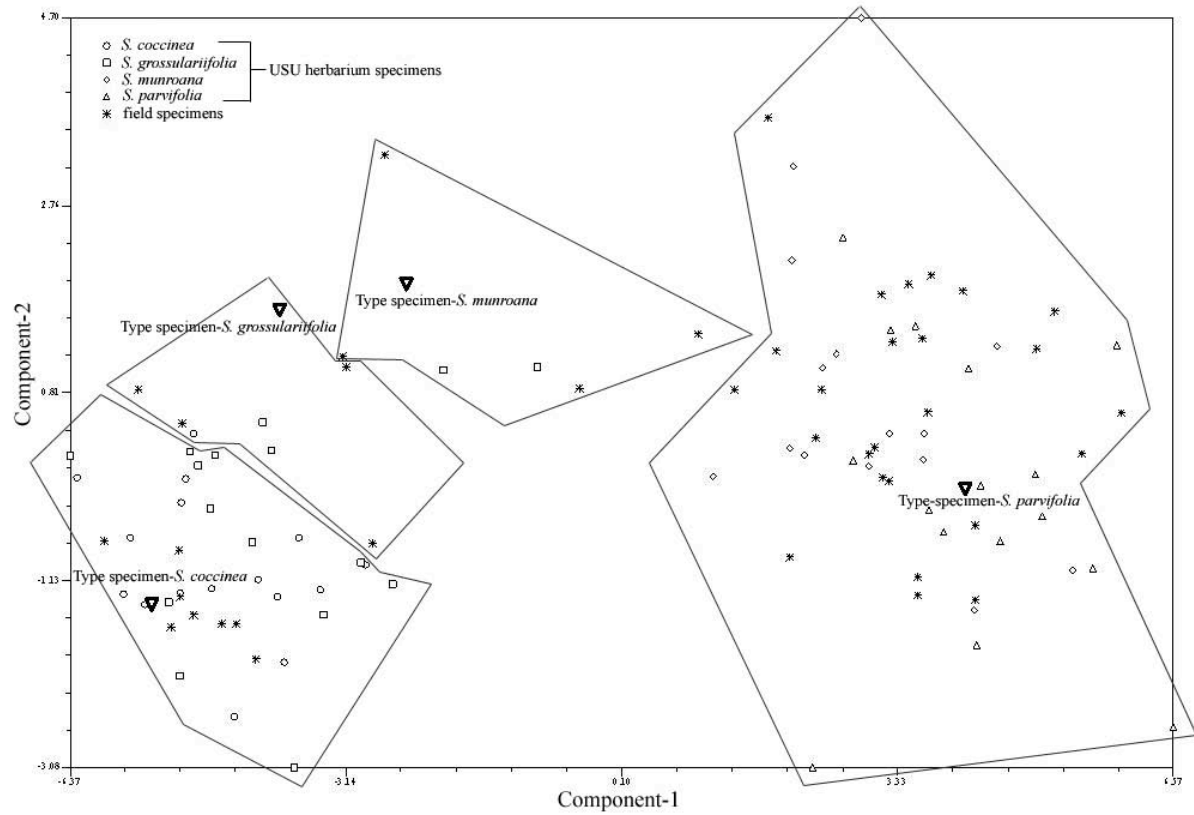
Fig. 3-2. Putative species name assignment of herbarium specimens and field collected specimens on the basis of the classification

model generated based on morphological characteristics of the type specimens by means of canonical variate analysis

S. munroana were assigned to the type group of *S. parvifolia* based on the classification model, and all of the USU herbarium specimens of *S. parvifolia* fell into their type group.

The results from morphological studies support the suggestion by Holmgren et al. (2005) that *S. grossulariifolia* can be easily confused with *S. coccinea*; and *S. munroana* can be confused with *S. parvifolia*. All of the USU herbarium specimens of *S. munroana* fell into the type group of *S. parvifolia*, supporting the suggestion by Holmgren et al. (2005) that *S. munroana* and *S. parvifolia* may be no more than varietally different. On the basis of morphological characteristics, these four putative species may be distinguished into two groups, including the composite group of *S. coccinea* and *S. grossulariifolia* and the composite group of *S. munroana* and *S. parvifolia*. However, some of the USU specimens of *S. grossulariifolia* collected in Cache County in northern Utah where *S. munroana* is co-occurring (Shultz et al., 2010) fell into the type group of *S. munroana*. The overlap in morphological characteristics between these two putative species may be a result of inter-specific hybridization. Holmgren et al. (2005) reported that some collections from the distributional range where *S. munroana* overlaps that of *S. grossulariifolia* appear to be first generation hybrids between the two putative species.

GENETICS. The field population assigned as *S. grossulariifolia* on the basis of the classification model of the type specimens (Fig. 3-2) grouped together with populations assigned as *S. coccinea*, while the population assigned as *S. munroana* grouped together with populations assigned as *S. parvifolia* in the Bayesian cluster analysis (Fig. 3-3A-C). Separation of the *S. coccinea* and *S. grossulariifolia* composite group from the *S. munroana* and *S. parvifolia* composite group occurred when testing a two population
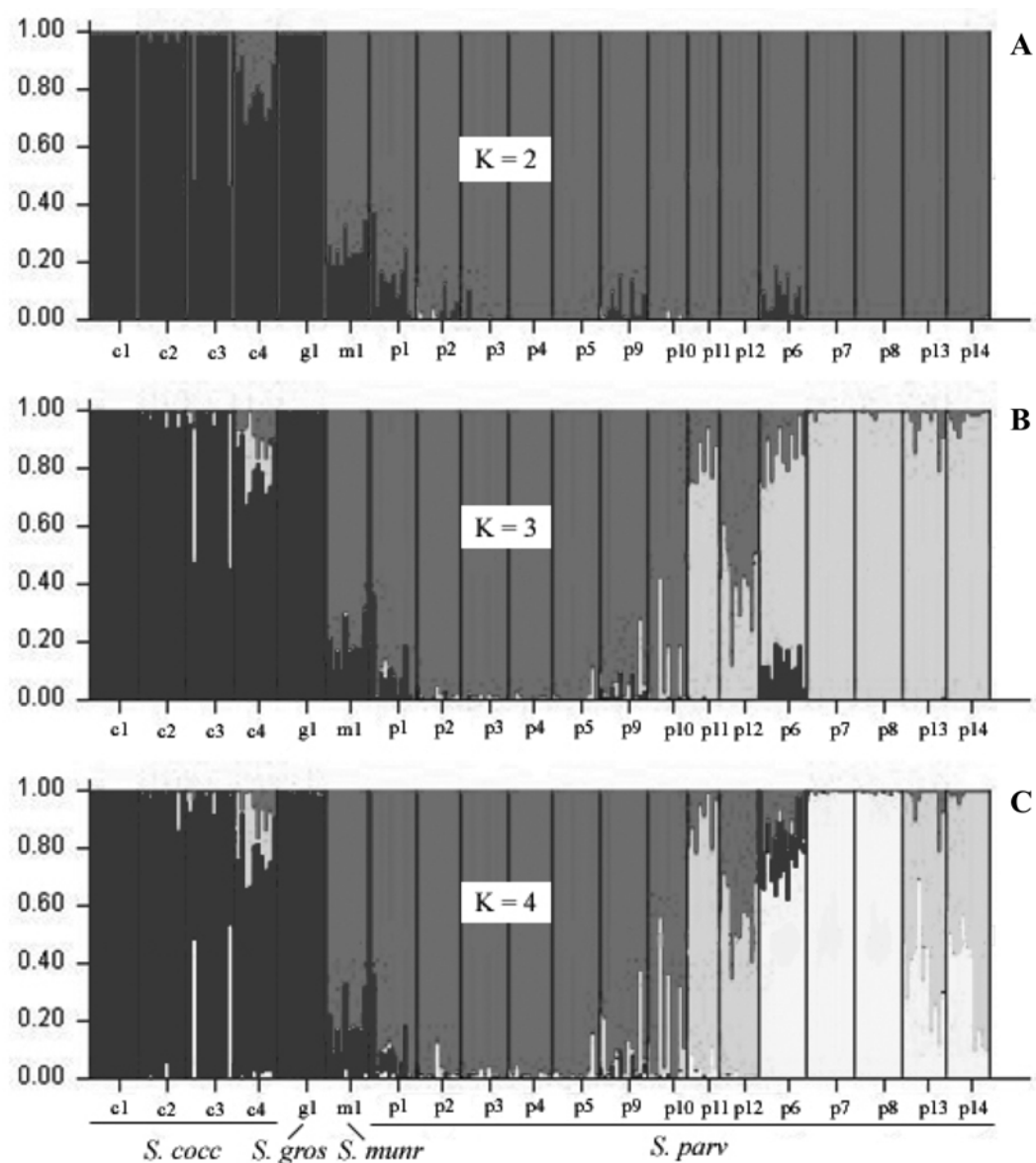
Fig. 3-3. Inferred population structure of *Sphaeralcea* AFLP genotypes from the field specimens when; (A) testing a two population model (K = 2); (B) testing a three population model (K = 3); and (C) testing a four population model (K = 4); a thin vertical line represents each individual, black lines separate individuals of different populations; *S. coc = S. coccinea*; *S. gros = S. grossulariifolia*; *S. mun = S. munroana*; *S. par = S. parvifolia*

model (K = 2) in the structure analysis, and remained separated when increasing the number of test populations in the model to three and four. Genetic variation was relatively greater within the composite group *S. munroana* and *S. parvifolia*, specifically within populations of *S. parvifolia* when the presumed number of populations in the model was increased. The population assigned as *S. munroana* also shared approximately 20% genetic similarity with the composite group of *S. grossulariifolia.*

The genetic distinctiveness between the two composite groups was consistent with morphology. These results strongly support the suggestions by Holmgren et al. (2005) that *S. munroana* may be no more than varietally different from *S. parvifolia*, as all of the USU herbarium specimens of *S. munroana* fell into the type group of *S. parvifolia*, and the field population assigned as *S. munroana* grouped genetically with *S. parvifolia*. Similarly, the results from morphological and genetic studies suggest that *S. grossulariifolia* and *S. coccinea* may be no more than varietally different, as 75% of the USU herbarium specimens of *S. grossulariifolia* fell into the type group of *S. coccinea*, and the field population assigned as *S. grossulariifolia* grouped genetically with *S. coccinea* in the structure analysis. Morphological and genetic distinctions appeared to be greatest between *S. coccinea* and *S. parvifolia*. It is possible that putative species, *S. coccinea* and *S. parvifolia* have evolved in different ecological niches, leading to genetic distinctions. This is similar to the findings of Tate (2002) who classified other *Sphaeralcea* species into North America and South America groups based on ITS genetic data.

The Bayesian cluster analysis showed that the field population assigned as *S. munroana* shared approximately 20% ancestry with the composite group of *S.*

grossulariifolia (Fig. 3-3A-C), which supports the possibility of inter-specific

hybridization between *S. grossulariifolia* and *S. munroana* suggested by Holmgren et al.

(2005), resulting in morphological overlapping between the two, as some of the USU

herbarium specimens of *S. grossulariifolia* fell into the type group of *S. munroana*.

Only one population out of 20 populations from the field collection was assigned

as *S. grossulariifolia*; and only one population was assigned as *S. munroana* based on the

classification model (Fig. 3-2). The small sample sizes of these species may be because

there were not many populations of the putative species *S. grossulariifolia* or *S.*

munroana, even though we used locations of the existing USU Herbarium specimens of

the four putative species to collect the field specimens.

CORRELATIONS. The field population assigned as *S. grossulariifolia* grouped

morphologically and genetically with the populations assigned as *S. coccinea* in cluster

analysis (Fig. 3-4A and B). The field population assigned as *S. munroana* was

morphologically closely related to the composite group of *S. coccinea* and *S.*

grossulariifolia, but grouped genetically with the populations assigned as *S. parvifolia*.

This result was consistent with the results from the morphological and genetic studies

about the distinction of the composite group of *S. coccinea* and *S. grossulariifolia* from

the composite group of *S. munroana* and *S. parvifolia*, and supports the possibility of

hybridization between *S. grossulariifolia* and *S. munroana*.

There was a correlation between morphological and genetic distances when all

putative species pooled together in Mantel's correlation tests (Table 3-4), but the

correlation was not significant within each composite group. The correlation between

geographical and genetic distances was significant within both composite groups, but
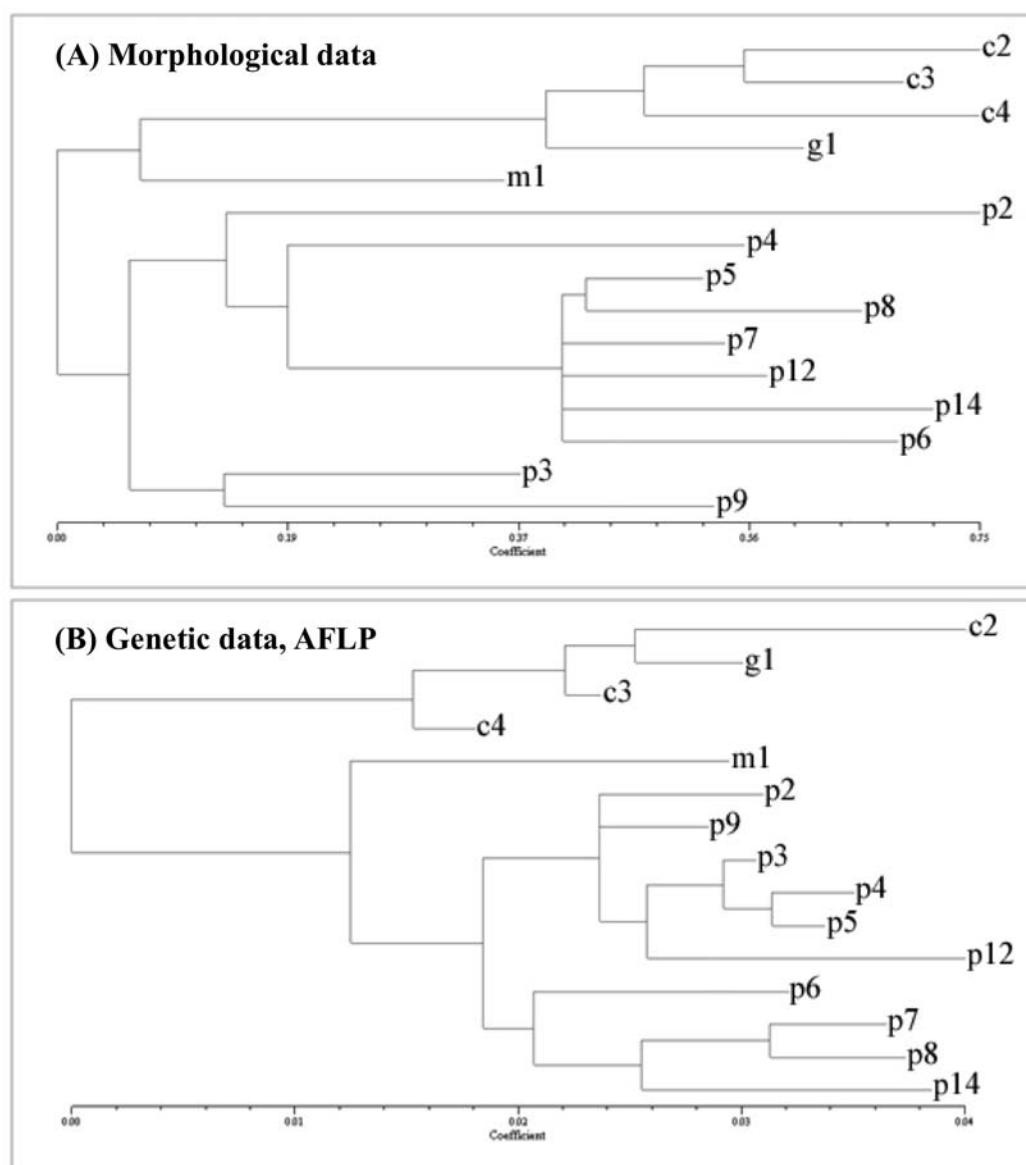
Fig. 3-4. Neighbor-Joining clustering trees constructed from morphological and AFLP genetic data of field collection of *Sphaeralcea* species; (A) the tree constructed from Euclidean distances of morphology; (B) the tree constructed from NEI-72 genetic distances

Table 3-4. Mantel's correlation tests performed with 999 permutations of morphological (Euclidean distances of morphology), genetic

(NEI-72 genetic distances), and geographic distances of the field specimens assigned on the basis of the classification

model as *S. coccinea*, *S. grossulariifolia*, *S. munroana*, and *S. parvifolia*

	All (n = 15)	*S. coccinea* (n = 3) & *S. grossulariifolia* (n = 1)	*S. parvifolia* (n = 10) & *S. munroana* (n = 1)
Geographical – Genetic distances	NS	$p = 0.038$; r = 0.978	$p = 0.024$; r = 0.384
Geographical – Morphological distances	NS	NS	$p = 0.011$; r = 0.437
Morphological – Genetic distances	$p = 0.001$; r = 0.665	NS	NS

not significant when all species pooled together. Only within the *S. munroana* and *S. parvifolia* composite group was the correlation between geographical and morphological distances significant.

The Mantel's correlation test between AFLP genetic- and geographical distances was used by Larson et al. (2010) for species delimitation tests of endemic *Lepidium papilliferum*. Similar to their approach, the correlation between AFLP genetic distance and geographical distance was not significant when all putative species pooled together (Table 3-4). This result is interpreted, as described by Good and Wake (1992) regarding species delimitation, as being different species. However, the correlation was significant when tested within each composite group, suggesting putative species within each composite group as being con-specific species and isolated by distance (Good and Wake, 1992). In addition, the significant correlation between geographical and morphological distances within the *S. munroana* and *S. parvifolia* composite group supports the Atwood and Welsh (2002) morphological isolation-by-distance findings within this group.

Utah is separated by the Rocky Mountains into two major ecoregions, including the Northwest ecoregion (Great basin) and the Southeast ecoregion (Colorado plateau). The Colorado Plateau differs from the Great Basin in having greater amounts of summer rainfall due to frontal systems moving northward from the Gulf of California, sandier soils, and streams which drain into river systems rather than closed basins and salt playas, resulting in a relatively more suitable growing season on the Colorado Plateau and somewhat greater diversification of plant habit, phenology, and physiology (Comstock and Ehleringer, 1992). According to the map of distribution (Fig. 3-1), the populations assigned as *S. parvifolia* (p1, p2, p3, p4, p5, p9, p10) and the population assigned as *S.*

munroana (m1) fell into the Great Basin ecoregion, and shared genetic similarity and grouped together in the structure analysis (Fig. 3-3C). The other populations of the field specimens assigned as *S. parvifolia* fell into the Colorado Plateau ecoregion according to their geographical distribution.

On the basis of genetic similarity, the populations assigned as *S. parvifolia* within the Colorado Plateau ecoregion group suggested by the structure analysis can be separated into two subgroups (Fig. 3-3C). The first subgroup included *S. parvifolia*, population p6, p7, and p8. All populations in this subgroup were collected in Emery County, in the central east section of Utah (Fig. 3-1). The other subgroup included *S. parvifolia,* population p11, p12, p13, and p14. The populations p11 and p12 were collected from south-western Utah, and the populations p13 and p14 were collected from south-eastern Utah. The populations p13 and p14 also shared genetic similarity with the other subgroup (p6, p7, p8) (Fig. 3-3C).

Morphological adaptation to different environmental settings may account for leaf morphological variation among populations of the *S. parvifolia* and *S. munroana* composite group. Leaf dissection could be a trade-off with pubescence, as both reduce heating, dissection through greater convection, hairs through reflection, with the hairs having the additional benefit of reducing boundary layer conductance. Anecdotally, leaves of *S. parvifolia* in the Colorado plateau were relatively densely pubescent but less lobed than *S. parvifolia* in the Great basin. Within the Colorado plateau group, leaf pubescence of the populations from the south (p11, p12, p13, p14) was relatively dense compared to that of the populations from the central east section of Utah (p6, p7, p8). The

dense pubescence may be an adaptation to the relatively hotter temperatures in the south, thus a lower transpiration rate and less rapid soil water depletion.

The results from morphological, genetic, and correlation studies support two pure types among the four putative species. *Sphaeralcea grossulariifolia* grouped morphologically and genetically with *S. coccinea*, while *S. munroana* grouped morphologically and genetically with *S. parvifolia*. The morphological variation among populations of *S. munroana* and *S. parvifolia* correlated with geographical distances. Resource identification is critical for using these *Sphaeralcea* species for low-water landscaping, especially within the composite group of *S. parvifolia* and *S. munroana*, because of morphological isolation-by-distance. Uses of plant materials from a similar ecological setting may provide more confidence in native plant production being brought to market.

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CHAPTER 4

ECOLOGICAL ADAPTATION OF *SHEPHERDIA ROTUNDIFOLIA*[1,2]

ABSTRACT. In addition to biodiversity conservation, the Colorado plateau's native and endemic shrub, *Shepherdia rotundifolia*, has potential to be used for sustainable urban low-water landscapes (LWL). However, it appears to be difficult to establish in irrigated urban landscapes. Little is known about this species in its native habitats that would help to facilitate use in LWL. The goal of this study was to investigate variability in terms of environmental conditions, morphology, and genetics among populations of *S. rotundifolia* in its native habitats, which may help to inform its limits to establishment in LWL. Six selected populations of *S. rotundifolia* occurred at elevation ranging from 1,200 to 2,500 m. The annual precipitation (199-409 mm/yr), average maximum temperature (17-25°C), average minimum temperature (3-9°C), and relative light intensity (RLI) (31-100%) were significantly different among populations. The air temperature was highest in July and dropped at least 10°C in December. Soil properties varied among the six populations, in terms of soil salinity (0.7-1.2 dS/m), pH (6.5-7.8), phosphorus (P) (1.6-27.1 mg/Kg), potassium (K) (148.5-373.5), and organic matter (OM) (0.7-8.7%). Leaf area, specific leaf area (SLA), and leaf trichome structure exhibited adaptive traits to shady environments as well as hot and dry summer and cold winter environments in its native habitats. The SLA was significantly correlated with RLI, OM, and K. A high level of K in its native habitats and a correlation of K with SLA may suggest potassium deficiency in LWL soils.

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[2] Additional index words: Roundleaf buffaloberry, SEM, AFLP

Water conservation is critical for urban systems, particularly in the U.S. Intermountain West (IMW) due to limited water supply. Low-water landscaping (LWL), specifically using drought-tolerant native species, is a key element for successful water conservation (Kjelgren et al., 2009). Drought-tolerant native species use minimal water, provide a natural look to the urban landscape, and support local native plant industries (Kjelgren et al., 2009; McKinney, 2002). The low-water landscape using drought adapted native species not only has potential to conserve water, it also has potential to increase biodiversity in urban environments.

Biodiversity is important for sustainable ecosystems. Preservation of species diversity endemism is typically focused on natural habitats in biodiversity hotspots, national parks, wildlife refuges, and national forests (Brooks et al., 2006; Myers et al., 2000). In addition to preservation of species in their natural habitats, wise use of the species in urban landscapes can create a commercially viable asset and urban biodiversity (Alvey, 2006) that creates more sustainable urban systems, educates the public about natural systems, and honors the species' natural habitat.

The IMW harbors large number of endemic species (Intermountain Native Plant Growers Association, 2011; Mee et al., 2003; Meyer et al., 2009) that have potential as urban landscape plants, since most are found in dry habitats. These species may be suited to LWL. Roundleaf buffaloberry (*Shepherdia rotundifolia*) in the family Elaeagnaceae, is an evergreen shrub that has a number of aesthetic qualities and apparent drought tolerance as well as nitrogen fixation that could be well suited to LWL. Aesthetically, it has a hemispherical-shaped canopy and silvery green, evergreen foliage that could accent the LWL (Mee et al., 2003). The Utah Division of Wildlife Resources encourages

creating landscapes for wildlife using native plants, such as *S. rotundifolia* as its fruit is food and cover for quail and small mammals (Nordstrom, 2001), therefore creating more urban biodiversity.

Even though this species has potential for use in the landscape and has been desired by plant growers, *S. rotundifolia* plants appear to be sensitive to over-watering and difficult to establish in irrigated urban landscapes (Mee et al., 2003), which constrains risk-averse native plant growers. Little is known about this species, especially the mechanisms underlying the difficulty to establish in urban landscapes. Temperature, irrigation, and fertilization increase N_2O and NO emission from urban soils (Bijoor et al., 2008; Hall et al., 2008; Livesley et al., 2010), which may lead to nitrogen deficiency in plants. However, this species is associated with *Frankia* for nitrogen fixation, thus N-deficiency may not be a limiting factor. Other urban environmental conditions may also limit establishment of *S. rotundifolia* in irrigated urban landscapes. Variability among populations of *S. rotundifolia* in their native habitats has not been studied enough to understand and resolve the limits of *S. rotundifolia* for landscape use in urban areas. Understanding how this species adapts to the different environmental conditions in their natural habitats may help to understand mechanisms underlying sensitivity to irrigated LWL.

The species is an endemic native to the Colorado Plateau, from southern Utah into the Grand Canyon region of Arizona (Schmutz et al., 1967; United States Department of Agriculture, 2011). The species naturally occurs on hillsides and bases of cliffs on very well drained rocky soils (Mee et al., 2003). Elevation and precipitation appear to be a strong environmental gradient for this species in Utah. Its natural habitat has been

reported to range from 170 to 480 mm yearly rainfall (Brotherson et al., 1983), from 1,500 up to 2,400 m. elevation (Kearney and Peebles, 1960). Variability in environmental conditions in their native habitat may have affected variation in adaptability among populations of this species. Leaf traits, specifically leaf area (LA) and specific leaf area (SLA) have been recognized as adaptive adjustments of plants to variations in environmental conditions (Gomes et al., 2011; Jin et al., 2011; Rieger et al., 2003), as well as contributing to genotype discrimination within species (Gomes et al., 2011). Low SLA is considered as an important strategy for plants living in harsh environmental conditions (Ceriani et al., 2009; Liu et al., 2011; Reich et al., 1998; Wilson et al., 1999). Variability among populations of *S. rotundifolia* has not been described, in particular the correlation between leaf traits and environmental conditions, but if it were described the information could facilitate *S. rotundifolia* use in LWL.

The goal of this study was to investigate variability in terms of environmental conditions, morphology, and genetics among populations of *Shepherdia rotundifolia* in its native habitats, which may help to understand mechanisms underlying sensitivity to irrigated LWL.

Materials and Methods

Since *S. rotundifolia* is only found in far southern Utah and northern Arizona, we chose to collect from populations from southwestern across southeastern Utah. The total number of populations selected was six, representing different ecological settings in their natural habitats. Elevation and precipitation variation were specifically selected for environmental, morphological, and genetic studies. The six populations included three

populations at different elevation in the town of Torrey in central Utah (Tor-2500, Tor-2300, and Tor-1600), one population in Bluff (Bluff), Utah and one population by Natural Bridges Monument (Nat. Bridge) in southeastern Utah, and one population in Springdale by Zion National Park (Springdale) in southwestern Utah (Fig 4-1).

ENVIRONMENT. Populations of *S. rotundifolia* were measured for elevation, precipitation, relative light intensity (RLI), temperature, evapotranspiration (ET_o), and soil properties. Relative light intensity, as a percent of total incoming solar radiation below the canopy compared to total incoming solar radiation above the canopy, was estimated from three canopy images taken with CI-110 Plant Canopy Digital Imager (CID Inc., Camas, WA) at each collection site. The canopy images were then analyzed with HemiView Canopy Analysis Software 2.1 (Delta-T Devices Ltd., Burwell, Cambridge). Air temperature and precipitation over 30-year records (1981-2010) at existing weather stations close to each site were downloaded from the Utah Climate Center website (http://climate.usurf.usu.edu/products/data.php), and reported in Table 4-1. The three populations located in the town of Torrey (Fig. 4-1), were used to represent variation in terms of environmental conditions due to differences in terms of elevation and canopy closure. Environmental Monitoring Systems-HOBO-U30 (Onset Computer Corporation, Pocasset, MA) was used at each site to monitor air temperature and solar radiation over time (May 2009-December 2009). ET_o was calculated on the basis of maximum and minimum air temperature at each site (Hargreaves and Allen, 2003).

Two soil samples from six populations were collected from soil surface to 30 cm depth. The soil samples were analyzed, in terms of salinity, pH, phosphorus (P), potassium (K), and organic matter (OM), at Utah State University (USU) Analytical Labs
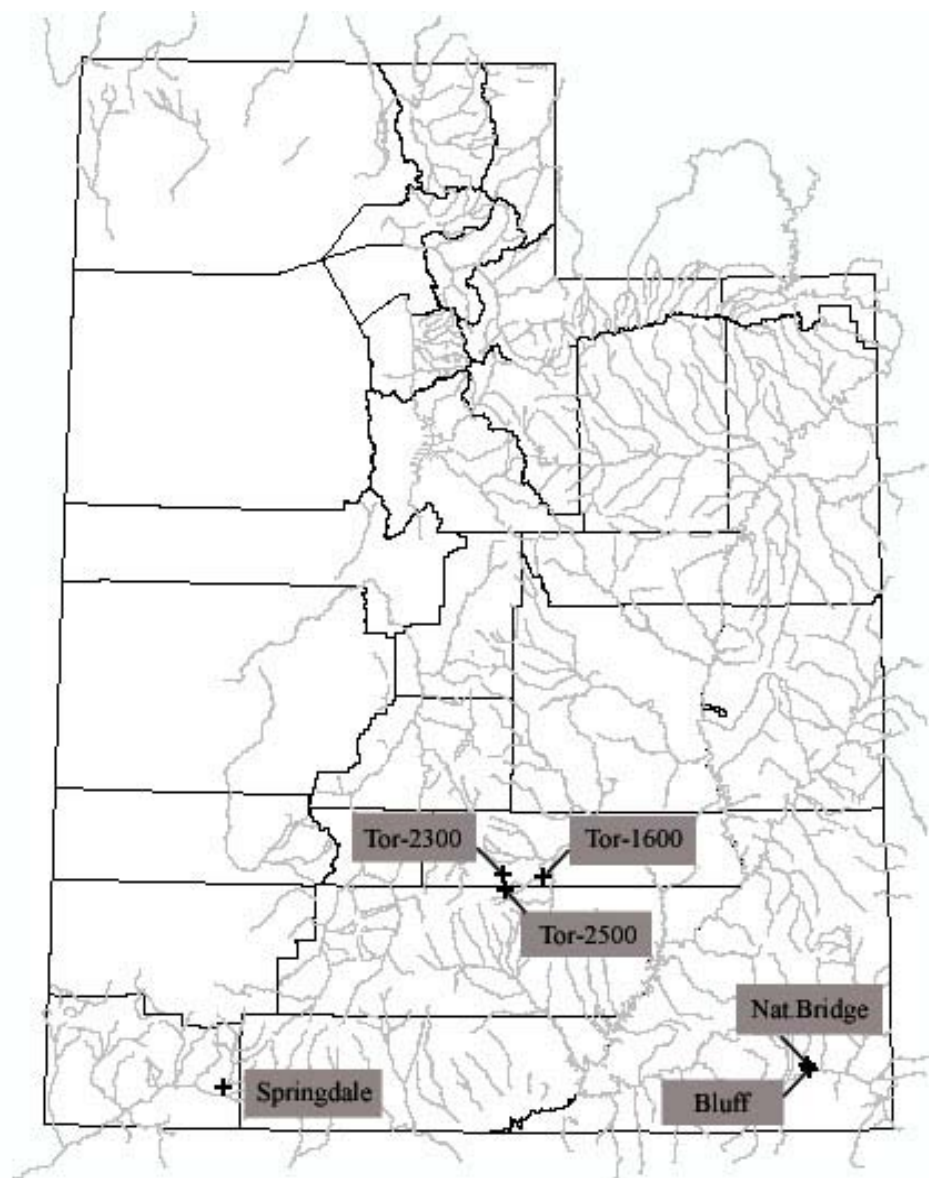
Fig. 4-1. Location of *Shepherdia rotundifolia* populations collected in southern Utah

Table 4-1. Environmental conditions among populations of *Shepherdia rotundifolia* in southern Utah, including elevation; average 30-year records (1981-2010) of annual precipitation and average maximum and minimum air temperature; and relative light intensity (RLI), and soil properties

Pop. ID	Location Latitude, Longitude Elevation, Nearest town	Average over 30 yrs[z] (1981-2010)* (n = 30)			RLI[z] (%) (n = 3)
		Precip. (mm/yr)	T_{max} (°C)	T_{min} (°C)	
Tor-2500	38.13N, 111.33W 2,507 m Torrey	285 ± 13.9^b	17 ± 0.2^d	3 ± 0.1^d	31 ± 4.3^b
Tor-2300	38.20N, 111.35W 2,295 m Torrey				98 ± 0.2^a
Tor-1600	38.19N, 111.10W 1,642 m Torrey	210 ± 11.3^c	19 ± 0.1^b	6 ± 0.1^b	100 ± 0.0^a
Nat. Bridge	37.30N, 109.54W 1,342 m Blanding	327 ± 14.5^b	17 ± 0.1^c	4 ± 0.1^{cd}	99 ± 0.1^a
Bluff	37.28N, 109.53W 1,342 m Bluff	199 ± 11.5^c	17 ± 0.2^d	4 ± 0.2^c	94 ± 0.6^a
Springdale	37.19N, 113.00W 1,188 m Springdale	409 ± 23.2^a	25 ± 0.2^a	9 ± 0.1^a	88 ± 5.5^a

[z] Values within a column with different letters indicate statistical significance at $\alpha = 0.05$

*Average precipitation, maximum and minimum temperatures over a 30 year record at each site obtained from an existing weather station closest to the site; the weather station Boulder (lat./long. 37.9 N 111.4W, elev. 2,036 m.) was used for the populations Tor-2500 and Tor-2300; the weather station Capital Reef NP (lat./long. 38.3 N 111.3W, elev. 1,676 m.) was used for the population Tor-1600; the weather station Natural Bridge NM (lat./long 37.6 N 110.0 W, elev. 1,981 m.) was used for the population Nat. Bridge; the weather station Bluff (lat./long. 37.3 N 109.6 W, elev. 1,317 m.) was used for the population Bluff; and the weather station Zion NP (lat./long. 37.2 N 113.0 W, elev. 1,234 m.) was used for the population Springdale

(USU, Logan, UT). The environmental variables among populations were subjected to Analysis of Variance (ANOVA) in PROC GLM in SAS software (SAS Institute, Cary, NC).

MORPHOLOGY. Morphological variation among populations of *S. rotundifolia* to different environmental conditions in their habitats was measured on leaf area (LA), specific leaf area (SLA), leaf thickness, and leaf pubescence. Because some populations were relatively small (approximately 5-10 individuals per population), specifically the populations Tor-2500, Tor-2300, and Tor-1600, a total number of five plants was used to represent each population. Approximately 15-20 mature sun leaves were randomly sub-sampled from each individual plant summer 2009. Specific leaf area was calculated as single-sided leaf area determined using a leaf area meter (LI-3100, Li-Cor, Lincoln, NE) divided by the leaf weight after drying at $65^{o}C$ for 24 hours. Leaf thickness and leaf pubescence were measured on leaf samples collected from three populations located in Torrey, Utah; Tor-2500, Tor-2300, Tor-1600. Leaf punches were collected from each site, and directly fixed in formalin-aceto-alcohol (FAA) solution in the field. The fixed leaf tissue was subjected to critical point drying using Samdri-PVT-3D (Tousimis, Rockville, MD). Two fixed leaf tissues were used to observe leaf trichome on the adaxial and abaxial surfaces, and the other fixed tissue was used to observe leaf thickness on cross-section surface under a scanning electron microscopy (SEM) (Hitachi S4000, Pleasanton, CA).

Leaf area and specific leaf area among populations were subjected to ANOVA using PROC GLM in SAS software (SAS Institute, Cary, NC) and were analyzed as correlation with environmental data at the six sites, including elevation, precipitation, air

temperature, relative light intensity, and soil property using Pearson's correlation

coefficient in PROC GLM.

GENETICS. Leaf samples (2-3 leaves per plant) collected from each field

population (5 plants per population) were dried in 28-200 mesh silica gel (Fisher

Scientific, Pittsburgh, PA). DNA was extracted with the Dneasy 96 Plant Kit (QAIGEN,

Valencia, CA). Amplified fragment length polymorphisms (AFLPs) were assayed as

described by Vos et al. (1995) with described modifications. The DNA samples were pre-

amplified EcoRI +1 / MseI +1 using A/C selective nucleotides. Selective amplification

primers consisted of five EcoRI +3 / MseI +3 primer combinations using AAC/CAA,

AAG/CAG, ACC/CAT, ACG/CTA, AGG/CTA, AGA/CCC selective nucleotides. The

EcoRI selective amplification primers included a fluorescent 6-FAM (6-carboxy

fluorescein) label on 5' nucleotides. Selective amplification products were combined with

GS600 LIZ internal lane size standard and fractionated using an ABI 3730 instrument

with 50-cm capillaries and sized between 50 and 600 bp with Genescan software

(Applied Biosystems, Foster City, CA). Although DNA molecules vary in length by

increments of 1 bp, the relative mobility of bands is also affected by sequence

composition. Thus, non-homologous bands of the same length may not have the same

relative mobility. Genescan trace files for each individual were visually analyzed for the

presence or absence of DNA bands in bins that were at least 0.3 bp or more apart using

Genographer software (available free at http://hordeum.oscs.montana.edu/genographer/

or directly from the author, Tom Blake, at blake@hordeum.oscs.montana.edu). Bayesian

clustering of individual plants without a priori assignment of individuals to hierarchical

groups was used to determine genetic structure and to test for possible admixture between

taxa, which might otherwise confound phylogenetic analysis, using Structure v2.1

(Pritchard et al., 2000). Three analyses used of each model with 100,000 iterations and

10,000 burn-in or 200,000 iterations and 20,000 burn-in with the dominant-

allele, admixture model of Structure v2.2 (Falush et al., 2007; Pritchard et al., 2000).

Results and Discussion

ENVIRONMENT. The six collection sites were generally found on hill slopes at

elevations ranging approximately from 1,200 to 2,500 m (Table 4-1). These populations

could be tentatively classified into two groups on the basis of elevation gradient,

including the high elevation populations (Tor-2500 and Tor-2300) and the low elevation

populations (Tor-1600, Nat. Bridge, Bluff, and Springdale). Most populations occurred in

an open area associated with pinyon pine community; except the population Tor-2500

which occurred underneath the canopy of a ponderosa pine forest, resulting in the lowest

relative light intensity and suggesting a degree of shade tolerance.

On the basis of a 30-year record (1981-2010), average annual precipitation at the

six collection sites ranged from approximately 200 to 400 mm/year (Table 4-1). The

population near Springdale, located adjacent to Zion National Park, had the highest

annual precipitation, approximately two times greater than annual precipitation at the

populations Tor-1600 and Bluff due to summer monsoonal subtropical ridge from the

Gulf of California. Average maximum temperature ranged from 17-25°C, while average

minimum temperature ranged from 3-9°C. Average maximum and minimum

temperatures were also highest near the Springdale population, due to its relatively lower

elevation. Habitats of the two high elevation populations had lower temperature compared to the low elevation populations.

Weather data recorded in the field in the year 2009 at the three sites in the town of Torrey showed average temperature to be highest in the month of July (~ 40°C at the site Tor-1600) and lowest in the month of December (~ -20°C at the site Tor-2500). In July 2009, average maximum air temperature at the site Tor-1600, which was located on a sandy hill slope habitat adjacent to Capital Reef National Park, was relatively higher than temperatures at the sites Tor-2300 and Tor-2500, respectively, and consistent with average minimum air temperature (Fig. 4-2). Maximum and minimum air temperatures at the three sites dropped down at least 10°C in the month of December compared to temperatures in the month of July, specifically at the site Tor-1600 where the maximum and minimum air temperatures dropped to be as cold as the temperatures at the site Tor-2500. The site Tor-2300 had the warmest winter maximum and minimum temperatures, probably because it surrounded by dense tree cover. Meanwhile the site Tor-1600 surrounded by sparsely perennial herbaceous, resulting in greater maximum and minimum differences between summer and winter at this site.

During the growing season (May-September), overall solar radiation intensity was highest at the site Tor-1600 and lowest at the site Tor-2500 (Fig. 4-3A) although peak evapotranspiration was highest at the site Tor-2500 (Fig. 4-3B). The lowest daily solar radiation at Tor-2500 and Tor-2300 compared to Tor-1600 may be a result of being underneath a canopy of ponderosa pine forest at 2500 m as well as being cloudier both the two high elevation sites (Figure 4-3B and C). Average reference evapotranspiration (ET$_o$) over year 2009 was also greatest at the site Tor-1600 (781 mm year^{-1}), where
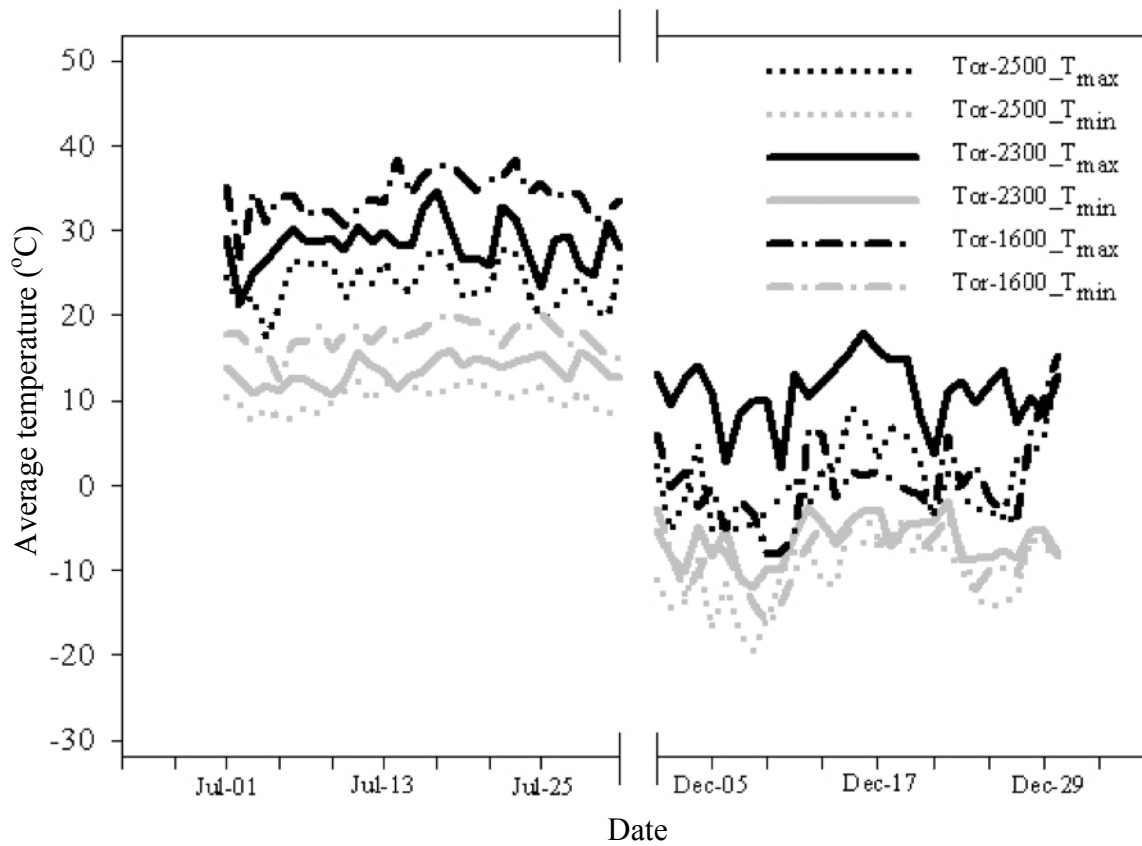
Fig. 4-2. Average maximum and minimum air temperature recorded from May 2009 to December 2010 at three populations in Torrey, Utah; Tor-2500, Tor-2300, and Tor-1600
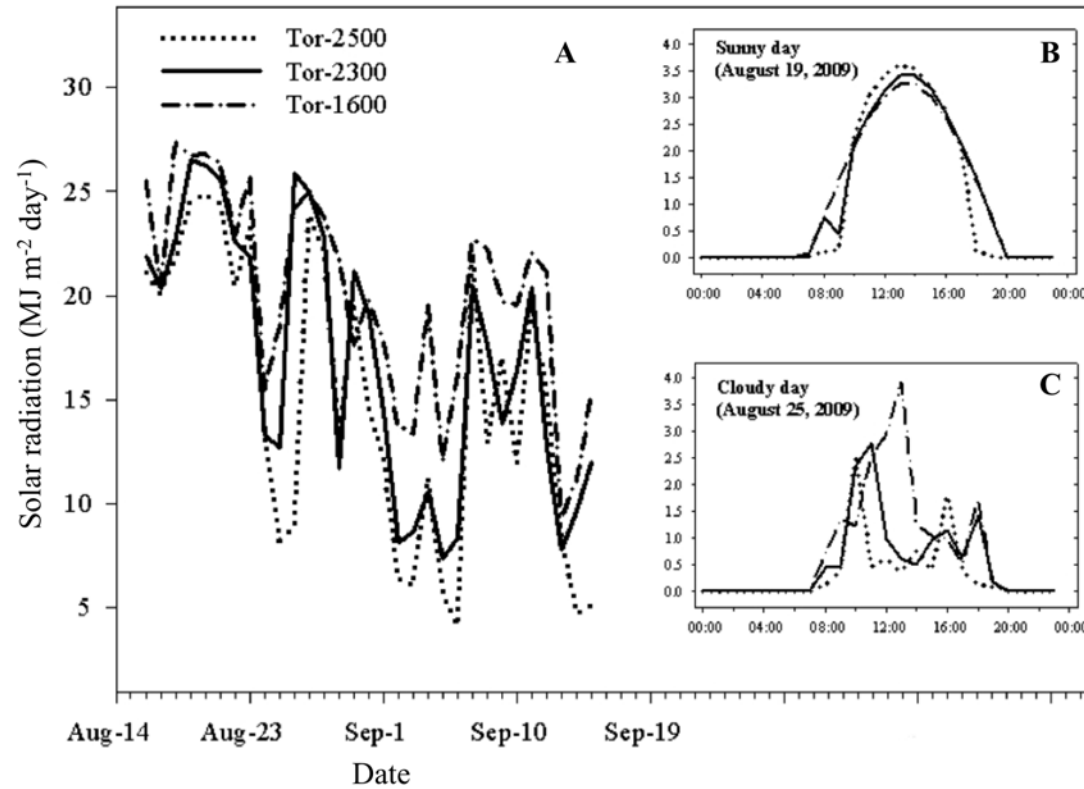
Fig. 4-3. Solar radiation intensity recorded at Tor-2500, Tor-2300, and Tor-1600; (A) daily solar radiation from August 16, 2009 to September 16, 2009; (B) hourly solar radiation on a sunny day (August 19, 2009); and (C) hourly solar radiation on a cloudy day (August 25, 2009)

precipitation was lower and average air temperature higher in the summer than at the sites Tor-2300 and Tor-2500, where ET_o were 705 and 564 mm year^{-1}, respectively.

Soil properties also varied among the six collection sites (Table 4-2). Soil parent material can be different among the sites (Stohlgren et al., 2005), but in general, *S. rotundifolia* occurs in very well drained sandy soils (Mee et al., 2003). *Shepherdia* species are symbiotic nitrogen-fixing shrubs with *Frankia*. Well-drained soils tend to have higher nitrogen mineralization and nitrification rates than poorly drained soil conditions (Ullah and Moore, 2009). Soil salinity levels at the sites were relatively low compared to salinity tolerant levels of crop species (Aragues et al., 2010; Dai et al., 2008), ranging from 0.7-1.2 dS/m. Soil pH of most sites was slightly alkaline, except at the site Tor-2500, where soil pH was slightly acid due to relatively high content of organic matter (OM) from ponderosa leaves. Alkaline condition also favors the activity of *Frankia*-nitrogen fixation (Bai et al., 2010).

Nutrient levels in the native soils of *S. rotundifolia* habitats varied among populations of *S. rotundifolia*. Even though phosphorus (P) level in soils at the site Tor-2500 was significantly higher than the other sites, the P level was low compared to the normal soil P level recommended for crop production lands (He et al., 2009). Meanwhile, potassium (K) level at all sites was very high compared to the level of K recommended in crop production soils (Franzen, 2007). The high concentration of potassium ions seemed to be common in soils beneath populations of *S. rotundifolia* compared to levels in soils adjacent to the shrub. This result was similar to findings of Fairchild and Brotherson (1980), who found that nitrogen and phosphorus were in greater concentration beneath the species' canopy than in open areas adjacent to the shrub.

Table 4-2. Soil properties; including soil texture, salinity (EC), pH, phosphorus (P), potassium (K), and organic matter (OM) at the six collection sites in Utah (n=2)

Pop ID	Texture	EC[NS]	pH[z]	P[z]	K[NS]	OM[NS]
		(dS/m)		(mg/kg)	(mg/kg)	(%)
Tor-2500	Silt loam	0.7 ± 0.2	6.5 ± 0.1^b	27.1 ± 7.9^a	373.5 ± 105.5	8.7 ± 2.1
Tor-2300	Sandy loam	1.2 ± 0.5	7.5 ± 0.2^a	5.2 ± 1.2^b	148.5 ± 48.0	1.8 ± 0.8
Tor-1600	Sandy clay	0.8 ± 0.1	7.7 ± 0.1^a	8.5 ± 5.5^{ab}	193.5 ± 3.5	0.7 ± 0.2
Nat. Bridge	Sandy loam	1.0 ± 0.1	7.6 ± 0.0^a	1.6 ± 0.0^b	243.0 ± 15.0	2.0 ± 0.4
Bluff	Sandy loam	1.1 ± 0.4	7.4 ± 0.0^a	3.3 ± 0.5^b	190.0 ± 103.0	3.9 ± 2.8
Springdale	Sandy loam	0.8 ± 0.1	7.9 ± 0.2^a	8.3 ± 1.2^{ab}	360.0 ± 73.0	6.3 ± 3.3

[z] Values within a column with different letters indicate statistical significance at $\alpha = 0.05$

The range of environmental conditions in terms of RLI, precipitation, temperature, solar radiation, ET_o, and soil properties among native habitats of *S. rotundifolia* suggests that the species adapted to full sun conditions and very well drained soils with low OM, and also tolerate shady environments as well as very hot and dry summer and cold winter environments. To tolerate such environmental conditions, *S. rotundifolia* may exhibit remarkable adaptive morphological characteristics.

MORPHOLOGY. Plants of *S. rotundifolia* appeared to have adaptive morphological characteristics to tolerate hot and dry conditions in its native habitats. Leaf morphological

characteristics of *S. rotundifolia* varied among populations (Table 4-3). Variation in leaf size and leaf thickness of *S. rotundifolia* may suggest different mechanisms at leaf level to tolerate different environmental conditions in their habitats. Plants at the site Tor-2500 exhibited adaptive traits to shady environments by having a relatively large leaf area (LA) and a relatively high specific leaf area (SLA) to increase surface area to absorb light for photosynthetic carbon assimilation under low light conditions (Schumacher et al., 2008). Meanwhile, plants at the site Tor-1600 adapted to a hot and dry habitat by having a relatively small LA and a relatively low SLA, although LA and SLA were not significantly different. Small leaf size of plants at the site Tor-1600 helps to reduce transpiration surface against high ambient temperature and ET_o, thus reducing water loss.

The leaf trichome layer in *S. rotundifolia* appeared to largely contribute to the leaf thickness (Fig. 4-4A-C). The abaxial trichome thickness of plants from the site Tor-1600 was almost two times greater than plants from the site Tor-2500. Even though we only had one measurement on leaf thickness for plants at the three sites, the relatively thick leaf of plants from the site Tor-1600 was consistent with low SLA (Table 4-3). Leaf trichomes, peltate and stellate (Cooper, 1932), were present on adaxial and abaxial surfaces, respectively (Fig. 4-5A and B). Abaxial surface trichome density was approximately five times higher than adaxial density, similar to the findings of Bissett et al. (2009) on *Elaeagnus umbellata,* also in the Eleagnaceae family. The peltate trichome on the upper surface helps to reflect excess radiation to protect the underlying tissues against ultraviolet-B radiation damage (Karabourniotis et al., 1993). The thicker underside stellate trichome helps with insulation and provides a moisture trap on leaf surface to protect against heat loss and reduce water loss, and increase leaf boundary

Table 4-3. Morphological characteristics of *S. rotundifolia* from different populations,

including leaf area; specific leaf area (SLA), leaf thickness in mesophyll layer,

and leaf trichome thickness

Pop. ID	Leaf Area[NS] (cm^2) (n = 5)	SLA[NS] (cm^2/g) (n = 5)	Mesophyll- Thickness (mm)	Trichome- Thickness (μm)
Tor-2500	2.3$\pm$0.5	48.6$\pm$5.5	0.15	0.46
Tor-2300	2.2$\pm$0.3	37.0$\pm$1.2	0.23	0.39
Tor-1600	1.3$\pm$0.2	37.8$\pm$1.5	0.21	0.75
Nat. Bridge	1.8$\pm$0.2	40.0$\pm$1.8	-	-
Bluff	1.5$\pm$0.1	40.4$\pm$3.0	-	-
Springdale	1.6$\pm$0.2	41.7$\pm$2.2	-	-

layer, thus reducing transpiration and impact of wind on plant energy budget (Press, 1999). The relatively thick stellate trichome at the population Tor-1600 may suggest regulating transpiration is more important for plants at this site than at the site Tor-2500. Specific leaf area is a measure of plant adaptation to environmental stresses, specifically heat and soil stresses. The SLA of plants at the six sites exhibited significant correlation with RLI (Pearson's r = 0.886; p = 0.019), K (Pearson's r = 0.832; p = 0.040), and OM (Pearson's r = 0.854; p = 0.030) in the Pearson's correlation test (Fig. 4-6A-C). The environmental conditions with high RLI, low K and OM, as appeared at the site Tor-1600, tend to force *S. rotundifolia* to invest in producing a thick abaxial leaf trichome
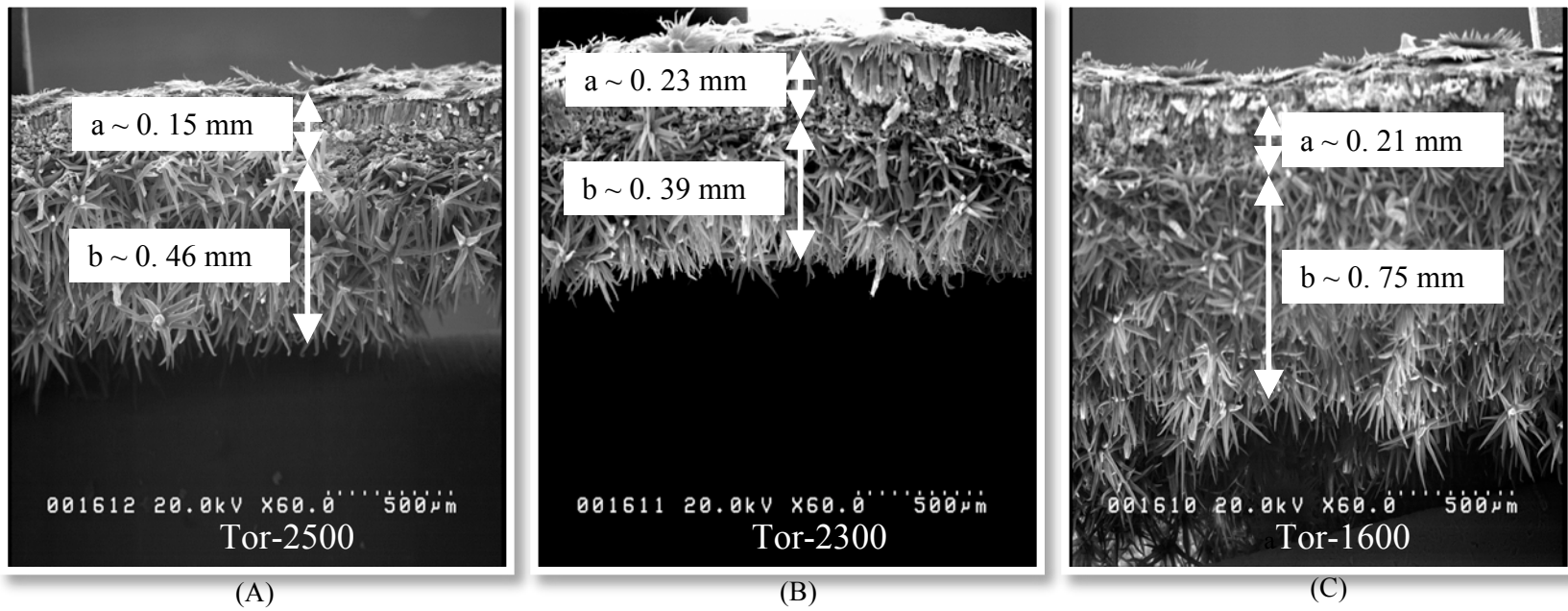
Fig. 4-4. Cross section of leaves observed under scanning electron microscope (SEM) of plants from three populations in

Torrey, Utah, including Tor-2500 (A); Tor-2300 (B), and Tor-1600 (C)
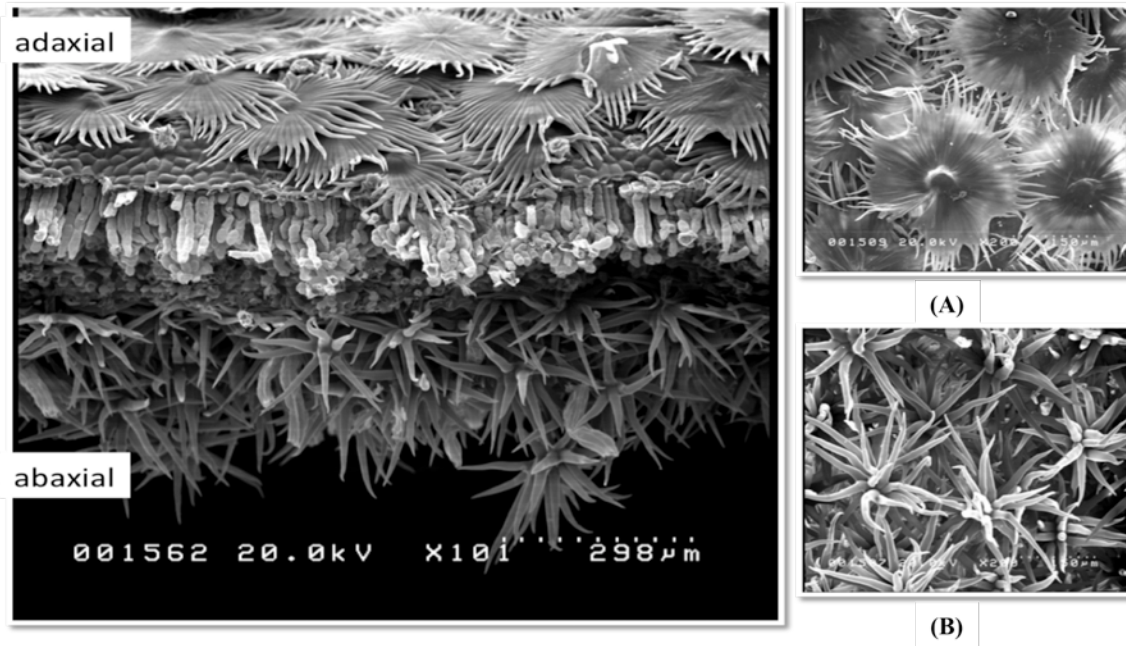
Fig. 4-5. Leaf trichome morphological characteristics observed under scanning electron microscope (SEM); (A): adaxial

trichome (upper) and (B) abaxial trichome (lower) sides of the leaf
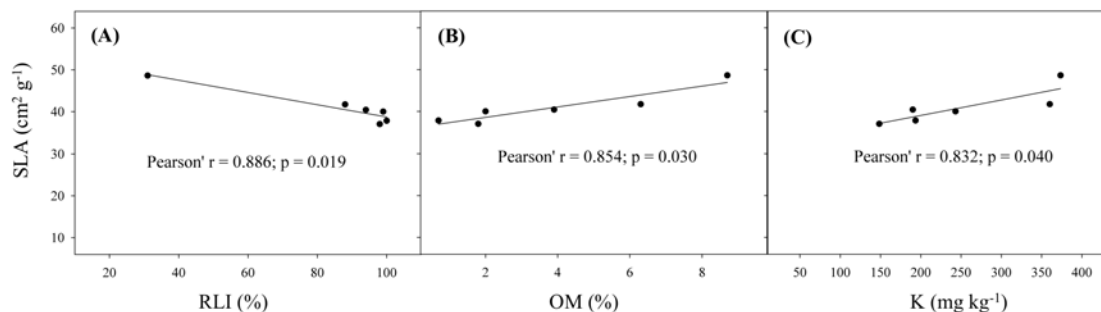
Fig. 4-6. Pearson's correlation of specific leaf area (SLA) with (A) relative light intensity

(RLI), (B) soil organic matter (OM), and (C) soil potassium (K)

layer, resulting in low SLA. The variation in SLA, specifically between plants at the site Tor-2500 and Tor-1600, suggests morphological plasticity of the species to the range from shady environments to hot and dry environments.

The responses of *S. rotundifolia* in terms of SLA responses to RLI, OM, and K, but no relationship to EC, P, and N in its native habitat conditions may suggest similar responses in urban landscape conditions. Increasing of RLI tends to increase heat stresses, thus decreasing SLA (Fig. 4-6A). Urban landscape conditions can range from shady environments to fully exposed to full sun, and similar to native habitats of *S. rotundifolia*. Increasing of OM tended to reduce environmental stresses, thus increasing SLA (Fig. 4-6B). Organic matter contents in urban soils can be as low as less than 1% in densely populated urban areas (Jim, 1998). The low OM in urban soils is consistent with

soil OM in native habitats of *S. rotundifolia*, specifically at the site Tor-1600 (Table 4-2). Low organic matter can lead to nutrient deficiency, particularly N and P (Jim, 1998). *Shepherdia* species are symbiotic nitrogen-fixing shrubs with *Frankia*, which is found to be abundant in dried sand dune habitats (Batzli et al., 2004). This may help *S. rotundifolia* to cope with soil N insufficient in its native soils.

Similar to responses to OM, increasing K tended to increase SLA (Fig. 4-6C). Even though concentrations of K in urban landscape soils can meet or exceed the recommended concentrations for horticultural soils (Pickett et al., 2008), temperature and irrigation can affect soil chemical and biological properties (Groffman et al., 2006), potentially leading to K deficiency in urban soils. Potassium is involved in many physiological processes such as photosynthesis, assimilate transport and enzyme activation, and plant water relation (Pettigrew, 2008) through lack of control over turgor (Amtmann and Armengaud, 2007). Water use efficiency is critical for plants, such as *S. rotundifolia*, that occur in dry habitats, such as the very dry site at Tor-1600. The high level of K in the native habitats of *S. rotundifolia* may have improved efficiency of plant water use (Egilla et al., 2005), thus possibly contributing to survival in such hot and dry habitats. Anecdotally, *S. rotundifolia* plants growing in urban soil conditions often show a yellowing in old leaves that progressively spreads to new growth and ultimately kills the whole plant. This response appears to be consistent with K^+ deficiency symptoms reported to appear first in older leaves (Amtmann and Armengaud, 2007). Sangakkara et al. (2011) found that fertilizer K can enhance root development, growth, and N-use efficiency of maize and mungbean, and may play a role in *S. rotundifolia* health.

GENETICS. Environmental variation, specifically elevation, appeared to be a strong environmental gradient for genetic variation among populations of *S. rotundifolia*. AFLP genetics of the high elevation populations, Tor-2500 and Tor-2300 was distinctive from the lower elevation populations in the Bayesian cluster analysis (Fig. 4-7A-D). The distinctiveness between the two population groups occurred when testing a three-population model (K = 3) using *S. argentea* as an out-group. Genetic variation among the lower elevation populations appeared to be greater than genetic variation among the high elevation populations when testing a three-population model in the structure analysis, and remained separated when increasing the number of test populations in the model to seven. The genetic distinction between the high elevation populations and the lower elevation populations may suggest genetic isolation by elevation of the two population groups and may further suggest underlying adaptive morphological characteristics that allow the species to tolerate a range of environmental conditions in its native habitats.

Anecdotally, seedling recruitment was minimal to nonexistent at all of the six collection sites, and may suggest species decline. A study on Holocene vegetation at Capitol Reef National Park using packrat middens by Cole et al. (1997) revealed that *S. rotundifolia* and other native species were severely reduced during the past 200 years to their lowest levels over a 5,400-year record. This dramatic decline may have been caused by grazing history in that area (Fisher et al., 2009), which may be cause for concern regarding species long-term sustainability. The high elevation populations have low genetic diversity, as no evidence of genetic polymorphism was detected based on AFLP analysis. In situ conservation of the species may enable greater gene flow among populations, thus increasing fitness to avoid potential species population decreases. In
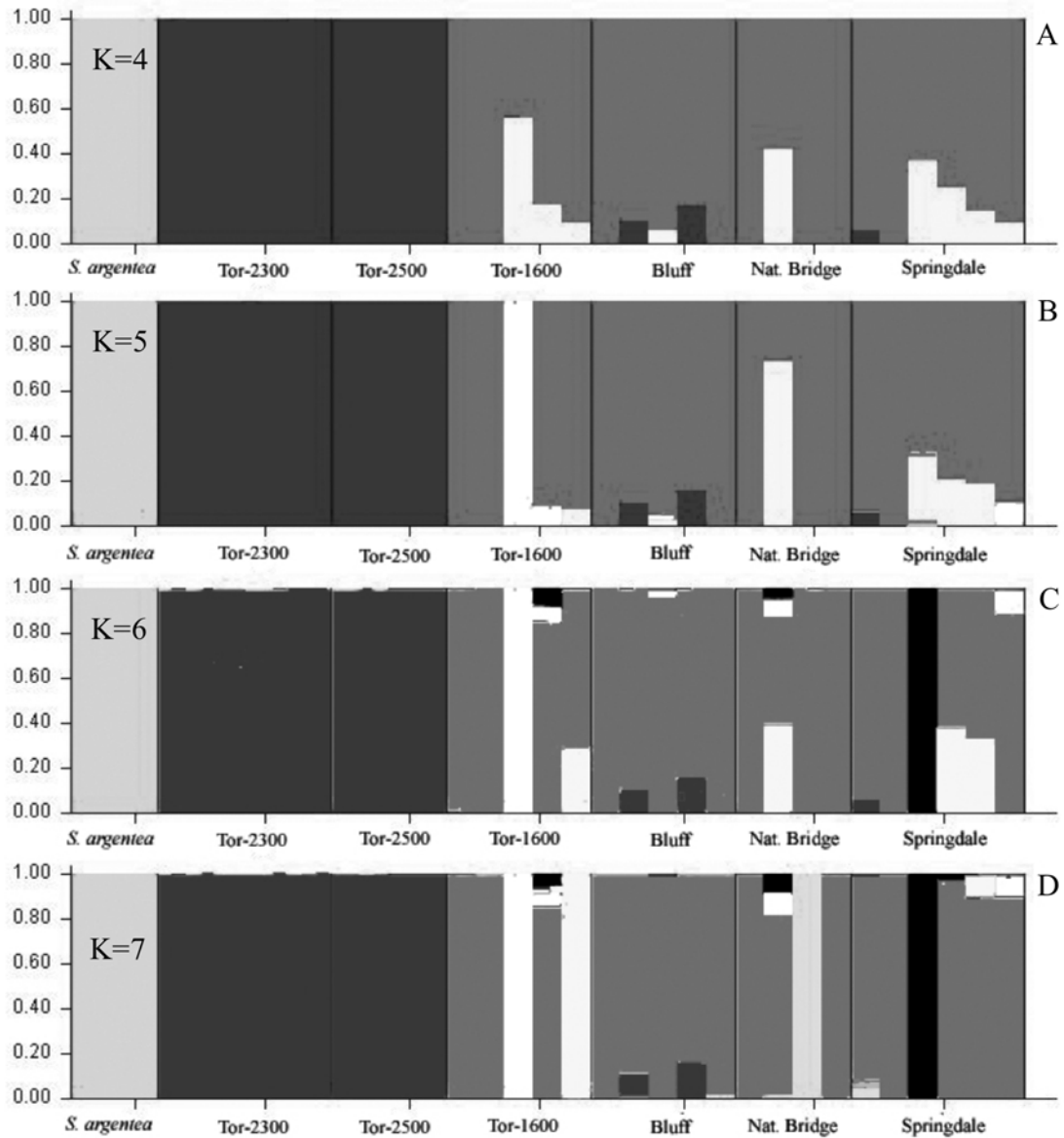
Fig. 4-7. Inferred population structure of *Shepherdia rotundifolia* AFLP genotypes from six populations in the field when; (A) testing a four population model (K = 4); (B) testing a five population model (K = 5); (C) testing a six population model (K = 6); and (D) testing a seven population model (K = 7); a thin vertical line represents each individual, black lines separate individuals of different populations

addition, species conservation for biodiversity ex-situ, including wise use of species to

create diverse and sustainable urban low-water landscapes may decrease the potential of

species decline.

Variability in native habitats of *S. rotundifolia* suggests a species tolerance of a

range of environments: from full sun to canopy shade and relatively cloudy conditions, as

well as tolerance to a range of soil organic matter and drought, hot summer, and cold

winter environments. The species appears particularly well adapted to drought,

specifically leaf thickness and trichome structure that minimizes leaf temperature and

transpiration; to poor nutrient and organic matter (OM) soils but with high potassium (K)

compared to K in crop production areas. The variability in environmental conditions in

the native habitats may facilitate its use in low-water landscaping, specifically the use of

plants adapted to environmental settings that are similar to a given landscape

environment. Genetic variation is also important for superior selection for future

development. AFLP of six populations selected in southern Utah was distinguished

between the high elevation populations and low elevation populations.

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CHAPTER 5

POTENTIAL OF *SHEPHERDIA ROTUNDIFOLIA* X *ARGENTEA* HYBRID FOR
LOW-WATER LANDSCAPING[1,2]

ABSTRACT. The species, *Shepherdia rotundifolia* native to southern Utah and northern Arizona has apparent aesthetic and ostensible drought tolerant qualities desirable for use in low-water urban landscapes (LWL). However, anecdotally the species appears to be sensitive to higher soil water conditions typical of landscape soils, thus difficult to produce in nursery production and establish in urban landscapes. We hand crossed *S. rotundifolia* with the riparian relative, *S. argentea* to develop an interspecific hybrid with desirable aesthetic characteristics of *S. rotundifolia* and tolerance of *S. argentea* to irrigated soil conditions. We compared genetic, morphological, and gas exchange characteristics of the hybrid with that of the two parents. The interspecific hybrid exhibited intermediate AFLP genetics, approximately 50% genetic similarity to both parents in Bayesian cluster analysis. Leaf morphological characteristics of the hybrid were also intermediate to its parents. Trichome structure of the hybrid was more similar to that of the female parent, *S. argentea* with peltate trichome on both leaf surfaces. Physiological responses, in terms of diurnal stomatal conductance (g_s), light response curve, midday P_n, and midday PhiPS2 of the hybrid were more similar to *S. argentea* than *S. rotundifolia*. The morphological characteristics and physiological responses of the hybrid compared to its parents may suggest tolerance to wet soil condition. The aesthetic qualities and physiological responses of the hybrid may lend them well to use in LWL.

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[2] Additional index words: Roundleaf buffaloberry, Silver buffaloberry, hand cross pollination, SEM, AFLP, morphology, physiology

Water conservation in the Intermountain West is becoming critically important as the population grows but water supplies do not. Large amounts of water are often applied to irrigated landscapes where turfgrass is prevalent (Kjelgren et al., 2009). Low-water use landscaping (LWL) is an increasingly important conservation tool in reducing water use in irrigated urban landscapes (Kjelgren et al., 2009). Use of drought tolerant plant material is an essential element of LWL, specifically native species because they use minimal water, require low maintenance, and provide a natural look to the urban landscape (Meyer et al., 2009). Native plant species also enhance biodiversity, thus creating a more sustainable urban system.

Several drought-tolerant species native to the Intermountain West have potential for use in LWL, but such taxa will need genetic improvement in order to meet both aesthetic and functional expectations in urban landscapes. The genus *Shepherdia,* in the *Eleagnaceae* family, contains *S. rotundifolia* (roundleaf buffaloberry), endemic to southern Utah and northern Arizona (United States Department of Agriculture, 2011). The species is dioecious (Nelson, 1935) naturally occurring on very well drained hillside slopes (Mee at al., 2003) at 1,500 to 2,400 m. elevation (Kearney and Peebles, 1960). *Shepherdia rotundifolia* has many aesthetic and practical desirable qualities for low-water landscapes, including apparent drought tolerance based on its native habitat in dry, rocky slopes in the 170 to 480 mm/year precipitation range (Brotherson et al., 1983), and evergreen gray-blue foliage which forms multiple clusters of attractive rosettes in a hemispherical crown shape (Mee et al., 2003). The Utah Division of Wildlife Resources encourages creating landscapes for wildlife using native plants, including *S. rotundifolia,*

as its fruit is food and cover for quail and small mammals (Nordstrom, 2001), thus

create more biodiversity in urban landscape.

Even though the species has potential for use in LWL, it appeared to be sensitive

to overwatering and difficult to establish in irrigated urban landscapes (Mee et al., 2003).

A study of variability among species, in terms of environmental and morphological

variations in their native habitats may help in cultivar selection as well as in

understanding its limit in the LWL. However, as an alternative to cultivar selection,

genetical improvement by crossing with related species better adapted to tolerate wet

soils may help to develop a new taxon that meets both aesthetic and functional

expectations in LWL. Interspecific hybridization plays an important role in plant adaptive

evolution, and the process often results in phenotypic novelty in the hybrid (Scascitelli et

al., 2010; Soltis and Soltis, 2009; Stelkens et al., 2009). A related species, *S. argentea*

(Silver buffaloberry), is a riparian shrub found throughout the western United States, that

tolerates a wide range of conditions from wet and heavy to dry soils (Mee et al., 2003).

However, it has thorns and an indistinct rangy growth habit, which makes it less

aesthetically desirable for low-water use landscapes compared to *S. rotundifolia* (Mee et

al., 2003).

Potential interspecific hybridization between *S. rotundifolia* and *S. argentea* may

create a hybrid that contains the aesthetic quality of *S. rotundifolia* and wet soil tolerance

of *S. argentea*. The goal of this study was to compare genetic, morphological

characteristics, and physiological responses of the interspecific hybrid between *S.

rotundifolia* and *S. argentea* with its parents.

Materials and Methods

We hand cross-pollinated *S. rotundifolia* and *S. argentea* (dioecious) in their
natural habitats in central Utah near the town of Torrey where both *S. rotundifolia* and *S.
argentea* plants could be found in close proximity. Five female plants from a population
of *S. argenetea*, located by the Fremont river on the roadside of Highway 12 (38.3 N,
111.4 W), were bagged with pollination bags, five bags per plant including a control bag
with no pollination, on April 11th, 2008 before their flowers opened to prevent
unexpected pollination. Meanwhile, five female plants of *S. rotundifolia* from a
population located on rocky hillside of Boulder mountain on Highway 12 (38.2 N, 111.4
W), were bagged the same way as for the female of *S. argentea* on the same day. On
April 30th, 2008, when flowers of both female *S. argentea* and *S. rotundifolia* in the
pollination bags were open, we collected fresh pollen from male plants of both species
from a population that was located closest to the bagged female plants for reciprocal
crosses. The *S. argentea* pollen used to pollinate the female *S. rotundifolia* was collected
from male plants at the same location with bagged female *S. argentea*, whereas the *S.
rotundifolia* pollen used to pollinate the female *S. argentea* were collected from male
plants from a population located adjacent to Capital Reef National Park.

In May 29th, 2008, we collected seeds from all pollination bags. Only one
pollination bag collected from a bagged female *S. argentea* produced seeds, with the total
number of five seeds. No seeds were produced from bagged female *S. rotundifolia*. These
seeds were subjected to stratification and germination, as described by Beddes and
Kratsch (2009). One week after seed germination, seedlings were grown in a growing

substrate designed for native plants in the Utah State University research greenhouse. We watered these seedlings every other day. In the winter 2008, we put the hybrid seedlings in a walk-in cooler to prevent the seedlings from outdoor freezing damage. Seeds of *S. rotundifolia* and *S. argentea* were collected from both female parent populations, geminated, and grown under the same conditions with the hybrid for future comparison.

However, *S. rotundifolia* is slow growing compared to *S. argentea* and the hybrid plants, therefore we used cuttings taken from the same bagged female population of *S. rotundifolia* that were rooted, instead of plants grown from seeds for this study. In April 2008, stem bases of *S. rotundifolia* cuttings from plants growing in their natural habitats were rooted and held on a propagation bench for 12 weeks under shade cloth and irrigated using intermittent mist. In summer 2009, plants of the hybrid and its parents were transplanted into three-gallon pot filled with a native mix soil. These plants were then grown in a pot-in-pot system in a common garden setting at the Utah State University (USU) Research Farm (Logan, UT). We watered these plants once a week.

To clarify the effect of age difference on physiological responses between plants of *S. rotundifolia* grown from cuttings and plants of *S. argentea* and the hybrid grown from seeds, we measured differential stomatal conductance (g_s) in situ between *S. rotundifolia* and *S. argentea*. On June 7th, 2009, g_s of both species was measured on five plants, three leaves per plants, from each species using leaf porometers (SC-1 porometer, Decagon Device, Pullman, WA) at 09:00 am, 11:00 am, and 01:00 pm, respectively. Two leaf porometers were calibrated before making measurements to eliminate errors due to porometer difference. These data were considered when comparing common garden

physiological responses between the two species and their hybrid. In the winter, we put

these plants back in to the walk-in cooler, and put them out again in the common garden

at the Greensville Research Farm for the physiological study in summer 2010. Four out

of five hybrid plants survived and were used for the physiological study.

GENETICS. Leaf samples (2-3 leaves per plant) were collected from five to seven

plants at a field population of *S. rotundifolia* and *S. argentea*, and from newly grown

hybrid plants. These leaf samples were dried in 28-200 mesh silica gel (Fisher Scientific,

Pittsburgh, PA). DNA was extracted with the Dneasy 96 Plant Kit (QAIGEN, Valencia,

CA). Amplified fragment length polymorphisms (AFLPs) were assayed as described by

Vos et al. (1995) with described modifications. The DNA samples were pre-amplified

EcoRI +1 / MseI +1 using A/C selective nucleotides. Selective amplification primers

consisted of five EcoRI +3 / MseI +3 primer combinations using AAC/CAA, AAG/CAG,

ACC/CAT, ACG/CTA, AGG/CTA, AGA/CCC selective nucleotides. The EcoRI

selective amplification primers included a fluorescent 6-FAM (6-carboxy fluorescein)

label on 5' nucleotides. Selective amplification products were combined with GS600 LIZ

internal lane size standard and were fractionated using an ABI 3730 instrument with 50-

cm capillaries and sized between 50 and 600 bp with Genescan software (Applied

Biosystems, Foster City, CA). Although DNA molecules vary in length by increments of

1 bp, the relative mobility of bands is also affected by sequence composition. Thus, non-

homologous bands of the same length may not have the same relative mobility. Genescan

trace files for each individual were visually analyzed for the presence or absence of DNA

bands in bins that were at least 0.3 bp or more apart using Genographer software

(available free at http://hordeum.oscs.montana.edu/genographer/ or directly from the

author, Tom Blake, at blake@hordeum.oscs.montana.edu). Bayesian clustering of

individual plants without a priori assignment of individuals to hierarchical groups was

used to determine genetic structure and test for possible admixture between taxa, which

might otherwise confound phylogenetic analysis, using Structure v2.1 (Pritchard et al.,

2000). Three analyses used of each model with 100,000 iterations and 10,000 burn-in or

200,000 iterations and 20,000 burn-in with the dominant-allele, admixture model of

Structure v2.2 (Falush et al., 2007; Pritchard et al., 2000).

MORPHOLOGY. Morphological characteristics of *S. rotundifolia* x *argentea* hybrid

compared to its parents were described on the basis of leaf morphological characteristics.

Morphological measurements included petiole length, leaf length and width, and leaf

length/leaf width on two year-old plants of the hybrid plants, which were then compared

to its parents. In addition to these leaf morphological characteristics, fine scale

morphological differences were compared through scanning electron microscope (SEM)

images. Three leaf punches per plant were collected from three plants from each species

and the hybrid, and directly fixed in formalin-aceto-alcohol (FAA) solution in the field.

The fixed leaf tissue was subjected to critical point drying using Samdri-PVT-3D

(Tousimis, Rockville, MD). Microscopic images of leaf structure were collected from the

SEM at the Nanoelectronics laboratory (USU, Logan, UT). Characteristics measured

included average leaf thickness and leaf trichome structures.

PHYSIOLOGY. Physiological responses of the hybrid in comparison to its parents

were investigated under well-watered conditions in summer 2010 on four individuals for

each parent species and their hybrid. Diurnal stomatal conductance (g_s) was measured on

three mature leaves for each individual plant, four plants from each species using a leaf

porometer (SC-1 Porometer, Decagon Device, Pullman, WA), every hour from 08:00 am-05:00 pm on a sunny day (28th June 2010).

Photosynthesis light response curves were measured on three consecutive days (30th June 2010 – 2nd July 2010) using a portable photosynthesis system (LI-6400, Li-Cor Corp., Lincoln, NE). The leaf chamber fluorometer was used as light source to vary photosynthetically active radiation (*PAR*) incident on leaf (*PAR* = 2000, 1500, 1000, 500, 200, 100, 50, 20, 0 μmol m^{-2}s^{-1}). The leaf chamber environment was maintained under the following conditions during measurements of all populations: CO_2 concentration 400 μmol mol^{-1}; airflow 500 μmol s^{-1} (Monaco et al., 2005); block temperature 24°C (somewhat variable depending on ambient temperature during measurement). Net photosynthesis (P_n) was measured between 11:00 am-01:00 pm when the intensity of solar radiation is most consistent and when stomata were most active.

From July 5th –July 26th, 2010, we measured midday (11:00 am – 01:00 pm) physiological responses of the hybrid compared to its parents. The midday measurements included P_n, quantum efficiency in photosystem II (PhiPS2), g_s, and leaf water potential (Ψ_{leaf}). The midday P_n and PhiPS2 were measured using a portable photosynthesis system (LI-6400, Li-Cor Corp., Lincoln, NE), midday g_s was measured using a leaf porometer (SC-1 Porometer, Decagon Device, Pullman, WA), and midday Ψ_{leaf} was measured using a pressure chamber. Variances in diurnal g_s and light response curve between the hybrid and its parents were subjected to a repeated measures analysis in PROC MIXED in SAS software (SAS Institute, Cary, NC). Midday measurements, including midday g_s, P_n, PhiPS2, and Ψ_{leaf} were subjected to analysis of variance (ANOVA) in PROC GLM in SAS software.

Results and Discussion

GENETICS. The AFLP genetic data support that five plants grown from seeds collected from the pollination bag on the *S. argentea* female parent are interspecific hybrids between female *S. argentea* and male *S. rotundifolia* parents. The hybrid plants exhibited intermediate genetic to its parents (Kuhlman et al., 2008). Even though there was some genetic variation, the F_1-generation of *S. rotundifolia* x *argentea* hybrid plants exhibited approximately 50% of genetic similarity to the male parent (*S. rotundifolia*), and the other 50% of genetic similarity to the female parent (*S. argentea*) on the basis of Bayesian cluster analysis (Fig. 5-1A and B). The genetic distinction of the hybrid from its parents remained consistent when the test population increased in number from two populations (K = 2) into three populations (K = 3). The intermediate genetics in the hybrid may result in intermediate morphological characteristics in the hybrid plants compared to its parents.

MORPHOLOGY. Indeed**,** overall morphological characteristics of the *S. rotundifolia* x *argentea* hybrid were intermediate to the parents (Table 5-1; Fig. 5-2). The hybrid plants appeared to be evergreen similar to the male parent, *S. rotundifolia* based on observations during winter 2010 where plants were housed in a cold frame. Leaf length and leaf width of the hybrid were intermediate to the parents, with leaf length of the hybrid more similar to the female parent, *S. argentea,* and leaf width more similar to the male parent, *S. rotundifolia,* based on ANOVA analysis. This resulted in the intermediate leaf shape in the hybrid. Leaf shape of *S. rotundifolia* was revolute-oval with approximately equally leaf length and leaf width (leaf length/width = 1.0).

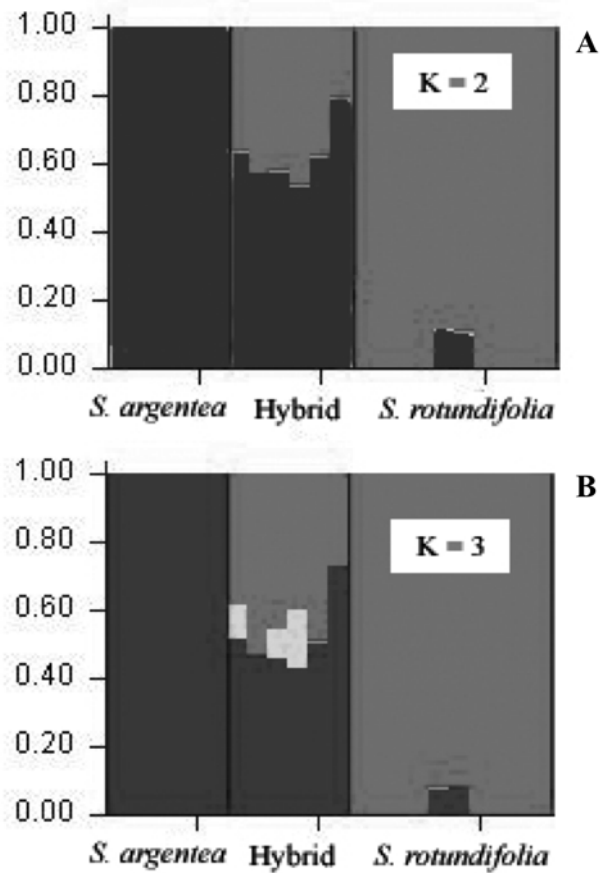Fig. 5-1. Inferred population structure of *S. rotundifolia, S. argentea,* and hybrid *S. rotundifolia* x *argentea* AFLP genotypes when; (A) testing a two population model (K = 2); and (B) testing a three population model (K = 3); a thin vertical line represents each individual, black lines separate individuals of different populations

Table 5-1. Leaf morphological characteristics of *S. rotundifolia*, *S. argentea*, and the

interspecific hybrid *S. rotundifolia* x *argentea*

	Species		
Morphology	*S. rotundifolia*[z]	*S. rotundifolia* x *argentea*	*S. argentea*
	(n = 2)	(n = 2)	(n = 2)
Petiole length (mm)	5.3 ± 0.0^b	10.5 ± 1.2^a	7.0 ± 0.0^b
Leaf length (mm)	24.0 ± 0.7^b	39.3 ± 3.3^a	42.5 ± 0.5^a
Leaf width (mm)	23.3 ± 0.3^a	24.5 ± 1.2^a	12.7 ± 0.3^b
Leaf length/width	1.0 ± 0.0^c	1.6 ± 0.1^b	3.4 ± 0.1^a

[z]Values within a column with different letters indicate statistical significance at $\alpha = 0.05$

Meanwhile leaf shape of *S. argentea* was entire-lanceolate (leaf length/width = 3.4). Leaf

shape of the hybrid plant was entire-ovate (leaf length/width = 1.6), which was

intermediate to the parents. Even though most morphological characteristics of the

hybrids appeared to be intermediate between its parents, petiole length of the hybrid was

greater than petiole length of either of the parents.

The intermediate morphological characteristic of the hybrid (Table 5-1; Fig. 5-2)

was consistent with the AFLP genetic differences, as the hybrid shared approximately

half-half of genetic similarity to its parents in the Bayesian cluster analysis (Fig. 5-1).

These results are similar to findings of Kuhlman et al. (2008) in the interspecific hybrid

of *Sorghum bicolor* x *macrospermum* and Sun et al. (2010) in the interspecific hybrid of
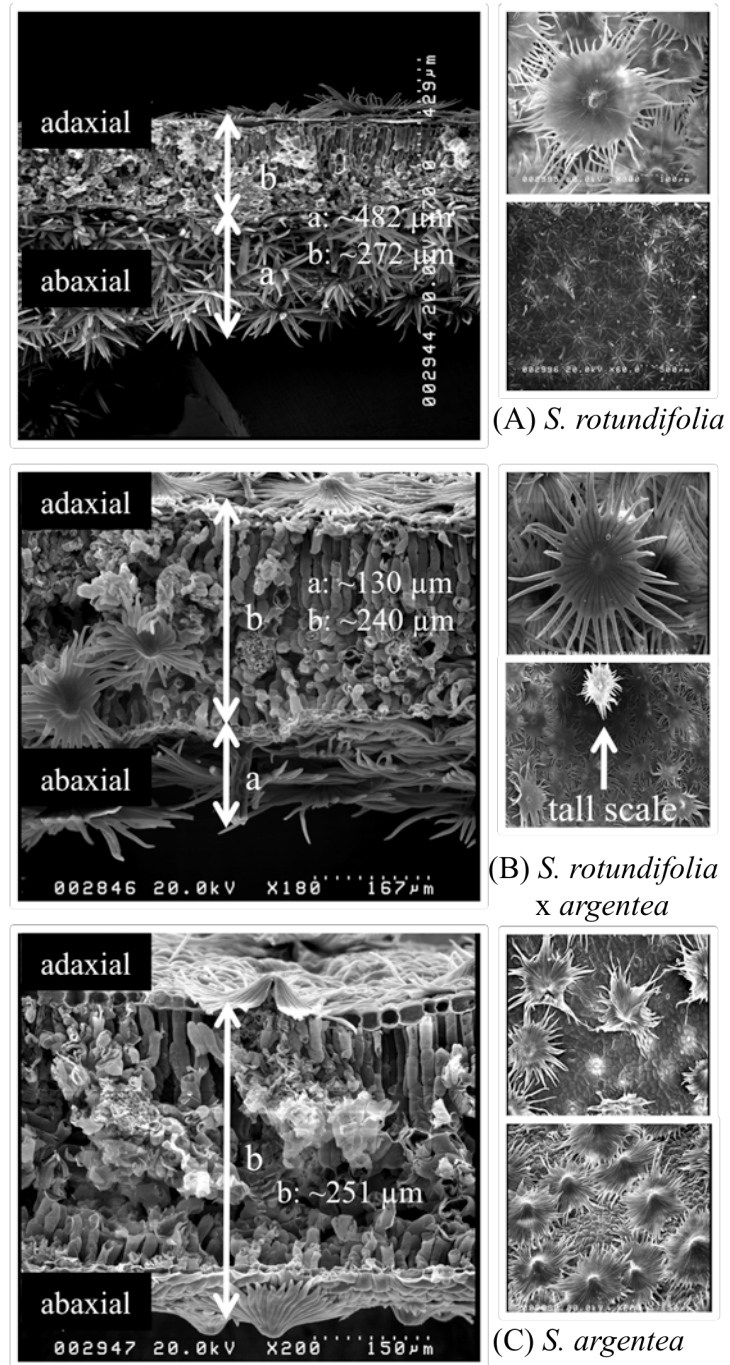
Fig. 5-2. Leaf scanning electron microscopic images (SEM) of *S. rotundifolia* (A); hybrid *S. rotundifolia* x *argentea* (B); and *S. argentea* (C)

Chrysanthemum grandiflorum x *indicum*. However, morphological traits of an interspecific hybrid can be significantly different from one or both parents (Jakesova et al., 2011; Jun et al., 2009). Morphological variation in a hybrid can result in phenotypic novelty (Scascitelli et al., 2010; Soltis and Soltis, 2009; Stelkens et al., 2009), such as theinterspecific hybrid between orchid *Ophrys arachnitiformis* and *O. lupercalis* producing more and different compounds in their floral odour, triggering fewer inspecting flights and contacts by male bees than those of the parental orchid species (Vereecken et al., 2010).

Leaf thickness of the hybrid appeared to be intermediate (~ 370 μm) to the parents; *S. rotundifolia* (~ 754 μm) and *S. argentea* (~ 251μm) (Fig. 5-2A-C). Leaf trichomes on the adaxial (upper side) of the male parent, *S. rotundifolia*, are peltate, meanwhile trichomes on the abaxial (lower side) are stellate (Fig. 5-2A). Leaf trichome of both surfaces of the female parent, *S. argentea*, are peltate (Fig. 5-2C). The female parent leaf structure appeared to dominate that of the hybrid, which had peltate trichomes on both leaf surfaces (Fig. 5-2B). However, the abaxial trichome of the hybrid was relatively thicker than *S. argentea* and there were some tall scales emerging from the peltate trichome coverage of the adaxial surface of the hybrid not observed in either parent. Emergent phenotypic features generated in the hybrid may be rare but have potential to create a new lineage and lead to evolutionary divergence (Johnston et al., 2004).

The stellate trichome on the abaxial surface of *S. rotundifolia* was densely packed, resulting in thicker leaves compared to *S. argentea* and the hybrid (Fig. 5-2A-C), likely to serve the function of reducing water loss from transpiration in hot and dried habitats

(Press, 1999). The peltate structure is an advantage in reflecting sunlight (Karabourniotis et al., 1993), but may not limit transpiration to the same extent as the stellate trichomes. Lack of the stellate trichome in *S. argentea* may be due to infrequent drought since it is typically found in riparian habitats (Mee et al., 2003). The leaf trichome structure of the hybrid was more similar to the female parents, *S. argentea*, than to the male parent, *S. rotundifolia*. This may suggest similar adaptation to wet soil conditions between the hybrid and *S. argentea*.

PHYSIOLOGY. The interspecific hybrid, *S. rotundifolia* x *argentea,* exhibited similar physiological responses to *S. argentea* in a common garden environment under well watered conditions through stomatal opening (Fig. 5-3). Even though diurnal stomatal conductance was not significantly different among the hybrid and its parents (F-value = 1.76, P = 0.20), *S. rotundifolia* appeared to have the relatively lowest dawn to dusk stomatal conductance compared to the hybrid and *S. argentea*. *Shepherdia argentea* and the hybrid appeared to have a more aggressive water use strategy, close to anisohydric, characteristic of species adapted to a regularly available water supply (Franks et al., 2007).

The differential between g_s of *S. rotundifolia* (grown from cuttings) and *S. argentea* (grown from seeds) in the common garden study (Fig. 5-3) was similar to the in situ stomatal conductance measurement of the two species. Average in situ g_s of *S. rotundifolia* at 09:00 am, 11:00 am, and 01:00 pm, were 138, 249, and 216 mmol $m^{-2} s^{-1}$, respectively. Meanwhile, average g_s of *S. argentea* at 09:00 am, 11:00 am, and 01:00 pm, were 284, 263, and 207 mmol $m^{-2} s^{-1}$, respectively. In both in situ and common garden
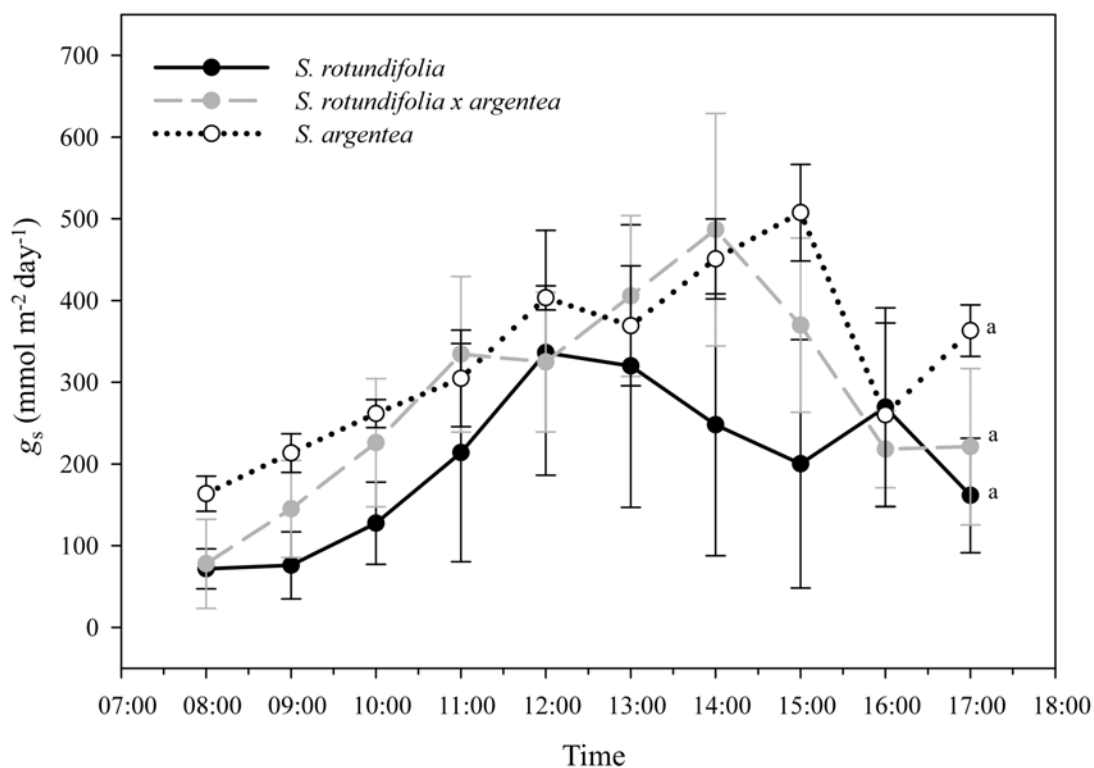
Fig. 5-3. Diurnal stomatal conductance (g_s) of *S. rotundifolia*, *S. argentea*, and the interspecific hybrid *S. rotundifolia* x *argentea*

measurements, g_s of *S. argentea* were higher than g_s of *S. rotundifolia* in the morning (at 09:00 am), then at 11:00 am to 01:00 pm, g_s of both species were close to each other. The similar pattern of g_s of both species between the measurements in a common garden and in situ suggests that the use of *S. rotundifolia* plants grown from cuttings did not appear to have effects of age-difference to compare physiological responses with plants of *S. argentea* and the hybrids grown from seeds.

Overall P_n in response to different levels of *PAR* of the hybrid was relatively higher than its parents, even though P_n among species was not significantly different (F-value = 0.39, P = 0.69) (Fig. 5-4). The light-independent reaction of *S. rotundifolia*, which is adapted to very limited water supply in its dry habitat has reached their maximum (light saturation point) at lower light level. The light response curve of *S. argentea*, which is adapted to regular water supply in its riparian habitat exhibited relatively higher P_n at high light level, than P_n of *S. rotundifolia* but lower than P_n of the hybrid. A study by Montgomery and Givnish (2008) on Hawaiian lobeliad species, whose habit ranges from open alpine bogs to densely shaded rainforest interiors, demonstrated the potential adaptive nature of species differences. They found that P_n in responses to different light regimes among lobeliad species appeared to maximize in its native environments. In this study, P_n in responses to different light levels of both *S. rotundifolia* and *S. argentea* were measured under well-watered conditions and open to full sun conditions, which more similar to native environments of *S. argentea*. The higher light response curve of *S. argentea* than *S. rotundifolia* may similar to finding of Montgomery and Givnish (2008) in lobeliad species. Therefore, the more similar light response curve of the hybrid to *S. argentea* may suggest the efficiency to adapt to a regular water supply, as in irrigated urban landscapes. The relatively higher P_n and g_s in the hybrid than its parents is similar to findings of Rieger et al. (2003) in interspecific hybrids between *Prunus ferganesis* and *P. persica*.

Overall midday physiological responses in terms of P_n and quantum efficiency (PhiPS2) of the hybrid exhibited responses closer to those of *S. argentea* than to *S. rotundifolia* under well water conditions over this study (Fig. 5-5A and B). Plants of *S.*
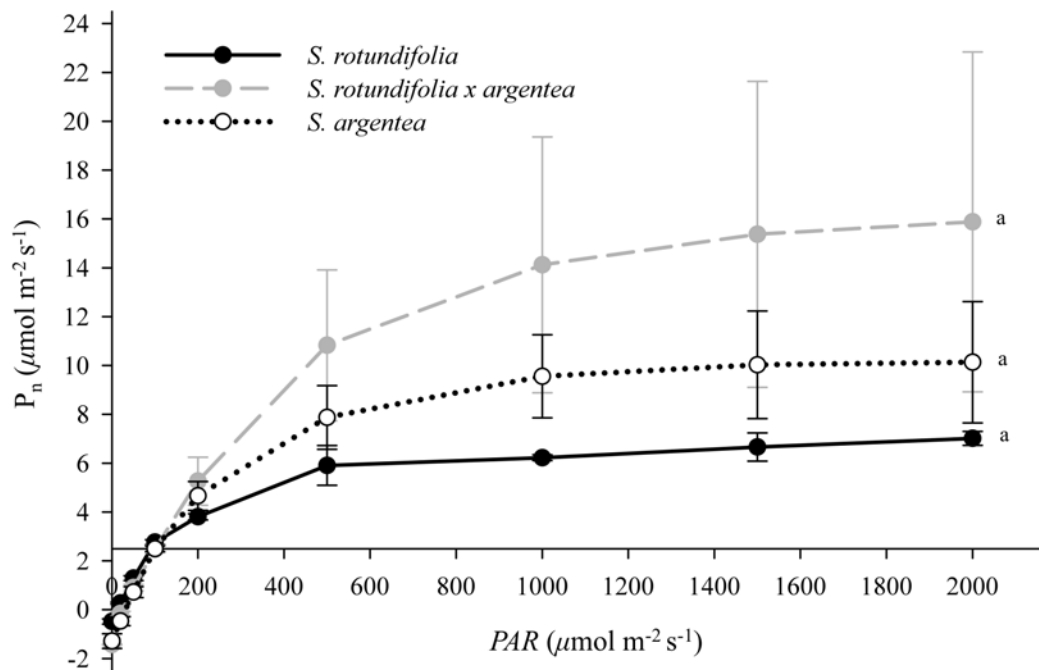
Fig. 5-4. Light response curve of *S. rotundifolia*, *S. argentea*, and the interspecific hybrid

   S. rotundifolia x *argentea*

rotundifolia exhibited lowest midday P_n and PhiPS2 and were consistent with light

response curve (Fig. 5-4). Plants of *S. rotundifolia* naturally occurred in hot and dry

habitats, thus transpiration regulation is critical in order to tolerate such drought

conditions. Drought stress inhibits P_n by decreased stomatal aperture (Cornic, 2000) in *S.*

rotundifolia. The relatively thick abaxial trichome layer (Fig. 5-2A) helps to prevent

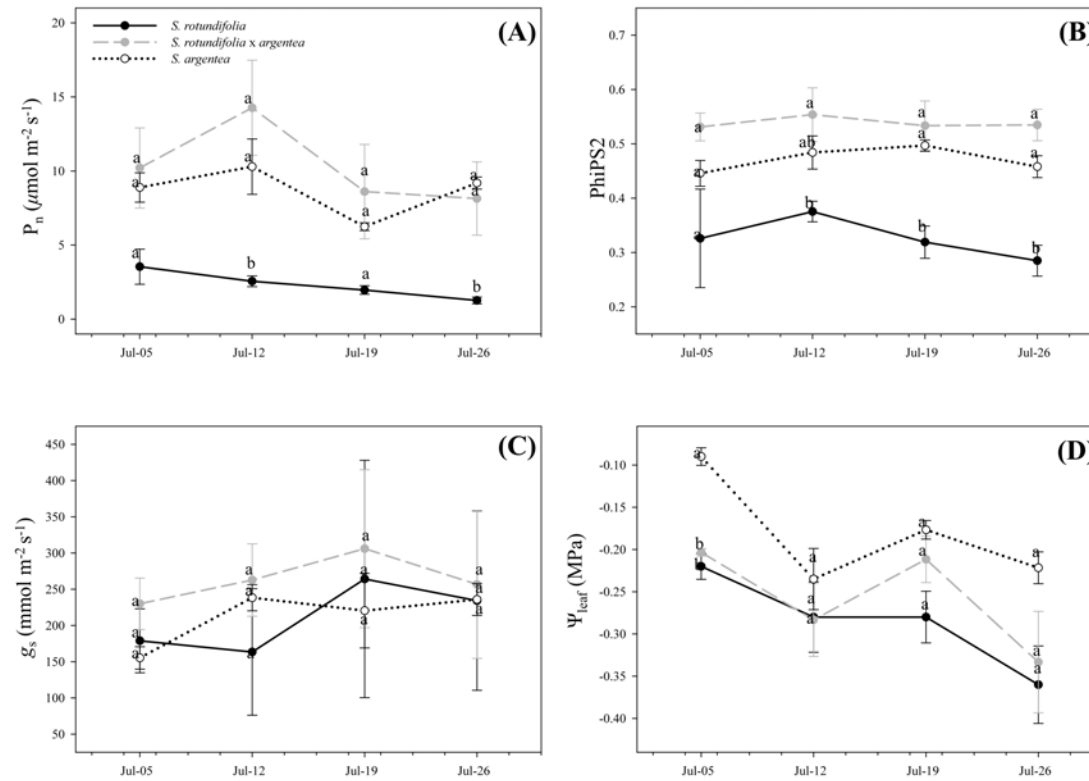water loss through transpiration. The low midday Ψ_{leaf} in *S. rotu ndifolia* may suggest

Fig. 55. Midday physiological responses of *S. rotundifolia*, *S. argentea*, and the interspecific hybrid *S. rotundifolia* x

argentea; (A) midday photosynthesis (P_n), (B) midday quantum efficiency of photosystem 2 (PhiPS2), (C) midday

stomatal conductance (g_s), and (D) leaf water potential (Ψ_{leaf})

high leaf hydraulic resistance to conserve water by limiting transpiration rates under high evaporative conditions, which associate with drought tolerance (Sinclair et al., 2008). Even though overall midday g_s and Ψ_{leaf} of the hybrid were not significantly different (at significant level $\alpha = 0.05$) from its parents (Fig. 5-5C and D), its diurnal g_s, light response curve, midday P_n, and midday PhiPS2 were more similar to those of *S. argentea* and may suggest greater tolerance to wet soil condition than *S. rotundifolia*. The interspecific hybrid, *S. rotundifolia* x *argentea* obtained genetic and morphological characteristics from both parents. Physiological responses under well water conditions of the hybrid appeared to be more similar to responses of *S. argentea* than *S. rotundifolia*, suggesting it may be more adapted to wet soil conditions. The aesthetic morphological characteristics and physiological responses of the hybrid may lend them well to use in LWL. A study on physiological responses of the hybrid to drought conditions compared to its parents may be necessary before use of the hybrid under drought conditions in urban landscapes.

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CHAPTER 6

CONCLUSION

Evaluation of ecophysiological, morphological, and genetic distinctions within the two IMW native plant genera containing species with high urban low-water landscape potential suggests genetic variation is important in developing superior selections of both *Sphaeralcea* and *Shepherdia* genera. Within the genus *Sphaeralcea*, even though the four putative species appeared to be only two pure types; *S. coccinea* and *S. parvifolia*, there is genetic variation among populations of the type *S. parvifolia* correlated with geographical distribution. Meanwhile, within the genus *Shepherdia*, the high elevation populations were genetically different from the low elevation populations. The genetic variation among populations of *S. rotundifolia* may underlie variations in terms of morphological characteristics and ecophysiological responses to variable environments in their native habitats.

Genetic improvement by hybridization may be an alternative to developing native plant species for use in the LWL. The interspecific hybrid, *S. rotundifolia* x *argentea*, appeared to obtain aesthetic qualities, specifically leaf morphological characteristics from both parents. Physiological responses of the hybrid under high water availability compared to its parents may also suggest superior adaptation, and lend them well to use in the LWL.

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