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Phenotypic and Genetic Characterization of Wildland Collections of Western and Searls Prairie Clovers for Rangeland Revegetation in the Western USA

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PHENOTYPIC AND GENETIC CHARACTERIZATION OF WILDLAND COLLECTIONS OF WESTERN AND SEARLS PRAIRIE CLOVERS FOR RANGELAND REVEGETATION IN THE WESTERN USA

by

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ABSTRACT

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Western prairie clover [*Dalea ornata* (Douglas ex Hook.) Eaton & J. Wright] is a perennial legume that occurs in the northern Great Basin, Snake River Basin, and southern Columbia Plateau, whereas Searls prairie clover [*Dalea searlsiae* (A. Gray) Barneby], also a perennial legume, occurs in the southern Great Basin and surrounding areas. Understanding the genetic and ecotypic variation of these prairie clovers is a prerequisite for developing populations suitable for rangeland revegetation in the western USA. DNA sequences of internal transcribed spacer (ITS/5.8S) and *trn*K/*mat*K were used to study the phylogeny of these species. The species were distinguished by DNA sequences from both regions and conserved haplotypes were observed between and within species. Common-garden plots of 22 collections of western prairie clover from Idaho, Oregon, and Washington and 20 collections of Searls prairie clover from Utah and

Nevada were established in northern Utah for phenotypic evaluation. Significant variation was detected among the collections for all traits measured in the common gardens for both species. Flowering date was correlated with collection-site temperature and elevation in western prairie clover collections, whereas biomass-related traits were closely related with collection-site precipitation in Searls prairie clover. Population structure from amplified fragment length polymorphism (AFLP) markers resulted in two distinct, genetically differentiated groups and a third admixed group in western prairie clover, and flowering date played a significant role in discriminating those genetic-based groupings of collections. For western prairie clover, two populations are recommended for development, one from the Deschutes River watershed and another from the remaining collections. For Searls prairie clover, two genetically different groups of collections were identified from southern Utah and eastern Nevada and from northwestern Utah. Three western Nevada collections exhibited close association with eastern Nevada and southern Utah groups for AFLP-markers but with collections from northwestern Utah for phenotypic traits. Strong isolation by distance was observed for Searls prairie clover collections suggesting that genetic drift and gene flow are major factors for determining population structure in this species. As a result, two regional seed sources should be developed for Searls prairie clover, one from northwestern Utah and the other from eastern Nevada and southern Utah.

This dissertation is dedicated to the loving memories of my brother

Kiran Bhattarai

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CHAPTER 1

BACKGROUND

The objectives of my research were to evaluate phenotypic and DNA-marker variation in 22 and 20 wildland collections of western prairie clover [*Dalea ornata* (Douglas ex Hook.) Eaton & J. Wright] and Searls prairie clover [*D. searlsiae* (A. Gray) Barneby], respectively. In my research, I tested the following four null hypotheses:

1. the DNA sequences of the maturase region of lysine gene (*trn*K/*mat*K) in the chloroplast genome and internal transcribed spacer region (ITS/5.8S) of ribosomal DNA in the nuclear genome do not differ between the two prairie clover species
2. phenotypic measurements and DNA markers do not differ among collections within both prairie clover species
3. genetic structure differences do not exist within populations of each species
4. genetic differences are not correlated with phenotypic and geographic differences.

Phenotypic measurements were assessed for forage production and quality, flowering date, inflorescence weight, and growth habit in two common gardens, and DNA marker variation was estimated using amplified fragment length polymorphisms (AFLP). The results of this research can be used to corroborate phylogenetic relationships between these two prairie clovers and identify regional seed sources for each species, which if used could minimize outbreeding depression and maladaptation when these species are used for revegetation/restoration programs, yet provide a tractable number of seed sources for germplasm development.

About 400 million ha (42%) of the total land area in the USA are grazed (Bovey 1987), out of which 177 million ha are used as public rangelands in the western USA (Baca 1995). The Great Basin lies in the Intermountain Region of the western USA and is located between the Rocky Mountains to the east and the Sierra-Nevada and Cascade Mountains to the west. About 60% of the Great Basin is public land that is managed by the Bureau of Land Management (BLM 2008). Sagebrush steppe and more arid semi-desert ecosystems cover an estimated total land area of 40 million ha in the Great Basin (MacMahon 1979). Vegetation in the Great Basin ranges from salt desert shrub, sage brush, and pinyon-juniper in valley zones to coniferous forest and subalpine/ alpine vegetation in montane zones. The Great Basin is a semiarid system where about 14% of the land receives less than 120 mm and about 50% of the land receives less than 230 mm of precipitation each year (Wernstedt 1960). Winter minimum temperature reaches below 0 °C in almost the entire region, whereas summer maximum temperature reaches above 38 °C in most of the region, with some localities in the Great Basin reaching temperatures above 49 °C (Wernstedt 1960).

Changes in disturbance regimes since European settlement have resulted in concomitant changes in floristic composition in western rangelands of the USA with many diverse native plant communities now dominated by invasive weeds (Miller et al. 1994). Increases in exotic annuals such as *Bromus tectorum* L. were reported as a cause of increased fire frequency (Whisenant 1990), which has also decreased native perennials. A large portion of the Great Basin burns each year (4.97×10^5 ha, average from 1998 to 2007; Mike Pellant, personal communication) and if left untreated, weed invasions will likely accelerate (Jessop and Anderson 2007). Post-fire treatment in the

Great Basin requires large amounts of seed for revegetation (BLM seed purchase in 2007 exceeded 3 X 10^6 kg), and a mix of species is preferred in seed mixtures because functionally diverse plant species are more likely to reduce weed invasion risk and increase ecosystem function (Pokorny et al. 2005; Sheley and Carpinelli 2005; Walker and Shaw 2005).

With an increased emphasis on the restoration of ecosystem integrity on western rangelands of the USA (Roundy et al. 1995), demand for native plant species for revegetation and restoration projects has increased (Richards et al. 1998). In this context, the Great Basin Native Plant Selection and Increase Project was initiated in 2000 with funding through the Bureau of Land Management and the Forest Service's Rocky Mountain Research Station. The goal of this project is to increase the availability and success of Great Basin native plants, especially forbs, in restoring native plant communities. The incorporation of legumes into pasture and rangeland ecosystems is a sustainable approach that can increase soil N levels, improve forage N content (Haynes 1980; Legard and Steele 1992; Posler et al. 1993; Aydin and Uzun 2005), improve wildlife forage and habitat (Madison and Robel 2001), and increase biodiversity. Legumes can increase productivity of non-leguminous neighbors by releasing symbiotically fixed N (Hogh-Jensen and Schjoerring 2000). Similarly, van der Heijden et al. (2006) documented that *Lotus corniculatus* L., *Ononis repens* L., and *Trifolium repens* L. increased productivity of associated grasses and forbs by reducing the competition for limited soil N. Because legumes contain more protein and less fiber than grasses at similar stages of growth (Cherney and Allen 1995), they provide high quality forage to herbivores. Native legume seed often costs more because of the lack of

commercial seed production, which is a major reason for their limited use in revegetation programs (Walker and Shaw 2005). The USDA Forest Service's National Seed Laboratory has identified 19 legume species native to the Great Basin for use in rangeland reclamation projects. Their priority list includes basalt milkvetch (*Astragalus filipes* Torr. ex A. Gray), Utah milkvetch [*Astragalus utahensis* (Torr.) Torr. & A. Gray], western prairie clover, Utah sweetvetch (*Hedysarum boreale* Nutt.), lupine (*Lupinus* L.), and silvery lupine (*Lupinus argenteus* Pursh).

Dalea L. is a widespread genus of the legume family (Fabaceae) in North America. Sixty-two species of *Dalea* have been reported from North America (USDA 2008), of which five species have been reported from the Great Basin. These include: white prairie clover (*D. candida* Michx. ex Willd.), Canyonlands prairie clover [*D. flavescens* (S. Watson) S.L. Welsh], wooly prairie clover (*D. lanata* Spreng.), western prairie clover, and Searls prairie clover. Specific to this project, western prairie clover is reported to occur in northeastern California, northwestern Nevada, Oregon, Idaho and southern Washington, whereas Searls prairie clover has a more southern distribution and found in southeastern California, Nevada, Utah, and northwestern Arizona. *Dalea* has been separated as a genus in Amorphae within the Fabaceae based on two collateral ovules and a basic chromosome complement of seven or (rarely) eight (Barneby 1977). Historically, there was a confusion concerning whether western prairie clover and Searls prairie clover may be one species (Barney 1977). However, Barneby (1977) argued that phylogenetic data based on morphological characteristics warranted their separation as two different species. No molecular data have been collected concerning these two species, which could provide insightful information for phylogenetic study (McMahon

and Hufford 2004). Genes encoded in the chloroplast DNA and nuclear internal transcribed spacer (ITS) region of ribosomal DNA are popular DNA sequences for studying phylogenetic relationships within legume genera (Hu et al. 2000; Lavin et al. 2001; McMahon and Hufford 2004).

Common-garden studies of phenotype and DNA-marker studies are complementary tools to unravel the roles of various evolutionary forces that determine population structure of a species (McKay and Latta 2002; Ouborg et al. 2006). Common-garden studies are limited by the choice of phenotypic traits measured and the phenotypic plasticity of certain genotypes. Similarly, DNA marker analysis primarily represents putatively neutral traits and is, therefore, a questionable tool for identifying adaptive zones. For example, neutral DNA markers (SSRs) did not detect variation among 12 naturalized alfalfa (*Medicago sativa* L.) populations collected from Manitoba, Canada and 10 alfalfa cultivars, whereas phenotypic traits measured in a common garden showed differences among populations indicating the role of divergent selection in those populations (Bagavathiannan et al. 2010). Therefore, reciprocal transplant studies are recommended to detect adaptive traits in species; however, comparing many collections in reciprocal transplant studies is very costly and intractable. One way to identify potential adaptive traits is to correlate phenotypic traits measured in common gardens with collection site environmental variables (Endler 1986), which is a common practice in forestry (Johnson et al. 2004).

Common-garden plots provide the same environment to evaluate phenotypic variation, such that the differences among populations are primarily due to genetic differences and/or the interaction between genetics and the environment. Because size,

fecundity, and morphology are important quantitative traits, variation in these traits when measured in common-garden studies represent quantitative genetic variation as well as phenotypic plasticity. In my studies, I measured dry-matter yield (DMY), inflorescence weight, number of stems and inflorescences, plant height, foliage diameter, forage quality traits [acid detergent fiber (ADF), neutral detergent fiber (NDF), crude protein (CP)], and flowering date in two common-garden locations for both prairie clover species. Inflorescence weight was measured as a surrogate for potential seed yield because the indeterminate growth of inflorescences combined with scattering of mature seeds from inflorescences by wind made it difficult to harvest seeds of these species.

Upright plant growth and potential seed yield are desirable traits for commercial seed production, whereas greater DMY is desirable when one of the revegetation goals is to provide forage for livestock and wildlife. Reproductive potential determines the probability of plant recruitment (Harper 1979) and is thus an important trait in evaluating species for revegetation/restoration programs. High forage quality is a desirable trait for livestock grazing. According to Buxton and Mertens (1995), forage quality is a function of nutrient concentration, intake or rate of consumption, digestibility, and proportion of metabolized product remaining within the animal. Forage quality is often estimated by in vitro or chemical methods because these techniques are less expensive and easier to conduct than animal evaluations. The Great Basin has a semiarid climate with most of the precipitation occurring as snow or early spring rain. As a result, early emergence is critical for a plant to utilize available water from snowmelt and early spring precipitation, which otherwise would be limited later in the season.

Various DNA marker techniques have been developed and used to study genetic diversity in plants (Nybom 2004), one of which is amplified fragment length polymorphism (AFLP). The AFLP technique is a dominant marker technique that requires no previous DNA sequence information yet produces robust and accurate results (Schlötterer 2004; Bonin et al. 2007). In brief, the AFLP technique involves the digestion of genomic DNA with endonucleases, one frequent cutter with a six-base-pair sequence recognition (common: *Mse*I) and the other a rare cutter with a four-base-pair sequence recognition (common: *Eco*RI) (Vos et al. 1995). A pair of PCR primers is used to selectively amplify DNA fragments that are complementary to adaptors and have additional three nucleotides for selective amplification. As such, the AFLP technique is able to amplify 1/64 of the total DNA fragments generated by two endonucleases. DNA fragments are visualized using electrophoresis, and similarities among individuals and groups are assessed based on the presence and absence of DNA fragments.

Although AFLP markers are commonly used to assess genetic diversity and define population structures in plants (Meudt and Clarke 2007), few AFLP-based studies, or other DNA marker analyses, have been conducted on rangeland legumes. Examples of such studies involved Utah sweetvetch and basalt milkvetch (Bushman et al. 2007; Bushman et al. 2010). AFLP markers were able to detect population structure for both species, as both species have a wide range of geographic distribution. Because gene flow barriers, drift, selection, founder effect, range expansion/contraction, and adaptation are responsible for population structure in a species, distinct population structures are expected to occur in a species that is found across a wide geographical range.

DNA marker variation primarily reflects the effects of gene flow and genetic drift (McKay and Latta 2002). Functional connectivity through gene flow is critical for species existence in a fragmented habitat (Fischer and Lindenmayer 2007). Some studies have identified that gene flow constrains adaptive divergence of populations due to its homogenizing effect (Stanton and Galen 1997; Sambatti and Rice 2006), whereas other studies have found that gene flow actually increases adaptive divergence by counteracting inbreeding depression in small populations (Hedrick 1995; Swindell and Bouzat 2006). These studies indicate that the role of gene flow in adaptive divergence of populations depends on the selection regime and population size. When selection pressure is high, gene flow cannot swamp adaptive genes because non-adaptive genes are selected against. When population size is small, the probability of inbreeding increases, which decreases genetic variability. Gene flow can counteract inbreeding depression and increase genetic diversity, which in turn increases the likelihood of adaptation to changing environments. In addition, gene flow provides beneficial mutations and adaptive genes among populations (Peck et al. 1998) or it can break co-adapted gene complexes (McKay et al. 2005) and thus can interfere with adaptive divergence of populations.

The phrase “isolation by distance” (IBD) was first used by Wright (1943) who suggested that genetic variation within a species increased as the spatial distance among its populations increased due to restricted gene flow. When the distribution of populations is larger than the gene flow capability, IBD can occur (Johnson and Black 1998). The IBD is usually stronger for populations that are at equilibrium for drift and gene flow, which may take considerable time to achieve (Slatkin 1993). Occasional long

distance dispersal of seeds can establish isolation by distance in a species. In such cases low genetic diversity would be expected in peripheral populations. IBD has been observed in many plant species (Raspe and Jacquemart 1998; Stöcklin et al. 2009); however, several studies failed to detect this relationship in many other plant species (Franceschinelli and Kesseli 1999; Albaladejo et al. 2009). The failure to detect IBD in naturally occurring populations can be due to interactions between environmental factors and a non-equilibrium state between genetic drift and gene flow (Kittlein and Gaggiotti 2008).

Loss of species dispersal corridors due to habitat loss and fragmentation in an isolated population can make the population genetically depauperate (Aguilar et al. 2008). Such isolated populations, therefore, have a lower evolutionary potential (Rice and Emery 2003) unless some form of gene flow is maintained. Assisted migration can mimic natural dispersal of species in severely fragmented landscapes and thus help to increase the chance of survival of populations in rapidly changing environments by providing new genetic materials for adaptation (Vitt et al. 2010). In the meantime, assisted migration has associated risks such as unintended hybridization, maladaptation and outbreeding depression. When a non-adapted population is introduced to a novel environment, restoration/revegetation programs will probably fail. The challenge for assisted migration is to ensure that population fitness does not decrease either with maladaptation and outbreeding depression, otherwise species may not evolve as an invasive species in a new environment (Vitt et al. 2010).

Anthropogenic induced rapid climatic change and habitat loss and fragmentation have threatened the existence and evolutionary potential of many species (Ouborg et al.

2006). Plant species respond to climate change by acclimatization through phenological and/or physiological adjustments, adaptation due to selection through generations, migration of populations to suitable habitats, or simply extinction (Davis and Shaw 2001). Acclimatization is genotype specific because genotypes vary for their ecological amplitudes that operate within specific boundaries. Adaptation can be limited by the amount of genetic material available for selection to act upon (Barrett and Schluter 2007). Sources of new genetic material for a population are: gene flow from immigration of new individuals and genetic materials, new allelic combinations by cross overs, and mutation. Short-lived plants species (such as annuals) have a greater chance to evolve in rapidly changing environments because of a fast generation turnover; however, populations of long-lived species (such as big trees and perennials) can have difficulty in adapting to rapidly changing environments without greater phenotypic plasticity, migration, and gene flow. As both western and Searls prairie clover species are perennials, assisted migration may be more critical for maintaining their evolutionary potential in the context of fragmented habitat and climate change.

Although local plant materials are considered best for restoration (McKay et al. 2005), "local is best" may not hold true in the context of highly fragmented habitats and rapidly changing climate (Broadhurst et al. 2008). In addition, having too many unwarranted local populations of a species for revegetation programs will likely increase the cost of seed. Genetic similarity among local populations and climatic similarity in their habitats can be used as a foundation to pool groups of "local" collections, thereby increasing the likelihood of the success of revegetation programs by allowing for lower priced seeds and appropriate genetic materials for a site. Seed transfer zones (STZ) are

meta-population boundaries of a species within which plant materials can be transferred with minimal risk of maladaptation and outbreeding depression (Bower et al. 2010). Ideally STZs for a species should encompass geographic areas of similar temperature, precipitation, elevation, soil, community composition, and historical gene flow because these factors are responsible for genetic structure. However, this approach defines a large number of STZs, which may be impractical for commercial seed production.

Seed transfer zones are also species specific due to gene flow variation in each species when climatic, topographic, and edaphic variables are equal. Gene flow can be based on species breeding system, dispersal mechanism, and life history. Because it is relatively easy to obtain data for precipitation, elevation, and temperature, many proposed STZs exist for plants (Cathey 1990; Vogel et al. 2005), sometimes standardized for two categories: (1) trees/shrubs/woody plants, and (2) forbs/grasses (Bower et al. 2010). Use of these STZs are recommended when genetic information about adaptive traits are not available (Bower et al. 2010), but these generalized STZs are likely not be the best for individual species use. Indeed, species may respond to different environmental extremes, and thus their population boundary may be constrained by different factors.

Having too many unwarranted STZs for a species will likely increase seed cost for revegetation/restoration. On the other hand, having seed transfer zones spread across different adaptive zones may cause maladaptation and outbreeding depression (McKay et al. 2005), which may lead to revegetation/restoration failure. Therefore, delineation of STZs based on phenotypic traits and DNA markers within a species may represent a feasible strategy for recommending plant species for revegetation/restoration programs.

Results of this research can provide information for delineating STZs for western and Searls prairie clover species.

LITERATURE CITED

Aguilar, R., M. Quesada, L. Ashworth, Y. Herrerias-Diego, and J. Lobo. 2008. Genetic consequences of habitat fragmentation in plant populations: susceptible signals in plant traits and methodological approaches. *Molecular Ecology* 17:5177-5188.

Albaladejo, R. G., L. F. Carrillo, A. Aparicio, J. F. Fernández-Manjarrés and J. P. González-Varo. 2009. Population genetic structure in *Myrtus communis* L. in a chronically fragmented landscape in the Mediterranean: can gene flow counteract habitat perturbation? *Plant Biology* 11:442-453.

Aydin, I., and F. Uzun. 2005. Nitrogen and phosphorus fertilization of rangelands affects yield, forage quality and the botanical composition. *European Journal of Agronomy* 23:8-14.

Baca, S. V. 1995. Managing rangelands for an improved environment and for production of food, fiber, recreation, and aesthetic values. *In*: N. E. West [ed.]. Proceedings-rangelands in a sustainable biosphere. Denver, CO: Society for Range Management. p. 3-4.

Bagavathiannan, M. V., B. Julier, P. Barre, R. H. Gulden, and R. C. Van Acker. 2010. Genetic diversity of feral alfalfa (*Medicago sativa* L.) populations occurring in Manitoba, Canada and comparison with alfalfa cultivars: an analysis using SSR markers and phenotypic traits. *Euphytica* 173:419-432.

Barneby, R. C. 1977. *Dale*a imagines. *Memoirs of the New York Botanical Garden* 27:1-892.

Barrett, R. D. H., and D. Schluter. 2007. Adaptation from standing genetic variation. *Trends in Ecology and Evolution* 23:38-44.

Bonin, A., D. Ehrich, and S. Manel. 2007. Statistical analysis of amplified fragment length polymorphism data: a toolbox for molecular ecologists and evolutionists. *Molecular Ecology* 16:3737-3758.

Bovey, R. W. 1987. Weed control problems, approaches, and opportunities in rangeland. *Review of Weed Science* 3:57-91.

Bower, A., B. St. Clair, and V. Erickson. 2010. Generalized provisional seed transfer zones for native plants. Available at: http://www.nativeseed.info/presentations/Bower.pdf. Accessed 10 August 2010.

Broadhurst, L. M., A. Lowe, D. J. Coates, A. A Cunningham, M. McDonald, P. A. Vesk, and C. Yates. 2008. Seed supply for broadscale restoration: maximizing evolutionary potential. *Evolutionary Application* 1:587-597.

Bureau of Land Management [BLM]. 2008. The Great Basin. Available at: http://www.blm.gov/nifc/st/en/prog/fire/more/gb. Accessed 4 April 2008.

Bushman, B. S, K. Bhattarai, and D. A. Johnson. 2010. Population structures of *Astragalus filipes* collections from western North America. *Botany* 88:565-574.

Bushman, B. S., S. L. Larson, M. D. Peel, and M. E. Pfrender. 2007. Population structure and genetic diversity in North American *Hedysarum boreale* Nutt. *Crop Science* 47:1281-1286.

Buxton, D. R., and D. R. Mertens. 1995. Quality-related characteristics of forages. *In*: R. F. Barnes, D. A. Miller, and C. J. Nelson [eds.]. Forages: the science of grassland agriculture. Volume II. Ames, IA: Iowa State University Press. p. 83-96.

Cathey, H. M. 1990. USDA plant hardiness zone map. [map] Washington, DC.: U.S. Department of Agriculture. USDA Miscellaneous Publication No. 1475. Available at: http://www.usna.usda.gov/Hardzone/ushzmap.html. Accessed 12 May 2010.

Cherney, J. H., and V. G. Allen. 1995. Forages in a livestock system. *In*: R. F. Barnes, D. A. Miller, and C. J. Nelson [eds.]. Forages: the science of grassland agriculture. Volume I. Ames, IA, USA: Iowa State University Press. p. 175-188.

Davis, M. B., and R. G. Shaw. 2001. Range shifts and adaptive responses to quaternary climate change. Science 292:673-679.

Endler, J. A. 1986. Natural selection in the wild. Princeton, NJ, USA: Princeton University Press. 358 p.

Fischer, J., and D. B. Lindenmayer. 2007. Landscape modification and habitat fragmentation: a synthesis. *Global Ecology and Biogeography* 16:265-280.

Franceschinelli, E. V., and R. Kesseli. 1999. Population structure and gene flow of the Brazilian shrub *Helicteres brevispira*. *Heredity* 82:355-363.

Harper, K. T. 1979. Some reproductive and life history characteristics of rare plants and implications for management. The endangered species: a symposium. *Great Basin Naturalist Memoirs* 3:129-137.

Haynes, R. J. 1980. Competitive aspects of the grass-legume association. *Advances in Agronomy* 33:227-261.

Hedrick, P. W. 1995. Gene flow and genetic restoration-the Florida panther as a case-study. *Conservation Biology* 9**:**996-1007.

Hogh-Jensen, H., and J. K. Schjoerring. 2000. Below-ground nitrogen transfer between different grassland species: direct quantification by N-15 leaf feeding compared with indirect dilution of soil N-15. *Plant and Soil* 227:171–183.

Hu, J.-M., M. Lavin, M. Wojciechowski, and M. J. Sanderson. 2000. Phylogenetic systematics of the tribe Millettieae (Leguminosae) based on chloroplast *trnK*/*matK* sequences, and its implications for evolutionary patterns in Papilionoideae. *American Journal of Botany* 87:418-430.

Jessop, B. D., and V. J. Anderson. 2007. Cheatgrass invasion in salt desert shrublands: benefits of postfire reclamation. *Rangeland Ecology and Management* 60:235-243.

Johnson, G. R., F. C. Sorensen, J. B. St. Clair, and R. C. Cronn. 2004. Pacific Northwest forest tree seed zones: a template for native plants? *Native Plant Journal* 5:131-140.

Johnson, M. S., and R. Black. 1998. Effects of isolation by distance and geographical discontinuity on genetic subdivision of *Littoraria cingulata. Marine Biology* 132:295–303.

Kittlein, M. J., and O. E. Gaggiotti. 2008. Interactions between environmental factors can hide isolation by distance patterns: a case study of *Ctenomys rionegrensis* in Uruguay. *Proceedings of the Royal Society B* 275:2633-2638.

Lavin, M., R. T. Pennington, B. B. Klitgaard, J. I. Sprent, H. C. De Lima, and P. E. Gasson. 2001. The dalbergioid legumes (Fabaceae): delimitation of a pantropical monophyletic clade. *American Journal of Botany* 88:503–533.

Legard, S. F., and K. W. Steele. 1992. Biological nitrogen fixation in mixed legume/grass pastures. *Plant and Soil* 14:137-153.

MacMahon, J. A. 1979. North American deserts: Their floral and faunal components. *In*: D. W. Goodall and R. A. Perry [eds.]. Arid-land ecosystems: structure, functioning, and management. Cambridge, United Kingdom: Cambridge University Press. p. 21-82.

Madison, L. A., and R. J. Robel. 2001. Energy characteristics and consumption of several seeds recommended for northern bobwhite food plantings. *Wildlife Society Bulletin* 29:1219-1227.

McKay, J. K., C. E. Christian, S. P. Harrison, and K. J. Rice. 2005. "How local is local?" - A review of practical and conceptual issues in the genetics of restoration. *Restoration Ecology* 1:432-40.

McKay, J. K., and G. Latta. 2002. Adaptive population divergence: markers, QTL and traits. *Trends in Ecology and Evolution* 17:185-291.

McMahon, M., and L. Hufford. 2004. Phylogeny of Amorphae (Fabaceae: Papilionoideae). *American Journal of Botany* 91:1219-1230.

Meudt, H. M., and A. C. Clarke. 2007. Almost forgotten or latest practice? AFLP applications, analyses, and advances. *Trends in Plant Science* 12:106-117.

Miller, R. F., T. J. Svejcar, and N. E. West. 1994. Implications of livestock grazing in the Intermountain sagebrush region: Plant composition. *In*: M. Vavra, W. A.

Laycock, and R. D. Pieper [eds.]. Ecological implications of livestock herbivory in the West. Denver, CO: Society for Range Management. p. 101-146.

Nybom, H. 2004. Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. *Molecular Ecology* 13:1143-1155.

Ouborg, N. J., P. Vergeer, and C. Mix. 2006. The rough edges of the conservation genetics paradigm for plants. *Journal of Ecology* 94:1233-1248.

Peck, S. L., S. P. Ellner, and F. Gould. 1998. A spatially explicit stochastic model demonstrates the feasibility of Wright's shifting balance theory. *Evolution* 52**:**1834-1839.

Pokorny, M. L., R. L. Sheley, C. A. Zabinski, R. E. Engel, T. J. Svejcar, and J. J. Brokowski. 2005. Plant functional group diversity as a mechanism for invasion resistance. *Restoration Ecology* 13:448-459.

Posler, G. L., A. W. Lenssen, and G. L. Fine. 1993. Forage yield, quality, compatibility and persistence of warm-season grass-legume mixtures. *Agronomy Journal* 85:554-560.

Raspe, O., and A. L. Jacquemart. 1998. Allozyme diversity and genetic structure of European populations of *Sorbus aucuparia* L. (Rosaceae: Maloideae). *Heredity* 81:537-545.

Rice, K. J., and N. C. Emery. 2003. Managing microevolution: restoration in the face of global change. *Frontiers in Ecology and the Environment* 1:469-478.

Richards, R. T., J. C. Chambers, and C. Ross. 1998. Use of native plants on Federal lands: policy and practice. *Journal of Range Management* 51:625-632.

Roundy, B. A., E. D. McArthur, J. S. Haley, and D. K. Mann. [eds.]. 1995. Proceedings of the wildland shrub and arid land restoration symposium. Ogden, UT: USDA Forest Service, Gen. Rep. INT-GTR-315. 384 p.

Sambatti, J. B. M., and K. J. Rice. 2006. Local adaptation, patterns of selection, and gene flow in the Californian serpentine sunflower (*Helianthus exilis*). *Evolution* 60:696-710.

Schlötterer, C. 2004. The evolution of molecular markers - just a matter of fashion? *Nature Reviews Genetics* 5:63-69.

Sheley, R. L., and M. F. Carpinelli. 2005. Creating weed-resistant plant communities using niche-differentiated nonnative species. *Rangeland Ecology and Management* 58:480-488.

Slatkin, M. 1993. Isolation by distance in equilibrium and non-equilibrium populations. *Evolution* 47:264-279.

Stanton, M. L., and C. Galen. 1997. Life on the edge: adaptation versus environmentally mediated gene flow in the snow buttercup, *Ranunculus adoneus*. *American Naturalist* 150:143-178.

Stöcklin, J., P. Kuss, and A. R. Pluess. 2009. Genetic diversity, phenotypic variation and local adaptation in the alpine landscape: case studies with alpine plant species. *Botanica Helvetica* 119:125-133.

Swindell, W. R., and J. L. Bouzat. 2006. Gene flow and adaptive potential in *Drosophila melanogaster*. *Conservation Genetics* 7:79-89.

USDA, NRCS. 2008. The plants database. National Plant Data Center, Baton Rouge, LA, USA. Available at: http://plants.usda.gov/java/profile?symbol=DALEA. Accessed 30 November 2009.

van der Heijden, M. G. A., R. Bakker, J. Verwaal, T. R. Scheublin, M. Rutten, R. van Logtestijn, and C. Staehelin. 2006. Symbiotic bacteria as a determinant of plant community structure and plant productivity in dune grassland. *FEMS Microbiology Ecology* 56:178-187.

Vitt, P., K. Havens, A. T. Kramer, D. Sollenberger, and E. Yates. 2010. Assisted migration of plants: changes in latitudes, changes in attitudes. *Biological Conservation* 143:18-27.

Vogel, K. P., M. R Schmer, and R. B. Mitchell. 2005. Plant adaptation regions: ecological and climatic classification of plant materials. *Rangeland Ecology and Management* 58:315-319.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, and M. Kuiper. 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research* 23:4407-4414.

Walker, S. C., and N. L. Shaw. 2005. Current and potential use of broadleaf herbs for reestablishing native communities. *In*: N. L. Shaw, M. Pellant, and S. B. Monsen [eds.]. Proceedings, Sage Grouse Habitat Restoration Symposium. Fort Collins, CO, USA: USDA Forest Service, Rocky Mountain Research Station. Proceedings RMRS-P-38. p. 46-61.

Wernstedt, F. L. 1960. Climatic fluctuations in the Great Basin, 1931-56. *Journal of Range Management* 4:173-178.

Whisenant, S. G. 1990. Changing fire frequencies on Idaho's Snake River Plains: Ecological and management implications. *In*: E. D. McArthur, E. M. Romney, S. D. Smith, and P. T. Tueller [eds.]. Proceedings, Symposium on Cheatgrass Invasion, Shrub Die-off, and Other Aspects of Shrub Biology and Management. Ogden, UT: USDA Forest Service, Gen. Tech. Rep. INT-276. p. 4-7.

Wright, S. 1943. Isolation by distance. *Genetics* 28:114-138.

CHAPTER 2

CHARACTERIZATION OF ITS/5.8S SEQUENCE AND *trn*K/*mat*K SEQUENCE FOR PHYLOGENY AND BIOGEOGRAPHY OF WESTERN AND SEARLS PRAIRIE CLOVERS

ABSTRACT

Historically there was a controversy regarding western prairie clover [*Dalea ornata* (Douglas ex Hook.) Eaton & J. Wright] and Searls prairie clover [*Dalea searlsiae* (A. Gray) Barneby] should be treated as one or two species. Although they are currently treated as two species based on morphological data, no genotypic-based phylogenetic data are available for these groups. Internal transcribed spacer (ITS) of ribosomal DNA (rDNA) in nuclear genome and maturase coding gene (*mat*K) located within the intron of the chloroplast lysine gene (*trn*K) were used to study the phylogeny of western and Searls prairie clovers. The *trn*K/*mat*K of the chloroplast region separated both groups in a maximum parsimony tree with 99% of bootstrap support, whereas ITS/5.8S of rDNA separated both groups in a maximum parsimony tree with a weak (59%) bootstrap support. Conserved haplotypes were observed between and within both groups, suggesting gene flow restriction might have played an important role in their speciation, as both groups occur geographically apart. Based on the conserved DNA polymorphic sequences, species-specific diagnostic primers were designed for both *trn*K/*mat*K region of the chloroplast genome and ITS/5.8S region of the ribosomal DNA.

INTRODUCTION

Western prairie clover [*Dalea ornata* (Douglas ex Hook.) Eaton & J. Wright] and Searls prairie clover [*Dalea searlsiae* (A. Gray) Barneby] are perennial forbs in the legume family Fabaceae. They grow 25 to 55 cm tall with many stout and erect stems. Both prairie clovers are diploid (n = 7, or rarely 8) and are primarily insect-pollinated (Barneby 1977; Jim Cane 2009, personal communication). Historically, taxonomists have disagreed whether or not western and Searls prairie clovers are two distinct species (Barneby 1977). Barneby (1977) argued that phylogenetic data based on morphological characteristics warranted separating these taxa as two different species.

Barneby (1977) characterized Searls prairie clover as a species of loose and narrow spikes (without petals 8 to 11 mm in diameter) with loosely arranged flowers having partly visible floral axis. He characterized western prairie clover as a species of dense and conelike spikes (without petals 13 to 16 mm in diameter) with compactly arranged flowers having invisible floral axes. Western prairie clover was reported to be coarsely leafy with glabrous stems, whereas Searls prairie clover was leafy with less than 1/2 to 1/3 of its stems bearing axillary leafy spurs at most nodes and commonly glabrous stems with ciliolate stipules (Barneby 1977). Western prairie clover occurs in the Columbia-Snake River Basin and northern Great Basin and is primarily found on soft clay and sandy soils derived from weathered of basalt and volcanic ash, whereas Searls prairie clover occurs on calciphile soils and sometimes on sandstones and other bedrocks in the southern portion of Nevada, Utah, and adjoining California and Arizona (Barneby 1977).

The identification of diversity and relatedness of biological samples by use of molecular markers has been a common practice for the last few decades. First generation molecular markers included protein-based allozymes and random amplifying nucleic acid based DNA markers. With innovations in the polymerase chain reaction (PCR) and new sequencing technologies, second generation markers were developed that comprised microsatellite markers (also called Simple Sequence Repeats or SSRs) (Varshney et al. 2005), amplified fragment length polymorphisms (AFLPs) (Bonin et al. 2007), and single nucleotide polymorphisms (SNPs). These markers showed greater capacity, accuracy, and affordability, and their use expanded the number of reports of molecular biological diversity and relatedness of biological samples. Although these marker methods have proven invaluable for estimating variation within species, they are often too polymorphic for deeper phylogenetic inference.

Chloroplast DNA sequences are commonly used in taxonomic and phylogenetic studies because of their lack of recombination, maternal inheritance, ability to be sequenced from poor quality herbarium tissues, and their ability to be sequenced regardless of ploidy levels. The *mat*K gene is more informative than other chloroplast genes for similar numbers of nucleotides sequenced because of its greater variability and differing patterns of molecular evolution that result in comparatively low levels of homoplasy (Hilu et al. 2003). The maturase coding (*mat*K) gene is situated within the group II intron of the tRNA gene for lysine (*trn*K) and encodes a maturase enzyme that is involved in splicing out introns (Neuhaus and Link 1987). Some studies have documented the role of *mat*K gene in splicing other introns (Liere and Link 1995; Vogel et al. 1997). Compared to other protein coding genes, *mat*K also deviates by a much

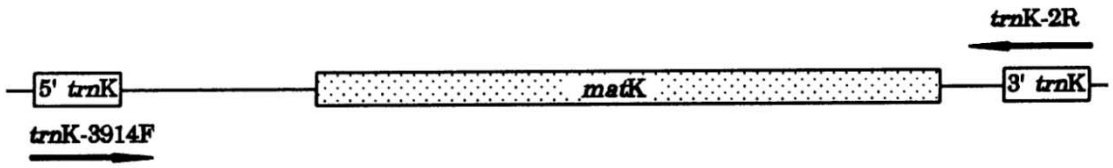

Figure 2-1. A diagram of chloroplast *trn*K showing *mat*K as a shaded box and non-shaded boxes are two primer positions that are used to amplify DNA samples in PCR. Introns are shown by solid lines. Diagram is not drawn to scale.

greater number of non-synonymous mutations and a higher frequency of indels (Olmstead and Palmer 1994; Johnson and Soltis 1995; Hilu and Liang 1997; Müller and Borsch 2005). The *trn*K intron with *mat*K gene was found to be informative for phylogenetic study of papilionoid genera, tribes, and in some cases species and Amorphae genera (Hu et al. 2000; Lavin et al. 2001; McMahon and Hufford 2004).

An internal transcribed spacer (ITS) is a non-translatable segment of an rRNA transcript that is found both in prokaryotic and eukaryotic genomes. In Eukaryotic rRNA transcript, there are two ITS regions, ITS1 and ITS2. ITS1 is flanked by 18S and 5.8S ribosomal genes, and ITS2 is flanked by 5.8S and 28S ribosomal genes. By using 5.8S as a universal primer bridge, ITS can be amplified into two smaller fragments of ITS1 and ITS2, which is advantageous for studying degraded samples (Kress et al. 2005). Some additional advantages of the ITS regions as a DNA barcode include simplicity, universality, high copy numbers, biparental inheritance, and greater mutational accumulation because of its neutrality. Consequently, the ITS region has been widely used for studying phylogenetic relationships among closely related individuals such as among species in a genus (Baldwin et al. 1995; Wojciechowski et al. 1999; Sha et al.

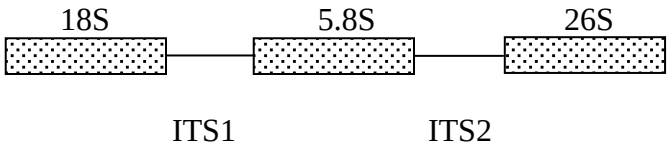

Figure 2-2. A partial diagram of eukaryotic rDNA showing given exons as shaded boxes, which are not drawn to scale. Internal transcribed spacers are shown by solid lines and are drawn approximately to scale.

2008). However, the inheritance and sequence homogenization of the ITS regions are still unclear, such that precise interpretation of ITS sequence-based clustering is not always possible (Wendel et al. 1995).

To address the phylogenetic question and estimate the amount of diversity among western and Searls prairie clover at the inter-species level, the ITS/5.8S and *mat*K genes were sequenced using congeneric markers (McMahon and Hufford 2004). In this study, I tested the hypothesis that unique DNA polymorphisms occur in the ITS/5.8S and *trn*K/*mat*K gene of western prairie clover and Searls prairie clover, which can be used to compare their relatedness. Further, haplotype diversity and distribution within each species were assessed to determine if unique haplotypes exist and if they exhibit a localized geographical pattern. Geographically local haplotypes are indicative of recent mutation, whereas geographically widespread haplotypes are of ancient mutations (Templeton et al. 1995; Schaal et al. 1998). A set of diagnostic markers have been shown among the two prairie clovers that take advantage of conserved DNA polymorphisms between them.

MATERIALS AND METHODS

Plant Materials

Twenty two seed collections of western prairie clover and 20 seed collections of Searls prairie clover were used for this study. Western prairie clover collections were from Idaho, Oregon, and Utah; Searls prairie clover collections were from Utah and Nevada (Fig. 2-3). Collected seeds were germinated and seedlings were grown in a greenhouse at the USDA-ARS Forage and Range Research Laboratory, Logan, UT under a 30/15ºC day/night temperature regime. Plant tissues were collected from the shoot apical region. Tissues were lyophilized and stored at -20ºC before use. Three samples for each collection were used for ITS/5.8S DNA sequencing, and one sample for each collection was used for *mat*K gene sequencing of western and Searls prairie clover.

DNA Extraction and Amplification

Approximately 17 mg of dried plant tissue were used for each sample, and DNA was extracted using the Qiagen DNeasy 96-well procedure (Qiagen, Valencia, CA) following the manufacturer's protocol. The concentration and quality of genomic DNA were tested spectrophotometrically and by agarose gel electrophoresis. The genomic DNA concentration was adjusted to 30 ng/µL.

Two primers, ITS-5a (Stanford et al. 2000) and ITS-4 (Baldwin 1993) were used to amplify and sequence the ITS/5.8S region (Table 2-1). JumpStart Taq DNA polymerase (Sigma, St. Louis, MO) and Platinum High-Fidelity polymerase (Invitrogen, Carlsband, CA) were used to amplify DNA in a PCR reaction of 35 cycles conditioned at

Table 2-1. Base composition and source of ITS/5.8S and trnK/matK primers used for the study. Amplicon length was obtained for two consecutive forward (F) and reverse (R) primer pairs given in the table.

Primer name	5' Sequence 3'	Source	Amplicon length
ITS-5a (F)	CCTTATCATTTAGAGGAAGGGAG	Stanford et al. 2000	
ITS-4 (R)	TCCTCCGCTTATTGATATGC	Baldwin 1993	705
trnk-3914F	TGGGTTGCTAACTCAATGG	Johnson and Soltis 1994	
matK820R	AATTCGATTTGGTCAAAGGA	McMahon and Hufford 2004	709-724
matK710F	GTATCGCACTATGTWTCATTTGA	Johnson and Soltis 1995	
matK-1470R	AAGATGTTGATYGTAAATGA	Johnson and Soltis 1994	742
matK1221F	GATGTACAAATACCCTATCC	McMahon and Hufford 2004	
matK-2340R	GAAGAAGCTCTTGGAAAGATC	McMahon and Hufford 2004	944
trnK-2R†	AACTAGTCGGATGGAGTAG	Johnson and Soltis 1994	

†Only used to amplify DNA fragment prior to sequencing reaction.

94°C for 30 s, 58°C for 30 s, and 72°C for 60 s, with a final extension at 72°C for 7 min. Similarly, two primers were used to amplify the *trn*K region of the chloroplast, 3194 as a forward primer and *trn*K2R as a reverse primer (Table 2-1) with 2 min. of extension time at 72°C for each PCR cycle. Subsequent PCRs were done with other closely located *trn*K primer pairs, 3194F-820R, 710F-1470R, and 1221F-2340R (Table 2-1).

Sequencing PCR

The PCR products were purified prior to sequencing with MinElute 96 UF PCR purification (Qiagen, Valencia, CA). Sequencing PCR was done sequentially by using each primer used for DNA amplification (except *trn*K2R). Sequencing reactions used BigDye version 3.1 (Applied Biosystems, Foster City, CA) in a PCR reaction of 45 cycles conditioned at 94°C for 5 s, 50°C for 10 s, and 60°C for 4 min. Subsequent dye terminator removal was done with Performa v3 96-well plates (Edge Biosystems, Gaithersburg, MD). Sequencing was done using an ABI 3730 capillary instrument (Applied Biosystems, Foster City, CA) at CIB (Logan, UT).

Sequence Alignment and Analyses

Individual sequences were checked and trimmed for quality before aligning them using Sequencher software (Gene Codes, Ann Arbor, MI). Eighty-five individuals were retained for the ITS/5.8S, and 42 individuals were retained for *trn*K/*mat*K analyses. The three primer pair amplified regions of the *trn*K/*mat*K were combined into one contiguous sequence. Heuristic searches were conducted to obtain a parsimony tree for the ITS/5.8S and the *trn*K/*mat*K derived trees in PAUP version 4.0b10 (Swofford 2002). Parsimony analysis was done on the sequences with TBR branch swapping and 100 permutations in

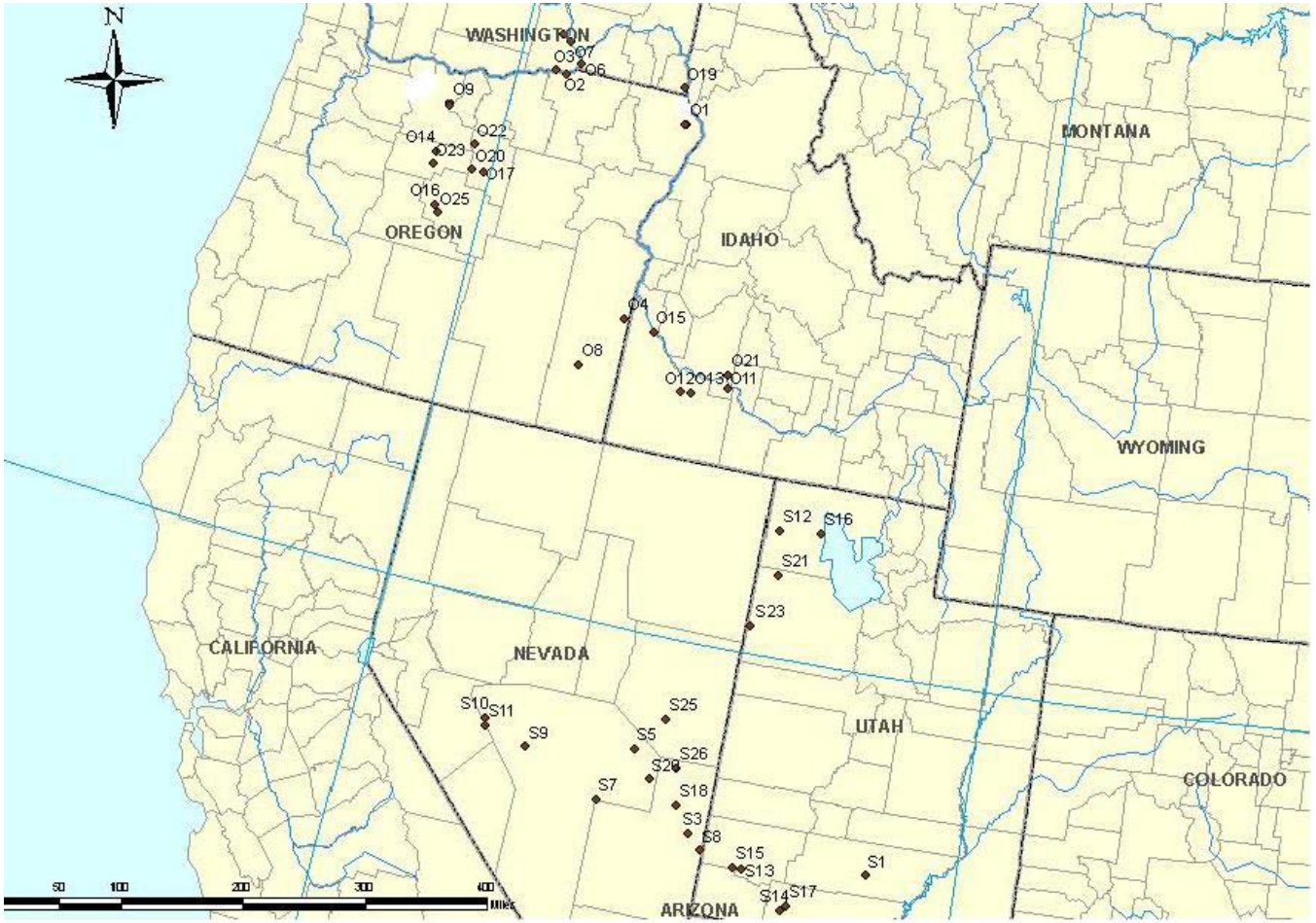

Figure 2-3. Seed collection sites showing western prairie clover collections (indicated by a letter ‘O’ with a collection number) and Searls prairie clover collections (indicated by a letter ‘S’ with a collection number).

PAUP. Diagnostic markers were designed using Primer-Blast software available at NCBI website (NCBI, Bethesda, MD).

RESULTS

Chloroplast *trn*K/*mat*K

A DNA fragment of length 2161 bp was obtained for the *trn*K/*mat*K region, out of which 40 (1.9%) characters were variable and parsimoniously informative. Thirty-nine of the 40 variable characteristics were SNPs. An indel of six base pairs (5'-TTCGAT-3') was observed at position 557 to 562, which is most probably a duplication of the DNA segment of position 551 to 556. When western and Searls prairie clovers were compared for variable characteristics, all Searls prairie clover collections lacked the six base pair indel. In addition, two transitional mutations were observed in western and Searls prairie clovers at positions 215 (A and G) and 475 (G and A) (Table 2-2), and two transversional mutations were observed in both groups at positions 253 (T and G) and 2,110 ('C' and 'T'). Consequently, out of 45 variable characters, 10 characters showed discriminating power between western and Searls prairie clovers. The remaining 35 variable characters were SNPs distributed across these two taxa.

Within each taxon, two SNPs exhibited geographical patterns in western prairie clover collections, and three SNPs exhibited geographical patterns in Searls prairie clover collections. A base pair 'T' was deleted from position 480 in western prairie clover collections from Idaho, Washington, and eastern Oregon compared to the collections from north central Oregon and the Do-1 collection from eastern Oregon. A transitional mutation was observed at position 1,785 between north central Oregon with base 'C' and

the remaining collections of western prairie clover collections with base ‘T’. In Searls prairie clover collections, northern Utah collections exhibited a transition mutation with ‘A’ base compared to the other remaining collections of ‘G’ base at position 1,473. Similarly, four Searls prairie clover collections (Ds-5, Ds-7, Ds-20, and Ds-26) exhibited two base pairs ‘TT’ deletion from their sequence at positions 481 and 482, whereas northern Utah collections, Ds-9 and Ds-3, exhibited one base pair ‘T’ addition at position 482. Ds-14 exhibited a unique 7 base pair insertion (5’-TTATATA-3’) at position 648-654. Remaining variable characteristics were randomly distributed across geographic area of collections and across both western and Searls prairie clover collections.

The *trn*K/*mat*K sequence based parsimony tree separated western and Searls prairie clovers into different clades with a 100% bootstrap value (Fig. 2-4), supporting the two taxa as two distinct species. Within Searls prairie clover, four collections (Ds-12, Ds-16, Ds-21, and Ds-23) from northwestern Utah were separated as a unique clade with bootstrap support of 64%. The two most western collections from Nevada (Ds-10 and Ds-11) were identified as a unique clade with 62% bootstrap support, which later linked with one eastern Nevada collection Ds-18 to form a clade with 63% bootstrap support. Two southern Utah collections Ds-13 and Ds-17 exhibited a clade with 64% bootstrap support. Within western prairie clover collections, eight collections (Do-9, Do-14, Do-16, Do-17, Do-20, Do-22, Do-23, and Do-25) from north central Oregon formed a separate clade with 63% bootstrap support. Within the north central Oregon clade, three collections (Do-14, Do-23, and Do-25) from the Deschutes River watershed formed a clade with 64% bootstrap support (Fig. 2-4).

Based on the conserved indel segments, three primers have been proposed as

Table 2-2. DNA sequence polymorphisms of western and Searls prairie clover collections observed in *trn*K/*mat*K region for the given locations.

	148	215	253	429	468	480	481	482	557	558	559	560	561	562	648	649	650	651	652	653	654	745	754	757	760	762	764	880	1215	1419	1431	1471	1473	1475	1493	1752	1785	1810	1835	1890	1974	1984	1995	2110	2132
DO_1	G	A	T	G	A	T	-	-	T	T	C	G	A	T	-	-	-	-	-	-	-	T	-	G	G	A	A	G	T	T	T	-	G	G	T	G	T	G	G	C	G	G	A	C	T
DO_2	G	A	T	G	A	-	-	-	T	T	C	G	A	T	-	-	-	-	-	-	-	T	A	G	G	A	A	G	T	T	T	-	G	G	T	G	T	G	G	C	G	G	A	C	T
DO_3	G	A	T	G	A	-	-	-	T	T	C	G	A	T	-	-	-	-	-	-	-	T	-	G	G	A	A	G	T	T	T	-	G	G	T	G	T	G	G	C	G	G	A	C	T
DO_4	G	A	T	G	A	-	-	-	T	T	C	G	A	T	-	-	-	-	-	T	A	T	-	G	G	A	A	G	T	T	T	-	G	G	T	G	T	G	G	C	G	G	A	C	T
DO_5	G	A	T	G	A	-	-	-	T	T	C	G	A	T	-	-	-	-	-	-	-	T	-	G	G	A	A	G	T	T	T	-	G	G	T	G	T	G	G	C	G	G	A	C	T
DO_6	G	A	T	G	A	-	-	-	T	T	C	G	A	T	-	-	-	-	-	-	-	T	-	G	G	A	A	G	T	T	T	-	G	G	T	G	T	G	G	C	G	G	A	C	T
DO_7	G	A	T	G	A	-	-	-	T	T	C	G	A	T	-	-	-	-	-	-	-	T	-	G	G	A	A	G	T	T	T	-	G	G	T	G	T	G	G	C	G	G	A	C	T
DO_8	G	A	T	G	A	-	-	-	T	T	C	G	A	T	-	-	-	-	-	-	-	T	-	G	G	A	A	G	T	T	T	-	G	G	T	G	T	G	G	C	G	G	A	C	T
DO_9	G	A	T	G	A	T	-	-	T	T	C	G	A	T	-	-	-	-	-	-	-	T	-	G	G	A	A	G	T	T	T	-	G	G	T	G	C	G	G	C	G	G	A	C	T
DO_11	G	A	T	G	A	-	-	-	T	T	C	G	A	T	-	-	-	-	-	-	-	T	-	G	G	A	A	G	T	T	T	-	G	G	T	G	T	G	G	C	G	G	A	C	T
DO_12	G	A	T	G	A	-	-	-	T	T	C	G	A	T	-	-	-	-	-	-	-	T	A	G	G	A	A	G	T	T	T	-	G	G	T	G	T	G	G	C	G	G	A	C	T
DO_13	G	A	T	G	A	-	-	-	T	T	C	G	A	T	-	-	-	-	-	-	-	T	A	G	G	A	A	G	T	T	T	-	G	G	T	G	T	G	G	C	G	G	A	C	T
DO_14	G	A	T	G	A	T	-	-	T	T	C	G	A	T	-	-	-	-	-	-	-	T	-	G	G	A	A	G	T	C	T	-	G	G	T	G	C	G	G	C	G	G	A	C	T
DO_15	G	A	T	G	A	-	-	-	T	T	C	G	A	T	-	-	-	-	-	-	-	T	-	G	G	A	A	C	T	T	T	-	G	G	T	G	T	G	G	C	G	G	A	C	T
DO_16	G	A	T	G	A	-	-	-	T	T	C	G	A	T	-	-	-	-	-	-	-	T	-	G	G	A	A	G	C	T	T	-	G	G	T	G	C	G	G	C	G	G	A	C	T
DO_17	G	A	T	G	A	T	-	-	T	T	C	G	A	T	-	-	-	-	-	-	-	T	-	G	G	A	A	G	T	T	T	-	G	G	T	G	C	G	G	C	G	G	A	C	T
DO_19	G	A	T	G	A	-	-	-	T	T	C	G	A	T	-	-	-	-	-	-	-	T	-	G	G	A	A	G	T	T	T	-	G	G	T	G	T	G	G	C	G	G	A	C	T
DO_20	G	A	T	G	A	T	-	-	T	T	C	G	A	T	-	-	-	-	-	-	-	-	A	-	A	T	C	G	T	T	T	-	G	G	T	G	C	G	G	C	G	G	A	C	T
DO_21	G	A	T	G	A	-	-	-	T	T	C	G	A	T	-	-	-	-	-	-	-	T	-	G	G	A	A	G	T	T	T	-	G	G	T	G	T	G	G	C	G	G	A	C	T
DO_22	G	A	T	G	C	T	-	-	T	T	C	G	A	T	-	-	-	-	-	-	-	T	-	G	G	A	A	G	T	T	T	-	G	G	T	G	C	G	G	C	G	G	A	C	C
DO_23	G	A	T	G	A	T	-	-	T	T	C	G	A	T	-	-	-	-	-	-	-	T	-	G	G	A	A	G	T	C	T	-	G	G	T	G	C	G	G	C	G	G	A	C	T
DO_25	G	A	T	G	A	T	-	-	T	T	C	G	A	T	-	-	-	-	-	-	-	T	-	G	G	A	A	G	T	C	T	-	G	G	T	G	C	G	G	C	G	G	A	C	T
DS_1	G	G	G	G	A	T	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	A	G	G	A	A	G	T	T	C	-	G	A	T	G	T	G	G	C	G	G	A	T	T
DS_3	T	G	G	G	A	T	T	T	-	-	-	-	-	-	-	-	-	-	-	-	-	T	A	G	G	A	A	G	T	T	C	-	G	A	T	G	T	G	G	C	G	G	A	T	T
DS_5	G	G	G	G	A	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	A	G	G	A	A	G	T	T	C	-	G	A	T	G	T	G	G	C	G	G	A	T	T
DS_7	G	G	G	G	A	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	A	G	G	A	A	G	T	T	C	-	G	A	T	G	T	A	G	C	G	G	A	T	T
DS_8	G	G	G	G	A	T	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	A	G	G	A	A	G	T	T	C	-	G	A	T	G	T	G	G	C	G	G	A	T	T
DS_9	G	G	G	G	A	T	T	T	-	-	-	-	-	-	-	-	-	-	-	-	-	T	A	G	G	A	A	G	T	T	C	-	G	A	T	T	T	G	G	C	G	G	A	T	T
DS_10	G	G	G	A	A	T	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	A	G	G	A	A	G	T	T	C	-	G	A	C	G	T	G	G	C	G	T	A	T	T
DS_12	G	G	G	G	A	T	T	T	-	-	-	-	-	-	-	-	-	-	-	-	-	T	A	G	G	A	A	G	T	T	C	-	A	A	T	G	T	G	G	C	G	G	A	T	T
DS_11	G	G	G	A	A	T	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	A	G	G	A	A	G	T	T	C	-	G	A	C	G	T	G	G	C	G	G	A	T	T
DS_13	G	G	G	G	A	T	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	A	G	G	A	A	G	T	T	C	-	G	A	T	G	T	G	A	C	G	G	A	T	T
DS_14	G	G	G	G	A	T	T	-	-	-	-	-	-	T	T	A	T	A	T	A	T	A	G	G	G	A	A	G	T	T	C	-	G	A	T	G	T	G	G	C	G	G	A	T	T
DS_15	G	G	G	G	A	T	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	A	G	G	A	A	G	T	T	C	-	G	A	T	G	T	G	G	C	T	G	A	T	T
DS_16	G	G	G	G	A	T	T	T	-	-	-	-	-	-	-	-	-	-	-	-	-	T	A	G	G	A	A	G	T	T	C	T	A	A	T	G	T	G	G	C	G	G	A	T	T
DS_17	G	G	G	G	A	T	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	A	G	G	A	A	G	T	T	C	-	G	A	T	G	T	G	A	C	G	G	A	T	T
DS_18	G	G	G	G	A	T	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	A	G	G	A	A	G	T	T	C	-	G	A	C	G	T	G	G	C	G	G	A	T	T
DS_20	G	G	G	G	A	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	A	G	G	A	A	G	T	T	C	-	G	A	T	G	T	G	G	C	G	G	A	T	T
DS_21	G	G	G	G	A	T	T	T	-	-	-	-	-	-	-	-	-	-	-	-	-	T	A	G	G	A	A	G	T	T	C	-	A	A	T	G	T	G	G	C	G	G	G	T	T
DS_23	G	G	G	G	A	T	T	T	-	-	-	-	-	-	-	-	-	-	-	-	-	T	A	G	G	A	A	G	T	T	C	-	A	A	T	G	T	G	G	C	G	G	A	T	T
DS_25	-	-	-	G	A	T	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	A	G	G	A	A	G	T	T	C	-	G	A	T	G	T	G	G	C	G	G	A	T	T
DS_26	G	G	G	G	A	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	A	G	G	A	A	G	T	T	C	-	G	A	T	G	T	G	G	C	G	G	A	T	T

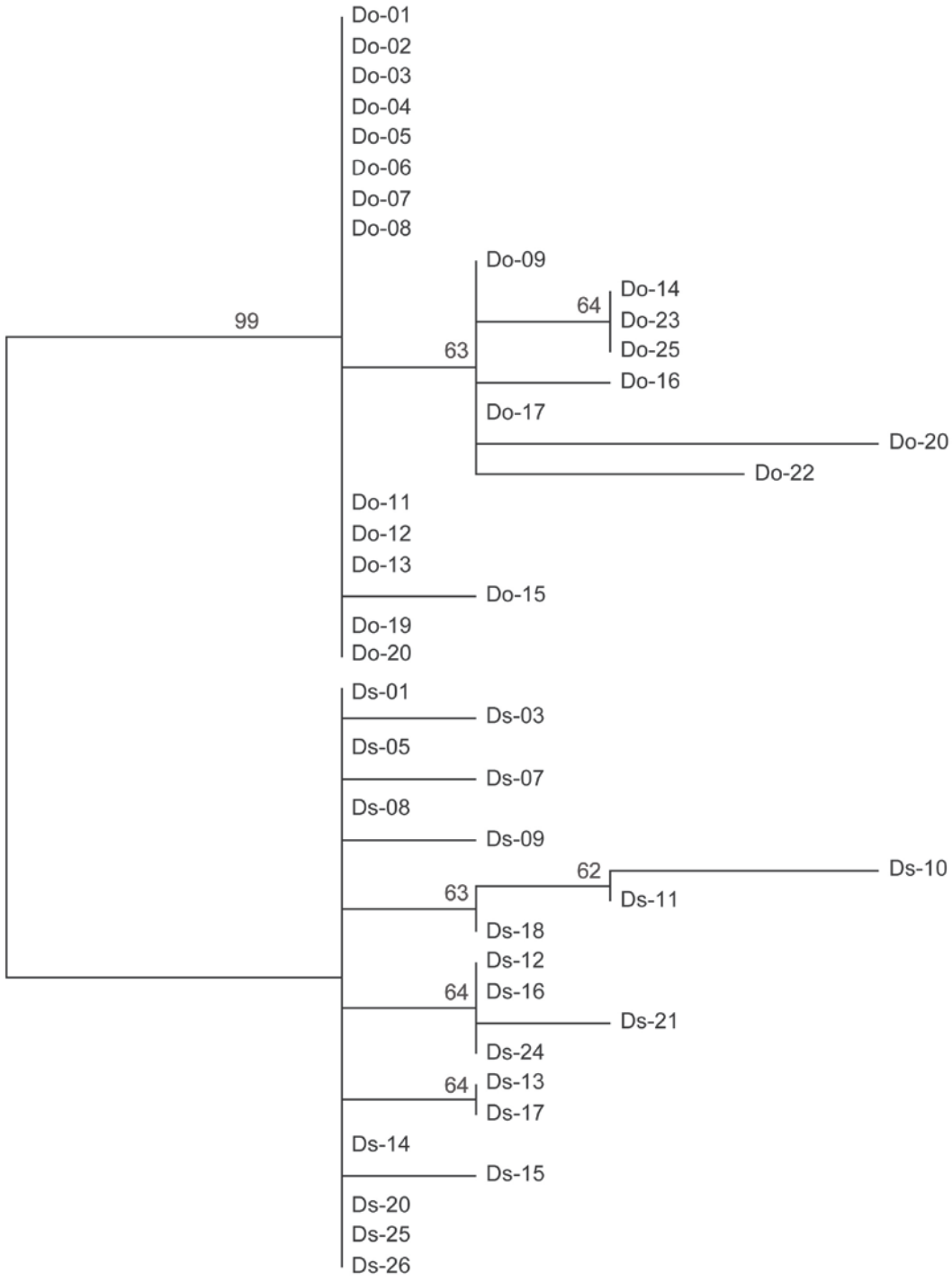

Figure 2-4. Parsimony dendrogram of western and Searls prairie clover collections based on *trn*K/*mat*K region of the chloroplast genome.

diagnostic primers with their melting temperature (Tm) in a range of 57.2 to 58.1 °C and 'GC' content in a range of 40.7 to 44.4% (Table 2-3). These proposed primers should only amplify western prairie clover, but not Searls prairie clover.

Nuclear ITS/5.8S

A DNA fragment of length 705 bp was obtained for the ITS/5.8S region, out of which 14 (2.6%) characters were variable and parsimoniously informative. Thirteen of the 14 variable characteristics were SNPs. An indel of five base pairs (5'-GCAWT-3') was observed at position 611 to 615. When western and Searls prairie clovers were compared, all western prairie clover collections lacked the five base pair indel from their ITS/5.8S. In addition, one transversion mutation was observed in both groups at position 139 (T and G).

Within each group, some SNPs exhibited geographical pattern in their distribution. For western prairie clover, collections from the Deschutes River watershed showed two transversion mutations with other collections at positions 280 (A for T) and 460 (C for A). Within the Deschutes River collections, two collections of geographical proximity Do-14 and Do-23 showed transition mutation (C for T) at position 617. However, collection Do-14 also exhibited 'T' base at the same position exhibiting polymorphisms for the locus. In Searls prairie clover, southern Utah collections (Ds-13, Ds-14, Ds-15) and two southeastern Nevada collections near the Utah border (Ds-3 and Ds-8) exhibited a transition mutation (T for C) at position 634 and two transversion mutations (A for T for both cases) at positions 584 and 614 (Table A-3). In addition, these collections exhibited similar mutations to the two most western collections from

Nevada, Ds-10 and Ds-11, for positions 117 (transversion mutation, G for C) and 175 (transversion mutation, C for A). Collections Ds-13, Ds-14, Ds-15, Ds-3, Ds-8, and Ds-18 exhibited a transition mutation at the 498 position (C for T) compared to other Ds collections. Meanwhile, Ds-18 exhibited polymorphisms at position 498 by having both 'C' and 'T' haplotypes.

The ITS/5.8S sequence based parsimony tree separated western and Searls prairie groups into two different clades with a low bootstrap value (65%) (Fig. 2-5). Within Searls prairie clover collections, closely located collections from eastern Nevada and southern Utah (Ds-03, Ds-08, Ds-13, Ds-14, and Ds-15) were separated as a unique clade with a high bootstrap support of 97%. Collection Ds-17 from southern Utah exhibited a separate clade indicating DNA polymorphisms in southern Utah collections on ITS/5.8S region. Within western prairie clover collections, five collections (Do-9, Do-14, Do-16, Do-23, and Do-25) from the Deschutes River watershed in north central Oregon formed a separate clade from other western prairie clover collections including collections from the adjoining John Day River watershed with 83% bootstrap support. Within the Deschutes River clade, two collections (Do-14 and Do-23) formed a separate clade with 68% bootstrap support. Meanwhile, Do-14 from Deschutes River watershed exhibited polymorphisms at ITS/5.8S (Fig. 2-5).

Three species-specific diagnostic primers were designed based on the conserved indels, ITS-DS1, ITS-DS2, and ITS-DS3 (Table 2-3). Melting temperature and 'GC' content for the primers ranged from 59.5 to 60.3°C and 57 to 65%, respectively. These primers should only amplify Searls prairie clover, but not western prairie clover.

DISCUSSION

The *trn*K/*mat*K of the chloroplast genome and ITS/5.8S region of the nuclear genome in western and Searls prairie clovers exhibited ample DNA polymorphisms to support the two species conclusion. A greater degree of support for the separation of the two groups was observed from *trn*K/*mat*K of chloroplast (100% bootstrap support) compared to the ITS/5.8S of ribosomal DNA (65%). The magnitude of a bootstrap value can be affected by the number of characters that support a particular tree and the total number of characters used to construct the tree. In addition, the bootstrap value for a node decreases when the numbers of non-informative characters within the node are high (Soltis and Soltis 2003). In our case, we found greater numbers of polymorphic haplotypes within each collection or collections from a geographical area in the ITS/5.8S compared to the polymorphisms obtained in the *trn*K/*mat*K. Meanwhile, closely related species that have not diverged extensively could get lower bootstrap support (Soltis and Soltis 2003). For example, McMahon and Hufford (2004) found relatively lower variability among several *Dalea* species for the ITS/5.8S and *mat*K region, in which they argued that the *Dalea* clade may be too young for the ITS and the *mat*K region to accumulate many changes. In the present study, we found two indels and a number of SNPs conserved to both groups in nuclear and chloroplast genomes, suggesting that these mutations may possibly have occurred after speciation events (Golding 1987; Templeton et al. 1995).

Western and Searls prairie clovers have not been found to overlap in their distribution (Welsh et al. 2003). Some haplotypes and indels exhibited a pattern of

Table 2-3. Diagnostic primers for western and Searls prairie clovers designed for *trn*K/*mat*K of chloroplast genome and ITS/5.8S of nuclear ribosomal DNA.

Diagnostic primer	5' Sequence 3'	Length	Tm (°C)	GC (%)
*trn*K/*mat*K				
trnK-DO1	ACG ATT CGA TTT CGA TGG AAA AAG CG	26	57.2	42.3
trnK-DO2	ACG ATT CGA TTT CGA TGG AAA AAG CGA	27	58.1	40.7
trnK-DO3	CGA TTC GAT TTC GAT GGA AAA AGC GAG	27	57.7	44.4
ITS/5.8S				
ITS-DS1	GGA CCG GTC GTG TGC GCA TT	20	60.3	65.0
ITS-DS2	GAC CGG TCG TGT GCG CAT TG	20	59.4	65.0
ITS-DS3	CGG TCG TGT GCG CAT TGCA TT	21	59.5	57.1

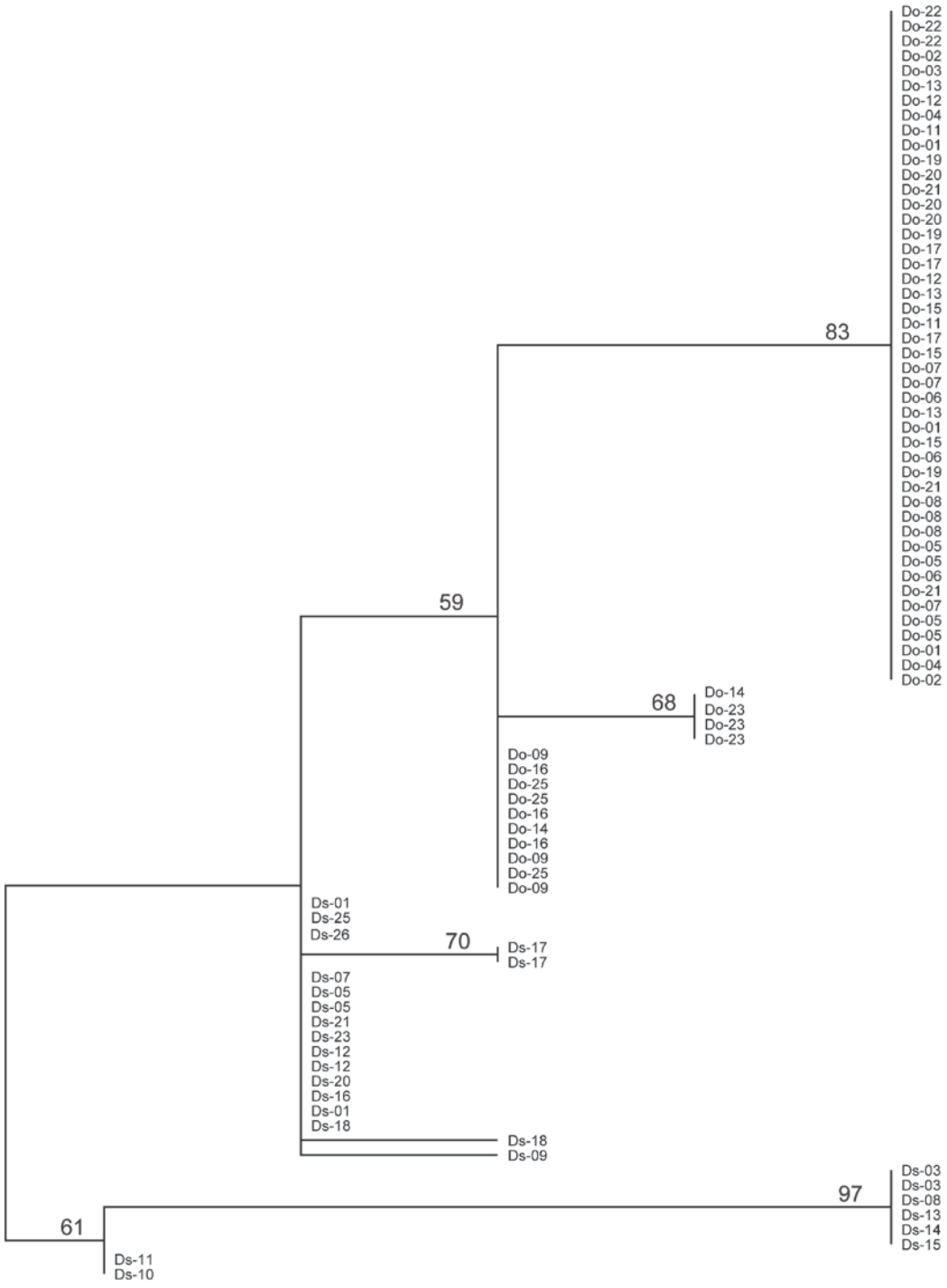

Figure 2-5. Bootstrapped parsimony dendrogram of western and Searls prairie clover collections based on ITS/5.8S region of ribosomal DNA.

"congruence" with geography (Figs. 3 and 4) (Schaal et al. 1998; Jorgensen et al. 2003) both within and between each groups, suggesting that a gene flow barrier might have played a strong role in the speciation of these two groups. In addition, differences of chloroplast haplotypes (*trn*K/*mat*K) and nuclear haplotypes (ITS/5.8S) with geography in both groups may be indicative of differential dispersal pattern of seeds and pollen grains as well as "selective wash" in the collections (Schaal et al. 1998), because the chloroplast is mainly maternally inherited in angiosperms. In this study, *trn*K/*mat*K placed northwestern Utah collections of Searls prairie clover in a separate clade, but there was not enough resolution of ITS for this group to be a separate clade. Similarly, western prairie clover collections from the Deschutes and John Day River watersheds were identified as a separate clade based on ITS/5.8S, but not based on the chloroplast. Both groups are insect-pollinated. Because John Day River collections and Deschutes River collections are similar in chloroplast but not in nuclear ITS/5.8S, the discrepancy may have resulted from "selective wash" (Schaal et al. 1998), because a "selective wash" due to recurrent gene flow from pollen grains can create populations with different nuclear genomes yet similar organellar genomes.

It is difficult to separate western and Searls prairie clovers at early stages of growth because their inflorescence characteristics are a major diagnostic feature (Barneby 1977). The diagnostic primers proposed in this study can be used to verify unidentified or ambiguous samples for both western and Searls prairie clover. Because these proposed primers were based on the conserved DNA polychromic sequences for chloroplast (*trn*K/*mat*K region) and nuclear genome (ITS/5.8S of rDNA), respectively,

they should be able to discriminate western and Searls prairie clovers species. Diagnostic primers for chloroplast genome (*trn*K-DO1, *trn*K-DO2, and *trn*K-DO3) would only amplify western prairie clover but not Searls prairie clover, whereas diagnostic primers for nuclear rDNA (ITS-DS1, ITS-DS2, and ITS-DS3) would only amplify Searls prairie clover but not western prairie clover.

LITERATURE CITED

Baldwin, B. G. 1993. Molecular phylogenetics of *Calycadenia* (Compositae) based on ITS sequences of nuclear ribosomal DNA: chromosomal and morphological evolution re-examined. *American Journal of Botany* 80:222-238.

Baldwin, B. G., M. J. Sanderson, J. M. Porter, M. F. Wojciechowski, C. S. Campbell, and M. J. Donoghue. 1995. The ITS region of nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny. *Annals of Missouri Botanical Garden* 82:247-277.

Barneby, R. C. 1977. *Dalea* imagines. *Memoirs of the New York Botanical Garden* 27:1-892.

Bonin, A., D. Ehrich, and S. Manel. 2007. Statistical analysis of amplified fragment length polymorphism data: a toolbox for molecular ecologists and evolutionists. *Molecular Ecology* 16:3737-3758.

Golding, G. B. 1987. The detection of deleterious selection using ancestors inferred from a phylogenetic history. *Genetics Research* 49:71-82.

Hilu, K. W., T. Borsch, K. Müller, D. E. Soltis, P. S. Soltis, V. Savolainen, M. Chase, M. Powell, L. A. Alice, R. Evans, H. Sauquet, C. Neinhuis, T. A. Slotta, J. G.

Rohwer, C. S. Campbell, and L. Chatrou. 2003. Angiosperm phylogeny based on *mat*K sequence information. *American Journal of Botany* 90:1758–1776

Hilu, K. W., and H. Liang. 1997. The *mat*K gene: sequence variation and application in plant systematics. *American Journal of Botany* 84: 830-839.

Hu, J.-M., M. Lavin, M. Wojciechowski, and M. J. Sanderson. 2000. Phylogenetic systematics of the tribe Millettieae (Leguminosae) based on chloroplast *trnK*/*matK* sequences, and its implications for evolutionary patterns in Papilionoideae. *American Journal of Botany* 87: 418-430.

Johnson, L. A., and D. E. Soltis. 1994. *mat*K DNA sequences and phylogenetic reconstruction in Saxigragaceae s. str. *Systematic Botany* 19:143-156.

Johnson, L. A., and D. E. Soltis. 1995. Phylogenetic inference in Saxifragaceae s.str. and *Gilia* (Polemoniaceae) using *mat*K sequences. *Annals of the Missouri Botanical Garden* 82:149–175.

Jorgensen, J. L., I. Stehlik, C. Brochmann, and E. Conti. 2003. Implications of ITS sequences and RAPD markers for the taxonomy and biogeography of the *Oxytropis campestris* and *O. arctica* (Fabaceae) complexes in Alaska. *American Journal of Botany* 90:1470-1480.

Kress, W. J., K. J. Wurdack, E. A. Zimmer, L. A. Weight, and D. H. Janzen. 2005. Use of DNA barcodes to identify flowering plants. Proceedings of the National Academy of Science, USA 102:8369-8374.

Lavin, M., R. T. Pennington, B. B. Klitgaard, J. I. Sprent, H. C. De Lima, and P. E. Gasson. 2001. The dalbergioid legumes (Fabaceae): delimitation of a pantropical monophyletic clade. *American Journal of Botany* 88:503–533.

Liere, K., and G. Link. 1995. RNA-binding activity of the *mat*K protein encoded by the chloroplast *trn*K intron from mustard (*Sinapis alba* L.). *Nucleic Acids Research* 23:917-921.

McMahon, M., and L. Hufford. 2004. Phylogeny of Amorphae (Fabaceae: Papilionoideae). *American Journal of Botany* 91:1219-1230.

Müller, K., and T. Borsch. 2005. Phylogenetics of *Utricularia* (Lentibulariaceae) and molecular evolution of the *trn*K intron in a lineage with high substitutional rates. *Plant Systematics and Evolution* 250:39-67.

Neuhaus, H., and G. Link. 1987. The chloroplast $tRNA^{Lys}$(UUU) gene from mustard (*Sinapsis alba* L.) contains a class II intron potentially coding for a maturase-related polypeptide. *Current Genetics* 11:251-257.

Olmstead, R. G., and J. D. Palmer. 1994. Chloroplast DNA systematics: a review of methods and data analysis. *American Journal of Botany* 81:1205-1224.

Schaal, B. A., D. A. Hayworth, K. M. Olsen, J. T. Rauscher, and W. A. Smith. 1998. Phylogeographic studies in plants: problems and prospects. *Molecular Ecology* 7:465-474.

Sha, L., R. Yang, X. Fan, X. Wang, and Y. Zhou. 2008. Phylogenetic analysis of *Leymus* (Poaceae: Triticeae) inferred from nuclear rDNA ITS sequences. *Biochemical Genetics* 46:605-619.

Soltis, P. S., and D. E. Soltis. 2003. Applying the bootstrap in phylogeny reconstruction. *Statistical Science* 18:256-267.

Stanford, A. M., R. Harden, and C. R. Parks. 2000. Phylogeny and biogeography of Juglans (*Juglandaceae*) based on *mat*K and ITS sequence data. *American Journal of Botany* 87:872-882.

Swofford, D. L. 2002. PAUP*. Phylogenetic analysis using parsimony (*and other methods), version 4.0b10 (Alvitec). Sinauer Associates, Sunderland, MA, USA.

Templeton, A. R., E. Routman, and C. A. Phillips. 1995. Separating population structure from population history: a cladistic analysis of the geographical distribution of mitochondrial DNA sequence data. III. Cladogram estimation. *Genetics* 140:767-782.

Varshney, R. K., A. Graner, and M. E. Sorrells. 2005. Geneic microsatellite markers in plants: features and applications. *Trends in Biotechnology* 23:48-55.

Vogel, J., T. Hubschmann, T. Börner, and W.R. Hess. 1997. Splicing and intron-internal RNA editing of *trn*K-*mat*K transcripts in barley plastids: support for *mat*K as an essential splicing factor. *Journal of Molecular Biology* 270:179-187.

Welsh, S. L., N. D. Atwood, S. Goodrich, and L. Higgins. 2003. A Utah flora. Brigham Young University, Provo, UT. 912 p.

Wendel, J. F., A. Schnabel, and T. Seelanan. 1995. Bidirectional interlocus concerted evolution following allopolyploid speciation in cotton (*Gossypium*). *PNAS* 92:280-284.

Wojciechowski, M. F., M. J. Sanderson, and J.-M. Hu. 1999. Evidence on the monophyly of *Astragalus* (Fabaceae) and its major subgroups based on nuclear ribosomal DNA ITS and chloroplast DNA *trnL* intron data. *Systematic Botany* 24:409–437.

CHAPTER 3

PHENOTYPIC AND GENETIC CHARACTERIZATION OF WESTERN PRAIRIE CLOVER COLLECTIONS FROM THE WESTERN USA

ABSTRACT

Few North American legumes are available for rangeland revegetation in the semi-arid western USA. Western prairie clover [*Dalea ornata* (Douglas ex Hook.) Eaton & J. Wright] is a perennial legume with desirable forage characteristics and is distributed in the northern Great Basin, Snake River Basin, and southern Columbia Plateau. Understanding the genetic and ecotypic variability of this species is a prerequisite for developing populations suitable for revegetation purposes. To address this need, we established two common-garden plots of western prairie clover collected from 22 sites in Idaho, Oregon, and Washington. Significant variation was detected among the collections for all traits measured. Among the measured traits, flowering date was correlated with collection-site temperature and elevation. Population structure estimates from 474 amplified-fragment length polymorphism markers resulted in two distinct, genetically differentiated groups and a third admixed group, and flowering date played a significant role in discriminating those genetic-based groupings of collections. Positive correlations were observed between phenotypic and genetic distance matrices ($r = 0.33$, $P = 0.005$), phenotypic and geographic distance matrices ($r = 0.35$, $P = 0.002$), and genetic and geographic distance matrices ($r = 0.31$, $P = 0.009$). Based on these results, we recommend that two germplasm sources of western prairie clover be developed for use in

rangeland revegetation, one from the Deschutes River region and the other encompassing Idaho, Washington, and eastern Oregon collection sites.

INTRODUCTION

The western U.S.A. has some of the highest numbers of endemic and imperiled species in the USA (Stein et al. 2000; Nachlinger et al. 2001). Use of a diversity of species in rangeland revegetation programs can help minimize weed invasion, because they occupy available niches that could otherwise be colonized by invasive weeds (Pfisterer et al. 2004; Walker and Shaw 2005; Brown et al. 2008). Legumes are of particular interest as they provide biologically fixed nitrogen to associated species, increase plant production, enhance forage quality, and provide food sources for herbivores and pollinators (Cherney and Allen 1995; Madison and Robel 2001; Aydin and Uzun 2005; Walker and Shaw 2005). In addition, legumes can maintain and restore natural successional trajectories (Richards et al. 1998).

High seed cost and limited seed availability of western North American legumes have restricted their use in revegetation/restoration programs (Walker and Shaw 2005). Seed of wildland-collected legumes is expensive, often several-fold higher than agronomically grown seed. Wildland-collected seed is of variable quality and may not be available when needed. Also, these legumes may be over-collected from specific wildland sites, thus disrupting the local population. Although seeds of western North American legumes are considerably more costly than grass seed, public land managers are interested in utilizing these legumes for reseeding and restoration because of their unique role in the ecosystem. Commercial seed production of these legumes could make

seed more readily available at a lower cost for rangeland revegetation/restoration programs in the semi-arid western USA.

Dalea L. is a widespread genus of the legume family (Fabaceae) comprising 62 species of prairie clovers in North America (USDA, NRCS 2009). *Dalea* is separated from other genera in the Amorphae tribe of Fabaceae based on a base chromosome number (*x*) of 7 (rarely 8) and two collateral ovules (Barneby 1977). Western prairie clover [*Dalea ornata* (Douglas ex Hook.) Eaton & J. Wright] is an insect-pollinated legume that is naturally distributed throughout the northern Great Basin, southern Columbia River Plateau, and Snake River Plain (USDA, NRCS 2009). Its distribution, lack of toxicity to herbivores, and relatively upright growth habit make western prairie clover a candidate for commercial seed production.

In reseeding disturbed rangelands, there can be the potential for introducing genes from different populations into local gene pools. This could potentially reduce population fitness by increasing the frequency of maladapted genes or cytological differences in local populations or disrupting co-adapted gene complexes (McKay et al. 2005). For outcrossing species that are sensitive to inbreeding depression, it may be important to increase genetic diversity in depauperate populations to increase population fitness (Ouborg et al. 2006) or to provide greater opportunities for adaptation to global change (Rice and Emery 2003). Therefore, identifying the amount and distribution of genetic variation in naturally occurring populations is important to make informed management decisions related to determination of conservation units.

Determining conservation units is not always straightforward, and includes analyses that infer short- and long-term evolutionary potential. The presence of local

adaptation is suggested by growing collections in common gardens and identifying traits that are correlated to environmental variables at the collection sites (St. Clair et al. 2005). The quantitative traits correlated to collection site environments are indicative of the phenotypic variation necessary to respond to short-term environmental changes (McKay and Latta 2002). Long-term evolutionary potential is suggested by the use of molecular markers to infer population structure (McKay and Latta 2002). The resulting marker-based population structures identify gene flow barriers that can enable allelic differentiation, and can be correlated with higher differentiation for quantitative traits (Merila and Crnokrak 2001). Genetic structures, if detected by a sufficient number of markers and in a sufficient number of populations, indicate underlying genetic differences of groups of collections that can be used to preserve evolutionary potential. Genetic structures have been identified for several Great Basin and other semi-arid western USA species (Larson et al. 2004; Bushman et al. 2007; Philips et al. 2008; Jones et al. 2008).

Amplified fragment length polymorphism (AFLP) is a genetic fingerprinting technique useful for determining genetic diversity and population structure in various plant species (Meudt and Clarke 2007). The AFLP markers do not require extensive genetic information on the species of interest when generated, are largely considered neutral, and are robust in their ability to differentiate populations (Schlötterer 2004). Although AFLP markers are used to assess genetic diversity and define population structures in plants, few AFLP-based studies, or other DNA marker analyses, have been conducted on rangeland legumes.

The objectives of this study were to evaluate phenotypic and molecular marker diversity of 22 wildland collections of western prairie clover. Our hypothesis was that genetic population structure(s) would exist within western prairie clover collections, and that variation of some of the phenotypic characters would be associated with the genetic variation. We assessed forage production and quality, flowering date, inflorescence weight and growth habit in two common-garden settings, and genetic diversity among and within collections using AFLP markers. This information is useful in determining optimum release strategies of this species for commercial seed production.

MATERIALS AND METHODS

Seed Collections

Based on available literature and herbarium specimen data, 22 seed collections of western prairie clover were obtained from Washington, Idaho, and Oregon during the summer of 2005 (Fig. 3-1). Collection site data obtained at each collection included elevation, latitude, longitude (Table 3-1), and associated plant species. The collection sites ranged in elevation from 110 to 1 163 m above sea level. Mean monthly temperature and precipitation data (average from 1961 to 1990) for each collection site were obtained from the Moscow Forestry Sciences Laboratory (2009). A 5- to 15-g section of insecticide strip [active ingredient Dochlorvos (2, 2-dichlorovinyl dimethyl phosphate) 18.6% and related compounds 1.4%, Hotshot No-Pest strip, United Industries Corp., St. Louis, MO] was placed in each bag of collected seed to reduce seed weevil damage. Seeds were air-dried in a greenhouse, threshed in a Wintersteiger seed thresher

(Model LD180, Des Moines, IA), and cleaned with sieves and a seed blower. Cleaned seeds were stored in a dark room maintained at 3ºC with a relative humidity of 20-25%.

Common-Garden Studies

Seeds were germinated in plastic boxes with moistened blotter paper at room temperature. After germination, seedlings were transplanted into Q-Plugs (International Horticulture Technologies, Hollister, CA) placed within Ray Leach stubby conetainers (Stuewe and Sons, Corvallis, OR). The seedlings were grown in a greenhouse at the USDA-ARS Forage and Range Research Laboratory, Logan, UT under a 30/15ºC day/night temperature regime. A single seedling was grown in each conetainer. Adequate fertilizer and watering was provided for 90 days to the plants before transplanting to field plots at Millville (lat 41˚39'N, long 111˚48'W, elevation 1 350 m above sea level) and Hyde Park (lat 41˚47'N, long 111˚48'W, elevation 1 380 m above sea level) locations in northern Utah. Soil at the Millville location is a Millville silt loam (coarse-loamy over sandy or sand-skeletal, mixed, superactive mesic, Calcic Haploxerolls). Soil at the Hyde Park location is a McMurdie silt loam (fine, montmorillonitic, mesic, Calcic Pachic Argixerolls). The experimental design at each of the common-garden locations was a randomized complete block with eight replications, and collections were assigned randomly within each replication. Each replication included five individual plants of each collection, for a total of 40 plants of each collection at each location. Plants were spaced 0.5-m apart within rows and between rows. Plants of commercially available purple prairie clover (*Dalea purpurea* Vent.) were included as checks at both locations. Seed for purple prairie clover came from

Table 3-1. Twenty-two western prairie clover collections with their collection site information, number of individuals sampled for genetic assays, percent polymorphic loci, and expected heterozygosity. Purple prairie clover (Dp) was used as a check and outgroup.

Collection ID	County and State	Latitude	Longitude	Elevation (m)	Temperature (°C)	No. of individuals	Polymorphic loci (%)	Expected heterozygosity
1	Wallowa, OR	N 45°41'	W 116°48'	631	10.5	8	34.4	0.12
2	Umatilla, OR	N 45°55'	W 119°07'	136	12.0	8	34.2	0.14
3	Benton, WA	N 45°56'	W 119°20'	131	11.9	8	36.7	0.14
4	Malheur, OR	N 43°24'	W 117°07'	1 163	8.2	8	38.0	0.14
5	Benton, WA	N 46°21'	W 119°21'	148	11.8	8	41.6	0.15
6	Walla Walla, WA	N 46°04'	W 118°54'	110	12.3	8	40.7	0.16
7	Franklin, WA	N 46°17'	W 119°11'	160	11.8	8	36.9	0.14
8	Malheur, OR	N 42°47'	W 117°43'	1 122	9.5	8	37.3	0.12
9	Sherman, OR	N 45°17'	W 121°01'	258	11.2	8	35.0	0.13
11	Elmore, ID	N 42°53'	W 115°07'	884	10.5	8	34.2	0.12
12	Owyhee, ID	N 42°44'	W 115°54'	945	10.4	8	34.2	0.12
13	Owyhee, ID	N 42°45'	W 115°43'	815	11.0	8	32.9	0.11
14	Jefferson, OR	N 44°43'	W 121°02'	696	9.6	8	32.1	0.10
15	Canyon, ID	N 43°19'	W 116°35'	713	11.5	8	33.5	0.12
16	Crook, OR	N 44°08'	W 120°48'	948	8.4	6	35.4	0.12
17	Wheeler, OR	N 44°37'	W 120°07'	1 001	8.3	8	44.9	0.16
19	Asotin, WA	N 46°04'	W 116°59'	259	12.5	7	44.1	0.16
20	Wheeler, OR	N 44°37'	W 120°20'	699	10.0	8	45.1	0.17
21	Elmore, ID	N 43°02'	W 115°09'	960	9.9	8	30.8	0.09
22	Wheeler, OR	N 44°54'	W 120°24'	465	11.2	8	41.6	0.15
23	Jefferson, OR	N 44°34'	W 121°02'	941	8.2	7	28.5	0.09
25	Crook, OR	N 44°03'	W 120°44'	1 038	8.0	8	29.7	0.10
Dp	-	-	-	-	-	8	40.1	0.15

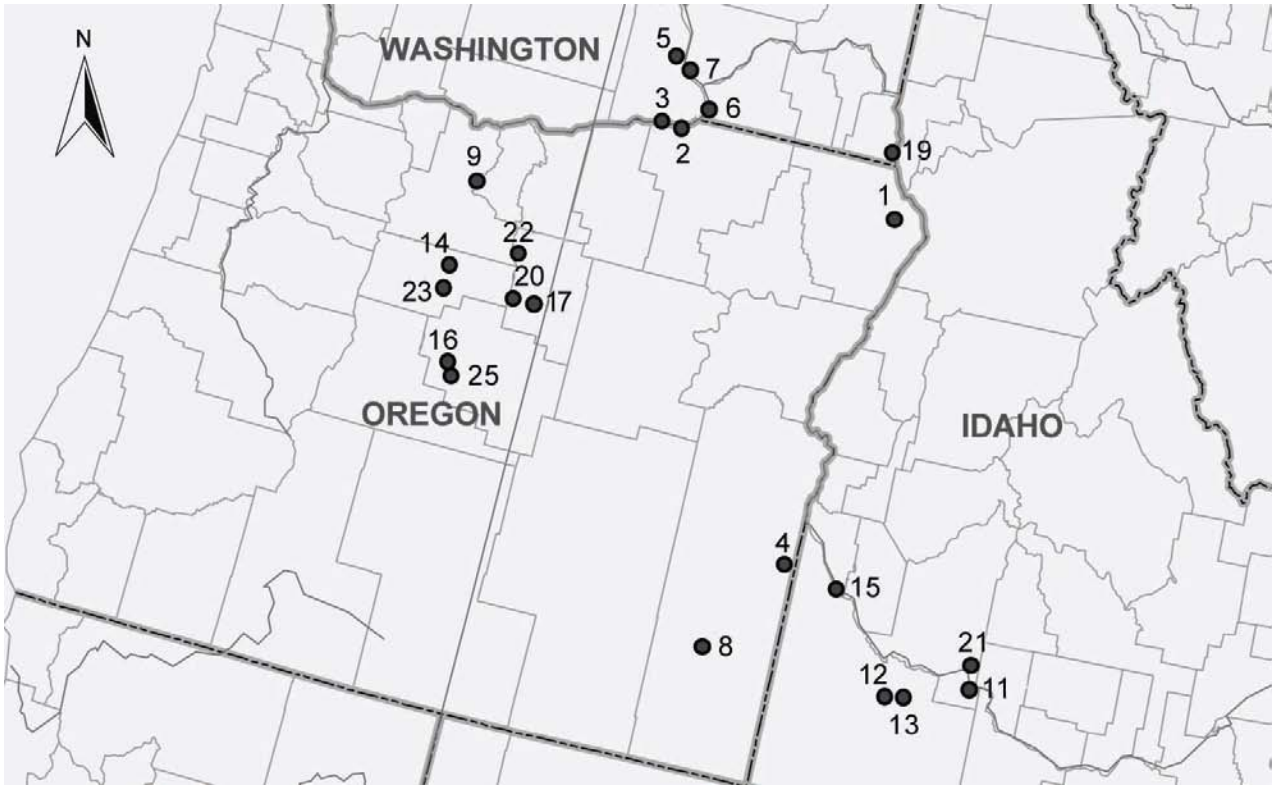

Figure 3-1. Map depicting collection sites for western prairie clover.

transplants originated from a 16.2-ha commercial production field, started from a 0.5-kg pool of seed harvested from five prairie-remnant sites in southern Wisconsin (Oak Prairie Farm, Pardeeville, Wisconsin). Plots were planted in May 2006 and were routinely weeded and watered during the establishment year.

During the second and third year after establishment (2007 and 2008), collections at both locations were evaluated for dry-matter yield (DMY) and number of inflorescences. For the DMY harvest at Hyde Park, plants were harvested at approximately 50% bloom (June 19 to 25) according to Miller (1984), and then after the first frost in October (October 17 to 25). At Millville, DMY was obtained after mature inflorescence harvest (26 July 2007, 4 August 2008), and then again after the first frost in October (October 17 to 26). The summer and fall DMY harvest values were combined in each year at each location. The number of inflorescences was determined at 50% bloom at Hyde Park and immediately before inflorescence harvest at Millville in both years.

To minimize bias between early- and late-flowering collections, inflorescences were harvested sequentially from late-June to late-July each year at the Millville location. Because western prairie clover has racemose inflorescences and its mature seeds disperse from the inflorescence easily, plants were checked daily and the mature seed portion were collected until the entire inflorescence was harvested. The inflorescences were bulked by replication, dried in a greenhouse setting, and weighed. Flowering date was measured at Millville location for both years. The flowering date was recorded as the number of days from January 1 when the first flower emerged on each plant, and was averaged across plants for each collection on a replication basis.

Additional measurements of plant height, foliage diameter, crude protein (CP), acid detergent fiber (ADF) and neutral detergent fiber (NDF) were taken at the Hyde Park location. Inflorescence weight was not measured at Hyde Park because samples were harvested for DMY at 50% of the field bloom. Plant height and foliage diameter were obtained immediately prior to DMY harvest. Values of NDF, ADF, and CP were determined from the first DMY harvest from Hyde Park in 2007. Samples were ground (Cyclotec 1093 Sample Mill) to pass through a 1-mm diameter screen. Ground samples were analyzed for CP using total combustion procedures (LECO TruSpec C/N analyzer, LECO Corp., St. Joseph, MI). ADF and NDF were analyzed at Agri Analysis Inc. (Leola, PA) following procedures of AOAC (1990) and Mertens (2002), respectively, as adapted to the Ankom A200 filter bag technique.

Analysis of variance was conducted using the MIXED procedure of SAS (SAS Institute 2004) with collection considered a fixed factor and replication a random factor. Data were checked for normality and homoscedasticity before analysis, and subsequently transformed when needed. Significance was determined at $\alpha = 5\%$, and mean separations were conducted using the Tukey Test. Pearson correlation coefficients and Spearman's rank coefficients were calculated using the CORR procedure of SAS.

Relationships among phenotypic traits and environmental and topographic variables at each collection site were evaluated by canonical correlation analysis (CCA) using the CANCORR procedure of SAS. Forage quality measurements were excluded from canonical correlation analysis, principal component analysis, and Mantel's test, because of their low variation among the collections, which reduced the power of discrimination for other phenotypic traits. Additionally, separate CCA, PCA, and

Mantels analyses were conducted on forage quality traits, and no significance was found. Therefore forage quality traits were not included in these analyses. The CCA procedure generated pairs of linear variable combinations with the highest correlations among a set of dependent (phenotypic) and independent (environmental and topographic) variables, which are termed canonical variables (Mardia et al. 1979).

Principal component analysis was conducted on the correlation matrix of phenotypic data using the FACTOR procedure of SAS. Significance of principal component axes was considered for components with eigenvalues greater than one, and an additional significance test was conducted with parallel analysis (PA) (Horn 1965) using 'paran' package in R (Dinno 2009). Significance for grouping of collections in the first two principal component axes obtained scatter plot was obtained using multiresponse permutation procedure (mrpp) using 'vegan' package in R (Oksanen et al. 2010).

Genetic Diversity and Population Structure

Plant tissues were collected from the shoot apical regions of eight individual plants from each collection prior to field transplanting. An additional eight plants of purple prairie clover were sampled for use as an outgroup. Tissues were lyophilized and stored at -20ºC. About 17 mg of dried plant tissue was used for each sample, and DNA was extracted using the Qiagen DNeasy 96-well procedure (Qiagen, Valencia, CA) following the manufacturer's protocol. The concentration and quality of genomic DNA were tested spectrophotometrically and by gel electrophoresis. Genomic DNA concentration was adjusted to approximately 30 ng/µL.

The AFLP procedure followed the method published in Vos et al. (1995), but was modified by resolving AFLP products on an ABI3730 capillary instrument (Life Technologies, Foster City, CA). Five selective primer pairs were chosen for analysis: E.AGG/M.CAT, E.AGA/M.CTG, E.AGC/M.CTA, E.AGT/M.CAC, and E.ACG/M.CTG. Amplified products were analyzed with Genescan (ABI, Foster City, CA) at the Center for Integrated Biosystems (Utah State University, Logan, UT). The AFLP bands were scored visually for presence or absence using Genographer software (Benham 2001).

The percentage of polymorphic loci and within-collection expected heterozygosity (H_E) was estimated in AFLP-SURV v1.0 (Vekemans et al. 2002) using the binary AFLP data. A matrix of pairwise genetic distance (*F*st), also obtained using AFLP-SURV, was used to construct a neighbor-joining (NJ) tree with Phylip software v3.69 (Felsenstein 2009). To assess model strength, bootstrap analysis was conducted using 1 000 permutations. Bayesian clustering analysis was conducted to assess population structures using STRUCTURE v2.2 (Falush et al. 2007), without advanced assignment of collections into populations. The raw binary data were analyzed with the RECESSIVE ALLELES option and the admixture model. Probabilities of K = 1 through K = 9 groupings were tested with three replications for each level of K. The Markov chain Monte Carlo (MCMC) procedure was used with 30 000 burn-in and 300 000 MCMC steps after burn-in to determine the probability of each structure model. The average estimated log probability of the data was plotted against the K-values to observe structural fit of the data. Differences between the K and K-1 average log probability values were also plotted to show changes in model fit, wherein a small change is an indication of the optimum number of robust population structures.

Important groups found in the STRUCTURE program and NJ tree were further tested with hierarchical Analysis of Molecular Variance (AMOVA), using Arlequin v3.1 software (Excoffier et al. 2005). First, a pairwise similarity matrix was generated among collections by estimating Dice's coefficient (Dice 1945) from the binary data of AFLP. Dice's coefficient was preferred over the simple matching coefficient because it estimates similarity based only on the band presence in at least one of the two individuals and thus reduces the bias in similarity estimates due to band absence in both individuals (Wong et al. 2001). The Dice's distance matrix was then used as an input file for the hierarchical AMOVA.

Mantel's *Z* statistic (Mantel 1967) was used to detect relationships among the genetic, phenotypic, and site characteristic distance matrices using the MXCOMP procedure of NTSYSpc (Rohlf 1998). For the genetic distance matrix, an F_{st} matrix obtained from AMOVA was used. A phenotypic matrix was constructed by using all phenotypic traits except forage quality traits. Collection-site characteristics (geographic distance, elevation, temperature, and precipitation) were each used to create site matrices. The ability of phenotypic traits to discriminate groups of collections identified by STRUCTURE or PCA was assessed by first using the STEPDISC procedure of SAS to determine the appropriate variables. Then the DISCRIM procedure of SAS was used to test significance of the appropriate discriminatory variables.

RESULTS

Common Gardens

The interaction between collections and years for dry-matter yield (DMY) was not significant for either common-garden location (Hyde Park: $F_{22,307} = 0.37$, $P = 0.99$; Millville: $F_{22,307} = 0.96$, $P = 0.47$). Therefore, DMY was combined across years within each location. The DMY was not combined across locations because of a significant interaction between locations and collections ($F_{22,667} = 2.88$, $P < 0.0001$). Collections differed significantly for DMY at both locations (Table 3-2), ranging from 85 to 242 g plot^{-1} at Hyde Park and 28 to 188 g plot^{-1} at Millville (Table 3-3). Collections Do9 and Do19 produced the greatest DMY at Hyde Park and Millville locations, respectively, but they were not significantly different from many other collections (Table 3-3). The 12 collections of western prairie clover with the highest average DMY values at Hyde Park were significantly higher in DMY than purple prairie clover (82 g plot^{-1}); however, at Millville they did not differ from purple prairie clover (135 g plot^{-1}). Collections Do1 and Do12 exhibited the lowest average DMY at both locations.

At Hyde Park, the collection by year interaction was not significant for number of inflorescences ($F_{22,300} = 0.81$, $P = 0.72$), hence these data were combined across years. At Millville, a significant interaction was observed between collections and years for number of inflorescences ($F_{22,307} = 2.24$, $P = 0.0014$). However, rankings among collections were consistent as indicated by the Spearman rank coefficients ($r = 0.63$, $P = 0.0013$), and did not change among the highest and lowest collections. Therefore, we also combined the data across years for number of inflorescences. At both locations,

number of inflorescences varied significantly (Table 3-2) with values ranging from 12 to 86 inflorescences per plant (Table 3-3). Collections with the highest number of inflorescences were Do14 (Hyde Park) and Do22 (Millville), both of which originated in central Oregon (Fig. 3-1). Purple prairie clover had the fewest inflorescences (9) at Hyde Park, and an intermediate number of inflorescences (63) at Millville. Collections Do1 and Do12 had the lowest numbers of inflorescences among the collections.

For inflorescence weight at Millville, the collection by year interaction was not significant ($F_{22,305} = 1.55$, $P = 0.06$), and the trait was combined across years. Collections differed significantly for inflorescence weight ($F_{22,318} = 15.76$, $P < 0.0001$) (Table 3-2) with collections from central Oregon generally having the highest values (Table 3-3). Collection Do9 had the greatest inflorescence weight (114 g plot^{-1}), yet did not differ from 10 other collections or from purple prairie clover (Table 3-3). Collection Do1 had the lowest inflorescence weight (20 g plot^{-1}); however, it was not significantly different from Do12 and Do11. Flowering dates ranged from 163 days (Do23) to 178 days (Do6) among Do collections, with average flowering date occurring about 21 June. Collection Do23 exhibited the earliest flowering, but did not differ from Do16, Do14, and Do25. All of these collections were from the Deschutes River watershed in central Oregon. The latest flowering collection was Do6, which did not differ from 11 other collections and purple prairie clover. Purple prairie clover was later (180 days) in flowering than all western prairie collections (Table 3-3).

The collection by year interaction was not significant for plant height ($F_{22,300} = 1.05$, $P = 0.40$) or for foliage diameter ($F_{22,300} = 0.72$, $P = 0.82$) at the Hyde Park location. Therefore, these traits were combined across years. Significant variation was

detected among the collections for plant height and foliage diameter (Table 3-2). Plant height ranged from 26 cm to 40 cm with Do19 having the greatest height, though it did not differ from seven other collections or from purple prairie clover (33 cm) (Table 3-3). Collection Do17 was the shortest, but it did not differ from seven other collections. Foliage diameter ranged from 45 cm to 68 cm for the collections. Collection Do22 had the greatest foliage diameter, but it did not differ from eight other collections. Collection Do12 had the least foliage diameter (45 cm) among collections, but it did not differ from seven other collections and purple prairie clover. The purple prairie clover check had a smaller foliage diameter (42 cm) than all the western prairie clover collections.

Differences were detected for ADF, NDF, and CP among the collections (Table 3-2). The ADF ranged from 30.0 to 37.1%, NDF ranged from 39.3 to 49.5%, and CP ranged from 16.7 to 19.2% (Table 3-3). Collection Do22 exhibited the greatest ADF, which differed from Do21 and Do6 but not from 19 other collections or from purple prairie clover. Collection Do8 had the greatest NDF, but it differed significantly only from five other collections (Table 3-3). Similarly, Do7 exhibited the greatest CP, which differed only from Do1 and Do9.

Correlations of Phenotypic Characters

A high positive correlation ($r = 0.84$, $P < 0.0001$) was observed for DMY (Table 3-4) and number of inflorescences ($r = 0.82$, $P < 0.0001$) between collections at Hyde Park and Millville. The DMY at Hyde Park was also correlated with inflorescence weight ($r = 0.85$, $P < 0.0001$), numbers of inflorescences at both locations (Hyde Park: $r = 0.61$, $P = 0.0027$; Millville: $r = 80$, $P < 0.0001$), and foliage diameter ($r = 0.62$, $P =$

Table 3-2. Means, standard deviations (SD), and *F*-statistics of traits in western prairie clover collections measured at Hyde Park, Millville, and combined across both locations.

Trait	Mean	SD	*F* statistic
Hyde Park			
Dry-matter yield, g plot^{-1}	165.0	48.3	$F_{22,322} = 11.08^{***}$[1]
Plant height, cm	32.7	3.2	$F_{22,321} = 8.58^{***}$
Number of inflorescences	36.2	13.1	$F_{22,322} = 12.13^{***}$
Foliage diameter, cm	56.5	6.7	$F_{22,321} = 10.81^{***}$
Acid detergent fiber (ADF), %	34.0	1.5	$F_{22,67} = 2.53^{**}$
Neutral detergent fiber (NDF), %	43.1	2.4	$F_{22,67} = 3.91^{***}$
Crude protein (CP), %	17.8	0.7	$F_{22,63.1} = 2.24^{**}$
Millville			
Dry-matter yield, g plot^{-1}	113.5	33.4	$F_{22,322} = 15.83^{***}$
Inflorescence weight, g plot^{-1}	66.8	25.8	$F_{22,318} = 15.76^{***}$
Number of inflorescences	61.0	17.4	$F_{22,322} = 12.30^{***}$
Flowering date[2]	172.8	4.4	$F_{22,318} = 17.72^{***}$

[1] $^{**} = P < 0.01$, $^{***} = P < 0.001$.
[2] Flowering date starts from January 1.

0.0023). The DMY at Millville was also correlated with inflorescence weight ($r = 0.71$, $P = 0.0002$) and numbers of inflorescences at Millville ($r = 0.71$, $P = 0.0002$). Flowering date was negatively correlated with number of inflorescences (Hyde Park: $r = -0.76$, $P < 0.0001$; Millville: $r = -0.58$, $P = 0.005$) and inflorescence weight (Millville: $r = -0.50$, $P = 0.018$), which indicated that earlier flowering plants produced more flowers and greater inflorescence weights. Among forage quality traits, NDF showed a moderate correlation to the number of inflorescences (Hyde Park: $r = 0.43$, $P = 0.046$), and ADF showed a

Table 3-3. Means and differences for dry-matter yield (DMY), number of inflorescences, plant height, foliage diameter, acid detergent fiber (ADF), neutral detergent fiber (NDF), crude protein, inflorescence weight, and flowering date in western prairie clover collections and purple prairie clover (Dp) at Hyde Park and Millville locations. Values followed by the same letter within a column are not significantly different at $P = 0.05$.

	Hyde Park							Millville			
Collection ID	DMY (g)	No. of infl.	Plant height (cm)	Foliage diameter (cm)	ADF (%)	NDF (%)	Crude protein (%)	DMY (g)	No. of infl.	Infl. weight (g)	Flowering date[1]
1	85.7^{G}	19.4^{FGH}	26.3^{F}	64.2^{ABC}	35.6 ^{AB}	39.3^{D}	17.0^{B}	27.9^{G}	20.8^{H}	20.0^{I}	173.8^{ABCDEF}
2	180.1^{ABCD}	32.6^{CDEF}	30.5^{DEF}	51.2^{FGH}	33.3 ^{AB}	41.1^{ABCD}	17.6^{AB}	108.8^{CDE}	55.4^{BCDEF}	46.3^{DEFGH}	176.6^{AB}
3	180.9^{ABCD}	41.5^{ABCD}	34.0^{ABCDE}	56.2^{BCDEFG}	33.4 ^{AB}	41.7^{AB}	16.8^{AB}	120.2^{BCDE}	67.8^{ABCDE}	56.0^{CDEFG}	173.8^{ABCDEF}
4	187.5^{ABC}	56.8^{AB}	38.4^{AB}	57.5^{BCDEFG}	34.8 ^{A}	47.0^{AB}	17.7^{AB}	113.0^{BCDE}	81.5^{AB}	86.1^{ABC}	169.2^{FGH}
5	174.0^{ABCD}	38.3^{ABCDE}	33.8^{ABCDE}	52.3^{EFGH}	33.6 ^{AB}	41.5^{ABCD}	18.1^{AB}	98.3^{DE}	51.8^{CDEF}	45.1^{EFGH}	177.1^{AB}
6	186.2^{ABC}	29.6^{CDEF}	31.3^{CDEF}	53.8^{DEFGH}	31.9 ^{B}	42.1^{ABCD}	18.1^{AB}	154.3^{ABC}	66.8^{ABCDE}	66.1^{BCDEFG}	177.9^{A}
7	164.7^{ABCDEF}	29.2^{CDEF}	31.6^{CDEF}	53.5^{DEFGH}	33.0 ^{AB}	42.2^{ABCD}	19.2^{A}	130.9^{BCD}	48.7^{CDEFG}	44.6^{EFGH}	174.9^{ABCDE}
8	115.6^{DEFG}	39.7^{BCDE}	33.1^{BCDE}	49.2^{GH}	35.2 ^{AB}	49.5^{A}	18.4^{AB}	91.5^{DEF}	60.0^{ABCDEF}	52.2^{DEFGH}	170.6^{DEFGH}
9	242.3^{A}	43.0^{ABCD}	34.5^{ABCDE}	65.7^{AB}	35.6 ^{AB}	47.0^{ABC}	16.7^{B}	156.4^{AB}	73.6^{ABC}	114.2^{A}	170.4^{EFGH}
11	98.0^{FG}	27.6^{DEFG}	33.1^{BCDE}	50.7^{GH}	33.7 ^{AB}	41.7^{ABCD}	18.3^{AB}	78.9^{EF}	39.6^{FGH}	36.8^{GHI}	175.8^{ABC}
12	84.9^{G}	12.4^{GH}	33.0^{BCDE}	45.1^{H}	32.8 ^{AB}	40.9^{CD}	16.9^{AB}	55.6^{FG}	27.3^{GH}	27.3^{HI}	176.8^{AB}
13	123.1^{CDEFG}	20.9^{EFGH}	36.7^{ABC}	55.7^{CDEFG}	34.2 ^{AB}	43.6^{ABCD}	18.3^{AB}	98.7^{DE}	41.5^{EFGH}	43.4^{FGH}	176.6^{AB}
14	203.4^{AB}	59.5^{A}	30.4^{DEF}	58.8^{ABCDEFG}	33.5 ^{AB}	43.9^{ABCD}	18.3^{AB}	113.7^{BCDE}	68.4^{ABCD}	77.4^{ABCDE}	167.4^{GHI}
15	132.3^{CDEFG}	33.6^{CDEF}	34.2^{ABCDE}	55.6^{CDEFG}	34.3 ^{AB}	45.4^{AB}	17.1^{AB}	92.7^{DEF}	43.9^{DEFG}	44.2^{EFGH}	175.4^{ABCDE}
16	195.6^{ABC}	44.6^{ABCD}	32.0^{CDE}	61.0^{ABCDEF}	34.4 ^{AB}	45.0^{ABCD}	17.8^{AB}	114.6^{BCDE}	74.3^{ABC}	74.1^{ABCDE}	165.3^{HI}
17	170.5^{ABCDE}	39.8^{ABCDE}	26.2^{F}	61.7^{ABCDE}	34.6 ^{AB}	43.1^{ABCD}	18.6^{AB}	115.2^{BCDE}	80.2^{AB}	94.0^{AB}	169.5^{FGH}
19	226.9^{AB}	30.8^{CDEF}	39.5^{A}	63.3^{ABCD}	35.1 ^{AB}	43.6^{ABCD}	17.3^{AB}	187.6^{A}	67.6^{ABCDE}	82.8^{BCDEF}	175.8^{ABCD}
20	210.8^{AB}	37.8^{BCDE}	29.5^{EF}	62.2^{ABCD}	36.0 ^{AB}	42.3^{ABCD}	17.7^{AB}	114.6^{BCDE}	66.6^{ABCDE}	99.4^{AB}	171.2^{CDEFG}
21	155.1^{BCDEF}	37.1^{BCDE}	35.6^{ABCD}	57.2^{BCDEFG}	30.0 ^{B}	40.8^{BCD}	17.2^{AB}	130.8^{BCD}	64.0^{ABCDEF}	70.9^{BCDEFG}	172.6^{BCDEFG}
22	240.1^{A}	44.5^{ABCD}	31.8^{CDE}	67.8^{A}	37.1 ^{A}	44.1^{ABCD}	17.8^{AB}	147.3^{ABC}	86.2^{A}	101.8^{AB}	175.1^{ABCDE}
23	166.7^{ABCDEF}	55.7^{AB}	31.6^{CDEF}	51.2^{FGH}	34.2 ^{AB}	42.0^{BCD}	18.7^{AB}	114.6^{BCDE}	83.1^{A}	75.7^{ABCDE}	162.7^{I}
25	188.4^{ABC}	48.7^{ABC}	31.7^{CDE}	63.5^{ABC}	32.0 ^{AB}	42.6^{BCD}	17.2^{AB}	110.5^{BCDE}	69.9^{ABCD}	83.4^{ABCD}	167.5^{GHI}
Dp	82.4^{EFG}	9.1^{H}	33.3^{ABCDE}	41.5^{H}	32.8 ^{AB}	42.1^{ABCD}	18.7^{AB}	134.8^{ABCDE}	63.2^{ABCDEF}	95.1^{ABC}	179.5^{A}

[1]Flowering date is measured from January 1.

moderate correlation to foliage diameter (r = 0.45, P = 0.036). No correlations were detected among ADF, NDF, and crude protein (Table 3-4).

Associations between phenotypic traits and environmental and topographic characteristics of the collection sites were assessed using canonical correlation analysis to identify traits with possible local adaptive significance. The first and second pairs of canonical variables contained 67 and 27% of the variation in the overall dataset, respectively, and only these two pairs of canonical variables exhibited significant correlations (P < 0.0001 and P = 0.0049, respectively). The first canonical variable for the phenotypic data set (PHEN1) primarily represented flowering date (r = -0.87, P < 0.0001). The first canonical variable for the environmental and topographic data set (CLIM1) was represented primarily by temperature (r = -0.99, P < 0.0001) and elevation (r = 0.86, P < 0.0001) (Table 3-5). There was a high negative correlation between PHEN1 and temperature (r = -0.95, P < 0.0001) and a positive correlation between PHEN1 and elevation (r = 0.82, P < 0.0001), indicating that flowering date was positively correlated with temperature and negatively correlated with elevation. The second canonical variable for the phenotypic data set (PHEN2) was correlated with DMY at Hyde Park (r = -0.69, P = 0.0004) and the number of inflorescences at both locations (Hyde Park: r = -0.55, P = 0.008; Millville: r = -0.54, P = 0.009) (Table 3-5). The second canonical variable for the environmental and topographic dataset (CLIM2) correlated with elevation (r = 0.49, P = 0.021) but not with precipitation or temperature (Table 3-5). Thus, the phenotypic character with the greatest correlation to collection site environment was flowering date, which responded to elevation and temperature.

Principal Component Analysis of Phenotypic Characters

Eigenvalues of the first three principal components were greater than one and described 89% of the total variation, while the first principal component was significant using parallel analysis (Table 3-6). The first principal component described 57% of the phenotypic variation, the second described 19%, and the third described 13%. Correlations for the first principal component were significant for DMY (Hyde Park: r = 0.91, $P < 0.0001$; Millville: $r = 0.76$, $P < 0.0001$), inflorescence weight ($r = 0.95$, $P < 0.0001$), number of inflorescences (Hyde Park: $r = 0.80$, $P < 0.0001$; Millville: $r = 0.94$, $P < 0.0001$), foliage diameter ($r = 0.63$, $P < 0.0018$), and flowering date ($r = -0.57$, $P = 0.0057$) (Table 3-6). Correlations for the second principal component were significant for DMY at Millville ($r = 0.56$, $P = 0.0065$), plant height ($r = 0.74$, $P < 0.0001$) and flowering date ($r = 0.69$, $P = 0.0004$), and the factor loading for the third principal component was significant for foliage diameter ($r = 0.64$, $P = 0.0014$) (Table 3-6). The scatter plot of the collections based on the first and second principal component scores showed a clustering of collections with two outlying collections (Fig. 3-2). Collections originating from the Deschutes River and John Day River watersheds in central Oregon, as well as Do4 from eastern Oregon, exhibited significant grouping compared to the remaining collections (Fig. 3-2, circled), with an observed delta value of 1.307 and an expected delta value of 1.784 ($P = 0.001$). The Do19 collection had the highest loadings for both principal components (i.e., high values for both sets of phenotypic traits), while Do1 had the lowest loadings for both components. Remaining collections from southeastern Washington, eastern Oregon, and Idaho were a large heterogeneous group.

Table 3-4. Pearson correlation coefficients (*r*) for dry-matter yield (DMY), inflorescence weight, number of inflorescences (No. of infl.), plant height, foliage diameter, flowering date, acid detergent fiber (ADF), neutral detergent fiber (NDF), and crude protein of 22 western prairie clover collections.

	DMY (Hyde Park)	DMY (Millville)	Infl. weight	No. of infl. (Millville)	No. of infl. (Hyde Park)	Flowering date	Plant height	Foliage diameter	ADF	NDF
DMY (Millville)	0.84***[1]									
Infl. Weight	0.85***	0.71***								
No. of infl. (Millville)	0.80***	0.71***	0.88***							
No. of infl. (Hyde Park)	0.61**	0.37^{ns}	0.66***	0.82***						
Flowering date	-0.26^{ns}	-0.05^{ns}	-0.50*	-0.58**	-0.76***					
Plant height	0.15^{ns}	0.4^{ns}	0.07^{ns}	0.09^{ns}	0.04^{ns}	0.19^{ns}				
Foliage diameter	0.62**	0.38^{ns}	0.66***	0.42^{ns}	0.30^{ns}	-0.25^{ns}	-0.12^{ns}			
ADF	0.24^{ns}	0.00^{ns}	0.30^{ns}	0.17^{ns}	0.11^{ns}	-0.11^{ns}	-0.11^{ns}	0.45*		
NDF	0.27^{ns}	0.28^{ns}	0.41^{ns}	0.41^{ns}	0.43*	-0.32^{ns}	0.37^{ns}	0.12^{ns}	0.42^{ns}	
Crude protein	-0.06^{ns}	0.07^{ns}	0.04^{ns}	0.16^{ns}	0.17^{ns}	-0.16^{ns}	0.22^{ns}	-0.30^{ns}	0.01^{ns}	0.08^{ns}

[1]ns = not significant, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

Table 3-5. Correlations between original variables and canonical variables for traits. The PHEN1 and PHEN2 are canonical variables obtained from phenotypic traits, and CLIM1 and CLIM2 are canonical variables obtained from environmental and topographic variables.

Original variables	PHEN1	PHEN2
Dry-matter yield, Millville	-0.27^{ns1}	-0.39^{ns}
Dry-matter yield, Hyde Park	-0.07^{ns}	-0.69***
Inflorescence weight	0.34^{ns}	-0.42^{ns}
No. of inflorescence, Hyde Park	0.53*	-0.55**
No. of inflorescence, Millville	0.38^{ns}	-0.54**
Plant height	-0.27^{ns}	0.39^{ns}
Foliage diameter	0.17^{ns}	-0.30^{ns}
Flowering date	-0.87***	0.38^{ns}
	CLIM1	CLIM2
Precipitation	0.45*	0.05^{ns}
Temperature	-0.99***	0.02^{ns}
Elevation	0.86***	0.49*

[1]ns = not significant, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

Genetic Diversity and Population Structure

Genetic diversity and population structure for the western prairie clover collections were determined with 474 AFLP bands. The percentage of polymorphic loci ranged between 29 to 45% with a mean of 36%, and the expected heterozygosity ranged from 0.09 to 0.17 with a mean of 0.13 (Table 3-1). Analysis of molecular variance (AMOVA) apportioned 31% of the variation among collections and 69% of variation within collections. Based on F_{st} distances, a NJ tree was used to investigate genetic structures, which showed two broad groups. The first group comprised five collections from the Deschutes River watershed (Fig. 3-3). The second group consisted of collections from geographically diverse areas in Idaho, Washington, and Oregon. Within

the larger second group, three collections from the John Day River watershed, collection Do19, and collection Do1 each formed subgroups with bootstrap support. Additionally, several two-group subgroups of geographically approximate collections with high bootstrap support were detected.

Bayesian clustering was used to test for population structures, wherein 1 to 9 groups were tested. The increase in average log probability values was minimal after the third group test, and the rate of change in the log probability values decreased sharply after that. These data indicated that little improvement in the model was obtained by including more than three groups (Fig. 3-4A). Five collections from the Deschutes River watershed in central Oregon constituted one genetically differentiated group (Fig. 3-4B). Fourteen collections from Idaho, Washington, and Oregon formed a second group. The

Table 3-6. Phenotypic traits used in principal component analysis with their respective correlations in the first three principal component axes. Eigenvalues, adjusted eigenvalues from parallel analysis (PA), and the cumulative proportion of the total variation of the measured trait data set were given for each principal component axis.

Traits	PC 1	PC2	PC 3
Dry-matter yield (Hyde Park)	0.91^{***}[1]	0.24^{ns}	0.18^{ns}
Dry-matter yield (Millville)	0.76^{***}	0.56^{**}	0.02^{ns}
Inflorescence weight	0.95^{***}	0.01^{ns}	0.13^{ns}
No. of infl. (Hyde Park)	0.80^{***}	-0.35^{ns}	-0.37^{ns}
No. of infl. (Millville)	0.94^{***}	-0.05^{ns}	-0.16^{ns}
Plant height	0.12^{ns}	0.74^{***}	-0.53^{*}
Foliage diameter	0.63^{**}	-0.04^{ns}	0.64^{**}
Flowering date	-0.57^{**}	0.69^{***}	0.32^{ns}
Eigenvalue	4.56	1.52	1.01
Adjusted eigenvalues (PA)	3.27	0.77	0.59
Cumulative proportion	0.57	0.76	0.89

[1]ns = not significant, $^{*} = P < 0.05$, $^{**} = P < 0.01$, $^{***} = P < 0.001$.

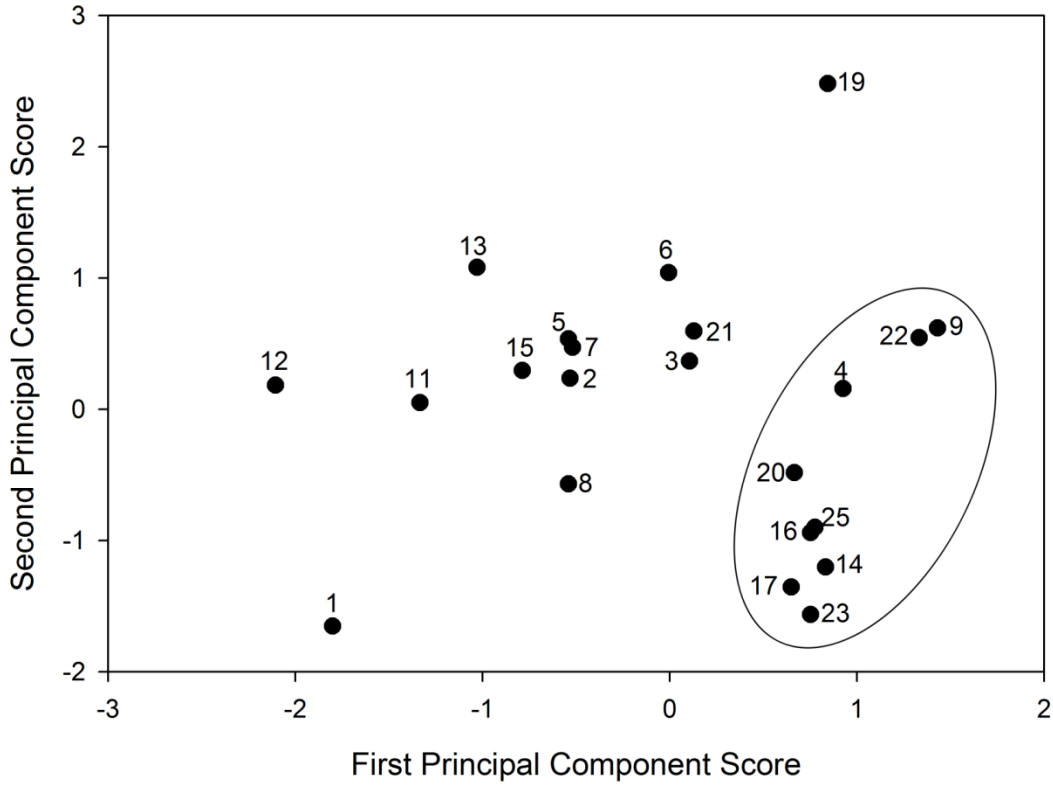

Figure 3-2. Scatter plots of the first principal component score with the second principal component score obtained from the western prairie clover phenotypic data. The circle indicates a significant group designation from principal component analysis.

Do17, Do20, and Do22 collections from the John Day River watershed were detected as a third group, but these three collections showed substantial admixture in that a large proportion of their AFLP markers were shared with the second group (Fig. 3-4B).

To test the significance of the three groups identified by Bayesian clustering, hierarchical analysis of molecular variance (AMOVA) was used. Thirty-one percent of the total genetic variation was apportioned among the three groups, 10% ($P < 0.001$) of the variation was apportioned among collections within groups, and the remaining 59% ($P < 0.001$) was apportioned within collections. In comparison, when only two major

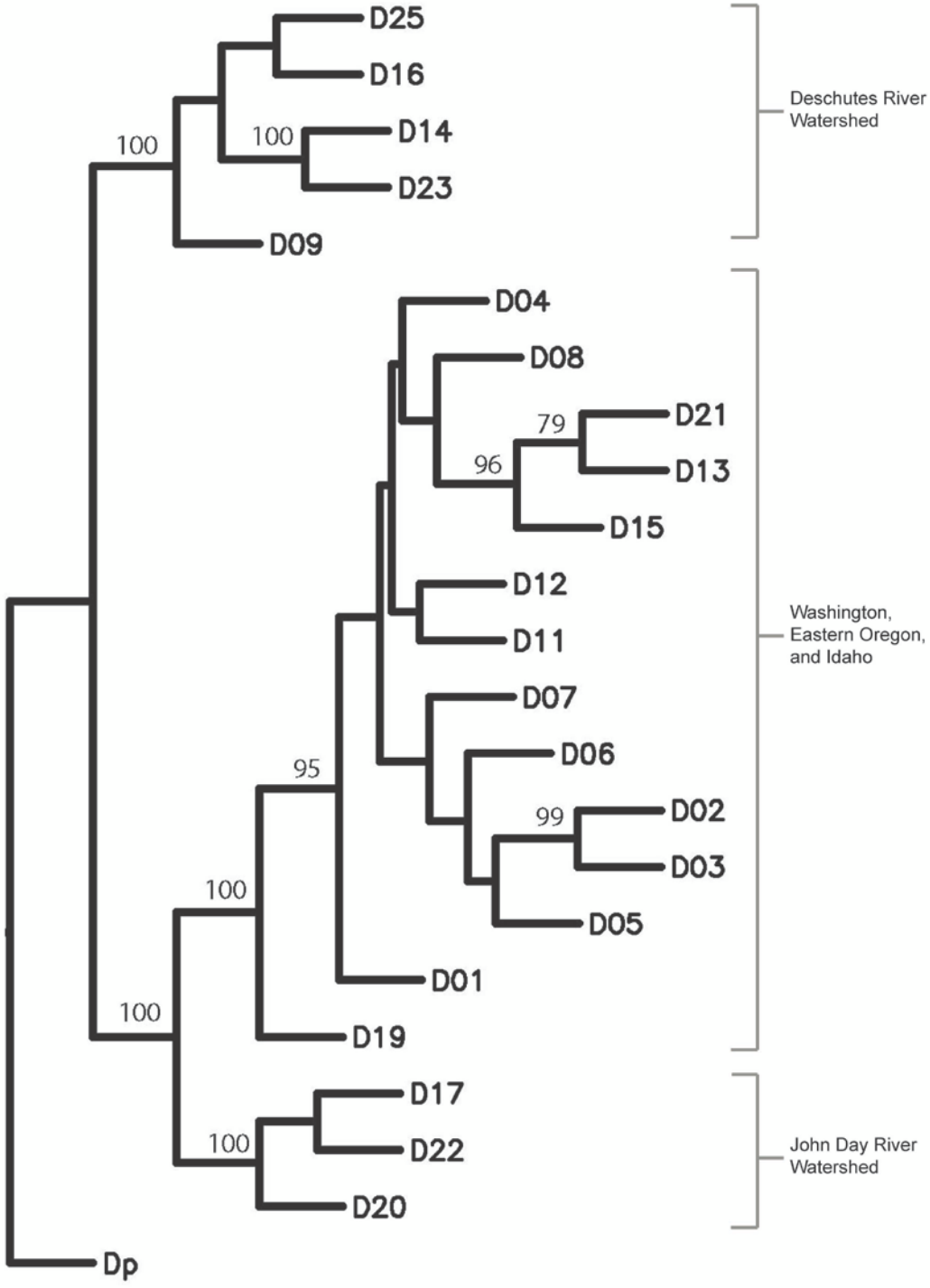

Figure 3-3. A consensus neighbor-joining dendrogram constructed using an F_{st} distance matrix of 22 western prairie clover collections, with associated bootstrap values (%). Only bootstrap values equal to or higher than 75% are shown.

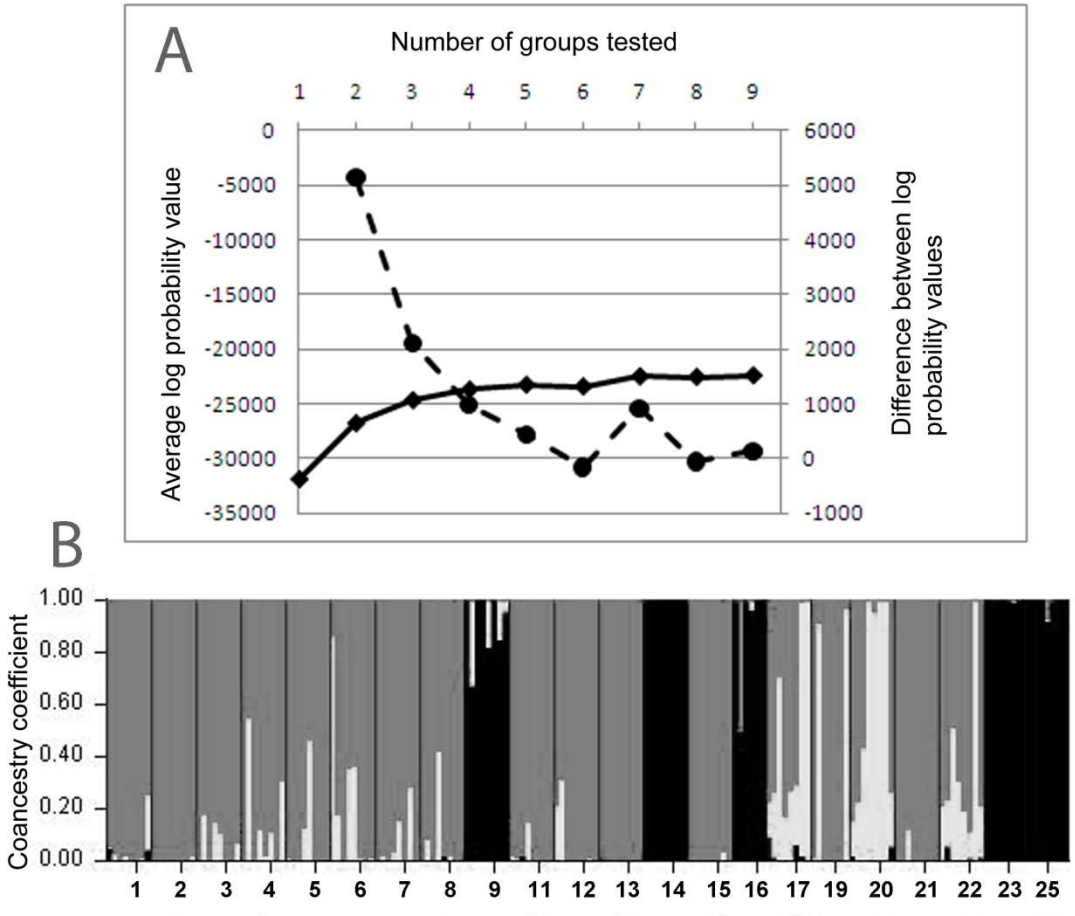

Figure 3-4. (A) The average log probability values of Bayesian clustering (solid line), and the differences between average log probability values (dashed line) of successive groups, testing groups 1-9. (B) Bar plot showing Bayesian clustering results, where western prairie clover collections were fit into three *a priori* groups. Collection numbers correspond to those in Table 3-1 and smaller bars within collections represent the individual plants.

groups, the Deschutes River watershed and the remaining collections (now including the John Day collections), were tested in hierarchical AMOVA, 34% ($P < 0.001$) of the total genetic variation was apportioned among the two groups and 12% ($P < 0.001$) among collections within groups. The slight difference of 3% of the variation apportioned among groups when comparing three-group and two-group scenarios suggests that the separation of the collections from the John Day River watershed as a population structure was not strong.

To determine if the marker-based genetic distances were correlated to the phenotypic, environmental, and geographic matrices, Mantel's correlation test statistic (Z) was applied. Significance was found for the correlation between the phenotypic and genetic distance matrices ($r = 0.33$, $P = 0.005$), the phenotypic and geographic distance matrices ($r = 0.35$, $P = 0.002$), and the genetic and geographic distance matrices ($r = 0.31$, $P = 0.009$). Unlike some of the phenotypic characters, the genetic distance matrix did not correlate with precipitation or elevation differences (data not shown). However, the genetic distance matrix was correlated to mean annual temperature differences ($r = 0.28$, $P = 0.004$).

Because the genetic matrix was correlated with the phenotypic distance matrix, individual phenotypic characters were tested for their ability to discriminate the groups of collections identified by genetically differentiated groups (Fig. 3-4B), and the phenotypic group (Fig. 3-2). When the three genetically differentiated groups were tested (Deschutes River watershed collections, John Day River watershed collections, and remaining collections from Idaho, Washington, and eastern Oregon; Fig. 3-4B), flowering date, inflorescence weight, and plant height discriminated the three groups with 100%

accuracy, with a Wilk's Lambda value of $F_{6,34} = 14.30$ ($P < 0.0001$). When only two genetically differentiated groups were compared (Deschutes River group and all the other collections including John Day), flowering date alone was retained in the stepwise regression model and discriminated with 100% accuracy ($F_{1,20} = 29.33$, $P < 0.0001$). When the phenotypic-based group of Deschutes and John Day River collections (with collection Do4) were compared against the Idaho, Washington, and eastern Oregon collections (Fig. 3-2), inflorescence weight, flowering date, and dry-matter yields at both locations were identified as discriminating variables with 100% accuracy ($F_{4,17} = 46.37$, $P < 0.0001$). Finally, when comparing just the three John Day River collections against those in Idaho, Washington, and eastern Oregon, inflorescence weight, plant height, and the number of inflorescences at the Millville location, but not flowering date, were discriminatory variables with 100% accuracy ($F_{3,13} = 28.67$, $P < 0.0001$).

DISCUSSION

Legumes for rangeland revegetation should ideally: 1) have a broad distribution to minimize unanticipated crossing with related species, 2) be adapted to and compatible in the range of sites they will be planted in, 3) be amenable to agronomic seed production, and 4) preferably be safe for grazing animals. Western prairie clover meets those requirements. It has a distribution in central and eastern Oregon, southeastern Washington, western Idaho, and several northern counties in Nevada and California (USDA NRCS, 2009); which precludes the risk of unanticipated crossing in those areas because hybridization with related species is likely to have already occurred naturally. Plant collections were often based on existing herbarium records, and seed from at least

100 individual plants were collected at each site. The collections we made of western prairie clover constituted many, but not all, of the counties reported to contain this species, and thus the phenotypic and genetic assessments reported herein may be limited to the geographical regions from which collections were obtained. As future populations of this species are reported, it would be interesting to extend phenotypic and genetic assessments to those populations.

The presence of western prairie clover from a range of elevations, ecoregions, temperature gradients, and precipitation regimes suggests broad adaptability, and phenotypic assessments in the common-garden plots found significant variation for all characters (Tables 3-2, 3-3). The number of inflorescences, inflorescence weight, and plant height were used as general indicators of the potential for seed production and mechanical harvest. For all three characters, some western prairie clover collections had values greater than purple prairie clover, a related species that is commercially grown in the mid-western USA.

Dry-matter yield and forage quality are important traits for livestock and wildlife grazing. Western prairie clover collections varied widely for DMY (28 to 188 g $plot^{-1}$ at Millville and 85 to 242 g $plot^{-1}$ at Hyde Park), and on average greater DMY was obtained at Hyde Park than Millville (165 g $plot^{-1}$ versus 114 g $plot^{-1}$, respectively). Although a significant collection by site interaction was observed for DMY, collections with the greatest DMY at Hyde Park were generally high-yielding collections at Millville, with the exception of purple prairie clover (Table 3-3). Dry-matter yields were not associated with forage quality traits, suggesting that selection for high DMY would not necessarily

lead to reduced forage quality, although some studies have shown otherwise (White and Wight 1984; van der Wal et al. 2000; Bhattarai et al. 2008).

Despite significant differences among collections for forage quality traits, the differences were relatively small (Table 3-3). On average, western prairie clover exhibited promising forage quality trait when compared to other legumes. Western prairie clover exhibited greater forage quality compared to basalt milkvetch (*Astragalus filipes* Torr ex. A Gray), a western North American legume evaluated at similar location (Bhattarai et al. 2008). In addition, purple prairie clover exhibited higher forage quality compared to 14 other legumes that were evaluated in Missouri, U.S.A (McGraw et al. 2004). Our study showed that western prairie clover has comparable forage quality value with the purple prairie clover (Table 3-3).

Strong positive correlations were detected among DMY, inflorescence weight, and number of inflorescences in western prairie clover collections (Table 3-4). This suggests that selection for high DMY would likely lead to greater seed yields, and that the number of inflorescences could be used to indirectly select for seed yield. Similar results were reported for basalt milkvetch, another legume from the Intermountain West, USA (Bhattarai et al. 2008). Collections with the greatest inflorescence weight and DMY were Do9, Do22, and Do19, yet these were not significantly different than half of the other collections (Table 3-3). Interestingly, these three collections each represented one of the three different genetically differentiated groups: Do9 from the Deschutes River watershed, Do22 from the John Day River watershed, and Do19 from the remaining collections (Figs. 3-1 and 3-4B). These collections could be excellent sources for release as individual germplasms or development of breeding populations (Jones 2009). It would

be interesting, therefore, to expand locality tests within geographic boundaries of those genetically differentiated groups.

Both NJ dendrogram and Bayesian clustering analyses separated the collections from the Deschutes River watershed as a homogeneous and distinct group (Figs. 3-3 and 3-4B). This homogeneity was detected at the two-group (K = 2) test in the STRUCTURE program and remained consistent through successive tests (Fig. 3-4B and data not shown). Thus, the Deschutes River watershed collections comprise a genetically differentiated group of collections. Among the remaining collections, both Bayesian clustering and the NJ dendrogram identified significant grouping of the John Day River watershed collections (Figs. 3-3 and 3-4B). The John Day collections, however, showed shared coancestry (genetic admixture) with the group of collections from Washington, eastern Oregon, and Idaho. This admixture also remained consistent throughout all further Bayesian clustering tests, and indicated that the collections from the John Day River watershed are genetically more similar to collections from more distant sites in this study than to those from the nearby Deschutes River watershed. For revegetation purposes, the presence of admixture and small (albeit significant) differences in molecular variance may not necessitate treating the John Day collections as a separate genetically differentiated group.

Although collections from the Deschutes and John Day River watersheds are genetically differentiated, they are in close geographic proximity and no apparent seed barrier exists between them. Major differences are not apparent in the climate or soils of the two watersheds (P. Pedone, personal communication, October 2009), and principle component analysis of phenotypic measurements was not able to separate the two groups

(Fig. 3-2). The causes of genetic differentiation among such groupings often cannot be succinctly determined, but possibilities include genetic drift, migration, selection/ adaptation, and range expansion. The AFLP markers can represent the cumulative effects of such processes and show evidence of gene-flow barriers. As molecular markers grouped the John Day River collections either separately or with distant collections in Idaho, eastern Oregon, and Washington (Fig. 3-3), while phenotypic measurements group the John Day River collections with nearby Deschutes River collections (Fig. 3-2), further studies are needed to determine the placement of John Day River watershed collections into a conservation unit.

One possible explanation for the genetic differences between the John Day River and Deschutes River watershed collections may be a differential historical disturbance of the Deschutes River watershed, which might have reduced its population size. This hypothesis is supported by the trend of relatively low expected heterozygosity (H_E) in collections from the Deschutes River watershed (average < 11%) compared to collections from the John Day River watershed (average = 16%) (Table 3-1). Additionally, the homogeneous grouping of individuals (Fig. 3-4B) and north-south distribution along the Deschutes River suggests that these collections may have originated from the same ancestral population. The Deschutes River was a popular route for Native Americans to travel to and from the Columbia River (Ray et al. 1938), and seed migration may have occurred along this corridor.

Traits with adaptive significance may be correlated with environmental variables of the collection sites because environmental variables partly determine the selective factors that will give rise to parallel variation in traits (Endler 1986). In our study,

flowering date was positively correlated with temperature (r = 0.83, P < 0.0001) and negatively correlated with elevation (r = -0.55, P = 0.0079). Because high-elevation sites typically are colder than low-elevation sites, plants from high elevations generally grow and flower at relatively lower ambient temperatures than plants from lower elevations. Consequently, when grown in a common garden, western prairie clover collections from high-elevation, low-temperature sites had an earlier flowering date than those from low-elevation sites.

In this study a correlation (r = 0.33, P = 0.005) was observed between genetic and phenotypic distance matrices. This suggests that some similar evolutionary processes may have been involved in the diversification of the measured phenotypic traits and neutral genetic markers. Flowering date, inflorescence weight, and plant height accurately discriminated the three genetically differentiated groups among the collections, while flowering date was the sole discriminatory variable of the two genetically differentiated groups. Deschutes River collections exhibited a trend of difference for flowering date and plant height with John Day River collections, but not for other phenotypic traits (Table 3-3). Flowering date was not a significant variable for discriminating the John Day River collections from those in eastern Oregon, Washington, and Idaho. Flowering date is considered a life history trait (Crnokrak and Roff 1995), which has been shown to generally reflect neutral genetic diversity more than morphological traits (Merila and Crnokrak 2001). Additionally, flowering date was also the phenotypic character most highly correlated with environmental and topographic characteristics of the collection sites in this study. As a result, flowering date is likely to

play a substantial role in defining both genetic structure and local adaptation of western prairie clover.

IMPLICATIONS

Western prairie clover is a non-toxic perennial legume native to the northern Great Basin, Snake River Basin, and southern Columbia Plateau in the western USA. Distribution and occurrence of western prairie clover in a variety of habitats makes it a potential candidate for use in rangeland revegetation programs. Based on genetic differences and phenotypic traits with possible adaptive significance, we recommend that separate seed sources (conservation units) be developed and locality tested from each of the two groups of collections described herein: one from the Deschutes River watershed and one from southeastern Washington, eastern Oregon, and Idaho. Further studies are needed to determine the placement of John Day River watershed collections into an optimal conservation unit.

LITERATURE CITED

Association of Official Analytical Chemists [AOAC]. 1990. Official methods of analysis. Arlington, VA, USA: Association of Official Analytical Chemists. 1141 p.

Aydin, I., and F. Uzun. 2005. Nitrogen and phosphorus fertilization of rangelands affects yield, forage quality and the botanical composition. *European Journal of Agronomy* 23:8-14.

Barneby, R. C. 1977. *Dalea* imagines. *Memoirs of the New York Botanical Garden* 27:1-892.

Benham, J. J. 2001. Genographer- AFLP/Microsatellite Software, v. 1.6.0. Available from http://hordeum.oscs.montana.edu/genographer/. Accessed 14 October 2007.

Bhattarai, K., D. A. Johnson, T. A. Jones, K. J. Connors, and D. R. Gardner. 2008. Physiological and morphological characterization of basalt milkvetch (*Astragalus filipes*): basis for plant improvement. *Rangeland Ecology and Management* 61:444-455.

Brown, C. S., V. J. Anderson, V. P. Claassen, M. E. Stannard, L. M. Wilson, S. Y. Atkinson, J. E. Bromberg, T. A. Grant III, and M. D. Munis. 2008. Restoration ecology and invasive plants in the semiarid West. *Invasive Plant Science Management* 1:399-413.

Bushman, B. S., S. L. Larson, M. D. Peel, and M. E. Pfrender. 2007. Population structure and genetic diversity in North American *Hedysarum boreale* Nutt. *Crop Science* 47:1281-1286.

Cherney, J. H., and V. G. Allen. 1995. Forages in a livestock system. *In*: R. F. Barnes, D. A. Miller, and C. J. Nelson [eds.]. Forages: the science of grassland agriculture. Volume I. Ames, IA, USA: Iowa State University Press, p. 175-188.

Crnokrak, P., and D.A. Roff. 1995. Dominance variance: associations with selection and fitness. *Heredity* 75:530-540.

Dice, L. R. 1945. Measures of the amount of ecologic association between species. *Ecology* 26:297-302.

Dinno. A. 2009. paran: Horn's test of principal component/factors. Available at: http://CRAN.R-project.org/package=paran. Accessed 5 May 2010.

Endler, J. A. 1986. Natural selection in the wild. Princeton, NJ, USA: Princeton University Press. 358 p.

Excoffier, L., G. Laval, and S. Schneider. 2005. Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online* 1:47-50.

Falush, D., M. Stephens, and J. K. Pritchard. 2007. Inference of population structure using multilocus genotype data: dominant markers and null alleles. *Molecular Ecology Notes* 7:574–578.

Felsenstein, J. 2009. PHYLIP (Phylogeny Inference Package) version 3.69. Distributed by Author, Department of Genome Sciences: University of Washington, Seattle, WA, USA.

Horn, J. L. 1965. A rationale and test for the number of factors in factor analysis. *Psychometrika* 30:179-185.

Jones, T. A. 2009. Conservation biology and plant breeding: special considerations for development of native plant materials for development of native plant materials for use in restoration. *Ecological Restoration* 27:8-11.

Jones, T. A., S. R. Larson, and B. L. Wilson. 2008. Genetic differentiation and hybridization among *Festuca idahoensis*, *F. roemeri*, and *F. ovina* detected from AFLP, ITS, and chloroplast DNA. *Botany* 86:422-434.

Larson, S. R., T. A. Jones, and K. B Jensen. 2004. Population structure in *Pseudoroegneria spicata* (Poaceae: Triticeae) modeled by Bayesian clustering of AFLP genotypes. *American Journal of Botany* 91:1789-1801.

Madison, L. A., and R. J. Robel. 2001. Energy characteristics and consumption of several seeds recommended for northern bobwhite food plantings. *Wildlife Society Bulletin* 29:1219-1227.

Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. *Cancer Research* 27:209-220.

Mardia, K. V., J. T. Kent, and J. M. Bibby. 1979. Multivariate analysis. New York, NY, USA: Academic Press. 518 p.

McGraw, R. L., F. W. Shockley, J. F. Thompson, and C. A. Roberts. 2004. Evaluation of native legume species for forage yield, quality, and seed production. *Native plants Journal* 5:152-159.

McKay, J. K., C. E. Christian, S. P. Harrison, and K. J. Rice. 2005. "How local is local?" - A review of practical and conceptual issues in the genetics of restoration. *Restoration Ecology* 1:432-40.

McKay, J. K., and G. Latta. 2002. Adaptive population divergence: markers, QTL and traits. *Trends in Ecology and Evolution* 17:185-291.

Merila, J., and P. Crnokrak. 2001. Comparison of genetic differentiation at marker loci and quantitative traits. *Journal of Evolutionary Biology* 14:892-903.

Mertens, D. R. 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. *Journal of AOAC International* 85:1217-1240.

Meudt, H. M., and A. C. Clarke. 2007. Almost forgotten or latest practice? AFLP applications, analyses and advances. *Trends in Plant Science* 12:106-117.

Miller, D. A. 1984. Forage crops. New York, NY, USA: McGraw-Hill. 530 p.

Moscow Forestry Sciences Laboratory. 2009. Custom climate data requests. Available at: http://forest.moscowfsl.wsu.edu/climate/customData/. Accessed 6 October 2009.

Nachlinger, J., K. Sochi, P. Comer, G. Kittel, and D. Dorfman. 2001. Great Basin: an ecoregion-based conservation blueprint. Reno, NV, USA: The Nature Conservancy. 160 p.

Oksanen, J., F. G. Blanchet, R. Kindt, P. Legendre, R. B. O'Hara, G. L. Simpson, P. Solymos, M. Henry, H. Stevans, and H. Wagner. 2010. vegan: community ecology package. Available at: http://CRAN.R-project.org/package=vegan. Accessed 5 May 2010.

Ouborg, N. J., P. Vergeer, and C. Mix. 2006. The rough edges of the conservation genetics paradigm for plants. *Journal of Ecology* 94:1233-1248.

Pfisterer, A. B., J. Joshi, B. Schmid, and M. Fischer. 2004. Rapid decay of diversity-productivity relationships after invasion of experimental plant communities. *Basic and Applied Ecology* 5:5-14.

Phillips, N. C., S. R. Larson, and D. T. Drost. 2008. Detection of genetic variation in wild populations of three *Allium* species using amplified fragment length polymorphisms. *HortScience* 43:637-643.

Ray, V. F., G. P. Murdock, B. Blyth, O. C. Stewart, J. Harris, E. A. Hoebel, and D. B. Shimkin. 1938. Tribal distribution in eastern Oregon and adjacent regions. *American Anthropologists* 40:384-415.

Rice, K. J., and N. C. Emery. 2003. Managing microevolution: restoration in the face of global change. *Frontiers in Ecology and the Environment* 1:469-478.

Richards, R. T., J. C. Chambers, and C. Ross. 1998. Use of native plants on Federal lands: policy and practice. *Journal of Range Management* 51:625-632.

Rohlf, F. J. 1998. NTSYSpc numerical taxonomy and multivariate analysis system version 2.0. Setauket, NY, USA: Exeter Software. 31 p.

SAS Institute. 2004. SAS/STAT 9.1 user's guide. Cary, NC, USA: SAS Institute, Inc. 5136 p.

Schlötterer, C. 2004. The evolution of molecular markers - just a matter of fashion? *Nature Reviews Genetics* 5:63-69.

St. Clair, J. B., N. L. Mandel, and K. W. Vance-Borland. 2005. Genecology of Douglas fir in western Oregon and Washington. *Annals of Botany* 96:1199-1214.

Stein, B., L. S. Kutner, and J. S. Adams. 2000. Precious heritage: the status of biodiversity in the United States. New York, NY, USA: Oxford University Press. 399 p.

USDA, NRCS. 2009. The Plants Database. National Plant Data Center, Baton Rouge, LA, USA. Available at: http://plants.usda.gov/java/profile?symbol=DALEA. Accessed 30 November 2009.

van der Wal, R. S., C. van Lieshout, C. Dormann, R. Langvatn, and S. D. Albon. 2000. Trading forage quality for quantity? Plant phenology and patch choice by Svalbard reindeer. *Oecologia* 123:108-115.

Vekemans, X., T. Beauwens, M. Lemaire, and I. Roldan-Ruiz. 2002. Data from amplified fragment length polymorphism (AFLP) markers show indication of size homoplasy and of a relationship between degree of homoplasy and fragment size. *Molecular Ecology* 11:139-151.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, and M. Kuiper. 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research* 23:4407-4414.

Walker, S. C., and N. L. Shaw. 2005. Current and potential use of broadleaf herbs for reestablishing native communities. *In*: N. L. Shaw, M. Pellant, and S. B. Monsen [eds.]. Proceedings, Sage Grouse Habitat Restoration Symposium. Fort Collins, CO, USA: USDA Forest Service, Rocky Mountain Research Station. Proceedings RMRS-P-38. p. 46-61.

White, L. M., and J. R. Wight. 1984. Forage yield and quality of dryland grasses and legumes. *Journal of Range Management* 37:233-236.

Wong, A., M. R. Forbes, and M. L. Smith. 2001. Characterization of AFLP markers in damselflies: prevalence of codominant markers and implications for population genetic applications. *Genome* 44:677-684.

CHAPTER 4

SEARLS PRAIRIE CLOVER FOR RANGELAND REVEGETATION: PHENOTYPIC AND GENETIC EVALUATIONS

ABSTRACT

Few North American legumes are available for use in rangeland revegetation in the western USA, but Searls prairie clover [*Dalea searlsiae* (A. Gray) Barneby] is one that holds promise. Commercial-scale seed production of this species could address issues of unreliable seed availability and high seed costs associated with its wildland seed collection. To evaluate its utility for revegetation, we collected Searls prairie clover at 20 locations across Utah and Nevada. Amplified fragment length polymorphisms (AFLP) and morphological and phenological traits (measured in common-garden plots) were used to clarify the role of evolutionary forces responsible for its genetic structure. Collections were evaluated for dry-matter yield, inflorescence weight, number of inflorescences, plant height, foliage diameter, flowering date, acid detergent fiber, neutral detergent fiber, and crude protein at two common-garden locations in northern Utah. Based on principal component analysis of morphological and phenological data, collections from southern Utah and eastern Nevada grouped together, whereas collections from western Nevada and northwestern Utah formed a separate group. Based on AFLP analyses, collections from northwestern Utah were differentiated from those of southern Utah and Nevada. Strong isolation by distance among collections suggests that genetic drift and gene flow are important factors in determining population structure in Searls prairie clover.

INTRODUCTION

Spread of invasive weeds in the Great Basin region has resulted in its identification as the third most endangered ecosystem in the USA (Stein et al., 2000). A large portion of rangelands in this region burns each year (4.97×10^5 ha, average from 1998 to 2007; Mike Pellant, 2009, personal communication) and if left untreated, weed invasions will likely accelerate (Jessop and Anderson, 2007). Post-fire revegetation after burning requires large amounts of seed. For example, in 2007 the BLM purchased more than 3×10^6 kg of seed for revegetation. A mix of species is preferred in seed mixtures for revegetation because functionally diverse plant species are more likely to minimize weed invasion risk and increase ecosystem function (Pokorny et al., 2005; Sheley and Carpinelli, 2005; Walker and Shaw, 2005).

North American legumes are of particular interest for inclusion in seed mixtures because they biologically fix nitrogen, thus enhancing forage nitrogen content (Cherney and Allen, 1995; van der Heijden et al., 2006), as well as improve wildlife forage and habitat (Madison and Robel, 2001). Seeds of North American legumes are usually wildland harvested for use in revegetation programs, resulting in unreliable seed availability and high seed prices, which limit their use (Walker and Shaw, 2005). Commercial seed production could reduce seed costs and make such species more readily available for rangeland revegetation.

Plant materials for rangeland revegetation are considered to be appropriate if they are adapted to and compatible with the local revegetation environment, do not pose the risk of gene transfer to related species that would not already occur naturally, are non-

toxic to herbivores because a large portion of western rangelands are used for livestock grazing, and are amenable to commercial seed production to ensure a reliable quantity and quality of seeds. Searls prairie clover, *Dalea searlsiae* (A. Gray) Barneby, is a perennial legume that naturally occurs in the southern Great Basin, southwestern Colorado Plateau, and northern Arizona (USDA, NRCS, 2010). It is a diploid (2n = 14, or rarely 16) and primarily insect-pollinated (Barneby, 1977; Jim Cane, 2009, personal communication). Its natural distribution within the southern Great Basin ameliorates gene transfer concerns in that region because hybridization with related species would have occurred naturally, if at all. Compounds that are toxic to livestock and wildlife, found in some other legumes, were below detectable levels in Searls prairie clover (D.A. Johnson, unpublished data). Additionally, its relatively upright growth habit makes Searls prairie clover a potential candidate for commercial seed production.

Local germplasm has been recommended for use in revegetation to reduce the possibility of maladaption or outbreeding depression (Lesica and Allendorf, 1999; McKay et al., 2005). However, maintaining and producing many local germplasm accessions for seed production can be difficult for seed growers (Broadhurst et al., 2008). In addition, this may lead to increased seed costs because demand for local germplasm is unpredictable given the sporadic nature of wildfires and subsequent variable need for local germplasm. By using phenotypic and genomic assessments to expand seed sources for native species to a larger regional level, the number of seed-sources required for use in revegetation/restoration programs and the risk of maladaptation and outbreeding depression can be minimized.

The appropriateness of regional seed sources can be determined by phenotypic and genotypic comparisons. Common-garden plots provide a relatively uniform environment for phenotypic evaluation, such that the differences among populations are primarily due to genetic differences and/or the interaction between genes and the environment. Phenotypic differences, if correlated with climatic variables of source collection sites, suggest the possibility of local adaption (Endler, 1986). Climatic variables that correlate with common-garden phenotypes can be used to delineate regional seed sources, as has been done for tree species (Johnson et al., 2004). Neutral DNA markers (e.g., AFLPs) measure overall genetic diversity due to evolutionary forces, such as gene flow, founder effects, and genetic drift. As a result, genetic diversity data obtained from DNA markers indicate the evolutionary potential of populations (McKay and Latta, 2002). In addition, DNA marker analyses provide estimations of inbreeding depression based on within-collection diversity, and group differentiation based on among-collection variation (Ouborg et al., 2006; Bonin et al., 2007).

When a distribution of populations is greater than the gene flow capability, isolation by distance can occur (Johnson and Black, 1998). Isolation by distance is usually detected by a positive correlation between genetic and geographic distances among individuals using the Mantel test (Mantel, 1967). Strong isolation by distance indicates that populations are at equilibrium with respect to gene flow and genetic drift (Slatkin, 1993). However, other factors such as precipitation, temperature, elevation, or disturbance can influence isolation by distance (Kittlein and Gaggiotti, 2008). Therefore, a partial Mantel test is often used to account for the influence of other factors when estimating isolation by distance (Telles and Diniz-Filho, 2005).

The objective of this study was to evaluate phenotypic and DNA-marker based variation of 20 wildland collections of Searls prairie clover. We assessed forage production and quality, flowering date, inflorescence weight, and growth habit in two common gardens, and we evaluated genetic diversity among and within collections using AFLP markers. Using these data, we evaluated the role of genetic drift and gene flow among populations. We also identified potentially adaptive phenotypic traits and regional seed sources that minimize the possibility of outbreeding depression and maladaptation, while providing a tractable number of seed sources for germplasm development.

MATERIALS AND METHODS

Seed Collections

Seed collections of Searls prairie clover were made from 20 sites in Utah and Nevada during the summer of 2005 (Fig. 4-1). Elevation, latitude, and longitude data were obtained for each collection site (Table 4-1). Mean annual temperature and precipitation (average from 1961 to 1990) of collection sites were obtained from the Moscow Forestry Sciences Laboratory (2009). A 5- to 15-g section of insecticide strip [active ingredient Dochlorvos (2, 2-dichlorovinyl dimethyl phosphate) 18.6% and related compounds 1.4%, Hotshot No-Pest strip, United Industries Corp., St. Louis, MO] was placed in each bag of collected seed to reduce seed weevil damage. Seeds were air-dried in a greenhouse, threshed by a Wintersteiger seed thresher (Model LD180, Des Moines, IA), and cleaned with hand-held sieves and a seed blower. Cleaned seeds were stored in a dark room maintained at 3 ºC with a relative humidity of 20-25%.

Common-Garden Studies

Seeds were germinated at room temperature in plastic boxes with moistened blotter paper. After germination, seedlings were transplanted into Q-Plugs (International Horticulture Technologies, Hollister, CA) placed within Ray Leach stubby conetainers (Stuewe and Sons, Corvallis, OR), and grown in a greenhouse at the USDA-ARS Forage and Range Research Laboratory, Logan, UT under a 30/15 ºC day/night temperature regime. A single seedling was grown in each conetainer. Fertilization and watering were provided for 90 days before the seedlings were transplanted to field plots at Millville (lat 41˚39', long 111˚48', 1,350 m above sea level) and Hyde Park (lat 41˚47', long 111˚48', 1,370 m above sea level) in northern Utah. Because of poor seedling establishment in the greenhouse, collection Ds-01 was not included at Hyde Park. Soil at Millville is a Millville silt loam (coarse-loamy over sandy or sand-skeletal, mixed, superactive mesic, Calcic Haploxerolls), whereas soil at Hyde Park is a McMurdie silt loam (fine, montmorillonitic, mesic, Calcic Pachic Argixerolls). The experimental design at each location was a randomized complete block with eight replications of five individual plants, for a total of 40 plants of each collection at each location. Plants were spaced 0.5-m apart within rows and between rows. Plots were planted in May 2006, routinely weeded and watered during the establishment year, and regularly weeded without irrigation in subsequent years. Plants of a commercially available purple prairie clover (*Dalea purpurea* Vent.), a related species indigenous to the Great Plains of the USA, were included as checks (designated as Dp) at both locations. Seed for purple prairie clover came from transplants originating from a 16.2-ha commercial production field that was initiated from a 0.5-kg pool of seed harvested from five prairie-remnant sites in

Table 4-1. Site information and number of individuals used for average similarity value and polymorphic loci for 20 collections of Searls prairie clover (*Dalea searlsiae*).

Collection	County and State	Latitude	Longitude	Elevation (m)	Mean annual temperature (°C)	Mean annual precipitation (mm)	No. of individuals	Average similarity value (S)	Polymorphic loci (%)
Ds-01	Garfield, UT	N37°46'	W111°25'	1,604	11.3	197	8	0.70	28.0
Ds-03	Lincoln, NV	N37°53'	W114°19'	1,614	10.2	255	8	0.69	33.4
Ds-05	Nye, NV	N38°42'	W115°25'	1,695	9.0	214	8	0.64	39.0
Ds-07	Nye, NV	N38°03'	W115°52'	1,890	9.0	238	8	0.65	37.4
Ds-08	Lincoln, NV	N37°43'	W114°05'	1,796	8.9	291	8	0.64	38.4
Ds-09	Nye, NV	N38°28'	W117°08'	2,000	8.2	185	6	0.66	28.6
Ds-10	Nye, NV	N38°41'	W117°53'	1,646	9.9	152	7	0.65	32.6
Ds-11	Mineral, NV	N38°36'	W117°51'	1,888	8.6	171	8	0.68	30.8
Ds-12	Box Elder, UT	N41°25'	W113°48'	1,413	8.8	209	8	0.73	24.8
Ds-13	Iron, UT	N37°36'	W113°22'	1,943	8.3	372	8	0.69	29.4
Ds-14	Kane, UT	N37°13'	W112°41'	1,695	10.7	347	8	0.64	32.6
Ds-15	Washington, UT	N37°36'	W113°31'	1,732	9.5	336	8	0.66	31.6
Ds-16	Box Elder, UT	N41°28'	W113°06'	1,597	8.3	267	8	0.77	25.0
Ds-17	Kane, UT	N37°17'	W112°36'	1,749	10.1	343	8	0.66	35.2
Ds-18	Lincoln, NV	N38°10'	W114°35'	1,834	9.4	305	8	0.66	37.6
Ds-20	Nye, NV	N38°24'	W115°06'	1,597	9.9	238	8	0.67	33.8
Ds-21	Toole, UT	N40°56'	W113°41'	1,326	10.0	172	8	0.74	27.6
Ds-23	Toole, UT	N40°19'	W113°57'	1,419	9.7	178	8	0.75	25.6
Ds-25	White Pine, NV	N39°06'	W115°02'	1,950	7.2	249	8	0.66	32.8
Ds-26	Lincoln, NV	N38°35'	W114°42'	2,036	7.6	321	8	0.67	32.6
Dp[†]	-	-	-	-	-	-	8	0.67	31.8

[†] Purple prairie clover (Dp) was used as an outgroup.

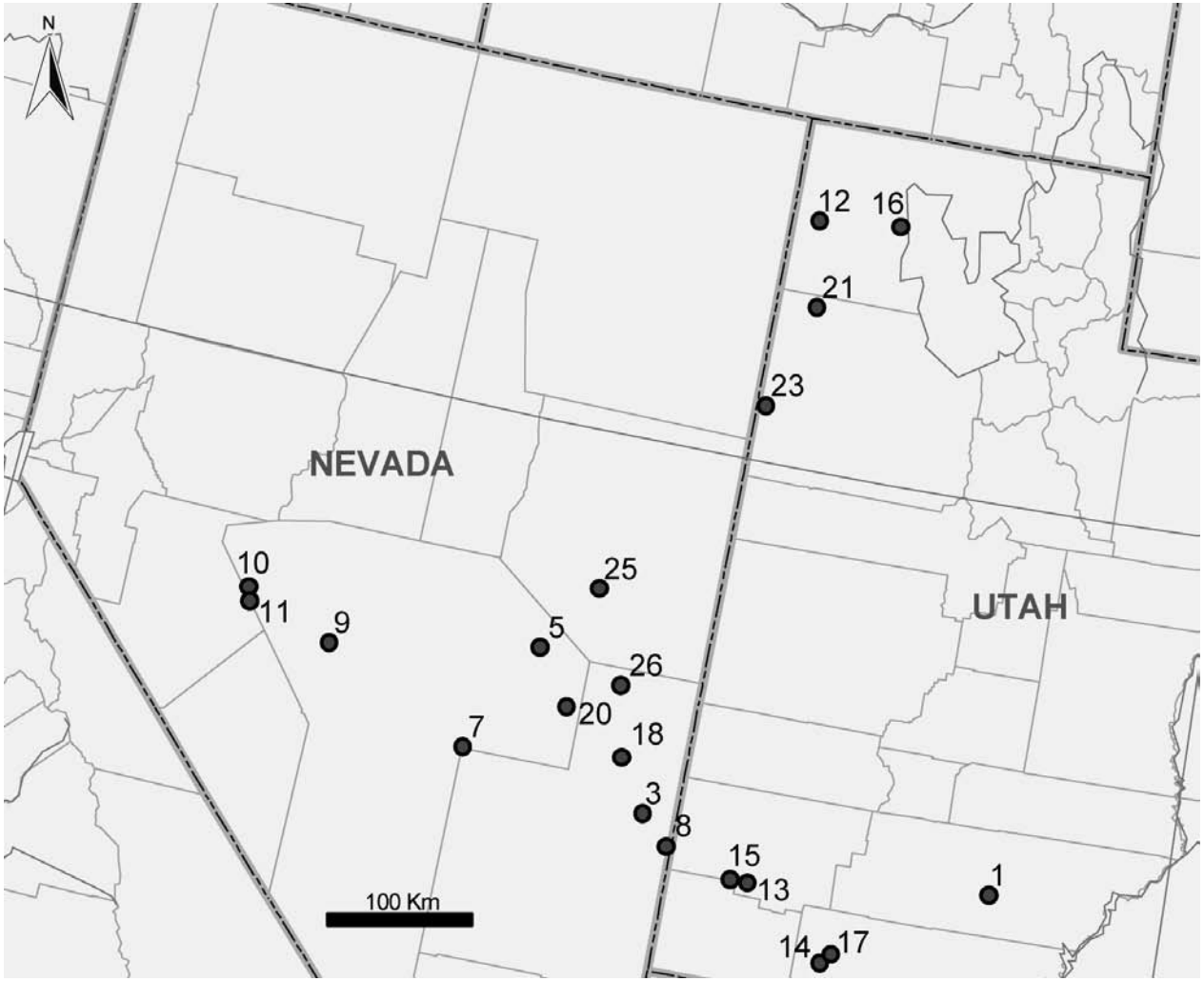

Figure 4-1. Map depicting collection sites of Searls prairie clover (*Dalea searlsiae*).

southern Wisconsin (Oak Prairie Farm, Pardeeville, WI). Measurements were taken during the second and third years after establishment (2007 and 2008).

Dry-matter yield (DMY), number of inflorescences, and flowering date were measured at both locations. Forage quality characteristics, including acid detergent fiber (ADF), neutral detergent fiber (NDF), and crude protein (CP), were evaluated at Hyde Park. Plant height, number of stems, and foliage diameter were also measured at Hyde Park. Potential seed production was estimated at Millville by determining inflorescence weights.

Collections at both locations were evaluated for DMY with two harvests taken during each growing season and combined at each location. At Hyde Park, plants were harvested at approximately 50% bloom (June 19 to 25), and then after the first frost in October (October 17 to 25). At Millville, DMY was obtained after mature inflorescence harvest (26 July 2007, 4 August 2008), and then after the first frost in October (17-26 October). The number of inflorescences was determined at 50% bloom at Hyde Park, and immediately before inflorescence harvest at Millville, in both years. The flowering date was recorded as the number of days from January 1 until the first flower emerged on each plant, and was averaged across the five plants for each replication.

At Hyde Park, plant height, number of stems, and foliage diameter were obtained immediately prior to the first DMY harvest. Foliage diameter was measured at the top of the canopy. Values of NDF, ADF, and CP were determined from the first DMY harvest in 2007. Samples were ground (Cyclotec 1093 Sample Mill, Tecator AB, Hoganas, Sweden) to pass through a 1-mm diameter screen. Ground samples were analyzed for CP using total combustion procedures (LECO TruSpec C/N analyzer, LECO Corp., St.

Joseph, MI). ADF and NDF were analyzed at Agri Analysis Inc. (Leola, PA) following procedures of the AOAC (1990) and Mertens (2002), respectively, as adapted to the Ankom A200 filter-bag technique.

Inflorescence weight was used to estimate potential seed yield at Millville. To minimize bias between early- and late-flowering collections, inflorescences were harvested sequentially from late June to late July each year. Because Searls prairie clover has racemose inflorescences and mature seeds easily disperse from the inflorescence, plants were checked daily, and the mature seed portion was collected until the entire inflorescence was harvested. The inflorescences were bulked by replication, dried in a greenhouse, and weighed.

Analysis of variance was conducted using the MIXED procedure of SAS (SAS Institute Inc., 2004) with collection considered a fixed factor and replication a random factor. Data were checked for normality and homoscedasticity before analysis and subsequently transformed when required. Significance was determined at $\alpha = 5\%$, and mean separations were conducted using the Tukey test. Pearson correlation and Spearman rank correlation coefficients were calculated using the CORR procedure of SAS. Principal component (PC) analysis was conducted on the phenotypic data using the FACTOR procedure of SAS.

Genetic Diversity and Population Structure

Plant tissues were collected from the shoot apical regions of eight individual plants from each collection prior to field transplanting. An additional eight plants of purple prairie clover were sampled for use as an out-group. Tissues were lyophilized and

stored at -20ºC. Approximately 17 mg of dried plant tissue were used for each sample, and DNA was extracted using the Qiagen DNeasy 96-well procedure (Qiagen, Valencia, CA) following the manufacturer's protocol. The concentration and quality of genomic DNA were tested spectrophotometrically and by agarose gel electrophoresis. Genomic DNA concentration was adjusted to 30 ng/µL.

The AFLP procedure followed the method of Vos et al. (1995), but was modified by resolving AFLP products on an ABI3730 capillary instrument (Life Technologies, Foster City, CA). Five selective primer pairs were chosen for analysis: E(*Eco*RI).AGG/M(*Mse*I).CAT, E.AGA/M.CTG, E.AGC/M.CTA, E.AGT/M.CAC, and E.ACG/M.CTG. Amplified products were analyzed with Genescan (ABI, Foster City, CA) at the Center for Integrated Biosystems (Utah State University, Logan, UT). AFLP bands between 50 to 500 bp were scored visually for presence or absence using Genographer software (Benham, 2001).

The percentage of polymorphic loci was estimated using the binary AFLP data. Average within-collection diversity of the collections was estimated with the Dice similarity coefficient (Dice, 1945), and their variances were computed using a SAS macro (Leonard et al., 1999). The similarity value of groups of collections from northwestern Utah and the remaining collections were tested for significance using the MIXED procedure in SAS. This identified genetic diversity differences among collections based on geographical groupings. In addition, the similarity values were correlated with PC scores for each collection using the CORR procedure in SAS.

Hierarchical analysis of molecular variance (AMOVA) was conducted using Arlequin v3.1 software (Excoffier et al., 2005). First, a Φ_{st} pairwise distance matrix was

generated among collections using Dice's coefficient from the binary AFLP data. Dice's coefficient was preferred over the simple matching coefficient because it estimates similarity based only on the band presence in at least one of the two individuals and thus reduces the bias in similarity estimates due to band absence in both individuals (Wong et al., 2001). The pairwise distance matrix was then used as an input file for the AMOVA procedure. In addition, the binary matrix was used to estimate percentage polymorphic loci and a pairwise F_{st} distance matrix with 1,000 bootstrap iterations in AFLP-SURV (Vekemans et al., 2002). The F_{st} matrix was used as an input file for constructing a neighbor-joining (NJ) dendrogram with node bootstrap support using 'NEIGHBOR' and 'CONSENSUS' programs sequentially in PHYLIP software (Felsenstein, 2009).

A model-based Bayesian clustering analysis was conducted to assess population structures, using STRUCTURE v2.2 (Falush et al., 2007) without advanced assignment of collections into populations. The raw binary data were analyzed with the RECESSIVE ALLELES option and the admixture model with correlated marker frequencies, but without population flags. Probabilities of the dataset for a given group from K = 1 through K = 9 were tested with three replications for each level of K. The Markov chain Monte Carlo (MCMC) procedure was used with 30,000 burn-in and 300,000 MCMC steps after burn-in to determine the probability of each structure model. The average estimated log probability of the data was plotted against the K-values to observe structural fit of the data. Additionally, the second-order rate of change of log probability (ΔK) between two successively tested models was plotted against the corresponding number of groups tested as described by Evanno et al. (2005). Because the ΔK procedure requires subtractions of former and latter structures, only groups K = 2 through K = 8 can

be shown when 1 to 9 structures are tested. Groupings of collections found in the STRUCTURE program and NJ tree were further tested with hierarchical AMOVA using Arlequin v3.1.

The spatial distribution of genetic structure was tested for isolation by distance analysis using the Mantel test in the collections. A pairwise linear geographic distance matrix was constructed with Geographic Distance Matrix Generator (Ersts, 2009). The pairwise population genetic distance matrix was constructed as a Φ_{st} matrix, using Arlequin v3.1. Both of these distance matrices were used to estimate a correlation statistic between genetic and geographic distances with IBDWS v3.16 (Jensen et al., 2005), with and without controlling elevation and site characteristics. Statistical significance of the Mantel test was determined by using 1,000 permutations. Distance matrix correlations were also made for phenotypic, elevation, temperature, and precipitation differences. The phenotypic matrix of pairwise Euclidean distances, based on the values of all phenotypic traits, was constructed using the DISTANCE procedure in SAS. Geographic distance, elevation, temperature, and precipitation distance matrices were also created using the DISTANCE procedure in SAS.

RESULTS

Common Gardens

Year by collection interactions were not significant for phenotypic traits measured at Hyde Park and Millville locations, except for the number of inflorescences at the Millville location ($F_{20,271} = 2.37$, $P = 0.0011$). However, for the number of inflorescences at Millville, the Spearman rank correlation was significant ($r = 0.64$, $P = 0.003$), and the

collection by year interaction term was not significant when the purple prairie clover (Dp) check and Ds-11 were removed from the analysis ($F_{18,251} = 1.58$, $P = 0.07$). Therefore, all data were combined across years. Additionally, for flowering date, the collection by location interaction term was not significant. Hence, collections were also combined across locations for this trait. Significant variation was detected for each trait (Table 4-2).

Dry-matter yield, number of inflorescences, and flowering date were measured at both locations. The Dp check exhibited the greatest DMY at both locations (Hyde Park: 227.2 g plot^{-1}, Millville: 184.5 g plot^{-1}), but it did not differ from several Searls prairie clover collections at both locations (Table 4-3). Of the Searls prairie clover collections, Ds-13 had the highest DMY at Hyde Park (197.5 g plot^{-1}), while Ds-16 exhibited the least DMY. At Millville, Ds-14 had the greatest DMY (158.2 g. plot^{-1}), and Ds-07 exhibited the least DMY (38.0 g plot^{-1}). For the number of inflorescences at Hyde Park, Ds-15 had the greatest number of inflorescences (35.9) and Ds-16 had the least number of inflorescences (8.8). At Millville, the Dp check had the greatest number of inflorescences (80.5), but it did not differ from eight Searls prairie clover collections. Among the Searls prairie clover collections, Ds-15 exhibited the greatest number of inflorescences (60.5), and Ds-21 had the least number of inflorescences (22.3). For flowering date, Ds-25 exhibited the earliest flowering at 160.1 days, while the Dp check exhibited the latest flowering date at 173.2 days. As with all traits, the high and low mean values did not differ from those of several other collections (Table 4-3). At Millville, the Dp check had the greatest inflorescence weight (137.1 g plot^{-1}), but it did not differ from Ds-15, Ds-14, Ds-13, and Ds-26 collections. Collections Ds-01 and Ds-

05 had the least inflorescence weight (20.7 g plot^{-1}), but they did not differ from nine other collections.

At Hyde Park, the Dp check had the greatest plant height (43.1 cm), number of stems (23.1), and foliage diameter (59.8 cm), but it did not differ from several other collections (Table 4-3). Among the Searls prairie clover collections, Ds-14 exhibited the greatest plant height (39.0 cm), one of the largest numbers of stems (11.6), and one of the largest foliage diameters (58.2 cm). Collections Ds-07 and Ds-16 had the lowest values for these three traits. As with other traits, these high and low values did not differ from several other collections. Forage quality traits exhibited significant variation among collections (ADF: $P < 0.0001$; NDF: $P = 0.01$; CP: $P = 0.0004$). Values of ADF ranged from 27.9 to 38.7%, NDF from 40.2 to 49.5%, and CP from 16.8 to 20.7% (Table 4-3). In particular, collections Ds-21 and Ds-23 (originating from northwestern Utah, Fig. 4-1) had comparatively high CP values and lower than average ADF and NDF values.

Phenotypic Correlations and Principal Component Analysis

A majority of the field phenotypic traits were significantly correlated with each other (Table 4-4). A high positive correlation was observed between DMY at the two locations ($r = 0.78$, $P < 0.0001$), and DMY at Hyde Park was correlated with all other traits, except for flowering date and NDF. Dry-matter yield at Millville was also correlated with all traits, except for flowering date, ADF, and NDF (Table 4-4). The number of inflorescences at both locations showed a high positive correlation with DMY, inflorescence weight, plant diameter, and ADF, and a negative correlation with flowering date and CP (Table 4-4). Aside from number of inflorescences, flowering date was

Table 4-2. Means and standard deviations (SD) of 20 collections of Searls prairie clover (*Dalea searlsiae*) and a purple prairie clover (*Dalea purpurea*) check with the associated *F*-statistic and *P*-value for phenotypic traits as measured at Hyde Park and Millville, UT, and combined over both locations.

Trait	Mean	SD	*F* statistic[†]
Hyde Park			
Dry-matter yield, g plot^{-1}	121	52.9	$F_{19,277} = 20.26^{**}$
Plant height, cm	31.7	5.5	$F_{19,277} = 11.19^{**}$
Number of stems	10.6	1	$F_{19,277} = 7.26^{**}$
Number of inflorescences	26.8	10.4	$F_{19,277} = 14.07^{**}$
Foliage diameter, cm	48.6	8.2	$F_{19,276} = 11.70^{**}$
Acid detergent fiber (ADF), %	35	2.5	$F_{19,55.1} = 4.82^{**}$
Neutral detergent fiber (NDF), %	46.5	2.5	$F_{19,55.1} = 2.20^{*}$
Crude protein (CP), %	18.3	1.2	$F_{19,55} = 3.71^{**}$
Millville			
Dry-matter yield, g plot^{-1}	94.8	13.7	$F_{20,292} = 13.86^{**}$
Inflorescence weight, g plot^{-1}	46.3	28	$F_{20,289} = 14.81^{**}$
Number of inflorescences	39.5	13.7	$F_{20,292} = 7.52^{**}$
Combined			
Flowering date[†]	164.8	3.7	$F_{20,427} = 8.30^{**}$

* = $P < 0.05$
** = $P < 0.01$
[†]Flowering date is measured from January 1.

correlated with ADF. Plant height was positively correlated with DMY, number of stems, foliage diameter, ADF, and NDF, but negatively correlated with crude protein (Table 4-4). Among forage quality traits, ADF and NDF were positively correlated with each other ($r = 0.74$, $P = 0.0003$); however, CP was negatively correlated with ADF ($r = -0.73$, $P = 0.0003$) and NDF ($r = -0.65$, $P = 0.0028$).

Because of the high correlations among traits, PC analysis was conducted on the phenotypic measurements to reduce the dimension of the dataset and visualize the similarities among the collections. The first three PC axes had eigenvalues greater than one, and each axis was represented by a different set of traits. The three axes explained 87% of the dataset variability (PC1 = 63%, PC2 = 13%, and PC3 = 11%) (Table 4-5). The PC1 axis exhibited correlations with inflorescence weight ($r = 0.95$, $P < 0.0001$), DMY (Hyde Park: $r = 0.78$, $P < 0.0001$; Millville: $r = 0.86$, $P < 0.0001$), number of inflorescences (Hyde Park: $r = 0.61$, $P = 0.0004$; Millville: $r = 0.76$, $P < 0.0001$), foliage diameter ($r = 0.69$, $P = 0.0008$), plant height ($r = 0.48$, $P = 0.032$), and number of stems ($r = 0.46$, $P = 0.041$). The PC2 axis exhibited correlations for forage-quality traits (ADF: $r = 0.76$, $P = 0.0001$; NDF: $r = 0.94$, $P < 0.0001$; CP: $r = -0.72$, $P = 0.0003$) and plant height ($r = 0.45$, $P = 0.044$), and PC3 for flowering date ($r = 0.92$, $P < 0.0001$), number of inflorescences (Hyde Park: $r = -0.64$, $P = 0.0024$; Millville: $r = -0.57$, $P = 0.0092$), and ADF ($r = -0.45$, $P = 0.045$) (Table 4-5).

The three collections with the highest PC1 scores were Ds-15, Ds-14, and Ds-13(Fig. 4-2A), suggesting these southern Utah collections had greater values of DMY, inflorescence weight, number of inflorescences, foliage diameter, plant height, and number of stems from these collections (Table 4-5). Other collections with PC1 scores above the mean (i.e., zero) (Ds-03, Ds-26, Ds-17, Ds-08, and Ds-18) originated from eastern Nevada and southern Utah (Fig. 4-1). Collections Ds-05, Ds-20, Ds-12, and Ds-07 formed a subgroup with the lowest PC1 scores as well as lower forage quality (high PC2 scores). The scatter plot of PC1 and PC3 scores indicated that collections varied widely for flowering date across the range of PC1 (Fig. 4-2B; Table 4-3). Collections

Table 4-3. Means for dry-matter yield (DMY), number of stems, number of inflorescences, plant height, foliage diameter, acid detergent fiber (ADF), neutral detergent fiber (NDF), crude pro tein, inflorescence weight, and flowering date for 20 collections of Searls prairie clover and a purple prairie clover check (Dp) at Millville, Hyde Park, and combined across both locations. Values followed by the same letter within a column are not significantly different at $P = 0.05$.

	Hyde Park								Millville			Combined
Collection	DMY ($g.plot^{-1}$)	No. stems	No. infl.	Plant height (cm)	Foliage diameter (cm)	ADF (%)	NDF (%)	Crude protein (%)	DMY ($g.plot^{-1}$)	No. infl.	Infl. weight ($g.plot^{-1}$)	Flowering date†
Ds-01	-		-	-	-	-	-	-	79.8^{DEFGHI}	29.9^{DEFG}	20.7^{G}	172.9^{ABC}
Ds-03	$134.3^{BCDEF‡}$	10.1^{BC}	32.9^{ABC}	35.6^{ABC}	50.8^{ABCD}	38.7^{A}	48.5^{AB}	17.9^{ABC}	140.0^{ABC}	44.0^{ABCDE}	57.6^{BCD}	$164.0^{ABCDEFG}$
Ds-05	84.3^{FGH}	8.0^{BCD}	15.3^{EFG}	35.0^{ABCD}	40.0^{DEF}	35.4^{ABC}	48.2^{AB}	16.8^{C}	70.4^{GHI}	26.1^{DEFG}	20.7^{G}	168.0^{A}
Ds-07	40.4^{HI}	6.4^{CD}	18.2^{DEFG}	21.2^{FG}	35.6^{EF}	36.1^{ABC}	46.4^{AB}	18.8^{ABC}	38.0^{J}	23.9^{FG}	22.1^{FG}	161.6^{EFG}
Ds-08	126.9^{CDEFG}	10.9^{B}	35.5^{AB}	31.4^{CDE}	59.1^{A}	36.4^{ABC}	47.8^{AB}	17.1^{ABC}	108.8^{BCDEFG}	45.2^{ABCDE}	49.1^{BCDE}	163.1^{BCDEFG}
Ds-09	74.3^{GH}	9.1^{BCD}	20.4^{BCDE}	26.7^{EFG}	49.9^{ABCD}	33.3^{BCD}	40.2^{B}	19.3^{ABC}	63.1^{HIJ}	33.8^{CDEFG}	32.6^{DEFG}	164.4^{ABCDEF}
Ds-10	108.6^{EFG}	11.5^{B}	24.6^{BCDE}	26.1^{EFG}	51.3^{ABCD}	36.6^{ABC}	45.6^{AB}	17.3^{ABC}	78.0^{DEFGHI}	43.1^{ABCDEF}	33.5^{CDEFG}	162.2^{DEFG}
Ds-11	81.2^{FGH}	9.4^{BCD}	24.1^{BCDE}	29.8^{CDEF}	45.9^{BCDEF}	35.3^{ABC}	47.6^{AB}	20.2^{ABC}	65.2^{HIJ}	35.2^{CDEFG}	27.9^{EFG}	165.7^{ABCDEF}
Ds-12	109.0^{EFG}	10.8^{B}	25.4^{BCDE}	32.4^{BCDE}	46.0^{BCDEF}	36.2^{ABC}	47.6^{AB}	17.4^{ABC}	71.4^{FGHI}	32.2^{CDEFG}	29.6^{EFG}	162.4^{DEFG}
Ds-13	195.7^{AB}	10.8^{B}	45.0^{A}	29.3^{CDEFG}	54.7^{ABC}	36.9^{AB}	48.1^{AB}	17.8^{ABC}	101.0^{CDEFGH}	52.3^{ABC}	65.4^{AB}	162.8^{CDEFG}
Ds-14	178.3^{ABCD}	11.6^{B}	25.8^{BCDE}	39.0^{AB}	58.2^{A}	36.2^{ABC}	46.5^{AB}	17.0^{BC}	158.2^{AB}	42.0^{BCDEF}	72.7^{AB}	166.5^{ABCD}
Ds-15	187.8^{ABC}	11.3^{B}	45.9^{A}	33.6^{BCDE}	54.8^{ABC}	36.0^{ABC}	45.3^{AB}	17.3^{ABC}	117.2^{ABCDEF}	60.5^{AB}	82.5^{AB}	161.9^{EFG}
Ds-16	35.1^{I}	5.9^{D}	8.8^{G}	21.3^{G}	34.2^{F}	31.2^{CD}	45.6^{AB}	19.5^{ABC}	48.5^{IJ}	26.8^{EFG}	21.7^{G}	165.1^{ABCDEF}
Ds-17	118.8^{DEFG}	10.1^{BC}	21.1^{CDEF}	33.0^{BCDE}	48.0^{ABCDE}	33.9^{ABCD}	45.1^{AB}	17.7^{ABC}	120.8^{ABCDE}	34.1^{CDEFG}	58.4^{BCD}	167.0^{ABC}
Ds-18	151.8^{ABCDE}	11.0^{B}	35.2^{AB}	35.5^{ABC}	54.5^{ABC}	36.6^{ABC}	46.3^{AB}	18.4^{ABC}	95.9^{CDEFGH}	44.6^{ABCDE}	51.3^{BCD}	161.2^{FG}
Ds-20	116.7^{DEFG}	11.7^{B}	32.6^{ABC}	35.3^{ABC}	44.8^{CDEF}	37.0^{AB}	49.3^{A}	17.1^{ABC}	73.8^{EFGHI}	33.6^{CDEFG}	29.5^{DEFG}	162.5^{DEFG}
Ds-21	58.4^{HI}	8.3^{BCD}	9.8^{FG}	27.2^{DEFG}	38.4^{EF}	27.9^{D}	41.1^{AB}	20.7^{AB}	87.9^{DEFGH}	22.3^{G}	22.7^{G}	169.4^{A}
Ds-23	79.1^{FGH}	8.6^{BCD}	16.3^{DEFG}	29.9^{CDEF}	36.3^{EF}	32.6^{BCD}	43.9^{AB}	20.7^{A}	69.9^{FGHI}	27.8^{DEFG}	30.2^{DEFG}	166.6^{ABCDE}
Ds-25	151.5^{ABCDE}	11.5^{B}	32.5^{ABC}	33.2^{BCDE}	52.7^{ABC}	34.6^{ABCD}	48.5^{AB}	18.1^{ABC}	98.5^{CDEFGH}	44.7^{ABCDE}	46.3^{BCDEF}	160.1^{G}
Ds-26	160.5^{ABCDE}	11.0^{B}	35.2^{AB}	35.3^{ABC}	56.7^{AB}	36.5^{ABC}	49.5^{A}	17.9^{ABC}	120.5^{ABCD}	46.7^{ABCD}	61.3^{ABC}	160.4^{FG}
Dp	227.2^{A}	23.1^{A}	30.8^{ABCD}	43.1^{A}	59.8^{AB}	32.7^{ABCD}	48.6^{AB}	18.7^{ABC}	184.5^{A}	80.5^{A}	137.1^{A}	173.2^{AB}

†Flowering date was measured from January 1.

‡Values followed by the same letter within a column are not significantly different at $P = 0.05$.

Table 4-4. Pearson correlation coefficients (*r*) with their associated *P* values for 20 collections of Searls prairie clover (*Dalea searlsiae*) for dry-matter yield (DMY) and the number of inflorescences (NFL) at Hyde Park (HP) and Millville (MV), inflorescence weight (INFLW), flowering date (FLD), plant height (HT), number of stems (ST), foliage diameter (FD), ADF, NDF, and crude protein (CP).

	DMY_HP	DMY_MV	NFL_HP	NFL_MV	INFLW	FLD	HT	ST	FD	ADF	NDF
DMY_MV	0.78***†										
NFL_HP	0.88***	0.53*									
NFL_MV	0.89***	0.64**	0.92***								
INFLW	0.90***	0.86***	0.76***	0.86***							
FLD	-0.43^{ns}	-0.02^{ns}	-0.67**	-0.58**	-0.30^{ns}						
HT	0.71***	0.74***	0.50*	0.45^{ns}	0.58^{ns}	-0.05^{ns}					
ST	0.83***	0.64**	0.76***	0.74***	0.64**	-0.44^{ns}	0.71***				
FD	0.86***	0.74***	0.80***	0.86***	0.80***	-0.46*	0.60**	0.84***			
ADF	0.62**	0.41^{ns}	0.71***	0.58**	0.49*	-0.60**	0.52*	0.58**	0.57*		
NDF	0.36^{ns}	0.30^{ns}	0.39^{ns}	0.23^{ns}	0.24^{ns}	-0.37^{ns}	0.50*	0.33^{ns}	0.31^{ns}	0.74***	
CP	-0.59**	-0.46*	-0.52*	-0.47*	-0.45^{ns}	0.36^{ns}	-0.54*	-0.58**	-0.57*	-0.73***	-0.65**

$^{*}P < 0.05$.
$^{**}P < 0.01$.
$^{***}P < 0.001$.
†ns, not significant ($P > 0.05$).
‡Flowering date is measured from January 1.

Table 4-5. Phenotypic traits used in principal component analysis with their respective loadings for the first three principal component axes. Eigenvalues and the cumulative proportion of the total variation of the measured trait data set are given for each principal component axis.

Traits	PC1	PC2	PC3
Eigenvalue	4.28	2.54	2.32
Cumulative proportion	0.63	0.76	0.87
Dry-matter yield, Hyde Park	0.78***†	0.25^{ns}	-0.33^{ns}
Dry-matter yield, Millville	0.86***	0.25^{ns}	0.17^{ns}
No. of inflorescences, Hyde Park	0.61**	0.26^{ns}	-0.64**
No. of inflorescences, Millville	0.76***	0.10^{ns}	-0.57**
Inflorescence weight	0.95***	0.16^{ns}	-0.19^{ns}
Flowering date	-0.07^{ns}	-0.26^{ns}	0.92***
Plant height	0.48*	0.45*	0.17^{ns}
No. of stems	0.46*	0.20^{ns}	-0.37^{ns}
Foliage diameter	0.69***	0.18^{ns}	-0.39^{ns}
Acid detergent fiber	0.29^{ns}	0.75***	-0.45*
Neutral detergent fiber	0.08^{ns}	0.94***	-0.11^{ns}
Crude protein	-0.24^{ns}	-0.72***	0.20^{ns}

$^{*}P < 0.05$.
$^{**}P < 0.01$.
$^{***}P < 0.001$.
†ns, not significant ($P > 0.05$).
‡Flowering date is measured from January 1.

Ds-20, Ds-12, and Ds-07 remained grouped with an earlier flowering date than Ds-05 (Fig. 4-2B; Table 4-3). A strong positive correlation ($r = 0.76$; $P = 0.0002$) was observed between PC1 scores and precipitation at the collection sites (Fig. 4-2C). Significant correlations were also found between PC3 scores and collection-site elevation ($r = -0.50$, $P = 0.0285$) and temperature ($r = 0.50$, $P = 0.03$). The PC2 score was not correlated with any collection site characteristic (Table 4-6).

Genetic Diversity and Population Structure

The AFLP fingerprinting generated 474 polymorphic DNA bands. The correlation between band sizes and frequencies was not significant ($r = 0.03$, $P = 0.45$), suggesting the absence of size homoplasy in AFLP markers (Vekemans et al., 2002). The number of bands per collection ranged from 91 to 111, and the percentage of polymorphic loci within collections varied from 25 to 39% (Table 4-1). Four collections from northwestern Utah (Ds-12, Ds-16, Ds-21, and Ds-23) exhibited the lowest percentage of polymorphism. Similarly, the mean similarity index (S-value) among individuals within each collection ranged from 0.64 to 0.77, with the collections from northwestern Utah (Ds-12, Ds-16, DS-21, and Ds-23) having the highest within-collection similarity (or low genetic diversity) (Table 4-1). The analysis of variance of S-values of groups indicated significant variation ($F_{1,18} = 63.9$, $P < 0.001$) between the four collections from northwestern Utah compared to all other collections. In addition, the amount of within-collection genetic similarity, the opposite of heterozygosity, exhibited no correlations with PC axes (Table 4-6).

Analysis of molecular variance apportioned 24% ($P < 0.0001$) of the genetic variation among collections and 76% ($P < 0.0001$) of the variation within collections. Genetic relationships among collections were visualized using a neighbor-joining (NJ) tree of pairwise *F*st values. Three groups of collections were found to have bootstrap support: one from northwestern Utah (Ds-12, Ds-16, Ds-21, and Ds-23), one from western Nevada (Ds-09, Ds-10, and Ds-11), and one from southern Utah (Ds-13, Ds-15, and Ds-17) (Fig. 4-3). The northwestern Utah, southern Utah, and western Nevada groups were supported by bootstrap value of 100, 86, and 75%, respectively.

Table 4-6. Pearson correlation coefficients (*r*) with the associated *P* value for 20 collections of Searls prairie clover (*Dalea searlsiae*) for the first principal component score (PC1), second principal score (PC2), and third principal component score (PC3) associated with collection-site elevation, temperature, and precipitation, and within-collection genetic similarity.

	Elevation	Temperature	Precipitation	Similarity[‡]
PC1	0.26[ns†]	0.17[ns]	0.76[***]	-0.23[ns]
PC2	0.11[ns]	0.15[ns]	0.31[ns]	-0.39[ns]
PC3	-0.50[*]	0.50[*]	-0.11[ns]	0.24[ns]

[*] $P < 0.05$.
[***] $P < 0.001$.
[†] ns, not significant ($P > 0.05$).
[‡] Similarity coefficients were estimated based on AFLP markers following Leonard et al. 1999.

Bayesian clustering of collections based on AFLP data showed a smaller increase in average log-likelihood value after the two-group test (Fig. 4-4A), and the second-order rate of change in the log-likelihood values showed a mode at the K = 2 structure (Fig. 4-4B). Of the two groups, the first included the four collections from northwestern Utah and the second included all other collections from southern Utah and Nevada (Figs. 4-1 and 4-4C). Little indication of admixed coancestry coefficients was detected between the two groups (Fig. 4-4C). When hierarchical AMOVA was used to test the partitioning of genetic variation among these two groups, the amount of variation apportioned among the two groups was 14% ($P < 0.001$), among populations within groups was 17% ($P < 0.001$), and within populations was 69% ($P < 0.001$).

Because both the NJ dendrogram and the Bayesian clustering results indicated that geographic distances tended to separate genetic structures, isolation by distance was examined using the genetic and linear geographic distances. The genetic-distance matrix was highly correlated with the geographic-distance matrix ($r = 0.77$, $P < 0.001$), and the

correlation was still highly significant after controlling for the effect of elevation (r = 0.76, $P < 0.001$), precipitation (r = 0.71, $P < 0.001$), or temperature (r = 0.75, $P < 0.001$). Conversely, after controlling for the linear geographic-distance effect, the genetic-distance matrix was not correlated with precipitation (r = -0.01, P = 0.49) and temperature (r = 0.16, P = 0.12), but a low correlation (r = 0.30, P = 0.011) was found with collection-site elevation. A significant correlation (r = 0.37, P = 0.005) was also observed between genetic and phenotypic distance matrices.

DISCUSSION

Currently, few forbs from western North America are available for rangeland revegetation (Walker and Shaw, 2005), especially legumes for semi-arid rangelands within the Great Basin. Land managers are interested in legumes for revegetation because of their ability to fix nitrogen, their high-quality forage for wildlife and livestock, and the unique ecological niche they occupy (Pokorny et al., 2005; Walker and Shaw, 2005). One large hurdle in providing legumes for revegetation is the economical production of seed of reliable quality and quantity in commercial settings, which requires mechanical harvest. Our Searls prairie clover collections exhibited significant variation for each of the traits measured in the common-garden studies (Table 4-2), and several exhibited agronomic traits comparable to commercially harvested purple prairie clover (Table 4-3). Greater plant height and potential seed yield are desirable traits for commercial seed production, whereas greater DMY is desirable when the revegetation goal is to provide forage for livestock and wildlife. These three traits had high loadings in PC1 and PC3 and these PC axes explained a majority of the total variation in the data

(Table 4-5). Collections with high PC1 scores could be used for germplasm release or selection and breeding when the goal is to increase seed and forage availability to livestock and wildlife. Interestingly, PC1 and PC3 also represented other highly correlated traits such as the number of inflorescences, inflorescence weight, number of stems, and foliage diameter (Table 4-4). These high positive correlations of foliage diameter or number of stems with DMY and inflorescence weight suggests that foliage diameter or number of stems could be used as surrogate traits to select for DMY and seed yield potential.

Flowering date for the collections ranged from 160 to 173 days (Table 4-3), i.e., from June 8 to 20. Because most precipitation in the Great Basin comes in the form of snow or early-spring rain, early growth can be critical for utilizing available water. Two collections from eastern Nevada (Ds-25 and Ds-26) exhibited the earliest flowering dates compared to other collections and may, therefore, be good candidates for revegetation in drier areas of the Great Basin. Flowering date also showed a high loading for PC3 (Table 4-5), which in turn was negatively correlated with elevation (r = -0.50, P = 0.0285) and positively correlated with mean annual temperature (r = 0.50, P = 0.03). These relationships suggest that earlier flowering generally occurred in collections that originated from sites at high elevation and low mean annual temperature.

Greater DMY is associated with lower forage quality within a species for many plant species (White and Wight, 1984; van der Wal et al., 2000; Bhattarai et al., 2008). Crude protein concentration is generally negatively correlated with DMY, ADF, and NDF. As plants mature, their leaf-to-shoot ratios decrease, increasing DMY, ADF, and NDF, but decreasing CP (Romero et al., 1987). Additionally, as plants age, the

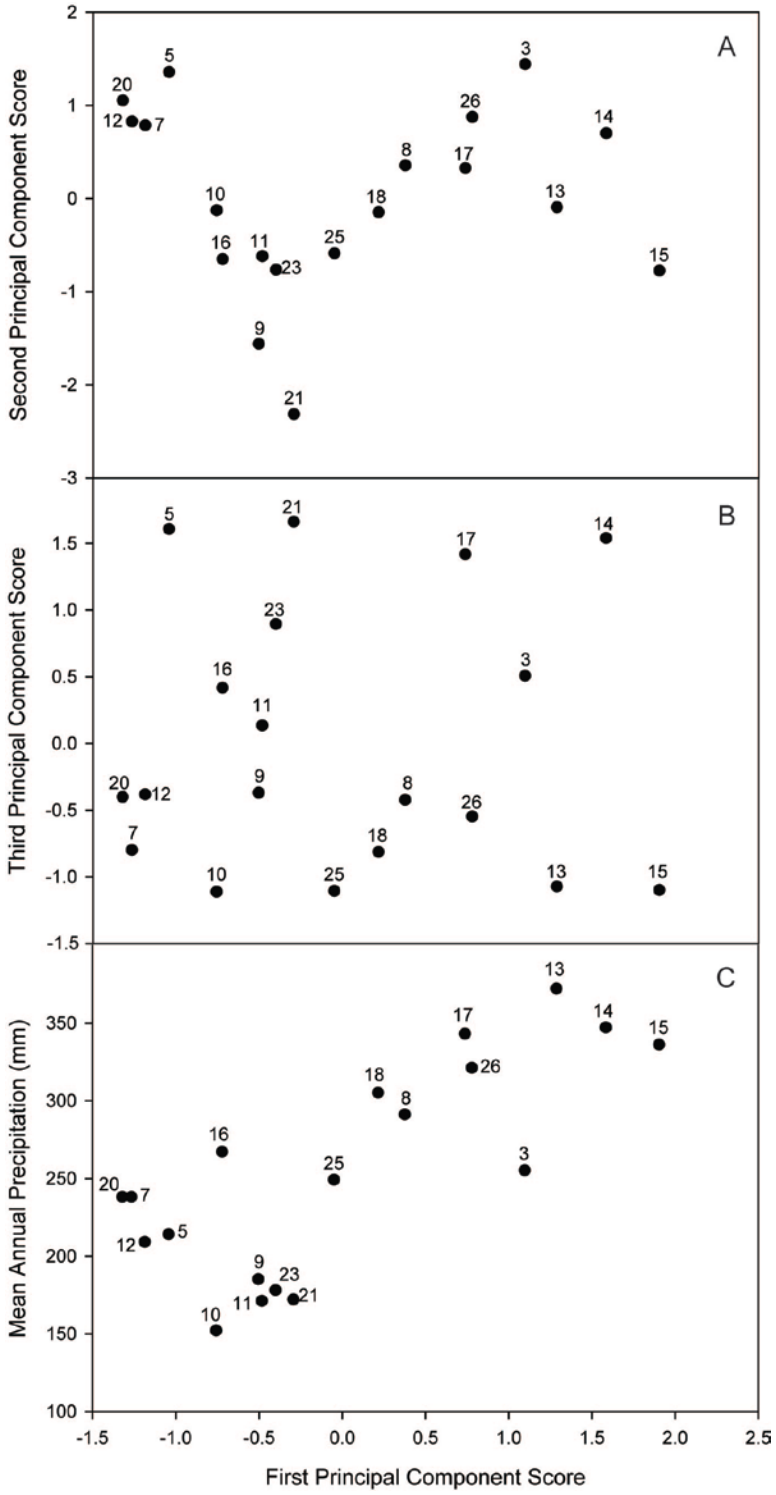

Figure 4-2. Scatter plots of the first principal component score with: A) second principal component score, B) third principal component score, and C) mean annual precipitation of the collection sites for Searls prairie clover (*Dalea searlsiae*).

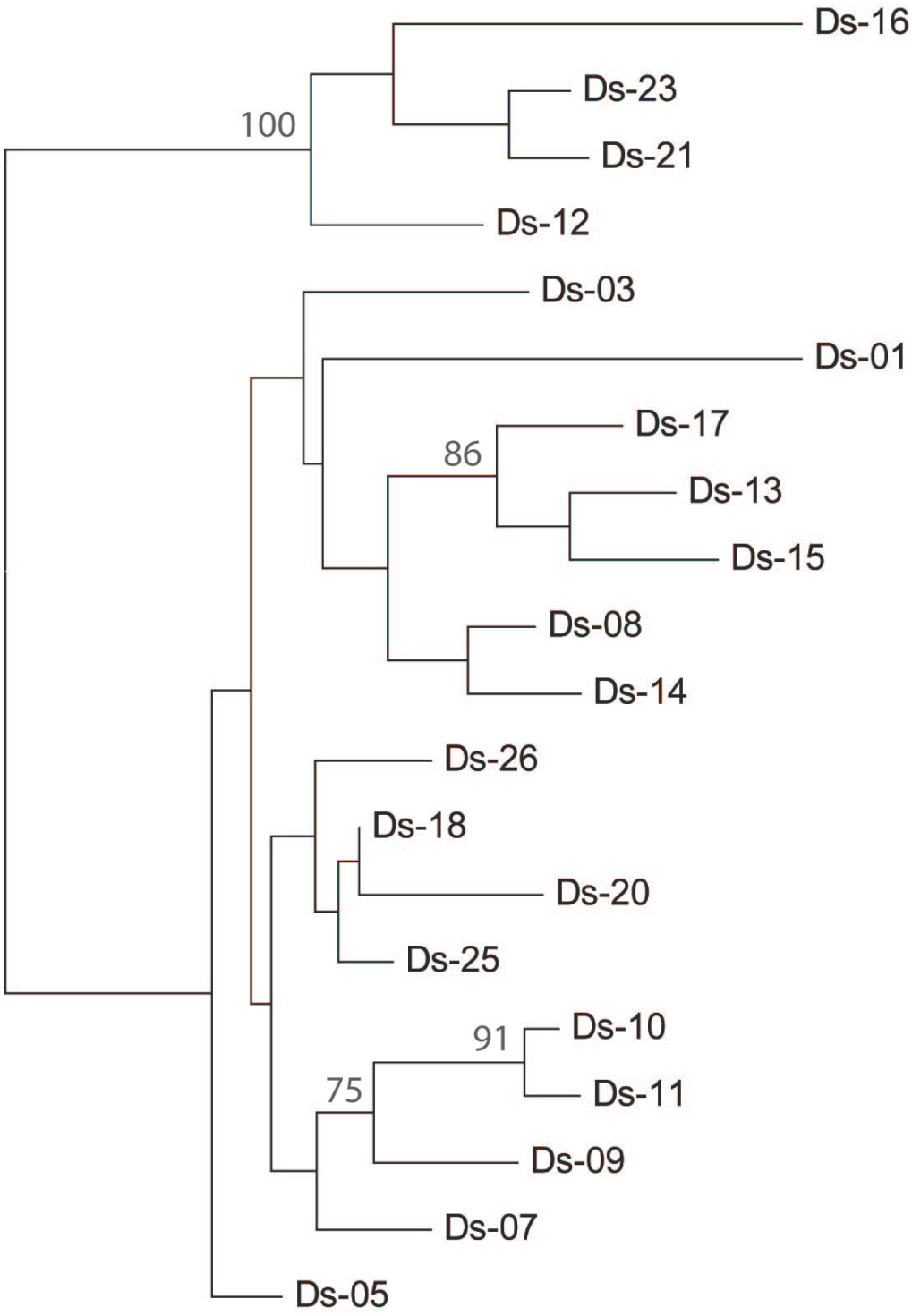

Figure 4-3. Neighbor-joining dendrogram obtained from a Φst genetic distance matrix among 20 collections of Searls prairie clover (*Dalea searlsiae*) with associated bootstrap values shown when greater than 75%.

proportion of ADF in a plant increases more rapidly than NDF (Hart et al., 1983). Because collections with greater DMY did not exhibit variation in ADF (15 out of 19 collections did not differ from each other), and NDF was not correlated with DMY, reductions in forage quality should not be a major concern when selecting for high DMY in Searls prairie clover.

In our study, 76% ($P < 0.0001$) of the genetic variation was apportioned within collections, highlighting that the majority of genetic variation is present within populations for this primarily outcrossing species (Jim Cane, 2009, personal communication). Using a within-collection genetic similarity index, collections from northwestern Utah exhibited lower genetic diversity (higher genetic similarity) compared to the other collections (Table 4-1 and Fig. 4-1). Collections from northwestern Utah represented the northern distribution of Searls prairie clover, and the lower genetic diversity among them suggests a possible founder effect or environmental stochasticity (Ray, 2001).

Although a general trend of a significant positive correlation was reported between genetic diversity and population fitness in meta-analysis studies (Reed and Frankham, 2003; Leimu et al., 2006), no correlation was also observed between genetic diversity and population fitness in *Clematis acerifolia* Maxim. (Lopez-Pujol et al., 2008). For the Searls prairie clover collections, correlation coefficients between PC scores obtained from phenotypic measurements and within-collection genetic similarity, the opposite of genetic diversity, obtained from AFLP analysis were not significant. This suggests that greater genetic diversity was not associated with greater plant size and fecundity (or fitness) in Searls prairie clover. However, the four collections from

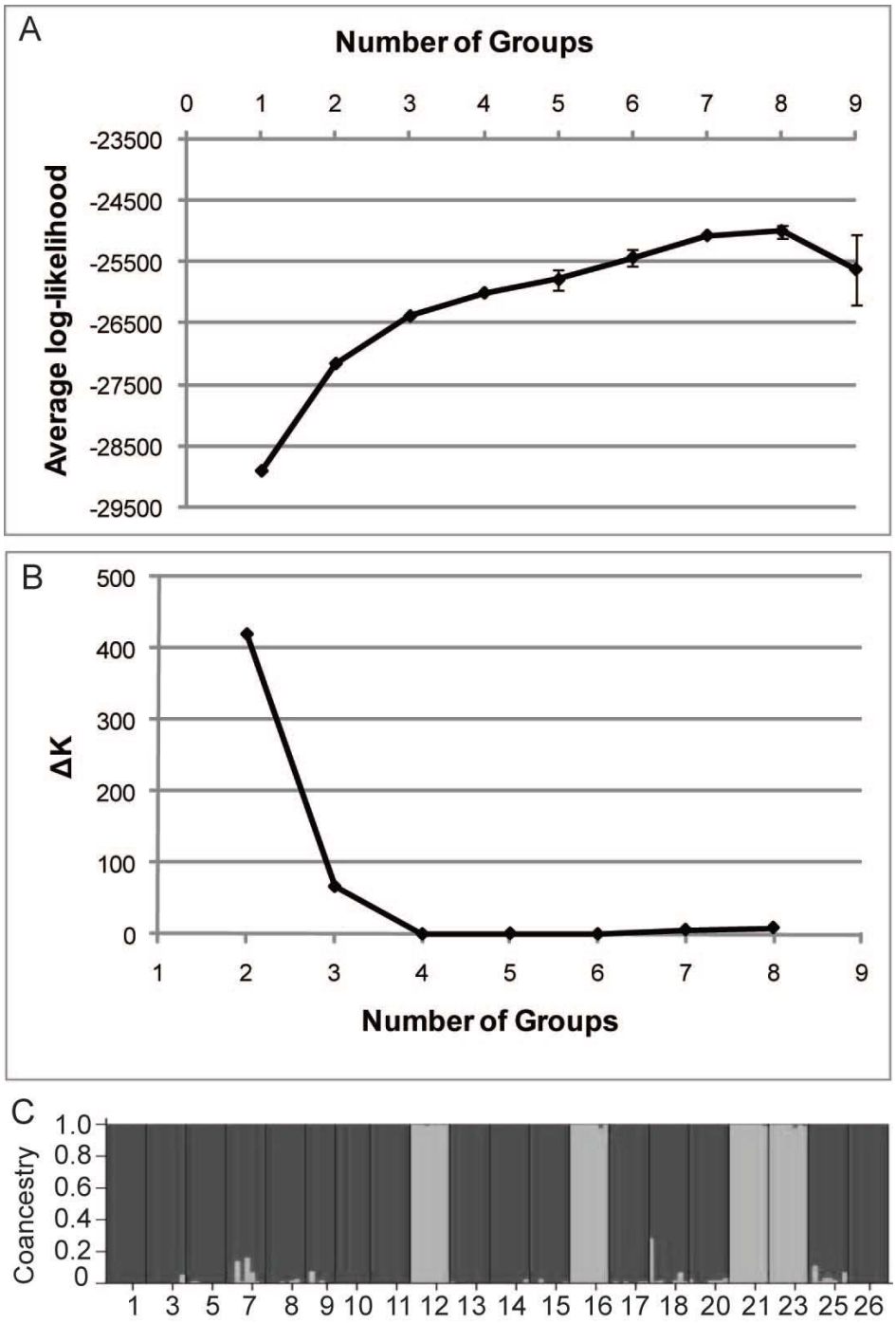

Figure 4-4. Bayesian clustering of Searls prairie clover (*Dalea searlsiae*) collections, testing from 1 to 9 groups. A) average log-likelihood values of each tested group, B) the second order of rate of change in the log-likelihood value of the subsequently tested group, and C) the bar plot of collections grouped at K = 2.

northwestern Utah (Ds-12, Ds-16, Ds-21, and Ds-23) exhibited the least genetic diversity and correspondingly showed lower fitness (smaller size and lower fecundity) (Tables 4-1 and 4-3).

Significant differences were also detected among populations with an overall Φst of 24% ($P < 0.0001$), indicating the presence of population structure. Three groups of collections with bootstrap support were observed in the NJ dendrogram (Fig. 4-3), and the group comprising the four northwestern Utah collections was also detected with Bayesian clustering (Fig. 4-4C). The northwestern Utah group was further supported by hierarchical AMOVA, with 14% ($P < 0.0001$) of the variation apportioned among groups when compared to the remaining 16 collections in Nevada and southern Utah. Thus, our data indicate that collections from northwestern Utah formed a genetically differentiated group and that this group has lower within-collection genetic diversity (higher similarity) when compared to the other collections.

The most diverse (lowest within-collection similarity) collections of Searls prairie clover were found across Nevada and southern Utah (Table 4-1 and Fig. 4-1). Within these collections, two groups located in western Nevada (Ds-09, Ds-10, and Ds-11) and southern Utah (Ds-13, Ds-15, and Ds-17) were identified with bootstrap support in the NJ tree (Figs. 4-1 and 4-2), but were not distinctive with the Bayesian clustering (Fig. 4-4C). The three southern Utah collections had some of the highest PC1 scores among all 20 collections, while the western Nevada collections had relatively low PC1 scores (Fig. 4-2). These two groups of collections were on the east-west periphery of our collection sites, and showed a trend toward isolation. Indeed, the overall significant isolation by distance found among these Searls prairie clover collections highlights this trend.

Although isolation by distance can occur when a distribution of populations is greater than gene flow (Johnson and Black, 1998), it may take populations considerable time to achieve this state (Slatkin, 1993). Isolation by distance has been reported for many plant species (Raspe and Jacquemart, 1998; Stöcklin et al., 2009), while several other studies failed to find this relationship (Franceschinelli and Kesseli, 1999; Albaladejo et al., 2009). The strong genetically differentiated group in northwestern Utah and the indication of isolation by distance for east and west peripheral collections indicates that gene flow is playing an important role in shaping the existing genetic structure in these Searls prairie clover collections.

If phenotypic traits are correlated with environmental characteristics at the collection site, these traits are considered to have potential adaptive significance (Endler, 1986). A high correlation between the PC1 scores and collection-site precipitation (r = 0.76, P = 0.0002) suggests that, if plants from high-precipitation sites are transplanted to sites with low precipitation, the traits represented by PC1 may be adversely affected. One challenge with common-garden plots is their inability to represent all the environments wherein the species is distributed, due to the need for intensive data collection and practical limitations and some confounding with phenotypic plasticity is inevitable. In this study, the mean annual precipitation at the collections sites varied from 152 to 372 mm, while precipitation at the common-garden locations was approximately 340 mm. The strongest genetically differentiated group (four collections from northwestern Utah) came from areas with low relative precipitation and had low PC1 scores (Table 4-1 and Fig. 4-2), such that a seed source developed within this area would represent a distinct genetic structure and environmental character. The larger group

comprising Nevada and southern Utah included collections from a range of precipitation, and therefore further testing should be conducted to determine if gene flow within that group has constrained local adaptive potential in areas with extremely low precipitation (McKay and Latta, 2002).

The genetic distance matrix was not associated with differences in precipitation and temperature, but it was correlated with phenotype ($r = 0.37$, $P = 0.005$) and elevation ($r = 0.30$, $P = 0.01$) differences (Table 4-6). The positive but weaker correlation between phenotypic and AFLP-marker based genetic distance matrices, indicated that evolutionary forces responsible for diversifying AFLP markers may be partly involved in diversifying the measured phenotypic traits among the collections. Perhaps one source of the positive correlation stems from the low phenotypic values (Table 4-3) found in the strongest genetically differentiated group (four collections from northwestern Utah). Indeed, the weak correlation between genetic distance and elevation was also probably observed because the genetically differentiated group of collections from northwestern Utah was from lower-elevation sites. However, within the larger genetic group of collections from southern Utah and Nevada, elevation did not appear to play a role in genetic variation when the four northwestern Utah collections were removed from the analysis ($r = 0.11$, $P = 0.82$).

In summary, a portion of collections of Searls prairie clover exhibited comparable agronomic traits to commercially available purple prairie clover, suggesting that Searls prairie clover has potential for commercial seed production. From a restoration and/or revegetation perspective, the development of regional seed sources would attenuate concerns of outbreeding depression and maladapation. The two genetically differentiated

groups identified by AFLP markers defined two seed sources, one from northwestern Utah and the other encompassing Nevada and southern Utah. However, the three westernmost Nevada collections (Ds-9, Ds-10, and Ds-11) shared low phenotypic values and low collection-site precipitation with the northwestern Utah collections, and precipitation was identified as an environmental character with possible local adaptive significance. Thus the three western Nevada collections may reside in different seed source groups based upon molecular or phenotypic information, such that further reciprocal transplant research may be necessary to place the western Nevada area into an appropriate regional seed source.

LITERATURE CITED

Albaladejo, R.G., L.F. Carrillo, A. Aparicio, J.F. Fernández-Manjarrés and J.P. González-Varo. 2009. Population genetic structure in *Myrtus communis* L. in a chronically fragmented landscape in the Mediterranean: can gene flow counteract habitat perturbation? Plant Biol. 11:442-453.

Association of Official Analytical Chemists [AOAC]. 1990. Official methods of analysis. Arlington, VA, USA: Association of Official Analytical Chemists. 1141 p.

Barneby, R. C. 1977. *Dalea* imagines. Memoirs of the New York Botanical Garden 27:1-892.

Benham, J.J. 2001. Genographer- AFLP/Microsatellite Software, v. 1.6.0. Available from http://hordeum.oscs.montana.edu/genographer/. Accessed 14 October 2007.

Bhattarai, K., D.A. Johnson, T.A. Jones, K.J. Connors, and D.R. Gardner. 2008. Physiological and morphological characterization of basalt milkvetch (*Astragalus filipes*): basis for plant improvement. Rangeland Ecol. Manag. 61:444-455.

Bonin, A., D. Ehrich, and S. Manel. 2007. Statistical analysis of amplified fragment length polymorphism data: a toolbox for molecular ecologists and evolutionists. Mol. Ecol. 16:3737-3758.

Broadhurst, L.M., A. Lowe, D.J. Coates, A.A Cunningham, M. McDonald, P.A. Vesk, and C. Yates. 2008. Seed supply for broadscale restoration: maximizing evolutionary potential. Evol. Appl. 1:587-597.

Cherney, J.H., and V.G. Allen. 1995. Forages in a livestock system. p. 175-188. *In*: R. F. Barnes, D. A. Miller, and C. J. Nelson (ed.). Forages: the science of grassland agriculture. Volume I. Iowa State University Press, Ames, IA, USA.

Dice, L.R. 1945. Measures of the amount of ecologic association between species. Ecology 26:297-302.

Endler, J.A. 1986. Natural selection in the wild. Princeton University Press, Princeton, NJ, USA.

Ersts, P.J. 2009. Geographic Distance Matrix Generator. Available at: http://biodiversityinformatics.amnh.org/open_source/gdmg. Accessed 8 October 2009.

Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCUTRE: a simulation study. Mol. Ecol. 14:2611-2620.

Excoffier, L., G. Laval, and S. Schneider. 2005. Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evol. Bioinform. Online 1:47-50.

Falush, D., M. Stephens, and J.K. Pritchard. 2007. Inference of population structure using multilocus genotype data: dominant markers and null alleles. Mol. Ecol. Notes 7:574–578.

Felsenstein, J. 2009. PHYLIP (Phylogeny Inference Package) version 3.69. Distributed by Author, Department of Genome Sciences: University of Washington, Seattle, WA.

Franceschinelli, E.V., and R. Kesseli. 1999. Population structure and gene flow of the Brazilian shrub *Helicteres brevispira*. Heredity 82:355-363.

Hart, R.H., O.M. Abdalla, D.H. Clark, M.B. Marshall, M.H. Hamid, J.A. Hager, and J.W. Waggoner, Jr. 1983. Quality of forage and cattle diets on the Wyoming High Plains. J. Range. Manage. 36:46-51.

Jensen, J.L., A.J. Bohonak, and S.T. Kelley. 2005. Isolation by distance, web service. BMC Genet. 6:13.

Jessop, B.D., and V.J. Anderson. 2007. Cheatgrass invasion in salt desert shrublands: benefits of postfire reclamation. Rangeland Ecol. Manag. 60:235-243.

Johnson, M.S., and R. Black. 1998. Effects of isolation by distance and geographical discontinuity on genetic subdivision of *Littoraria cingulata.* Mar. Biol. 132:295–303.

Johnson, G.R., F.C. Sorensen, J.B. St Clair, and R.C. Cronn. 2004. Pacific Northwest forest tree seed zones: a template for native plants? Native Plant J. 5:131-140.

Kittlein, M.J., and O.E. Gaggiotti. 2008. Interactions between environmental factors can hide isolation by distance patterns: a case study of *Ctenomys rionegrensis* in Uruguay. Proc. Royal Soc. B 275:2633-2638.

Leimu, R., P. Mutikainen, J. Koricheva, and M. Fischer. 2006. How general are positive relationships between plant population size, fitness and genetic variation? J. Ecol. 94:942-952.

Leonard, A.C., S.E. Franson, V.S. Hertzberg, M.K. Smith, and G.P. Toth. 1999. Hypothesis testing with the similarity index. Mol. Ecol. 8:2105-2114.

Lesica, P., and F.W. Allendorf. 1999. Ecological genetics and the restoration of plant communities: mix or match? Restor. Ecol. 7:42-50.

Lopez-Pujol, J., P.M. Zhang, and S. Ge. 2008. No correlation between heterozygosity and vegetative fitness in the narrow endemic and critically endangered *Clematis acerifolia* (Ranunculaceae). Biochem. Genet. 46:433-445.

Madison, L.A., and R.J. Robel. 2001. Energy characteristics and consumption of several seeds recommended for northern bobwhite food plantings. Wildlife Soc. B 29:1219-1227.

Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. Cancer Res. 27:209-220.

McKay, J.K., C.E. Christian, S.P. Harrison, and K.J. Rice. 2005. "How local is local?" - A review of practical and conceptual issues in the genetics of restoration. Restor. Ecol. 1:432-40.

McKay, J. K., and G. Latta. 2002. Adaptive population divergence: markers, QTL and traits. Trends Ecol. Evol. 17:185-291.

Mertens, D.R. 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. J. AOAC Int. 85:1217-1240.

Moscow Forestry Sciences Laboratory. 2009. Custom climate data requests. Available at: http://forest.moscowfsl.wsu.edu/climate/customData/. Accessed 6 October 2009.

Ouborg, N.J., P. Vergeer, and C. Mix. 2006. The rough edges of the conservation genetics paradigm for plants. J Ecol. 94:1233-1248.

Pokorny, M.L., R.L. Sheley, C.A. Zabinski, R.E. Engel, T.J. Svejcar, and J.J. Brokowski. 2005. Plant functional group diversity as a mechanism for invasion resistance. Restor. Ecol. 13:448-459.

Raspe, O., and A.L. Jacquemart. 1998. Allozyme diversity and genetic structure of European populations of *Sorbus aucuparia* L. (Rosaceae: Maloideae). Heredity 81:537-545.

Ray, C. 2001. Maintaining genetic diversity despite local extinctions: effects of population scale. Biol. Conserv. 100:3-14.

Reed, D.H., and R. Frankham. 2003. Correlation between fitness and genetic diversity. Conserv. Biol. 17:230-237.

Romero, F., H.H. Van Horn, G.M. Prine, and E.C. French. 1987. Effect of cutting interval upon yield, composition, and digestibility of Florida 77 Alfalfa and Florigraze rhizome peanut. J. Anim. Sci. 65:786-796.

SAS Institute. 2004. SAS/STAT 9.1 user's guide. Cary, NC, USA: SAS Institute, Inc.

Sheley, R.L., and M.F. Carpinelli. 2005. Creating weed-resistant plant communities using niche-differentiated nonnative species. Rangeland Ecol. Manag. 58:480-488.

Slatkin, M. 1993. Isolation by distance in equilibrium and non-equilibrium populations. Evolution 47:264-279.

Stein, B., L.S. Kutner, and J.S. Adams. 2000. Precious heritage: the status of biodiversity in the United States. Oxford University Press, New York.

Stöcklin, J., P. Kuss, and A. R. Pluess. 2009. Genetic diversity, phenotypic variation and local adaptation in the alpine landscape: case studies with alpine plant species. Bot. Helv. 119:125-133.

Telles, M.P.C, and J.A.F. Diniz-Filho. 2005. Multiple Mantel tests and isolation-by-distance, taking into account long-term historical divergence. Genet. Mol. Res. 4:742-748.

USDA, NRCS. 2010. The Plants Database. National Plant Data Center, Baton Rouge, LA, USA. Available at: http://plants.usda.gov/java/profile?symbol=DALEA. Accessed 30 February 2010.

van der Heijden, M.G.A., R. Bakker, J. Verwaal, T.R. Scheublin, M. Rutten, R. van Logtestijn, and C. Staehelin. 2006. Symbiotic bacteria as a determinant of plant community structure and plant productivity in dune grassland. FEMS Microbiol. Ecol. 56:178-187.

van der Wal, R.S., C. van Lieshout, C. Dormann, R. Langvatn, and S.D. Albon. 2000. Trading forage quality for quality? Plant phenology and patch choice by Svalbard reindeer. Oecologia 123:108–115.

Vekemans, X., T. Beauwens, M. Lemaire, and I. Roldan-Ruiz. 2002. Data from amplified fragment length polymorphism (AFLP) markers show indication of size

homoplasy and of a relationship between degree of homoplasy and fragment size. Mol. Ecol. 11:139-151.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, and M. Kuiper. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. 23:4407-4414.

Walker, S.C., and N.L. Shaw. 2005. Current and potential use of broadleaf herbs for reestablishing native communities. p. 46-61. *In*: N. L. Shaw, M. Pellant, and S. B. Monsen (ed.). Proceedings, Sage Grouse Habitat Restoration Symposium. Fort Collins, CO: USDA Forest Service, Rocky Mountain Research Station. Proceedings RMRS-P-38.

White, L.M., and J.R. Wight. 1984. Forage yield and quality of dryland grasses and legumes. J. Range. Manage. 37:233–236.

Wong, A., M.R. Forbes, and M.L. Smith. 2001. Characterization of AFLP markers in damselflies: prevalence of codominant markers and implications for population genetic applications. Genome 44:677-684.

CHAPTER 5

CONCLUSIONS

Currently, few forbs from western North America are available for revegetation efforts, and this applies especially to legumes for the semi-arid rangelands of the Great Basin. Legumes for rangeland revegetation should ideally have a broad distribution to minimize unanticipated crossing with related species, be adapted to and compatible in the range of sites they will be planted in, be amenable to agronomic seed production, and preferably be safe for grazing animals. These criteria, when met, will minimize the concern relating to out-breeding depression, introduction of maladapted genes, and reduction of forage quality due to introduction of toxic plants. Western prairie clover [*Dalea ornata* (Douglas ex Hook.) Eaton & J. Wright] and Searls prairie clover [*Dalea searlsiae* (A. Gray) Barneby] hold promise because they are not toxic to livestock and they possess a wide range of natural habitats. One large hurdle in providing legumes for revegetation is the economical production of seed of reliable quality and quantity in commercial settings, which requires mechanical harvest. Having to produce many populations of seeds in commercial settings can be costly for rangeland species, while there is a need to address a genuine risk of outbreeding depression and introduction of maladapted genes while pooling collections into a group without scientific information. One way to address this issue is to identify the genetic structures of a species across its distribution based on DNA markers and phenotypic measurements in common-garden studies, and categorize regional seed sources based on their genetic structure.

In Chapter 2, results from ITS/5.8S and *trn*K/*mat*K DNA sequences separated western and Searls prairie clovers, thus providing molecular evidence that these two taxa are different species. Based on the two conserved indels, diagnostic primers were designed for both ITS/5.8S DNA of the nuclear genome and *trn*K/*mat*K of the chloroplast genome. Occurrence of many conserved DNA polymorphic sequences in both species and congruence haplotypes within each species suggested that speciation might have occurred due to gene dispersal restriction, as the range of both western and Searls prairie clovers are geographically separated. No hybrids between these groups are reported yet; however, having more or less similarity in appearance between these two groups may make it harder to identify hybrids between them. It would be interesting to conduct gene-flow study in a common garden to see if their genetic architecture prevents gene flow between them. This information might be important for revegetating areas when two groups are in close proximity.

In Chapter 3, 22 wildland collections of western prairie clover were evaluated for morphological and agronomical traits in two common-garden locations (Hyde Park and Millville), and for AFLP markers in a plant genetic laboratory, respectively. Western prairie clover collections varied widely for DMY (28 to 188 g plot^{-1} at Millville and 85 to 242 g plot^{-1} at Hyde Park). Although a significant collection by site interaction was observed for DMY, collections with the greatest DMY at Hyde Park were generally high-yielding collections at Millville as indicated by a significant correlation between them. Dry-matter yields were not associated with forage quality traits, suggesting that selection for high DMY would not necessarily lead to reduced forage quality. Despite significant differences among collections for forage quality traits, the differences were relatively

small. On average, western prairie clover exhibited the most promising forage quality compared to other legumes. Strong positive correlations were detected among DMY, inflorescence weight, and number of inflorescences in western prairie clover collections. This suggests that selection for high DMY would likely lead to greater seed yields, and that the number of inflorescences could be used to indirectly select for seed yield. Both neighbor joining (NJ) dendrogram and Bayesian clustering analyses of AFLP markers separated the collections from the Deschutes River watershed as a homogeneous and distinct group from other western prairie clover collections. Among the remaining collections, both Bayesian clustering and the NJ dendrogram identified significant grouping of the John Day River watershed collections. The John Day River collections, however, showed shared coancestry (genetic admixture) with collections from Washington, eastern Oregon, and Idaho.

Flowering date was identified as having potential adaptive significance in western prairie clover, as it was positively correlated with temperature and negatively correlated with elevation. As flowering date accurately discriminated the two genetic groups of collections (Deschutes River watershed and the remaining collections), designating a seed transfer zone for these two regions could address both the evolutionary history and adaptive zone issues.

In Chapter 4, 20 wildland collections of Searls prairie clover were evaluated for morphological and agronomical traits in two common-garden locations (Hyde Park and Millville) and for AFLP markers in a plant genetic laboratory. Searls prairie clover collections exhibited variation for all measured traits. Collections from eastern Nevada and southern Utah exhibited greater morphological and agronomical traits compared to

collections from northwestern Utah and western Nevada. Significant positive correlations of foliage diameter or number of stems with dry-matter yield and inflorescence weight suggests that foliage diameter or number of stems could be used as surrogate traits to select for dry-matter yield and seed yield potential. Two collections from eastern Nevada (Ds-25 and Ds-26) exhibited the earliest flowering dates compared to other collections and may, therefore, be good candidates for revegetation in drier areas of the Great Basin.

Significant differences were detected among collections for AFLP markers, indicating the presence of population structure. Three groups of collections with bootstrap support were observed in the NJ dendrogram, and one of the groups comprising the four northwestern Utah collections was also detected with Bayesian clustering. Thus, the results indicated that collections from northwestern Utah formed a genetically differentiated group and that this group has lower within-collection genetic diversity (higher similarity) when compared to the other collections. The most diverse (lowest within-collection similarity) collections of Searls prairie clover were found across Nevada and southern Utah.

A significant genetic isolation by geographical distance was observed among the collections of Searls prairie clover, suggesting an important role of gene flow barriers responsible for the observed population structure. In addition, collection site precipitation was closely associated with the biomass related traits in Searls prairie clover, indicating an important role of precipitation in determining possible adaptive zones.

In conclusion, this study provides molecular evidence to that western and Searls prairie clover are two different species, in which speciation might have occurred due to geographic isolation. Ample variations in the measured phenotypic traits and AFLP markers was observed among the wildland collections of both species, with both species containing traits with promising agronomic potential. For western prairie clover, two geographic regions, one from Deschutes River and another from remaining collections except from the John Day River watershed can be developed as two seed transfer zones. However, a reciprocal translocation study is necessary to confirm whether to keep the John Day River collections with the Deschutes River collections (based on phenotypic traits measured in common gardens and *trn*K/*mat*K DNA sequences) or to unite them with the remaining collections (based on AFLP-markers and ITS/5.8 DNA sequences). In Searls prairie clover, a range of variation was observed among all the wildland collections. Based on the two genetic groups identified in the AFLP study and common-garden studies, two regional seed sources can be developed; one from northwestern Utah and the other from eastern Nevada and southern Utah. These two seed transfer zones for Searls prairie clover should address the concern of possible out-breeding depression and introduction of maladapted genes through revegetation. However, three western Nevada collections exhibited closer association with eastern Nevada and southern Utah groups for AFLP-markers and northwestern Utah for phenotypic traits. Therefore, a reciprocal transplant study is necessary to determine whether the western Nevada group warrants a separate seed zone or whether it is affiliated closely enough with the northwestern Utah or eastern Nevada/southern Utah.

APPENDIX

Table A-1. A consensus sequence obtained from the ITS/5.8S of ribosomal DNA of western and Searls prairie clovers. Numbers represent nucleotide positions.

5’ ⟶ 3’

1 AAGTCGTAAC AAGGTTTCCG TAGGTGAACC TGCGGAAGGA TCATTTATGT

51 TGCCTAACAA TCAGATCGAC CCGCGAACAT GTTATATTTA CCACTGATGG

101 GTGGCTTGGT TCTCCACTGC CCCCATAAGC CGGGAGGGTG TCGCTCAACG

151 CGGTCTCCTC TCGGTGTAAC AAAAACCCCG GCGCTGAATG TGCCAAGGAT

201 GTTGGAATTG TTCTGTGCAC CCTCGTCGCT GACCTGGAAA CGGATCTCGG

251 GCGTGTGGAT GTGCAAACAC TTGAAATCTT AATGACTCTC GACAACGGAT

301 ATCTTGGCTC TTGCATCGAT GAAGAACGTA GCGAAATGCG ATACTTGGTG

351 TGAATTGCAG AATCCCGTGA ACCATCGAGT TTTTGAACGC AAGTTGCGCC

401 TGAAGCCATT AGGCTGAGGG CACGCCTGCC TGGGTGTCAC GCATCGTTGC

451 TCCAAAATCA AAGCCCATGT ATACCATGAG CGTGGCTGGG GTGAATGCTG

501 GCCTCCCGTG AGCTTTGTCT CACGGTTGGT TGAAAACTTA GCCTGCTTGT

551 TGCTTGCGTC AGGACATACG GTGGTTGAGT GAATTTCTCT GGACCGGTCG

601 TGTGCGCATT :::::ATACA GTGGCAGACT CTTCGACCCA TGAGTGTGAC

651 TTGCTCGCAC CCACAACGCG ACCTCAGGTC AGGCGGGGCT ACCCGCTGAG

701 TTTAA

Table A-2. A consensus sequence obtained from the *trn*K/*mat*K region of the chloroplast genome of western and Searls prairie clovers. Numbers represent nucleotide positions.

5’ ⟶ 3’

1	TCGGCTTTTA	AGCGCGATTC	CATTTTTTAC	ACATTTCTAT	GAAGCAATTG
51	GTTCATCCAT	ACTATCGGTA	GAGTTTGTAA	AACCACGACT	GATCCAGAAA
101	GGAATGAATG	GAAAAAACAG	CATGTCGTAT	CAATGTCGAA	TTCGAAAGAT
151	TTTTCATTCT	TATTATTGGA	TCCGGCCGAT	TTTGTTGTTT	GAATTCTAGG
201	TTCGGAACAA	AAAAAATTAA	GTTGGGTTAA	ATTAATAAAG	GGATAGAGCT
251	TGTCGGCTCC	AATTATAGGG	AAACAAAAAG	CAACGAGCTT	TCATTTTAAA
301	TTGGAATGAT	TACCCCATCT	AATTGTACGT	TAAAAATGGA	TTAGTACTTG
351	ATGCGGGAAA	AGCTTTTCCC	ACGAATGGAT	TATTGATTTT	TTTTATGAGT
401	CCTAACTATT	AGTTATTCTC	CATTATGGGG	TGGCGATAAA	TGTGTAGAAG
451	AAAGAGTATA	TTGATAAAGA	TTTTTTTTTT	::CCAAAATC	AAAAGAGCGA
501	TTGGGCTGAG	AAAATAAAGG	ATTTCTAACC	AGCTTGTTAT	CCTAGAACGA
551	TTCGATTTCG	ATGGAAAAAG	CGAGGAGAGA	GAATCCGTTG	ATGGGTTTTA
601	CTTGTTTTCT	GGGTATCCAT	TCGTATTCGT	TCTAATTCGA	ATATATA:::
651	::::TATAAT	AGAGTATCCC	ATTTTTTTGC	CTGTATCGCA	CTATGTATCA
701	TTTGAAAATC	CAATGAATCC	CTGATCCTTT	GACCAAATCG	AATTTCAAAA
751	AAAATGGAGG	AATATCAAGT	ATATTTAGAA	CTAGATAGAT	CTCGCCAACA
801	GGACTTCCTA	TACCCATTTA	TTTTTCAGGA	GTATATTTAT	GGACTTGTTT
851	ATGGTCATGA	TTTAAATGGA	TCCATTTTGG	TAGAAAATGT	GGATTATGAT
901	AACAAATCTA	GTTTACTAAT	TGTAAAACGT	TTAATTACTC	GAATGTATCA
951	ACAGAATCAT	TTGATTGTTT	CTGCTAATGA	TTTTAACAAA	AATCAACAAT
1001	TTTGGGGATA	TAACAAGAAT	TTGTATTCTC	AAATAATATC	AGAGGCTTTT
1051	GCCATCGTCG	TGGAAATTCC	ATTTTACTCA	CAATTACGTT	CTTCCTTAGA
1101	GGGGGCAGAA	GTAATAAAAT	CTTATAATAA	GTTGCGATCA	ATTCATGCTA
1151	TTTTTCCTTT	TTTCGAGGAT	AAATTTACAT	ATTTAAATTA	TGTGTCAGAT
1201	GTACAAATAC	CCTATCCTAT	CCATTTGGAA	ATCTTGGTTC	AAATCCTTCG

1251 ATACTGGGTA AAAGATCCCC CCCTCTTTCA TTTATTAAGG TCCTTTCTTT

1301 ACCAGTATTG TAATTGGAAT AGTTTTATTA ATCCAAAAAA ATCGATTTCT

1351 AGTTTTTCAA AAAGTAATCC AAGATTTTTC TTCTTCCTAT ATAATTTTTA

1401 TGTATGTGAA TACGAATCTA TCTTCCTTTT TCTACGTAAA AAATCCTCTC

1451 ATTTACGATT AACATCTTTT :AGCGTTCTT TTTGAGCGAA TCTATTTCTA

1501 TGCAAAAATA GAACATCTTG TGGAAGTTTT TCCCAAGGAT TTTTTGTCCA

1551 CCTTATCATT GTTCAAGGAT CCCTTGATTC ATTATGTTAG ATATCAAGGA

1601 AAATCCATTC TGGCTTCAAA GAATGCGCCT CTTTTGATGA ATAAGTGGAA

1651 ATACTATCTT ATCTCTTTAT GGCAATGTTA TTTTAATGTT TGGTCTCAAC

1701 CGGGAACGAT CTATATAAAC CAATTATCCG ATCATTCATT TCACTTTTTT

1751 TGGGGGGGCT ATTTTTCAAA TGTGCGGCTA AATCTTTCAG TAGTACGGAG

1801 TCAAATGCTG GAAAATTCAT TTCTAATCGA AATTGTTATG AAAAAGCTTG

1851 ATACAATAGT TCCAATTATT CCTATAATTA GATCATTAGC TAAAGCGAAA

1901 TTTTGTAATG TATTAGGGCA TCCCATTAGT AAGCCGGTCT GGGCCGATTT

1951 ATCTGATTTT GGTATTATTG ACCGATTTTT GCGGATCCGC AGAAAAATTT

2001 CTCATTATTA CAATGGATCC TCAAAAAAAA AGAGTTTGTA TCGAATAAAA

2051 TATATCCTTC GGCTTTCTTG TATTAAAACT TTGGTTCGTA AACACAAAAG

2101 TACTGTACGC GCTTTTTTGA AAAGATTGGG TTCAGAAGAA TTATGAAAGA

2151 ATTTACAGAG A

Table A-3. DNA sequence polymorphisms of western (Do) and Searls (Ds) prairie clover collections observed in ITS/5.8S of rDNA region for the corresponding locations given in table A-1.

	19	117	139	145	175	274	280	460	498	584	611	612	613	614	615	617	634	700
Do_01	C	C	T	T	A	A	T	A	C	T	-	-	-	-	-	T	C	G
Do_01_2[†]	-	C	T	T	A	A	T	A	C	T	-	-	-	-	-	T	C	-
Do_01_3	C	C	T	T	A	A	T	A	C	T	-	-	-	-	-	T	C	G
Do_02	-	C	T	T	A	A	T	A	C	T	-	-	-	-	-	T	C	G
Do_02_2	C	C	T	T	A	A	T	A	C	T	-	-	-	-	-	T	C	G
Do_03	C	C	T	T	A	A	T	A	C	T	-	-	-	-	-	T	C	G
Do_03_2	-	C	T	T	A	A	T	A	C	T	-	-	-	-	-	T	C	-
Do_04	C	C	T	T	A	A	T	A	C	T	-	-	-	-	-	T	C	G
Do_04_2	-	C	T	T	A	A	T	A	C	T	-	-	-	-	-	T	C	G
Do_05	C	C	T	T	A	A	T	A	C	T	-	-	-	-	-	T	C	G
Do_05_2	-	C	T	T	A	A	T	A	C	T	-	-	-	-	-	T	C	G
Do_05_3	C	C	T	T	A	A	T	A	C	T	-	-	-	-	-	T	C	G
Do_06	C	C	T	T	A	A	T	A	C	T	-	-	-	-	-	T	C	G
Do_06_2	C	C	T	T	A	A	T	A	C	T	-	-	-	-	-	T	C	G
Do_06_3	C	C	T	T	A	A	T	A	C	T	-	-	-	-	-	T	C	G
Do_07	C	C	T	T	A	A	T	A	C	T	-	-	-	-	-	T	C	G
Do_07_2	C	C	T	T	A	A	T	A	C	T	-	-	-	-	-	T	C	G
Do_07_3	C	C	T	T	A	A	T	A	C	T	-	-	-	-	-	T	C	G
Do_08	C	C	T	T	A	A	T	A	C	T	-	-	-	-	-	T	C	G
Do_08_2	C	C	T	T	A	A	T	A	C	T	-	-	-	-	-	T	C	G
Do_08_3	C	C	T	T	A	A	T	A	C	T	-	-	-	-	-	T	C	G
Do_09	C	C	T	T	A	A	A	C	C	T	-	-	-	-	-	T	C	G
Do_09_2	C	C	T	T	A	A	A	C	C	T	-	-	-	-	-	T	C	G
Do_09_3	C	C	T	T	A	A	A	C	C	T	-	-	-	-	-	T	C	G

Table A-3 continued….

	19	117	139	145	175	274	280	460	498	584	611	612	613	614	615	617	634	700
Do_11	C	C	T	T	A	A	T	A	C	T	-	-	-	-	-	T	C	G
Do_11_2	C	C	T	T	A	A	T	A	C	T	-	-	-	-	-	T	C	G
Do_12	C	C	T	T	A	A	T	A	C	T	-	-	-	-	-	T	C	G
Do_12_2	C	C	T	T	A	A	T	A	C	T	-	-	-	-	-	T	C	G
Do_13	C	C	T	T	A	A	T	A	C	T	-	-	-	-	-	T	C	G
Do_13_2	C	C	T	T	A	A	T	A	C	T	-	-	-	-	-	T	C	G
Do_13_3	C	C	T	T	A	A	T	A	C	T	-	-	-	-	-	T	C	G
Do_14	C	C	T	T	A	A	A	C	C	T	-	-	-	-	-	C	C	G
Do_14_2	C	C	T	T	A	A	A	C	C	T	-	-	-	-	-	T	C	G
Do_15	C	C	T	T	A	A	T	A	C	T	-	-	-	-	-	T	C	G
Do_15_2	C	C	T	T	A	A	T	A	C	T	-	-	-	-	-	T	C	G
Do_15_3	C	C	T	T	A	A	T	A	C	T	-	-	-	-	-	T	C	G
Do_16	C	C	T	T	A	A	A	C	C	T	-	-	-	-	-	T	C	G
Do_16_2	C	C	T	T	A	A	A	C	C	T	-	-	-	-	-	T	C	G
Do_16_3	C	C	T	T	A	A	A	C	C	T	-	-	-	-	-	T	C	G
Do_17	C	C	T	T	A	A	T	A	C	T	-	-	-	-	-	T	C	G
Do_17_2	C	C	T	T	A	A	T	A	C	T	-	-	-	-	-	T	C	G
Do_17_3	C	C	T	T	A	A	T	A	C	T	-	-	-	-	-	T	C	G
Do_19	C	C	T	T	A	A	T	A	C	T	-	-	-	-	-	T	C	G
Do_19_2	C	C	T	T	A	A	T	A	C	T	-	-	-	-	-	T	C	G
Do_19_3	C	C	T	T	A	A	T	A	C	T	-	-	-	-	-	T	C	G
Do_20	C	C	T	T	A	A	T	A	C	T	-	-	-	-	-	T	C	G
Do_20_2	C	C	T	T	A	A	T	A	C	T	-	-	-	-	-	T	C	G
Do_20_3	C	C	T	T	A	A	T	A	C	T	-	-	-	-	-	T	C	G
Do_21	C	C	T	T	A	A	T	A	C	T	-	-	-	-	-	T	C	C

Table A-3 continued…

	19	117	139	145	175	274	280	460	498	584	611	612	613	614	615	617	634	700
Do_21_2	C	C	T	T	A	A	T	A	C	T	-	-	-	-	-	T	C	G
Do_21_3	T	C	T	T	A	A	T	A	C	T	-	-	-	-	-	T	C	C
Do_22	C	C	T	T	A	A	T	A	C	T	-	-	-	-	-	T	C	G
Do_22_2	C	C	T	T	A	A	T	A	C	T	-	-	-	-	-	T	C	G
Do_22_3	C	C	T	T	A	A	T	A	C	T	-	-	-	-	-	T	C	G
Do_23	C	C	T	T	A	A	A	C	C	T	-	-	-	-	-	C	C	G
Do_23_2	C	C	T	T	A	A	A	C	C	T	-	-	-	-	-	C	C	G
Do_23_3	C	C	T	T	A	A	A	C	C	T	-	-	-	-	-	C	C	G
Do_25	C	C	T	T	A	A	A	C	C	T	-	-	-	-	-	T	C	G
Do_25_2	C	C	T	T	A	A	A	C	C	T	-	-	-	-	-	T	C	G
Do_25_3	C	C	T	T	A	A	A	C	C	T	-	-	-	-	-	T	C	G
Ds_01	-	C	G	T	A	A	A	C	C	T	G	C	A	T	T	T	C	-
Ds_01_2	-	C	G	T	A	A	A	C	C	T	G	C	A	T	T	T	C	-
Ds_03	-	G	G	T	C	A	A	C	T	A	G	C	A	A	T	T	T	-
Ds_03_2	-	G	G	T	C	A	A	C	T	A	G	C	A	A	T	T	T	-
Ds_05	-	C	G	T	A	A	A	C	C	T	G	C	A	T	T	T	C	-
Ds_05_2	-	C	G	T	A	A	A	C	C	T	G	C	A	T	T	T	C	-
Ds_07	-	C	G	T	A	A	A	C	C	T	G	C	A	T	T	T	C	-
Ds_08	-	G	G	T	C	A	A	C	T	A	G	C	A	A	T	T	T	-
Ds_09	-	C	G	A	A	A	A	C	C	T	G	C	A	T	T	T	C	-
Ds_10	-	G	G	T	C	A	A	C	C	T	G	C	A	T	T	T	C	-
Ds_11	-	G	G	T	C	A	A	C	C	T	G	C	A	T	T	T	C	-
Ds_12	-	C	G	T	A	A	A	C	C	T	G	C	A	T	T	T	C	-
Ds_12_2	-	C	G	T	A	A	A	C	C	T	G	C	A	T	T	T	C	-
Ds_13	-	G	G	T	C	A	A	C	T	A	G	C	A	A	T	T	T	-

Table A-3 continued….

	19	117	139	145	175	274	280	460	498	584	611	612	613	614	615	617	634	700
Ds_14	-	G	G	T	C	A	A	C	T	A	G	C	A	A	T	T	T	-
Ds_15	-	G	G	T	C	A	A	C	T	A	G	C	A	A	T	T	T	-
Ds_16	-	C	G	T	A	A	A	C	C	T	G	C	A	T	T	T	C	-
Ds_17	-	C	G	T	A	T	A	C	C	T	G	C	A	T	T	T	C	-
Ds_17_2	-	C	G	T	A	T	A	C	C	T	G	C	A	T	T	T	C	-
Ds_18	-	C	G	T	A	A	A	C	C	T	G	C	A	T	T	T	C	-
Ds_18_2	-	C	G	T	A	A	A	C	T	T	G	C	A	T	T	T	C	-
Ds_20	-	C	G	T	A	A	A	C	C	T	G	C	A	T	T	T	C	-
Ds_21	-	C	G	T	A	A	A	C	C	T	G	C	A	T	T	T	C	-
Ds_23	-	C	G	T	A	A	A	C	C	T	G	C	A	T	T	T	C	-
Ds_25	-	C	G	T	A	A	A	C	C	T	G	C	A	T	T	T	C	-
Ds_26	-	C	G	T	A	A	A	C	C	T	G	C	A	T	T	T	C	-

†Number of replicates for the given collection. Only high quality DNA sequences were retained for the analysis.

CURRICULUM VITAE

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Education:

PhD; Plants, Soils, and Climate, USU Nov. 2010
Major: Plant Science (GPA 3.86/4.0)

MS; Wildland Resources, USU May 2007
Major: Ecology (GPA 3.83/4.0)

MSc. (2 yrs) and BSc. (2 yrs) Dec 2001
Tribhuvan University, Kathmandu, Nepal

Experience:

Graduate Research Assistant May 2007 – Dec 2010
USDA-FRRL, Plant Genetics Lab
Utah State University, Logan, Utah

Graduate Research Assistant Aug. 2004 – May 2007
USDA-FRRL, Plant Physiology Lab
Utah State University, Logan, Utah

Assistant Lecturer Mar. 2002 – July 2004
Amrit Science College, Institute of Science and Technology
Tribhuvan University, Kathmandu, Nepal

Documentary Producer 2002
5 documentaries (15 minute each) in Nepali Language;
For: Society of Environmental Journalists, Nepal
(Aired on Nepal Television)

Awards:

- School of Graduate Studies Dissertation Fellowship for 2009/2010. Utah State University, Logan, UT ($5,000)
- Second place; Plants, Soils, and Climate, Intermountain Graduate Student Symposium Oral presentation (2010)

- Third place; College of Agriculture, Intermountain Graduate Student Symposium Poster presentation (2009)
- Center for Integrated Biosystems (CIB) Graduate Student Research Grant (2008) ($5,400)
- Full scholarship for 'Gene Expression and Micro-Array Analysis' training, CIB, USU (August 2006) ($1,600)
- Cornell Nepal Study Program Research Grant, Tribhuvan University, Nepal (2000)

Publications:

Bhattarai, K., D.A Johnson, T.A. Jones, K.J Connors, and D.R. Gardner. 2008. Physiological and morphological characterization of basalt milkvetch (*Astragalus filipes*): Basis for plant improvement. *Rangeland Ecology and Management* 61:444-555.

Bushman, B.S., K. Bhattarai, and D.A. Johnson. 2010. Population structures of *Astragalus filipes* collections from western North America. *Botany* 88:565-574.

Johnson, D.A., T.A Jones, K.J. Connors, K. Bhattarai, B.S. Bushman, and K.B. Jensen. 2008. Notice of release of NBR-1 Germplasm basalt milkvetch. *Native Plant Journal* 9:127-133.

Under Review:

Bhattarai, K., B. S. Bushman, D. A. Johnson, and J. G. Carman. Phenotypic and genetic characterization of the wildland collections of western prairie clover from the western USA. (Rangeland Ecology and Management).

Bhattarai, K., D. A. Johnson, and J. Kosanke. Rhizobial strains for basalt milkvetch: a North American legume for rangeland restoration. (Western North American Naturalist).

In Preparation:

Bhattarai, K., B. S. Bushman, D. A. Johnson, and J. G. Carman. Phenotypic and genetic characterization of wildland collections of Searl's prairie clover from the western USA. (for Crop Science)

Bhattarai, K., B. S. Bushman, D. A. Johnson, and J. G. Carman. Characterization of the phylogenetic relationship of western prairie clover and Searls prairie clover based on DNA sequence from internal transcribed spacer (ITS) and the *Maturase* in chloroplast.

Specialized Training:

- International Teaching Graduate Assistant, Utah State University. 10-20 August 2009.
- Gene Expression and Micro-Array Analysis. Utah State University. 1-4 August 2006.

Presentations:

Oral:

Bhattarai, K., B. S. Bushman, D. A. Johnson, and J. G. Carman. Evaluation of wildland collections of Searls prairie clover for revegetation in the Great Basin, western USA.

Presented at the Intermountain Graduate Student Symposium, Utah State University (31 March 2010).

Bhattarai, K., D. A. Johnson, B. S. Bushman, and J. G. Carman. Western and Searls prairie clovers: North American legumes for rangeland revegetation. Presented at the 63rd Society of Range Management annual meeting, Denver, CO (10 February 2010).

Bhattarai, K., B. S. Bushman, D. A. Johnson, and J. G. Carman. Genetic diversity of western prairie clover in the Great Basin and southern Columbia Plateau, USA. Oral presentation. Utah Plant Genetics Conference. University of Utah, Salt Lake City, UT (14 November 2009).

Bhattarai, K., B. S. Bushman, D. A. Johnson, and J. G. Carman. Characterization of the extent and the distribution of genetic diversity in western prairie clover. Plants, Soils and Climate Departmental Seminar, Utah State University (9 November 2009).

Bhattarai, K., B. S. Bushman, D. A. Johnson, and J. G. Carman. Estimation of population structures in two North American wildland legumes. Plants, Soils and Climate Departmental Seminar, Utah State University (27 October 2008).

Bhattarai, K., and D. A. Johnson. Physiological and morphological evaluations of various collections of *Astragalus filipes* for restoring degraded rangelands in the western U.S.A. Intermountain Graduate Student Symposium, Utah State University (2 April 2007).

Poster:

Bhattarai, K., B. S. Bushman, D. A. Johnson, and J. G. Carman. Germplasm release strategy of Searls prairie clover for the Great Basin. National Native Seed Conference, Snowbird, UT (18 May 18 2010).

Bhattarai, K., B. S. Bushman, D. A. Johnson, and J. G. Carman. Identification of the cryptic population structures in *D. searlsiae* and *D. ornata* in wildland collections from the U.S. Intermountain West. Presented at the Intermountain Graduate Student Symposium, Utah State University (4 April 2009).

Bhattarai, K., B. S. Bushman, D. A. Johnson, and J. G. Carman. Estimation of the genetic diversity in *D. searlsiae* and *D. ornata* in wildland collections from the U.S. Intermountain West. Presented at the 62nd Society of Range Management annual meeting, Albuquerque, NM (9 February 9 2009).

Bhattarai, K., B. S. Bushman, D. A. Johnson, and J. G. Carman. Phenotypic and genotypic characterization of wildland seed collections of two North American wildland legumes. Plants, Soils and Climate Departmental Seminar, Utah State University (6 October 6 2008).

Bhattarai, K., D. A. Johnson, and T. A. Jones. Basalt milkvetch: candidate species for rangeland restoration in the western U.S. Poster presented at the Ecological Society of America Annual Meeting, San Jose, CA (10 August 2007).

Software Skills:

Microsoft Office, SAS, R, SigmaPlot, PAUP, JoinMap, MapQTL, Sequencher, GeneMarker, Genographer, GenAlEx, Arlequin, STRUCTURE, AFLP-SURV, and Phylip

Memberships:

Society for Range Management (2008 - Current)

Ecological Society of America (2006/2007)

American Academy of Arts and Science (2005/2006)