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Assessment of AFLP-based Genetic Variation in Three Native Plant Species across the State of Minnesota

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Analysis of genetic diversity and population differentiation determines how diverse natural populations are and how closely related they are to one another, which can provide clues concerning adaptation for restoration projects. This research analyzed the genetic diversity of three native species across their range in Minnesota. Using Amplified Fragment Length Polymorphisms, the genetic diversities of three species–prairie cordgrass (*Spartina pectinata*), purple prairie clover (*Dalea purpurea*), and spotted Joe-pye weed (*Eupatorium maculatum*)–were examined. The diversity for all the species had more disjunct relationships rather than displaying geographic or ecological patterns. The genotypic variation may be due to ecotypic variation or to genetic drift as a result of habitat fragmentation. The species had Gst values, a measure of how much populations differ, that ranged from 0.18 up to 0.27, indicating clear population differentiation. Analysis of molecular variance results concurred. The natural populations of all these species showed moderate levels of genetic diversity. This information is helpful in ensuring adequate diversity in seed sources for restorations. Additional research on these populations by performing common garden and reciprocal transplantation experiments would be a useful supplementation to the molecular marker data. For restorations in Minnesota, the best option may be to use seed that is as close as possible.

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Prepared by:

Kristine Moncada

Nancy Jo Ehlke

Gary Muehlbauer

Craig Sheaffer

Donald Wyse

Department of Agronomy and Plant Genetics

University of Minnesota

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Executive Summary

The intrinsic genetic diversity of native plant populations is important to a species' ability to adapt to the environment. In projects involving ecosystem restoration or planting of native species, suitably adapted plants are important to the success of the project. Analysis of genetic diversity and population differentiation of a species determines how diverse natural populations are and how closely related they are to one another, which can provide clues concerning adaptation. Until recently, there has been a lack of information available on the genetic diversity present in native plants in general, especially in Minnesota. This research analyzed the genetic diversity of three native species across their range in Minnesota. The goal was to investigate the geographical range of relatedness and, presumably, adaptation of native species across the state of Minnesota. Presently, somewhat arbitrary guidelines are used by the Minnesota Department of Transportation (MN/Dot) and other state and conservation agencies for distances for which seed can be collected to plant and restore a new site. Knowledge of patterns of genetic diversity is critical in forming future conservation and restoration plans.

Using Amplified Fragment Length Polymorphisms (AFLPs), a method of DNA fingerprinting, the genetic diversities of three species–prairie cordgrass (*Spartina pectinata*), purple prairie clover (*Dalea purpurea*), and spotted Joe-pye weed (*Eupatorium maculatum*)–were examined. The species were sampled throughout their ranges within the state of Minnesota. One hypothesis was that the patterns of variation would relate to simple geographic proximity. Another potential relationship that was considered was that diversity would vary according to the three biomes in Minnesota of the Ecological Classification System (ECS), which classifies environments on the basis of common traits. Neither hypothesis was supported by the data. The diversity had a disjunct relationship rather than displaying clear geographic or strong ecological patterns of the ECS system. The genotypic variation may be due to ecotypic variation or to genetic drift as a result of habitat fragmentation. The species had Gst values (a measure of how much populations differ) that ranged from 0.18 up to 0.27, indicating clear population differentiation. Analysis of molecular variance (AMOVA) results concurred with the Gst values. The natural populations, no matter the size, of all these species showed moderate levels of genetic diversity. Small populations may be as useful as larger ones from which to acquire seed supplies. This information is helpful in ensuring adequate diversity in seed sources for restorations.

At this time, with the erratic patterns of diversity that were observed, few recommendations for seed collections zones can be made. Even within the new field of restoration genetics, there are conflicting opinions as to whether molecular markers are useful indicators of adaptability. The patterns of genetic variation may be due to genetic drift cause by population fragmentation or due to selection. Additional research on these populations by performing common garden and reciprocal transplantation experiments would be a useful supplementation to the molecular marker data. This would provide a more definitive answer to the question of adaptability of potential seed sources. Based on the results of this study, the current methods of seed collection used by Mn/DOT for

restorations cannot be either validated or discounted. Until the underlying causes for the variation are determined, the best option at this time may be to err on the side of caution and use seed that is as close as possible to the restoration site.

Chapter 1. Introduction

Background

The genetic diversity of native plant populations is important to a species' ability to adapt to the environment. In native restorations, suitably adapted plants are important to the success of the project. Analysis of genetic diversity and population genetics of natural plant populations determines how closely related natural populations are to one another, which can provide clues concerning adaptation. Until recently, there has been a lack of information available on the genetic diversity present in native plants in general, especially in Minnesota. Presently, guidelines that have not been fully confirmed are used by the state and other conservation agencies to determine distances for which seed can be collected to plant and restore a new site. The field of restoration genetics is still developing and there is presently little consensus on what distances to use for seed collection. Knowledge of patterns of genetic diversity and population genetics is a critical component in forming future conservation and restoration plans.

Objectives

This research analyzed the genetic diversity and population differentiation of three native species across their range in Minnesota. The goal was to investigate the geographical range of relatedness and presumably adaptation of native species across the state of Minnesota. Three species were chosen based on their use in restorations and roadside planting by the Minnesota Department of Transportation (Mn/DOT), because of their ubiquity in the natural landscape and due to the wide-ranging environments in which the plants occur. Using Amplified Fragment Length Polymorphisms (AFLPs), the genetic diversities of three species–prairie cordgrass (*Spartina pectinata*), purple prairie clover (*Dalea purpurea*), and spotted Joe-pye weed (*Eupatorium maculatum*)–were examined. These species occur in various environments where they have different roles. Prairie cordgrass is a wetland grass species. Purple prairie clover is a prairie legume species. Spotted Joe-pye weed is a wetland forb species. Big bluestem (*Andropogon gerardii*) was originally included in the study, but was dropped due to technical difficulties in the laboratory. Once sites for plant collection were chosen throughout the state of Minnesota, plant material was then collected from the sites. Primers for AFLP analysis were identified and laboratory analysis was performed. The amplified products were scored to develop haplotypes, which were used in subsequent statistical analyses.

Two hypotheses were considered for this research. One hypothesis was that the patterns of variation would relate to simple geographic proximity so that populations that were nearer would be more closely related than those farther. Another potential relationship that was considered was that diversity would vary according to the three biomes in Minnesota of the Ecological Classification System (ECS), which classifies environments on the basis of common traits – populations within a biome would be more closely related to each other than they would be to those in other biomes. The null hypothesis was that there would be no discernible pattern to the genetic diversity. In more general terms, there are three questions that this research can address that are related to

restoration issues. Are the populations significantly different from one another? If they are different, what are the patterns of variation? And, finally, what are the underlying reasons for the patterns? By examining the genetic diversity of these three species across their range in Minnesota, it is hoped that this information can be used in developing seed collection zones for state agencies like Mn/DOT for their restoration projects.

Chapter 2. Species Descriptions

Prairie cordgrass

Prairie cordgrass (*Spartina pectinata* Link), also known as slough grass and ripgut, is a long-lived perennial belonging to the Poaceae family (Gleason and Cronquist, 1991; USDA, NRCS, 2005). It is stout-stemmed, growing up to three meters tall. The shiny, flat blades taper and are up to 1.2 meters long and 1.3 centimeters wide. The blade edges are rough (Ladd, 1995). The inflorescence is a panicle with straw-colored, one-sided spikes (Eggers and Reed, 1997). The individual spikes within an inflorescence number between seven and twenty-seven (Gleason and Cronquist, 1991) and are three to fifteen centimeters long (Ladd, 1995). The second glume of each spikelet has an awn three to ten mm in length (Gleason and Cronquist, 1991). It blooms in the midsummer to early fall (Ladd, 1995). In the fall, the leaves first turn reddish then light yellow (Eggers and Reed, 1997).

The scaly rhizomes of prairie cordgrass form dense stands of monotypic clones that are between one and ten meters across (Eggers and Reed, 1997). The rhizomes can grow 1.5 to 3 meters per year (USDA, NRCS, 2005). Members of this genus produce germinating seed only sparingly and can be a tetraploid, hexaploid, or octoploid (Gleason and Cronquist, 1991). Precise information for the breeding system of prairie cordgrass is not known, but another member of this genus (*S. alterniflora*) was found to have significant levels of out-crossing (Travis et al, 2002).

Prairie cordgrass occurs in wet prairies, prairie marshes, and on the shores of lakes and rivers throughout the tallgrass region (Ladd, 1995). Prairie cordgrass can tolerate alkaline conditions, but not prolonged flooding (USDA, NRCS, 2005). The geographic distribution of prairie cordgrass within the United States is shown in Figure 2-1 (USDA, NRCS, 2005), and its distribution in Minnesota is shown in Figure 2-2 (University of Minnesota Herbarium, 2002). It also can be found in mesic prairies, but usually only in the low spots (Eggers and Reed, 1997).

Prairie cordgrass provides cover for wildlife (USDA, NRCS, 2005). Other uses include having been used by pioneers and Native Americans as thatching and fuel (Ladd, 1995). It is also used as a horticultural plant, particularly as a shore binder for erosion control. One cultivar is ‘Aureo-marginata’ that is distinguishable from the native species by its yellow marginal stripes (Still, 1994). While there are not many other cultivars in commercial production, there are two potential releases from NRCS Plant Materials Centers (USDA, NRCS, 2005).

Purple prairie clover

Purple prairie clover (*Dalea purpurea* Vent) is a moderately long-lived perennial warm-season legume that belongs to the Fabaceae family (Gleason and Cronquist, 1991; USDA, NRCS, 2005). It also goes by the name of violet prairie clover (USDA, NRCS, 2005). Plants are slender, upright, and around 0.6 meter tall (Ladd, 1995). The leaves

are compound, alternate with 3 to 9 leaflets that are 2.5 centimeters long and 0.3 centimeter wide. The inflorescence is a dense, cylindrical, terminal spike. The individual flowers are purple with bright orange protruding stamens that flower from the bottom up. The normal bloom time is late spring to summer (Ladd, 1995). Gleason and Cronquist (1991) state that purple prairie clover is a diploid with 2n equal to 14. Purple prairie clover is primarily cross-pollinated by insects (USDA, NRCS, 2005).

Purple prairie clover can often be found growing in patches (Tekiela, 1999). Its habitat is dry to mesic prairies throughout the tallgrass region (Ladd, 1995). It can also grow in woodland openings, sand prairies, gravel-hill prairies and along railroads (USDA, NRCS, 2005). The geographic distribution of purple prairie clover within the United States shown in Figure 2-3 (USDA, NRCS, 2005), with its distribution in Minnesota is shown in Figure 2-4 (University of Minnesota Herbarium, 2002). Another similar species (*D. candida*) has a similar range, but can be differentiated by its wider leaves, taller form, and white flowers (Moyle and Moyle, 1977).

Being a member of the legume family, this species does fix nitrogen (Tekiela, 1999) and would presumably be an important contributor to a restored ecosystem. Purple prairie clover is a host for the Blues and Dog Face butterflies (Tekiela, 1999). It is utilized by many other species of bees and butterflies as a pollen source. This species is somewhat aromatic and was used by Native Americans to make tea (Ladd, 1995). Purple prairie clover is palatable and nutritious, but decreases under heavy grazing (USDA, NRCS, 2005). This species may also have value as an ornamental plant in the landscape (Lindgren, 1992). Cultivars include 'Kaneb' (from South Dakota) and 'Bismarck' (South Dakota) which was released for its vigor and higher seed yield (USDA, NRCS, 2005).

Spotted Joe-pye weed

Spotted Joe-pye weed (*Eupatorium maculatum* L.) is a moderately long-lived perennial that belongs to the Asteraceae family (Gleason and Cronquist, 1991; USDA, NRCS, 2005). It can be up to 1.5 meters tall. The stems are unbranched and usually have purple spots. The leaves are coarsely toothed, up to 23 centimeters long and six centimeters wide, lance-ovate in shape, and are whorled around the stem (Ladd, 1995). The flower heads are discoid, meaning there are no ray flowers, and occur in corymbiform inflorescences consisting of many purplish tubular flowers (Gleason and Cronquist, 1991). It blooms from late spring to early fall (Ladd, 1995).

Gleason and Cronquist (1991) list spotted Joe-pye weed as a tetraploid (2n=20), but state that diploid forms can also occur. This species hybridizes with closely related species within the genus (Gleason and Cronquist, 1991). Another species that occurs in eastern Minnesota is *E. purpureum* (Sweet Joe-pye weed) that can be distinguished from spotted Joe-pye weed by its purple stems that lack spots and its flower color. Since the genus is in the Asteraceae family and attractive to pollinating insects, it is probable that there is a certain level of outcrossing present. Spotted Joe-pye weed prefers permanently moist sites (Ladd, 1995) and occurs in sedge meadows, calcareous fens, and shallow marshes (Tekiela, 1999). The geographic distribution of spotted Joe-pye weed within the United

States is shown in Figure 2-5 (USDA, NRCS, 2005), with its distribution in Minnesota shown in Figure 2-6 (University of Minnesota Herbarium, 2002).

Spotted Joe-pye weed was purportedly named after a Native America doctor who used this plant medicinally (Moyle and Moyle, 1977). The species is used as a landscape plant and is popular in Great Britain (Still, 1994). The nomenclature of the cultivars in the literature is confusing since there is hybridization that occurs within the genus. Two cultivars, 'Atropurpureum' and 'Gateway,' are listed as either/both *E. maculatum* and *E. purpureum*. These two species may hybridize in nature. There are three subspecies or varieties (depending on the authority) that occur in Minnesota: *E. maculatum* var. *bruneri, E. maculatum* var. *foliosum* and *E. maculatum* var. *maculatum* (USDA, NRCS, 2005). *E. maculatum* var. *bruneri* occurs in the western U.S., with *E. maculatum* var. *maculatum* occurring in the eastern U.S., while *E. maculatum* var. *foliosum* is the least widespread occurring in the upper tier of the northeastern states (USDA, NRCS, 2005).

Figure 2-1. Prairie cordgrass – U.S. distribution. The shaded states indicate the areas where prairie cordgrass is found according to the USDA NRCS PLANTS Database, 2005.

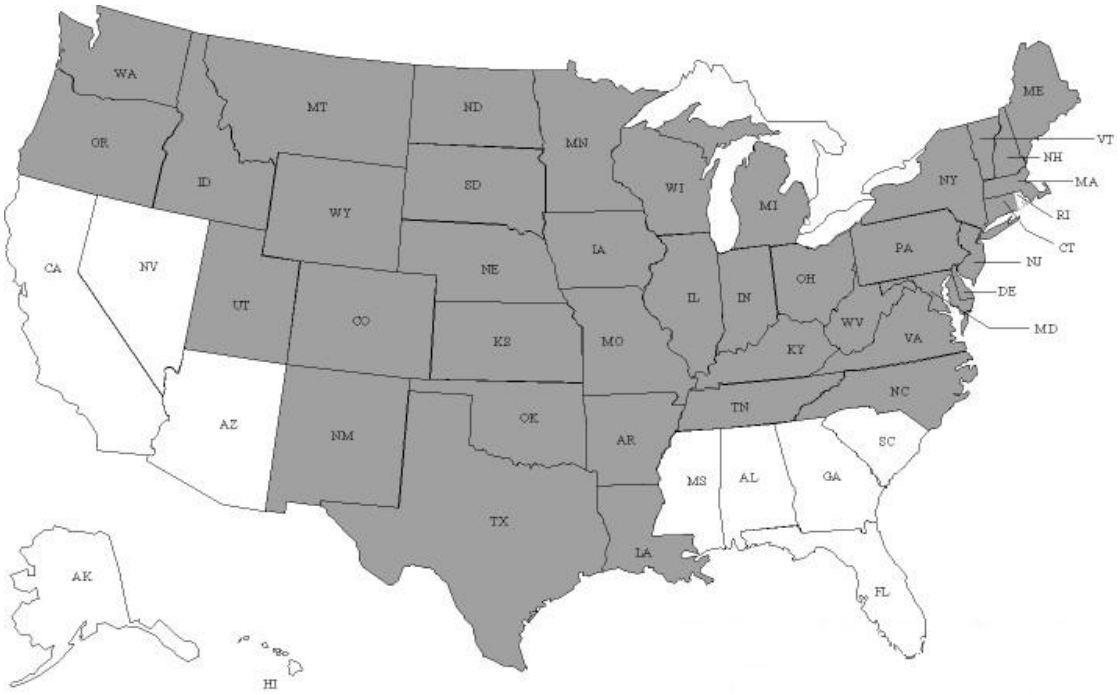


Figure 2-2. Prairie cordgrass – Minnesota distribution. The distribution of prairie cordgrass in Minnesota according to the University of Minnesota Herbarium records. Shaded counties indicate where this species has been found historically.

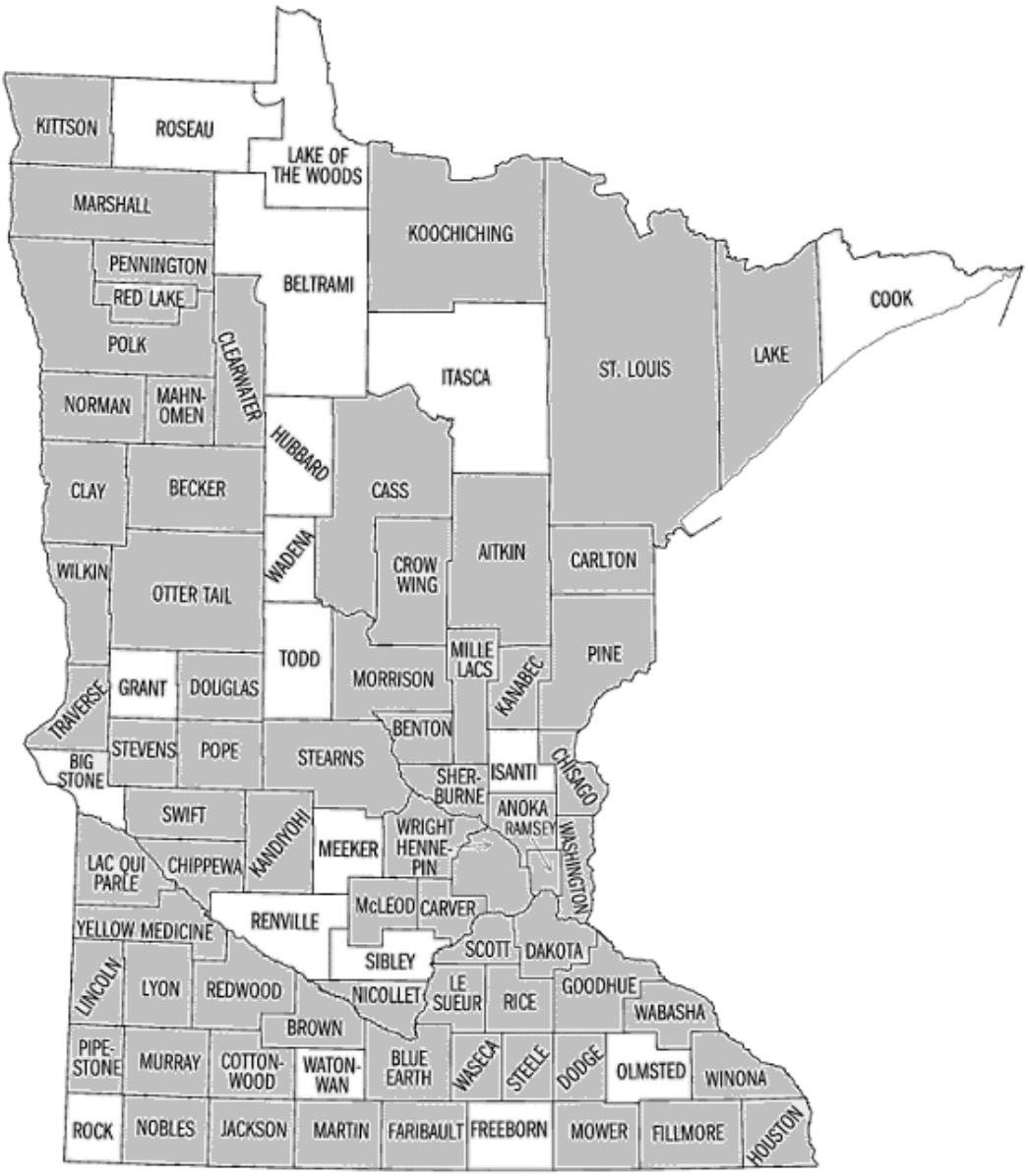


Figure 2-3. Purple prairie clover – U.S. distribution. The shaded states indicate the areas where purple prairie clover is found according to the USDA NRCS PLANTS Database, 2005.

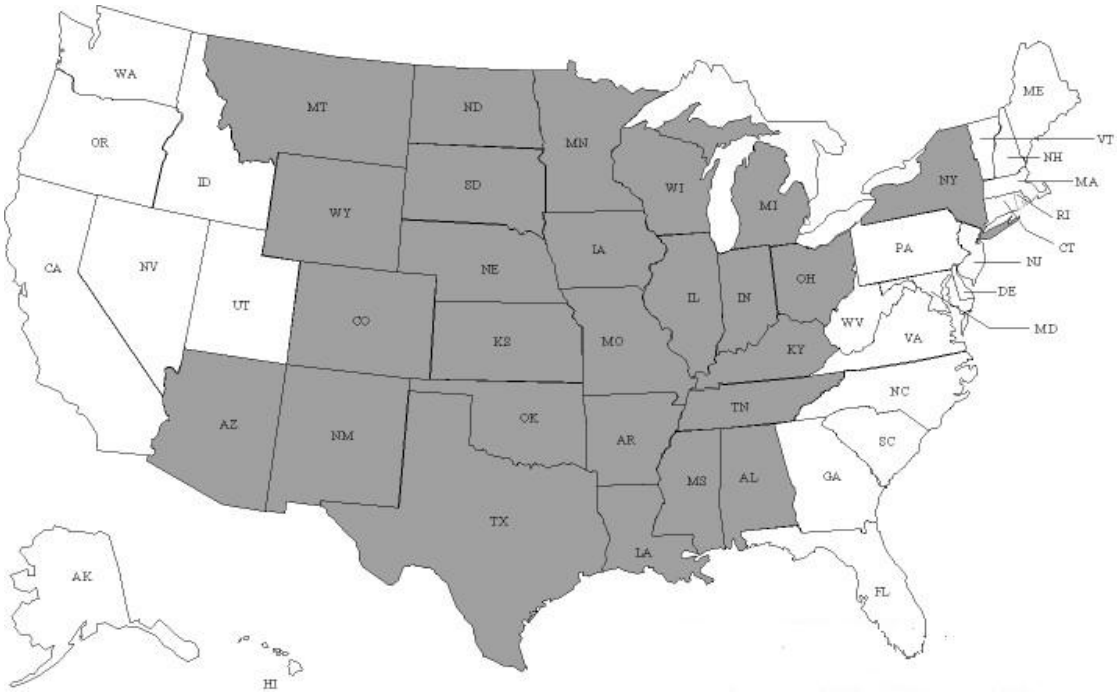


Figure 2-4. Purple prairie clover – Minnesota distribution. The distribution of purple prairie clover in Minnesota according to the University of Minnesota Herbarium records. Shaded counties indicate where this species has been found historically.

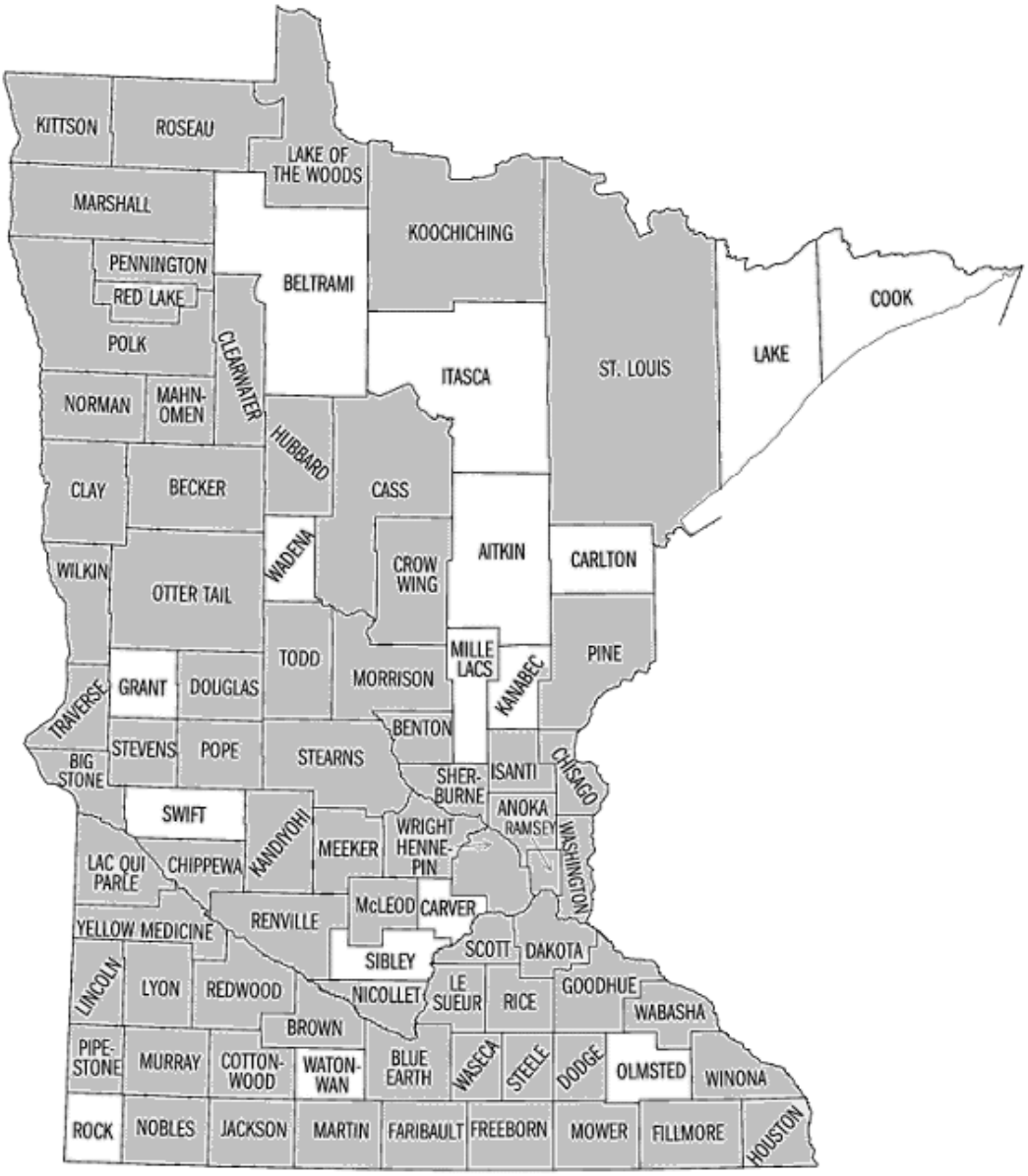


Figure 2-5. Spotted Joe-pye weed – U.S. distribution. The shaded states indicate the areas where spotted Joe-pye weed is found according to the USDA NRCS PLANTS Database, 2005.

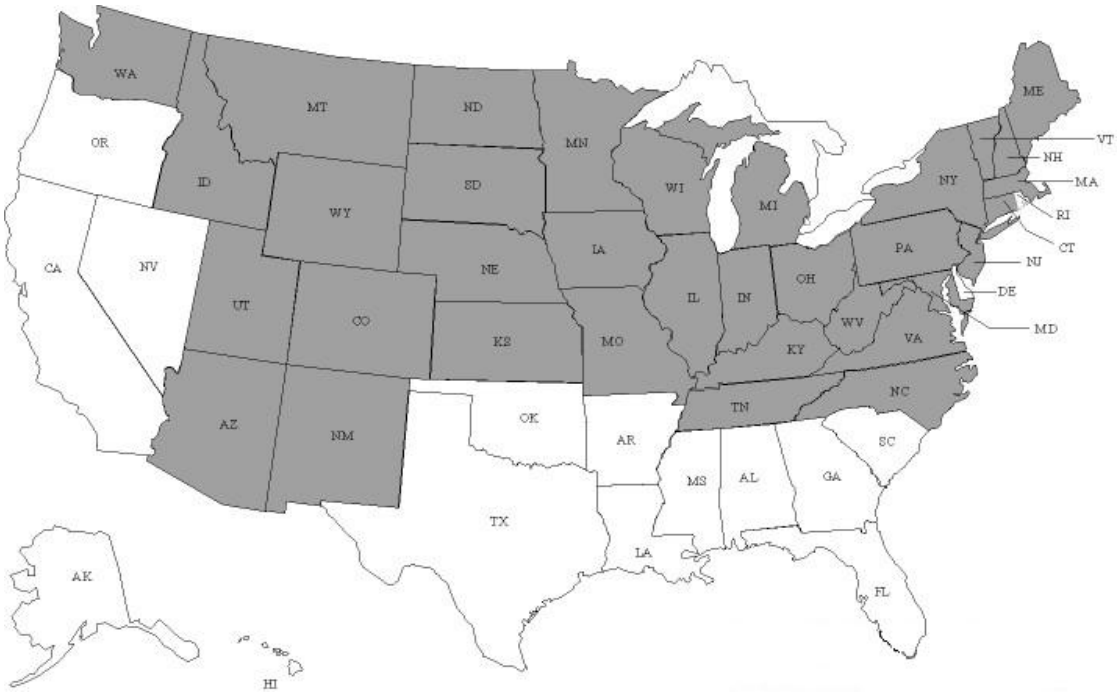
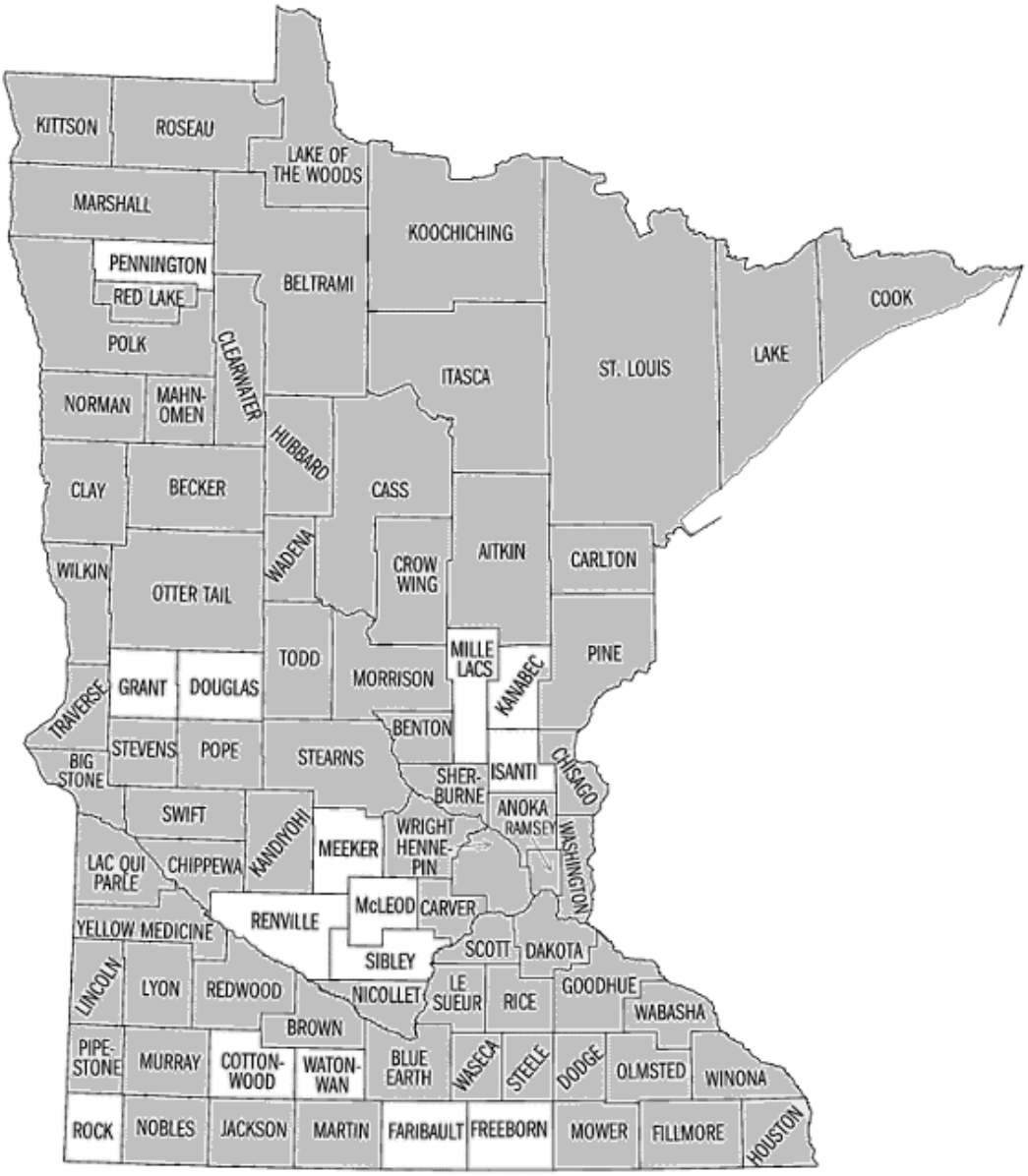


Figure 2-6. Spotted Joe-pye weed – Minnesota distribution. The distribution of spotted Joe-pye weed in Minnesota according to the University of Minnesota Herbarium records. Shaded counties indicate where this species has been found historically.



Chapter 3. Minnesota Ecology

Minnesota has a variety of environments within its borders. One way to categorize these environments is through the Ecological Classification System (ECS), which is a nationwide system that combines information on climate, geology, hydrology, topography, soil, and vegetation (Mn/DNR, 1999). Biomes, based on major climate zones and native vegetation, are the highest level of classification in the system. Three biomes or provinces occur in Minnesota: Laurentian Mixed Forest, Eastern Broadleaf Forest, and Prairie Parkland. (A fourth biome, Tallgrass Aspen Parklands, a mix of prairie and aspen savanna, is now defined as separate from the Eastern Broadleaf Forest.) It is unique to have three biomes within one non-mountainous state, which is an indication of how diverse the environments within Minnesota are. Laurentian Mixed Forest is the most highly forested area comprised of conifer, conifer-hardwood, or hardwood vegetation. The Eastern Broadleaf Forest is the transitional area between the prairie and the true forest. The Prairie Parkland is the area that once was primarily tallgrass prairie (Mn/DNR Web site, 2005). These three biomes are divided into sections, of which Minnesota has ten. Sections are further divided into subsections. See Figure 3-1 for the biomes, sections, and subsections of Minnesota.

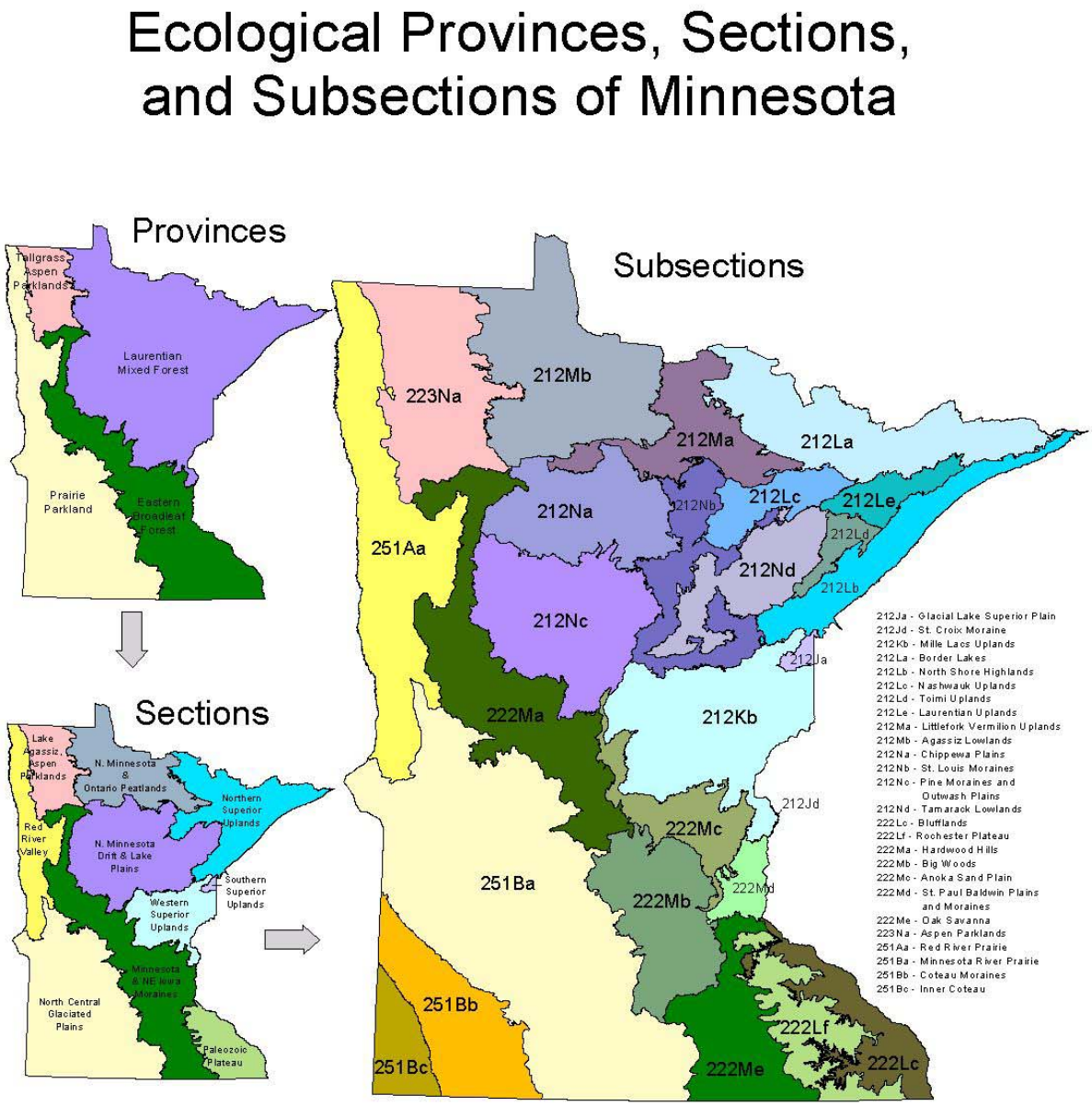
Of the differing environments that Minnesota has, much of the original natural habitat is gone. Less than 1% of the prairie ecosystem is left in Minnesota; less than 4% of the original old growth forest remains; and only 47% of wetlands in Minnesota remain (Allmann, 1997). Numerous agencies, like the Department of Natural Resources (DNR) Scientific and Natural Areas, the Nature Conservancy, and the DNR State Park Systems, protect the remaining natural areas that are still left in Minnesota. All three agencies are conducting their own restoration projects to increase the level of native habitat.

The Minnesota Department of Resources preserves undisturbed sites like prairies, old growth forest, peatlands, and the habitats of rare species in the Scientific and Natural Areas (SNA) program. The mission statement of the program, which was started in the mid-1960s, is to "preserve and perpetuate the ecological diversity of Minnesota's natural heritage" (Mn/DNR, 1999). There are over 100 sites at this time.

The Minnesota Chapter of the Nature Conservancy (TNC) has over fifty sites throughout the state (TNC, 2000), many of them jointly owned with the SNA program. While the Nature Conservancy is a private organization rather than a state agency, the goals are similar: to protect unique natural habitats. The Nature Conservancy was established nationally in 1951, and state chapters were developed in the 1970s (TNC, 2000).

The Minnesota State Park system is another source of natural areas in the state. These areas tend to be more disturbed than SNA or TNC sites, but are also much larger in size. There are 66 state parks in the system (MN State Park System Web site, 2005). Several state parks have been protected for a much longer period of time relative to other types of sites. Minnesota's oldest state park is Itasca, which was established in 1891 (MN State Park System Web site, 2005).

Figure 3-1. Ecological Classification System



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For more information contact:
Dan Hanson
MN DNR, Division of Forestry
Resource Assessment Program
413 SE 13 Street
Grand Rapids, MN 55744
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Chapter 4. Native Plants and Restoration

The Minnesota Department of Transportation – Mitigation and roadside plantings

The Minnesota Department of Transportation (Mn/DOT) has two different uses for native plant seed: mitigation restorations and roadside plantings. Mitigation is performed when Mn/DOT displaces a habitat during the building of a road or other structures. A roadside planting is the vegetation of the areas immediately adjacent to the road like ditches or embankments. The process for these two types of restorations varies (Robert Jacobson, personal communication).

Mitigation has different goals than roadside plantings. Mitigated sites may not be as disturbed as roadsides and are designed to more closely emulate natural environments. The Minnesota Wetland Conservation Act of 1991 (WCA Rule, Chapter 8420) required the restoration or creation of displaced wetlands (Mn/DOT Web site, 2005). Prior to this law, Mn/DOT had been replacing some wetlands since 1984. Seed for mitigated sites currently use local seed sources to ensure adaptation (Robert Jacobson, personal communication).

The other type of restoration project done by Mn/DOT is roadside vegetation. The use of native plants in roadside plantings has been advocated for over 40 years (Aikman, 1960). Using native materials has been experimented with in Minnesota starting at least thirty years ago with sideoats grama (*Bouteloua curtipenula*), Indian grass (*Sorghastrum nutans*), little bluestem (*Schizachyrium scoparium*), and big bluestem (White and Smithberg, 1972). Prior to that, exotic species, including Kentucky bluegrass (*Poa pratensis*), timothy (*Phleum pretense*), brome grass (*Bromus* sp.), various fescues (*Festuca* spp.), birdsfoot trefoil (*Lotus corniculatus*) and crown vetch (*Coronilla varia*), were used almost exclusively (White et al, 1972). It has been found that the exotics sometimes did not perform well on the roadside leading to dead spots, which led to susceptibility to invasion by noxious weeds like leafy spurge (*Euphorbia escula*), spotted knapweed (*Centaurea maculosa*) and Canada thistle (*Cirsium arvense*) (Robert Jacobson, personal communication).

More intensive research by Mn/DOT using native plants was begun in the 1980s with a more formal plan activated in the mid 1990s (Mn/DOT Web site, 2005). Major advantages for using native plants could include less mowing and decreased maintenance costs, better establishment by the native plants, and a higher diversity of species in the roadside native mixes which may diminish susceptibility to invasion. Another benefit to using native plants along the roadsides is invasive weed control, at least for those weed species that establish in disturbed conditions (Blumenthal et al, 2005). This may be due partly to species richness of native restored sites (Case, 1990) or better competition due to adaptability by limiting resources and limiting establishment (Blumenthal et al, 2005). Prairie plants with their extremely deep roots could help detoxify roadsides and improve control of water, sediments, and nutrients. Native grasses such as big bluestem can provide long-term erosion control because they have much more extensive root systems than exotics such as brome and Kentucky bluegrass. Big bluestem has roots that can

reach ten feet in depth, while brome roots may only go down to one foot in depth (Mn/DOT Web site, 2005). An additional advantage to using native plants on the roadside is the benefit to wildlife. Up to 19 mammals and 23 bird species use roadsides for their homes; native plantings can provide better habitat for their needs (Mn/DOT Web site, 2005).

The Minnesota Department of Transportation manages 195,000 acres of vegetated roadside area with 25% being forested and the rest consisting of non-native plantings, native plantings, or native remnants (Mn/DOT Web site, 2005). The Mn/DOT Integrated Roadside Vegetation Management Program has the goals of managing roadside vegetation to promote safety, economic accountability, environmental responsibility, and aesthetic appearance. Objectives include better erosion control, wildlife habitat shelterbelts, and reduced chemical inputs for noxious weed management (Mn/DOT Web site, 2005). The roadside can be quite different from the natural environments in which the native species generally occur. During highway construction, the existing topsoil and vegetation are usually removed. Sometimes clay soil is used (rather than reusing the existing topsoil) for its structural characteristics. Highway roadsides are as disturbed as an environment can possibly get and are among the ecosystems most susceptible to invasions by noxious species (Baker, 1986). The conditions after construction remain harsh with soil compaction from the initial construction; continuing compaction from mowers and other vehicles; competition from noxious weeds; chemicals such as road salt, brake dust, oils; and steep slopes that shed water quickly. Some native plants are able to grow on these poor soils (Mn/DOT Web site, 2005).

Mn/DOT recommends using native species on the roadsides, but will still utilize non-native species under certain circumstances (Mn/DOT Seeding Manual, 2003). The department has developed numerous seed mixes designed for different purposes like for wetlands, dry prairie, or forest edge. The mixes include cover crops because native species are slow to establish. The mixes can include up to over forty different species. Roadsides, unlike mitigation sites, use a regional seed source that encompasses a larger range (Robert Jacobson, personal communication). Prairie cordgrass, purple prairie clover, and spotted Joe-pye weed are currently among the species being used by Mn/DOT (Mn/DOT Seeding Manual, 2003).

The Department of Transportation has specific requirements for the source of seed for their projects. The Minnesota Crop Improvement Association has certification standards for native grasses and forbs endorsed by Mn/DOT (Mn/DOT website, 2005). The certification program ensures that the plant material is properly identified. Yellow Tag certification is seed that is source-identified from native stands or seed production fields, and is assumed to be relatively genetically diverse. The material is from a definable geographic region, the size of which is not regulated, but the region must be labeled on the tag (Mn/DOT Web site, 2005). DNA fingerprinting could aid in source identification and determining adequate diversity.

Guidelines for seed used on restoration projects

Restoration genetics is a new field combining restoration ecology and population genetics. At this time, few guidelines exist for self-sustaining adaptive restored populations (Hufford and Mazer, 2003). There are very wide-ranging ideas on how to choose seed for restoration. Cultivars, composites of different genotypes, and local ecotypes are among the possible sources for seed in a restoration. The goal is to have adequate diversity to ensure that there is future ability to adapt, while still having locally adapted genotypes. There can be detrimental effects of erring too far in either direction.

In the past, cultivars have been recommended for restorations. However, the breeding/selection process for cultivars is most likely not going to provide adaptive traits as well as local, native seed can. Visser and Reheul (2001), in a study of North-African perennial bunch grass (*Stipa lagascae*), analyzed populations in a search to find appropriate seed sources. They suggested that the populations were only different because of geographic isolation, not area-specific adaptation. They came to the conclusion in their restoration efforts that the seed sources should be chosen on the basis of vigor and highest level of genetic diversity (Visser and Reheul, 2001), even going so far as to suggest following conventional breeding practices of selection for vigor in restoration seed supplies. While using cultivars or selections for restorations may be sound reasonable, it seems unlikely that most conservation agencies in this state would follow this controversial method given the prevailing opinions.

Different restorations may require differing seed sources, or even a mixture of genotypes, rather than a single, local genotype. Lesica and Allendorf (1999) suggest that the choice of native plant material for restoration should be dependent on site size and level of disturbance. Highly disturbed areas, such as highway roadsides, are not representative of habitat to which the local plants are adapted, regardless of proximity. They recommend using a mixture of genotypes to ensure successful plant establishment. The introduced populations with higher genetic diversity may adapt more quickly in the disturbed areas. One problem is that nearby local populations could be genetically contaminated by gene transfer, which could result in a loss of indigenous diversity. Using only plants with selfing breeding systems could offset this problem (Lesica and Allendorf, 1999). Crop species, which could be considered a model for gene flow in native species, have been found to transfer genes to weed species (Lesica and Allendorf, 1999). In big bluestem, it has been found that there is the potential for introgression from introduced Conservation Reserve Program plantings to natural populations (Selbo and Snow, 2005), but further research is necessary to determine whether cross-pollination actually is occurring. Outbreeding depression under research settings has been found in several native species (Hufford and Mazer, 2003).

The last option for seed collection is also the most broadly supported: seed should be collected as close as possible to the site to be restored (Packard and Mutel, 1997). But, there is no consensus on what constitutes “local” or adequately close. As an example of how specific adaptation can be, big bluestem populations from Kansas and Illinois were

grown in each other's soils with and without their matching mycorrhizae. Each ecotype grew better in its own soil and had differences in interactions with their mycorrhizae that seemed to correlate with the nutrient level of their native soil (Schultz et al, 2001). There may be similar associations in Minnesota where seed from one site may be less able to thrive at an alternative site. In a native annual legume *Chamaecristra fasciculata* from Kansas, Minnesota, and Oklahoma, seed production was significantly decreased when grown in nonnative environments of the other states (Etterson and Shaw, 2001).

A more extreme view on seed sources for restoration is that natural plant populations can demonstrate biologically significant genetic differentiation on a very small spatial scale in the range of 10 to 100 m (Linhart and Grant, 1996). Therefore, what some would consider a single population would consist of smaller distinct populations adapted to their small locales. Li et al (1999) found differences in RAPD polymorphisms in wild emmer wheat (*Triticum dicoccoides*) that corresponded to sunny and shady microniches. Based on results of research such as this, Linhart (1995) recommends a maximum distance for seed collection of 100 m for herbaceous species and 1 km for trees. He advocates that despite similarity of environments, the use of plant materials from distant sites can lead to out-breeding depression. Seed quantities may be scarce due to intense fragmentation of the natural environment or small collection sites. Having adequate seed is an important issue, especially for large restorations. For very large restorations, using seed sources at this small scale would be extremely impractical.

One general guideline for seed collection is 100 miles north or south and 200 miles east or west (Packard et al, 1997). As mentioned previously, for the purposes of Mn/DOT, native plant restoration in Minnesota depends on the type of site. Mitigated sites must use a local source, while roadside plantings use a regional source (Robert Jacobson, personal communication). Another state agency, the Minnesota Department of Natural Resources (Mn/DNR) recommends that landowners use seed that is not more than 100 miles from their restoration site (Mn/DNR, 2000). The guideline for the DNR Scientific and Natural Areas (SNAs) is that seed for restoration for those sites is to be collected immediately adjacent to the site (Mn/DNR, 1999). Knapp and Rice (1994) suggest that collecting within a predetermined radius does not take into consideration environmental patchiness. The distance should not be the only consideration, but that the habitat within that distance should also be recognized. Many species do not show a simple relationship between geographical and genetic distance (Krauss and Koch, 2004).

There are several possible repercussions of using non-adapted seeds for restoration. One is that the seed is not adapted to a site and the restoration is less successful. Introgression, which is contamination of indigenous populations with foreign genes, could damage local populations by passing on deleterious genes. Additionally, outbreeding depression can occur when two differently adapted populations hybridize, resulting in breaking up of co-adapted gene complexes and dilution of locally adapted loci which leads to offspring adapted to neither of the original environments. One of the most serious consequences could be the permanent loss of biodiversity in the original populations already affected by habitat loss (Krauss and Koch, 2004). Founder effects occur when small source populations are used to restore other sites resulting in genetic

bottlenecks. One example is that of eelgrass (*Zostera marina*) where the restored populations had lower genetic diversity than natural populations. The restored populations had lower germination and reproductive shoots (Hufford and Mazer, 2003). Many studies have been performed on geographically isolated populations to detect outbreeding depression with negative effects that may be dependent on reproductive system (Hufford and Mazer, 2003). There are also forces that work in opposition to adaptation. Gene flow, founder effects, and phenotypic plasticity might lessen the effects of local adaptation. Gene flow homogenizes variation while founder effects and drift may make for chance variation that is unrelated to adaptation (Rice and Knapp, 2000).

In addition to local adaptation, the level of diversity in a population is important. Lande and Shannon (1996) propose that increasing genetic variability does not always correspond to better adaptability of a population, in particular when there is a constant environment. The "excess", or nonadaptive variability, must be selected against, initially to the detriment of the population while it reaches equilibrium. This does not necessarily hold true for random, unpredictable environments. Larger amounts of genetic variation may be important when fluctuations are high and occur for a long period (Lande and Shannon, 1996). It is not known whether natural selection will eliminate maladapted alleles reducing the need to consider outbreeding depression (Hufford and Mazer, 2003).

Chapter 5. DNA Fingerprinting

FTA technology

Prior to DNA fingerprinting, DNA must be isolated from the plant samples. FTA (Fast Technology for Analysis) technology is a method designed to simplify the collection and purification of DNA from plant tissues. FTA cards are imbedded with chemicals that lyse cell membranes and denature proteins upon contact. Nucleic acids, which are protected from nucleases, oxidation, UV damage, and microbial and fungal attack, are stabilized within the card for storage at room temperature. The stability of genomic DNA on FTA cards for at least 11 years has been demonstrated (Whatman, 2002). Researchers are increasingly using FTA cards in plant molecular genetics including species such as barley (Drescher and Graner, 2002), *Arabadopsis*, corn, tomato, soybean, sugarbeet, petunia, rice, poppy (Lin et al 2000), and cassava (Ndunguru et al, 2005). Fresh plant material is pressed into the FTA card. The plant cells, now within the card, are lysed and the DNA within them is isolated, bound, and preserved (Whatman, 2002). The three main benefits are the ease of transport (no dry ice or silica gel), the ease of storage (at room temperature for up to twelve years), and less time spent isolating DNA (it takes 1 day to process 96 samples with FTA). It can be especially helpful in field research in remote areas such as the study performed on *Impatiens* species in the mountain forests of Indonesia (Tsukaya, 2004). Once the DNA is captured within the card, a punch of the paper is taken. The punch is washed with FTA reagent to isolate the DNA for further processing. Compounds such as chlorophyll and other PCR inhibitors are removed along with the compounds that stabilize DNA (Whatman, 2003). This technique had not been previously been used with AFLPs, but has been used for restriction fragment length polymorphisms (Whatman, 2002).

AFLP markers

Molecular markers are useful tools to quantify large amounts of biological information, which can reveal details about diversity and population structure. They are a means to analyze differences in sequences of DNA from areas of the genome that are known to vary, which leads to a unique "fingerprint" for an individual plant. Common types of molecular markers used in DNA fingerprinting are restriction fragment length polymorphism (RFLP), randomly amplified polymorphic DNA (RAPD), microsatellites, and amplified fragment length polymorphism (AFLP). AFLPs are a major type of marker used in systematics, population genetics, DNA fingerprinting, and quantitative trait loci (QTL) mapping. They have been found to be equal to other types of molecular markers in time/cost efficiency, reproducibility, and resolution (Mueller and Wolfenbarger, 1999). Like other polymerase chain reaction (PCR) – based methods, AFLP have co-migrating band homology, which means that bands that have migrated the same distance on the gel are considered equivalent. The absence of a band does not imply anything and the presence indicates common ancestry (Sharbel, 1999). The AFLP technique is useful because it generates many markers from previously uncharacterized genomes, only small amounts of DNA are required, it is not technically demanding (Sharbel, 1999), and it is repeatable (Vos et al, 1995). This technique is especially

valuable when working with native plants that have not been previously characterized genetically. AFLPs are most useful for distinguishing populations within a species, rather than distinguishing species across a genus. Using AFLPs in different *Echinacea* species within the genus, Merchanda et al (2004) sequenced co-migrating bands across the genus. They found that 50% of similarly migrating bands are actually falsely homologous (cases where bands that are not related appear to be) (Merchanda et al, 2004). This may indicate that at least for some species, the high levels of polymorphisms that are generated using AFLPs do not translate to comparisons between species within a genus.

Many plant species are polyploids. With AFLP analysis, a marker could be present four times in a sample for a tetraploid and be compared to another sample where the marker is present only once. They would both have equivalent scores of 1. The degree of relatedness would be read as 100% when it should be 25% similar. With dominant markers, heterozygotes cannot be differentiated from amplified homozygotes. In order to overcome dosage effects of dominant markers where in a polyploid species a sample that has a single copy of a marker is scored the same as a sample with more than one copy, which can underestimate diversity, large numbers of markers should be used (Milbourne et al, 1997). In any case, care should be taken when estimating genetic diversity (Milbourne et al, 1997). To attain the same level of efficiency, dominant markers like AFLPs need 4 to 10 times as many markers as co-dominant ones like microsatellites. Compared to other marker systems, AFLP generates 10 to 100 times more bands (Sharbel, 1999). Another suggestion to eliminate bias is removing loci whose frequency is less than 3/N where N equals sample size (Sharbel, 1999).

AFLPs have been found to sample loci randomly throughout the whole genome, both nuclear and organellar, and are able to distinguish between populations (Mueller and Wolfenbarger, 1999). With the AFLP technique, markers are scattered throughout the genome, which is an issue in validating neutrality (Sharbel, 1999). There is no guarantee that bias will not exist as localized amplification can happen, but this will vary depending on the technique and species studied (Sharbel, 1999). It has been found in numerous other species like rice (Virk et al, 1998), barley (Waugh et al, 1997), and sugar beet (Schondelmaier et al, 1996) that AFLP markers are distributed throughout the whole genome. The assumption of selective neutrality, where the markers are not under natural selection pressure, is also not guaranteed because the AFLP markers are spread throughout the whole genome and may include sampling of functional genes (Sharbel, 1999).

One of the assumptions used for studies of molecular variance is that markers are neutral. Theoretical studies have found that genetic polymorphisms could not be maintained in a population just based on heterozygotic advantage. Because of this, they are considered neutral, which means that differences in variation are equivalent in function and have a similar relative fitness (Hedrick, 2000). Some scientists (Linhart and Grant, 1996) do not believe that molecular markers are neutral, and the variation found is a result of small-scale natural selection, but their view is in the minority. There is a contradiction in relating markers to adaptive traits, since molecular markers are considered neutral. If

they were not, the neutral assumption would be violated, and the estimates would be subject to uncertainty (McRoberts et al, 1999).

There are some concerns over the use of molecular diversity estimates in the field of ecology. Molecular markers are generally not considered a good method to correlate genetic variation with adaptive variation. Molecular markers are good for determining founder effects, genetic swamping, and population divergence (Hufford and Mazer, 2003). McRoberts et al (1999) noted that there are problems linking eco-physiological traits with molecular markers. Since molecular markers are not reflective of local adaptation, common garden comparisons could be more useful (Rice and Knapp, 2000). One issue with molecular markers is whether genetic diversity measures can be correlated with adaptive variation. Demissie et al (1998) found that RFLP diversity in barley did not correlate with morphological variation and suggested RFLPs may not be appropriate to use molecular markers to define ecotypes. In a meta-analysis of 71 data sets, Reed and Frankham (2001) found that molecular markers do not find differentiation due to natural selection and primarily determine the effects due to genetic drift. They state that neutral molecular markers like AFLPs are not useful to conservation biologists as a way to reveal population differentiation due to local adaptation. These results were also corroborated with research performed on a native California grass (*Nassell pulchra*) where no relationship between molecular diversity and quantitative traits was found (Rice and Knapp, 2000). One suggestion may be to use molecular markers along with additional measures of diversity. Reciprocal transplantation is still the best way to discriminate local adaptation effects (Latta, 2004).

AFLP methodology

Amplified fragment length polymorphism (AFLP) is a technique for DNA fingerprinting. After DNA has been isolated, it is digested with two restriction endonucleases EcoR 1 and Mse 1. EcoR 1 cuts frequently being a 4-base pair cutter, while Mse 1 cuts more infrequently as a 6-base pair cutter (Vos et al, 1995). Fragments of varying lengths are generated, but only those with two different restriction sites (one Eco R1 end and one Mse 1 end) are amplified (Vos et al, 1995). The endonucleases are inactivated, and the fragments ligated to EcoR 1 and Mse 1 adapters to generate template DNA for amplification.

There are two amplification steps: pre-amplification and selective amplification. Pre-amplification decreases the relative amount of DNA templates for the next step because the fragments have to match exactly or they are not amplified (Vos et al, 1995). Pre-amplification uses primers with one selective nucleotide. Selective amplification uses the same adapters with 3 extra nucleotides. The EcoR 1 primer, but not the Mse 1, in this step is labeled with a fluorescently-labeled probe for detection in the PCR gel. The use of different primers generates the number of markers necessary for the research. AFLP bands are scored for presence and absence to generate the haplotypes or DNA fingerprints, which are generally unique for each individual.

Chapter 6. Genetic Diversity and Population Structure

Terminology

A population is defined as a collection of interbreeding individuals together in space and time (Hedrick, 2000). There is much variation within natural populations at the molecular level. Population genetics study the amount and patterns of variation in populations (Hedrick, 2000). The genetic variation within a population is determined by selection, migration, mutation, genetic drift, and the mating system. Genetic variation within a species depends on soil types, growing season temperatures, length of growing season, and seasonal distribution of precipitation. Generally, genetic variation changes gradually across populations when a species is cross-pollinating, has continuous distribution, and/or is a long-lived perennial. Variation among populations is greater with self-pollination, with disjunct distributions, and with annuals or short-lived perennials (Millar and Libby, 1989). Geographic range and breeding system explain the largest proportion of genetic variation (Jasieniuk and Maxwell, 1991). Gene flow can be a big factor in the amount of diversity between populations. The more that genes are shared between populations via cross-pollination or seed dispersal, the less the diversity between the populations without affecting variation within a population. In determining genetic variation, populations are assumed to be in Hardy-Weinberg equilibrium with the assumptions that there is random mating, no migration, and normal gene segregation. This means that without change, allelic proportions will not change over time when the population is large and mating is random (Hedrick, 2000). Many population genetic analyses make these assumptions, although populations under natural conditions often fail to meet them.

Statistical approaches

Once the initial DNA fingerprinting is finished, haplotypes for each plant sample are generated. Haplotypes are a list of 1s and 0s depending on the presence and absence of a given polymorphic marker. Analyses to address different characteristics of the populations now can be executed. Common types of data analysis for population genetics include diversity estimates, genetic distance, cluster analysis and population differentiation.

Nei's (1987) diversity estimate is one way to analyze the data, which measures the proportion of polymorphic loci and average heterozygosity per locus. A locus is considered polymorphic when the most common allele is less than or equal to 0.95. This statistic measures the diversity of each marker and then calculates the average of all the markers over the population. The measurements generated are Ht, which is average gene diversity for the groups, and Hs, which is within population gene diversity. Average gene diversity is analogous to the chance that two random loci from a population will be different (Nei, 1987). This diversity estimate assumes a randomly mating population (Nei, 1987). These statistics can indicate the genetic "health" of populations. Low diversity estimates can indicate a genetically depauperate population. These estimates can also be compared to other species that have similar life histories, though there are

limitations when comparing results from different molecular marker systems (Krutovsii et al 1999).

Nei's unbiased genetic distance is an estimate of gene differences per locus (Nei, 1978). This statistic calculates the genetic distance of the populations on the basis of how dissimilar the haplotypes are. The numerical values fall between 0 (completely dissimilar) to 1 (completely alike). A genetic distance matrix is generated when comparing several populations and all their distances to one another. This estimate can be used to make dendrograms and principle coordinate (PCO) analysis to determine population dynamics.

Dendrograms or cluster diagrams show the relationships between populations by grouping them together. One method that generates dendrograms is the unweighted arithmetic average clustering algorithm (UPGMA). Using this algorithm, the most similar populations are clustered together with pairwise distances being recalculated as each consecutive population is added. UPGMA uses distance information from Nei's distance matrix. With the distance matrix, it begins by clustering the two smallest distances. Then new distances are calculated with the two closest as one new unit. Some information can be lost in the sometimes-arbitrary groupings (Legendre and Legendre, 1998). To check what effect this lost information has, dendrograms can be cross-referenced back to the original data by recreating the distance matrix from the dendrogram using a cophenetic correlation to find goodness of fit.

Another method that can be used to examine populations is principal coordinate (PCO) analysis. This analysis also used Nei's genetic distance matrix. PCO analysis plots the populations in two or three dimensions, which can be more informative than dendrograms. PCO analysis looks at the data set as a matrix (Legendre and Legendre, 1998). In the case of three dimensions, the three axes of a PCO analysis represent the largest fractions of variability with the three-dimensional data matrix. Principle coordinate analysis can sum up the majority of the variability of the matrix in a few dimensions of the principal axes (Legendre and Legendre, 1998).

An important component of population genetics is determining whether populations are the same or not. In 1921, Sewall Wright quantified the fixation index or F-statistic. The Fst is a measure of genetic differentiation. It is a number between 0 and 1 and utilized the diversity measures. It is given by the formula:

$$Fst = (Ht - Hs)/Ht$$

Wright developed guidelines for interpreting Fst (Hartl and Clark, 1997).

0 – 0.05 = little genetic divergence
0.05 – 0.15 = moderate genetic divergence
0.15 – 0.25 = great genetic divergence
0.25 and above = very great genetic divergence

Figures of much greater than 0.25 do occur, though Wright did not give interpretations for these figures. When a population is divided into isolated populations, there is less heterozygosity than there would be if the populations were undivided. The decline in heterozygosity within a population is quantified using an index known as Wright's F statistic or the fixation index (Hartl and Clark, 1997). Another measure of population differentiation is Nei's Gst. Nei developed this statistic for multi-allelic marker systems. Gst is the coefficient of gene differentiation and gives the relative magnitude of gene differentiation among subpopulations (Nei, 1978). However, Gst is dependent on gene diversity. When Ht is very small, Gst may be artificially large. In the case of bi-allelic molecular markers like AFLPs, Fst and Gst are equivalent.

Another important statistic of population differentiation is the analysis of molecular variance (AMOVA) model (Excoffier et al, 1992), a general design for hierarchical analysis of molecular variance similar to analysis of variance (ANOVA). The main difference is the substitution of SSD, the sum of squared difference between all haplotype pairs for SS, the sum of squares. The MSD is the mean of the squared differences instead of the mean square. AMOVA uses a matrix of squared distances between all pairs of haplotypes. These variance components are generated by equating the mean squared differences to the expected deviations similar to the F-statistic. The Φst or phi(st) generated in AMOVA is a correlation of random haplotypes within populations. Φst is analogous to Fst and Gst, which involve nonlinear transformation into estimates of genetic diversity. F-statistic assumptions are almost never met in natural populations, which also applies to Φ-statistics. The significance is tested using a permutational approach, unlike the F-statistic which assumes normality because this is more appropriate for molecular data (Excoffier et al, 1992).

Chapter 7. Materials and Methods

Sample collection

Big bluestem (*Andropogon gerardii* Vitman*)*, prairie cordgrass (*Spartina pectinata* Link), slender wheatgrass (*Elymus trachycaulus* (Link) Gould), purple prairie clover (*Dalea purpurea* Vent), false sunflower (*Heliopsis helianthoides* Linneaus), and spotted Joe-pye weed (*Eupatorium maculatum* L.) were the target species for collection. False sunflower and slender wheatgrass were taken out of consideration because of lack of populations found. Big bluestem was dropped due to technical issues with the DNA isolation and AFLP procedures.

In order to sample natural populations of the target species, natural, undisturbed sites were chosen. The primary goal in the site selection was to have access to indigenous populations with as low of a probability of introgression as possible. There were three categories of natural areas from which sites were chosen: Scientific and Natural Areas (SNA) Program, The Nature Conservancy (TNC), and the Minnesota State Park system. This research used SNA sites as much as possible because of the relative lack of disturbance and their mission for research. Second in preference were sites from the Nature Conservancy. This research utilized TNC sites primarily from the prairie region of Minnesota. Sites within the Minnesota State Park system were also utilized. However, since some state parks have introduced plantings and exotic species, caution was exercised in choosing sites to assure that the plant populations were indigenous. State park naturalists and rangers were consulted at these sites to identify undisturbed locations.

Sites were chosen based on probability or knowledge that the target species would be present. Prior to visiting sites, herbarium records were consulted to determine the likelihood of finding the target species (University of Minnesota Herbarium Web site, 2002). Because the species tend toward different environments (like spotted Joe-pye weed or cordgrass in wetlands to purple prairie clover in dry prairies), it was not expected that each site would contain all species. One of the hypotheses of this experiment was that populations would be differentiated according to the relationships defined by the Ecological Classification System. Another was that there would be a simple relationship with variation and geographic distances. Sites were chosen when possible within as many sections as possible and secondarily as spread out from one another as possible. Sites were identified based on species availability and ecoregion. The collection sites were distributed across biomes as much as possible.

Information was collected on each site, including topography (hill or low-lying area), other species present, density of target species, phenotypic notes, quality of site, and combined with any other information obtained from sources such as the DNR and TNC. Examples of the type of information include soil surveys, type of prairie or wetland and topography. A total of 45 sites were chosen initially, but only 21 sites are included in the final results. Some sites that were not included did not have the target species. There were also some sites dropped in order to reduce the number of samples. Table 7-1 lists

each site with owner of site, date visited, and GPS coordinates. Table 7-2 provides the county where the site is located; the type of community; the acreage of the site; the hardiness zone; and the biome, section, and subsection of the Ecological Classification System. As Minnesota is a diverse state, the sites varied greatly within a species. For instance, prairie cordgrass was collected from a calcareous fen, a wet meadow, and a mesic prairie. Purple prairie clover collection sites ranged from a mesic prairie to a goat prairie to a jack pine savanna. Spotted Joe-pye weed collection sites ranged from the shores of Lake Superior to a bog to a peatland. Figures 7-1, 7-2, and 7-3 show site collection maps for each species. Much more information was collected about each site, but is not included for the purposes of brevity.

The sample size per species, per site chosen for this experiment was 20. Natural populations in this study did not always contain 20 individuals, resulting in fewer than 20 specimens were collected. The number of samples actually collected for each site for each species is included in Tables 7-3, 7-4 and 7-5. Where there were less than 20 individuals collected, it was due to small populations present at the site or lack of quality DNA isolation for those samples. These tables also include a general indication of overall population size given in terms of less than 25 individuals (<25), between 25 and 50 individuals (25 – 50), and greater than 50 individuals (>50).

Coordinates were recorded using GPS and photos of each site were taken with a digital camera. At each site, twenty random samples from twenty different plants were collected when possible for each species to represent a population. Ten meters were paced between each sample collected except for prairie cordgrass, which was collected at twenty meters to ensure that samples from the same clone were not collected. Tissue from each plant was preserved on site with FTA micro cards using the direct leaf press method. Gloves were worn while collecting and alcohol was swabbed over all equipment to prevent cross-contamination. Leaf material was placed upon the FTA card, covered with parafilm, and pressed into the card using needlenose pliers until extract was drawn through the back of the card. Samples were stored in barrier pouches with silica gel packets in coolers with ice to protect from high outdoor summer temperatures until arrival in the lab where the samples were stored at room temperature.

Laboratory procedures

The DNA was isolated and purified on 2mm FTA card punches using the Whatman protocol for downstream RFLP analysis. Modifications included an additional final wash using 85% ethanol followed by an hour of air drying. The procedure was modified slightly in order to work with the three species; at times, more washes with the FTA purification reagent were necessary. Rather than having the DNA eluted off the punch, the DNA on the punch was digested and retained throughout the PCR steps up until the selective amplification step.

The AFLP technique used was that of Groh et al (2001). The procedure was adapted to work with the FTA cards. Template DNA, which remains on the purified punch until the selective amplification step, was digested using 5 U of the restriction enzymes MseI and

EcoRI. Digested DNA was then ligated to 25 pmol of MseI and 2.5 pmol EcoRI adaptors in a volume of 50 µl. Pre-amplification was performed using the ligated/digested punch and 37.5 ng pre-amplification primers (MseI + one selective nucleotide and EcorRI + one selective nucleotide). The product was diluted by adding 10 µl of ddH2O. Two µl of this dilution and 30 ng of selective amplification primers (MseI + three selective nucleotides and EcoRI + three selective nucleotides) were used in the selective amplification. Two selective primers from each restriction site combined to form four primer pair combinations. Table 7-6 shows the list of adaptors and primers used. The EcoR I-site selective primers were labeled with the fluorophores JOE, 6-FAM, or VIC. Prairie cordgrass samples were labeled with JOE with the primer pairs run individually. Purple prairie clover and spotted Joe-pye weed samples were labeled with either 6-FAM or VIC depending on the primer. Because 6-FAM and VIC are different colored, these samples were run together in the same lane at the same time using the multiplexing technique. The AFLP procedure was performed twice on each individual sample with all primer pairs. Table 7-6 shows the numbers of AFLP markers and levels of polymorphisms generated for each species with the four primer pairs.

For gel analysis of the amplified fragments, the samples were sent to the Advanced Genetic Analysis Center (AGAC) at the University of Minnesota. Amplified products were separated on the Applied Biosystems' ABI Prism 3100 Genetic Analyzer, which uses capillary electrophoresis. This system is less susceptible to contamination than the older system, and the output is in the form of data rather than gels. The peak detection threshold was set at 100 in order to eliminate background noise. ABI Prism Genescan Analysis Software detected fragments from the ABI Prism 3100. The files from this step were analyzed with ABI Prism's Genotyper software, which puts the data into text format and was used to generate electropherograms. The electropherograms were visualized for proper fluorescence level and to eliminate false peaks using Genoprofiler (2005).

The text files, after being prepped using Genoprofiler (You and Luo, 2003), were imported into Excel. Peakmatcher (DeHaan et al, 2002), an Excel macro, was used to score the presence or absence of the fragments and to generate haplotypes in the form of 1s and 0s. The 1s and 0s corresponded to presence or absence of a marker. All markers for the three species were in the range of 50 to 500 base pair or relative migration units (RMUs) in the terminology of Peakmatcher. The repeatability in Peakmatcher was set to 90%. Each loci that did not have a repeatability of 90% across all the replicates was removed to eliminate ambiguous fragments. To remove some of the possible bias, loci with a null frequency of less than 0.03 of the number of samples were removed. These haplotypes were used in all the subsequent data analyses.

Statistical analysis

POPGENE V 1.31 software (Yeh et al, 1997) was used to estimate Nei's unbiased measure of genetic distance. POPGENE was also used to estimate Nei's total average gene diversity (H_t), within population gene diversity (H_s), and Nei's coefficient of gene differentiation (Gst). Cluster analysis of the individuals was performed with POPGENE using unweighted arithmetic average clustering algorithm (UPGMA). The genetic

distances from POPGENE were analyzed using NTSYSpc Version 2.1 (Rohlf, 2000) for PCO analysis and dendrograms using UPGMA. For AMOVA analysis, Arlequin software (Schneider et al, 2000) was used. This software computes genetic diversity indices based on haplotype frequencies up to four hierarchy levels. For this study, two-level and three-level hierarchies were used; among populations and among individuals within populations, and with the clusters that were apparent in the dendrograms and PCO analysis. AMOVA was also used to test the original hypothesis that the patterns of genetic variance would correspond to the Ecological Classification System. AMOVA was also used to determine if the diversity patterns were related to ecological factors. The number of permutations for significance testing was set at 1000. The longitude and latitude was used to create a geographic distance matrix, which was compared to the genetic distance matrix using NTSYS with a Mantel test. Distance matrices of the data divided into two separate primer pairs were compared using a Mantel test in NTSYS to ensure that adequate numbers of markers were used. A cophenetic comparison of the dendrogram-derived distance matrices were compared to the original distance matrices. Correlations between diversity and population size were compared using Microsoft Excel. Cluster analysis (dendrograms not shown) was performed on the individuals of the populations. Clustering of individuals was evident when the individual plants of a population were grouped together.

Table 7-1. Site GPS coordinates and collection dates

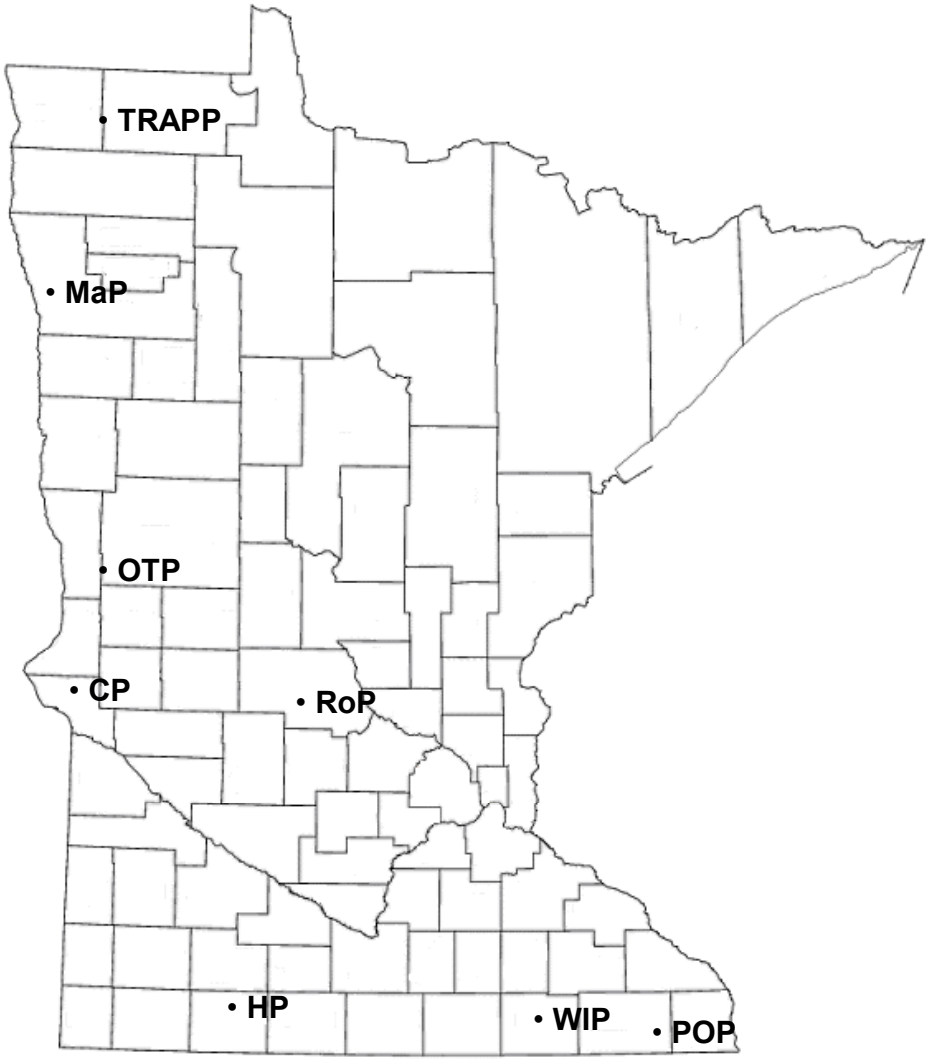
Site	Owned by	Collection Date	Latitude	Longitude	Elevation
Clinton Prairie	SNA	8/16/2003	N 45 27.437	W 096 33.884	1168
Gooseberry Falls	State Park	7/28/2003	N 47 08.357	W 091 28.007	620
Holthe Prairie	SNA	8/11/2003	N 43 45.179	W 095 04.445	1394
Lake Bemidji State Park	State Park	9/7/2003	N 47 32.291	W 094 49.004	1142
Lake Louise State Park	State Park	8/21/2003	N 43 31.645	W 092 31.755	1255
Malmberg Prairie	SNA/TNC	9/6/2003	N 47 43.888	W 096 49.163	885
Mound Prairie	SNA	7/30/2003	N 43 45.741	W 091 25.415	915
Norway Dunes	TNC	8/23/2003	N 48 41.296	W 096 34.379	1034
Old Mill State Park	State Park	8/23/2003	N 48 21.942	W 096 34.044	913
Ottertail Prairie	SNA	8/17/2003	N 46 09.125	W 096 13.592	1060
Paul Bunyan Savanna	TNC	8/27/2003	N 46 21.736	W 094 13.554	1200
Pin Oak Prairie	SNA	8/7/2003	N 43 47.617	W 092 12.955	990
Prairie Coteau	SNA	8/10/2003	N 44 06.844	W 096 09.036	1790
Red Lake Peatland	SNA	7/27/2003	N 48 20.277	W 094 33.242	1000
Roscoe Prairie	SNA/TNC	8/15/2003	N 45 25.081	W 094 40.871	1188
Saint Croix Savanna	SNA	7/17/2003	N 44 59.872	W 092 46.861	805
Saint Croix State Park	State Park	8/28/2003	N 45 57.012	W 092 38.586	921
Schaefer Prairie	TNC	8/26/2003	N 44 43.420	W 094 18.114	1042
Two Rivers Aspen Prairie	SNA	8/22/2003	N 48 39.588	W 096 20.638	1040
Verlyn Marth	SNA	8/17/2003	N 45 44.693	W 096 00.186	1127
Wild Indigo Prairie	SNA	8/21/2003	N 43 43.562	W 092 54.561	1239

State Park = Minnesota State Park System
TNC = The Nature Conservancy of Minnesota
SNA = Scientific and Natural Areas

Table 7-2. Site descriptions

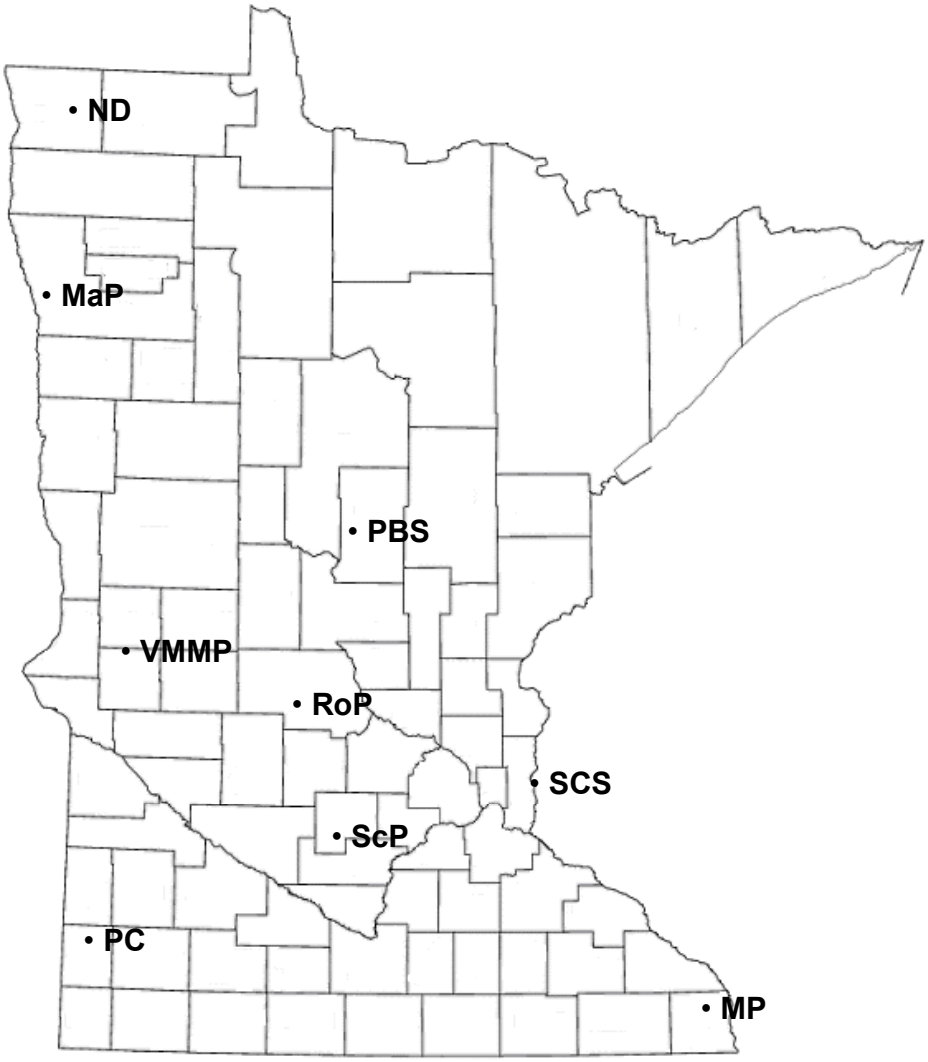
Site	County	Community types	Acres	Zone	Biome	Section	Subsection
Clinton Prairie	Big Stone	glacial till hill prairie	160	4A	Prairie	N. Central Glaciated Plains	Mn River Prairie
Gooseberry Falls	Lake	mixed evergreen forest	1,675	4B	Coniferous	N. Superior Uplands	North Shore
Holthe Prairie	Jackson	calcareous seepage fen fen-meadow-shrub-swamp	148	4B	Prairie	N. Central Glaciated Plains	Coteau Moraines
Lake Bemidji State Park		tamarack bog	6,765	3A	Coniferous	N. Mn Drift & Lake Plains	Chippewa Prairie
Lake Louise State Park		hardwoods wetlands	1,168	4B	Deciduous	Mn & NE IA morainal	Oak Savannah
Malmberg Prairie	Polk	mesic prairie wet prairie	80	3B	Prairie	Red River Valley	Red River Prairie
Mound Prairie	Houston	goat prairie	257	4B	Deciduous	Paleozoic Plateau	Blufflands
Norway Dunes	Kittson	mesic sand dune oak savanna	320	3B	Deciduous	Lake Agassiz Aspen Parklands	Aspen Parklands
Old Mill State Park	Marshall	riverine forest brushland dry-mesic woods	406	3B	Prairie	Red River Valley	Red River Prairie
Ottertail Prairie	Ottertail	wet to wet mesic prairie	320	4A	Prairie	Red River Valley	Red River Prairie
Paul Bunyan Savanna	Crow Wing	jack pine savanna	160	3A	Coniferous	N. Mn Drift & Lake Plains	Pine Moraines & Outwash Plains
Pin Oak Prairie	Fillmore	blufflands rare-in-blufflands wet meadow	184	4B	Deciduous	Paleozoic Plateau	Blufflands
Prairie Coteau	Pipestone	dry hill prairie wet prairie	329	4B	Prairie	N. Central Glaciated Plains	Inner Coteau
Red Lake Peatland	Beltrami	peatland	87,580	2B	Coniferous	N. Mn & Ontario Peatlands	Agassiz Lowlands
Roscoe Prairie	Stearns	blacksoil tallgrass prairie sedge marsh	57	4A	Deciduous	Mn & NE IA morainal	Hardwood Hills
Saint Croix Savanna	Washington	oak savanna	112	4A	Deciduous	Mn & NE IA morainal	St. Croix Moraines or Blufflands
Saint Croix State Park	Pine	aspen-conifer forest	34,037	3B	Coniferous	W. Superior Uplands	Mille Lacs Uplands
Schaefer Prairie	McLeod	tallgrass prairie wet mesic prairie dry prairie	160	4A	Prairie	N. Central Glaciated Plains	Mn River Prairie
Two Rivers Aspen Prairie	Roseau	prairie parkland wet to wet-mesic brush prairie	1,333	3B	Deciduous	Lake Agassiz Aspen Parklands	Aspen Parklands
Verlyn Marth	Stevens	dry-mesic prairie knolls cattail marsh/sedge meadow	34	4A	Prairie	Red River Valley	Red River Prairie
Wild Indigo Prairie	Mower	mesic tallgrass prairie	150	4A	Deciduous	Mn & NE IA morainal	Oak Savannah or Blufflands

Figure 7-1. Map of Prairie cordgrass sites



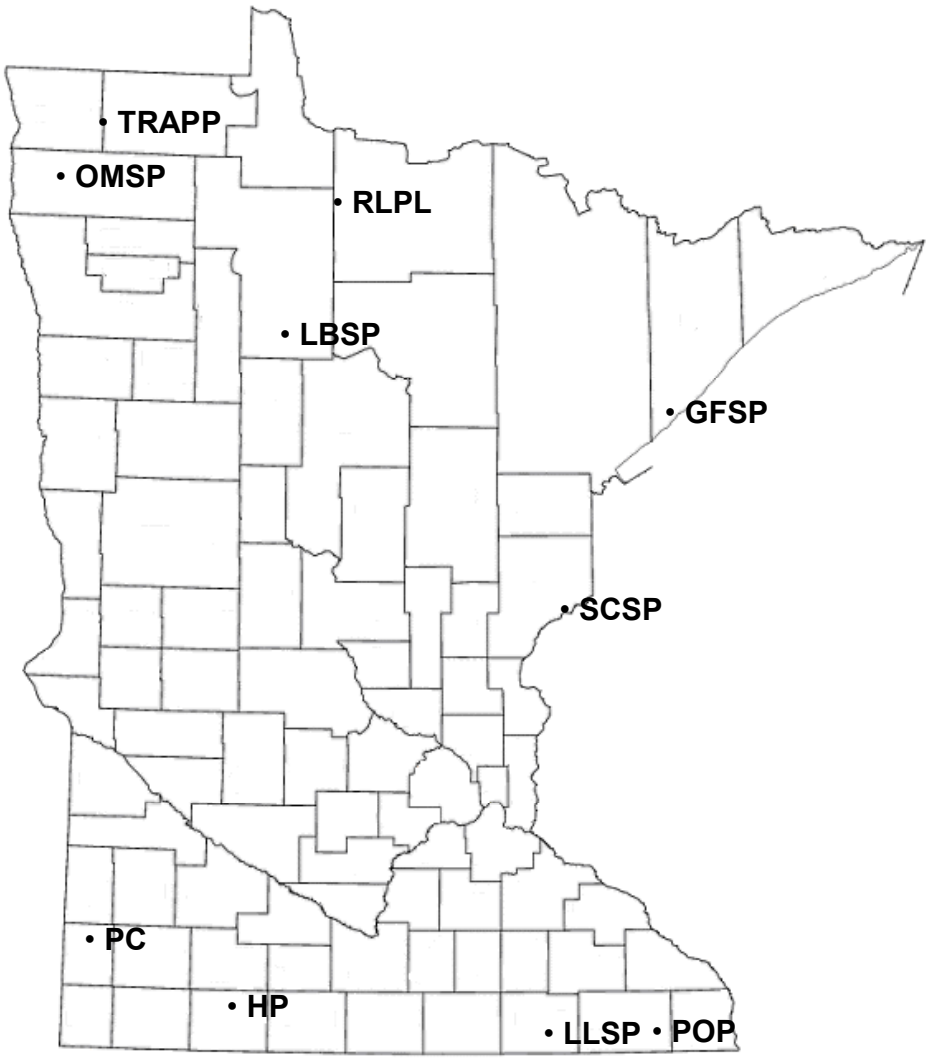
MAP = Malmberg Prairie
MP = Mound Prairie
ND = Norway Dunes
PBS = Paul Bunyan Savanna
PC = Prairie Coteau
ROP = Roscoe Prairie
SCP = Schafer Prairie
SCS = St. Croix Savanna
VMMP = Verlyn Marth Prairie

Figure 7-2. Map of Purple prairie clover sites



MAP = Malmberg Prairie
MP = Mound Prairie
ND = Norway Dunes
PBS = Paul Bunyan Savanna
PC = Prairie Coteau
ROP = Roscoe Prairie
SCP = Schafer Prairie
SCS = St. Croix Savanna
VMMP = Verlyn Marth Prairie

Figure 7-3. Map of Spotted Joe-pye weed sites



GFSP = Gooseberry Falls State Park
HP = Holthe Prairie
LBSP = Lake Bemidji State Park
LLSP = Lake Louise State Park
OMSP = Old Mill State Park
PC = Prairie Coteau
POP = Pin Oak Prairie
RLPL = Red Lake Peatland
SCSP = St. Croix State Park
TRAPP = Two Rivers Aspen Parklands

Table 7-3. Prairie cordgrass – Sample size, population size (number of individuals), and total gene diversity (Ht) within each population

Population	Sample size	Population size	Total Gene Diversity (Ht)
Clinton Prairie	20	25 - 50	0.2636 +/- 0.0302
Holthe Prairie	11	< 25	0.1715 +/- 0.0350
Malmberg Prairie	13	< 25	0.1638 +/- 0.0348
Ottertail Prairie	20	25 - 50	0.1941 +/- 0.0317
Pin Oak Prairie	11	< 25	0.1801 +/- 0.0357
Roscoe Prairie	5	< 25	0.1940 +/- 0.0420
Two Rivers Aspen Prairie	13	< 25	0.2122 +/- 0.0333
Wild Indigo Prairie	7	< 25	0.2220 +/- 0.0366

Table 7-4. Purple prairie clover – Sample size, population size (number of individuals), and total gene diversity (Ht) within each population

Population	Sample size	Population size	Total Gene Diversity (Ht)
Malmberg Prairie	20	25 - 50	0.1182 +/- 0.0224
Mound Prairie	18	> 50	0.1143 +/- 0.0216
Norway Dunes	19	> 50	0.1387 +/- 0.0273
Paul Bunyan Savanna	13	< 25	0.1411 +/- 0.0261
Prairie Coteau	12	> 50	0.1591 +/- 0.0327
Roscoe Prairie	19	25 - 50	0.1508 +/- 0.0273
Saint Croix Savanna	16	25 - 50	0.1049 +/- 0.0214
Schaefer Prairie	15	25 - 50	0.2068 +/- 0.0285
Verlyn Marth Prairie	20	< 25	0.1212 +/- 0.0219

Table 7-5. Spotted Joe-pye weed – Sample size, population size (number of individuals), and total gene diversity (Ht) within each population

Population	Sample size	Population size	Total Gene Diversity (Ht)
Gooseberry Falls State Park	15	25 - 50	0.1693 +/- 0.0239
Holthe Prairie	19	> 50	0.1613 +/- 0.0245
Lake Bemidji State Park	16	> 50	0.1814 +/- 0.0249
Lake Louise State Park	7	<25	0.1524 +/- 0.0335
Old Mill State Park	11	25 - 50	0.1636 +/- 0.0283
Pin Oak Prairie	17	> 50	0.1737 +/- 0.0234
Prairie Coteau	14	25 - 50	0.1744 +/- 0.0286
Red Lake Peatland	9	> 50	0.1436 +/- 0.0310
Saint Croix State Park	9	< 25	0.1954 +/- 0.0323
Two Rivers Aspen Prairie	10	< 25	0.2037 +/- 0.0311

Table 7-6. List of AFLP adaptors and primers. The four selective primer pairs used in this study–M47E33, M47E45, M61E33, and M61E45–are the combination of the two *MseI* site-selective primers with the two *EcoRI* site-selective primers.

Primers/Adaptors	Sequences
MseI adaptor	5'-GACGATGAGTCCTGAG-3' 3'-TACTCAGGACTCAT-5'
EcoRI adaptor	5'-CTCGTAGACTGCGTACC-3' 3'-CTGACGCATGGTTAA-5'
MseI pre-selective primer (M02)	GATGAGTCCTGAGTAAC
EcoRI pre-selective primer (E01)	GACTGCGTACCAATTCA
MseI selective primer (M47)	GATGAGTCCTGAGTAACAA
MseI selective primer (M61)	GATGAGTCCTGAGTAACTG
EcoRI selective primer (E33)	*GACTGCGTACCAATTCAAG
EcoRI selective primer (E45)	*GACTGCGTACCAATTCATG

a Sequences given in the 5' to 3' orientation unless otherwise noted
b Pre-selective primers have one selective nucleotide each (underlined)
c Selective primers have two additional selective nucleotides each (underlined)
* *EcoRI* site-selective primers fluorescently labeled green or blue

Chapter 8. Results and Discussion

Polymorphic markers

The populations of the three species have very high levels of polymorphisms with the primer pairs used. This research found polymorphism levels for the markers ranging from 95 to 100% (Table 8-1). Other native plant populations have shown similarly high levels especially in out-crossing grass species (Fu et al, 2004). Fu et al (2004) found 100% polymorphic bands in little bluestem while Merchanda et al (2004) found over 99% rates of polymorphism in *Echinacea* spp. In another study of *Echinacea* spp., high levels of polymorphisms between 90 – 94% per primer set were also found (Kim et al, 2004). It is not surprising that these natural populations that were sampled over large ranges would have high levels of polymorphisms with AFLPs. The high levels of polymorphisms are most likely due to the wide level of species distribution, the outcrossing nature of these species, and characteristics of the primers themselves.

Some of the populations of prairie cordgrass had private markers, meaning that they are unique to individual populations. Eleven private markers were found in the Clinton Prairie population. Two private markers were found in the Wild Indigo Prairie population, with one private marker each in Malmberg Prairie and Two Rivers Aspen Prairie Parkland. Purple prairie clover and spotted Joe-pye weed populations had no private loci, but there were several markers that were present in only two populations. Prairie cordgrass with its higher number of private markers may be displaying a greater population divergence than the other two species.

Genetic distance and similarity

Tables 8-2, 8-3, and 8-4 show Nei's unbiased measures of genetic identity and genetic distance. The numbers above the diagonal show the similarity between each population and the numbers below the diagonal show the distance between each population. Spotted Joe-pye weed demonstrates the highest similarities of the three species for all the populations. The similarity values ranged from 0.9318 for the least similar to 0.9914 for the most similar. High genetic similarity of populations could be due to many factors. The populations may only be recently separated, migration or gene flow has occurred between them, or the populations were large with little genetic drift or similar selection pressures affected loci in the same way (Hedrick, 2000). Prairie cordgrass and purple prairie clover had wider ranges of similarity. Prairie cordgrass ranged from 0.8484 to 0.9611. Purple prairie clover ranged from 0.8754 to 0.9915.

Diversity

Healthy natural populations can be analyzed for their levels of genetic diversity in order to compare to the genetic diversity of seed sources for restorations. The level of genetic diversity within a population may have important implications in seed or plant sources for restoration projects. All three species in this study have average gene diversities (Ht) that show moderate values. Table 8-5 shows total gene diversity (Ht) and within

population gene diversity (Hs). Prairie cordgrass has the highest total value at 0.2780 +/- 0.0207. Spotted Joe-pye weed was at 0.2100 +/- 0.0160, while purple prairie clover has the lowest at 0.1928 +/- 0.0161. The within population diversity (Hs) for prairie cordgrass was 0.2016 +/- 0.0097. Purple prairie clover had the lowest 0.1402 +/- 0.0060, and spotted Joe-pye weed had a value of 0.1716 +/- 0.0086. High genetic diversity in the range of 0.300 would be expected with all of these species due to their wide geographic range, outcrossing nature, and long-lived perennial life history (Hamrick and Godt, 2000). The lower values found in this study may be related to fragmentation or to unknown levels of outcrossing. The rate of decline in genetic diversity due to reasons such as habitat fragmentation is slower in polyploids than in diploids (Gustafson et al, 2004). Species like prairie cordgrass and spotted Joe-pye weed would be expected to have higher retention of diversity than would a diploid such as purple prairie clover. It has been found that for the purple prairie clover in Illinois, there was a low level of diversity using RAPDs and allozymes compared to other members of Fabaceae (Gustafson et al, 2002). The values could also be due to the marker system. AFLPs have a tendency to underestimate diversity because they do not detect the number of copies for a given locus (Milbourne et al, 1997). Thus, the diversity estimates found in this study cannot be compared directly to co-dominant markers like allozymes and microsatellites.

Hypothetically, smaller populations would be expected to have lower diversity. In their simulation models, Lesica and Allendorf (1991) found that small populations might not necessarily be of lower diversity, especially those small populations that are under environmental stress. This research included many small populations. Tables 7-3, 7-4, and 7-5 show the results from POPGENE for the total gene diversity (Ht) of each population for each species along with each population size. No correlation was found between population size and diversity of the individual population for any of the species. Preservation of genetic variation is very important to the continuation of a population; so determining the levels of diversity is essential to conservation and to restorations. This research supports the idea that small populations are worth preserving and could serve as diverse seed sources for restorations. Research in other native species, like big bluestem, Indian grass, and purple prairie clover, has found that small sites do not necessarily correlate with lower diversity (Gustafson et al, 1999; Gustafson et al, 2002; Gustafson et al, 2004).

Population differentiation

There are numerous measures of differentiation that will tell whether populations are different from one another. This research found, by two different statistics, that populations of these native plants in Minnesota are significantly different from one another. Table 8-5 shows the population differentiation (Gst) for each species. Spotted Joe-pye weed is the least differentiated with a value of 0.18, which is moderately to greatly differentiated. Prairie cordgrass and purple prairie clover both have values around 0.27 indicating great differentiation. Tables 8-6, 8-7, and 8-8 are AMOVA tables for each species generated from Arlequin. The differentiation value derived from the Φ statistics show differentiation of 0.23 for prairie cordgrass, 0.27 for purple prairie clover, and 0.12 for spotted Joe-pye weed. Again, prairie cordgrass and purple prairie clover

would be considered greatly differentiated, while spotted Joe-pye weed would be moderately differentiated.

Using allozymes, these species would be expected to have differentiation values in the range of 0.21 (Hamrick and Godt, 2000). As AFLPs are a dominant marker, the values cannot be compared directly. Estimates of population differentiation are always higher in AFLPs than in co-dominant markers like microsatellites (Gaudeul et al, 2004). They found Fst values for *Eryngium alpinum* that were almost twice as high with AFLPs as with microsatellites. In a study of purple prairie clover populations in Illinois and Kansas, slight to moderate population differentiation was found. They found values of 0.042 using the traditional Fst calculations and 0.14 and 0.23 using Shannon's diversity, which partitions variance similar to AMOVA. Big bluestem and Indian grass in Illinois using RAPDs had Fst values of 0.125 and 0.121, respectively (Gustafson et al, 2004). Travis et al (2002) found that *Spartina alternifolia* populations were differentiated by a range of Fst values of 0.0490 to 0.1101 using AFLPs, indicating little to moderate differentiation.

Patterns of variance

Two hypotheses that relate to the patterns of variation were considered for this research. One hypothesis was that the patterns of variation would relate to simple geographic proximity, meaning populations that were nearer would be more closely related than those farther. Another potential relationship that was considered was that diversity would vary according to the three biomes in Minnesota of the Ecological Classification System. If this were the case, populations within a biome would be more closely related to each other than they would be to those in other biomes. The null hypothesis was that there would be no discernible pattern to the genetic diversity. A second null hypothesis would be that populations are not significantly different from one another. As was stated in the previous section, this research has shown that with the species in this study, the populations are different across Minnesota.

A Mantel test using NTSYS was performed to test whether geographic distances were correlated to genetic distances. No correlation was apparent for any of the three species. AMOVA analyses were done to examine whether the genetic variation would correlate to each of the three biomes. Again, none of the three species demonstrated this type of relationship. This research did not find a relationship between genetic variation and geographic proximity for any of the species examined. The genetic diversity of some native species has been found to vary with geography, while others have not. In little bluestem (*Schizachryrium scoparium*), a prairie grass, Huff et al (1998) found that populations varied more between sites with high or low fertility, than varied with geography. In the same species, Fu et al (2004) using AFLPs found there was a relationship of variation with geographic origin. In another prairie grass, big bluestem, genetic relatedness did not correlate well with geographic distance (Gustafson et al, 1999). With smooth cordgrass (*Spartina alterniflora*) in Louisiana, Travis and Hester (2005) found there was a significant correlation between genetic differentiation and geographical distance over the range of 400 km. Gustafson et al (2002) found that the

genetic relationships correlated well with geographic proximity for purple prairie clover in Kansas and Illinois. Distance may not be the best gauge of genetic similarity, especially in cases of environmental heterogeneity (Hufford and Mazer, 2003). Discontinuous variation can also occur because of isolation of disjunct populations and genetic drift or natural selection.

Dendrograms for each respective species were generated from NTSYS (Figures 8-1, 8-2, and 8-3) and the principle coordinate analysis from NTSYS for each of the three species (Figures 8-4, 8-5, and 8-6). The eigenvalues of the first three PCO axes account for 92.70% of the variation for prairie cordgrass. The eigenvalues of the first two PCO axes account for 100% of the variation for purple prairie clover. The eigenvalues of the first three PCO axes account for 99.98% of the variation for spotted Joe-pye weed. While no relationships were found that corresponded to geographic distances or to the ECS, groupings are detectable on the dendrograms (Figures 8-1, 8-2, and 8-3) and on the PCO analysis (Figures 8-4, 8-5, and 8-6) for all the species. AMOVA analysis was conducted on the clusters detected by the PCO analysis and found that they account for a significant portion of the variance, with values of 13.04% for prairie cordgrass, 25.52% for purple prairie clover and 10.74% for spotted Joe-pye weed (Tables 8-6, 8-7, and 8-8). These clusters account for much of the variation among populations. With this evidence of substructure within these populations, the population dynamics could be associated with ecological and environmental factors. Other AMOVA analyses were performed to determine whether there were correlations of variance to ecotypic factors like soil type and moisture regime. No correlations were found for purple prairie clover or spotted Joe-pye weed. Prairie cordgrass did demonstrate one pattern of correlation; 7.70% of the variation was attributable to differences due to populations from mesic sites to populations from wet sites (Table 8-6). Mesic sites are Clinton Prairie, Malmberg Prairie, Roscoe Prairie and Wild Indigo Prairie, while the wet sites are Holthe Prairie, Ottertail Prairie, Pin Oak Prairie and Two Rivers Aspen Prairie Parklands. Patterns like this one that seem to fit a certain selection model do not necessarily mean that selection was acting because genetic drift can often generate the same patterns (Epperson, 2003). Strong patterns are only produced when selection is very intense. Prairie cordgrass is a wetland species so the moisture regime of a site could be considered a strong selection factor.

The maps of Minnesota along with population locations classified by clusters detected on the dendrograms are shown in Figures 8-7, 8-8, and 8-9. These figures show graphically how the populations do not vary by geography or by the ECS. One interesting pattern that was noticed in prairie cordgrass (Figure 8-7) and purple prairie clover (Figure 8-8) was that geographically distant populations appeared similar genetically. It was also somewhat evident in spotted Joe-pye weed (Figure 8-9). One theory as to why this is apparent may be due to the markers used. Highly polymorphic markers have a higher incidence of false homology. The more distant populations are from one another, the more likely false homologies occur (Merchanda et al., 2004). There is also the issue of higher homoplasy (why absence of band cannot be found) (Gaudeul et al., 2004), which again would be more prevalent in highly polymorphic AFLP markers. It could be possible that the relationships are somewhat skewed because of both issues.

High levels of phenotypic variation were observed in this study, some of which may translate to genotypic differences. All three species demonstrated differences in height from site to site. Plants in the north tended to be shorter and smaller. It was also noticed that flower color for purple prairie clover seemed to vary from purple to more of a pinkish color depending on the site. Dry sites for purple prairie clover had shorter, sparser plants. Prairie cordgrass would grow differently on dry sites, with clearly separated plants without many rhizomes, compared to the large, continuous masses that occur in the wetter areas. It was noticed that the bloom periods for the different species varied dramatically from site to site and seems to be dependent on moisture regimes and at one site, the light regime. Differing bloom time may lead to reproductively isolated cohorts that restrict gene flow.

Table 8-1. AFLP markers generated from each species with four primer pairs

Species	Primer pair	Number of markers	% Polymorphic
Prairie cordgrass	M47E33	103	97%
	M47E45	119	99%
	M61E33	113	99%
	M61E45	125	99%
Total		460	99%
Purple prairie clover	M47E33	92	96%
	M47E45	81	95%
	M61E33	84	100%
	M61E45	22	99%
Total		279	97%
Spotted Joe-pye weed	M47E33	67	97%
	M47E45	77	96%
	M61E33	85	99%
	M61E45	88	100%
Total		317	97%

Table 8-2. **Similarity of Prairie cordgrass populations.** Nei's unbiased measures of genetic identity (above diagonal) and genetic distance (below diagonal) for prairie cordgrass populations.

Population	CP	HP	MAP	OTP	POP	ROP	TRAPP	WIP
CP	*****	0.8600	0.8914	0.8987	0.8484	0.9189	0.8691	0.9368
HP	0.1508	*****	0.9192	0.9552	0.9161	0.8973	0.9194	0.8710
MAP	0.1150	0.0842	*****	0.9611	0.8842	0.8964	0.9167	0.8943
OTP	0.1068	0.0459	0.0397	*****	0.9165	0.8940	0.9395	0.8882
POP	0.1644	0.0876	0.1230	0.0872	*****	0.8743	0.9339	0.8581
ROP	0.0846	0.1083	0.1094	0.1121	0.1343	*****	0.8979	0.9524
TRAPP	0.1403	0.0840	0.0870	0.0624	0.0684	0.1077	*****	0.8841
WIP	0.0653	0.1381	0.1117	0.1186	0.1531	0.0487	0.1232	*****

CP = Clinton Prairie
HP = Holthe Prairie
MAP = Malmberg Prairie
OTP = Ottertail Prairie
POP = Pin Oak Prairie
ROP = Roscoe Prairie
TRAPP = Two Rivers Aspen Parkland
WIP = Wild Indigo Prairie

Table 8-3. **Similarity of Purple prairie clover populations.** Nei's unbiased measures of genetic identity (above diagonal) and genetic distance (below diagonal) for purple prairie clover populations.

Population	MAP	MP	ND	PBS	PC	ROP	SCP	SCS	VMMP
MAP	*****	0.9057	0.8964	0.9634	0.8776	0.9897	0.8955	0.9105	0.9915
MP	0.0990	*****	0.9868	0.9671	0.9376	0.9030	0.9588	0.9822	0.9191
ND	0.1094	0.0132	*****	0.9541	0.9336	0.8897	0.9566	0.9713	0.9092
PBS	0.0373	0.0335	0.0470	*****	0.9279	0.9572	0.9553	0.9701	0.9701
PC	0.1305	0.0645	0.0687	0.0748	*****	0.8754	0.9477	0.9531	0.8891
ROP	0.0103	0.1020	0.1169	0.0438	0.1331	*****	0.8889	0.9083	0.9870
SCP	0.1104	0.0421	0.0444	0.0457	0.0538	0.1178	*****	0.9656	0.9046
SCS	0.0938	0.0180	0.0291	0.0303	0.0480	0.0962	0.0350	*****	0.9217
VMMP	0.0085	0.0843	0.0952	0.0303	0.1175	0.0130	0.1003	0.0815	*****

MAP = Malmberg Prairie
MP = Mound Prairie
ND = Norway Dunes
PBS = Paul Bunyan Savanna
PC = Prairie Coteau
ROP = Roscoe Prairie
SCP = Schafer Prairie
SCS = St. Croix Savanna
VMMP = Verlyn Marth Prairie

Table 8-4. **Similarity of Spotted Joe-pye weed populations.** Nei's unbiased measures of genetic identity (above diagonal) and genetic distance (below diagonal) for prairie cordgrass populations.

Pop.	GFSP	HP	LBSP	LLSP	OMSP	PC	POP	RLPL	SCSP	TRAPP
GFSP	*****	0.9881	0.9887	0.9337	0.9418	0.9906	0.9495	0.9591	0.9374	0.9483
HP	0.0120	*****	0.9914	0.9393	0.9447	0.9882	0.9524	0.9558	0.9388	0.9442
LBSP	0.0114	0.0086	*****	0.9401	0.9471	0.9896	0.9517	0.9606	0.9420	0.9499
LLSP	0.0686	0.0627	0.0617	*****	0.9878	0.9472	0.9510	0.9676	0.9413	0.9318
OMSP	0.0600	0.0569	0.0543	0.0123	*****	0.9527	0.9609	0.9784	0.9516	0.9493
PC	0.0094	0.0119	0.0105	0.0542	0.0485	*****	0.9540	0.9680	0.9445	0.9521
POP	0.0519	0.0488	0.0495	0.0503	0.0399	0.0471	*****	0.9687	0.9780	0.9736
RLPL	0.0418	0.0452	0.0402	0.0329	0.0218	0.0325	0.0318	*****	0.9555	0.9571
SCSP	0.0646	0.0632	0.0598	0.0604	0.0496	0.0570	0.0223	0.0455	*****	0.9779
TRAPP	0.0530	0.0574	0.0514	0.0706	0.0520	0.0491	0.0267	0.0439	0.0223	*****

GFSP = Gooseberry Falls State Park
HP = Holthe Prairie
LBSP = Lake Bemidji State Park
LLSP = Lake Louise State Park
OMSP = Old Mill State Park
PC = Prairie Coteau
POP = Pin Oak Prairie
RLPL = Red Lake Peatland
SCSP = St. Croix State Park
TRAPP = Two Rivers Aspen Parklands

Table 8-5. Total gene diversity (Ht), within population diversity (Hs) and Nei's coefficient of gene differentiation (Gst) for each species.

Species	Ht	Hs	Gst
Prairie cordgrass	0.2780 +/- 0.0207	0.2016 +/- 0.0097	0.2749
Purple prairie clover	0.1928 +/- 0.0161	0.1402 +/- 0.0060	0.2726
Spotted Joe-pye weed	0.2100 +/- 0.0160	0.1716 +/- 0.0086	0.1828

Table 8-6. Analysis of molecular variance (AMOVA) for Prairie cordgrass. Three AMOVA analyses conducted on prairie cordgrass. The first is a two-level hierarchy that tests within and among populations. The second adds the clusters derived from the dendrogram (Figure 8-1) as a third level of partitioning of variance. The third adds difference between the mesic sites (CP, MaP, RoP and WIP) and wet sites (HP, OTP, POP and TRAPP) as a third level of partitioning of variance.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	p-value
<u>All populations</u>					
Among populations	7	1492.513	13.65869 Va	22.72	0.00000
Within populations	92	14274.597	46.46301 Vb	77.28	0.00000
<u>Two clusters (dendrogram)</u>					
Among clusters	1	548.796	8.37256 Va	13.04	0.02151
Among populations within clusters	6	943.717	9.39501 Vb	14.63	0.00000
Within populations	92	4274.597	46.46301 Vc	72.34	0.00000
<u>Mesic versus wet</u>					
Among clusters	1	440.989	4.78175 Va	7.70	0.03128
Among populations within clusters	6	1051.524	10.88901 Vb	17.53	0.00000
Within populations	92	4274.597	46.46301 Vc	74.78	0.00000

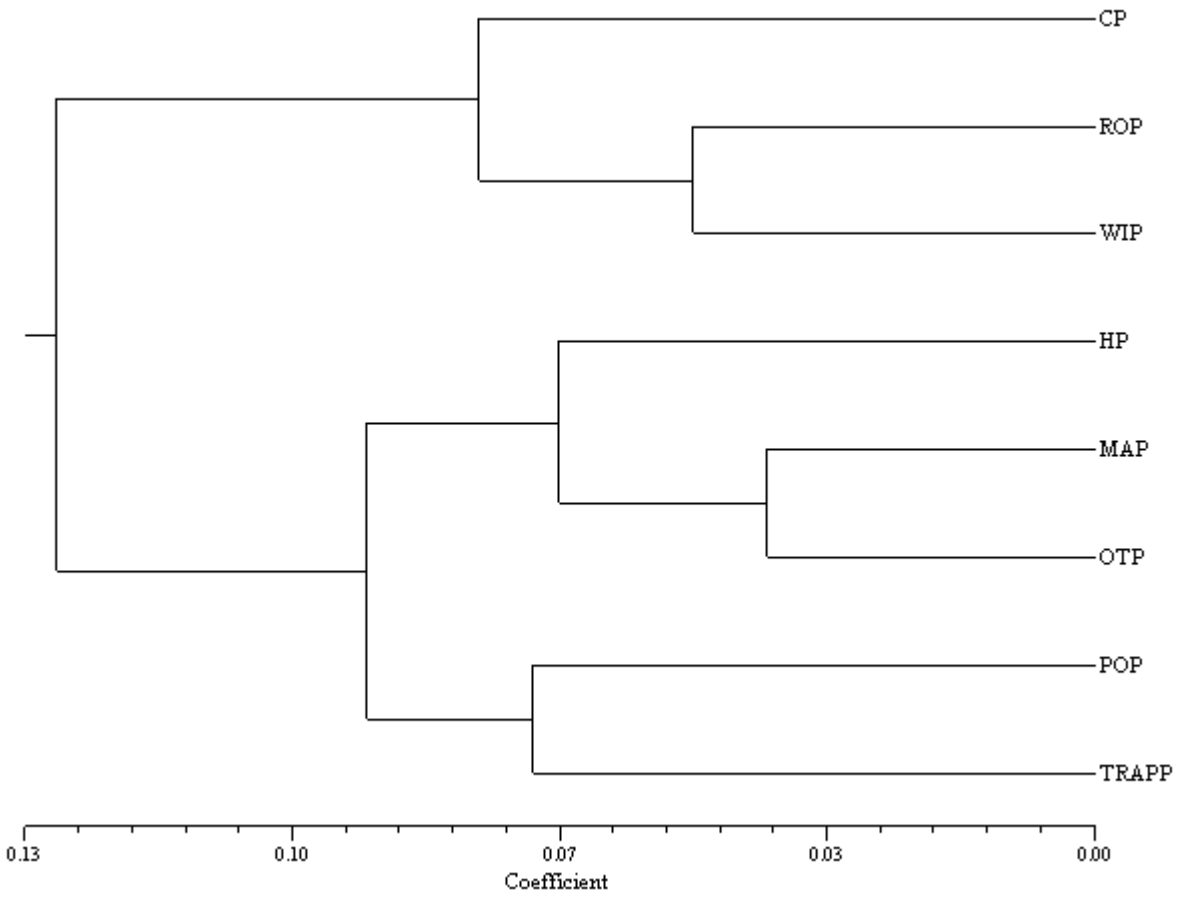
Table 8-7. Analysis of molecular variance (AMOVA) for Purple prairie clover. Two AMOVA analyses conducted on purple prairie clover. The first is a two level hierarchy that tests within and among populations. The second adds the clusters derived from the dendrogram (Figure 8-2) as a third level of partitioning of variance.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	p-value
<u>All populations</u>					
Among populations	8	1048.225	6.69802 Va	26.79	0.00000
Within populations	143	2617.972	18.30750 Vb	73.21	0.00000
<u>Two clusters (dendrogram)</u>					
Among clusters	1	592.132	7.23740 Va	25.52	0.00782
Among populations within clusters	7	456.092	2.81690 Vb	9.93	0.00000
Within populations	143	2617.972	18.30750 Vc	64.55	0.00000

Table 8-8. Analysis of molecular variance (AMOVA) for spotted Joe-pye weed. Two AMOVA analyses conducted on spotted Joe-pye weed. The first is a two level hierarchy that tests within and among populations. The second adds the clusters derived from the dendrogram (Figure 8-3) as a third level of partitioning of variance.

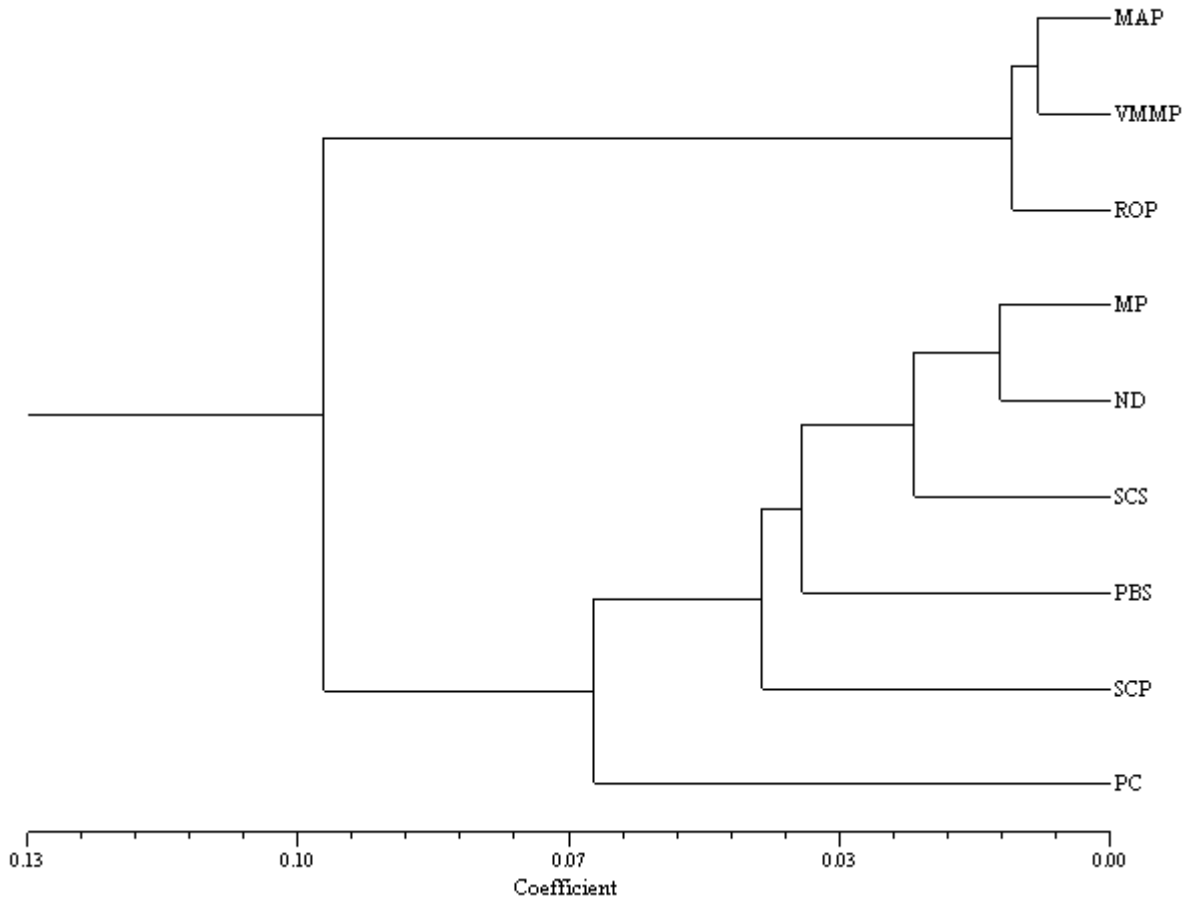
Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	p-values
<u>All populations</u>					
Among populations	9	649.821	3.56278 Va	11.50	0.00000
Within populations	117	3207.021	27.41044 Vb	88.50	0.00000
<u>Two clusters (dendrogram)</u>					
Among clusters	1	270.271	3.49336 Va	10.74	0.00000
Among populations within clusters	8	379.550	1.60825 Vb	4.95	0.00000
Within populations	117	3207.021	27.41044 Vc	84.31	0.00000

Figure 8-1. Prairie cordgrass dendrogram. UPGMA dendrogram of prairie cordgrass populations.



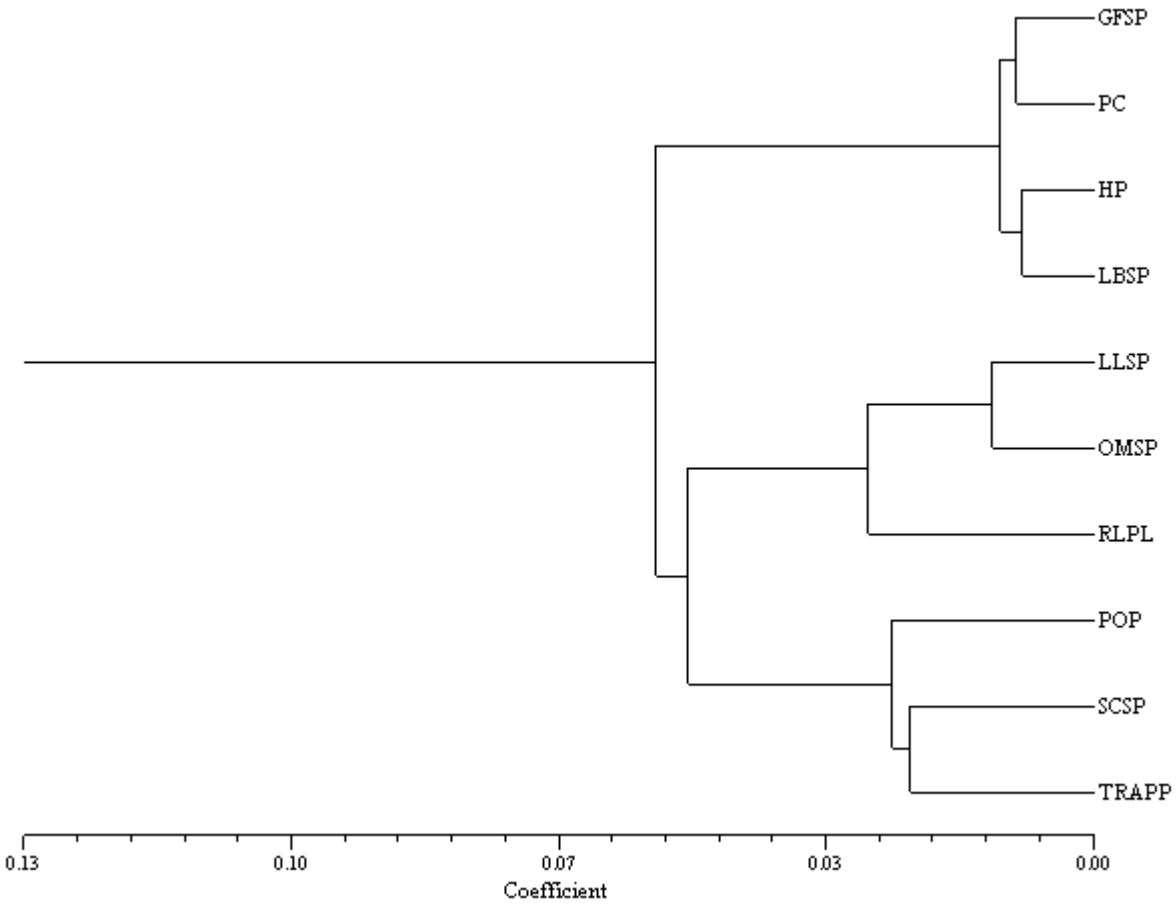
CP = Clinton Prairie
HP = Holthe Prairie
MAP = Malmberg Prairie
OTP = Ottertail Prairie
POP = Pin Oak Prairie
ROP = Roscoe Prairie
TRAPP = Two Rivers Aspen Parkland
WIP = Wild Indigo Prairie

Figure 8-2. Purple prairie clover dendrogram. UPGMA dendrogram of purple prairie clover populations.



MAP = Malmberg Prairie
MP = Mound Prairie
ND = Norway Dunes
PBS = Paul Bunyan Savanna
PC = Prairie Coteau
ROP = Roscoe Prairie
SCP = Schafer Prairie
SCS = St. Croix Savanna
VMMP = Verlyn Marth Prairie

Figure 8-3. Spotted Joe-pye weed dendrogram. UPGMA dendrogram of spotted Joe-pye weed populations.



GFSP = Gooseberry Falls State Park
HP = Holthe Prairie
LBSP = Lake Bemidji State Park
LLSP = Lake Louise State Park
OMSP = Old Mill State Park
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POP = Pin Oak Prairie
RLPL = Red Lake Peatland
SCSP = St. Croix State Park
TRAPP = Two Rivers Aspen Parklands

Figure 8-4. Prairie cordgrass PCO analysis. Prairie cordgrass populations plotted along the first two principal coordinate (PCO) axes. PCO analysis is based on Nei's unbiased measure of genetic distance.

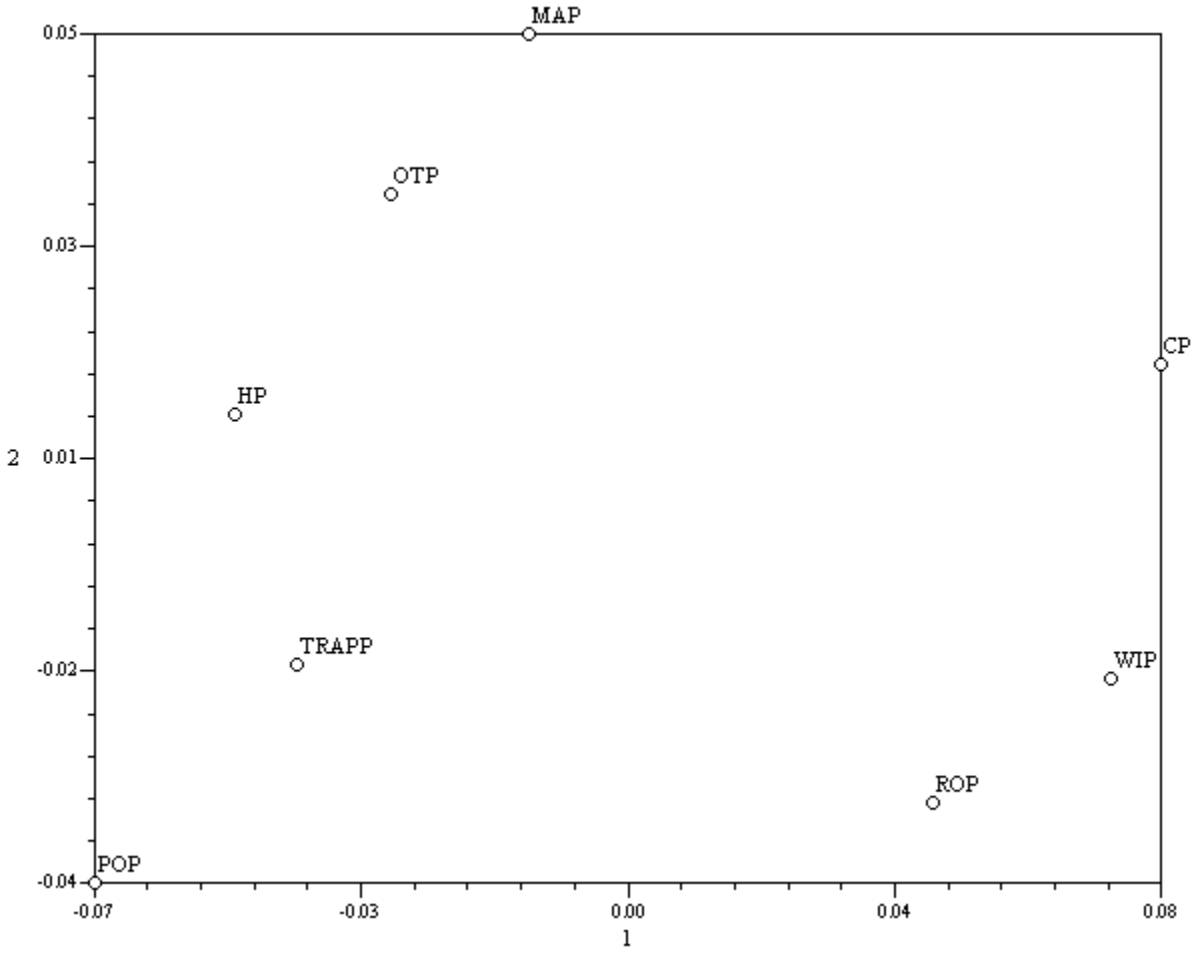

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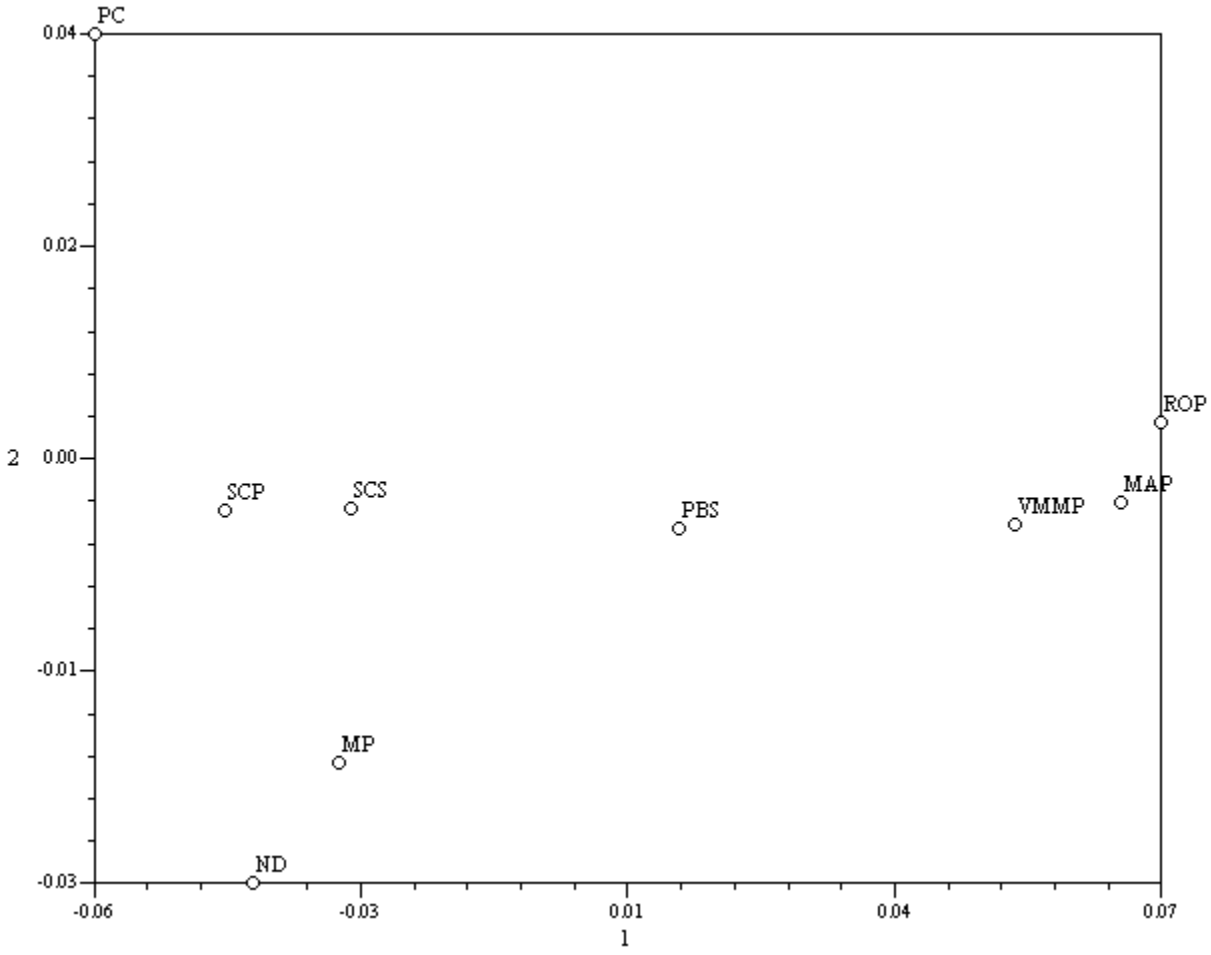
Figure 8-5. Purple prairie clover PCO analysis. Purple prairie clover populations plotted along the first two principal coordinate (PCO) axes. PCO analysis is based on Nei's unbiased measure of genetic distance.



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MP = Mound Prairie
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PC = Prairie Coteau
ROP = Roscoe Prairie
SCP = Schafer Prairie
SCS = St. Croix Savanna
VMMP = Verlyn Marth Prairie

Figure 8-6. Spotted Joe-pye weed PCO analysis. Spotted Joe-pye weed populations plotted along the first two principal coordinate (PCO) axes. PCO analysis is based on Nei's unbiased measure of genetic distance.

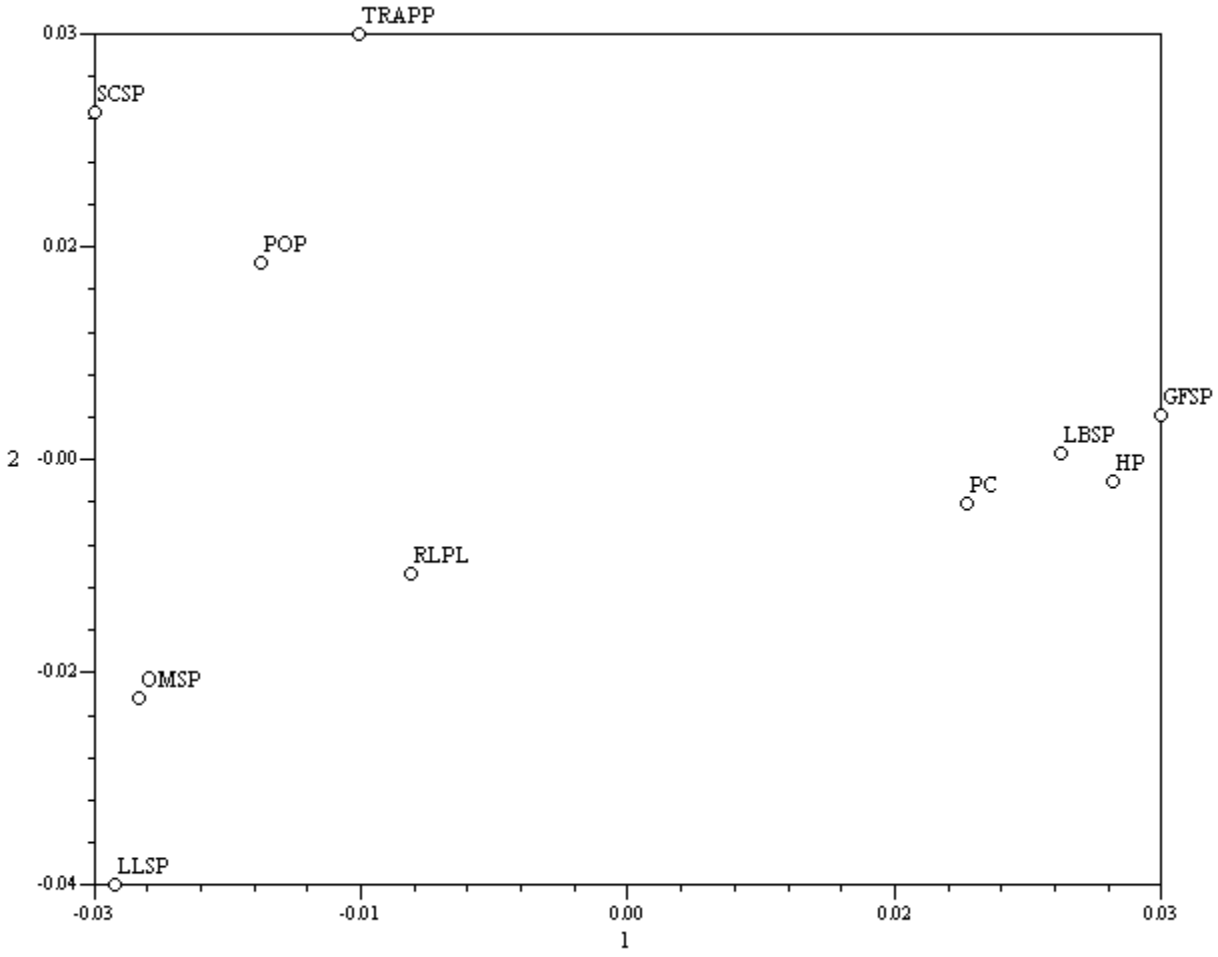

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Figure 8-7. Prairie cordgrass – Two clusters of dendrogram by site location. The filled squares correspond to populations belonging to one cluster of the dendrogram. The open triangles correspond to populations belonging to the second cluster of the dendrogram. These clusters account for much of the variation among populations.

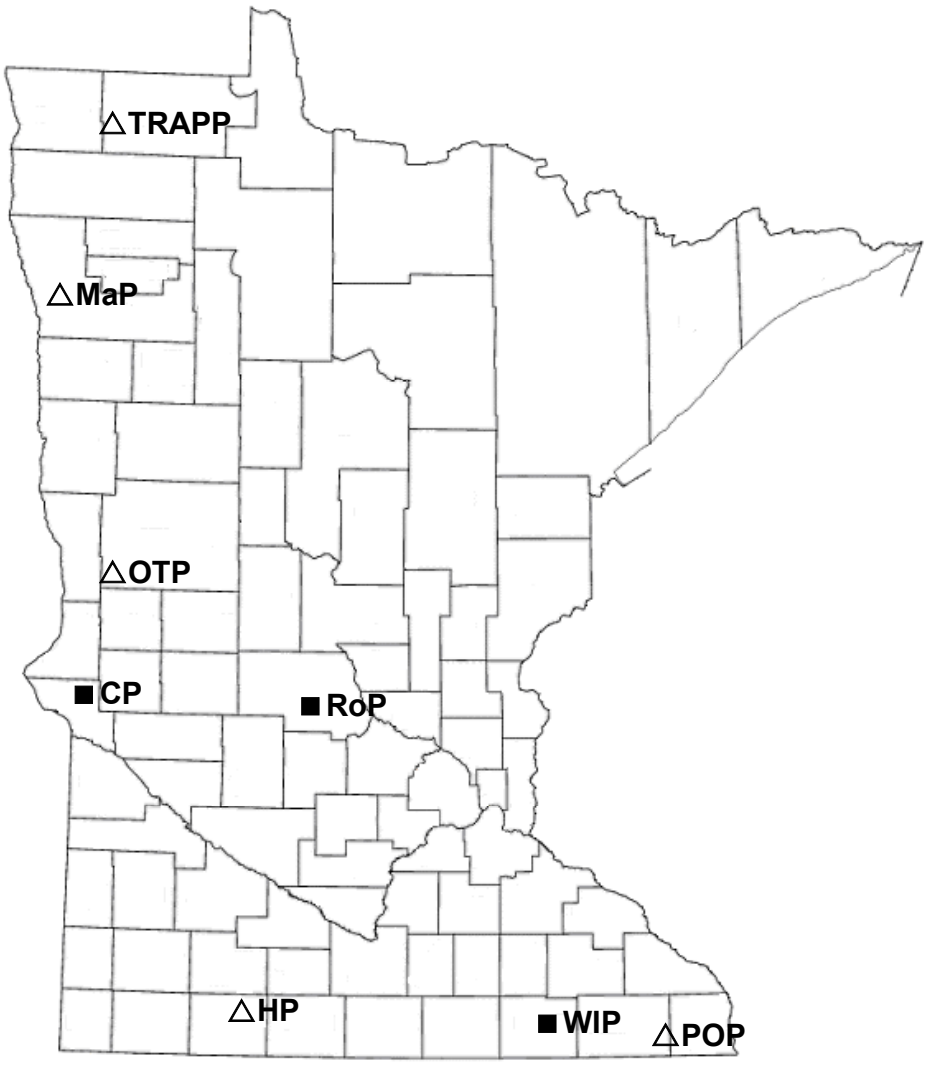

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Figure 8-8. Purple prairie clover – Two clusters of dendrogram by site location. The filled squares correspond to populations belonging to one cluster of the dendrogram. The open triangles correspond to populations belonging to the second cluster of the dendrogram. These clusters account for much of the variation among populations.

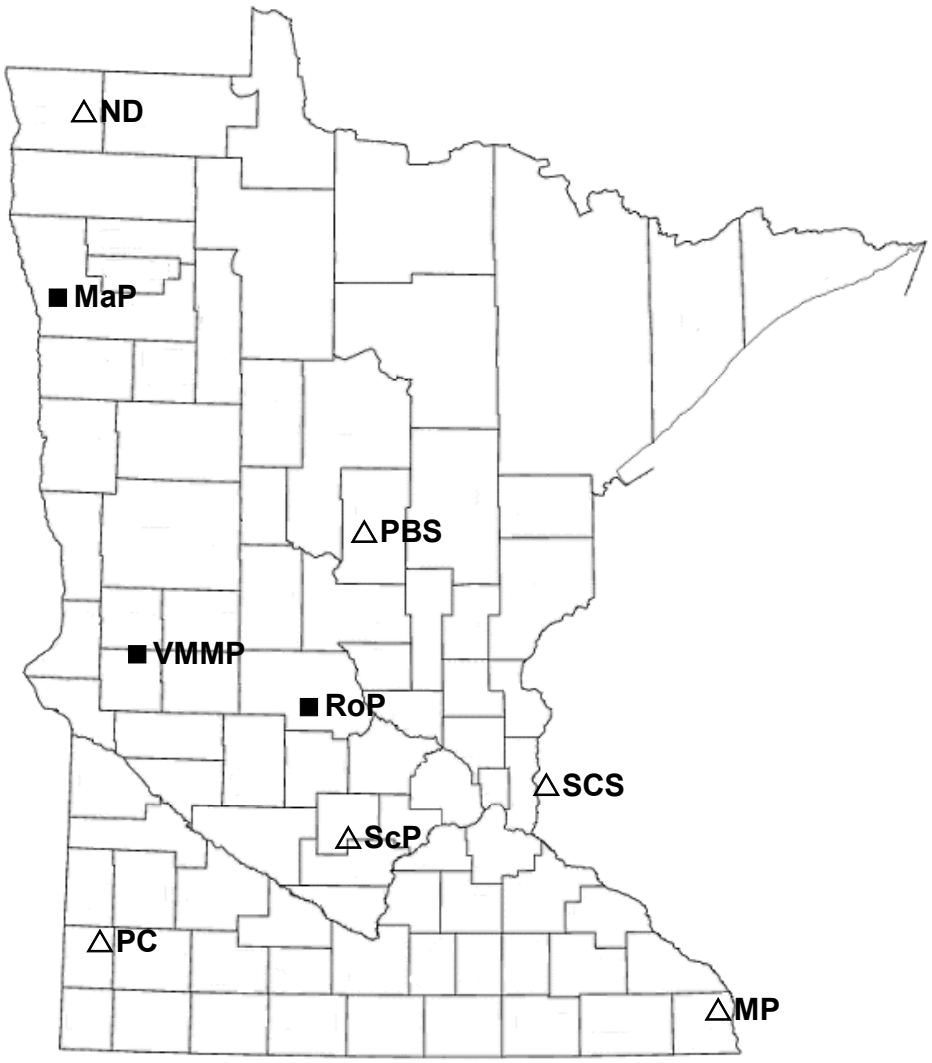

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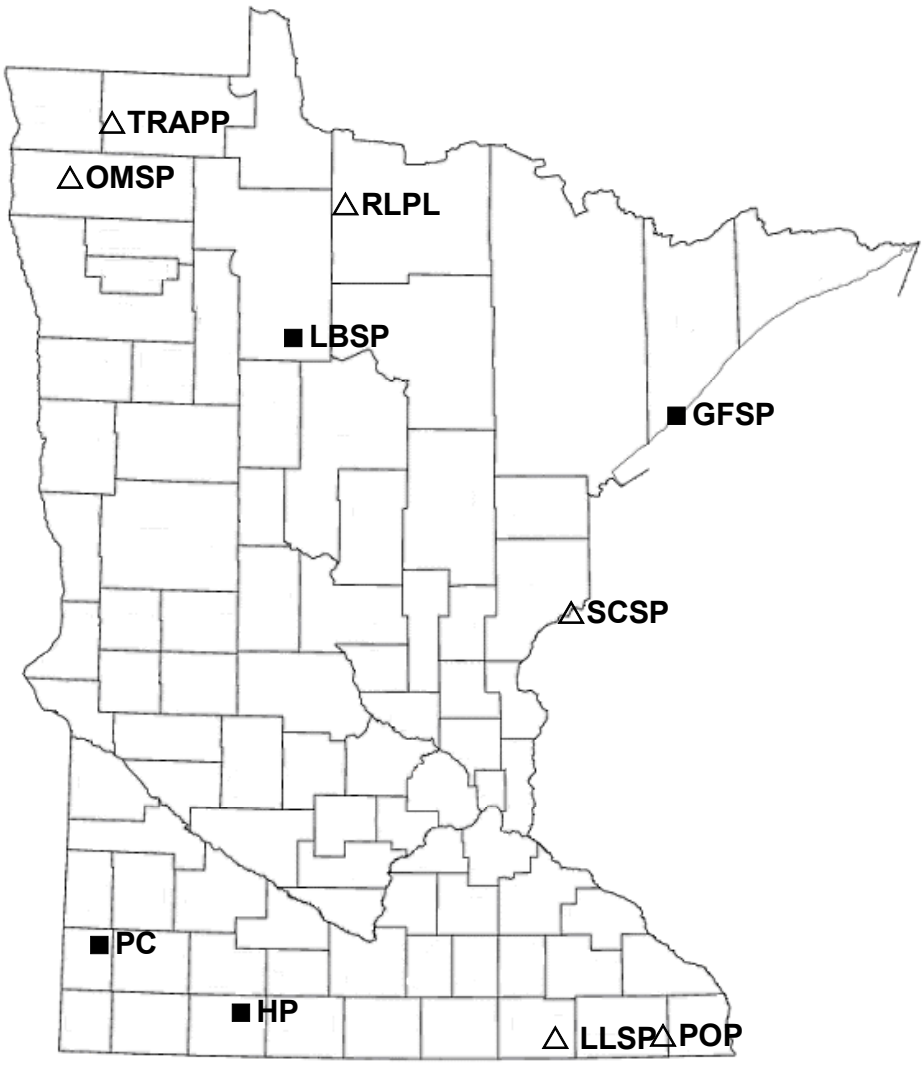
Figure 8-9. Spotted Joe-pye weed – Two clusters of dendrogram by site location. The filled squares correspond to populations belonging to one cluster of the dendrogram. The open triangles correspond to populations belonging to the second cluster of the dendrogram. These clusters account for much of the variation among populations.



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Chapter 9. Conclusions

The population genetics of native species is an important area of study with the issues of restoration and conservation. There are three basic questions that were considered for this research. Are the populations significantly different from one another? If they are different, what are the patterns of variation? What are the underlying reasons for these patterns?

It was found that the populations are significantly different from one another (although the exact level of differentiation may not be precise due to the dominant markers used). This is to be expected because these species, due to intense habitat fragmentation, are obviously not growing as a continuous population with high gene flow. Habitat fragmentation will have an effect on gene flow. Prairie, where many of the sites in this study were located, is especially fragmented with less than 1% of the prairie ecosystem left (Allmann, 1997).

Spotted Joe-pye weed populations across the state appear to be more genetically similar relative to the other two species. This could be due to the fact that spotted Joe-pye weed is not limited to pristine habitats as is a species like purple prairie clover. It may be found more often in disturbed places like ditches, which could lead to greater gene flow. Prairie cordgrass has greater population divergence, which could be partly due to its breeding system, clonal growth, and low seed viability. Purple prairie clover populations also seem to be more differentiated from one another, which may be due to the small population sizes and narrow range of habitat in which it can occur within a single prairie.

The second question concerns the way the genetic variation is partitioned. The patterns of genetic variation were found to be disjunct across the state of Minnesota. The original hypotheses that the variation may correlate to geography or to the ECS were not supported. Some researches with native species (little bluestem, purple prairie clover, and smooth cordgrass) have shown a geographic and genetic relationship, while other research has not (little bluestem and big bluestem). Given the fragmented status of native populations in Minnesota, populations here most likely would be more disjunct than continuous. Minnesota may be a unique case because of the confluence of three (or four) biomes in one state. Although a relationship between genetic variation and the Ecological Classification System was also not demonstrated in this research, there may be other ecological factors superseding this classification that are in effect. Distance may not be the best indicator of genetic similarity. Other factors like elevation, soil, climate, and life history of the species may have a greater impact (Hufford and Mazer, 2003).

Some authorities list two to three sub-species of spotted Joe-pye weed in Minnesota (Gleason and Cronquist, 1991). This could explain some of the discontinuous patterns of relationships with this species. Also, it is known that spotted Joe-pye weed hybridizes with others within the genus. *E. maculatum* subsp. *maculatum* and *E. maculatum* subsp. *bruneri* are the most prevalent subspecies in Minnesota (Anita Cholewa, personal communication). The samples taken for this research were not identified to subspecies so both may have been included. Further research would need to be done to find out if the

differentiation is due to sub-speciation. Given that these populations were not that highly differentiated in the first place, it may even be that the subspecies are not significantly different at the genetic level.

The third and final question of these species' population genetics is: what is behind these disjunct patterns of variation? There could be numerous reasons for this pattern, including issues with the dominant marker technique, random genetic drift, divergent selection forces, asymmetrical habitat fragmentation, or even introgression from past restorations. A certain genotype may be ascribed to a site by chance and not to adaptation (Rice and Knapp, 2000). AFLPs and molecular markers are most useful in detecting genetic drift, more so than differences due to adaptive selection. This research is only a start in determining why these relationships exist.

Chapter 10. Recommendations and Implications for Native Plant Restorations

The genetic diversity and population genetics of native plants is increasingly being examined for a multitude of species. Few studies have been performed in Minnesota at this time. One study that was completed in Minnesota involved examining phenotypic variation of big bluestem, little bluestem, bee balm (*Monarda fistulosa*), and three blazing star species (*Liatris* spp.) (Concibidio, 1995). The variation due to genetic differences was determined by planting different populations in a common garden experiment. Variation that was seen in the native environment was phenotypic while variation in the common garden was genetic. With big bluestem, a strong relationship between geographic distances and phenotypic distances without substantial differences was found. The results suggested that this species could readily adapt to wide geographic areas with latitude producing the greatest differences. Based on her results, the author suggests a radius of 300 to 400 miles for seed collection zones for big bluestem (Concibido, 1995). It was suggested that *Monarda fistulosa* and *Liatris aspera* could be collected at a distance radius of 200 to 300 miles. *Schizachyrium scoparium* was found to vary at distances of 200 miles.

The research above is an example of examining phenotypic differences, while this study examined genotypic differences. In regard to restoration genetics, there are three aspects to consider: genetic differences, phenotypic differences, and adaptive differences. To gain a complete picture and insight in determining seed restoration guidelines, all three aspects need to be examined. There are also phenotypic differences where there are morphological differences that do not affect adaptability. Different environmental conditions would lead to different phenotypes, even though the genotype remains constant. Further research would be necessary with common garden experiments to determine whether these differences are purely phenotypic or whether there is some genetic basis. Common garden experiments do not differentiate between differences due to drift or selection. They do find differences due to phenotype. Reciprocal transplant experiments are the best way to find differences due to adaptation. Plants are grown at each other's sites to determine "home-site advantage" (Hufford and Mazer, 2003). In restoration ecology, an ideal model would be to evaluate seed sources in the environments in which the restorations would occur (Gustafson et al, 2002).

This research focused on genetic differences, which may or may not be unrelated to adaptive differences. A useful supplement to this data would be to run common garden and reciprocal transplantation experiments on the same species on the same sites. The combination of all this information would provide more definitive answers for delineating collection zones for restorations. Determining whether the differences are due to geographic isolation or drift, versus adaptive selection would be important for determining seed collection zones. At this time, without any indications of strong geographic correlations, it is difficult to provide strong recommendations for seed collection zones. One strategy may be to ensure high levels of genetic diversity within the populations used as the source for the restorations. Based on the results of this study, the current methods of seed collection used by Mn/DOT for restorations cannot be either validated or discounted. Until the underlying causes for the variation found in this

research are determined, the best option at this time may be to err on the side of caution and use seed that is as close as possible to the restoration site.

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