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The use of AFLP to detect genetic differentiation within and among populations of two prairie plant species: Panicum virgatum and Coreopsis palmata

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THE USE OF AFLP TO DETECT GENETIC DIFFERENTIATION WITHIN AND AMONG POPULATIONS OF TWO PRAIRIE PLANT SPECIES: *PANICUM VIRGATUM* AND *COREOPSIS PALMATA*

An Abstract of a Thesis

Submitted

in Partial Fulfillment

of the Requirements for the Degree

Masters of Science

Christopher Andrew Hilker

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ABSTRACT

The degree of genetic diversity within any species is crucial to its survival with respect to environmental stresses and its ability to adapt. As native Iowa prairie plant populations continues to diminish, genetic diversity within the state becomes crucially important for restoration, reconstruction, and conservation efforts. This study seeks to determine the degree of genetic variation within native Iowa populations of *Panicum virgatum* (switchgrass) and *Coreopsis palmata* (prairie coreopsis, tickseed, prairie tickseed). Plants were obtained directly from the tallgrass prairie, from native seed plantings, and from greenhouse grown cultivated varieties (switchgrass). Amplified Fragment Length Polymorphisms (AFLP) provided genetic fingerprints of each individual plant, which allowed for each species to be compared and analyzed. Genetic variation within switchgrass populations was found to be high, with most genetic variations occurring among populations. Genetic variation within prairie coreopsis was found to be average with most genetic variations occurring within populations. The genetic structures and characteristics shown in this study may provide insight for future prairie plantings and restoration efforts to maintain and increase genetic diversity within remnant prairie populations.

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This Study by: Christopher Andrew Hilker

Entitled: THE USE OF AFLP TO DETECT GENETIC DIFFERENTIATION WITHIN AND AMONG POPULATIONS OF TWO PRAIRIE PLANT SPECIES: *PANICUM VIRGATUM* AND *COREOPSIS PALMATA*

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DEDICATION

This work is dedicated to my grandparents, whose lives have shown me what struggle is and the rewards of perseverance, and to my parents who have given me support and love and have pushed me to succeed in all of my endeavors.

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First and foremost I would like to thank my major advisor, Dr. James Jurgenson, for introducing me to the world of molecular biology and instilling in me a curiosity to explore new techniques and ideas. I am grateful for the instruction, insight, support, and advice through the ups and downs of research. Dr. Steve O'Kane was responsible for guiding me through population genetics and gave me advice and help despite my continuous pestering. Your help was invaluable. Dr. Daryl Smith introduced me to the wonders of the prairie and helping me understand the preciousness of the prairie ecosystem. Dr. Jim Demastas taught me systematics and gave advice that was of great help. Dr. Jeff Tamplin helped with figures which allowed this manuscript to have some visual stimuli. Dylan Baker, a friend and editing phenom, and Eric Berns, a friend who was always there when I needed to relieve a little stress, you guys provided the understanding and support only fellow graduate students could. Greg Houseal and Dave Williams provided expertise on prairie plants that resulted in the collection the right plants. Tara Poshusta, Shana Edwards, and Tanya Poshusta whose help as undergraduate researchers provided great assistance in helping with DNA extractions and lab work as well as good company. Finally, to friends and family for believing in me and supporting me through all of this, I can not express the gratitude I have for all of you. Thank-you!

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CHAPTER 1

INTRODUCTION

Iowa's Geological Landforms

Throughout the history of the Earth, the area of land we now refer to as Iowa has undergone a variety of geological influences. The bedrock underlying Iowa's surface is composed of sedimentary rock deposited by the ancient seas that covered the land (Thomson, 1992). Glaciation cycles of the Pleistocene stripped the land of vegetation and reformed the landscape. The advance and retreat of glaciers resulted in an array of different landforms across the state (Figure 1).

The Southern Iowa Drift Plain is Iowa's largest landform. Glacial drift was deposited by the Pre-Illinoian glaciers between 500,000 and 2,500,000 years ago. Deep meandering streams and well-established drainage systems on thick deposits of glacial drift attest to the advanced age of the landscape (Prior, 1991). The Southern Iowa Drift Plain is also characterized by steeply sloped hills carved out by years of erosion.

The Iowan Surface occupies a major portion of northeast Iowa and is characterized by rolling long slopes. Although this area used to be part of the Southern Iowa Drift Plain, periods of intense cold weathering and erosion between 16,500 and 21,000 years ago during the Wisconsinan glaciation, loosened, removed, and redeposited earth materials on the Iowan surface region (Prior, 1991).

The northwest corner of Iowa contains the Northwest Iowa Plains. The Northwest Iowa Plains was also once part of the Southern Iowa Drift Plain, but it underwent much of the same transformation as the Iowan Surface did during the

Wisconsinan glaciation. The Northwest Iowa Plains were affected by glacial movement along the eastern edge and thick deposits of wind-blown loess (soil) throughout this region created steepened hillsides and smoothed out various irregularities. In addition to these factors, this area is higher and drier with the most extensive prairie (Prior, 1991).

North central Iowa is the most recently glaciated portion of the state and is known as the Des Moines Lobe. This section of the state experienced glaciation as recent as 12,500-14,000 years ago during the advancement and retreat of the Wisconsinan Glacier (Prior, 1991). Glacial advance and retreat left moraine ridges throughout this region. Features such as fresh glacial drift, natural lakes, and a poorly drained surface are evidence of the recent glaciation.

The distinct Paleozoic Plateau of the northeast corner of Iowa is characterized by bedrock outcroppings throughout the region. The absence of glacial deposits indicates that this area was not glaciated. However, due to the massive amounts of erosion caused by glacial melt, it is difficult to be positive that this area did not experience glaciation. Nonetheless, the exposed bedrock outcroppings, the lack of loess, and the deeply carved drainage ways make this landform unique in Iowa (Prior, 1991).

The other three landforms within Iowa are the Loess Hills, the Mississippi Alluvian Plain, and the Missouri Alluvian Plain. The Alluvian plains were deposited as the two large rivers bordering Iowa carried extensive glacial melt. Warmer temperatures melted the glaciers and created floods of sediment-loaded water which carved the large flood plains and valleys associated with the Mississippi and Missouri rivers (Thompson, 1992). These waters carried large amounts of silt which were deposited along the edges of

the rivers. The Loess Hills are a distinctive landform created through the deposition of large amounts of wind blown silt along the Missouri River during the Wisconsinan period. Steep, ridged hills with unique biological characteristics were the result of the deposition of hundreds of feet of loess over thousands of years (Prior, 1991).

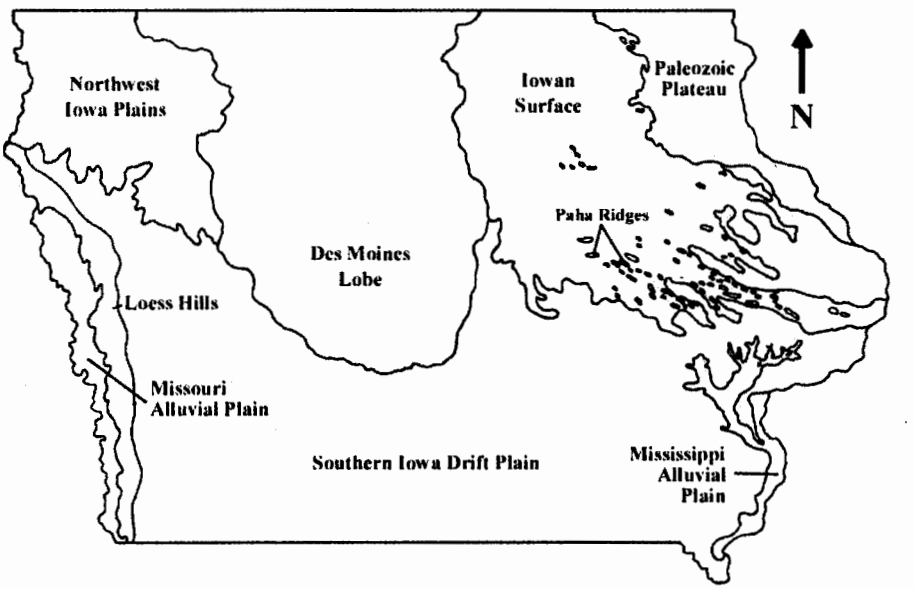

Figure 1. Iowa Landforms. The relative locations of landforms created during the geological history of Iowa (adapted from Prior, 1991)

The development of the Iowa landforms, in addition to the climate, rainfall patterns, and numerous other environmental influences, created numerous microenvironments across Iowa. As prairie established itself as the dominant plant community, these microenvironments may have influenced the genetic evolution of prairie plants. In the time preceding settlement, prairie developed in Iowa as the natural

ecological response to numerous factors that define the environment: geology, landforms, soils, climate, and other organisms interacting over time (Thompson, 1992).

Prairie

Prairie, meaning meadow, was the name the French explorers called the vast treeless landscape they found stretching throughout Middle America (Smith and Smith, 1980). The versatility of the prairie ecosystem allowed it to exist in the harsh climate of the Midwest, especially the hot and dry summers and winters which are freezing cold and dry. The extreme environment allowed for a variety of plants to adapt and evolve together. This created one of the most complex and balanced ecosystems on Earth (Smith and Smith, 1980).

At the end of the last glacial period, prairie emerged as North America's largest continuous ecosystem (Chadwick, 1995). The tallgrass prairie grew to cover 250 million acres (100 million hectares) and was maintained across North America for 8,000 years (Shirley, 1994). Tallgrass prairie stretched from Ohio to central Nebraska and from Manitoba to Texas (Figure 2) (Costello, 1969). Within the prairie, a wide array of plant life existed, with each species being a vital part of the ecosystem. Dominated by over 30 species of grasses and over 250 forbs, the tallgrass prairie maintained rich diversity (Shirley, 1994). Within Iowa, prairie developed and evolved as the dominate ecosystem over 80% of the state, with the remaining 20% containing scattered wetlands, savannas, and forests (Smith, 1998).

With the beginning of the 19th century, an era of change began which was to have a profound effect on the tallgrass prairie of Iowa. The vast , diverse, and complex

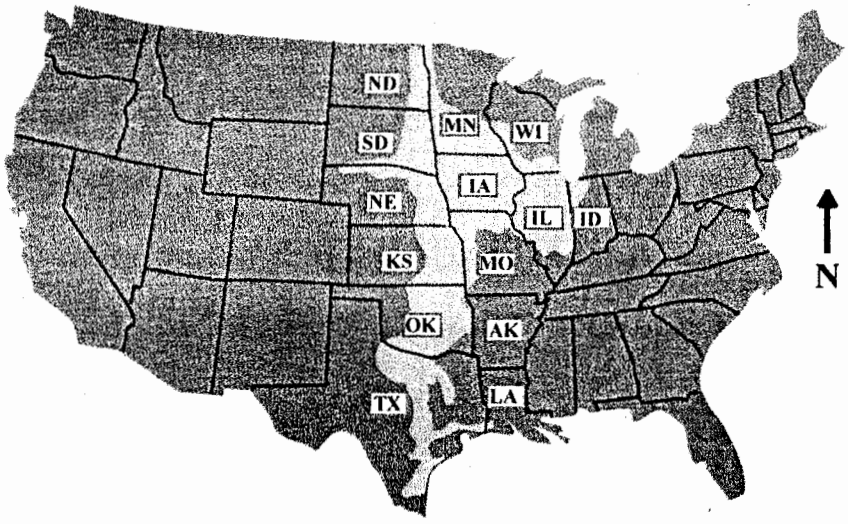

Figure 2. The original extent of the tallgrass prairie. The extent of the tallgrass prairie within the United States prior to settlement. Iowa is the only state that is completely encapsilated by prairie (adapted from Kurtz, 2001)

ecosystem that had existed for thousands of years, began to be destroyed. What took thousands of years to create, would all but be eliminated within one hundred years of human occupation.

Human settlement and technological advancements would cause the elimination of most of the tallgrass prairie (Figure 3). Between 1830 and 1900 nearly all of Iowa's prairie disappeared (Smith, 1981). Most of Iowa's 29 million acres of prairie was plowed up, overgrazed, or developed for settlement (Kurtz, 2001). Conversion of the original landscape to agriculture and urban use has eliminated more than 99.9% of Iowa's natural prairie communities (Smith, 1998). Grasslands have characteristics that readily allow for

agricultural exploitation (Knapp and Seastedt, 1998). Agricultural growth took priority over maintaining the complex interactions of the grasslands. "No one alive now has ever seen a complete U.S. prairie ecosystem and no one alive back when all the native wildlife was still around viewed the prairie as an ecosystem" (Chadwick, 1995, p 40).

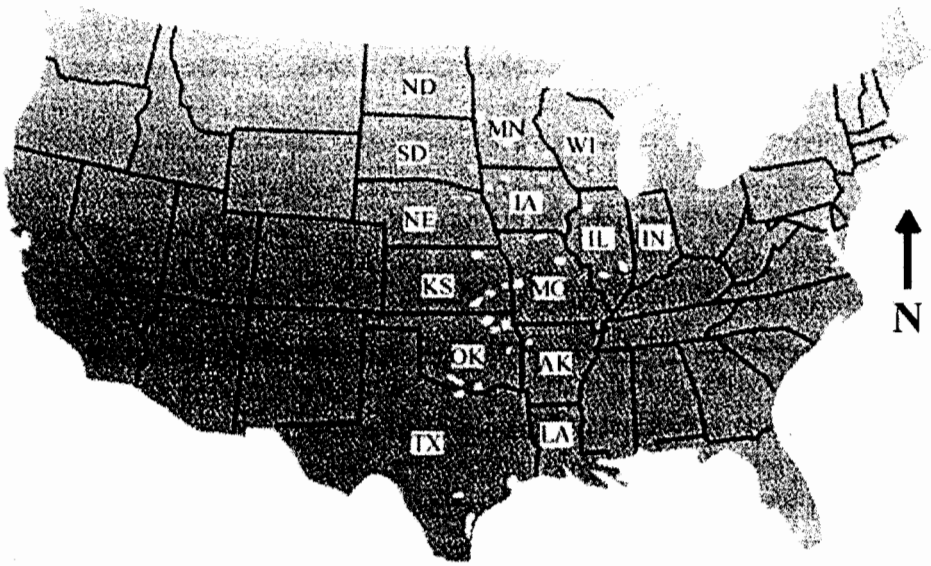

Figure 3. The current extent of the tallgrass prairie. Some of the larger prairie remnants that remained intact after settlement. Due to the small size of some of the remnants, not all can be visualized on such a large map (adapted from Kurtz, 2001)

The soils that allow for agricultural success were created by the prairie. The massive root systems of prairie plants extending downward ten feet or more serve several functions, one of which is to hold nutrients and water (Kurtz, 1996). Roots protect the plants from drought and cold as well as provide adequate nutrient exchange with the soil. Grassland ecosystems take energy from the sun and put it into the ground, storing twice

as much carbon as is in the soil of forests (Chadwick, 1995). With 75%-80% of a prairie's biomass underground (Chadwick, 1995), the microbes, invertebrates, and prairie plant roots act in concert to produce some of the highest quality soil on the Earth. Maintaining natural ecosystems and the genetic diversity they contain is often outweighed by economic, political, and individual priorities (Kurtz, 1996).

In the aftermath of human settlement, less than 0.1% of Iowa's original tallgrass prairie was intact (Smith, 1981). The prairie could no longer function as an ecosystem, but existed as small isolated fragments located on unfavorable land, railroad rights of way, cemeteries, and hidden corners of the landscape (Kurtz, 1996). Small remnant tallgrass prairies primarily remained in agriculturally unfavorable soil with steep slopes. These little tracts of unbroken sod are the remnant tallgrass prairies that we know today.

When the prairie was continuous across the landscape, the ecosystem functioned as one unit in a complex interchange of nutrients, energy, and genes. When the tallgrass prairie was broken up into remnant populations, each patch was forced to function on its own. This made remnant populations more susceptible to inbreeding, edge effects, and environmental stresses such as drought, disease, flood, and insect invasions. While functionally extinct, remnant tallgrass prairies may still be able to give us insight and knowledge on how the once massive ecosystem functioned (Chadwick, 1995). The plants that exist in remnants can not function in the same manner as they originally did, but they may still maintain the genetic variation that took a long time to accumulate.

The isolation of prairie remnants ultimately reduces the biodiversity within the prairie community. Natural control dynamics, such as grazing, browsing, and fire can no

longer function as they once did. Changes in the ecosystem's dynamics not only affect plants, but all of the interactions that occur within the prairie ecosystem. Animals that graze or browse no longer have substantial food sources; insects that rely on prairie plants for food need to find new sources of food; and microbes that co-exist with prairie plants become endangered in congruence with prairie depletion. An example of such an effect is seen in grassland dependent birds that have declined 25%-65% in recent decades (Chadwick, 1995). Plants and animals have occupied replacement niches in the artificial environment created by humans in place of prairies. The elimination of niches and the occupation of replacement niches create problems for mankind such as crop pests and uncontrolled animal populations.

The elimination of natural ecosystem dynamics causes prairie remnants to become degraded and undergo successional changes pushing prairies toward extinction. Without interventions such as restoration and reconstruction, prairies, as they once were, may be lost forever.

> The outstanding scientific discovery of the twentieth century is not television, or radio, but rather the complexity of the land organism. Only those who know the most about it can appreciate how little we know about it. The last word in ignorance is the man who says of an animal or plant: "What good is it?" If the land mechanism as a whole is good, then every part is good, whether we understand it or not. If the biota, in the course of aeons, has built something we like but do not understand, then who but a fool would discard seemingly useless parts? To keep every cog and wheel is the first precaution of intelligent tinkering. (Leopold, 1953, p.145-146)

Prairie Restoration and Reconstruction

As information about remnant prairies and the prairie ecosystem become more widely known, efforts to preserve and rebuild the tallgrass prairie have increased. Remaining tracts and remnants of tallgrass prairie have become more important as efforts intensify to conserve and rebuild the prairie ecosystems and to maintain their species (Knapp and Seastedt, 1998). In addition to repairing remnant prairies, new prairies are being planted in an effort increase the resource. Restoration and reconstruction of prairies can occur in a variety of places: private land, parks, roadsides, or even in a backyard. Prairies provide a range of benefits from beauty to medicinal value while reintroducing the natural vegetation back to the landscape.

Prairie restoration focuses on enhancing the ecological quality of a remnant prairie. The size and limited biological diversity that exist in small remnant prairies are insufficient for them to maintain themselves. As a result, edge effects, exotic species invasions, and undesirable succession occurs. Restoration attepmpt to return the prairie remnant to a level where it can function in an ecologically complete manner as it did in the past. Prairie reconstruction attempts to accomplish the same goal, building a prairie where it no longer exists.

Prairie restoration and reconstruction are intended to increase biodiversity through the accumulation of plants that once existed, but have since disappeared from the area. The addition of plants increases the gene pool and helps restore native dynamics to the prairie. The addition of plants, however, does not restore all of the dynamics that once naturally existed. Restored and reconstructed prairies may look like a prairie, but are

often a long way from functioning like one (Chadwick, 1995). Additional management must periodically be administered to maintain development of the prairie and insure survival. Such management practices are periodic burning, exotic species removal, and the addition of species.

Prairie restoration and reconstruction provide benefits beyond the reinstatement of biodiversity. Prairie plants are quite beautiful and different plants bloom at different times, maintaining an aesthetically pleasing appearance throughout the year. Historically, the Native Americans relied on the prairie plants for food and medicinal cures, some of which are still used today (Kindscher, 1992). Prairie species also have a large root system which helps to stop erosion and sequester carbon. In addition to these effects, prairie species naturally replace nutrients and out compete mal-adapted weeds. The ability of prairies to maintain themselves reduces the need for of chemical and mowing maintenance. Reasons such as this have prompted the Iowa Department of Transportation and counties to explore the use of prairies along Iowa's roadways. Iowa's roadsides total more than 600,000 (240,000 hectares).

Successful prairie reconstruction and restoration both require careful planning before planting can even begin. Assessing the site, a plan of action, and goals must all be considered before beginning. Once plans have been initiated, the numerous problems and considerations encompassed in the reconstruction or restoration site may present threats to success. Existing seed banks, land alterations such as terraces and drainage tiles, and not having the availability of the original fauna all may pose a threat to a reconstruction or restoration project. A major problem is the invasion and prevalence of exotic species

that disrupt natural processes and compete with the native plants for space, water, and nutrients. Often these species invade restoration and reconstruction projects due to their aggressiveness and ability to establish themselves in disrupted areas. Exotic species thus reduce native biodiversity while introducing foreign genes, a phenomenon that could be detrimental to the prairie.

Collection of the correct seed for the reconstruction or restoration project may present a large obstacle to the success of the reconstruction or restoration (Apfelbaum et al., 1997). First, the availability of seed presents a problem. Prairie seed is often hard to come by. This is due to the sparseness of prairie remnants as well as the manner in which seed can be collected. Hand collecting allows for seed separation and various species to be collected, but often it is laborsome and done by amateurs. Mechanical mechanisms for seed collecting provide more seed with less effort, but often only certain seeds are collected, resulting in a less diverse seed collection. Obtaining prairie seed with sufficient viability is another problem. The viability of seed must be high for a restoration or reconstruction to succeed.

> In the early years, seed was usually hand collected from local native prairie remnants. Since the collectors were usually amateurs and little was known about the biology of native prairie species, it was difficult to determine optimum seed maturity and proper seed storage techniques. This often resulted in seed collections with very few viable seeds and lots of chaff. (Smith, 1994, p 43)

These combined factors make the cost of prairie seed high. The cost of seed is a third concern facing most restoration and reconstruction efforts. Collecting, sorting, and cleaning viable native seed is so expensive that often cultivated varieties (cultivars) are used to meet the demand for seed in prairie restoration and reconstruction projects.

Commercially developed cultivars, however, present new problems to restorationists. The commercial growers of native prairie grasses in Nebraska and Kansas have provided prairie grass seed for range restoration in the western states and provide a ready source for large amounts of prairie grass seed. Many of these cultivars were developed at the United States Department of Agriculture Natural Resources Conservation Services (USDA NRCS) Plant Materials Center in Manhattan, Kansas to increase grazing productivity of rangelands. Consequently, plants that exhibited forage qualities of vigorous growth, high germination rate, good establishment and extended grazing capability were selected (Smith, 1994). The selection of specific growing characteristics and the propagation of some cultivars by rhizomious division (Fischer, 1996) resulted in the selection of specific genes and then the cloning of those genes. This limited the amount of genetic variability within a given cultivar and caused concern over their use in prairie restorations and reconstructions. The lack of genetic variability and the genetic differentiation of cultivars may produce deleterious effects in prairie plantings. Since cultivars were developed from a limited gene pool, they may create problems when introduced into different prairie ecotypes. The more vigorous cultivars may overwhelm the local species that are not as vigorous and reduce biodiversity over time. Debate over the use of cultivars and non-local ecotypes versus local ecotypes arose in the prairie restoration community and remains unsettled due to the lack of information about the genetic variability and diversity of prairie ecotypes.

Genetic Diversity

Within the scientific community, it is understood and accepted that genetic diversity needs to be maintained and preserved. Population genetics theory has long emphasized the importance of genetic variation within and between populations (Allendorf, 1983). To preserve a community's ecological and natural evolutionary processes, genetic variation must be kept intact to ensure speciation and or extinction (Frankel, 1983).

Long-term conservation is distinct from static preservation. Conservation implies a process of continuing evolution. The question that remains is whether or not nature reserves promote, restrict, or even inhibit conservation processes. In contrast to the wild continuous populations of the past, many populations of species now exist in small and disconnected patches. These factors increase the potency of genetic forces on relict populations: inbreeding, genetic drift, and random fixation of alleles. These forces result in a gradual weakening and genetic impoverishment of the species. "Wild species must have available a pool of genetic diversity if they are to survive environmental pressures exceeding the limits of developmental plasticity. If this is not the case, extinction would appear inevitable" (Frankel, 1983, p. 3). Without genetic variation, populations may become eliminated by a catastrophe such as drought, parasitism, infection, or countless other natural phenomenon that normally would have been absorbed by a diverse gene pool.

Two factors can work as a barrier to genetic exchange between plants (Chesser, 1983). First, geographic distance can reduce or stop the movement of seeds and pollen

among populations. Along the same lines, a physical barrier imposed by geographic formations such as rivers or mountains may inhibit gene flow. Second, different habitats or ecological differences may prevent or inhibit gene flow. Isolation by distance can have a dramatic effect on a population's genetic variability. Genetic drift may occur due to the lack of genetic exchange between populations.

When the prairies were settled and plowed up, founder populations were created which caused a genetic bottleneck that limited and isolated the genetic variability in prairie remnants. These prairie remnants became subject to founder effects and inbreeding, which increases genetic drift, reduces variability, and differentiates populations (Templeton et al., 1990). Inbreeding depression is the increased expression of deleterious alleles due to breeding by individuals that share genes by descent (Chambers, 1983). Harmful recessive alleles that may have persisted at a low frequency in a population gradually increase as the population becomes more homozygous.

The pattern of genetic diversity in a species is largely determined by three evolutionary forces: genetic drift, migration, and natural selection (Allendorf, 1983). These forces may differ between prairie plant species, due to evolutionary adaptation, and may be dramatically affected by prairie fragmentation. For this reason, genetic analysis must be performed on a variety of species to understand the biodiversity that remains in an ecosystem.

Genetic Analysis Techniques

Technological advances and increasing knowledge about DNA has led science to develop several methods of detecting genetic variability. To assess expressed genetic

variability, common garden techniques were developed. A common garden consists of a collection of a plant species from a variety of geographical sources and grown together in a common plot with all the same environmental influences the same. Plants can then be compared and noted differences can be attributed to the plant rather to the environment. Morphological and phenological differences are recorded. This technique is time consuming and does not assess neutral genetic differences that are present in the genome, but not expressed.

To reduce the time needed to assess genetic variation, isozyme variation began to be measured. Isozymes are various forms of enzymes within individual plants, which means there are different amino acid sequences and thus different genetic codes. To examine differences, enzymes are compared between plants. While this technique is much faster than common gardens, isozyme studies also focus strictly on expressed genetic differences and ignore the majority of DNA that is not expressed.

The introduction of the Polymerase Chain Reaction (PCR) to the scientific community begin a new era in assessing genetic variation. The polymerase chain reaction amplifies DNA exponentially through the use of DNA primers, a thermostable DNA polymerase, and temperature variation. Fragments of DNA between primers are replicated, and thus amplified after many PCR cycles and can be visualized by gel electrophoresis.

In the recent past, one of the most widely used techniques to assess genetic variability is randomly amplified polymorphic DNA (RAPD) analysis. In 1990, Welsh and McClelland used 10-12mer oligonucliotides to randomly amplify portions of five

Staphlococcus species genomes. Using low stringency PCR, followed by high stringency PCR, the 10-12mer oligonucleotides annealed randomly throughout the genome. Portions of the genome were then amplified if the 10-12mer oligonucleotides were located on opposite strands and close enough for amplification to occur. The RAPD technique provided a fast method of genetic analysis that assessed the entire genome with the benefit of not needing to know specific DNA sequences within the genome. The RAPD technique has since been widely used in genetic variability studies, linkage mapping, and gene flow studies (Chalmers et al., 1992; Heun and Helentjaris, 1993; Koller et al., 1993; Williams et al., 1993; Sharma et al., 1995; Yazdani et al., 1995). Despite its benefits, RAPD analysis lost favor because of problems with reproducibility and sensitivity (Heun and Helentjaris, 1993; Sharma et al., 1996; Lanham and Brennan, 1999).

The problems associated with RAPD analysis were eliminated when inter-simple sequence repeats (ISSR) analysis was developed. The benefit of a fast assay of the entire genome was maintained when ISSRs were used as primers instead of arbitrary sequences. Microsatellites are repeated nucleotide base sequences that occur randomly throughout genomes. The DNA between microsatellite sequences can be amplified via PCR to produce a random genetic fingerprint. The inter DNA length between microsatellite loci varies from individual to individual (Wu et al., 1994; Zietkiewicz, 1994) which allows for a genetic DNA profile to be created.

While these techniques use PCR alone to create a genetic fingerprint, the use of restriction endonucleases can also be a beneficial tool to assess genetic variability.

Restriction endonucleases function by cleaving DNA at specific sequences within the genome creating an array of different sized fragments. Restriction fragment length polymorphism (RFLP) was designed to use these different fragment lengths to identify and differentiate between individuals (Botstein et al. 1980). The creation of an RFLP genetic profile uses Southern hybridization to attach known probes to a smear of fragment lengths created by running digested DNA on a gel. Distinguishable markers obtained through RFLP can be used for a variety of genomic analysis. The usefulness of RFLP markers have allowed the production of genetic maps of several plant species (Berznatzky and Tanksley, 1986; Helentjaris, 1987; Heun et al., 1991; Liu and Tsunewaki, 1991). While RFLP is a highly reproducible genetic analysis tool, requirements such as clones, large amounts of DNA, and Southern hybridization make it expensive and time consuming.

In 1995, Vos et al. introduced a new genetic analysis tool that combined the advantages of RFLP and PCR derived techniques. Amplified Fragment Length Polymorphism (AFLP) analysis used PCR to amplify specific fragments of a digested genome. Disadvantages of RFLP such as the need for clones, large amounts of DNA and hybridization were eliminated in AFLP analysis with the use of DNA manipulation and PCR. These techniques allowed for AFLP to be fast and reproducible while randomly assessing the entire genome.

Genomic DNA fingerprints are produced through AFLP by selecting digested fragments of DNA and then using PCR to amplify them. The basis of AFLP rests on how restriction endonucleases cleave DNA. The restriction endonucleases that are generally

used in AFLP cut each strand at a different place, so "sticky ends" extend off of each fragment of the DNA. For example, *EcoR I* cuts the palindromic sequence (the other DNA strand has the same sequence in an antiparallel orientation) 5'...G⇓AATTC...3' and thus leaves the a four base extension, or "sticky end" of 5'...AATT...3' on each strand of DNA. Synthetic DNA adapters are then made that have complimentary "sticky ends" that anneal to the "sticky ends" left on the DNA fragments by the restriction endonucleases. After annealing, the adapters are ligated on to the DNA fragments using the enzyme ligase. The adapters also contain a core sequence that is complimentary to PCR primers designed for the AFLP procedure. The primers can then anneal to the adapters, which allows for the amplification of the digested DNA fragments.

Depending on the size of the genome, the number of restriction endonucleases may differ. Restriction enzymes may cut a genome frequently or infrequently due to the length of the cutting sequence. The more base pairs in the cutting sequence generally means the less often a restriction endonuclease will cut. On a small genome one infrequent cutter will probably produce enough fragments for analysis. However, with larger genomes one infrequent cutter would produce too many fragments to analyze. To clarify the banding pattern, two or three restriction endonucleases are used to produces a variety of different ended fragments which can be used to reduce the number of fragments analyzed. In a two enzyme digest, a frequent cutter and an infrequent cutter are used. The genome is cut up into many fragments, most of which have both ends cut by the frequent cutter. However, the AFLP procedure selects only for fragments that have at least one cut by the infrequent cutter. Thus only a random portion of the genome is used

making analysis possible. Recently, a triple enzyme AFLP (TE-AFLP) was introduced (van der Wurff, 2000). In this procedure, two infrequent cutters are used with one frequent cutter. Fragments that have infrequent cuts on both sides are selected for analysis. This analysis eliminates large portions of the genome quickly and easily simplifying analysis on large genomes.

Another method used to simplify analysis on large genomes, is the addition of a selective nucleotide base tail on to the end of the primers. The selective tail requires that the adjacent DNA fragment have complimentary bases for amplification to occur. Therefore, the addition of more selective bases reduces the number of fragments amplified. Different primer base extensions amplify different fragments, so different primer extensions provide different banding patterns for analysis. Therefore, one restriction digest can provide several different DNA banding patterns for analysis.

The Iowa Ecotype Project

The limitations of native seed availability and the high costs of harvesting seed from scattered remnants made locally collected prairie seed hard to find and expensive. In 1990, the Iowa Ecotype Project (IEP) was initiated at the University of Northern Iowa (UNI). This project's goal is to increase Iowa-origin prairie seed production in an effort to provide regional ecotypes (used loosely to mean a regional population or subpopulations) of Iowa prairie seed for reconstruction and restoration efforts at a lower cost (Smith, 1994; Houseal and Smith, 2000).

The Native Roadside Vegetation Center (NRVC) at UNI functions as the home of the IEP and the Roadside Program which assists Iowa counties in establishing and

maintaining Integrated Roadside Vegetation Management (IRVM) programs. In addition, the NRVC provides education and consultation on prairie restoration techniques and management.

The IEP has worked to address the concerns raised over genetically selected cultivars being used in Iowa prairie restoration and reconstruction projects. This is being accomplished through research on Iowa prairie species and increased production of Iowa origin prairie seed to provide an economically competitive alternative to cultivars. The first priority focused on producing enough seed for roadside plantings in Iowa (Smith, 1994). As production increases, Iowa origin seed will be available for restoration and reconstruction.

Growing season, day length, and temperature regimes are influenced by latitude, which may influence the development of prairie plants. Therefore, the state was divided into three latitudinal zones (Figure 4) from which seed would be collected from prairie remnants. The boundaries fall along political borders and not specifically biological (Houseal and Smith, 2000). The time of floral development and some isozyme work have suggested that this regional division may be correct for certain species of the Iowa prairie (Houseal and Smith, 2000). Species are collected separately, without bias toward characteristics. This ensures that species are not collected for specific traits and thus limit the gene pool (Smith, 1994; Houseal and Smith, 2000). According to the Genetic Certification Standards, seed collected in this manner is classified as "source identified."

To maximize the gene pool and counteract the loss of genetic variation during collection, each species' seed, within each zone, is mixed together. The division of the

state into more zones may be appropriate, but the number of seed growers, as well as the market demand for seed, may not support further divisions.

Collected seed is grown in separate plots for each zone to further increase the native Iowa species. When sufficient seed is available, it is distributed to commercial growers to allow them to increase and distribute native seed for roadside plantings, prairie restoration, and reconstruction projects at a reasonable cost to the consumer. The "source identified" seed insures that seed from Iowa is used in Iowa for prairie reconstruction projects.

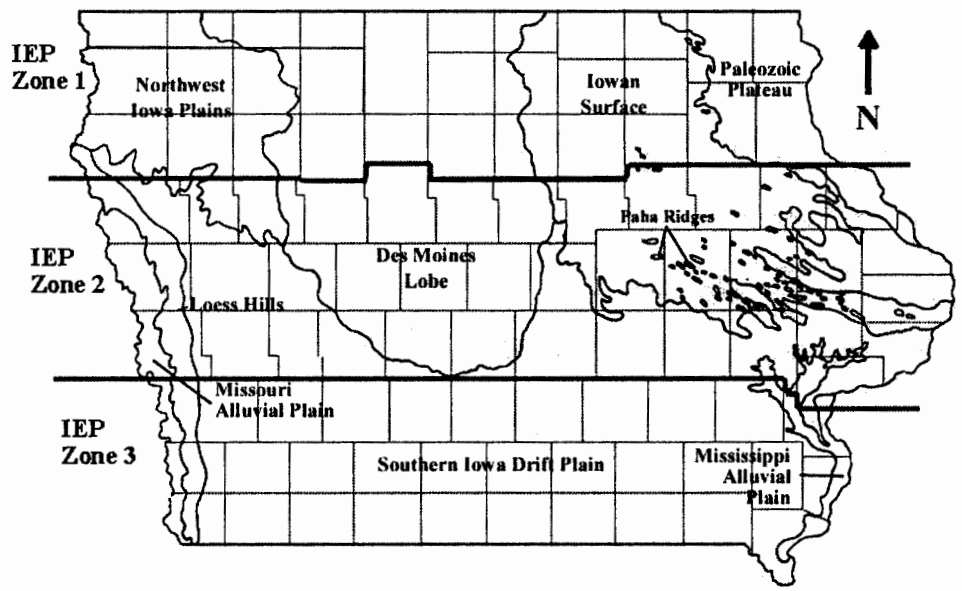

Figure 4. Iowa Ecotype Project Zones, Counties, and Landforms. A map of Iowa divided by counties, landforms, and the IEP zones. As is evident from the map, the Iowa Ecotype Project zone boundaries are strictly political and have no bias toward biological boundaries (adapted from Prior, 1991)

The Project

Different ecological and life history characteristics exist for different plant species, which thus affect the genetic structure of those species. It is, therefore, likely that different prairie species differ in their genetic structures. To verify the validity of the IEP, more extensive genetic studies were needed to examine the issues of regional ecotypes and the genetic structure of the Iowa prairie, its remaining remnants, and various prairie plant species.

Work associated with the IEP has provided some information to address the concerns over genetic variation in remnant prairies. Kitchen (1999) used RAPD analysis to analyze *Liatris aspera* (rough blazing star) and found that 78% of the genetic variability occurred within populations. Sadler (2000) used AFLP to analyze *Solidago rigida* (stiff goldenrod) and found that most variation occurred within populations as well. Sadler (2000) also found evidence supporting the division of *Solidago rigida* into two subspecies. AFLP research on IEP species has been utilized in undergraduate research projects. This research has examined several species, but differences have not been statistically analyzed. Isozyme research on *Panicum virgatum* has also been done and showed differentiation between several populations as well as cultivars (G. Houseal, pers. comm).

This project was initiated to determine genetic variation between populations of *P. virgatum* and between populations of *C. palmata*. Concern over the genetic variation within *Panicum virgatum* arose due to the extensive planting of *P. virgatum* cultivars and the uncertainty regarding the genetics of populations. *Coreopsis palmata* is a plant that

propagates more readily through division of adult plants than through seed, arousing suspicions of clonality (Smith and Smith, 1980). The degree of clonality and relatedness of *C. palmata* populations made it a good subject to test. This project used AFLP to assess the genetic variability within these two species and to detect the presence of cultivars in remnant prairies as well as within the IEP propagation plots. Common garden plots have been for further research of *Panicum virgatum* and *Coreopsis palmata.*

Panicum virgatum L.

Panicum virgatum (switchgrass) belongs to the *Poaceae* or grass family. *Panicum virgatum* L. var. *spissum* Linder grows in the New England states and extends as far south as Maryland. The more common type of switchgrass, and the one studied here, is *Panicum virgatum* L. var. *virgatum.* This variety of switchgrass extends as far west on the continental United States as Nevada, but also exits on Hawaii, and as far east as Massachusetts.

Panicum virgatum is a native, perennial, warm-season, tall grass that is wind pollinated and self-infertile. However, *P. virgatum* has been known to spread by division (rhizomes) which may show clumps to be clonal. One of the chief plants of the tallgrass prairie, *P. virgatum* was originally found in two thirds of North America (Fischer, 1996). It is most commonly found in prairie lowland, but will grow under a wide range of climatic conditions. *Panicum virgatum* can grow in sand, loam, or clay and thrives in moisture regimes from near drought to periodic flooding (Fischer, 1996). It is also tolerant of salty and acidic soils (Sharp, 1997). These characteristics have helped *P. virgatum* survive the destruction of the prairie.

Panicum virgatum var. *vigatum* exists as two main ecotypes: lowland and upland. The lowland ecotype is generally tetraploid (Barnett and Carver, 1967), erect, coarse-stemmed, without hairs on the leaf blades, robust, and stands 61-305 cm in height (Porter, 1966). The upland ecotype is generally hexaploid or octoploid (Barnett and Carver, 1967), fine-stemmed, broad based, semi-decumbent, have varying amounts of hairs on the leaf blades, and stands 92-152 cm in height (Porter, 1966).

Panicum virgatum exists in a variety of ploidy levels, from diploid ($2n = 18$) to duodecaploid ($2n = 108$) (Church, 1940; Nielson, 1944; Riley and Vogel, 1982; McMillan and Weiler, 1995). Studies using flow cytometry differ in assessment of upland switchgrass ploidy levels (Lu, 1995; Wullschleger et al., 1996). Octoploid chromosome sets have been reported in populations previously thought to be hexaploid (Taliaferro and Hopkins, 1994). Riley and Vogel (1982) found the cultivars Blackwell, Cave-in-Rock, and Pathfinder to be hexaploid. However, mitotic and meiotic cytogenetic analyses combined with flow cytometry has demonstrated that plants with 3 picograms (pg) of DNA per nucleus are tetraploid while those with 5.2-6 pg of DNA per nucleus are octoploid (Lu, 1995; Hopkins et al., 1996). These finding suggests that plants that were thought to be hexaploid are in fact octoploid in accordance with previous flow cytometry data (Hultquist et al., 1996).

Several *P. virgatum* cultivars have been developed by the Soil Conservation Service Plant Materials Center from several areas within the United States in order to restore grazing to rangelands after the dust bowl years (Figure 5). Six *P. virgatum* cultivars were examined in this study. Alamo is a tetraploid, lowland variety of *P.*

virgatum (Hopkins et al., 1996), originally collected in 1964 near George West, Texas, and released for use by the Plant Materials Center in 1978 (Forage Information System, 1996). Alamo is characterized by a coarse foliage and a late maturity (Sharp, 1997). Kanlow is also a tetraploid, lowland variety of *P. virgatum* (Hopkins et al., 1996) that is quite versatile and well suited to poorly drained sites, regions of periodic flooding, and upland soil (Sharp, 1997). Kanlow was originally collected near Wetumka, Oklahoma in 1957 and was released for use in 1963 (Forage Information System, 1996). Blackwell is an octoploid, upland variety of *P. virgatum* (Hopkins et al., 1996) that is characterized by lush foliage, disease resistance, and heavy, vigorous roots and stems (Sharp, 1997). Blackwell was originally collected near Blackwell, Oklahoma in 1934 and was released for use in 1944 (Forage Information System, 1996). Cave-in Rock is an octoploid, upland *P. virgatum* (Hopkins et al., 1996) variety. Cave-in-Rock was originally collected near Cave-In-Rock, Illinois in 1958, it was released for use in 1973 (Forage Information System, 1996). Cave-in-Rock is noted for its adaptability and tolerance to high humidity (Sharp, 1997). Pathfinder is an octoploid, upland *P. virgatum* cultivar (Hopkins et al., 1996) that matures late and survives winter well (Sharp, 1997). Pathfinder was originally developed from domestic collections from Nebraska and Kansas in 1953 and was released for use in 1967 (Forage Information System, 1996). Forestburg is an upland *P. virgatum* and research suggests it is octoploid (Hultquist et al., 1996). Forestburg originally came from a switchgrass stand near Forestburg, South Dakota and was released in 1987 for use (Forage Information System, 1996).

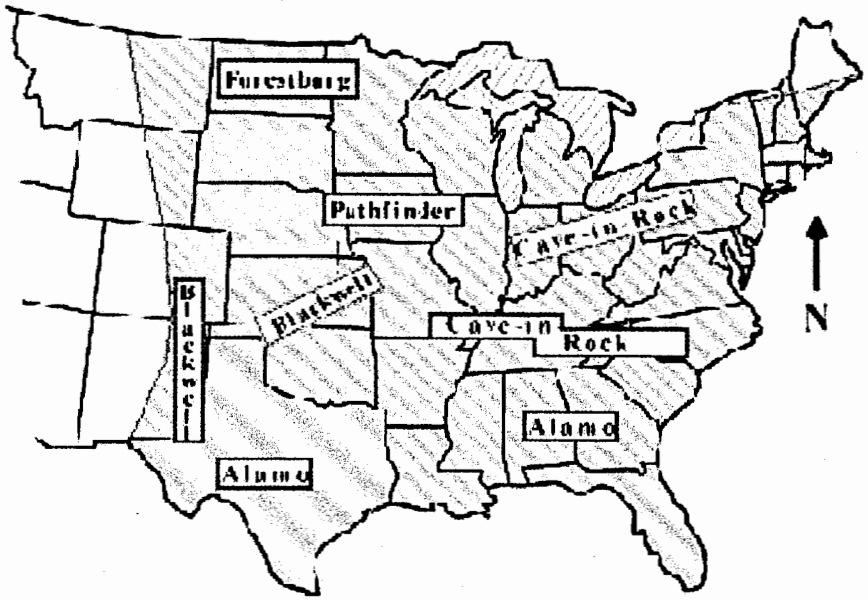

Figure 5. *Panicum virgatum* range and cultivar adaptation areas. The general areas where *P. virgatum* cultivars are the best adapted to. Kanlow variety is best adapted to lowlands of the lower two-thirds of the shaded area (adapted from Sharp Brothers Seed Company ©1997)

Coreopsis palmata

Not much is known about the plant *Coreopsis palmata* (prairie coreopsis or prairie tickseed). It is a perennial dicot belonging to the *Asteraceae,* or aster family. It is likely pollinated by bees and is self-infertile which promotes sexual reproduction. However, due to it's nature to preferably reproduce via rhizomes (Smith and Smith, 1980), it is thought to be a very clonal species. It is a common prairie species that exists from Minnesota to Louisiana and from Nebraska to Indiana in the United States. Chromosomal studies show a chromosome count of $n = 13$ and do not reveal any

polyploidy within the species (Smith, 1971). There is, however, some variation of chromosome number within the genus (Smith, 1975). The difference in chromosome number has raised some debate on the phylogeny of the *Coreopsis* genus (Smith, 1983; Jansen et al., 1986; Crawford et al.,1990; Crawford et al., 1991; Ryding, 1992; Seung - Chui et al., 1999). There are no *C. palmata* cultivars, but is grown in production plots by prairie seed growers for reconstruction and restoration projects.

CHAPTER 2

MATERIALS AND METHODS

Plots and Tissue Collection

The Iowa Ecotype Project currently maintains collections of several species of prairie plants at the University of Northern Iowa in three locations. The plants in each plot were grown from seed collected in one of three latitudinal zones of Iowa (Figure 4). The plots for each zone are separated by a sufficient distance to prevent cross-pollination between plants of different zones. Each plot contains collections from different remnant prairies within each zone. All species collected within a zone are planted in these plots and each collection is marked with a tag denoting the species and accession number.

Tissue samples were collected from these zone plots for genetic analysis. These samples are referred to as "plot" collections throughout this paper. Plant tissue was collected separately by accession, or population, and was placed in Ziploc™ bags, labeled with the accession and individual number, and placed in a cooler with ice. Stem and leaf tissue samples were collected and analyzed (Table 1, Figure 6) for *Panicum virgatum* as follows.

Northern Plot – 3 populations, each with 10 individuals
Central Plot – 4 populations, each with 10 individuals
Southern Plot – 2 populations, each with 10 individuals
Total = 9 populations and 90 individuals.

Stem and leaf tissue samples for *Coreopsis palmata* were collected and analyzed (Table 3, Figure 8) as follows:

Northern Plot – 4 populations; 8,8 (104, 131) 9,9 (132,140) individuals
(Original seed accession number denoting populations is in parentheses)
Central Plot – 4 populations, each with 8 individuals
Southern Plot – 3 populations, each with 10 individuals
Total = 11 populations and 96 individuals

Tissue was also field collected from remnant prairies in the northern and southern zones of Iowa. These samples are referred to as "field" collections throughout this paper. Plant tissue was collected separately by prairie, or population, and was placed in Ziploc™ bags labeled with the prairie name and a collection number. Tissue was then placed in a cooler with ice and transported back to UNI. The collection numbers of field samples were two-part numbers. The first number refers to a patch (*C. palmata*) or clump (*P. virgatum*) of plants located within the prairie. The number of patches or clumps found within a given prairie was variable. The second part of the collection number referred to the individual plant collected from within a given patch or clump. Plants collected from remnant prairies were collected in such a way to help distinguish the clonality of the plants.

P. virgatum tissue collected and analyzed (Table 2, Figure 7) for field populations were as follows:

Northern Iowa = 3 counties – 5 sites – 48 individuals
Kossuth county
Smith prairie – 4 areas (2 individuals/area)
Stinson prairie – 4 areas (2 individuals/area)
Winnebago county
Winnebago River Trail – 5 areas (3 individuals/area)

Cerro Gordo county
Hoffman prairie – 4 areas (2 individuals/area)
Wilkenson prairie – 4 areas (2 individuals/ area)

Southern Iowa = 4 counties – 4 sites – 48 individuals
- Warren county
  - Medora prairie – 4 areas (3 individuals/area)
- Clarke county
  - Flaherty prairie – 4 areas (3 individuals/area)
- Lucas county
  - Land Between Two Railroads – 4 areas (3 individuals/area)
- Ringgold county
  - Mt. Ayr Wildlife – 4 sites (3 individuals/area)

C. palmata tissue collected and analyzed (Table 4, Figure 9) for field populations were as follows:

Northern Iowa – 2 counties – 4 sites – 48 individuals
- Kossuth county
  - Smith prairie – 4 areas(3 individuals/area)
  - Stinson prairie – 4 areas (3 individuals/area)
- Cerro Gordo county
  - Hoffman prairie – 4 areas (3 individuals/area)
  - Wilkinson prairie – 4 areas (3 individuals/area)

Southern Iowa – 4 counties – 5 sites – 48 individuals
- Warren county
  - Medora – 2 areas (3 individuals/area)
  - Rolling Thunder – 2 areas (3 individuals/area)
- Clarke county
  - Flaherty – 4 areas (3 individuals/area)
- Lucus county
  - Land Between Two Railroads – 4 areas (3 individuals/area)
- Ringgold county
  - Sand Creek – 4 areas (3 individuals/area)

Accessions and the number of individuals collected and analyzed were selected to obtain populations representing different parts of the state. The number of individuals analyzed from each population were occasionally lowered to a total of 96 for analysis efficiency.

Plant tissue was then brought back to the lab and prepared for storage. Populations were prepared separately to ensure that there was no contamination. Individuals were removed from the bags, shaken to remove unattached leaves, and then examined for unattached tissue. The plant tissue was then placed in separate porcelain mortars with approximately 0.4 grams (g) of sterile sea sand (Fisher Scientific) and approximately 20 milliliters (ml) of liquid nitrogen. Using a pestle, each individual was separately ground into a fine powder. The powder was then placed in 4 ml Fisherbrand HDPE scintivials 03-337-40 (Fishers Scientific) and labeled to indicate the collection site and individual. Tissue was then stored at -80°Celsius.

Genomic DNA Isolation

P. virgatum DNA from Iowa Ecotype plots was extracted from the powdered leaf tissue using a modified Doyle and Doyle (1990) hexadecyltrimethyl-ammonium bromide (CTAB) (Fisher Scientific) / sevag [24:1 (v/v) chloroform Iso-amyl alcohol] protocol. Approximately 50 mg of ground tissue was placed in a sterile, 1.5 ml microcentrifuge tube containing 700 microliters (µl) of pre-warmed (65°C) 2% CTAB extraction buffer [100 mM Tris-HCl (tris hydroxymethyl aminomethane) pH 8.0, 2% (w/v) CTAB, 20 mM ethylenediamine tetraacetic acid (EDTA) pH 8.0, 1.4 M NaCl, 0.5% (w/v) sodium bisulfite (General Chemical Company), 0.5% (v/v) beta-mercaptoethanol]. The samples were mixed by inversion and incubated for two hours at 65°C with mixing every 15 minutes. The samples were then removed from the incubator and allowed to cool to room temperature. Seven hundred microliters of sevag was added, mixed to an emulsion, and

Table 1. *P. virgatum* Plot Tissue Collections. Locational information for each *Panicum virgatum* population collected from the IEP plots. In the population zone and accession number column the zone is represented by the first digit and the accession by the last two digits. These plots were grown from seed gathered at the respective location indicated by county, the landform it was found on, and UTM (Universal Transverse Mercator) coordinants of the center of each location. Accession sites can be visualized in Figure 6.

Original seed Accession Number	Original Location (Iowa County)	Iowa Landform	UTM x Coordinate	UTM y Coordinate	IEP Zone
119	Cherokee	Northwest Iowa Plains	289402	4750391	1
124	Pocahontas	Des Moines Lobe	343072	4717090	1
127	Dickinson	Des Moines Lobe	318223	4807378	1
206	BlackHawk	Iowan Surface	536777	4716017	2
216	Boone	Des Moines Lobe	432214	4655950	2
218	Webster	Des Moines Lobe	399169	4696512	2
221	Buchanan	Iowan Surface	601230	4711632	2
305	Monroe	Southern Iowa Drift Plain	497289	4538073	3
311	Union	Southern Iowa Drift Plain	400609	4548735	3

Table 2. *Panicum virgatum* Field Tissue Collections. Locational information for each *Panicum virgatum* population collected directly from their original sites. These plants were gathered from each respective location as indicated by accession site, Iowa county, the landform it was found on, and UTM (Universal Transverse Mercator) coordinants of the center of each site. Accession sites can be visualized in Figure 7.

Accession Site	Iowa County	Iowa Landform	UTM x Coordinate	UTM y Coordinate	IEP Zone
Northern Iowa					
Hoffman Prairie (Hof)	Cerro Gordo	Des Moines Lobe	463243	4775943	1
Smith Prairie (Sm)	Kossuth	Des Moines Lobe	399497	4764849	1
Stinson Prairie (St)	Kossuth	Des Moines Lobe	391861	4766856	1
Winnebago River Trail (WRT)	Winnebago	Des Moines Lobe	446855	4793350	1
Wilkinson Prairie (Wik)	Cerro Gordo	Iowan Surface	495392	4780851	1
Southern Iowa					
Medora Prairie (Med)	Warren	Southern Iowa Drift Plain	447364	4562202	3
Flaherty Prairie (Fla)	Clarke	Southern Iowa Drift Plain	422297	4548431	3
Mount Ayr (Mt. A)	Ringgold	Southern Iowa Drift Plain	388623	4505499	3
Land Between Two Railroads (2RR)	Lucas	Southern Iowa Drift Plain	465367	4542891	3

Table 3. *Coreopsis palmata* Plot Tissue Collections. Locational information for each *Coreopsis palmata* population collected from the Iowa Ecotype plots. In the population zone and accession number column the zone is represented by the first digit and the accession by the last two digits. These plots were grown from seed gathered at the respective location indicated by county, the landform it was found on, and UTM (Universal Transverse Mercator) coordinates of the center of each location. Accession sites can be visualized in Figure 8.

Original seed Accession Number	Original Location (Iowa County)	Iowa Landform	UTM x Coordinate	UTM y Coordinate	IEP Zone
104	Cerro Gordo	Iowan Surface	463243	4775943	1
131	Kossuth	Des Moines Lobe	391861	4766856	1
132	Howard	Iowan Surface	550137	4809822	1
140	Cherokee	Northwest Iowa Plains	301723	4735962	1
208	BlackHawk	Iowan Surface	565361	4702306	2
230	Audubon	Southern Iowa Drift Plain	345732	4627135	2
236	Webster	Des Moines Lobe	403640	4700845	2
242	Marshall	Southern Iowa Drift Plain	498563	4636214	2
320	Warren	Southern Iowa Drift Plain	436188	4591910	3
324	Clarke	Southern Iowa Drift Plain	422297	4548431	3
335	Montgomery	Southern Iowa Drift Plain	326411	4536414	3

Table 4. *Coreopsis palmata* Field Collections. Locational information for each *Coreopsis palmata* population collected directly from their original sites. These plants were gathered from each respective location as indicated by accession site, Iowa county, the landform it was found on, and UTM (Universal Transverse Mercator) coordinates of the center of each site. Accession sites can be visualized in Figure 9.

Accession Site	Iowa County	Iowa Landform	UTM x Coordinate	UTM y Coordinate	IEP Zone
Northern Iowa					
Hoffman Prairie (Hof)	Cerro Gordo	Des Moines Lobe	463243	4775943	1
Smith Prairie (Sm)	Kossuth	Des Moines Lobe	399497	4764849	1
Stinson Prairie (St)	Kossuth	Des Moines Lobe	391961	4766856	1
Wilkinson Prairie (Wik)	Cerro Gordo	Iowan Surface	495392	4780851	1
Southern Iowa					
Rolling Thunder Prairie (RTP)	Warren	Southern Iowa Drift Plain	444689	4561802	3
Medora Prairie (Med)	Warren	Southern Iowa Drift Plain	447364	4562202	3
Flaherty Prairie (Fla)	Clarke	Southern Iowa Drift Plain	422297	4548431	3
Sand Creek Prairie (SC)	Ringgold	Southern Iowa Drift Plain	413889	4514433	3
Land Between Two Railroads (2RR)	Lucas	Southern Iowa Drift Plain	465367	4542891	3

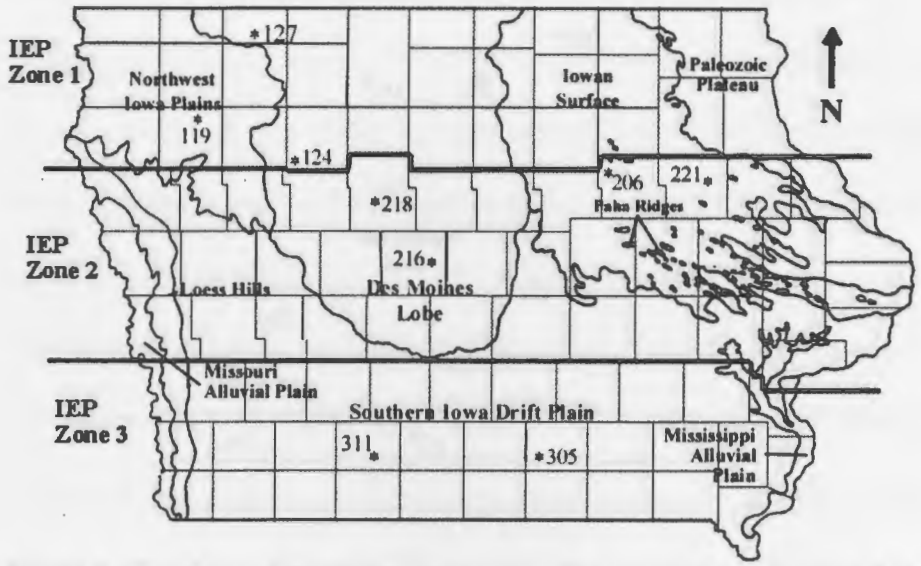

Figure 6. *P. virgatum* plot original location. The remnant prairie sites that *Panicum virgatum* seed was collected from before it was planted in the IEP plots located around the University of Northern Iowa.

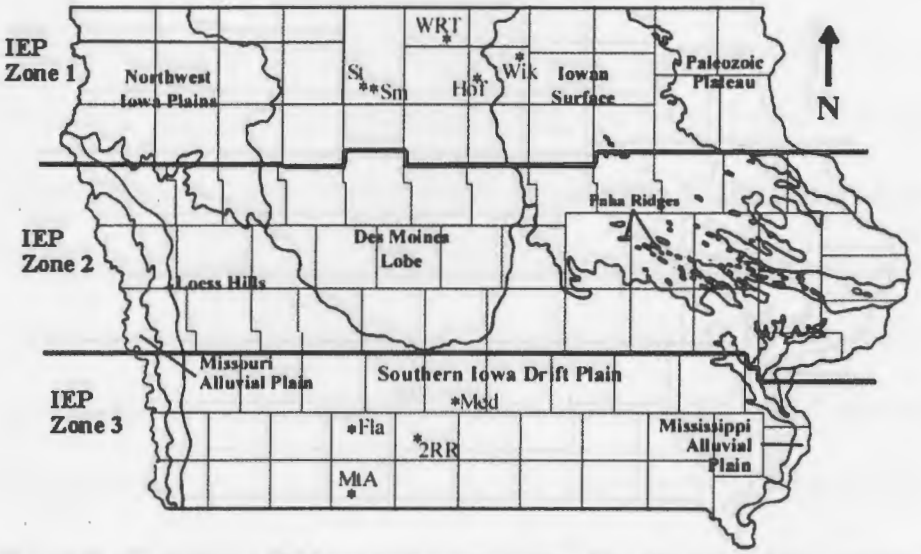

Figure 7. *P. virgatum* field accession sites. The location of the remnant prairie sites that *Panicum virgatum* tissue was collected from for analysis. Prairie sites are abbreviated as shown in Table 2.

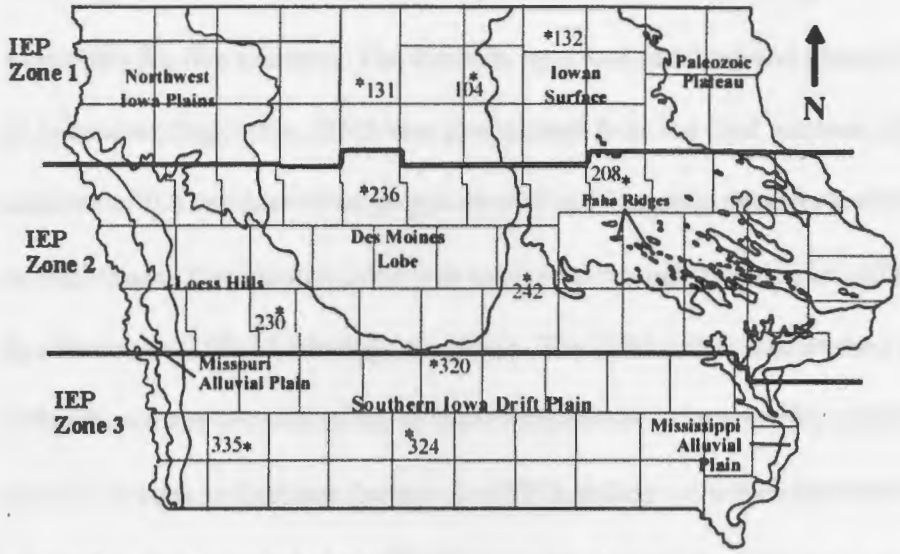

Figure 8. *C. palmata* plot original locations. The remnant prairie sites that *Coreopsis palmata* seed was collected from before it was planted in the IEP plots located around UNI.

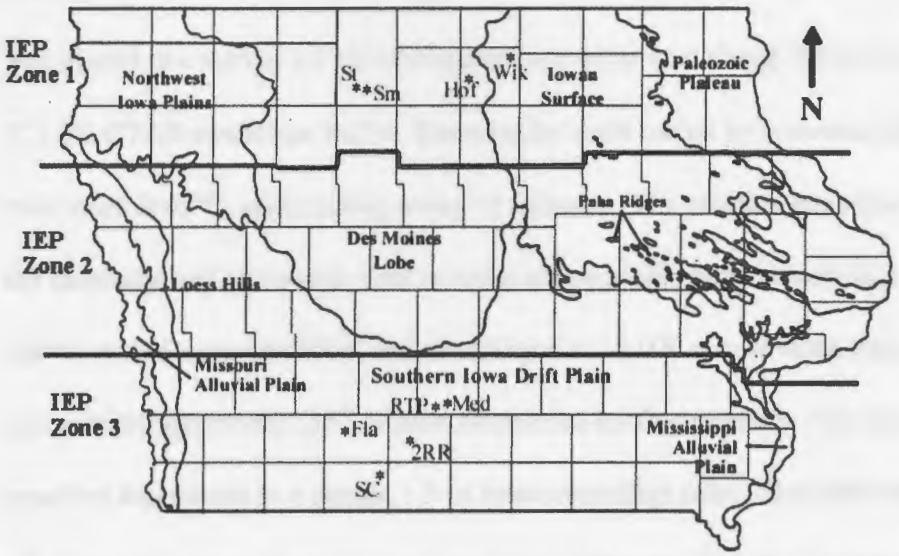

Figure 9. *C. palmata* field accession locations. The location of the remnant prairie sites that *Coreopsis palmata* tissue was collected from for analysis. Prairie sites are abbreviated as shown in Table 4.

centrifuged at 14,000 rpm at room temperature in a microcentrifuge model 235C (Fisher Scientific) for five minutes. The aqueous layer was removed and placed in a sterile, 1.5 µl microcentrifuge tube. DNA was precipitated from the final aqueous extract by the addition of 0.8 volumes of isopropyl alcohol and inverting the microcentrifuge tube several times. Precipitated DNA was recovered by centrifugation at 14,000 rpm at 4° C in a Beckman GSR-15 tabletop centrifuge. The DNA pellet was washed with 700 ml of 70% ethanol and allowed to dry at room temperature. Occasionally, pellets were placed in a 65° C oven to facilitate drying. Dry DNA pellets were then dissolved in 50 µL of TE (10mM Tris-HCl pH 8.0, 1mM EDTA pH 8.0) and stored at -20° C.

All DNA from *Coreopsis palmata* samples and field collected samples of *P. virgatum* was extracted from powdered tissue using a modified silica matrix protocol (Huang and Sun, 2000; S. O'Kane, pers. comm.). Approximately 50 mg of ground tissue was placed in a sterile, 1.5 ml microcentrifuge tubes containing 700 µl of pre-warmed (65 °C) 2% CTAB extraction buffer. The samples were mixed by inversion and incubated for two hours at 65°C, with mixing every 15 minutes. The samples were then removed from the incubator and allowed to cool to room temperature. Seven hundred µl of sevag was added, mixed to an emulsion, and centrifuged at 14,000 rpm at room temperature in a microcentrifuge model 235C (Fisher Scientific) for five minutes. The aqueous layer was removed and placed in a sterile, 1.5 µl microcentrifuge tube. The tube was then filled with approximately 850 µl adsorption buffer [5 M guanidine thiocyanate, 100mM Tris, 5 mM EDTA pH 8.0, adjusted to pH 6.5, and 1.8% w/v de-fined diatomaceous earth] and

mixed periodically for 10 minutes at room temperature. The diatomaceous earth was then pelleted by centrifugation at 14,000 rpm in a microcentrifuge model for 1 minute. The liquid layer was removed and disposed of in a waste container. The diatomaceous earth pellet was then re-suspended in wash buffer [80 mM potassium acetate, 8.4 mM Tris-HCl pH 7.4, and 40 µM EDTA pH 8.0] and mixed thouroughly. The diatomaceous earth was then pelleted by centrifugation at 14,000 rpm in a microcentrifuge for 1 minute. The liquid layer was then removed and discarded. The diatomaceous earth was then allowed to dry at room temperature. Drying was occasionally aided by a 65°C oven. Sixty-seven µl of TE was then added to the diatomaceous earth and incubated for 30 minutes at 65°C with finger vortexing every ten minutes. The diatomaceous earth was pelleted by centrifugation at 14,000 rpm in a microcentrifuge for 1 minute. The TE containing the DNA was collected and placed in a sterile, 1.5 ml microcentrifuge tube. Thirty-three microliters of TE was then added to the diatomaceous earth and incubated for 30 minutes at 65°C with finger vortexing every ten minutes. The diatomaceous earth was pelleted by centrifugation at 14,000 rpm in a microcentrifuge for 1 minute. The TE containing the DNA was collected and added to the previous TE collection. The TE containing the DNA was then stored at –20°C.

The concentration of the isolated DNA solutions were determined using ethidium bromide florescent intensities of the samples in comparison to a known standard. Two microliters of DNA solution were mixed with two microliters of 10X loading buffer (50% glycerol, 3 mM tartrazine yellow in 5X TAE) and 6 µl of water. The 10 µl solution was

loaded into a 0.7% agarose gel containing 1X TAE buffer (40mM Tris-acetate, 2 mM disodium EDTA) and 0.5 µg/ml ethidium bromide. Gels were run at 100 volts(v) for about 30 minutes in TAE buffer using a FisherBiotech Midi-Horizontal electrophoresis system FB-SB-1316 (Fisher Scientific) and Dan-Kar model DK300 power supply. Twenty-five micrograms of *Hind* III digested lambda phage DNA (Promega) was run as a standard marker in the two outer lanes of agarose gels containing unknown concentrations of isolated plant DNAs. The gels were observed using a ultraviolet transilluminator (UVP, model TM36) and a digital image was taken using a COHU high performance CCD camera connected to a Macintosh Quadra 840av computer with a Bit Image photography program (NIH Image version 1.51). The images of the plot sample gels were analyzed with NCSA GelReader (version 2.0.3x for Macintosh) for intensity of florescence from the samples and standard markers. The images of the field sample gels were analyzed with Kodak™ 1D (version 3.5.2 USB) for intensity of florescence for the samples and markers. The intensity data obtained from GelReader was placed into a Microsoft® Excel spreadsheet and DNA concentration was calculated using the known standard DNA bands. The DNA concentration for gels analyzed by Kodak™ 1D were computed by the program.

Preparation of DNA Templates For AFLP

After the DNA concentration for each plant sample was determined, the DNA was diluted to a standard concentration of 20 ng/µl. The DNA was then digested with restriction endonucleases and ligated with AFLP adapters. In a 10 µl reaction volume, 100 ng of DNA from each sample was double-digested with 1 unit each of *EcoR I*

(Promega) and *Mse I* (New England Biolabs Inc.) in One-Phor-All (OPA) (Promega) buffer (10 mM Tris-acetate pH 7.5, 10 mM Mg acetate, 50 mM K acetate) at 37°C for two hours. Specific double-stranded oligonucleotide adapters were then attached to the digested DNA fragments in a ligation reaction. This was accomplished by addition of a 10 µl reaction volume containing OPA, T4 ligase buffer (50 mM Tris-Cl pH 7.8, 10 mM MgCl, 20 mM dithiothreitol, 1 mM ATP, 50 µg/ml bovine serum albumin), 0.4 µl of 50 pm/µl *Mse I* adapter and 0.4 µl of 5 pm/µl *EcoR I* adapter was added to the 10 µl DNA digest and incubated from six hours to overnight at 20°C. The ligation was then diluted nine-fold in TE buffer for later use. Adapters were made by annealing two oligonucleotides designed to create a 5' overhang complementary to the *Mse I* or *EcoR I* "sticky end" created during the restriction digest. Oligonucleotides were annealed by mixing equimolar combinations of *EcoR I*-oligo-1 (5' CTCGTAGACTGCCTACC 3') and *EcoR I*-oligo-2 (5' AATTGGTACGCAGTC 3') or *Mse I*-oligo-1 (5' GACGATGAGTCCTGAG 3') and *Mse I*-oligo-2 (5' TACTCAGGACTCAT 3') in OPA, heating to 95°C, and cooling 0.25°C per minute to room temperature.

Primer Preparation

Primers were all prepared by Integrated DNA Technologies, Inc. (Coralville, IA) (Table 5). Prior to use, lyophilized primers were dissolved in 50 µl of TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). Absorbance at wavelength 260 nm was measured using a Unico™ UV-2102 PC spectrophotometer to determine concentration.

Table 5. Primers used in amplification reactions. The name of the primer as determined by the primer sequence contains and the specific sequence of each primer. These primers contain core sequences that anneal to the ligated adapter sequences, and a selective base tail. During the first amplification only one selective base was used (indicated by *Mse I*-Core+Adapt+C and *EcoR I*-Core+Adapt+A). The second amplifications then made use of the remaining primers with the four base selective tails (indicated by *Mse I* – CCGG, *EcoR I*-CAGT, etc.) and produced 12 separate fingerprints which were scored and analyzed.

Primer	Sequence
EcoR I-CORE+ADAPT+A	CTCGTAGACTGCGTACCAATTCA
EcoR I-AATG	AGACTGCGTACCAATTCAATG
EcoR I-ACGC	AGA CTG CGT ACC AAT TCACGC
EcoR I-AGGT	AGA CTG CGT ACC AAT TCAGGT
Mse I-CORE+ADAPT+C	GACGATGAGTCCTGAGTAAC
Mse I-CCGG	GATGAGTCCTGAGTAACCGG
Mse I-CAGG	GATGAGTCCTGAGTAACAGG
Mse I-CAGT	GATGAGTCCTGAGTAACAGT
Mse I-CCTT	GATGAGTCCTGAGTAACCTT

Oligonucleotides *EcoR I*-oligo-1, *EcoR I*-oligo-2, *Mse I*-oligo-1 and *Mse I*-oligo-2 were used to make the *EcoR I* and *Mse I* adapters, respectively. The primers *EcoR I*-CORE+ADAPT+A and *Mse I*-CORE+ADAPT+C were diluted to a concentration of 50 ng/µl. The primers *EcoR I*-AATG, *EcoR I*-ACGC and *EcoR I*-AGGT were diluted to 27.8 ng/µl in TE. Primers *Mse I*-CCGG, *Mse I*-CAGG, *Mse I*-CAGT and *Mse I*-CCTT were diluted to 6.7 ng/µl in TE with 0.89 mM dNTP's (0.222 mM each).

Amplificatoin of DNA

Adapter-modified DNA preparations were amplified in two separate PCRs, a preamplification and a specific amplification as described by Vos et al. (1995). The preamplification was run (in a Genemate® Genius thermocycler, Techme) in a 51 µl reaction volume consisting of 5 µl of the diluted ligation as template, 0.41 µL of 100 mM

dNTP's, 50 ng of *EcoR I*-CORE+ADAPT+A, 50 ng of *Mse I*-CORE+ADAPT+C, AFLP buffer (50 mM KCl, 10 mM Tris-HCl pH 8.41 at 25°C, 7.5 mM $MgCl_2$), and 0.14 µL Taq polymerase (7 U/µl). The reaction was run for 20 cycles of 94°C for 30 seconds, 56°C for 60 seconds and 72°C for 60 seconds. A 72°C hold for 5 minutes was added after the final cycle followed by a soaking at 4°C. Three microliters of this reaction mixture was diluted with 147 µl of TE for use as template in the second amplification reactions. For the second amplification reactions, the *EcoR I* primers were radiolabeled with ^{33}P. In a 50 µl volume, 500 ng of *EcoR I* specific primer (27.8 ng/µL) (*EcoR I*- AATG, *EcoR I*- ACGC, or *EcoR I*- AGGT), kinase buffer (New England Biolabs) (70 mM Tris-HCl pH 7.6, 10 mM $MgCl_2$ and 5 mM dithiothreitol), 10 µl of gamma labeled ATP^{33} at 3000 Ci/mmol and 20 units of T4 polynucleotide kinase (New England Biolabs) were incubated for one hour at 37°C for the labeling reactions and then at 70°C for 15 minutes to inactivate the enzyme in a Genemate® Genius thermocycler. The *Mse I* primer for the second reaction (*Mse I*-CCTT, *Mse I*-CAGG, *Mse I*-CAGT or *Mse I*-CCGG) was prepared as described earlier with dNTP's added. The second amplification reactions were performed in a 5 µl reaction mix containing: 1.3 µl of the diluted preamplification DNA, 1.925 µl of sterile water, 0.5 µl of 10X AFLP buffer, 1.125 µl of specific *Mse I* primer (*Mse I*-CCTT, *Mse I*-CAGG, *Mse I*-CAGT or *Mse I*-CCGG), 0.025 µl of Taq polymerase (7 U/µl) and 0.125 µl of the ^{33}P labeled *EcoR I* primer. A touchdown program that lowered the annealing temperature by 0.7°C every cycle was used in the first part of the specific amplification. Thirteen cycles were performed, starting at 94°C for 30 seconds, 65°C for 30 seconds and 72°C for 60 seconds. The touchdown program was

followed by 23 more cycles at 94°C for 30 seconds, 56°C for 30 seconds, and 72°C for 60 seconds with a 72°C hold for 5 minutes and a 4°C hold after the last cycle. These reactions were also run in thermocycler. The primers *EcoR I*-AATG, *EcoR I*-ACGC and *EcoR I*-AGGT were used in combination with each *Mse I*-CAGG, *Mse I*-CAGT, *Mse I*-CCGG and *Mse I*-CCTT for a total of twelve primer-pair combinations to produce fingerprints for all samples.

Electrophoresis

Amplified fragments were separated using denaturing polyacrlyamide gel electrophoresis (PAGE). Glass plates 18 inches x 14 inches were prepared as described by Maniatis et al. (1982). RainX® (Unelko Corporation) was used to make one plate hydrophobic to prevent the gel sticking during removal. A 6% gel solution was made up of 27 g of urea, 7.2 ml 50% Longranger™ (FMC), 6 ml 10X TBE (1 M Tris base, 0.9 M boric acid, 0.01 M EDTA), 30 µl N,N,N',N'-tetramethylethylenediamine (TEMED) and 300 µl of 10% ammonium persulfate to initiate polymerization. Gels were allowed to polymerize for one hour then placed in a vertical electrophoresis apparatus (Fisher Scientific) containing 1X TBE buffer in both resevoirs. Gels were connected to a power supply and warmed to 45°C by running 60 watts of current through the gel. The AFLP reactions were prepared for analysis through the addition of an equal volume (5 µl) of formamide loading buffer (10 ml deionized ultrapure formamide, 200 µl 0.5M EDTA pH 8, 10 mg bromophenol blue dye and 10mg xylene cyanol FF dye), heated to 94°C for 3 minutes and then placed immediately on ice. Gels were loaded with 1.4 µl of each sample and separated at 60 watts for approximately 2 hours.

Autoradiography

Following electrophoresis, one of the glass plates was removed and the gel blotted onto a 35 x 43 cm piece of Whatman 3MM filter paper. The gels were then dried to the paper in a gel dryer at 80°C under vacuum for 2 hours. After the gels were dried thoroughly, they were placed in a x-ray film cassette with a 35 x 43 cm piece of Classic Blue-sensitive ™ x-ray film (Molecular Technologies Inc.). The film was exposed to the gel for 72 hours and developed with Kodak™ developer and fixer according to the manufacturer's instructions.

Scoring Autoradiographs

After the films were developed they were placed on a light box to help visualize the bands. Bands occurring at different molecular weights were considered an allele and were scored as a character. Each character was recorded as band present (1) or band absent (0). The scores were then entered into MacClade 3.06 due to the ease and accuracy of entry. Data was exported as a space delimited text file for formatting and analysis.

Data Analysis

Data sets were created to represent all the characters of all individuals of each population so comparisons could be made between individuals, populations, and grouped populations. There were five data sets made: *Panicum virgatum* plot, *Panicum virgatum* field, *Panicum virgatum* commercial, *Coreopsis palmata* plot, and *Coreopsis palmata* field. Each *Panicum virgatum* data set had 430 characters representing the alleles scored

across all *P. virgatum* individuals. Each *Coreopsis palmata* data set consisted of 433 characters representing the alleles scored across all *C. palmata* individuals.

Population Differentiation

To test whether or not sets of samples are representative of the same gene pool, genetic heterozygozity was examined between collections. Pair-wise comparisons between accessions were made examining genetic heterozygosity. Chi-square significance tests were performed as demonstrated by Ryman, and Jorde (2001). Chi-square tests were performed on heterozygous alleles and then summed for each pair-wise comparison. The summed chi-squares were then tested for significance against the summed degrees of freedom using a chi-square contingency table.

Genetic Variability

The amount of genetic variability between individuals was assessed by making pairwise comparisons of all the individuals within each population. To assess the amount of genetic variation between populations or groups of populations, the data sets were reformatted to allow the computer program Popgene (v 1.31, Yeh et al. 1997) to identify any hierarchical structure.

The genetic identities (I) and genetic distances (D) were calculated for each individual, population, or group according to the formulas proposed by Nei (1972) using Popgene.

$$I = J_{XY}/\sqrt{J_X J_Y}$$

Where:

J_{XY} = The arithmetic mean over all loci of the probability of identity of a gene from population X and a gene from population Y.

J_X = The arithmetic mean over all loci, of the probability of identity of two randomly chosen genes in population X.

J_Y = The arithmetic mean over all loci of the probability of identity of two randomly chosen genes in population Y.

The genetic distance was then calculated as:

$D = -\ln(I)$

Genetic Differentiation

To assess the amount of genetic differentiation among populations or groups of populations, the data sets had populations removed and added to allow Popgene to make all population or group comparisons. To identify the degree of genetic differentiation between and within populations Nei's (1973) G-statistics were calculated. The gene differentiation relative to the total population is given by:

$G_{ST} = D_{ST}/H_T$

Where:

H_T = The total allelic diversity the total population.

D_{ST} = The average allelic diversity among sub-populations. Which is equal to the total allelic diversity (H_T) minus the average allelic diversity found within a sub-population (H_S).

G-statistics were calculated for all collections and all pair-wise comparisons of accessions for a given collection. Total allelic diversity (H_T) was also calculated for each individual population by treating each individual in a population as a separate population.

Minimum Evolution Tree

Using PAUP software (v. 4.0b4a, Swofford 1999), minimum evolution trees were created to illustrate the genetic distance relationships between all individuals of all populations, populations, or groups of populations based on Nei's 1972 formula for genetic distance. Nexus files were created to load Nei's genetic distances into Paup. Trees were created using full heuristic searchs with branch swapping and TBR options selected and are presented as an unrooted phenograms. They are unrooted due to the fact that no information is available regarding possible outgroups. A phenogram was created for all the individuals scored in the same manner.

Significance of Diversity

To determine if a correlation exists between the geographical distance and the genetic distance (D) (Nei, 1972), Mantel tests were performed using R-package software (Legendre and Vaudor, 1991) with 1000 iterations. Mantel tests (Mantel, 1967) between geographical distance and G_{ST}, determined by Nei's G-statistics (1973), were also performed. Geographical distances were calculated from the UTM coordinates (Tables 1-4) using Pythagorean's theorem. A Student T-test and correlation were used to determine the significance of the relationships. The null hypotheses of there being no relation was rejected by $p > 0.05$. Also calculated were adjusted r-values (Hubert, 1985) and adjusted probabilities (Hope, 1968) to compensate for insufficiencies within the data sets.

CHAPTER 3

RESULTS

To maximize the efficiency of the AFLP procedure, modifications to DNA isolation procedures were refined for the two plant species studied. Originally, the Doyle and Doyle (1992) method of DNA isolation was modified by eliminating the use of phenol and scaling the entire extraction down to a 1.5 ml size. This method was eventually discarded in favor of a silica matrix isolation (Huang and Sun, 2000). The Huang and Sun (2000) method was also modified by the replacement of Sephaglas with diatomaceous earth. The increased silica surface area offered by the diatomaceous earth increased DNA binding and provided high yields of clean DNA for both of the species in this study.

A two-step AFLP reaction was used to produce the clearest DNA profiles. The first amplification used a single selective nucleotide base extension on the end of both the *EcoR I* primer (A) as well as the *Mse* I primer (C) to reduce the number of amplified bands. The second amplification made use of four selective nucleotide base extensions (the single base extension from the first amplification plus three more nucleotides) on the end of the primers. Twelve primer pairs produced fingerprints for each species respectively and had between 35 and 40 bands per primer pair scored.

Panicum virgatum Plot

The genetic profile for *P. virgatum* consisted of 430 characters (bands). There were 9 populations that consisted of 10 individuals apiece collected from the IEP plots located around the campus of the UNI. These populations were compared with each

other and then grouped together with their most closely related population (as determined by PAUP analysis of Nei's (1972) genetic distances) respectively, and then analyzed as groups. Group analysis was done to reduce the bias of small sample size. *Panicum virgatum* was also compared to six greenhouse grown commercial cultivated varieties (cultivars) [Alamo (A), Blackwell (B), Cave-In-Rock (C), Forestberg (F), Kanlow (K), and Pathfinder (P)] to ensure that the populations collected were indeed remnant prairie plants and not products of cross-pollination with introduced cultivars nor cultivars established within the remnant.

Chi-square tests were performed on all loci for all pair-wise combinations of populations. Chi-squares were then summed (Table 6) and tested against the summed degrees of freedom (Table 6) for each pair-wise combination. Chi-square analysis of all of the *P. virgatum* plot populations and cultivars showed probabilities of essentially zero.

Genetic distances (Nei, 1972) and genetic identity (Nei, 1972) were then calculated pair-wise between populations (Table 7), grouped populations (Table 8), IEP zones (Table 9), and individuals (data not shown) for *P. virgatum*. Genetic distance (D) measures the allelic differences at each loci compared between two populations of individuals, while genetic identity (I) measures allelic similarity in the same manner. The smaller the genetic distance (the larger the genetic identity), the more alleles the two populations have in common. The genetic distances calculated for *P. virgatum* plot and

Table 6. *P. virgatum* plot populations and cultivars: Summed chi-squares of polymorphic loci (upper triangle) and number of polymorphic loci (lower triangle). The summed chi-squares (located in the upper triangle) and the polymorhpic loci (located in the bottom triangle) for all pair-wise combinations between plot populations and commercial cultivated varieties(cultivars) of *P. virgatum*. The number of polymorphic loci is equal to the summed degrees of freedom used to determine chi-square contingency. This is because chi-square is only calculated on polymorphic loci, each having one degree of freedom. Plot populations are designated by a three digit number with the first number referring to the IEP zone it was collected in and the last two numbers referring to it's accession number. The cultivars are abbreviated with the first letter of their name (A – Alamo, B – Blackwell, C – Cave-in-rock, F – Forestburg, K – Kanlow, and P – Pathfinder).

	119	124	127	206	216	218	221	305	311	A	B	C	F	K	P
119		785.50	1018.06	830.37	1089.08	985.58	988.68	1081.31	1183.33	1342.53	1124.86	1124.28	1120.19	1404.23	1183.22
124	230		857.21	799.33	899.27	1098.17	938.27	1186.17	1242.40	1467.46	1283.04	1296.18	1312.43	1418.66	1316.80
127	248	237		828.78	912.39	1106.07	1063.74	1212.58	1386.29	1553.04	1262.97	1232.09	1282.72	1518.59	1313.42
206	247	243	247		699.16	1004.54	955.74	1155.71	1234.46	1418.95	1099.80	1029.71	1098.17	1341.33	1130.75
216	255	246	247	248		1035.09	906.62	1186.52	1387.36	1569.28	1332.05	1323.00	1410.04	1516.76	1365.01
218	245	250	256	258	260		849.86	1243.11	1202.65	1594.60	1258.49	1276.26	1288.04	1512.84	1352.37
221	260	253	264	267	265	257		1176.37	1241.37	1554.18	1308.20	1380.99	1386.88	1511.99	1420.08
305	231	236	246	252	251	244	253		1041.75	1670.76	1228.93	1162.62	1188.17	1648.16	1346.06
311	234	243	253	257	260	248	256	211		1560.08	1207.99	1192.88	1281.49	1418.52	1329.42
A	226	236	239	247	251	246	257	224	221		1102.28	1151.53	1039.28	425.59	1126.05
B	215	224	227	232	240	232	246	201	203	160		435.38	447.77	959.58	382.28
C	210	219	215	220	234	225	245	193	197	158	96		537.95	998.16	475.32
F	209	223	219	229	240	227	246	193	206	145	98	98		989.38	417.35
K	226	232	239	240	245	240	255	225	211	88	146	141	141		1002.50
P	212	223	222	229	237	232	246	203	206	159	90	95	92	148	

cultivar populations had a maximum of 0.3266 (minimum genetic identity of 0.7272) which was found between population 305 and the Alamo cultivar, and a minimum of 0.0887 (maximum genetic identity of 0.9151) which was found between the Blackwell cultivar and the Pathfinder cultivar. The average genetic distance between all populations was found to be 0.2049, and the average genetic identity between all populations was 0.8170. When the cultivars were excluded, the maximum genetic distance droped to 0.1738 (minimum genetic identity of 0.8405) between populations 216 and 311and the minimum rises to 0.0925 (maximum genetic identity of 0.9116) which is found between populations 119 and 124. The average genetic distance for just the plot populations is 0.1273 (genetic identity of 0.8807) and 0.1980 (genetic identity of 0.8236) for just the cultivars.

When populations were grouped (Table 8) (cultivars excluded) the maximum genetic distance was 0.1091(minimum genetic identity of 0.8966) between groups 119 and 311,305; the minimum genetic distance was 0.0608 (maximum genetic identity of 0.941) between groups 124,127 and 216,206; and the average was 0.0925 (average genetic identity of 0.9117). When the populations were grouped further into IEP zones (Table 9) the maximum genetic distance was 0.0911 (minimum genetic identity of 0.9129) between zones 1 and 3, the minimum was 0.0424 (maximum genetic identity of 0.9585) between zones 1 and 2, and the average was 0.0726 (average genetic identity of 0.9302).

Table 7. *P. virgatum* plot populations and cultivars: Genetic Distances (lower triangle) and Genetic Identity (upper triangle). Nei's (1972) genetic distance and Nei's (1972) genetic identity numbers for all pair-wise comparisons between plot populations and commercial cultivated varieties(cultivars) of *P. virgatum*. Plot populations and cultivars are designated in the same manner as described in Table 6.

	119	124	127	206	216	218	221	305	311	A	B	C	F	K	P
119		0.9116	0.8883	0.9088	0.8753	0.8878	0.8895	0.8786	0.8652	0.7748	0.8103	0.8108	0.8105	0.7620	0.7983
124	0.0925		0.9050	0.9096	0.8988	0.8723	0.8925	0.8661	0.8597	0.7534	0.7837	0.7800	0.7783	0.7603	0.7769
127	0.1184	0.0998		0.9069	0.8948	0.8723	0.8789	0.8644	0.8411	0.7363	0.7874	0.7890	0.7819	0.7423	0.7760
206	0.0956	0.0948	0.0977		0.9219	0.8838	0.8897	0.8703	0.8596	0.7560	0.8121	0.8227	0.8120	0.7687	0.8051
216	0.1332	0.1067	0.1112	0.0814		0.8807	0.8954	0.8653	0.8405	0.7308	0.7714	0.7723	0.7576	0.7375	0.7638
218	0.1191	0.1367	0.1366	0.1235	0.1271		0.9042	0.8558	0.8643	0.7272	0.7866	0.7820	0.7804	0.7412	0.7682
221	0.1171	0.1137	0.1290	0.1168	0.1104	0.1007		0.8662	0.8576	0.7305	0.7731	0.7608	0.7591	0.7371	0.7530
305	0.1294	0.1438	0.1457	0.1390	0.1447	0.1557	0.1436		0.8819	0.7213	0.7963	0.8076	0.8024	0.7252	0.7745
311	0.1448	0.1512	0.1730	0.1513	0.1738	0.1458	0.1536	0.1257		0.7398	0.8011	0.8028	0.7897	0.7635	0.7791
A	0.2552	0.2832	0.3062	0.2797	0.3136	0.3186	0.3140	0.3266	0.3014		0.7434	0.7315	0.7589	0.9050	0.7381
B	0.2103	0.2437	0.2390	0.2081	0.2595	0.2400	0.2574	0.2278	0.2217	0.2966		0.9032	0.9015	0.7770	0.9151
C	0.2097	0.2485	0.2370	0.1952	0.2583	0.2459	0.2733	0.2137	0.2197	0.3127	0.1018		0.8781	0.7656	0.8922
F	0.2102	0.2506	0.2461	0.2083	0.2776	0.2480	0.2757	0.2201	0.2361	0.2759	0.1036	0.1300		0.7689	0.9087
K	0.2718	0.2741	0.2980	0.2631	0.3045	0.2995	0.3050	0.3213	0.2699	0.0998	0.2523	0.2671	0.2628		0.7666
P	0.2253	0.2525	0.2536	0.2168	0.2695	0.2637	0.2837	0.2556	0.2496	0.3037	0.0887	0.1140	0.0958	0.2658	

Table 8. *P. virgatum* plot populations grouped with their closest genetic relative: Genetic Distances (lower triangle) and Genetic Identity (upper triangle). Nei's (1972) genetic distance and Nei's (1972) genetic identity numbers for all pair-wise comparisons between grouped plot populations of *P. virgatum*. Plot populations are designated in the same manner as described in Table 6. Populations that were grouped were the most similar genetically (as determined by PAUP analysis of Nei's (1972) genetic distance) and are separated with a comma.

	119	124,127	216,206	218,221	311,305
119		0.9155	0.9041	0.9047	0.8966
124,127	0.0882		0.9410	0.9203	0.8988
216,206	0.1008	0.0608		0.9263	0.9004
218,221	0.1001	0.0830	0.0766		0.9093
311,305	0.1091	0.1067	0.1049	0.0951	

Table 9. *P. virgatum* Plot Zone Relationships: Genetic Distances (lower triangle) and Genetic Identity (upper triangle). Nei's (1972) genetic distance and Nei's (1972) genetic identity numbers for all pair-wise comparisons between all *P. virgatum* populations in each of the IEP zones. Plot populations are designated by a three digit number with the first number referring to the IEP zone it was originally collected in, thus the group designation of hundreds.

	100	200	300
100		0.9585	0.9129
200	0.0424		0.9193
300	0.0911	0.0842	

Nei's (1973) G-statistics are a measure of population differentiation in regard to allele frequencies within each respective population. These calculations compare allele frequencies within each population and group populations according to frequency commonality. These numbers tell us the degree of allelic differentiation among populations. The G_{ST} tells us how dissimilar the populations are and is calculated using H_T (total heterozygosity) which tells us the amount of heterozygosity that exists among

populations. A high H_T number signifies more heterozygosity within the populations that are compared. A G_{ST} of one means that two population have reached fixation from each other and they have no gene flow between them; so all genetic variation is found within each separate population respectively. A G_{ST} of zero means that the two populations are genetically identical and have complete gene flow to maintain it, so all genetic variation exists in both populations. The maximum G_{ST} value for *P. virgatum* plot analysis (Table 10) was found to be 0.7906 between the cultivars Alamo and Cave-in-Rock, while the minimum G_{ST} value was found to be 0.1553 between populations 216 and 206. The G_{ST} among all populations was found to be 0.5002, which was calculated from the mean H_T and the mean D_{ST} (calculated across all loci for all populations). When the cultivars were excluded the maximum G_{ST} drops to 0.3200 between populations 311 and 127. The G_{ST} for just the plot populations is 0.3623 and is 0.7955 for just the cultivars. The maximum amount of heterozygosity (H_T) that existed between two populations (Table 10) was 0.2633 within populations 218 and the Cave-in-Rock cultivar. The minimum amount of genetic heterozygosity (H_T) was 0.0793 between the Forestburg cultivar and the Pathfinder cultivar. The amount of genetic heterozygosity (H_T) was found to be 0.2633 among all populations. Without the cultivars, the maximum amount of heterozygosity drops to 0.2256 between two populations was 221 and 206 and the minimum amount of heterozygosity rises to 0.1826 between the two populations of 311 and 305. The amount of heterozygosity among the plot populations is 0.2417 and is 0.1781 among the cultivars. The amount of heterozygosity within each population (Table 11) ranges from 0.0324 in

Table 10. *P. virgatum* plot populations and cultivars: G_{ST} (upper triangle) and H_T (lower triangle). Nei's (1973) G-statistics for all pair-wise comparisons between plot populations and commercial cultivated varieties(cultivars) of *P. virgatum*. Plot populations and cultivars are designated in the same manner as described in Table 6.

	119	124	127	206	216	218	221	305	311	A	B	C	F	K	P
119		0.1952	0.2328	0.1834	0.2438	0.2284	0.2159	0.2682	0.2849	0.5195	0.4601	0.4763	0.4798	0.5344	0.4867
124	0.1915		0.2071	0.1838	0.2094	0.2542	0.2132	0.2905	0.2958	0.5463	0.4968	0.5181	0.5230	0.5406	0.5161
127	0.2027	0.1940		0.1868	0.2140	0.2523	0.2321	0.2910	0.3196	0.5587	0.4890	0.5041	0.5153	0.5547	0.5134
206	0.2069	0.2051	0.2075		0.1553	0.2179	0.2003	0.2613	0.2728	0.5039	0.4249	0.4259	0.4427	0.4919	0.4439
216	0.2143	0.2030	0.2059	0.2075		0.2310	0.1993	0.2789	0.3097	0.5459	0.4895	0.5045	0.5228	0.5409	0.5085
218	0.2065	0.2116	0.2129	0.2207	0.2155		0.1886	0.2971	0.2804	0.5564	0.4795	0.5011	0.5058	0.5447	0.5110
221	0.2132	0.2105	0.2175	0.2256	0.2168	0.2106		0.2698	0.2781	0.5328	0.4750	0.5033	0.5078	0.5278	0.5064
305	0.1943	0.1982	0.2002	0.2104	0.2060	0.2075	0.2105		0.2811	0.6118	0.5165	0.5215	0.5315	0.6098	0.5546
311	0.2024	0.2033	0.2124	0.2172	0.2189	0.2062	0.2164	0.1826		0.5875	0.5028	0.5194	0.5386	0.5656	0.5415
A	0.1977	0.2059	0.2148	0.2182	0.2233	0.2225	0.2280	0.2099	0.2037		0.7040	0.7906	0.7776	0.5751	0.7772
B	0.1868	0.1973	0.1968	0.1984	0.2101	0.2008	0.2140	0.1807	0.1808	0.1633		0.5361	0.5469	0.7292	0.4916
C	0.1815	0.1939	0.1910	0.1886	0.2047	0.2633	0.2145	0.1704	0.1749	0.1640	0.0867		0.6383	0.7695	0.5891
F	0.1807	0.1938	0.1934	0.1925	0.1925	0.1977	0.2144	0.1719	0.1800	0.1499	0.0865	0.0923		0.7725	0.5558
K	0.2031	0.2025	0.2117	0.2122	0.2122	0.2158	0.2247	0.2078	0.1925	0.0798	0.1468	0.1472	0.1447		0.7588
P	0.1887	0.1969	0.1985	0.1980	0.2101	0.2056	0.2196	0.1872	0.1874	0.1624	0.0827	0.0882	0.0793	0.1483	

the Alamo cultivar to 0.1803 in population 221. Without the cultivars, the minimum amount of heterozygosity within a population becomes 0.1187 in population 311.

When populations were grouped (Table 12) (cultivars excluded) the maximum G_{ST} was 0.1998 between groups 119 and 311,305; the minimum G_{ST} was 0.1013 between groups 216,206 and 124,127; and was 0.2262 when comparing all grouped populations. The maximum amount of heterozygosity (H_T) (Table12) was 0.2451 found between the two grouped populations of 216,206 and 218,221; the minimum of 0.2130 was found within the two grouped population of 119 and 124,127, and the amount of heterozygosity within all grouped populations was 0.2523. The amount of heterozygosity within each grouped population (Table 13) ranges from 0.1454 in population 119 to 0.2047 in population 221,218.

When the populations were grouped further into IEP plot zones (Table 14) the maximum G_{ST} was 0.1432 between zones 1 and 3, the minimum was 0.0617 between zones 1and 2, and the was 0.1072 between all three zones. The maximum amount of heterozygosity (H_T) (Table 14) was 0.2562 within zones 1 and 2, the minimum amount of 0.2418 was found within zones 1 and 3, and the amount of heterozygosity within all zones was found to be 0.2593. The amount of heterozygosity within each grouped population (Table 15) ranges from 0.1681 in zone 3 to 0.2195 in zone 2.

Unrooted phenograms were constructed from Nei's genetic distances (1972) to visualize how populations or individual plants are "related" to each other. Unrooted

Table 11. Genetic heterozygosity (H_T) within IEP plot and cultivar populations for *P. virgatum*. The amount of genetic heterozygosity found within each specific population of *P. virgatum*. Plot populations are designated in the same manner as described in Table 6. The cultivars are abbreviated with the first letter of their name.

Population	H_T
119	0.1454
124	0.1502
127	0.1503
206	0.1733
216	0.1737
218	0.1603
221	0.1803
305	0.1251
311	0.1187
Alamo	0.0324
Blackwell	0.0469
Cave-in-Rock	0.0391
Forestburg	0.0335
Kanlow	0.0380
Pathfinder	0.0387

phenograms for *P. virgatum* were constructed for populations (Figures 10,12, and 18), grouped populations (Figure 11) , and individual plants (Figure 16). The degree of genetic distance is expressed by branch length and thus the further the two populations or individuals are, the more allelic bands they do not have in common. These trees are unrooted because an outgroup was not available in this study.

Mantel tests were performed to see if any correlation existed for populations (cultivars excluded) between either the G_{ST}'s or genetic distances and their geographical distances. The r-value between the genetic distance and geographical distance for *P. virgatum* plot samples was 0.52 with the p-value of 0.0002 (Hope, 1968). A T-test was also calculated for the two matrices, which produced a probability of 0.001. The r-value

between the G_{ST} and geographical distance for *P. virgatum* plot samples was 0.49 with the p-value 0.001 (Hope, 1968). A T-test was also done on the same matrices and produced a probability of 0.0018.

Table 12. *P. virgatum* plot populations grouped with their closest genetic relative: G_{ST} (upper triangle) and H_T (lower triangle). Nei's (1973) G-statistics for all pair-wise comparisons between grouped plot populations of *P. virgatum*. Plot populations are designated in the same manner as described in Table 6. Populations that were grouped were the most similar genetically (as determined by PAUP analysis of Nei's genetic distance (1972)) and are separated with a comma.

	119	124,127	216,206	218,221	311,305
119		0.1636	0.1753	0.1728	0.1998
124,127	0.2130		0.1013	0.1309	0.1736
216,206	0.2244	0.2309		0.1179	0.1654
218,221	0.2258	0.2408	0.2451		0.1515
311,305	0.2145	0.2349	0.2409	0.2390	

Table 13. Genetic heterozygosity (H_T) within IEP plot grouped populations for *P. virgatum*. The amount of genetic heterozygosity found within each specific grouped population of *P. virgatum*. Plot populations are designated in the same manner as described in Table 6. Populations that were grouped were the most similar genetically (as determined by PAUP analysis of Nei's genetic distance (1972)) and are separated with a comma.

Grouped Population	H_T
119	0.1454
124,127	0.1864
206,216	0.2015
218,221	0.2047
311,305	0.1681

Table 14. *P. virgatum* Plot Zone Relationships: G_{ST}(upper triangle) and H_T (lower triangle). Nei's (1972) genetic distance and Nei's (1972) genetic identity numbers for all pair-wise comparisons between all *P. virgatum* populations in each of the IEP zones. Plot zones are designated as in Table 9.

	100	200	300
100		0.0617	0.1432
200	0.2562		0.1258
300	0.2418	0.2526	

Table 15. Genetic heterozygosity (H_T) within IEP plot zones for *P. virgatum*. The amount of genetic heterozygosity found within each specific IEP zone for *P. virgatum*. Plot populations are designated in the same manner as in Table 9.

IEP Zone	H_T
100	0.1962
200	0.2195
300	0.1681

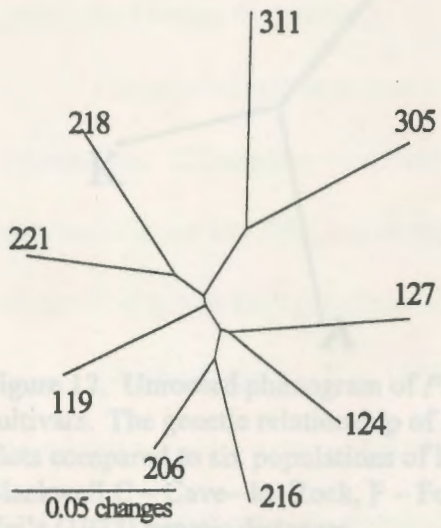

Figure 10. Unrooted phenogram of *P. virgatum* plot populations. The genetic relationships of *P. virgatum* populations collected from the IEP plots based on Nei's (1972) genetic distances.

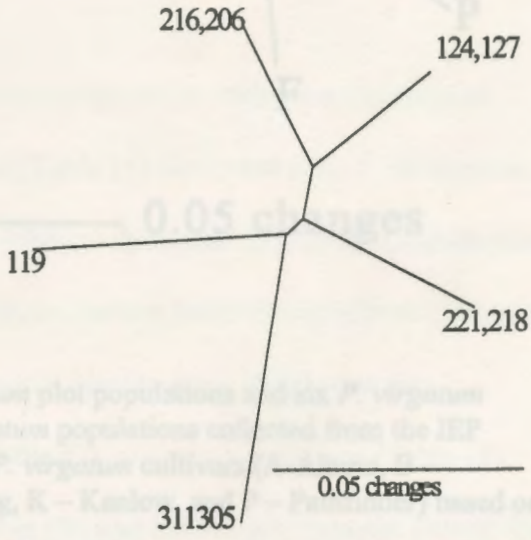

Figure 11. Unrooted phenogram of grouped *P. virgatum* plot populations. The genetic relationships of grouped *P. virgatum* populations collected from the IEP plots based on Nei's (1972) genetic distances.

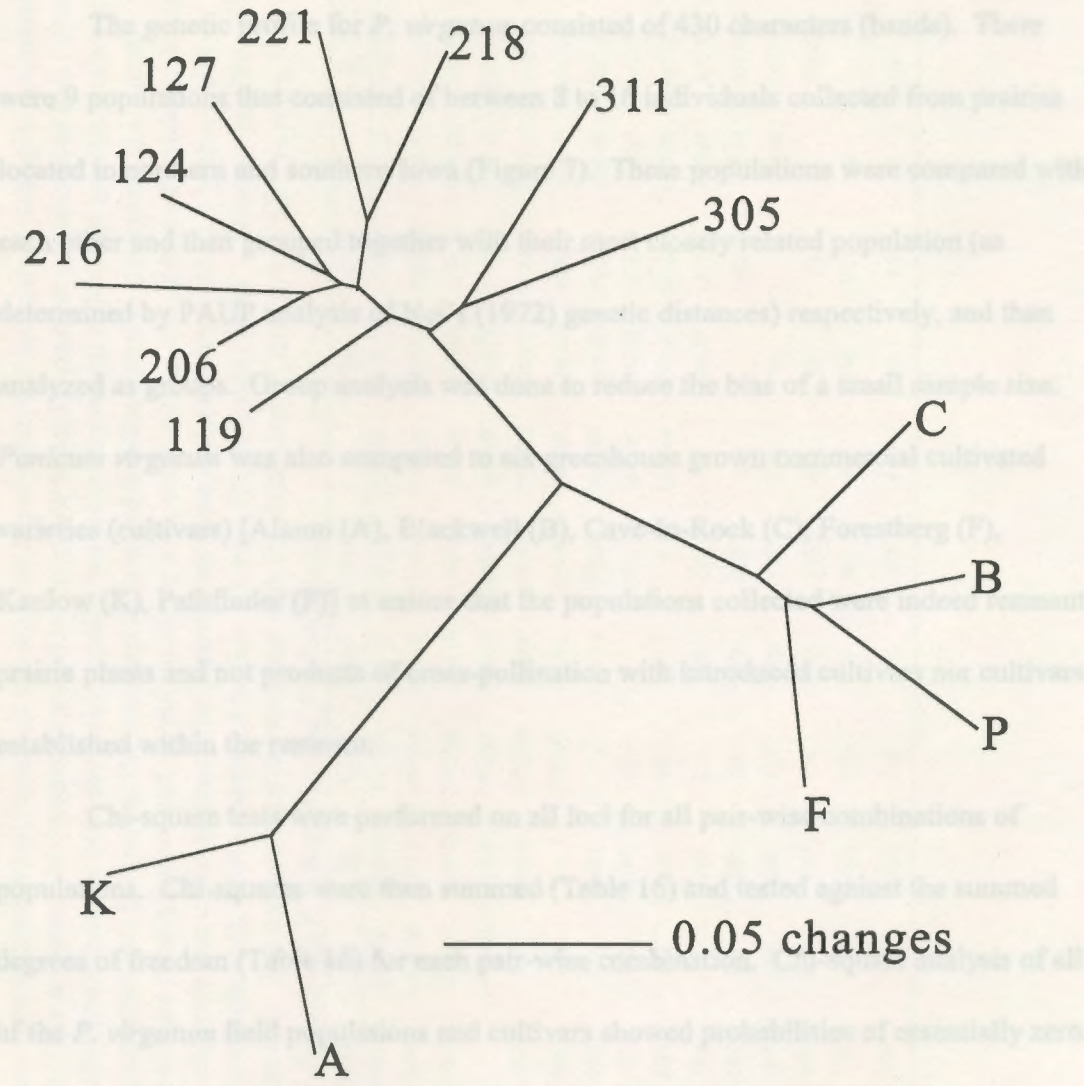

Figure 12. Unrooted phenogram of *P. virgatum* plot populations and six *P. virgatum* cultivars. The genetic relationship of *P. virgatum* populations collected from the IEP plots compared to six populations of known *P. virgatum* cultivars (A-Alamo, B - Blackwell C – Cave– In–Rock, F – Forestburg, K – Kanlow, and P – Pathfinder) based on Nei's (1972) genetic distances.

Panicum virgatum Field

The genetic profile for *P. virgatum* consisted of 430 characters (bands). There were 9 populations that consisted of between 8 to 16 individuals collected from prairies located in northern and southern Iowa (Figure 7). These populations were compared with each other and then grouped together with their most closely related population (as determined by PAUP analysis of Nei's (1972) genetic distances) respectively, and then analyzed as groups. Group analysis was done to reduce the bias of a small sample size. *Panicum virgatum* was also compared to six greenhouse grown commercial cultivated varieties (cultivars) [Alamo (A), Blackwell (B), Cave-In-Rock (C), Forestberg (F), Kanlow (K), Pathfinder (P)] to ensure that the populations collected were indeed remnant prairie plants and not products of cross-pollination with introduced cultivars nor cultivars established within the remnant.

Chi-square tests were performed on all loci for all pair-wise combinations of populations. Chi-squares were then summed (Table 16) and tested against the summed degrees of freedom (Table 16) for each pair-wise combination. Chi-square analysis of all of the *P. virgatum* field populations and cultivars showed probabilities of essentially zero.

Genetic distances (Nei, 1972) and genetic identity (Nei, 1972) were then calculated pair-wise between populations (Table 17), grouped populations (Table 18), northern and southern Iowa populations (Table 19), and individuals (data not shown) for *P. virgatum*. The genetic distance calculated for *P. virgatum* field and cultivar populations had a maximum of 0.3604 (minimum genetic identity of 0.6974) which was

Table 16. *P. virgatum* field populations and cultivars: Summed chi-squares of polymorphic loci (upper triangle) and number of polymorphic loci (lower triangle). The summed chi-squares (located in the upper triangle) and the polymorhpic loci (located in the bottom triangle) for all pair-wise combinations between field populations and commercial cultivated varieties(cultivars) of *P. virgatum*. The number of polymorphic loci is equal to the summed degrees of freedom used to determine chi-square contingency. This is because chi-square is only calculated on polymorphic loci, each having one degree of freedom. Field populations are designated by an abbreviation of the name of the prairie they were collected from (Med – Medora, Fla – Flaherty, 2RR – Land Between Two Railroads, MtA – Mount Ayr, Sm – Smith, St – Stinson, WRT – Winnebago River Trail, Hof – Hoffman, and Wik – Wilkinson). The cultivars are abbreviated with the first letter of their name (A – Alamo, B – Blackwell, C – Cave-in-rock, F – Forestburg, K – Kanlow, and P – Pathfinder).

	Med	Fla	2RR	MtA	Sm	St	Win	Hof	WRT	A	B	C	F	K	P
Med		956.80	1507.55	1685.16	1327.71	1176.31	2194.73	1442.47	1439.98	1810.64	1415.51	1513.44	1511.05	1730.51	1437.89
Fla	223		1624.94	1717.96	1359.98	1135.37	2299.94	1430.46	1417.38	1963.32	1639.65	1601.03	1703.83	1983.58	1613.58
2RR	225	216		965.17	1053.02	1218.18	1763.56	1154.19	1172.16	1849.34	1438.65	1409.96	1505.55	1762.01	1382.78
MtA	223	214	151		1000.49	1142.38	1764.05	1361.27	1234.25	1890.29	1305.55	1427.06	1470.59	1781.80	1418.29
Sm	223	217	164	151		688.30	1420.35	920.68	948.98	1278.23	1098.13	1174.36	1179.53	1253.43	1073.88
St	238	226	196	182	174		1379.70	974.61	936.00	1254.62	1069.69	1071.99	1147.17	1236.71	1127.09
WRT	226	218	170	154	158	184		1314.32	1358.69	2094.79	1641.99	1613.34	1691.46	1888.55	1633.03
Hof	230	218	175	172	161	190	154		770.84	1339.64	1219.26	1210.24	1252.51	1346.79	1178.79
Wik	235	222	173	166	170	195	160	157		1309.64	1131.41	1112.21	1157.08	1264.83	1159.62
A	248	243	193	186	180	205	178	178	185		1102.28	1151.53	1039.28	425.59	1126.05
B	229	227	178	165	170	200	161	177	180	160		435.38	447.77	959.58	382.28
C	231	221	173	167	169	194	157	170	172	158	96		537.95	998.16	475.32
F	227	225	176	169	169	197	158	172	173	145	98	98		989.38	417.35
K	240	237	188	179	177	202	168	178	181	88	146	141	141		1002.50
P	228	221	169	165	166	198	159	170	175	159	90	95	92	148	

found between the populations Flaherty and the Kanlow cultivar and a minimum of 0.0887 (maximum genetic identity of 0.9015) which was found between the Blackwell cultivar and the Pathfinder cultivar. The average genetic distance between all populations was found to be 0.2126 (average genetic identity of 0.8103). When the cultivars were excluded the maximum genetic distance drops to 0.2030 (minimal genetic identity of 0.8163) between populations Winnebago River Trail and Flaherty and the minimum rises to 0.0909 (maximum genetic identity of 0.9131) which is found between populations Mt. Ayr and Land Between Two Railroads. The average genetic distance for just the field populations is 0.1495 (genetic identity of 0.8615) and is 0.1980 (genetic identity of 0.8236) for just the cultivars.

When populations were grouped (Table 18) (cultivars excluded) the maximum genetic distance was 0.1807 (minimum genetic identity of 0.8347) between groups Medora, Flaherty and Winnebago River Trail; the minimum genetic distance was 0.0966 (maximum genetic identity of 0.9079) between groups Mt Ayr, Land Between Two Railroads and Smith, Stinson; and the average was 0.1234. When the populations were grouped further and compared northern and southern Iowa (Table 19) the genetic distance was 0.0678 (genetic identity of 0.9345).

The maximum G_{ST} value for *P. virgatum* field analysis (Table 20) was found to be 0.7906 between the cultivars Alamo and Cave-in-Rock, while the minimum G_{ST} value was found to be 0.1989 between populations Medora and Flaherty. The overall G_{ST} was found to be 0.6756, which was calculated from the average H_T and D_{ST} (calculated across

Table 17. *P. virgatum* field populations and cultivars: Genetic Distances (lower triangle) and Genetic Identity (upper triangle). Nei's (1972) genetic distance and Nei's (1972) genetic identity numbers for all pair-wise comparisons between field populations and commercial cultivated varieties (cultivars) of *P. virgatum*. Field populations and cultivars are designated in the same manner as Table 16.

	Med	Fla	2RR	MtA	Sm	St	WRT	Hof	Wik	A	B	C	F	K	P
Med		0.9098	0.8598	0.8427	0.8400	0.8603	0.8305	0.8266	0.8259	0.7186	0.7799	0.7636	0.7625	0.7293	0.7752
Fla	0.0945		0.8466	0.8391	0.8413	0.8697	0.8163	0.8321	0.8323	0.7025	0.7527	0.7575	0.7413	0.6974	0.7545
2RR	0.1511	0.1665		0.9131	0.8810	0.8637	0.8604	0.8707	0.8645	0.7266	0.7903	0.7933	0.7782	0.7403	0.7954
MtA	0.1711	0.1754	0.0909		0.8883	0.8764	0.8565	0.8453	0.8601	0.7257	0.8178	0.7976	0.7906	0.7425	0.7979
Sm	0.1744	0.1728	0.1267	0.1184		0.9056	0.8662	0.8702	0.8652	0.7664	0.8011	0.7852	0.7841	0.7707	0.8047
St	0.1505	0.1396	0.1466	0.1319	0.0991		0.8801	0.8623	0.8672	0.7647	0.8027	0.8016	0.7860	0.7677	0.7899
Win	0.1857	0.2030	0.1504	0.1549	0.1437	0.1278		0.8765	0.8733	0.7533	0.8087	0.8087	0.7990	0.7769	0.8074
Hof	0.1904	0.1838	0.1384	0.1681	0.1391	0.1482	0.1318		0.8938	0.7551	0.7780	0.7787	0.7704	0.7533	0.7847
WRT	0.1912	0.1835	0.1456	0.1507	0.1448	0.1425	0.1355	0.1122		0.7576	0.7933	0.7965	0.7872	0.7675	0.7865
A	0.3304	0.3531	0.3193	0.3206	0.2661	0.2682	0.2833	0.2809	0.2776		0.7434	0.7315	0.7589	0.9050	0.7381
B	0.2486	0.2840	0.2353	0.2012	0.2217	0.2198	0.2123	0.2511	0.2316	0.2966		0.9032	0.9015	0.7770	0.9151
C	0.2697	0.2777	0.2315	0.2262	0.2418	0.2212	0.2124	0.2501	0.2276	0.3127	0.1018		0.8781	0.7656	0.8922
F	0.2712	0.2994	0.2508	0.2350	0.2432	0.2408	0.2244	0.2609	0.2393	0.2759	0.1036	0.1300		0.7689	0.9087
K	0.3157	0.3604	0.3007	0.2977	0.2605	0.2644	0.2524	0.2833	0.2647	0.0998	0.2523	0.2671	0.2628		0.7666
P	0.2547	0.2817	0.2289	0.2258	0.2173	0.2358	0.2140	0.2425	0.2402	0.3037	0.0887	0.1140	0.0958	0.2658	

Table 18. *P. virgatum* field populations grouped with their closest genetic relative: Genetic Distances (lower triangle) and Genetic Identity (upper triangle). Nei's (1972) genetic distance and Nei's (1972) genetic identity numbers for all pair-wise comparisons between grouped field populations of *P. virgatum*. Field populations and cultivars are designated in the same manner as Table 16. Populations that were grouped were the most similar genetically (as determined by PAUP analysis of Nei's genetic distance (1972)) and are separated with a comma.

	Med,Fla	MtA,2RR	St,Sm	WRT	Wik,Hof
Med,Fla		0.8822	0.8912	0.8347	0.8701
MtA,2RR	0.1253		0.9079	0.8724	0.8974
St,Sm	0.1151	0.0966		0.8876	0.9052
WRT	0.1807	0.1365	0.1192		0.8930
Wik,Hof	0.1391	0.1082	0.0996	0.1132	

Table 19. *P. virgatum* field North versus South relationships: Genetic Distances (lower triangle) and Genetic Identity (upper triangle). Nei's (1972) genetic distance and Nei's (1972) genetic identity numbers for the comparison between northern Iowa (Smith, Stinson, Winnebago River Trail, Hoffman, and Wilkinson) grouped field populations and southern Iowa (Medora, Flaherty, Land Between Two Railroads, and Mt. Ayr) grouped field populations of *P. virgatum*.

	North	South
North		0.9345
South	0.0678	

all loci for all populations). The maximum amount of heterozygosity (H_T) (Table 20) that existed between two populations was 0.2236 between populations Wilkinson and Flaherty. The minimum amount of heterozygosity that existed between two populations was 0.0793 between the Forestburg cultivar and the Pathfinder cultivar. The amount of heterozygosity among all populations was 0.2426. Without the cultivars the maximum G_{ST} drops to 0.4741 between populations Mt. Ayr and Hoffman. The G_{ST} for just the field populations

Table 20. *P. virgatum* field populations and cultivars: G_{ST} (upper triangle) and H_T (lower triangle). Nei's (1973) G-statistics for all pair-wise comparisons between field populations and commercial cultivated varieties (cultivars) of *P. virgatum*. Field populations and cultivars are designated in the same manner as Table 16.

	Med	Fla	2RR	MtA	Sm	St	WRT	Hof	Wik	A	B	C	F	K	P
Med		0.1989	0.3161	0.3656	0.3522	0.2801	0.3819	0.3776	0.3651	0.5580	0.4815	0.5151	0.5191	0.5498	0.4976
Fla	0.1920		0.3632	0.4019	0.3790	0.2879	0.4327	0.4002	0.3849	0.6097	0.5472	0.5603	0.5792	0.6149	0.5576
2RR	0.1934	0.1864		0.3169	0.3653	0.3396	0.4254	0.3939	0.3874	0.6605	0.5765	0.5965	0.6162	0.6505	0.5857
MtA	0.1905	0.1793	0.1255		0.3839	0.3442	0.4703	0.4741	0.4286	0.7030	0.5875	0.6389	0.6505	0.6935	0.6285
Sm	0.1989	0.1856	0.1476	0.1335		0.2695	0.4259	0.4052	0.3958	0.6379	0.5765	0.6181	0.6235	0.6353	0.5880
St	0.2116	0.1948	0.1775	0.1613	0.1557		0.3363	0.3573	0.3347	0.5576	0.4957	0.5155	0.5375	0.5558	0.5237
WRT	0.1963	0.1899	0.1500	0.1411	0.1441	0.1601		0.4169	0.4031	0.6805	0.5971	0.6228	0.6389	0.6601	0.6161
Hof	0.2018	0.1868	0.1493	0.1503	0.1463	0.1719	0.1364		0.3491	0.6614	0.6155	0.6382	0.6509	0.6648	0.6242
Wik	0.2078	0.2236	0.1579	0.1493	0.1544	0.1756	0.1437	0.1384		0.6346	0.5745	0.5932	0.6079	0.6266	0.5975
A	0.2283	0.2057	0.1936	0.1841	0.1720	0.1939	0.1715	0.1744	0.1788		0.7540	0.7906	0.7776	0.5751	0.7772
B	0.2059	0.1986	0.1691	0.1458	0.1609	0.1817	0.1505	0.1688	0.1673	0.1633		0.5361	0.5469	0.7292	0.4916
C	0.2081	0.1986	0.1625	0.1501	0.1632	0.1771	0.1453	0.1633	0.1607	0.1640	0.0867		0.6383	0.7695	0.5891
F	0.2078	0.2051	0.1687	0.1525	0.1628	0.1833	0.1490	0.1664	0.1641	0.1499	0.0865	0.0923		0.7725	0.5558
K	0.2231	0.2256	0.1869	0.1758	0.1696	0.1922	0.1599	0.1749	0.1738	0.0798	0.1468	0.1472	0.1447		0.7588
P	0.2045	0.2015	0.1632	0.1516	0.1557	0.1840	0.1476	0.1621	0.1670	0.1624	0.0827	0.0882	0.0793	0.1483	

Table 21. Genetic heterozygosity (H_T) within field populations for *P. virgatum*. The amount of genetic heterozygosity found within each specific population of *P. virgatum*. Field populations and cultivars are designated by their names.

Population	H_T
Medora	0.1622
Flaherty	0.1316
Land Between Two Railroads	0.0973
Mount Ayr	0.0785
Smith	0.0802
Stinson	0.1308
Winnebago River Trail	0.0704
Hoffman	0.0820
Wilkinson	0.1012
Alamo	0.0324
Blackwell	0.0469
Cave-in-Rock	0.0391
Forestburg	0.0335
Kanlow	0.0380
Pathfinder	0.0387

is 0.5074 and is 0.7955 for just the cultivars. Without the cultivars, the minimum amount of genetic heterozygosity between two field populations drops to 0.1255 between populations Mt. Ayr and Land Between Two Railroads. The amount of heterozygosity among the field populations is 0.2170 and is 0.1781 among the cultivars. The amount of heterozygosity within each population (Table 21) ranges from 0.0324 in the Alamo cultivar to 0.1622 in the Medora population. Without the cultivars, the minimum amount of heterozygosity within a population becomes 0.0704. in the Winnebago River Trail population.

When populations were grouped (Table 22) (cultivars excluded) the maximum G_{ST} was 0.3602 between groups Mt. Ayr, Land Between Two Railroads and Winnebago

River Trail; the minimum G_{ST} was 0.1987 between groups Medora, Flaherty and Smith, Stinson; and was 0.3614 when comparing all populations. The maximum amount of heterozygosity (H_T) was 0.2248 between the two grouped populations of Medora, Flaherty and Smith, Stinson; the minimum was 0.1568 between the two groups Winnebago River Trail and Wilkinson, Hoffman; and the amount of heterozygosity among all grouped populations was 0.2213. The amount of heterozygosity within each grouped population (Table 23) ranges from 0.1318 in the Winnebago River Trail population to 0.1780 in the Medora, Flaherty population. When the populations were grouped further (Table 24) and northern and southern Iowa prairies were compared the G_{ST} was 0.1072 and H_T was 0.2406. The amount of heterozygosity existing in each zone (Table 25) was 0.1651 in the northern prairies and 0.1874 in the southern prairies.

Table 22. *P. virgatum* field populations grouped with their closest genetic relative: G_{ST} (upper triangle) and H_T (lower triangle). Nei's (1973) G-statistics all for pair-wise comparisons between grouped field populations of *P. virgatum*. Field populations are designated in the same manner as Table 16. Populations that were grouped were the most similar genetically (as determined by PAUP analysis of Nei's genetic distance (1972)) and are separated with a comma.

	Med,Fla	MtA,2RR	St,Sm	WRT	Wik,Hof
Med,Fla		0.2324	0.1987	0.3455	0.2408
MtA,2RR	0.2133		0.2134	0.3602	0.2464
St,Sm	0.2248	0.1847		0.2968	0.2092
WRT	0.2098	0.1595	0.1685		0.3059
Wik,Hof	0.2245	0.1800	0.1923	0.1568	

Table 23. Genetic heterozygosity (H_T) within grouped field populations for *P. virgatum*. The amount of genetic heterozygosity found within each specific grouped population of *P. virgatum*. Grouped field populations are labeled as the names of the prairies or areas that were grouped together.

Grouped Population	H_T
Medora,Flaherty	0.1780
Mount Ayr, Land Between Two Railroads	0.1221
Smith, Stinson	0.1453
Winnebago River Trail	0.0704
Wilkinson, Hoffman	0.1318

Table 24. *P. virgatum* field north versus south relationships: G_{ST} (upper triangle) and H_T (lower triangle). Nei's (1973) G-statistics for the comparison between northern Iowa (Smith, Stinson, Winnebago River Trail, Hoffman, and Wilkinson) grouped field populations and southern Iowa (Medora, Flaherty, Land Between Two Railroads, and Mt. Ayr) grouped field populations of *P. virgatum*.

	North	South
North		0.1072
South	0.2406	

Table 25. Genetic heterozygosity (H_T) within northern and southern prairies for *P. virgatum*. The amount of genetic heterozygosity found within the prairie that compose the Northern Iowa (Smith, Stinson, Winnebago River Trail, Hoffman, and Wilkinson) and Southern Iowa (Medora, Flaherty, Land Between 2 Railroads, and Mt. Ayr) field collection sites of *P. virgatum*.

Area	H_T
North	0.1651
South	0.1874

Unrooted phenograms were constructed from Nei's genetic distances (1972) to visualize how populations or individual plants are related to each other. Unrooted phenograms for *P. virgatum* were constructed for populations (Figures 13, 15, and 18), grouped populations (Figure 14), and individual plants (Figure 16, 16.1, and 16.2). The

degree of genetic distance is expressed by sum of branch lengths and thus the further apart the two populations or individuals are, the fewer allelic bands they have in common.

Mantel tests were performed to see if any correlation existed for populations (cultivars excluded) between either the G_{ST}'s or genetic distances and their geographical distances. The r-value between the genetic distance and geographical distance for *P. virgatum* field samples was 0.19 with the p-value of 0.178 (Hope, 1968). A T-test was calculated on the two matrices, which produced a probability of 0.143. The r-value between the G_{ST} and geographical distance for *P. virgatum* field samples was 0.296 with the p-value of 0.033 (Hope, 1968). A T-test was also done on the same matrices and produced a probability of 0.044.

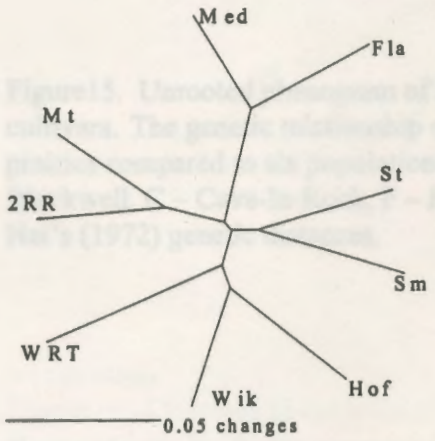

Figure 13. Unrooted phenogram of *P. virgatum* field populations. The genetic relationship of *P. virgatum* populations collected from remnant prairie based on Nei's (1972) genetic distances.

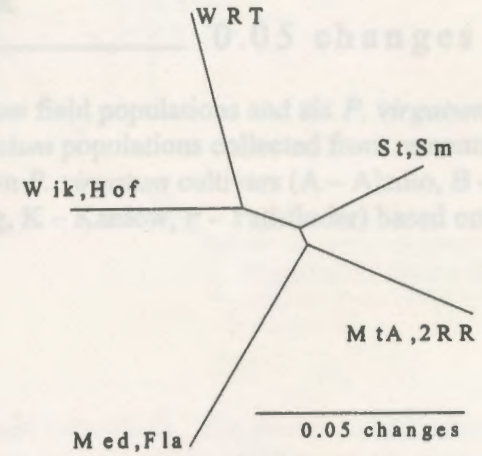

Figure 14. Unrooted phenogram of grouped *P. virgatum* grouped field populations. The genetic relationship of grouped *P. virgatum* populations collected from remnant prairies based on Nei's (1972) genetic distances.

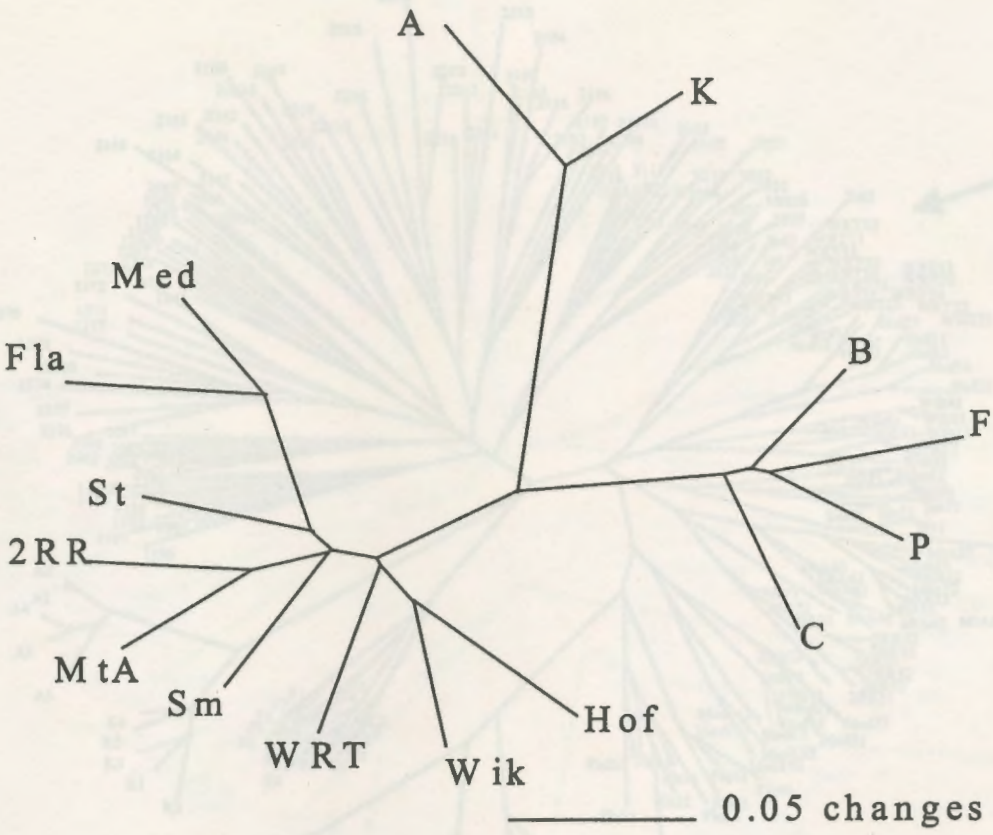

Figure15. Unrooted phenogram of *P. virgatum* field populations and six *P. virgatum* cultivars. The genetic relationship of *P. virgatum* populations collected from remnant prairies compared to six populations of known *P. virgatum* cultivars (A – Alamo, B – Blackwell, C – Cave-In-Rock, F – Forestburg, K – Kanlow, P – Pathfinder) based on Nei's (1972) genetic distances.

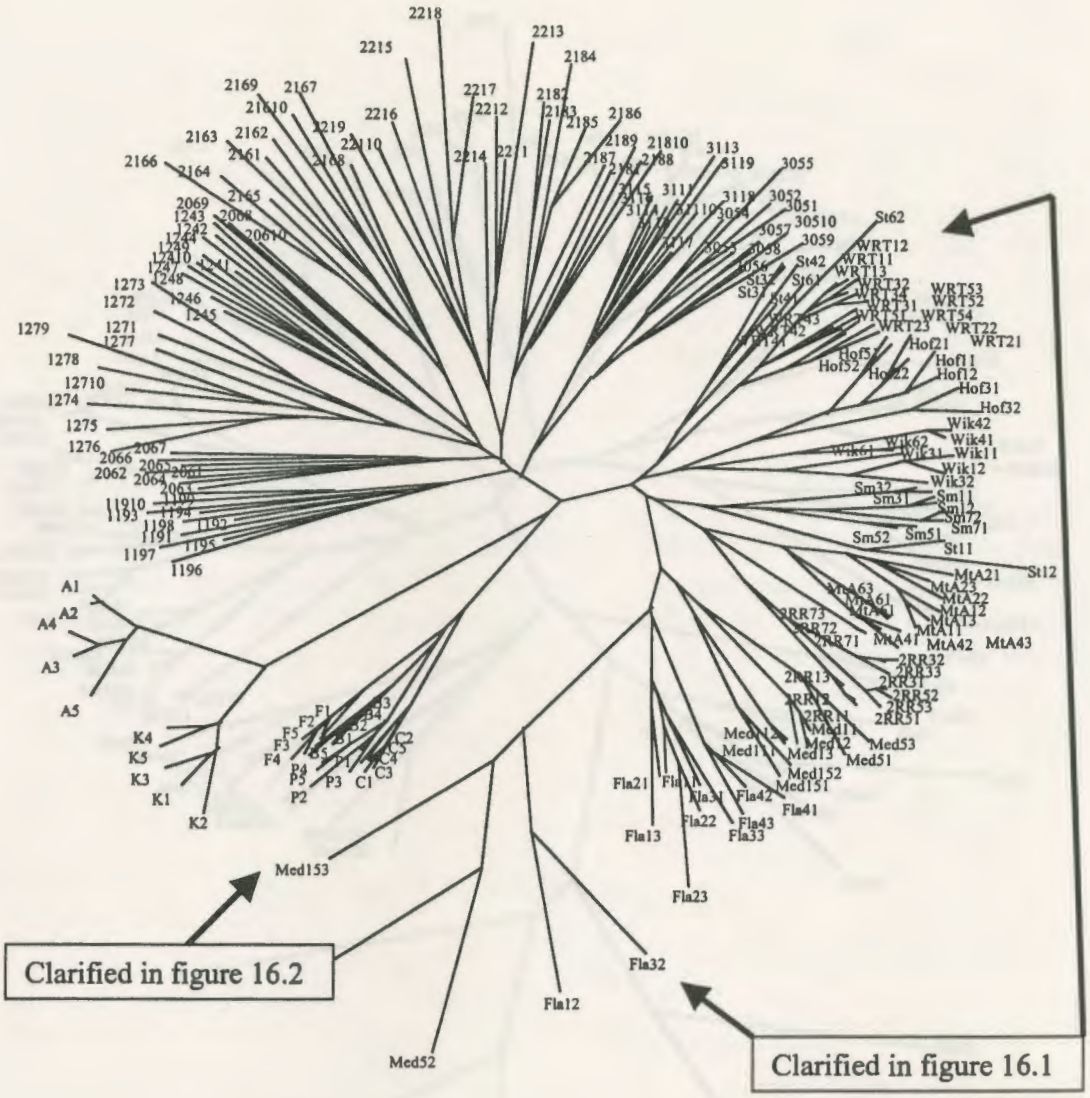

Figure 16. Unrooted phenogram of *P. virgatum* individuals. The genetic relationship of *P. virgatum* individuals compared to each other based on Nei's (1972) genetic distances. Plot populations are distinguished by a four or five digit number. The first three digits refer to the population while the last one or two numbers refer to the specific individual. Field populations are distinguised by a prairie abbreviation and then a two or three digit number. The first one or two digits refer to a specific clump of *P. virgatum* within the prairie, while the last digit refer to an individual plant collected from a specific clump. Cultivars are distinguished by a letter abbreviation for their name followed by a number depicting the individual plant.

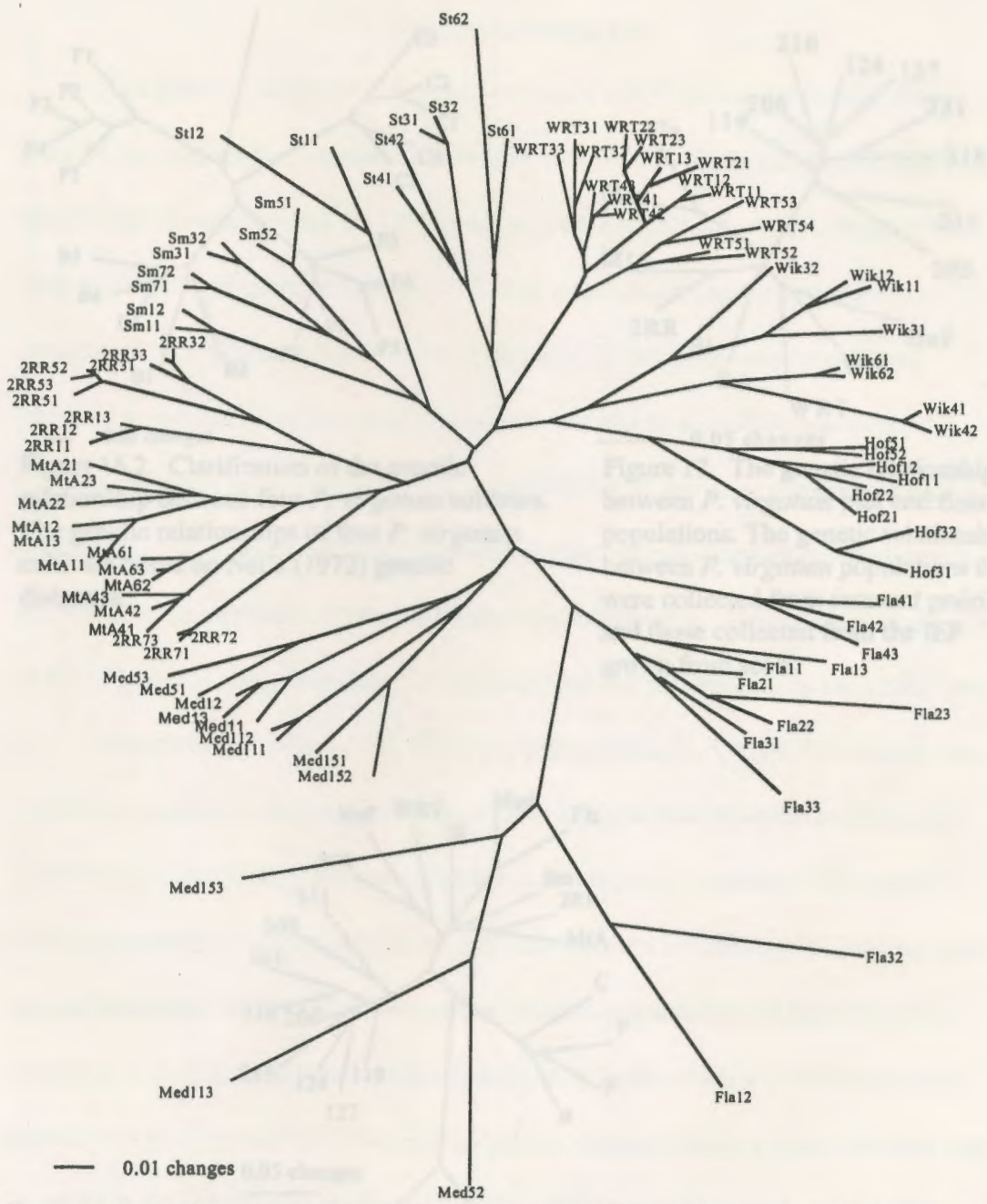

Figure 16.1. Clarification of the genetic relationship between *P. virgatum* field samples. The genetic relationships of *P. virgatum* individuals collected from remnant prairies based on Nei's (1972) genetic distances. Field populations are distinguished by a prairie abbreviation and then a two or three digit number. The first one or two digits refer to a specific clump of *P. virgatum* within the prairie, while the last digit refer to an individual plant collected from a specific clump.

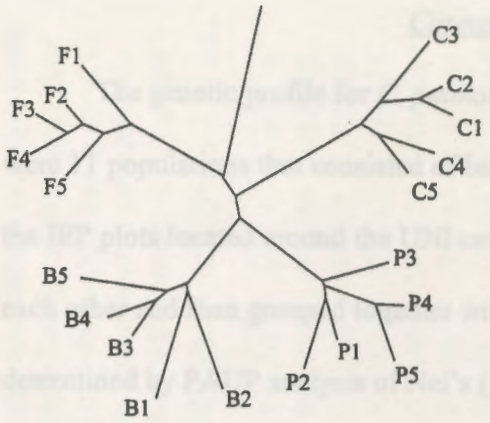

Figure 16.2. Clarification of the genetic relationship between four *P. virgatum* cultivars. The genetic relationships of four *P. virgatum* cultivars based on Nei's (1972) genetic distances.

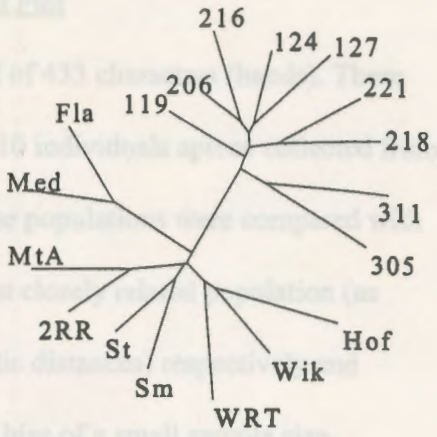

Figure 17. The genetic relationship between *P. virgatum* plot and field populations. The genetic relationship between *P. virgatum* populations that were collected from remnant prairies and those collected from the IEP grown from seed.

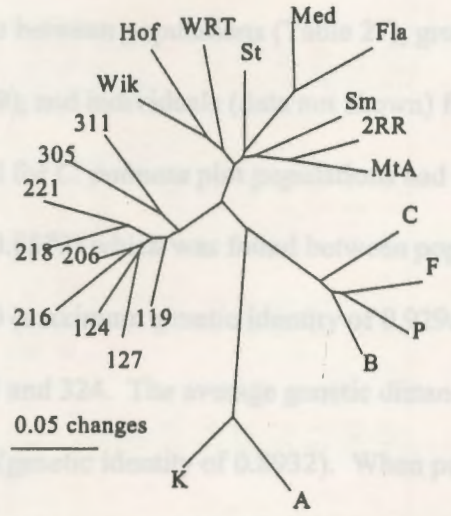

Figure 18. Unrooted phenogram of all *P. virgatum* populations. The genetic relationships of all populations of *P. virgatum* examined in this study. It should be noted that comparisons between field populations and plot populations should be simplistic in nature because they were collected at a different time and place than the plot populations.

Coreopsis palmata Plot

The genetic profile for *C. palmata* consisted of 433 characters (bands). There were 11 populations that consisted of between 8 to 10 individuals apiece collected from the IEP plots located around the UNI campus. These populations were compared with each other and then grouped together with their most closely related population (as determined by PAUP analysis of Nei's (1972) genetic distances) respectively and analyzed as groups. This was done to eliminate the bias of a small sample size.

Chi-square tests were performed on all loci for all pair-wise combinations of populations. Chi-squares were then summed (Table 26) and tested against the summed degrees of freedom (Table 26) for each pair-wise combination. Chi-square analysis of all of the *C. palmata* plot populations and cultivars showed probabilities of essentially zero.

Genetic distances (D) (Nei, 1972) and genetic identity (I) (Nei, 1972) were then calculated pair-wise between populations (Table 27), grouped populations (Table 28), IEP zones (Table 29), and individuals (data not shown) for *C. palmata*. The genetic distances calculated for *C. palmata* plot populations had a maximum of 0.1541 (minimal genetic identity of 0.8572) which was found between populations 324 and 208 and a minimum of 0.0730 (maximum genetic identity of 0.9296) which was found between populations the 335 and 324. The average genetic distance between all populations was found to be 0.1132 (genetic identity of 0.8932). When populations were grouped the maximum genetic distance was 0.1185 (minimum genetic identity of 0.8882) between groups 132,242 and 320,230; the minimum genetic distance was 0.0581(maximum

Table 26. *C. palmata* plot populations: Summed chi-squares of polymorphic loci (upper triangle) and number of polymorphic loci (lower triangle). The summed chi-squares (located in the upper triangle) and the polymorhpic loci (located in the bottom triangle) for all pair-wise combinations between plot populations of *C. palmata*. The number of polymorphic loci is equal to the summed degrees of freedom used to determine chi-square contingency. This is because chi-square is only calculated on polymorphic loci, each having one degree of freedom. Plot populations are designated by a three digit number with the first number referring to the IEP Zone it was collected in and the last two numbers referring to it's accession number.

	104	131	132	140	208	230	236	242	320	324	335
104		726.68	831.46	701.30	978.02	863.33	810.16	857.69	871.89	856.32	818.16
131	150		668.80	712.64	776.24	903.86	755.20	669.59	996.25	852.49	832.86
132	155	156		609.78	764.40	1024.81	738.57	648.49	1107.98	992.95	873.27
140	142	149	145		697.49	865.57	739.72	820.10	913.52	829.03	730.71
208	170	169	167	154		1063.27	754.57	818.71	1154.46	1151.28	1006.38
230	140	154	152	139	163		913.01	1072.60	713.25	1708.63	839.45
236	162	167	164	162	172	159		660.36	981.38	842.55	783.64
242	151	150	148	149	165	152	154		1146.67	996.32	867.44
320	135	153	155	138	159	112	154	145		700.83	771.65
324	137	150	152	139	164	125	155	142	117		650.43
335	141	148	146	134	162	128	152	140	119	117	

genetic identity of 0.9435) between groups 236,131,208 and 132,242; and the average was 0.0781 (genetic identity 0.9250). When the populations were grouped further into IEP zones the maximum genetic distance was 0.0614 (minimum genetic identity 0.9404) between zones 1 and 3, the minimum was 0.0424 (maximum genetic identity 0.9585) between zones 1 and 2, and the average was 0.0544 (genetic identity =0.9471).

The maximum G_{ST} value for *C. palmata* plot analysis (Table 30) was found to be 0.4899 between populations 242 and 230, while the minimum G_{ST} value was found to be 0.2578 between populations 140 and 132. The overall G_{ST} for all populations was found to be 0.5155, which was calculated from the average H_T and D_{ST} (calculated across all loci for all populations). The maximum amount of heterozygosity (H_T) (Table 30) that existed between two populations was 0.1572 between populations 104 and 208. The minimum amount of genetic heterozygosity (H_T) was 0.0931 between 320 and 324. The amount of genetic heterozygosity (H_T) was found to be 0.1727 among all populations. Within specific populations the amount of genetic heterozygosity (Table 31) ranged from 0.0531 in population 324 to 0.1068 in population 236.

When populations were grouped (Table 32) the maximum G_{ST} was 0.2978 between groups 320,230 and 132,242; the minimum G_{ST} was 0.1315 between groups 132,142 and 236,131,208; and the average was 0.2884. The maximum amount of heterozygosity (H_T) (Table 32) was 0.2095 between the two grouped populations of 320,230 and 324,335; the minimum of 0.1452 was among grouped population of 104,140 and 324,335; and the amount of heterozygosity among all grouped populations was 0.1822. Variation within each grouped population (Table 33) varied from 0.0915 in 242,

Table 27. *C. palmata* plot populations: Genetic Distances (lower triangle) and Genetic Identity (upper triangle). Nei's (1972) genetic distance and Nei's (1972) genetic identity numbers for all pair-wise comparisons between plot populations of *C. palmata*. Plot populations are designated in the same manner as Table 26.

	104	131	132	140	208	230	236	242	320	324	335
104		0.9002	0.8919	0.9108	0.8614	0.8818	0.8880	0.8815	0.8975	0.8966	0.9024
131	0.1052		0.9148	0.9071	0.8918	0.8758	0.8942	0.9080	0.8826	0.8985	0.8987
132	0.1144	0.0891		0.9276	0.8997	0.8630	0.9024	0.9161	0.8735	0.8859	0.8987
140	0.0934	0.0975	0.0752		0.9091	0.8871	0.9056	0.8925	0.8973	0.9071	0.9170
208	0.1492	0.1145	0.1057	0.0953		0.8495	0.8938	0.8847	0.8592	0.8572	0.8768
230	0.1258	0.1326	0.1473	0.1198	0.1631		0.8742	0.8503	0.9157	0.8997	0.8960
236	0.1187	0.1118	0.1027	0.0992	0.1123	0.1345		0.9088	0.8836	0.9015	0.9081
242	0.1261	0.0965	0.0876	0.1137	0.1225	0.1621	0.0956		0.8579	0.8764	0.8939
320	0.1081	0.1248	0.1353	0.1084	0.1518	0.0881	0.1238	0.1533		0.9259	0.9170
324	0.1092	0.1070	0.1211	0.0975	0.1541	0.1057	0.1038	0.1319	0.0770		0.9296
335	0.1027	0.1068	0.1068	0.0866	0.1315	0.1099	0.0964	0.1121	0.0866	0.0730	

Table 28. *C. palmata* plot populations grouped with their closest genetic relative: Genetic Distances (lower triangle) and Genetic Identity (upper triangle). Nei's (1972) genetic distance and Nei's (1973) G_{ST} numbers for all pair-wise comparisons between grouped plot populations of *C. palmata*. Plot populations are designated in the same manner as Table 26. Populations that were grouped were the most similar genetically (as determined by PAUP analysis of Nei's genetic distance (1972)) and are separated with a comma.

	104,140	236,131,208	132,242	320,230	324,335
104,140		0.9401	0.9301	0.9179	0.9344
236,131,208	0.0618		0.9435	0.9118	0.9293
132,242	0.0724	0.0581		0.8882	0.9140
320,230	0.0856	0.0923	0.1185		0.9406
324,335	0.0678	0.0733	0.0899	0.0612	

Table 29. *C. palmata* plot zone relationships: Genetic Distances (lower triangle) and Genetic Identity (upper triangle). Nei's (1972) genetic distance and Nei's (1972) genetic identity numbers for all pair-wise comparisons between all *C. palmata* populations in each of the IEP zones. Plot populations are designated by a three digit number with the first number referring to the IEP zone it was originally collected in, thus the group designation of hundreds.

	100	200	300
100		0.9585	0.9404
200	0.0424		0.9424
300	0.0614	0.0593	

132 to 0.1544 in 104, 140. When the populations were grouped further into IEP plot zones (Table 34) the maximum G_{ST} was 0.1512 between zones 1 and 3, the minimum was 0.0869 between zones 1and 2, and was 0.1593 among all zones. The maximum amount of heterozygosity (H_T) (Table 34) was 0.1961 between zones 1 and 2, the minimum amount of 0.1699 was found between zones 1 and 3, and the heterozygosity among all zones was found to be 0.1883. The amount of heterozygosity within each grouped population (Table 35) ranges from 0.1015 in zone 3 to 0.1411 in zone 1.

Table 30. *C. palmata* plot populations: G_{ST} (upper triangle) and H_T (lower triangle). Nei's (1973) G-statistics for all pair-wise comparisons between plot populations of *C. palmata*. Plot populations are designated in the same manner as Table 26.

	104	131	132	140	208	230	236	242	320	324	335
104		0.3329	0.3533	0.3242	0.3994	0.4385	0.3482	0.3966	0.4134	0.4033	0.3723
131	0.1364		0.2763	0.3060	0.3163	0.4150	0.3103	0.3099	0.4098	0.3647	0.3493
132	0.1393	0.1387		0.2578	0.3021	0.4418	0.2954	0.2926	0.4310	0.3949	0.3517
140	0.1258	0.1373	0.1272		0.2933	0.4119	0.3002	0.3606	0.3981	0.3630	0.3221
208	0.1572	0.1532	0.1488	0.1397		0.4509	0.3035	0.3509	0.4428	0.4356	0.3848
230	0.1253	0.1378	0.1429	0.1270	0.1531		0.4057	0.4899	0.4087	0.4368	0.4253
236	0.1456	0.1525	0.1480	0.1418	0.1560	0.1423		0.2984	0.3952	0.3464	0.3171
242	0.1370	0.1347	0.1302	0.1360	0.1485	0.1418	0.1381		0.4865	0.4395	0.3854
320	0.1156	0.1323	0.1357	0.1199	0.1464	0.0972	0.1357	0.1359		0.3748	0.3811
324	0.1192	0.1281	0.1330	0.1184	0.1503	0.1079	0.1305	0.1304	0.0931		0.3311
335	0.1212	0.1327	0.1318	0.1185	0.1459	0.1143	0.1322	0.1269	0.1020	0.0993	

Table 31. Genetic heterozygosity (H_T) within IEP plot populations for *C. palmata.* The amount of genetic heterozygosity found within each specific population of *C. palmata.* Plot populations are designated in the same manner as Table 21.

Population	H_T
104	0.0821
131	0.0987
132	0.0996
140	0.0862
208	0.1040
230	0.0615
236	0.1068
242	0.0793
320	0.0531
324	0.0597
335	0.0719

Table 32. *C. palmata* plot populations grouped with their closest genetic relative: G_{ST} (upper triangle) and H_T (lower triangle). Nei's (1973) G-statistics for all pair-wise comparisons between grouped plot populations of *C. palmata.* Plot populations are designated in the same manner as Table 26. Populations that were grouped were the most similar genetically (as determined by PAUP analysis of Nei's genetic distance (1972)) and are separated with a comma.

	104,140	236,131,208	132,242	320,230	324,335
104,140		0.1399	0.1868	0.2406	0.1999
236,131,208	0.1819		0.1315	0.2137	0.1776
132,242	0.1623	0.1821		0.2978	0.2437
320,230	0.1512	0.1793	0.1660		0.2095
324,335	0.1452	0.1731	0.1559	0.2095	

Table 33. Genetic heterozygosity (H_T) within IEP plot grouped populations for *C. palmata*. The amount of genetic heterozygosity found within each specific grouped population of *C. palmata*. Plot populations are designated in the same manner as Table 26. Populations that were grouped were the most similar genetically (as determined by PAUP analysis of Nei's genetic distance (1972)) and are separated with a comma.

Population	H_T
104,140	0.1544
236,131,208	0.1233
132,142	0.0915
320,230	0.1254
324,335	0.0930

Table 34. *C. palmata* plot zone relationships: G_{ST} (upper triangle) and H_T (lower triangle). Nei's (1973) G-statistics all pair-wise comparisons between all *C. palmata* populations in each of the IEP zones. Plot zones are designated in the same manner as Table 29.

	100	200	300
100		0.0869	0.1512
200	0.1961		0.1404
300	0.1699	0.1764	

Table 35. Genetic heterozygosity (H_T) within IEP plot zones for *C. palmata*. The amount of genetic heterozygosity found within each specific IEP zone for *C. palmata*. Plot zones are designated in the same manner as Table 29.

IEP Zone	H_T
100	0.1411
200	0.1638
300	0.1015

Unrooted phenograms were constructed from Nei's genetic distances (1972) to visualize how populations or individual plants are related to each other. Unrooted phenograms for *C. palmata* were constructed for populations (Figures 19 and 24),

grouped populations (Figure 20), and individual plants (Figure 23). The degree of genetic distance is expressed by branch length and thus the further the two populations or individuals are, the more allelic bands they do not have in common.

Mantel tests were performed to see if any correlation existed for populations between either the G_{ST}'s or genetic distances and their geographical distances. The r-value between the genetic distance and geographical distance for *C. palmata* plot samples was 0.097 with a p-value of 0.248 (Hope, 1968) being. A T-test was also done on the two matrices, which produced a probability of 0.232. The r-value between the G_{ST} and geographical distance for *C. palmata* plot samples was 0.037 with the p-value of that assessed by Hope (1968) being 0.404. A T-test was also done on the same matrices and produced a probability of 0.392.

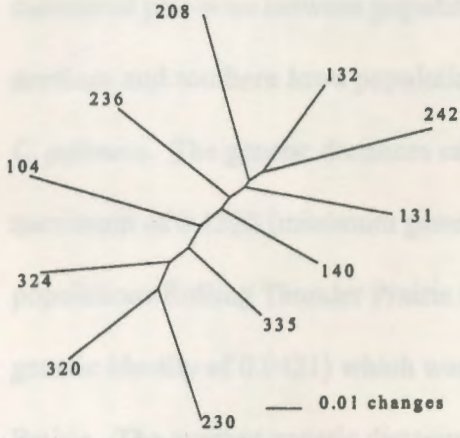

Figure 19. Unrooted phenogram of *C. palmata* plot populations. The genetic relationships of *C. palmata* populations collected from the IEP plots based on Nei's (1972) genetic distances.

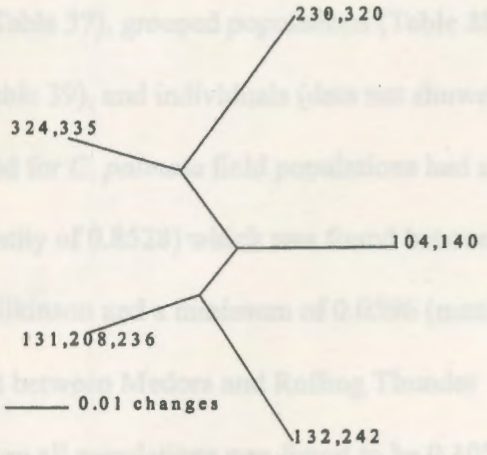

Figure 20. Unrooted phenogram of *C. palmata* grouped plot populations. The genetic relationships of grouped *C. palmata* populations collected from the IEP plots based on Nei's (1972) genetic distances.

Coreopsis palmata Field

The genetic profile for *C. palmata* consisted of 433 characters (bands). There were 11 populations consisting of between 6 to 12 individuals apiece. These populations were compared with each other and then grouped together with their most closely related population (as determined by PAUP analysis of Nei's (1972) genetic distances) respectively and analyzed as groups. This was done to eliminate the bias of small sample size.

Chi-square tests were performed on all loci for all pair-wise combinations of populations. Chi-squares were then summed (Table 36) and tested against the summed degrees of freedom (Table 36) for each pair-wise combination. Chi-square analysis of all of the *C. palmata* field populations and cultivars showed probabilities of essentially zero.

Genetic distances (D) (Nei, 1972) and genetic identity (I) (Nei, 1972) were then calculated pair-wise between populations (Table 37), grouped populations (Table 38), northern and southern Iowa populations (Table 39), and individuals (data not shown) for *C. palmata*. The genetic distances calculated for *C. palmata* field populations had a maximum of 0.1593 (minimum genetic identity of 0.8528) which was found between populations Rolling Thunder Prairie and Wilkinson and a minimum of 0.0596 (maximum genetic identity of 0.9421) which was found between Medora and Rolling Thunder Prairie. The average genetic distance between all populations was found to be 0.1057 (genetic identity of 0.9000). When populations were grouped the maximum genetic distance was 0.1012 (minimum genetic identity of 0.9038) between groups Rolling

Table 36. *C. palmata* field populations: Summed chi-squares of polymorphic loci (upper triangle) and number of polymorphic loci (lower triangle). The summed chi-squares (located in the upper triangle) and the polymorhpic loci (located in the bottom triangle) for all pair-wise combinations between plot populations of *C. palmata*. The number of polymorphic loci is equal to the summed degrees of freedom used to determine chi-square contingency. This is because chi-square is only calculated on polymorphic loci, each having one degree of freedom. Field populations are designated by an abbreviation of the prairie it was collected from (Med – Medora, RTP – Rolling Thunder Prairie, Fla – Flaherty, 2RR – Land Between Two Railroads, SC – Sand Creek, Sm – Smith, St – Stinson, Hof – Hoffman, Wik – Wilkinson).

	Med	RTP	Fla	2RR	SC	Sm	St	Hof	Wik
Med		316.42	501.44	589.02	833.76	690.88	840.73	713.82	959.00
RTP	48		546.27	730.89	838.95	811.68	958.65	756.34	1089.36
Fla	67	71		833.30	1071.01	1201.08	1423.11	1128.61	1486.23
2RR	86	92	94		836.25	1161.96	1250.15	917.63	1298.55
SC	113	113	117	118		1163.15	1272.08	1138.75	1493.66
Sm	92	99	111	119	135		758.38	919.92	1080.42
St	107	113	126	129	145	114		913.12	879.24
Hof	99	104	116	151	139	117	123		718.19
Wik	105	112	121	126	145	117	114	110	

Table 37. *C. palmata* field populations: Genetic Distances (lower triangle) and Genetic Identity (upper triangle). Nei's (1972) genetic distance and Nei's (1972) genetic identity numbers for all pair-wise comparisons between field populations of *C. palmata*. Field populations are designated in the same manner as Table 36.

	Med	RTP	Fla	2RR	SC	Sm	St	Hof	Wik
Med		0.9421	0.9340	0.9235	0.8885	0.9086	0.8881	0.9049	0.8704
RTP	0.0596		0.9294	0.9045	0.8875	0.8929	0.8721	0.9006	0.8528
Fla	0.0683	0.0732		0.9247	0.9031	0.8891	0.8674	0.8973	0.8588
2RR	0.0796	0.1004	0.0783		0.9266	0.8927	0.8850	0.9196	0.8787
SC	0.1182	0.1193	0.1019	0.0762		0.8942	0.8834	0.8971	0.8589
Sm	0.0959	0.1132	0.1176	0.1135	0.1118		0.9336	0.9172	0.8990
St	0.1187	0.1368	0.1423	0.1222	0.1240	0.0687		0.9191	0.9191
Hof	0.1000	0.1046	0.1084	0.0838	0.1086	0.0864	0.0844		0.9363
Wik	0.1388	0.1593	0.1522	0.1294	0.1521	0.1064	0.0844	0.0659	

Table 38. *C. palmata* field populations grouped with their closest genetic relative: Genetic Distances (lower triangle) and Genetic Identity (upper triangle). Nei's (1972) genetic distance and Nei's (1972) genetic identity numbers for all pair-wise comparisons between field grouped populations of *C. palmata*. Field populations are designated in the same manner as Table 36. Populations that were grouped were the most similar genetically (as determined by PAUP analysis of Nei's genetic distance (1972)) and are separated with a comma.

	RTP,Fla,Med	2RR,SC	Sm,St	Hof,Wik
RTP,Fla,Med		0.9375	0.9061	0.9038
2RR,SC	0.0646		0.9113	0.9106
Sm,St	0.0986	0.0929		0.9426
Hof,Wik	0.1012	0.0937	0.0591	

Table 39. *C. palmata* field north versus south relationships: Genetic Distances (lower triangle) and Genetic Identity (upper triangle). Nei's (1972) genetic distance and Nei's (1972) genetic identity numbers for the comparison between northern Iowa (Smith, Stinson, Hoffman, and Wilkinson) grouped populations and southern Iowa (Medora, Rolling Thunder Prairie, Flaherty, Land Between Two Railroads, and Sand Creek) grouped field populations of *C. palmata.*

	North	South
North		0.9264
South	0.0764	

Thunder Prairie, Flaherty, Medora and Hoffman, Wilkinson; the minimum genetic distance was 0.0591 (maximum genetic identity of 0.9426) between groups Smith, Stinson and Hoffman, Wilkinson; and the average was 0.0850 (genetic identity of 0.9187). When the populations were grouped further into Northern and Southern Iowa populations, the genetic distance was 0.0748 (genetic identity of 0.9264).

The maximum G_{ST} value for *C. palmata* field analysis (Table 40) was 0.6344 between populations Wilkinson and Rolling Thunder Prairie, while the minimum G_{ST} value was found to be 0.3176 between populations Stinson and Smith. The overall average G_{ST} was 0.6202, which was calculated from the average H_T and D_{ST} (calculated across all loci for all populations). The maximum amount of heterozygosity (H_T) that existed within two populations was 0.1189 between populations Flaherty and Wilkinson. The minimum amount of genetic heterozygosity (H_T) (Table 41) was 0.0461 between populations Medora and Rolling Thunder Prairie. The amount of genetic heterozygosity among all populations was 0.1386. The amount of heterozygosity that existed within any one population ranged from 0.0201 in Rolling Thunder Prairie to 0.0838 in Sand Creek.

Table 40. *C. palmata* field populations: G_{ST} (upper triangle) and H_T (lower triangle). Nei's (1973) G-statistics for all pair-wise comparisons between field populations of *C. palmata*. Field populations are designated in the same manner as Table 36.

	Med	RTP	Fla	2RR	SC	Sm	St	Hof	Wik
Med		0.6171	0.5265	0.4918	0.5115	0.5255	0.5448	0.4940	0.6028
RTP	0.0461		0.5457	0.5488	0.5151	0.5663	0.5790	0.5063	0.6344
Fla	0.0609	0.0629		0.4221	0.4247	0.5097	0.5293	0.4571	0.5632
2RR	0.0750	0.0839	0.0849		0.3246	0.4569	0.4526	0.3595	0.4819
SC	0.1039	0.1042	0.1073	0.1051		0.4033	0.4092	0.3734	0.4694
Sm	0.0838	0.0910	0.1033	0.1104	0.1217		0.3176	0.3603	0.4294
St	0.0984	0.1058	0.1184	0.1188	0.1314	0.0976		0.3376	0.3571
Hof	0.0922	0.0940	0.1061	0.1044	0.1269	0.1071	0.1110		0.2983
Wik	0.1033	0.1115	0.1189	0.1182	0.1393	0.1102	0.1056	0.0999	

Table 41. Genetic heterozygosity (H_T) within field populations for *C. palmata*. The amount of genetic heterozygosity found within each specific population of C. palmata. Field populations are designated by the name of the prairie they were gathered from.

Population	H_T
Medora	0.0221
Rolling Thunder Prairie	0.0201
Flaherty	0.0480
Land Between Two Railroads	0.0578
Sand Creek	0.0838
Smith	0.0655
Stinson	0.0720
Hoffman	0.0702
Wilkinson	0.0732

When populations were grouped (Table 42) the maximum G_{ST} was 0.2798 between groups Smith, Stinson and Rolling Thunder Prairie, Flaherty, Medora; the minimum G_{ST} was 0.2039 between groups Hoffman, Wilkinson and Smith, Stinson; and was 0.3268 when comparing all populations. The maximum amount of heterozygosity (H_T) was 0.2019 between grouped populations Hoffman, Wilkinson and Smith, Stinson; the minimum amount of heterozygosity was 0.1179 between grouped populations Land Between Two Railroads, Sand Creek and Rolling Thunder Prairie, Flaherty, Medora and the amount of heterozygosity among all grouped populations was 0.1509. The amount of heterozygosity within each grouped population (Table 43) ranges from 0.0699 in the Rolling Thunder Prairie, Medora, Flaherty population to 0.0987 in the Hoffman, Wilkinson population. When the populations were grouped further and northern and southern Iowa prairies were compared (Table 44) the G_{ST} was 0.1578 and H_T was 0.1621. The amount of heterozygosity existing in each zone (Table 45) was 0.1138 in the northern prairies and 0.1021 in the southern prairies.

Table 42. *C. palmata* field populations grouped with their closest genetic relative: G_{ST} (upper triangle) and H_T (lower triangle). Nei's (1973) G-statistics pair-wise comparisons between field grouped populations of *C. palmata*. Field populations are designated by an abbreviation of the prairie name they were collected from. Populations that were grouped were the most similar genetically (as determined by PAUP analysis of Nei's genetic distance (1972)) and are separated with a comma.

	RTP,Fla,Med	2RR,SC	Sm,St	Hof,Wik
RTP,Fla,Med		0.2254	0.2798	0.2609
2RR,SC	0.1179		0.2640	0.2355
Sm,St	0.1281	0.1466		0.2039
Hof,Wik	0.1284	0.1461	0.2019	

Table 43. Genetic heterozygosity (H_T) within grouped field populations for *C. palmata*. The amount of genetic heterozygosity found within each specific population of *C. palmata*. Field populations are designated by the name of the prairie they were gathered from and prairies in a group are separated by a comma.

Grouped Population	H_T
Rolling Thunder Prairie, Medora, Flaherty	0.0699
Land Between Two Railroads, Sand Creek	0.0951
Smith, Stinson	0.0913
Hoffman, Wilkinson	0.0987

Table 44. *C. palmata* field north versus south relationships: G_{ST} (upper triangle) and H_T (lower triangle). Nei's (1973) G-statistics for the comparison between northern Iowa (Smith, Stinson, Hoffman, and Wilkinson) grouped populations and southern Iowa (Medora, Rolling Thunder Prairie, Flaherty, Land Between Two railroads, and Sand Creek) grouped field populations of *C. palmata*.

	North	South
North		0.1578
South	0.1621	

Table 45. Genetic heterozygosity (H_T) within northern and southern field populations for *C. palmata*. The amount of genetic heterozygosity found within the prairie that compose the northern Iowa (Smith, Stinson,, Hoffman, and Wilkinson) and southern Iowa (Medora, Flaherty, Rolling Thunder Prairie, Land Between Two Railroads, and Mt. Ayr) field collection sites of *C. palmata*..

Area	H_T
North	0.1138
South	0.1021

Unrooted phenograms were constructed from Nei's genetic distances (1972) to visualize how populations or individual plants are related to each other. Unrooted phenograms for *P. virgatum* were constructed for populations (Figures 21 and 24), grouped populations (Figure 22), and individual plants (Figure 23, 23.1, and 23.2). The

degree of genetic distance is expressed by sum of branch lengths and thus the further apart the two populations or individuals are, the fewer allelic bands they have in common.

Mantel tests were performed to see if any correlation existed for populations between either the G_{ST}'s or genetic distances and their geographical distances. The r-value between the genetic distance and geographical distance for *C. palmata* field samples was 0.035 with the p-value of 0.442 (Hope, 1968) being. A T-test was calculated on the two matrices, which produced a probability of 0.411. The r-value between the G_{ST} and geographical distance for *C. palmata* plot samples was 0.211 with the p-value of 0.085 (Hope, 1968). A T-test was also done on the same matrices and produced a probability of 0.076.

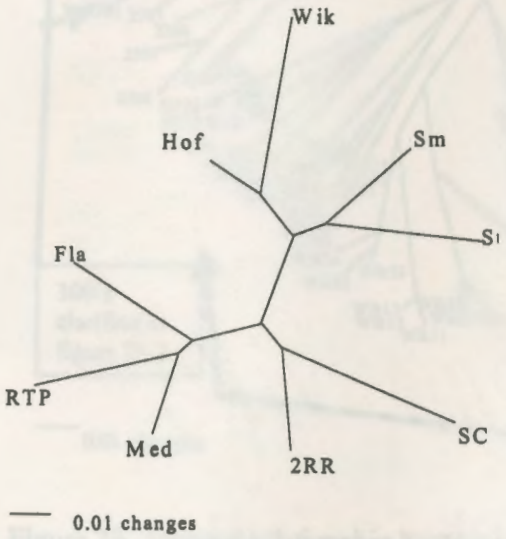

Figure 21. *C. palmata* field populations. The genetic relationship of *C. palmata* populations collected from remnant prairie based on Nei's (1972) genetic distances.

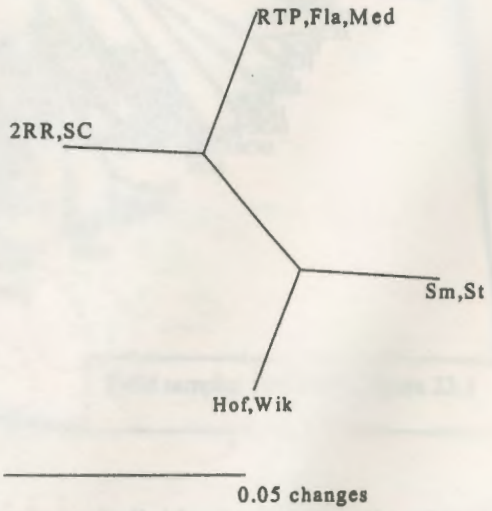

Figure 22. *C. palmata* grouped field populations. The genetic relationship of grouped *C. palmata* populations collected from remnant prairies based on Nei's (1972) genetic distances.

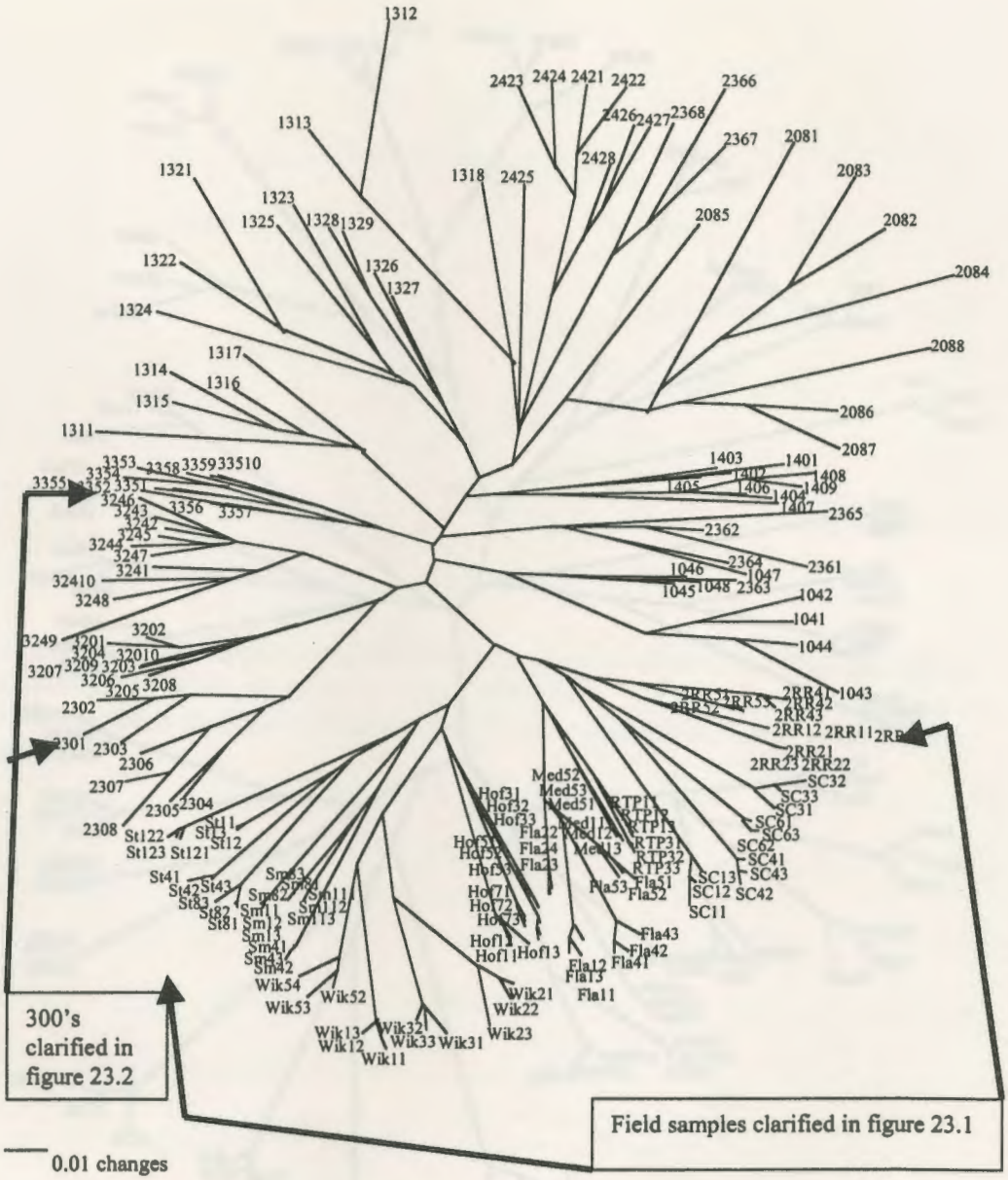

Figure 23. Genetic relationship between *C. palmata* individuals. The genetic relationship of *C. palmata* individuals compared to each other based on Nei's (1972) genetic distances. The first three digits refer to the population while the last one or two numbers refer to the specific individual. Field populations are distinguised by a prairie abbreviation and then a two or three digit number. The first one or two digits refer to a specific patch of *C. palmata* within the prairie, while the last digit refer to an individual plant collected from a specific patch.

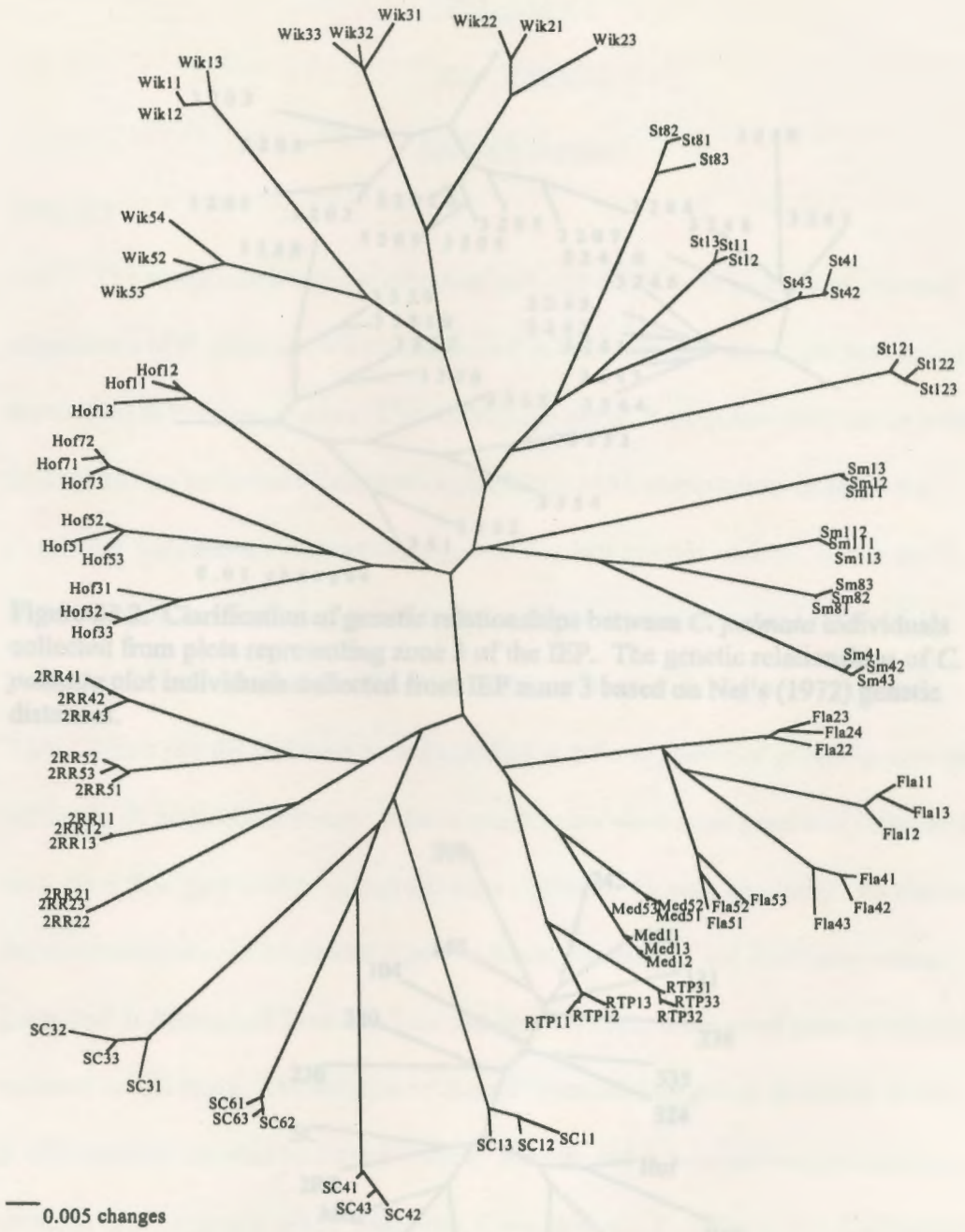

Figure 23.1. Clarification of the genetic relationship between *C. palmata* field samples. The genetic relationships of *C. palmata* individuals collected from remnant prairies based on Nei's (1972) genetic distances. The first one or two digits refer to a specific patch of *C. palmata* within the prairie, while the last digit refer to an individual plant collected from a specific patch.

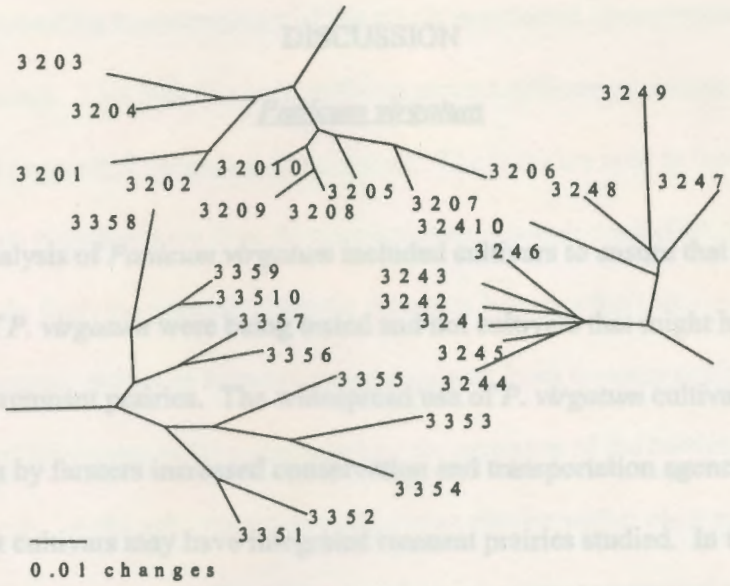

Figure 23.2. Clarification of genetic relationships between *C. palmata* individuals collected from plots representing zone 3 of the IEP. The genetic relationships of *C. palmata* plot individuals collected from IEP zone 3 based on Nei's (1972) genetic distances.

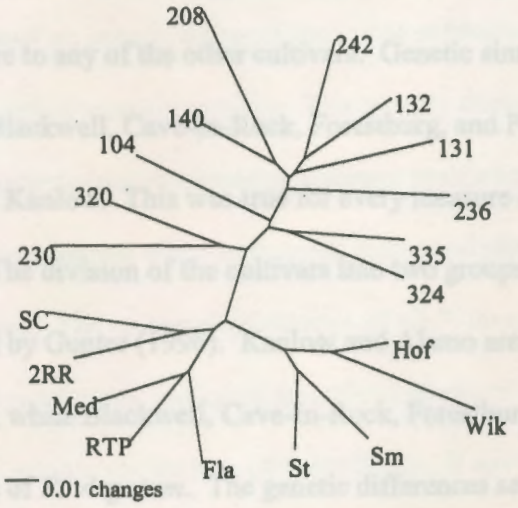

Figure 24. *C. palmata* field populations and plot populations. The genetic relationship between the *C. palmata* populations collected from remnant prairies and those collected from the IEP plots.

CHAPTER 4

DISCUSSION

Panicum virgatum

Cultivars

The analysis of *Panicum virgatum* included cultivars to ensure that remnant populations of *P. virgatum* were being tested and not cultivars that might have established themselves in remnant prairies. The widespread use of *P. virgatum* cultivars in prairie grass plantings by farmers increased conservation and transportation agencies the possibility that cultivars may have integrated remnant prairies studied. In this study, AFLP analysis greatly distinguished between remnant populations and cultivars, indicating that the remnant prairies studied did not contain *P. virgatum* cultivars.

When just the cultivars were examined, a definite pattern of grouping emerged within the *P. virgatum* cultivars. Alamo and Kanlow were more genetically similar to each other than they were to any of the other cultivars. Genetic similarity was likewise demonstrated between Blackwell, Cave-In-Rock, Forestburg, and Pathfinder when compared to Alamo and Kanlow. This was true for every measure of genetic variability assessed in this study. The division of the cultivars into two groups is similar to the RAPD analysis reported by Gunter (1996). Kanlow and Alamo are lowland ecotype varieties of *P. virgatum*, while Blackwell, Cave-In-Rock, Forestburg, and Pathfinder are upland ecotype varieties of *P. virgatum*. The genetic differences seen between these two groups are likely accentuated due to the difference in ploidy level, lowland varieties being tetraploid and highland varieties being octoploid.

When the cultivars were included in the data analysis, they tended to skew the overall analysis making measurements of the whole population appear more genetically diverse and distinct. This was due to significant genetic differences within the cultivars as compared to remnant *P. virgatum* populations. The cultivars tend to have fewer polymorphic loci between them than do remnant prairie populations (IEP plots or samples collected directly from the prairie). Genetic distances between the cultivars tended to be similar to the genetic distances between those collected from remnant prairie plants, with the exception of the commonalties displayed by the grouping of the cultivars into upland and lowland types. Although genetic distances were similar within each grouping, they were particularly high between the cultivars and populations representing remnant prairie. Since cultivars were obtained from a wide geographic range and were selected from a small sample size, it is likely that certain alleles were lost which would be evident as genetic differences distinguishing them from remnant populations. Examination of the unrooted phylograms based on genetic distance show that cultivars distinctly group away from populations representing remnant prairies. This genetic distinction makes it unlikely that any of the populations representing remnant prairie were composed of cultivars. This is further supported when examining individual plants and their genetic distances (data not shown). The unrooted phylogram of all *P. virgatum* individuals shows populational differentiations, indicated by individual plants grouped as populations. Due to the genetic similarities of the cultivars, if any single plant had been of cultivar seed origin it would have grouped with the cultivars examined.

Differentiation among cultivars and populations representing remnant prairies were further supported by the degree of genetic heterozygosity (H_T) and the genetic variation among remnant samples and cultivars (G_{ST}). The high G_{ST} of the cultivars (0.7955) and the lower genetic heterozygosity ($H_T = 0.1781$) (in comparison to remnant populations) indicates that these population have been significantly differentiated through selective cultivation and have likely lost alleles. The G_{ST} of the cultivars may be very high as a result of the absence of gene flow between cultivars after selection and development. When the cultivars are analyzed individually by ploidy level the G_{ST} between the upland varieties drops to 0.6573 and between the lowland varieties drops to 0.5751. While these G_{ST} values are considerably lower, they are still high in comparison to those values reported by Hamrick and Godt (1990) for a given plant species. The degree of genetic heterozygosity within each cultivar is very low, indicating high similarity among individuals. Since there are high G_{ST} values among cultivars, most of the genetic variation within a cultivar is unique to that cultivar. The specific propagation of cultivated varieties explains the differentiation. The selection of a cultivar form a small sample size (sometimes only one plant) and human manipulation of breeding that is prevalent in the production of cultivars to maintain desired traits eliminates gene flow between cultivars creating genetic drift and limits the degree of genetic variation. Cultivars were predominately propagated by division in initial selection and development (Fischer, 1996) which reduced the amount of genetic recombination that occurred and limited the amount of genetic variation among populations. The production of cultivar seed has likely increased the number of generations and offspring produced in

comparison to the native prairie populations of *P. virgatum,* which in turn accelerated genetic drift.

Iowa Ecotype Project Plot Populations and Native Prairie Populations

Panicum virgatum samples that were gathered from the Iowa Ecotype Project plots and directly in the field from remnant prairie exhibit a high degree of genetic variability as apparent by the number of polymorphic loci amplified (73% among all plot populations and 65.6% among all field populations). These values were high when compared to the average number of polymorphic loci in a plant species (Hamrick and Godt, 1990). The level of genetic heterozygosity among the plot populations (H_T = 0.2417) and field populations (H_T = 0.2170) were also high when compared to the average for a given plant species, as reported by Hamrick and Godt (1990). The high diversity is in part due to the underestimation of true diversity in isozyme studies, which Hamerick and Godt (1990) examined, that results from a limited number of sampled loci and the lack of a random sampling of the entire genome (Clegg, 1990). Individual populations also expressed a high degree of variation for the most part. The degree of variation within each population is likely a function of the population size, as demonstrated for populations of *Coreopsis integrifolia* (Cosner and Crawford, 1994) and *Sticherus flabellatus* (Keiper and McConchie, 2000)

The level of variation present is consistent with the life history of *P. virgatum*. Hamrick et al. (1979) found that the three life history variables of pollination mechanism, mating system, and fecundity had the highest correlation within the genetic variation of a given species. Wind pollination, outcrossing, high fecundity, and the plant being a

perennial were shown to generally provide or maintain the most genetic variation within a plant species. *Panicum virgatum* is self-infertile and wind pollinated (Moser and Vogel, 1995) which may in part explain the high degree of variation. While specific fecundity rates were unavailable, *P. virgatum* can grow in a wide variety of habitats (Fischer, 1996) which suggests the production of a large amount of seed and high variability within that seed.

Chi-square significance testing between pair-wise comparisons of populations tested for allele frequency homogeneity at each polymorphic locus and then were combined to compare populations. In all pair-wise comparisons (in each respective collection), the null hypothesis, having no allele frequencies difference, was rejected ($\alpha<0.05$). Therefore, allele frequencies among sampled populations were different enough to conclude that samples were taken from different gene pools and showed that all populations were genetically distinct from one another.

Although individuals did aggregate into populations, genetic distances and genetic identities did not indicate any extreme separation of populations, which would indicate a distinct genetic difference. The unrooted phylogram of the *P. virgatum* plot populations (Figure 10) shows the genetic distance relationships between the populations, from which some grouping of *P. virgatum* populations by IEP zones is apparent. However, inconsistencies in this separation and the lack of distinct separation make it unlikely that each zone represents a specific ecotype. For example, populations 206 and 216 from zone 2 are more closely related to populations 124 and 127 from zone one than to populations 221 and 218 from zone two. In addition, we see that populations do not

always have the smallest genetic distance with to those geographically closest to them. Populations 216 (Boone county) and 218 (Webster county) from zone two are geographically close to each other as are populations of 206 (BlackHawk county) and 221 (Buchanan county) from zone two, but the smaller genetic distances occur between populations 216 and 206 and between 218 and 221. This may be in part a result of habitat influence. Population 218 was collected from a wet prairie while population 206 was collected from a dry prairie. Population 216 was obtained from a wetter area and 221 was obtained from a mesic prairie, but has dryer areas. This pairing may be a result of due to wet and dry soil ecotype differences. Other explanations of grouping may be due to a number of factors (habitat, animal migration patters, landform similarity, etc.), which may have influenced seed dispersal and affected dispersal of ecotypes against plants during their establishment.

Unrooted phylograms of the field populations of *P. virgatum* show division of northern and southern populations, which is likely in part due to both distance and landform. East and west division was not apparent. Populations in northern Iowa indicate genetic relationships in accordance to geographic distance, however this relationship is not seen in southern Iowa populations. Medora prairie and Flaherty prairie were both fairly large, open and hilly as compared to the smaller isolated areas of Mount Ayr and Land Between Two Railroads. Remnant size and landform differences may account for some of the genetic similarities observed.

Population differentiation was further supported by the partitioning of genetic variation observed between populations of *P. virgatum*. When cultivars were excluded,

36.2% (G_{ST} = 0.3623) of the genetic variation existed among plot populations while 63.8% existed within populations. When cultivars were excluded from field sample analysis, 50.7% (G_{ST}= 0.5074) of the genetic variation existed among field populations while 49.3% of the variation existed within populations. The degree of genetic variation among *P. virgatum* plant populations was high compared to the average 22% for a given plant species found by Hamrick and Godt (1990). The higher degree of inter-population differentiation is likely explained because *P. virgatum* to reproduce via rhizomes as well as the geographic isolation of prairie remnants. These attributes were likely accentuated in established plants of the field collections whereas the plants of plot populations were planted from seed. In comparison to cultivars, native populations developed on native prairie with random selection, some sexual reproduction, and gene flow. These characteristic allowed more total diversity to exist among populations (H_T = 0.2417) while maintaining lower genetic differentiation (G_{ST} = 0.3623) as compared to cultivars.

Genetic structure within *P. virgatum* showed two different results obtained from the Mantel (1967) tests. Tests were run comparing both Nei's (1972) genetic distance and Nei's (1973) G_{ST} measure of genetic differentiation to geographical distance. In *P. virgatum* plot samples, both tests had positive correlation that were found to be significant ($\alpha < 0.05$) according to a T-test and Hope (1968). This indicates that the genetic structure for the *P. virgatum* plot collections is governed by isolation by distance. Mantel tests run on the same measures of genetic differentiation in field populations had positive correlations that were found not to be significant ($\alpha > 0.05$) for genetic distance and barely significant ($\alpha < 0.05$) for G_{ST} according to according to a T-test and

Hope's (1968) significance test. These results indicate that the genetic structure of *P. virgatum* may be a result of something other than distance. The discrepancies seen here may be a result of the different prairies or differences in sampling. The collection of multiple field samples from a given clump of *P. virgatum* increases the likelihood of collecting clones or very genetically similar plants as indicated by Figures 16 and 16.1. The individuals collected from the plots display distinctly more variation than those collected from a given clump of *P. virgatum*. This method of collection may have dramatically limited the variation sampled within each prairie, which may in turn limit the genetic structure analyzed.

When populations were grouped together, the amount of genetic differentiation was reduced within both the plot and field samples. The smaller genetic distance (and higher genetic identity respectively) can be accounted for by examining the alleles contained in a grouped "population." In AFLP studies, it is preferable to have 20 separate individuals per population (10 individuals is low on the accepted range and may be considered poor sampling). To reach the preferred number of 20, populations were grouped with their most similar genetic relative to reach this number. This grouping increased the number of alleles accounted for in each population and thus increased the number of shared alleles between populations, which in turn reduces the genetic distance (increases the genetic identity). The large decrease in genetic distance seen when populations were grouped may suggest that populations were not accurately sampled and more individuals may need to be tested per population to generate accurate results. However, this may also simply be a function of combining genetically separate

populations. Populations were grouped further into IEP zones (plot) and northern and southern populations (field) to determine how genetically distinct each was. The drop in genetic distance seen in the grouping into IEP zones was not as great, indicating that several alleles were already shared within the first grouping of populations. Measures of genetic differentiation among populations follow a similar pattern, as do genetic distances for both collections of *P. virgatum* studied. Grouping populations produces lower G_{ST} values, which tells us that grouped populations have more varying alleles in common. The genetic variation among groups increases slightly with each successive grouping. The measure of genetic heterozygosity (H_T) varies because grouping populations changed the allelic frequencies, which increased the probability of finding a varying allele in any group at any specific loci. Genetic differentiation among grouped populations thus exhibit lower proportions of interpopulation variation and higher proportions of intra population variation.

If the grouping does indeed give a more accurate picture of the *P. virgatum* prairie populations, then we see that there is, or was, much more gene flow originally present between populations of *P. virgatum*. Lower G_{ST} values (plot = 0.2262, field = 0.3614) and the slight increase in diversity (plot = 0.2593, field = 0.2213) supports the idea of more gene flow and sexual reproduction between populations of *P. virgatum*. These estimates of gene flow and genetic diversity indicate that there was more pollen and seed dispersal between populations and that reproduction by division may not have been a large part of *P. virgatum's* mode of reproduction. However, the grouping of populations would naturally have more genes in common and a smaller G_{ST} would be found.

Therefore, if inaccurate sampling did occur, it is likely that the true G_{ST} would be found somewhere between that found in the first analysis and the grouped analysis.

Conclusions for *P. virgatum*

Panicum virgatum prairie populations are distinctly different than cultivar populations according to AFLP analysis. The analysis of cultivars showed differentiation between lowland and upland types. Differentiation between upland and lowland types in remnant prairie populations is not as apparent. It is therefore unlikely that any remnant population is composed of cultivars. Evidence exists that multiple ploidy levels may exist within two of the remnant prairies studied. Examination of individuals collected from the field (Figure 16.1) show that five individuals (three from Medora and two from Flaherty) branch off separately from their respective populations, indicating a significant genetic difference within these individuals. This may be due to an difference in ploidy level, which should be resolved with more testing. Both of these prairies are hilly with wet valleys making them ideal candidates for both lowland and upland ecotypes. It is a possibility that a lowland ecotype variety or species is present within these prairies wet lowland regions. This possibility should be examined further with a more specific sampling record.

The levels of genetic variation detected in the *P. virgatum* are explained by their ecological and life history characteristics. The plot samples were planted from seed and are products of recent gene flow as a result of reproduction. Since they were allowed to establish in a controlled environment, the level of genetic diversity was higher than those collected directly from the prairie remnants, which show ecotypic differences and show

indications of clonality. Since cultivars were selected for specific reproducible traits, genetic variation was dramatically reduced in these populations as compared to remnant populations which propagate both sexually and asexually. These differences in reproduction explain the genetic differentiation expressed in Figures 17 and 18.

The overall genetic structure of *P. virgatum* has not been clarified in this study due to variability in Mantel test results. *Panicum virgatum* populations that are the most similar genetically do not seem to exhibit a definite pattern of gene flow. This study indicates that each prairie remnant is affected differently by different habitats and ecology. The exact mechanism of gene flow is unclear between populations, as indicated by pair-wise analysis with genetic distances and G_{ST}. When combining habitat commonalties that provide similar selective pressures, animal migration patterns (which may never be fully understood because of the prairie was destroyed before patterns could be studied) which may have influenced seed dispersal, and genetic barriers, it is difficult to understand the genetic interaction between populations. It is likely that many complex interactions of the prairie ecosystem varied in different regions of the prairie. These variations likely influenced the genetic similarities and differences that are shown in this study of remaining *P. virgatum* plants and populations throughout the prairie.

Due to the life history characteristics of *P. virgatum*, it is probable that *P. virgatum* had a smaller proportion of inter-population variation in the past. However, fragmentation and isolation has likely resulted in a founder effect, which has since accentuated the genetic diversity within *P. virgatum*. A study of the variation within a prairie and degree of sexual versus asexual reproduction by *P. virgatum* would enhance

the understanding of this species as well as what needs to be done to preserve the remaining natural diversity. As for now, the available seed sources present appear to contain a lot of variation, but for that variation to be collected and used in restoration and reconstruction, seed must be collected from a variety of genetically similar locations.

Coreopsis palmata

Iowa Ecotype Project Plot Populations and Native Prairie Populations

Coreopsis palmata samples gathered from the IEP plots and directly from the field exhibit a degree of genetic variation that is consistent with previously reported isozyme studies within the genus *Coreopsis* (Crawford and Smith, 1982; Crawford et al., 1984; Crawford and Whitkus, 1988; Cosner and Crawford, 1990; Cosner, 1991; and Cosner and Crawford, 1994). The number of polymorphic loci (50.0% between all plot populations and 42.7% for all field populations) are slightly low compared to the average for all plant species (Hamrick and Godt, 1990). The level of genetic heterozygosity among the plot populations ($H_T = 0.1727$) and field populations ($H_T = 0.1386$) were slightly high for the plot samples and slightly low for the field populations, as compared to average for a given plant species reported by Hamrick and Godt (1990). The differences in variability seen between the plot and field samples are likely explained by the differences in propagation. The plot samples were planted from seed while it is likely that field populations tend to be more clonal since *C. palmata* propagates easily by division (Smith and Smith, 1980).

The level of variation present is consistent with life history character variables of *C. palmata*. The three life history variables that Hamrick et al. (1979) found to correlate

highly with the genetic variation of a species explain the level of variation found within *C. palmata*. *Coreopsis palmata* is self-infertile, but tends to propagate via rhizomes, which limits sexual reproduction and thus variation. *Coreopsis palmata* is also likely pollinated by bees. Due to the social aspects of animals, pollination tends to be limited to a certain regions, which reduces the level of genetic variation as compared to wind-pollinated (Gunvor et al., 1998). When we consider the fragmentation of the prairie, it is likely that distance between prairies restricts gene flow via animal pollination. Specific fecundity rates were unavailable, but due to the limited diversity, it is unlikely that *C. palmata* produces large amounts of genetically variable seed. In spite of these limitations, variation in *C. palmata* is average due to characteristics which tend to increase variation such as being a late successional species, a perennial, and having a fairly wide spread geographic range (Hamrick and Godt, 1990).

Chi-square significance testing was done at each polymorphic locus then chi-square values were combined for pair-wise comparisons of populations which tested for allele frequency homogeneity. In all pair-wise comparisons (in each respective collection) the null hypothesis, having no allele frequencies difference, was rejected ($\alpha<0.05$). Therefore, allele frequencies between sampled populations were different enough to suggest that samples were taken from different gene pools and that all populations were genetically distinct from one another.

Genetic distances and genetic identities did not indicate any extreme separation of populations. The unrooted phylogram of the *C. palmata* plot populations (Figure 19) shows the genetic distance relationships between the populations. There seems to be no

definite grouping of *C. palmata* populations. When examining populations by IEP zones there appear to be some relationships, but they are unclear. The distinction between each population likely indicates that populations tend to be genetically differentiated due to lack of gene flow due to the life history characteristics discussed earlier.

Unrooted phylograms of the field populations (Figure 21) of *C. palmata* show division of northern and southern populations, but indicate no relationships between east and west populations. This is likely in part due to both distance and landform. Field populations of *C. palmata* also show more distinct grouping of populations. This likely suggests that gene flow between these locations was or is more prevalent than between the remnant prairies sampled from the plot. Northern populations indicate genetic relationships to southern Iowa populations in accordance to geographic distance. However, when comparing genetic relationships within northern or southern population groupings, a larger geographic distance does not always indicate a larger genetic distance. However, the groupings seen are similar to those seen in *P. virgatum* earlier (Medora and Flarety grouping together and Land Between Two Railroad grouping with a small, isolated prairie).

Population differentiation was further supported by the partitioning of genetic variation observed between populations of *C. palmata*. Genetic variation among plot populations was estimated at 51.6% ($G_{ST} = 0.5155$) while 47.4% existed within populations. Genetic variation among field samples was estimated at 62.0% ($G_{ST} = 0.6202$) while 38.0% existed within populations. The degree of genetic variation within *C. palmata* plant populations was very low compared to the average 78% found by

Hamrick and Godt (1990). In isozyme studies of species in the *Coreopsis* genus, a wide range of G_{ST} numbers were reported (0.039 - 0.519) (Crawford and Whitkus, 1988; Cosner and Crawford, 1990; Cosner, 1991; Cosner and Crawford, 1994) which was due to varying life history characteristics within each species. The extremely low degree of intra-population heterozygosity and high G_{ST} is likely explained by the ability of *C. palmata* to reproduce via rhizomes, limitations of pollen and seed dispersal, as well as the geographic isolation of prairie remnants. These attributes were likely accentuated in plant tissue from the field collections over the plot populations planted from seed. The level of heterozygosity in each population was low in comparison to the average plant species (Hamrick and Godt, 1990) and low in comparison to other species in the *Coreopsis* genus (Crawford and Smith, 1982; Crawford et al., 1984; Crawford and Whitkus, 1988; Cosner and Crawford, 1990; Cosner, 1991; Cosner and Crawford, 1994). The low level of heterozygosity is likely due to the isolation of populations without wide pollen and seed flow and the clonality found in *C. palmata*. Specific populations that have low heterozygosity within them are explained in three ways. First, some populations had a small sample size in the study (Medora and Rolling Thunder Prairie) which likely did not sample the population adequately. Second, some populations may have a small population size within the prairie and result in low heterozygosity due to size, as discussed before. Finally, it is likely that older populations (likely those established in the Southern Iowa drift plain rather than more recently glaciated Iowa landforms) have experience more genetic drift due to limited gene flow over a longer period of time.

Differences between populations, as well as those seen between plot and field samples, may also be accounted for by the difference in time and place of the sampling.

Genetic structure within *C. palmata* generally showed that there was no correlation between geographic distance and genetic differentiation. Mantel (1967) tests were run comparing both Nei's (1972) genetic distance and Nei's (1973) G_{ST} measure of genetic differentiation to geographical distance. In *C. palmata* plot samples, both tests had low positive correlations that were not significant ($\alpha > 0.05$) according to a T-test and Hope (1968). The genetic structure for the *C. palmata* plot samples indicates seed was collected from genetically isolated and differentiated prairies. Mantel tests run on the same measures of genetic differentiation in field populations had low positive correlations that were not significant ($\alpha > 0.05$) according to according to a T-test and Hope (1968). These results indicate that the genetic structure of *C. palmata* is greatly influenced by distances, which isolates populations by a lack of gene flow. These factors, along with a tendency to propagate by division, account for the genetic variation seen in *C. palmata.*

Populations were grouped together for the same reasons as discussed for *P. virgatum* previously, and reductions and increases in various measurements can be explained in the same manner. Since genetic distance differences in plot samples were variable and did not significantly separate specific groups of populations , conclusions drawn from the grouped data is likely to be biased and inaccurate. If the grouping does indeed give a more accurate picture of the *C. palmata* prairie populations, then we see that there is or was much more gene flow originally present between populations of *P.*

virgatum. Lower G_{ST} values (plot = 0.2978, field = 0.3268) and the slight increase in genetic diversity (plot = 0.1822, field = 0.1509) support the idea of more gene flow and sexual reproduction between populations of *C. palmata*, if grouping does represent a more accurate population. These estimates of gene flow and genetic variation indicate that there was more pollen and seed dispersal between populations in the past. However, the level of intra-population variation is still low compared to averages among plant species (Hamrick and Godt, 1990) which suggests that reproduction by rhizomes is still a major part of *C. palmata's* mode of reproduction. Examination of the genetic distance relationships seen in the original plot populations of *C. palmata* shows us that populations exhibit fairly similar genetic distances from each other. As, no two populations are extremely similar genetically, and genetic differentiation among populations is about 50%, it is unlikely that the grouping of populations gave a more accurate picture of the genetic structure of *C. palmata*. It is likely that these populations are specifically separated by distance and that little gene flow exists between them. This however may not be the case for the field populations examined. The relative close proximity of some of sites in this study and the genetic relationships between them suggest that gene flow at least was more prevalent between them. The high G_{ST} value does not support this, but this may be attributed to the method of sampling.

Conclusions for *C. palmata*

The overall genetic structure of *C. palmata* can not be completely clarified by this study. The method of collection of the field sample, the limited number of populations that could be found, and the lack of a knowledge about *C. palmata* in general make

conclusions difficult. This study does however suggest that *C. palmata* generally shows some characteristics of isolation. The overall low variation in association with the high G_{ST} suggests that little gene flow existed between populations of *C. palmata* in the remnant prairie. Gene flow that does exist, or did exist, likely only occurred between populations that are in close proximity. The fragmentation of the prairie has likely eliminated gene flow between populations of *C. palmata*. If this is the case, *C. palmata* will become increasingly more inbred and lose diversity in the near future. To maximize the variation that is present among patches of *C. palmata,* restoration and reconstruction efforts should collect seed from a variety of genetically similar prairies.

Levels of variation between plot and field populations are likely due to the plot samples coming from material that was planted from seed while those existing in the prairie likely came from material spreading through rhizomes. This becomes more evident when examining the unrooted phylogram of *C. palmata* individuals (Figures 23 and 23.1). The collection of multiple field samples from a given patch of *C. palmata* increases the likelihood of collecting clones or very genetically similar plants. The degree of similarity between plants from the same patch is very high, suggesting that the only variation may occur though point mutations in the rhizomes over long periods of time. The variation seen within plot individuals is larger suggesting that plant material from sexually reproduced seed is distinctively different. Three samples from the field and plot were taken from the same prairie (Stinson – 131, Hoffman – 104, and Flaherty – 324.) The differences between the samples are large, which indicate large changes. Large genomic changes have been known to occur in plant species that are subjected to

stress (Clegg, 1990). If fragmentation of the prairie is causing stress within populations of *C. palmata*, this could explain the differences seen in such populations.

Conclusions

The analysis of AFLP markers in two species of prairie plants gives insight to the genetic structure of *Panicum virgatum* and *Coreopsis palmata* the prairie. These species exhibit different genetic structures and levels of genetic variation. Different species that evolved within the prairie became specialized for different life characteristics and will show different genetic structures. The genetic analysis of several prairie plant species will give insight to how prairie plants functioned together in a complex ecosystem.

The two plant species studied show that remnant populations are and have been isolated genetically. The fragmentation of the prairie, which limited population numbers and likely cut off gene flow, will greatly affect remnant populations of *P. virgatum* and *C. palmata.* Without the gene flow that continuous prairie provided, these populations will experience increased genetic drift, especially due to their clonal natures. A continuous corridor of prairie, that may be provided by roadside plantings, would increase gene flow and diversity for these two species of prairie plants.

Finally, this study shows that seeded prairie plants exhibit more variation than is present within the existing remnants. The inability of some seed to establish in a given habitat limits the genetic diversity seen within the plants studied there. Genetic diversity will be increased through the harvesting and planting of sexually produced seed that carry genes that are typically unable to be expressed. The planting of prairies and interseeding of existing remnants with widely collected seed, will introduce more genes and increases

the diversity present as well as help prevent genetic drift. The mixing of ecotypes in the case of interseeding will likely not affect the prairie because seed collected from different ecotypes will not establish well. The differences in prairie plant genetic structure suggest that seed collection sites should be a major consideration. To maximize genetic variation during seed collection, one should consider the degree of gene flow that existed among the plant's populations. A clonal species such as *C. palmata* should be collected from a variety of sites to maximize variation within the seed, however, a less clonal species such as *P. virgatum* need not be collected from as many sites to capture the same degree of genetic variation.

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