

Assessment of Baseline Genetic Data on *Andropogon gerardii* (Big Bluestem), *Sorghastrum nutans* (Indian Grass), and *Dalea purpurea* (Purple Prairie Clover) among Remnant and Restored Illinois Tallgrass Mesic Prairies and Selected Grass Cultivars.

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By

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INTRODUCTION

Tallgrass prairies occur in the eastern portion of the North American Prairie biome, with *Andropogon gerardii* Vitman (big bluestem), *Sorghastrum nutans* (L.) Nash (Indian grass), and *Panicum virgatum* L. (switchgrass) dominating the vegetation (Anderson, 1991). Prior to settlement by Europeans, Illinois contained approximately 8.9 million hectares of tallgrass prairie. Despite its heritage and its sobriquet as the prairie state, less than 0.01% of the original high quality prairie remains (Robertson and Schwartz, 1994).

Prairies have been preserved because they are an important part of Illinois' natural and cultural heritage. The majority of the remnant prairies remaining today consist of abandoned pioneer cemeteries, railroad right-of-ways, and lands unsuitable for agriculture. A number of sites in the state have been restored by seeding prairie species, with more than 90% of the projects using local genotypes (ecotypes) (Schramm, 1990). Choice of ecotypes was desirable because: 1) restoration includes the genetic and structural components of the historical community, 2) natural selection has presumably determined the most fit ecotype, and 3) the preservation of local gene pools will maintain genetic diversity at the landscape level. However, very little empirical data are available documenting the existence of ecotypes in Illinois.

The objectives of this study were to: 1) determine the genetic diversity of *Andropogon gerardii*, *Sorghastrum nutans*, and *Dalea purpurea* Vent. in remnant and restored populations throughout Illinois, 2) relate genetic diversity to population size, 3) determine if Illinois populations were genetically different from Konza Prairie Research Area (KS), 4) evaluate the genetic relationships among Illinois populations of

Andropogon gerardii and *Sorghastrum nutans* with commonly purchased cultivars of *Andropogon gerardii* ('Roundtree', 'Kaw', and 'Pawnee') and *Sorghastrum nutans* ('Rumsey' and 'Cheyenne'), 5) determine if 'foreign' alleles were present in native prairie species located adjacent to non-native conspecifics, and 6) determine if there is an association between genetic diversity and competitive ability (which will be tested at a later date).

The three most important families of the tallgrass prairie community are Poaceae, Fabaceae, and Asteraceae. *Andropogon gerardii* and *Sorghastrum nutans* are perennial, outcrossing, wind pollinated, rhizomatous species of Poaceae. *Dalea purpurea* is a perennial, outcrosser with partial selfing, insect pollinated species, which was included because of differences in life history traits and membership in a different family (Fabaceae). Therefore, this study includes species from two of the three most important families of the tallgrass community.

METHODS

Sites: A list of potential sites was provided by Mr. Bill McClain (IDNR); however, in an effort to avoid complications implicit in comparing potential ecotypes associated with different edaphic conditions, only tallgrass mesic (black soil) prairies were selected (Table 1). During fall 1995, twenty five sites from across the state were sampled. In addition to Illinois populations, all three species were collected from the 3489 ha remnant Konza Prairie Research Natural Area (KS), area 004B, and used as a reference population. Five of the more commonly purchased grass cultivars, three commercial cultivars of *Andropogon gerardii* and two of *Sorghastrum nutans*, were included in this study (Illinois Agronomy Handbook, 1994).

Collection methods: In the fall of 1995, seeds were collected from 30 randomly selected individuals at each site, that were at least 10 meters apart. From select populations, bulk samples containing seeds from 100-300 additional plants were collected and used to augment the number of individuals derived from each site, in the event that individual collections had not produced adequate seed. Seeds were removed from the maternal plant, stored on ice, transported to Southern Illinois University at Carbondale, and cold stratified for three months. Seeds were then processed (chaff removed for the grasses and scarification for *Dalea purpurea*), germinated in petri dishes on moistened filter paper, and one seedling from each maternal plant was removed, potted into a 10.1 x 10.1 x 8.0 cm square pot containing a commercial growth media (Promix-HP, Hummert International, Earth City, MO), and grown in a greenhouse. Ambient lighting in the greenhouse was supplemented with Grow-Lux standard grow lamps from 07:00 to 19:00 hours (Hummert International, Earth City, MO). Plants were watered as needed and fertilized with 350 g m² of Peter's Fertilizer (N:P:K, 20:20:20; Grace-Sierra Horticultural Products, Milpita, CA) every three weeks.

Allozyme Methods: Approximately 1.0 g of fresh leaf material was homogenized in Wendel and Weeden extraction buffer 3 (Wendel and Weeden, 1989). The extract was then centrifuged at 10,000 r.p.m. for 15 min and the supernatant stored frozen (-80 °C) in 1.5 ml microcentrifuge tubes. Extracts were kept frozen until ready to load on the gel.

Enzyme separation was accomplished using 13% starch gels. Enzymes were assayed using three gel-electrode buffer systems: (A) Tris EDTA Borate pH. 8.0 (Ayala

et al., 1972), (B) Ridgeway pH 8.1 (Ridgeway et al., 1970), and (C) Lithium Hydroxide pH 8.1 (Selander et al., 1971).

A maximum of 25 enzyme systems and 12 gel/electrode buffer systems were surveyed for each species. Enzyme staining protocols were essentially as reported in Soltis and Soltis (1989). Five enzyme systems coding for eight putative loci were used with *Dalea purpurea*. The following enzyme systems (with locus abbreviations, enzyme commission numbers, and buffer systems in parentheses) were used: aspartate amino transferase (AAT-2, 2.6.1.1, A), glucose phosphate isomerase (GPI-2, 5.3.1.9, B), triose phosphate isomerase, (TPI-1, TPI-2, 5.3.1.1, A), 6-phosphogluconate dehydrogenase (6PGD-1, 6PGD-2, 1.1.1.44, A), and phosphoglucomutase (PGM-2, PGM-3, 2.7.5.1, B). Six enzyme systems coding for nine putative loci were used with *Sorghastrum nutans*: aspartate amino transferase (AAT-2, 2.6.1.1, B), glucose phosphate isomerase (GPI-2, 5.3.1.9, B), malic enzyme, (ME-1, 1.1.1.40, B), triose phosphate isomerase, (TPI-1, TPI-2, 5.3.1.1, B), 6-phosphogluconate dehydrogenase (6PGD-1, 6PGD-2, 1.1.1.44, A), and phosphoglucomutase (PGM-2, PGM-3, 2.7.5.1, A). Five enzyme systems coding for six putative loci were used with *Andropogon gerardii*: aspartate amino transferase (AAT-2, 2.6.1.1, A), diaphorase (DIA-1, 1.6.2.2, A), glucose phosphate isomerase (GPI-2, 5.3.1.9, B), malate dehydrogenase (MDH-1, 1.1.1.37, A) and phosphoglucomutase (PGM-2, PGM-3, 2.7.5.1, B).

Allozyme Data Analysis: Controlled crosses to document the inheritance of the allozyme patterns were not conducted, therefore genotypes inferred from the banding phenotypes should be considered putative. The most common allele at a locus was assigned a mobility number of 100 and all other bands given numbers relative to it. For

Dalea purpurea, these relative mobility numbers were then assigned genotypes (AA, AB, BB). Genotypes were used to calculate genetic diversity statistics with BIOSYS-1 (Swofford and Selander, 1981) including: mean number of alleles per locus (A), proportion of polymorphic loci per population (P) using the 95% criterion, unbiased expected heterogeneity (H_{exp}), and the inbreeding coefficient ($F=(H_{exp}-H_{obs})/H_{exp}$). Population relationships were investigated using unweighted pair-group method with arithmetic averaging (UPGMA) cluster analysis of Cavalli-Sforza and Edwards chord distance between populations. Principle components analysis (PCA) of allele frequencies among populations was accomplished using PROC PRINCOMP (SAS, 1989). Parallel analysis (PA) was used to estimate the 95th percentile eigenvalues with Longman's Method and only significant axes were retained for interpretation (Franklin et al., 1995).

Given that *Andropogon gerardii* ($2n=20-90$; Keeler and Kwankin, 1989) and *Sorghastrum nutans* ($2n=24, 40, 80$; Gleason and Cronquist, 1991) are polyploids, genotype and allelic frequencies were not determined. Frequencies of unique electrophoretic phenotype patterns (A thru J) were used to calculate the mean number of phenotypes per locus (A'), proportion of polymorphic loci per population (P'), and a measure of diversity ($1-\sum P_i^2$) based on one minus the proportion of homozygotes. Population relationships based on the electrophoretic phenotype frequencies, were investigated using UPGMA cluster analysis of genetic distances among populations, PCA, and PA of the correlation matrix of the PCA. Statistical testing for differences in diversity measures, among populations within species, were not performed due to the relatively small number of remnant, restored, and cultivar accessions (2-7).

RAPD Methods: Genomic DNA was isolated using a modified mini-prep CTAB procedure (Nickrent, 1996), quantified by comparison to standards of known concentration on agarose gels, and then diluted to a working concentration of ca. 5.0 µg/µl. Thirty ten-base oligonucleotide primers (Operon Technologies) were surveyed and six were selected for each species. Amplification reactions were performed in 25 µl volumes containing 10 mM Tris-HCL (pH 9.0), 50 mM KCl, 0.1% Triton X-100, 1.2 mM MgCl₂, 0.2 mM each dATP, dCTP, dGTP, and dTTP (United States Biochemical), 20 ng of primer, 0.2 µl Taq polymerase (Promega), and approximately 25 ng of genomic DNA. Samples were overlaid with 30 µl of mineral oil. Amplifications were performed in a Stratagene Robocycler (model #400860, Stratagene, La Jolla, CA) programmed with these parameters: 94 °C for 3 min, followed by 45 cycles of 94 °C for 1 min, 36 °C for 1 min, 72 °C for 2 min, and a final incubation at 72 °C for 10 min. Amplification products (5 µl of a 25 µl reaction) were electrophoresed in 1.0% agarose gels with Tris, Borate, EDTA (TBE) running buffer, stained with ethidium bromide, scanned, and stored as a digitized image file with the Gel-doc 1000 system (BioRad Laboratories, Hercules, CA). Molecular Analyst (BioRad Laboratories) software package was used to modify the image, altering the contrast and enhancing the visualization of the DNA bands. A DNA molecular weight marker (Promega, # G1741) was used to standardize band intensities and to estimate the size of the RAPD bands.

RAPD Data Analysis: Five to eight RAPD bands were scored as presence / absence data, for each primer. Gene frequencies were estimated following that of Lynch and

Milligan (1994). Relationships among populations were calculated using PCA and PA, based on these gene frequencies.

RESULTS AND DISCUSSION

Dalea purpurea: Purple prairie clover was collected from three remnant and three restored Illinois populations. The Illinois remnant populations possessed less alleles per locus and less polymorphic loci than the restored populations, albeit not statistically significant (Table 2). The Gensburg-Markham population was the least genetically diverse. The size of the remnant was not a good predictor of the inbreeding coefficient, given that Pellville Prairie was considerably smaller than Gensburg-Markham Prairie. Black Hawk State Historical Site (Black Hawk S.H.S.) and Morton Arboretum were the two inbred restored populations. Morton Arboretum was restored with 'local' seed from multiple seed source populations, within a 60 mile radius, between 1962-1972 (Schulenberg, personal communication). One would predict high levels of genetic diversity given multiple source populations, however this population is one of the least diverse. The seed source population for Black Hawk S.H.S. and Morton Arboretum restoration projects were not available for this study (Schramm, personal communication; Schulenberg, personal communication). Use of inbred populations as the seed source for restoration projects may result in a restored population that retains the desired 'ecotype', but which is genetically depauperate. Unfortunately, without genetic data from the specific source populations, we were unable to address this issue in this study.

In an isozyme study of the Federally Endangered *Dalea foliosa* (Gray) Barneby, Wiltshire (1994) determined that overall levels of genetic variability were low, with polymorphism ranging from 0-24% and heterozygosity from 0-0.081. In our study,

measures of genetic diversity of *Dalea purpurea* were comparable, with polymorphism ranging from 11-33% and heterozygosity from 0.047-0.089. The population fixation index of *Dalea purpurea* across all 9 loci ($F_{ST}=0.06$) indicated little genetic divergence among populations, which was different than *Dalea foliosa* apportioning most of its variation among populations. Both species show low genetic variability, however the difference in population divergence could reflect limited gene flow among *Dalea purpurea* populations.

Relationships among *Dalea purpurea* populations were investigated using allozyme and RAPD data. Cluster analysis based on allozyme data revealed predictable associations with Morton Arboretum associating with Gensburg-Markham (Fig. 1). The four remaining prairies formed two groupings, with the Pellville / Grant Creek and Mason County State Nursery (Mason County S.N.) / Black Hawk S.H.S. associations. These may reflect the geographical proximity of Pellville to Grant Creek and Mason County S.N. to Black Hawk S.H.S.. The second level associations linked the Mason County S.N. / Black Hawk S.H.S. group with the Grant Creek / Pellville group, suggesting a central Illinois grouping different than that of the Chicago metropolitan area. Principle components analysis of allele frequencies demonstrated the same relationships among populations (Fig. 2). The central Illinois, northeastern Illinois geographical designations were well resolved by the first three principle axes. Inclusion of Konza Prairie (KS) into the analysis showed Konza to be different from the Illinois populations, suggesting an Illinois type (Fig. 2). The results of the RAPD analysis were generally consistent with that of the allozyme data (Fig. 3). The Mason County S.N. / Black Hawk S.H.S. grouping was well resolved in the first two axes. The Gensburg-Markham /

Morton and the Grant Creek / Pellville groupings were statistically discernible on the third axes, but these relationships were not as clear as the allozyme results.

Sorghastrum nutans: Indian Grass was collected from 14 remnant and nine restored Illinois prairies (Table 1). A drought coupled with a fungal infection in the late summer / early fall of 1995 resulted in low seed set, thus only six Illinois populations, two cultivar collections, and Konza Prairie (KS) were included in this study. Nine putative allozyme loci were scored with 44 - 56% of the loci being polymorphic (P') (Table 3). Mean number of electrophoretic phenotypes per polymorphic locus (A') ranged from 2.2 - 3.3, with the cultivars, remnants, and restored accessions averaging 3.0, 2.7, and 2.5, respectively. Diversity ranged from 1.04 - 1.67, with the remnants (1.36) being more diverse than the restored (1.28) and cultivar (1.23) accessions. These results indicate that the Illinois remnants, Wheelock and DeSoto Railroad Prairies, were more genetically diverse than the restored Illinois populations or the cultivars. Hence, remnant Illinois populations of *Sorghastrum nutans* would provide a more genetically diverse seed source for future Illinois restoration projects.

The Konza *Sorghastrum nutans* accession had the lowest genetic diversity (0.6), yet Konza Prairie occupies over 3400 ha and shows no signs of decreased fitness resulting from lower allozyme diversity. This result was interesting because presumably the larger the population, the higher the expected genetic diversity. The sampling of this population maintained 30 m between each collection, reducing the probability of sampling clones. However, if the genetic neighborhood of this wind pollinated outcrosser was up to 1 km in this large contiguous prairie, then sampling error would account for the low genetic diversity (Crawley, 1997). Increased frequency of higher

polyploid levels in the western range of *Andropogon gerardii* have been documented by Keeler (1990) and were consistent with models of variable and disturbed conditions favoring polyploids (Lewin, 1980). Unfortunately, the geographic distribution of *Sorghastrum nutans* polyploid levels along an east - west transect have not been investigated.

Population level relationships were investigated using UPGMA cluster analysis with Cavalli-Sforza and Edwards chord distance among populations and PCA based on electrophoretic phenotype frequencies. A central Illinois group including Wheelock Railroad Prairie (remnant), Meadowbrook Park (restored), Weldon Springs (restored), and Mason County S.N. (remnant and restored) was distinct from the southern Illinois DeSoto Railroad Prairie (remnant) and the northeastern Illinois Fermi Prairie (restored) (Fig. 4). The PCA results were congruent with those of the allozyme UPGMA analysis (Fig. 5). Meadowbrook Park, Weldon Springs, and Mason County S.N. were entirely or partially restored prairies. Following the recommendations to use Illinois ecotypes (McClain, 1986), the seed source populations were probably located in the central - westcentral part of the state, which explains the central Illinois grouping. The 'Cheyenne' cultivar formed an association intermediate between the central Illinois group and DeSoto Railroad Prairie, while the 'Rumsey' cultivar was separate from all other populations in the study. 'Rumsey' was originally collected from south-central Illinois and developed at the Missouri Soil Conservation Service (Illinois Agronomy Handbook, 1996). The source population for our purchased seed was collected from a Missouri location. The distant relationship between 'Rumsey' and the Illinois accessions was unexpected, given that the original source of 'Rumsey' was from Illinois. These

differences may reflect the recent genetic history of 'Rumsey'. Developed and grown in Missouri, it is possible that there was gene flow between native Missouri populations and the 'Rumsey' cultivar. Alternatively, the original Illinois source of 'Rumsey' may have been different from the sampled Illinois sites (e.g. not mesic black soil prairies) and these differences were maintained and amplified through artificial selection during the development and propagation of this cultivar.

The relationships among the Illinois populations and the 'Cheyenne' cultivar were equally perplexing. The original source for the 'Cheyenne' cultivar was presumably located in the western Great Plains, but the source population for this seed lot was grown in Texas. Consequently, the genetic history of this accession was not as well known as 'Rumsey'. This notwithstanding, the association of 'Cheyenne' with the central Illinois grouping suggest two possible explanations. The seed source for the Mason County S.N., Weldon Springs, and / or Meadowbrook Park restorations was not from local ecotypes, but actually consisted of the 'Cheyenne' cultivar. This possibility seems unlikely given the strong emphasis on the use of local ecotypes. Another plausible explanation was that populations of 'Cheyenne' were planted near the restoration seed source populations or the actual restored site. The state of Illinois and private landowners have been known to use native grasses as cover crops for wildlife and erosion control (Schramm, 1990). In these situations, the need for large quantities of seed usually precludes the collection and processing of seed from 'local' populations. The resulting population derived from a non-Illinois ecotype would then be a source of pollen that could be wind dispersed into a remnant prairie. The introgression of non-native ecotypes has been documented in

several species and has the potential to be a larger problem than inbreeding depression (Rhymer and Simberloff, 1996).

Much like the 'Rumsey' accession, the Konza Prairie population of *Sorghastrum nutans* was different from the central Illinois group, 'Cheyenne' accession, and the Femi population of northeastern Illinois. These results suggest that an Illinois ecotype exists, although more non-Illinois accessions would be needed to further investigate this possibility.

Andropogon gerardii: Big Bluestem was collected from 24 populations across Illinois. The low seed set in 1995 reduced the number of populations included in this study to seven remnant and seven restored sites, for a total of 14 Illinois sites. In addition, three cultivars ('Roundtree', 'Kaw', and 'Pawnee') and Konza Prairie Research Natural Area (KS) were included in this study.

Six putative loci were scored with 50 - 100% of the loci being polymorphic (P') (Table 4). The mean number of electrophoretic phenotypes per polymorphic locus (A') ranged from 2.3 - 4.3, with little difference among remnant (3.3), restored (3.2) and cultivar (3.1) accessions. The genetic diversity ranged from 1.50 - 2.32, with the remnant accessions (1.78) being less diverse than the restored (1.97) and cultivar (1.91) accessions. Higher genetic diversity in restored populations compared to remnant populations may reflect: 1) truly higher genetic diversity, as a consequence of multiple seed source for the initial restoration, 2) reduced genetic variation at the remnant sites resulting from selection for the most fit ecotype, and 3) the remnant populations were experiencing higher levels of inbreeding than the restored populations. Additionally,

there is no relationship between habitat size and genetic diversity, with Tomlinson Prairie (0.4 ha) having higher levels of diversity than the 31.6 ha Grant Creek.

Examination of the population relationships among 14 Illinois population (seven remnant and seven restored), three cultivars, and the Kansas reference population (Konza Prairie) of *Andropogon gerardii* proceeded in four steps: 1) relationships among remnant accessions, 2) remnant and cultivar accessions, 3) restored and remnants accessions, and 4) associations among all remnant, restored, and cultivar accession.

Cluster analysis among the seven remnant populations demonstrated two groupings. A central Illinois association included Sunbury, Wheelock, and Weston prairies with the Iroquois C.C.A accession being more distantly related (Fig. 6). Grant Creek and Tomlinson prairies, which were two of the most eastern remnant populations, form the east-northeastern association. Principle components analysis confirm Iroquois C.C.A and DeSoto prairies as being separate for the central and east-northeastern remnant groupings (Fig. 7). Inclusion of Konza Prairie (KS) supported the contention that Iroquois C.C.A and DeSoto accessions were different from the other Illinois groupings and that remnant Illinois populations were different from the reference population (Fig. 8).

Principle component analysis of cultivar and remnant populations resulted in the association of 'Kaw' with the central Illinois group (Fig. 9). 'Roundtree' and 'Pawnee' cultivars formed associations with the east-northeastern Grant Creek / Tomlinson grouping, while Iroquois C.C.A. and DeSoto prairies remained distant from the other associations.

Analysis of the remnant and restored populations revealed three groupings, as well as the retention of DeSoto and Iroquois C.C.A as distinct populations (Fig. 10). The central Illinois remnant grouping of Sunbury, Weston, and Wheelock was retained. The east-northeastern Grant Creek / Tomlinson grouping was expanded to include Goose Lake and Fermi prairies, both of which were in close proximity to the original remnant populations. The last grouping includes four restored populations (Meadowbrook Park, Mason County S.N., Black Hawk S.H.S., and Albany Mounds) which were located in central Illinois. If the restored populations shared a common seed source (i.e. central Illinois), then one would predict that the central Illinois restored grouping should form a tight association with this remnant association. However, these restored central Illinois populations associated more closely with the east-northeastern grouping, suggesting that the genetic relationships among remnant and restored populations of *Andropogon gerardii* in Illinois were more complex than geographic proximity.

Principle component analysis of all Illinois populations, Konza Prairie (KS), and three commercial cultivars ('Kaw': KS, 'Roundtree': MO, and 'Pawnee': NE) failed to resolve distinct geographic associations within Illinois, with exception of Iroquois C.C.A and DeSoto Railroad Prairie being distinct from the other accessions (Fig. 11). Unlike the Konza Prairie population being somewhat different from that of Illinois populations, in the 3rd principle axis, the cultivars formed tight associations with the Illinois accessions. 'Kaw' was associated with the central Illinois remnant populations and the other Illinois populations were associated with 'Roundtree' and 'Pawnee'. These associations among the cultivars and the Illinois accessions could have been cause by: 1) introgression of cultivar genes into Illinois populations via pollen dispersal from roadside

plantings of cultivar seed, or 2) ecotypes based on different edaphic conditions, however unlike the Illinois collections that were restricted to mesic blacksoil prairies, the cultivar population substrates are unknown.

Relationships among 13 Illinois prairies, two remnant Arkansas prairies, and three cultivars were investigated using RAPD. RAPDs are a finer scale molecular marker than allozymes, and have been used to investigate genetic relationships within several grass species (Dawson et al., 1993; Gunter et al., 1996; Gustafson and Nickrent, 1997; Huff et al., 1993; M'Ribu and Hilu, 1994, 1996; Stiller and Denton, 1995; Sweeney and Danneberger, 1994). However, the relationships among Illinois populations were somewhat more perplexing (Fig. 12). Iroquois C.C.A and DeSoto Railroad Prairie were identified as different from other Illinois populations, based on allozyme data, yet the RAPD data do not give congruent results. RAPD analysis does identify two remnant Arkansas populations as different from the Illinois accessions, unfortunately, no allozyme data are available on these Arkansas populations. 'Kaw' was associated with central Illinois remnant populations based on allozyme data, but RAPD data suggest that 'Kaw', as well as 'Pawnee', were different from the Illinois populations. These differences in results may reflect differences between the two methodologies, with the allozyme data not being sensitive enough to detect differences between 'Kaw' and 'Pawnee' cultivars and the Illinois populations.

In conclusion, the *Andropogon gerardii* study illustrated three points. First, the associations among Illinois populations were not based upon simple geographic proximity. For example, Iroquois C.C.A would be predicted to be associated with the east-northeastern group, however Iroquois C.C.A was distinct from all Illinois accessions.

Second, 'Kaw', 'Pawnee', and 'Roundtree' cultivars were not different from most of the Illinois populations sampled, based on allozyme data. Third, Iroquois C.C.A (most eastern) and DeSoto (most southern) accessions were the most distantly related to the Illinois, Kansas, and cultivar accessions.

General Conclusions: There were no consistent patterns in genetic diversity, when comparing remnant and restored populations. Restored populations that were planted with seed from genetically depauperate populations were themselves low in genetic diversity. When comparisons between the two dominant grasses were made, *Andropogon gerardii* genetic diversity increased from the remnant (1.78), cultivar (1.91), to the restored (1.97) accessions, while the *Sorghastrum nutans* remnant (1.36) accessions were more diverse than the restored (1.28) and cultivar (1.23) accessions. The one consistent trend was that both grasses from Konza Prairie had the lowest levels of genetic diversity. Life history traits such as longevity, pollination system, and seed dispersal tend to be correlated with genetic diversity (Hamrick and Godt, 1989). *Andropogon gerardii* and *Sorghastrum nutans* are perennial, wind pollinated, outcrossing, rhizomatous, sod forming, co-dominant, C₄ grasses of the tallgrass prairie ecosystem, yet there was no consistent pattern of genetic diversity within Illinois. The lack of congruence may reflect differences in population histories, sampling error, or ploidy level.

To address differences in population histories, one would need genetic information of the presettlement condition in Illinois. Knowing whether the pre-European settlement Illinois prairie was one panmictic system or a mosaic of locally adapted populations, would aid in assessing the current condition. In addition, current restoration practitioners fail to adequately document their restoration efforts. Of the

information that would be important, seed source selection is critical. If the local ecotype is the goal, then knowledge of whether the source population is a true remnant or a cultivar (planted as a wildlife cover crop or erosion control) is essential. Unfortunately, the presettlement condition and most of the restoration information is lost forever and good documentation is rare.

Sampling error, in studies such as this, may be manifested in the form of an inadequate genetic survey, use of a genetically depauperate population as the source population, or failure to sample the entire population during specimen collection. First, in this study, we surveyed 18 to 25 enzyme systems and could only resolve six to nine loci. In addition, the ploidy levels of the grasses precludes scoring actual genotypes from the allozyme data. The scoring of the electrophoretic phenotypes provides less genetic information than actual genotypes, which is the outcome of working with these polyploids. Second, the sampling error related to the seed source for restored populations was illustrated with the *Dalea purpurea* data. Third, in an effort to adequately sample the entire population, sampling distances between individual collections were increased up to 35 m between each collection for larger sites and 10 m between collections at the smaller sites.

An increase in *Andropogon gerardii* ploidy levels from east to west has been documented, with the higher ploidy levels occurring in the western range of the species (Keeler, 1990). In Konza Prairie, Keeler (1992) documented intermingling of several polyploid cytotypes within most sampling locations. Higher polyploid levels tend to occur more frequently in harsher environments, inferring an increased fitness with an increase in ploidy level. It is possible that the frequency of higher ploidy variants,

octoploids were consistently larger than hexaploids (Keeler, 1992), within Konza Prairie compensate for the lack of allozyme diversity. Although investigation of the potential relationship between plant ploidy level and genetic diversity is an important research question, it is beyond the scope of this study.

The general population level relationships of *Andropogon gerardii*, *Sorghastrum nutans*, and *Dalea purpurea* in Illinois tallgrass mesic prairies suggest three associations; 1) central Illinois, 2) northeastern Illinois, and 3) southern Illinois. These associations were supported by the *Dalea purpurea*, *Sorghastrum nutans*, and the analysis of remnant *Andropogon gerardii* populations, although divergence among populations appears low. The lack of high levels of population divergence (i.e. Illinois populations dramatically different from non-Illinois populations) may reflect life history characteristics of these species. Long lived perennial, outcrossing, wind pollinated species tend to partition most of their allozyme diversity within populations (Hamrick and Godt, 1989). The complexities associated with the presumed *Andropogon gerardii* cultivar introgression into remnant and restored Illinois populations does not negate the distinction of three general associations within Illinois. These Illinois populations were different from Konza Prairie, Kansas (allozyme) and the Arkansas Prairies (RAPD).

Intraspecific introgression occurs in plant populations, especially in those species with high gene flow, and may be an important source of genetic variation. However, the intentional introduction of a non-local ecotypes may result in swamping the local population with a non-local gene pool, resulting in the loss of the historical genetic information (Rhymer and Simberloff, 1996). Higher ploidy variants of *Andropogon gerardii* tend to be larger and can produce viable intermediate ploidy variants ($2N=60$

crossed with $2N=80$ may result in a viable $2N=70$; Keeler, 1992). The introduction of a competitively aggressive polyploid variant (larger plants tend to be better competitors than smaller plants) may alter the community level dynamics of the remnant or restored prairie. Hence, the intentional introduction of a distinctly different gene pool could result in the loss of the historical genetic information and should be approached with caution.

Recommendations:

- 1) Specifically document the goals of a restoration, reclamation, or conservation project. Two possible alternatives are:
 - a. To restore a historical ecosystem / population / genotype.
 - b. To restore the physical structure with no regards for the historical genetic information.
- 2) Recognition of three general associations within Illinois, corresponding to central Illinois, northeastern Illinois, and a southern Illinois.
- 3) Establishment of regional seed source populations designed to maximize the regional genetic diversity, while retaining the regional genetic identity.
- 4) Establishment of a state database with proper documentation of Illinois restoration projects. Including, but not limited to, site history, goal of the project, species to be introduced / restored, specific seed source population location, and management practices to establish and maintain the proposed project.
- 5) State database / repository for genetic, demographic, and life history data collected during scientific investigations of Illinois ecosystems.

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Table 1. Populations sampled, counties, status, area, and species used in this study. (AG = *Andropogon gerardii* SN = *Sorghastrum nutans* DP = *Dalea purpurea*)

Population	County	Status	Area (ha)	AG	SN	DP
1 Green River Conservation Area	Lee	remnant				
2 Brownlee Cemetery Prairie	Mercer	remnant				
3 Lyndon-Agnew Railroad Prairie	Whiteside	remnant				
4 Munson Township Cemetery Prairie	Henry	remnant				
5 Albany Mound	Whiteside	restored	22.3	X		
6 Wheelock Railroad Prairie	Whiteside	remnant	16.2	X	X	
7 Black Hawk State Historical Site	Rock Island	restored	0.4	X		X
8 Grant Creek Prairie	Will	remnant	31.6	X		X
9 Goose Lake Prairie (NE source)	Grundy	restored	622*	X		
10 Goose Lake Prairie (local source)	Grundy	restored				
11 Gensburg - Markham Prairie	Cook	remnant	38.4			X
12 Fermi-Lab Tract #1	Du Page	restored	156**	X	X	
13 Fermi-Lab Railroad Prairie	Du Page	remnant				
14 Morton Arboretum (Schulenberg Prairie)	Du Page	restored	40.5			X
15 Iroquois County Conservation Area	Iroquois	remnant	506*	X		
16 Prospect Cemetery Prairie	Ford	remnant				
17 Tomlinson Pioneer Cemetery Prairie	Champaign	remnant	0.4	X		
18 Meadowbrook Park	Champaign	restored	12.2	X	X	
19 Weldon Springs State Park	De Witt	restored	6.8	X	X	
20 Sunbury Railroad Prairie	Livingston	remnant	5	X		
21 Weston Cemetery Prairie	McLean	remnant	2	X		
22 Mason County State Nursery	Mason	restored	97.2	X	X	X
23 Pellville Cemetery Prairie	Vermilion	remnant	0.6			X
24 Freeman United Mine	Perry	restored				
25 Desoto Railroad Prairie	Jackson	remnant	13.4	X	X	
Konza Prairie Research Natural Area (KS)		remnant	3487*	X	X	X
	Origin	Source				
'Roundtree'	MO	MO	cultivar	X		
'Pawnee'	NE	NE	cultivar	X		
'Kaw'	KS	NE	cultivar	X		
'Osage'	KS	MO	cultivar			
'Rumsey'	IL	MO	cultivar			X
'Cheyenne'	Great Plains	TX	cultivar			X

* Total area of the site. ** Area in the inner loop.

Table 2. *Dalea purpurea* allozyme results based on 8 putative loci. Mean number of alleles per locus (A), percent polymorphic loci (P), heterozygosity (expected and observed), and an inbreeding coefficient (F) with 1 = completely inbred.

Population	Status	n	A	P	H exp	H obs	F
Pellville	Remnant	26	1.3	11	0.046	0.047	-0.022
Grant Creek	Remnant	23	1.4	11	0.054	0.063	-0.167
Gensburg - Markham	Remnant	20	1.3	33	0.081	0.050	0.383
Black Hawk S.H.S.	Restored	18	1.6	22	0.090	0.080	0.110
Mason County S.N.	Restored	20	1.4	33	0.091	0.089	0.022
Morton Arboretum	Restored	20	1.4	33	0.102	0.061	0.402
Konza Prairie (KS)	Remnant	13	1.7	11	0.081	0.068	0.160
Illinois Remnant	Mean	23	1.4	19	0.060	0.053	0.117
Illinois Restored	Mean	19	1.5	30	0.094	0.077	0.181

Table 3. *Sorghastrum nutans* allozyme results based on nine putative loci. Mean number of electrophoretic phenotypes per locus (A'), percent polymorphic loci (P'), and diversity ($1 - \sum P_i^2$) with 0 = no variation.

Population	Status	n	A'	P'	diversity
DeSoto	Remnant	23	2.4	56	1.04
Wheelock	Remnant	18	3.0	44	1.67
Meadowbrook	Restored	33	2.2	56	1.18
Mason County S.N.	Restored	14	2.5	44	1.14
Ferni Tract 1	Restored	20	2.4	56	1.2
Weldon Springs	Restored	31	2.8	44	1.58
Cheyenne'	Cultivar	24	2.6	56	1.42
Rumsey'	Cultivar	24	3.3	44	1.04
Konza Prairie (KS)	Remnant	30	2.2	56	0.6
Illinois Remnant	Mean	21	2.7	50	1.36
Illinois Restored	Mean	25	2.5	50	1.28
Cultivar	Mean	24	3.0	50	1.23

Table 4. *Andropogon gerardii* allozyme results based on six putative loci. Mean number of electrophoretic phenotypes per locus (A'), percent polymorphic loci (P'), and diversity ($1 - \sum P_i^2$) with 0 = no variation.

Population	Status	n	A'	P'	diversity
DeSoto	Remnant	24	2.8	83	1.92
Grant Creek	Remnant	24	4.3	50	1.58
Iroquois C.C.A.	Remnant	31	3.4	67	1.77
Sunbury	Remnant	24	3.3	50	1.71
Tomlinson	Remnant	19	3.3	50	1.84
Weston	Remnant	11	2.3	67	1.91
Wheelock	Remnant	18	3.8	67	1.72
Goose Lake (NE)	Restored	23	3.0	83	2.09
Fermi Tract I	Restored	33	3.2	83	1.91
Meadowbrook	Restored	26	3.4	83	2.00
Weldon Springs	Restored	14	2.8	67	1.86
Mason County S.N.	Restored	34	3.6	83	1.85
Black Hawk S.H.S.	Restored	21	3.2	83	1.81
Albany Mounds	Restored	8	3.3	67	2.25
'Roundtree'	Cultivar	40	3.2	100	1.50
'Kaw'	Cultivar	24	3.0	67	1.92
'Pawnee'	Cultivar	22	3.2	83	2.32
Konza Prairie (KS)	Remnant	34	3.2	83	1.38
Illinois Remnant	Mean	22	3.3	62	1.78
Illinois Restored	Mean	23	3.2	78	1.97
Cultivar	Mean	29	3.1	83	1.91

FIGURES

Fig. 1. UPGMA phenogram of genetic relationships among three remnant and three restored *Dalea purpurea* populations in Illinois. Cavalli-Sforza and Edwards chord distance among populations based on allozyme allele frequencies.

Fig. 2. Principle component analysis of genetic relationships (allozyme data) among six *Dalea purpurea* population from Illinois and Konza Prairie Research Natural Area, KS. The first three principle axes accounted for 78% of the variation. Mason County S.N. (1), Black Hawk S.H.S. (2), Pellville (3), Grant Creek (4), Morton Arboretum (5), Gensburg - Markham (6), and Konza Prairie (7). Restored = underlined.

Fig. 3. Principle component analysis of genetic relationships among six *Dalea purpurea* population in Illinois, based on 37 RAPD bands. The first three principle axes accounted for 78% of the variation. Mason County S.N. (1), Black Hawk S.H.S. (2), Pellville (3), Grant Creek (4), Morton Arboretum (5), and Gensburg - Markham (6) Restored = underlined.

Fig. 4. UPGMA phenogram of *Sorghastrum nutans* genetic relationships among six Illinois populations, two cultivars, and Konza Prairie (KS). Cavalli-Sforza and Edwards chord distance among populations based on electrophoretic phenotype frequencies.

Fig. 5. Principle component analysis of *Sorghastrum nutans* genetic relationships among six Illinois populations, two commercial cultivars, and Konza Prairie (KS), based on electrophoretic phenotype frequencies. The first three principle axes accounted for 75% of the variation. Mason County S.N. (1), Meadowbrook (2), Weldon Springs (3), Wheelock (4), DeSoto (5), Fermi Tract I (6), 'Cheyenne' (VII), 'Rumsey' (VIII), and Konza Prairie (9). Restored = underlined and Cultivar = roman numbers.

Fig. 6. Cluster analysis of *Andropogon gerardii* genetic relationships among seven Illinois populations. Cavalli-Sforza and Edwards chord distance among populations based on electrophoretic phenotype frequencies.

Fig. 7. Principle component analysis of seven Illinois population of *Andropogon gerardii*, based on allozyme phenotype frequencies. The first three principle axes accounted for 72% of the variation. Weston (1), Wheelock (2), Sunbury (3), Grant Creek (4), Tomlinson (5), DeSoto (6), and Iroquois C.C.A. (7).

Fig. 8. *Andropogon gerardii* principle component analysis of seven remnant Illinois populations and Konza Prairie (KS), based on allozyme phenotype frequencies. The first three principle axes accounted for 64% of the variation. Weston (1), Wheelock (2), Sunbury (3), Grant Creek (4), Tomlinson (5), DeSoto (6), Iroquois C.C.A. (7), and Konza Prairie (8).

Fig. 9. *Andropogon gerardii* principle component analysis of seven remnant Illinois populations and three cultivars. The first three principle axes accounted for 55% of the variation. Weston (1), Wheelock (2), Sunbury (3), Grant Creek (4), Tomlinson (5), DeSoto (6), Iroquois C.C.A. (7), 'Kaw' (VIII), 'Pawnee' (IX), and 'Roundtree' (X).

Fig. 10. *Andropogon gerardii* principle component analysis of seven remnant and seven restored Illinois populations, based on allozyme phenotype frequencies. The first three principle axes accounted for 54% of the variation. Weston (1), Wheelock (2), Sunbury (3), Grant Creek (4), Tomlinson (5), DeSoto (6), Iroquois C.C.A. (7), Goose Lake (8), Weldon Springs (9), Fermi Tract I (10), Meadowbrook (11), Albany Mounds (12), Mason County S.N. (13), and Black Hawk S.H.S. (14). Restored = underlined.

Fig. 11. *Andropogon gerardii* principle component analysis of 14 Illinois populations, three cultivars, and Konza Prairie, based on allozyme phenotype frequencies. The first three principle axes accounted for 45% of the variation. Weston (1), Wheelock (2), Sunbury (3), Grant Creek (4), Tomlinson (5), DeSoto (6), Iroquois C.C.A. (7), Goose Lake (8), Weldon Springs (9), Fermi Tract I (10), Meadowbrook (11), Albany Mounds (12), Mason County S.N. (13), and Black Hawk S.H.S. (14), 'Kaw' (XV), 'Roundtree' (XVI), 'Pawnee' (XVII), and Konza (18). Restored = underlined.

Fig. 12. Principle component analysis of *Andropogon gerardii* RAPD data, from 13 Illinois populations, two remnant Arkansas populations, and three cultivars. The first three principle axes accounted for 43% of the variation. Illinois populations (not labeled), Roth Prairie Arkansas (1), Fairmont Prairie Arkansas (2), 'Kaw' (III), 'Roundtree' (IV), and 'Pawnee' (V).

Fig. 1. UPGMA phenogram of genetic relationships among three remnant and three restored *Dalea purpurea* populations in Illinois. Cavalli-Sforza and Edwards chord distance among populations based on allozyme allele frequencies.

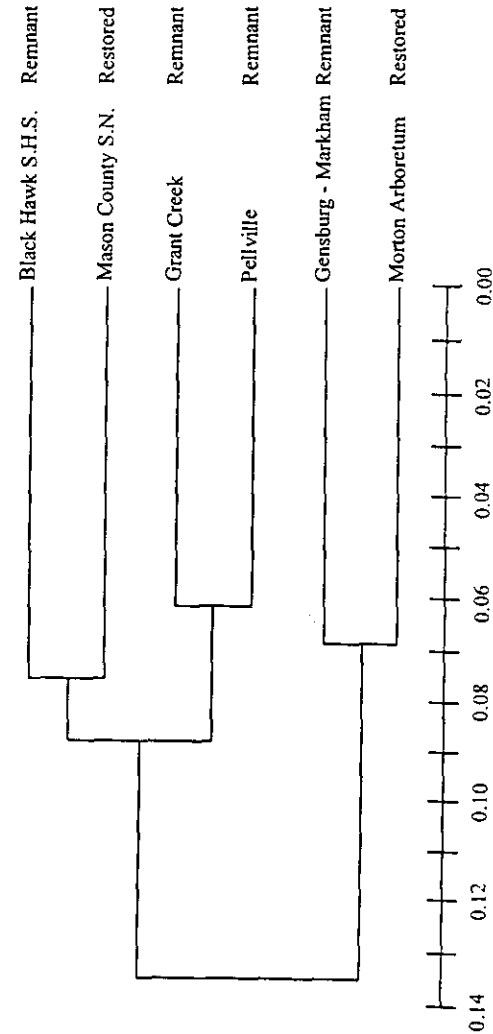


Fig. 2. Principle component analysis of genetic relationships (allozyme data) among six *Dalea purpurea* populations from Illinois and Konza Prairie Research Natural Area, KS. The first three principle axes accounted for 78% of the variation. Mason County S.N. (1), Black Hawk S.H.S. (2), Pellville (3), Grant Creek (4), Morton Arboretum (5), Gensburg - Markham (6), and Konza Prairie (7). Restored = underlined.

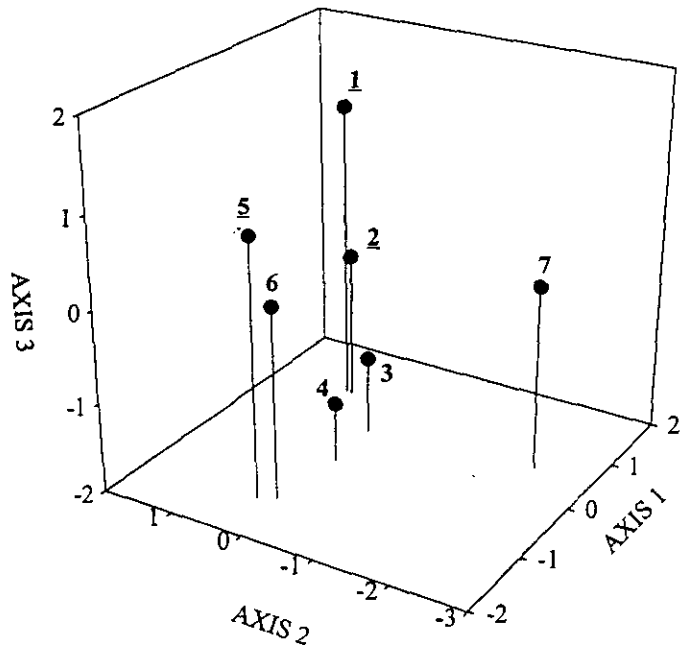


Fig. 3. Principle component analysis of six *Dalea purpurea* populations in Illinois, based on 37 RAPD bands. The first three principle axes account for 78% of the variation. Mason County S. N. (1), Black Hawk S.H.S. (2), Pellville (3), Grant Creek (4), Morton Arboretum (5), and Gensburg - Markham (6). Restored = underlined.

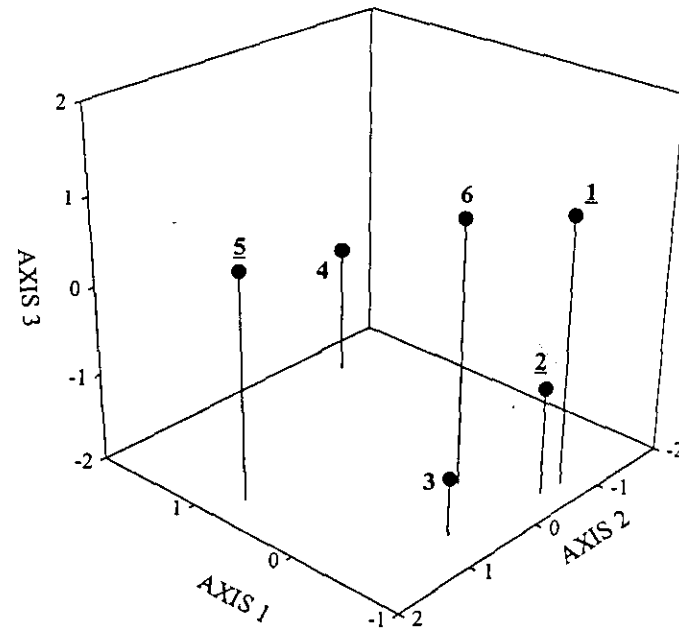


Fig. 4. UPGMA phenogram of *Sorghastrum nutans* genetic relationships among six Illinois populations, two cultivars, and Konza Prairie (KS). Cavalli-Sforza and Edwards chord distance among populations was based on electrophoretic phenotype frequencies.

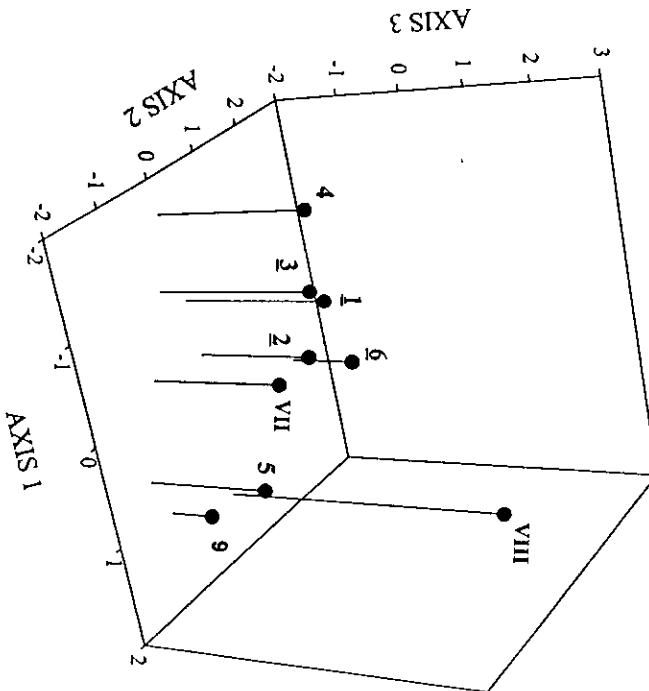
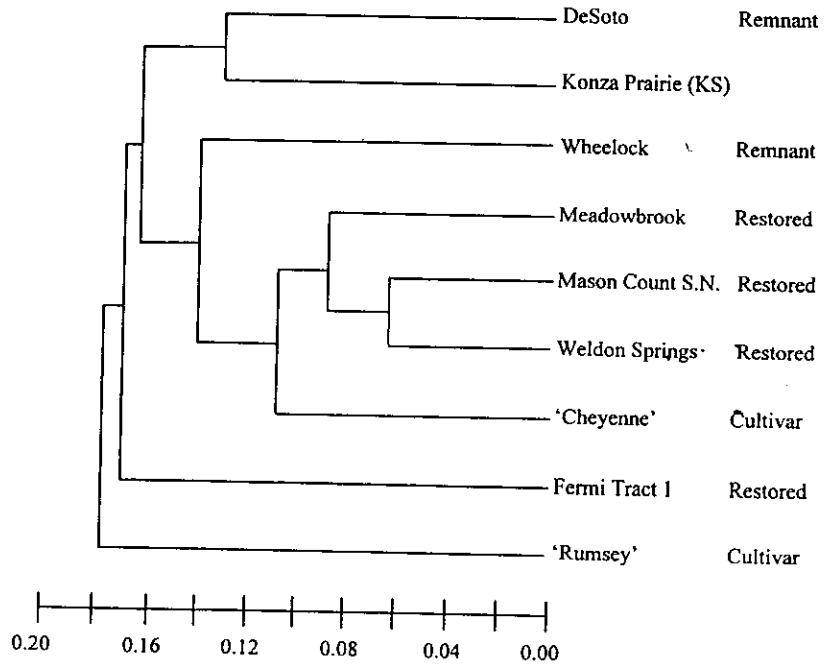


Fig. 5. Principle component analysis of *Sorghastrum nutans* genetic relationships among six Illinois populations, two commercial cultivars, and Konza Prairie (KS), based on allozyme electrophoretic phenotype frequencies. The first three principle axes accounted for 75% of the variation. Mason County S.N. (1), Meadowbrook (2), Weldon Springs (3), Wheelock (4), DeSoto (5), Fermi Tract 1 (6), 'Cheyenne' (VII), 'Rumsey' (VIII), and Konza Prairie (9). Restored = underlined and Cultivar = roman numbers.

Fig. 6. Cluster analysis of *Andropogon gerardii* genetic relationships among seven remnant Illinois populations. Rogers genetic distance between populations was based on the electrophoretic phenotype frequencies.

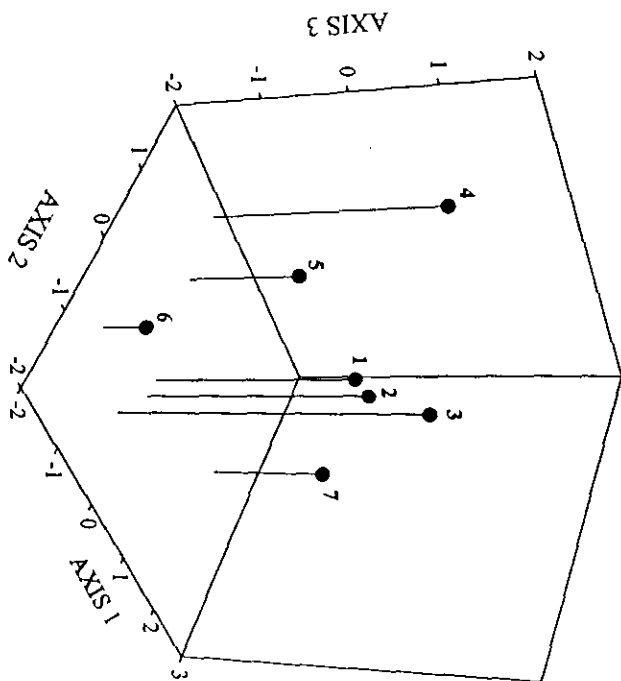
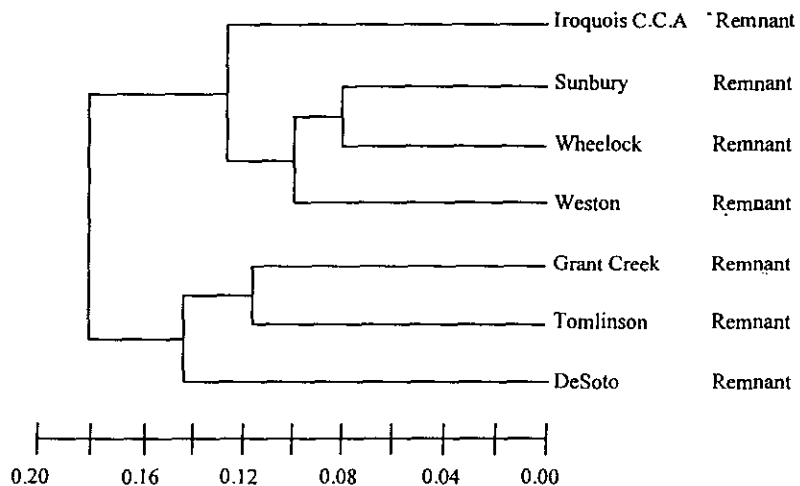


Fig. 7. Principle component analysis of seven Illinois populations of *Andropogon gerardii*, based on allozyme phenotype frequencies. The first three principle axes accounted for 72% of the variation. Weston (1), Wheelock (2), Sunbury (3), Grant Creek (4), Tomlinson (5), DeSoto (6), and Iroquois C.C.A. (7).

Fig. 8. *Andropogon gerardii* principle component analysis of seven remnant Illinois and Konza Prairie (KS). The first three principle axes accounted for 64% of the variation. Weston (1), Wheelock (2), Sunbury (3), Grant Creek (4), Tomlinson (5), DeSoto (6), Iroquois C.C.A. (7), and Konza Prairie (8).

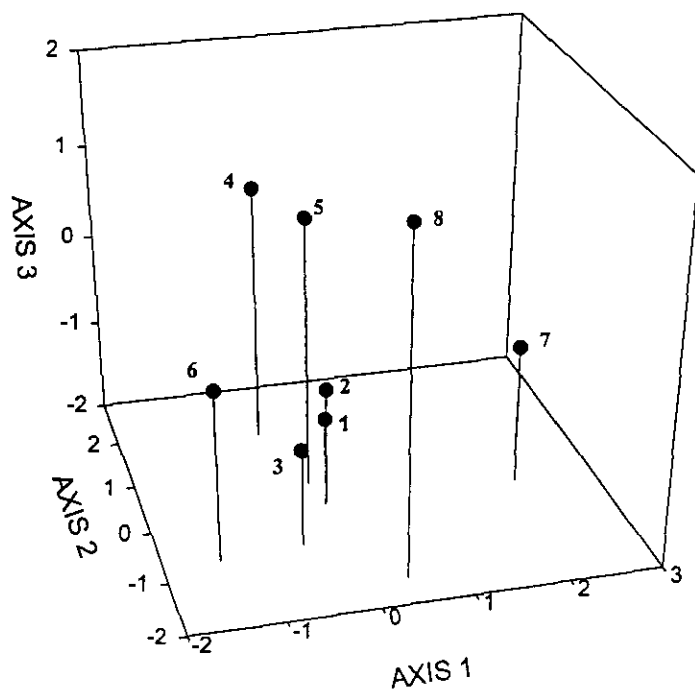


Fig. 9. *Andropogon gerardii* principle component analysis from seven remnant Illinois populations and three cultivars. The first three axes accounted for 55% of the variation. Weston (1), Wheelock (2), Sunbury (3), Grant Creek (4), Tomlinson (5), DeSoto (6), Iroquois C.C.A. (7), 'Kaw' (VIII), 'Pawnee' (IX), and 'Roundtree' (X).

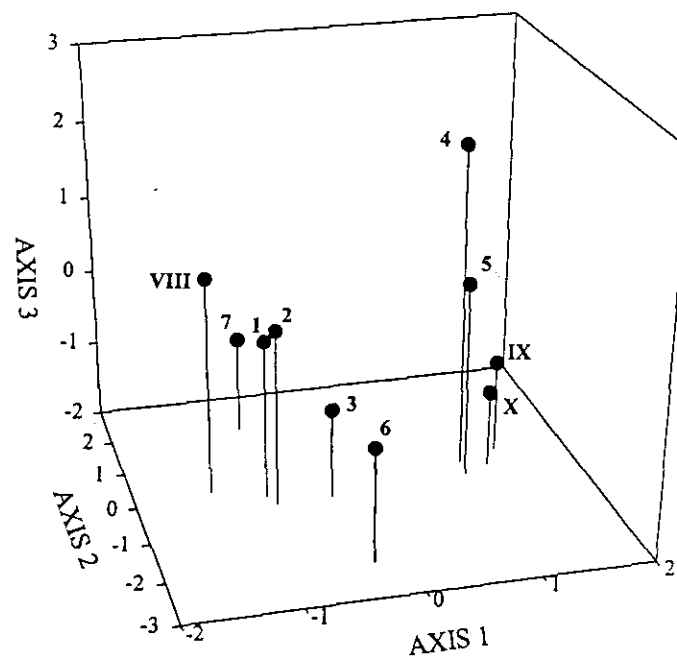


Fig. 10. *Andropogon gerardii* principle component analysis from seven remnant and seven restored Illinois populations, based on allozyme phenotype frequencies. The first three principle axes accounted for 54% of the variation. Weston (1), Wheelock (2), Sunbury (3), Grant Creek (4), Tomlinson (5), DeSoto (6), Iroquois C.C.A. (7), Goose Lake (8), Weldon Springs (9), Fermi Tract 1 (10), Meadowbrook (11), Albany Mounds (12), Mason County S.N. (13), and Black Hawk S.H.S. (14). Restored = underlined.

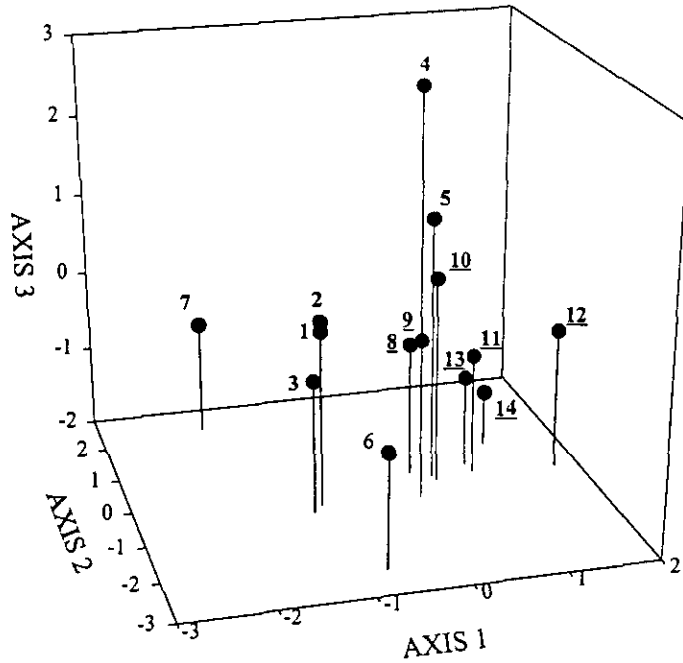
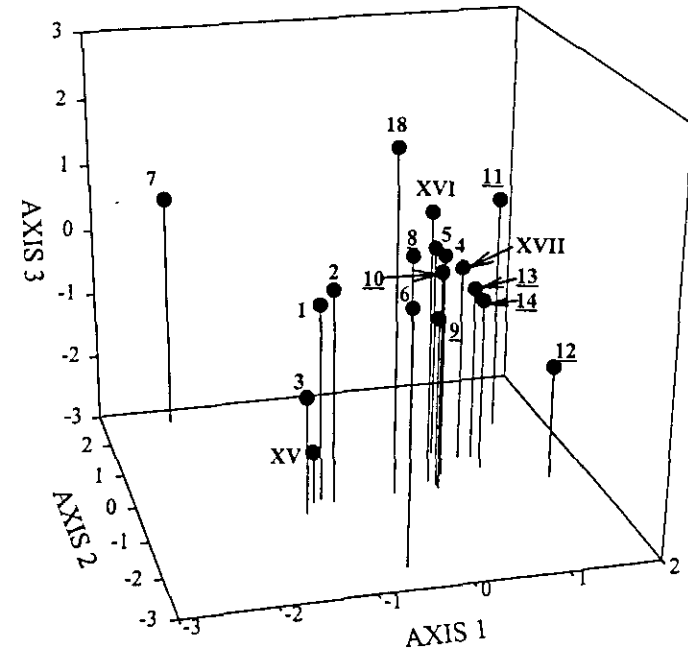
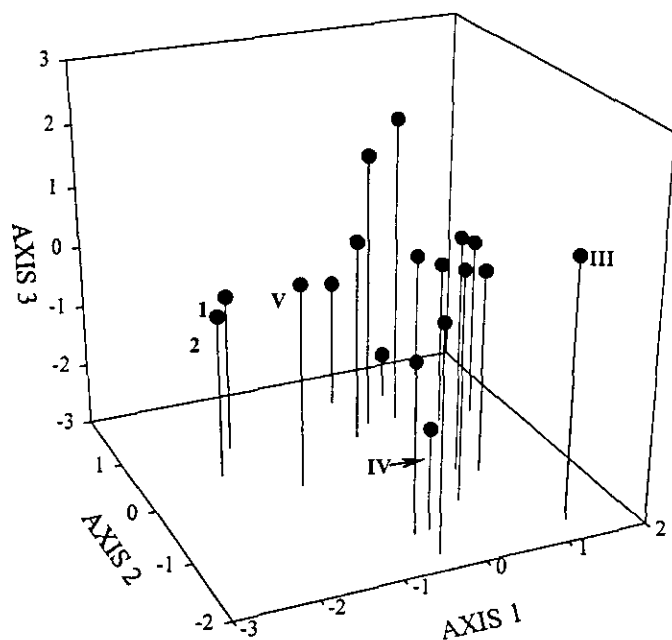


Fig. 11. *Andropogon gerardii* principle component analysis of 14 Illinois populations, three cultivars, and Konza Prairie. The first three principle axes accounted for 45% of the variation. Weston(1), Wheelock(2), Sunbury(3), Grant Creek(4), Tomlinson(5), DeSoto(6), Iroquois C.C.A.(7), Goose Lake(8), Weldon Springs(9), Fermi Tract 1 (10), Meadowbrook(11), Albany Mounds(12), Mason County S.N.(13), Black Hawk S.H.S.(14), 'Kaw' (XV), 'Roundtree' (XVI), 'Pawnee'(XVII), Konza(18). Restored = underlined.



Appendix A

Fig. 12. Principle component analysis of *Andropogon gerardii* RAPD data, from 13 Illinois populations, two remnant Arkansas populations, and three cultivars. The first three principle axes account for 43% of the variation. Illinois populations (not labelled), Roth Prairie Arkansas (1), Fairmont Prairie Arkansas (2), 'Kaw' (III), 'Roundtree' (IV), 'Pawnee' (V).

1) *Dalea purpurea* enzyme PCA with six Illinois populations and Konza Prairie (KS).

	<u>Eigenvalues</u>	<u>Cumulative Variation (%)</u>
Axis 1	6.41	40
Axis 2	3.61	63
Axis 3	2.48	78

2) *Dalea purpurea* RAPD PCA with six Illinois populations.

	<u>Eigenvalues</u>	<u>Cumulative Variation (%)</u>
Axis 1	12.08	33
Axis 2	9.55	58
Axis 3	7.45	78

3) *Sorghastrum nutans* enzyme PCA with six Illinois populations, two cultivars, and Konza Prairie (KS).

	<u>Eigenvalues</u>	<u>Cumulative Variation (%)</u>
Axis 1	5.89	29
Axis 2	5.37	56
Axis 3	3.90	75

4) *Andropogon gerardii* enzyme PCA with seven remnant Illinois populations.

	<u>Eigenvalues</u>	<u>Cumulative Variation (%)</u>
Axis 1	7.11	31
Axis 2	5.62	55
Axis 3	3.88	72

5) *Andropogon gerardii* enzyme PCA with seven remnant Illinois populations and Konza Prairie (KS).

	<u>Eigenvalues</u>	<u>Cumulative Variation (%)</u>
Axis 1	6.36	25
Axis 2	5.36	47
Axis 3	4.38	64

Appendix A

6) *Andropogon gerardii* enzyme PCA with seven remnant Illinois populations and three cultivars.

	<u>Eigenvalues</u>	<u>Cumulative Variation (%)</u>
Axis 1	5.27	21
Axis 2	4.71	40
Axis 3	3.87	55

7) *Andropogon gerardii* enzyme PCA with seven remnant and seven restored Illinois populations.

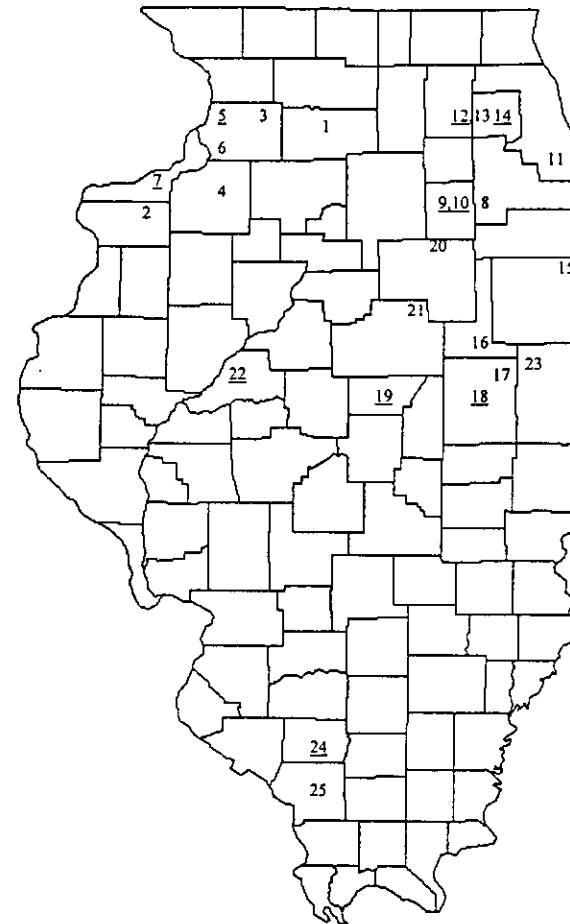
	<u>Eigenvalues</u>	<u>Cumulative Variation (%)</u>
Axis 1	5.85	23
Axis 2	4.42	41
Axis 3	3.32	54

8) *Andropogon gerardii* enzyme PCA with 14 Illinois populations, three cultivars, and Konza Prairie (KS).

	<u>Eigenvalues</u>	<u>Cumulative Variation (%)</u>
Axis 1	5.06	19
Axis 2	3.59	32
Axis 3	3.52	45

10) *Andropogon gerardii* RAPD PCA with 14 Illinois populations, three cultivars, and two remnant Arkansas Prairie.

	<u>Eigenvalues</u>	<u>Cumulative Variation (%)</u>
Axis 1	6.54	18
Axis 2	4.97	31
Axis 3	4.40	43



Appendix B. Map of Illinois with 25 collections sites (remnant and restored). Green River Conservation Area (1), Brownlee Cemetery Prairie (2), Lyndon-Agnew Railroad Prairie (3), Munson Township Cemetery Prairie (4), Albany Mounds (5), Wheelock Railroad Prairie (6), Black State Historical Site (7), Grant Creek Prairie (8), Goose Lake Prairie (NE : 9, local : 10), Gensburg-Markham Prairie (11), Fermi Laboratory (tract #1 : 12, railroad : 13), Morton Arboretum (14), Iroquois County Conservation Area (15), Prospect Cemetery Prairie (16), Tomlinson Cemetery Prairie (17), Meadowbrook Park (18), Weldon Springs (19), Sunbury Railroad Prairie (20), Weston Cemetery Prairie (21), Mason County State Nursery (22), Pellville Cemetery Prairie (23), Freeman United Mine (24), and DeSoto Railroad Prairie (25).