Goose Lake Prairie State Natural Area Project: *Andropogon gerardii* Vitman (Big Bluestem) Genetics and Gene Flow.

Danny J. Gustafson and Daniel L. Nickrent
Department of Plant Biology
Southern Illinois University
Carbondale, IL 62901-6509, U.S.A.
Correspondence: Danny J. Gustafson. Tel: 217-789-2749;
E-mail: dgustaf@siu.edu

Running Title: *Andropogon* Genetics and Gene Flow.
Keywords: *Andropogon gerardii*; gene flow, introgression, tallgrass prairie restoration.
*This project was funded by the Illinois Department of Natural Resources, Natural Heritage Division from contributions to the Illinois Wildlife Preservation.

Abstract

Genetic diversity, relatedness, and gene flow of *Andropogon gerardii* (big bluestem) were studied in three restored tallgrass prairies, using isozyme and agronomic traits. These three populations differed in seed source (Nebraska and local), time since establishment, and spatial distribution within the Goose Lake Prairie State Natural Area, Grundy County, Illinois. The results of this study show that the Nebraska plants were morphologically and genetically different from the local plants. Differences in outcrossing rates were attributed to pollen dispersal distance and not genetic structure or seed source. The established *Andropogon* populations maintained their genetic identity for more than 20 years, despite the intraspecific hybrid progeny being genetically different than the parental population. Seedling recruitment is rare in established *Andropogon* populations. Hence, the introgression of non-local genes into a remnant population through seedling establishment would likely take many generations, depending on the intraspecific hybrid fitness relative to the native genotypes. The use of this hybrid seed in a prairie restoration may affect the success of the project. If the hybrid progeny possess the agronomic traits for which forage grasses were developed, then establishment of more conservative prairie plants could be difficult.

Introduction

As defined by the Society of Ecological Restoration Board of Directors, restoration ecology is the process of repairing damage caused by humans to the diversity and dynamics of indigenous systems (Jackson et al. 1995). Prairie restorationist throughout the Midwest have made great strides in restoring the species composition and structural components of the tallgrass prairie, however the historical genetic composition of the indigenous tallgrass prairies is in question. A baseline genetic survey of *Andropogon gerardii* Vitman (big bluestem) from remnant and restored Illinois prairies and three cultivars showed that the restored populations sampled were more genetically similar to the

cultivars then the remnant Illinois populations (Gustafson et al. 1997). Given the strong emphasis to use 'local' seed, the intentional introduction of non-Illinois (cultivar) seed into restoration projects seems unlikely. It was speculated that pollen dispersal from cultivar populations into the native Illinois populations could be responsible for these associations. Gene flow among genetically divergent restored populations has become a major concern in the restoration of plant populations, because of the potential disruption of local adaptation by an increased influx of inappropriate genetic material (Montalvo et al. 1997). Rhymer and Simberloff (1996) suggested that introgression, rather than inbreeding depression, is the largest threat to our compromised ecosystems.

Andropogon gerardii (hereafter referred to as *Andropogon*) is a native, polyploid, warm-season, perennial prairie grass that is a widespread component of the North American prairie biome and is a dominant species of the tallgrass prairie (Weaver & Fitzpatric 1934; Keeler 1990; McKone et al. 1998). This species is considered a complex polyploid with two cytotype polyploid races (hexaploid 2n=6x=60 and enneaploid 2n=9x=90), with an increased frequency of the enneaploid in the western range of the species (Keeler 1990). The 9x cytotypes were larger and taller than the 6x cytotypes, with no difference in seed produced per unit area (Keeler & Davis 1999). These polyploid cytotypes interbreed and produce fertile euploid and aneuploids, although aneuploids were rarely observed in nature (Keeler 1992; Norrmann et al. 1997). *Andropogon* has a pre-zygotic incompatibility mechanism, failure of the pollen tube to penetrate and grow into the style, that results in low seed set (0.2 – 6%) following self-pollination (Norrmann et al. 1997). In addition, none of the self-pollinated progeny survived beyond the first growing season (post-zygotic incompatibility mechanism) (Norrmann et al. 1997).

In this study, we examined isozyme variation in three restored *Andropogon* populations. These populations differed in the origin of the seed (Nebraska or local) used in the restoration, time since establishment (1970's or

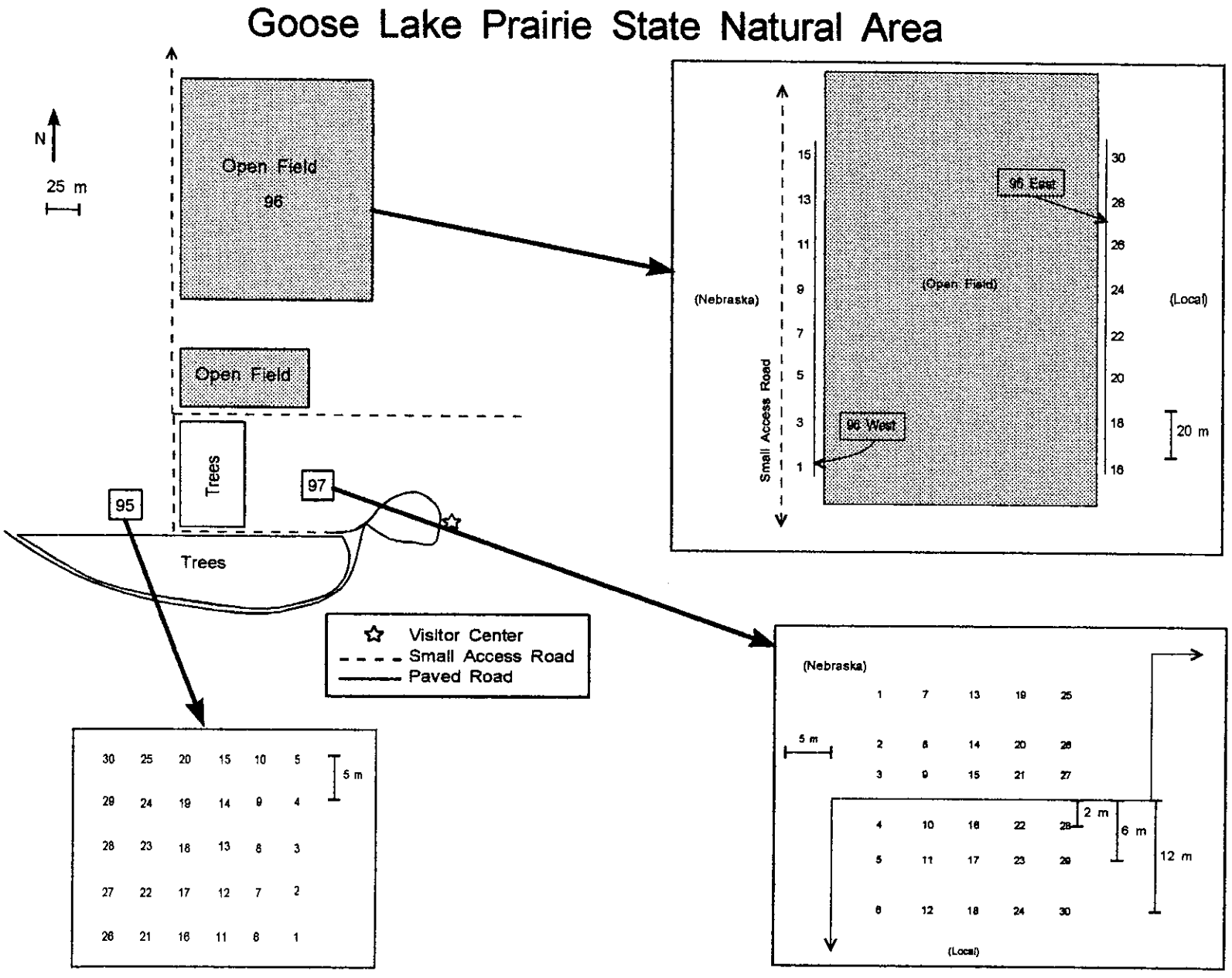
1990's), and spatial distribution within Goose Lake Prairie State Natural Area, Illinois. Second, we examined several performance measures of the local and Nebraska *Andropogon* plants growing in the field. Third, we examined isozyme variation in the progeny of several families from all three restored populations. The objectives of this study were to: 1) estimate genetic diversity of established *Andropogon* plants and their progeny; 2) test for genetic structuring within these restored populations; 3) estimate outcrossing rates; 4) determine if gene flow (via pollen) from the Nebraska genotype could alter the local genotype; and 5) compare performance measures of Nebraska and local *Andropogon* growing in the field.

Materials and Methods

Study sites and plant material: Goose Lake Prairie State Natural Area (hereafter referred to as Goose Lake Prairie), Grundy County, Illinois, was located approximately 50 miles southwest of Chicago and one mile southwest of the confluence of the Kankakee and Des Plaines rivers. The original 97 ha. was purchased by the State of Illinois in 1969, which now totals 1027 ha. of remnant and restored tallgrass prairie (IDNR information pamphlet).

Three sites were selected for this study, with vegetative material from flowering plants being collected 25 July 1998 (Fig. 1). The first location (Site 95) was a 17 ha. prairie restored in the 1970's with Nebraska seed. This site was used to investigate fine-scale genetic structuring, gene flow, and outcrossing rates in a homogeneous population of Nebraska *Andropogon*. Six transects were located in a north-south direction and spaced five meters apart. Along each transect, a single flowering *Andropogon* plant was randomly sampled every five meters (total of five plants per transect). These plants were marked with survey flags and fresh leaf material was collected. For all sites, fresh leaf material was stored on ice, transported to Southern Illinois University at Carbondale (SIUC), extracted, and stored at –80 ° C.

Figure 1. Site map with the location and sampling strategies illustrated for the three restored *Andropogon* populations.



The second location (Site 96) was a large restored (local seed) prairie abutting the Nebraska population (Site 95). This entire area was initially planted using a seed drill, which is evident by the spatial distribution (one meter intervals) of the established plants. However, a section (ca. 150m by 200m) did not become established with prairie plants, hereafter referred to as Open Field. Whether modifications to the soil chemistry (fertilizer or herbicides), hydrology (tiling), or soil compaction (plow pan), it appears that previous agricultural practices may have been responsible for the lack of prairie plant establishment. Site 96 was used to investigate large-scale genetic structure, gene flow, and outcrossing rates of local *Andropogon* planted adjacent to and 150 m from the Nebraska population. Two transects were established in a north-south orientation, with the west transect being located in the local *Andropogon* population directly adjacent to the Nebraska population and the east transect located 150 m to the east (Fig. 1). A total of fifteen flowering *Andropogon* plants were randomly sampled per transect (10 m intervals), marked with survey flags, and fresh leaf material was collected.

The third location (Site 97) was a restored in the 1990's with Nebraska *Andropogon* planted adjacent to local *Andropogon* (Fig. 1). The casual observer could see differences between the Nebraska and local plants, with a clear line of demarcation between the two plantings based on plant size (Site Manager, personal communication). This site was used to investigate fine-scale genetic structuring, gene flow, and outcrossing rates in a restored prairie with distinct Nebraska and local *Andropogon* populations. Five parallel transects (5 m apart) were established perpendicular to the east-west demarcation line separating the two populations, six plants (three of Nebraska and three local) were randomly sampled per transect, marked with survey flags, and fresh leaf material was collected.

On 27 September 1998, plant performance measures were recorded on marked plants in Site 95 and Site 97. These measures included the maximum

height, number of flowering culms, and the number of inflorescences. The presence of insect damage (usually borrowing into and destroying the immature inflorescence) and the presence of an inflorescence with green tissue (as a measure of maturity) were recorded as presence / absence data. Seeds were collected from the 30 marked plants in each of the three sites, transported to SIUC, and stored at 4 °C. In March 1999, *Andropogon* seeds were germinated, grown one week, then extracted for the isozyme analysis.

Isozyme electrophoresis: Approximately 0.5 g of fresh leaf material was homogenized in the Tris-HCl extraction buffer of Wendel and Weeden (1989), centrifuged at 10,000 r.p.m. for 15 min and the supernatant stored frozen (-80 °C) in 1.5 ml microcentrifuge tubes until needed. Enzyme separation was accomplished using 13% w / v starch gel (Starch Art hydrolyzed potato starch, Smithville, TX). Four enzyme systems, coding for five putative loci, were assayed using a Tris EDTA Borate pH. 8.0 gel / electrode buffer system. Enzyme staining protocols were essentially as reported in Wendel and Weeden (1989). The following enzyme systems (with locus abbreviations and enzyme commission numbers in parentheses) were used: aspartate amino transferase (AAT-2, 2.6.1.1), glucose phosphate isomerase (GPI-2, 5.3.1.9), malate dehydrogenase (MDH-1, 1.1.1.37) and phosphoglucomutase (PGM-2, PGM-3, 2.7.5.1).

The most common allele at a locus was assigned a mobility number of 100 and all other bands given numbers relative to it. Because *Andropogon* was a polyploid, gels were scored from banding phenotypes at putative loci. In addition to multilocus phenotype profiles for each individual, band frequencies were calculated for each locus and for all populations.

Statistical analyses: Descriptive measures of genetic diversity were calculated following Jonsson et al. (1996): (i) ramets (*R*) or seedlings (*S*) sampled; (ii) number of multilocus phenotypes (*MP*); and (iii) Simpson's diversity index (*D*) corrected for finite sample size. To test for genetic structure, Jaccard's similarity

and geographic distance between individuals within a population were compared using Mantel's test (PC-ORD 1995). This procedure used a Monte Carlo randomization test (1000 iterations) which calculates the standardized Mantel's statistic (Z) of the observed data and the probability of exceeding this value by random chance. The null hypothesis was that the degree of genetic similarity between individual plants and geographic distance between them were not related.

Multilocus (t_m) and single locus (t_s) outcrossing rates were calculated using MLTET, which is a generalized program for estimating inbreeding parameters and outcrossing rates in tetraploids (Ritland 1990). The multiple and single outcrossing rates represent the proportion of progeny that are the result of outcrossing, 1.00 being the maximum value. For this analysis, individual plants were scored as tetraploids, including unbalanced heterozygotes (i.e. AAAB), using dosage intensity of the banding patterns. This method of coding genotype data underestimates the genetic information per individual, because a hexaploid individual (AAABBC) would have to be coded as a tetraploid individual (AABC). Therefore, the results of the outcrossing analyses should be considered conservative estimates.

Population level relationships were investigated using euclidean distance, based on frequency data, and cluster analysis (unweighted pair group means analysis - UPGMA) (PC-ORD 1995).

Kruskal-Wallis one-way analysis of variance was used to test for differences in plant performance measures (maximum height, number of culms, number of inflorescences, insect damage, and maturity) among plants originally from the Nebraska and local seed sources (SigmaStat 1995).

Results

Diversity of established plants: Genetic diversity estimates of the established *Andropogon* populations ranged from 0.86 – 0.97, with Site 95 being the most diverse (Table 1). In Site 95, there were twenty-one distinguishable multilocus

Table 1. Maternal and progeny (seedling) diversity in three big bluestem populations at Goose Lake Prairie, quantified by the number of sampled maternal ramets (*R*), distinquishible multilocus phenotypes (*MP*), and Simpson's diversity index (*D*) corrected for finite sample size.

		MATERNAL			OFFSPRING			
Population	Seed Source	*R*	*MP*	*D*	Maternal Plants	Seedlings (*S*)	*MP*	*D*
Site 95								
	Nebraska	29	21	0.97	12	30	8	0.71
Site 96								
	West (local)	15	12	0.96	14	76	16	0.80
	East (local)	15	8	0.85	5	32	10	0.84
	Overall	30	18	0.95	19	108	19	0.85
Site 97								
	Nebraska	15	10	0.93	9	35	12	0.83
	Local	15	7	0.78	11	20	11	0.94
	Overall	30	15	0.86	20	55	19	0.86

phenotypes identified from 29 plants sampled. Having been established with Nebraska seed in the mid-1970's, Site 95 was also the oldest site sampled in this study.

The genetic diversity estimate for Site 96 (D = 0.95) was similar to Site 95 (D = 0.97), despite the eleven percent difference between the west and east sub-samples (Table 1). It is possible that the west transect was in the 10-15 m of uncultivated land between the original agricultural field and a small access road that parallels the Open Field's west boundary. If this were the case, the high genetic diversity estimates could be the result of the west section containing a mixture of remnant and planted *Andropogon*, while the east section consisted of only planted material.

Site 97 had the lowest genetic diversity (D = 0.86) of all sites sampled and the largest difference (16 %) between the two sub-samples (Table 1). The sampling scheme (spacing of sampling points and total area covered) and the method of establishing the prairie (via broadcasting seed) were similar to that of Sites 95, yet Site 97 genetic diversity was 11% less than Site 95. This result was surprising given that Site 97 contains Nebraska and local *Andropogon* in discrete blocks (Fig. 1). With the sample containing half Nebraska and half local *Andropogon*, one may expect higher levels of genetic diversity than a population established with a single seed source. The genetic composition of the seed initially planted in Site 97 could account for the low diversity estimates in the established population.

Diversity of the progeny: Site 95 offspring sample was restricted to $\leq$ three seedlings per maternal plant. There was a 27% decrease in genetic diversity from 0.97 to 0.71 in one generation (Table 1). Site 96 had an 11% decrease in genetic diversity mostly attributed to the 17% decreased in the west sub-sample. There was no change in overall genetic diversity estimates between the established *Andropogon* and their offspring in Site 97 (Table 1). Although the Nebraska population diversity estimate decreased by 11%, which was the

average decrease between generations, while the local population had the only increase in genetic diversity from 0.78 to 0.94 (Table 1).

Genetic Structure: The results of the Mantel's tests indicated no relationship between geographic location and genetic similarity in Site 95 (Z=0.39, p=0.28) and Site 96 (Z=0.01, p=0.41). There was a significant negative association (Z=0.28, p=0.03) in Site 97, suggesting that genetic similarity decreased as the distance between plants increased. This result was not surprising given that Site 97 contained discrete Nebraska and local populations adjacent to one another.

Outcrossing rates and inbreeding estimates: Outcrossing rates varied among the three sites. Multilocus estimates (t_m) ranged between 0.60 (Site 96 – east) to 1.00 (Site 95), with the mean single-locus estimates (t_s) similar to the multilocus estimates (Table 2). The west transect in Site 96, which was located 10 meters east of a Nebraska population, had higher outcrossing rates and lower pollen pool inbreeding estimates than the east transect. Only five of the 15 maternal plants along the east transect, which was 150 m east across the Open Field, produced seedlings. With a predominately west to east wind and pollen dispersal following a leptokurtic probability distribution (Nurminiemi et al. 1998), it appears that the low reproductive output of the east transect was the result of limited pollen dispersal and self-incompatibility mechanisms.

Site 97 outcrossing estimates were similar between the Nebraska (0.79) and local (0.77) populations. The close proximity of two genetically different pollen donor populations, as seen with Site 96 – west transect, would decrease the likelihood of parental (selfing) or bi-parental (e.g. sibling mating) inbreeding. The pollen pool inbreeding coefficients were twice as high as the other populations sampled, suggesting a limited number of *Andropogon* plants contributed disproportionately to the pollen pool (Table 2).

Population relationships: The genetic relationships among the Goose Lake Prairie populations (maternal and offspring) were investigated using band

Table 2. Multilocus (t_m) outcrossing rates, single-locus (t_s) outcrossing rates, and parental inbreeding coefficients (F) in three restored big bluestem populations.

Population	Seed Source	Maternal	Seedlings	t_m	t_s	$F_{(parental)}$	$F_{(pollen\ pool)}$
Site 95	Nebraska	12	30	1.00 (0.23)	1.00 (0.13)	0.40 (0.00)	0.32 (0.10)
Site 96							
	West (local)	14	76	0.81 (0.10)	0.87 (0.06)	0.40 (0.00)	0.46 (0.11)
	East (local)	5	32	0.60 (0.10)	0.50 (0.08)	0.40 (0.00)	0.55 (0.11)
	Overall	19	108	0.68 (0.05)	0.72 (0.04)	0.40 (0.00)	0.50 (0.09)
Site 97							
	Nebraska	9	35	0.79 (0.09)	0.77 (0.07)	0.40 (0.00)	0.88 (0.08)
	Local	11	20	0.77 (0.03)	0.78 (0.07)	0.40 (0.00)	1.00 (0.00)
	Overall	20	55	0.80 (0.06)	0.80 (0.05)	0.40 (0.00)	0.92 (0.05)

Standard errors are in parentheses, based on 500 bootstraps.

frequency data and UPGMA cluster analysis (Fig. 2). The maternal Nebraska populations grouped together, indicating that non-local genotypes that have become established can maintain the original genetic composition.

Offspring from Site 95 and Site 96 – west grouped together, even though the maternal populations were different. The close proximity of Site 96 – west to the 16.2 ha Nebraska population suggests that pollen from Site 95 is changing the genetic composition of the local population's seed. In the event that seedling recruitment were to occur, the next generation of *Andropogon* in Site 96 – west would be genetically different than the current population. Within Site 97, the Nebraska and local offspring clustered together and were then grouped with the offspring from Site 95 and Site 96 – west. In both situations, there was a Nebraska population planted adjacent to a local population. This offspring group clustered with the maternal Site 97 – local population.

The maternal and offspring from Site 96 – east were clustered together. Low outcrossing rates and only 33% of the established plants producing seedlings could be indicative of increased inbreeding relative to Site 96 – west. Located on the eastern edge of the Open Field and the distribution pattern of wind-dispersed pollen suggests an edge effect, with some distance limit to which pollen is effectively dispersed.

Plant performance: The Nebraska *Andropogon* plants were consistently shorter, produced fewer culms, and fewer inflorescences than plants of local origin (Table 3). These results were consistent with the site manager's personal observations, 'the Nebraska plants don't do as well as the local plants.' In addition to the difference in plant performance, the Nebraska plants were more frequently damaged by insects (23%) and matured later in the growing season than the local plants (Table 3).

Figure 2. Cluster analysis of Euclidean distances among populations, based on isozyme band frequencies.

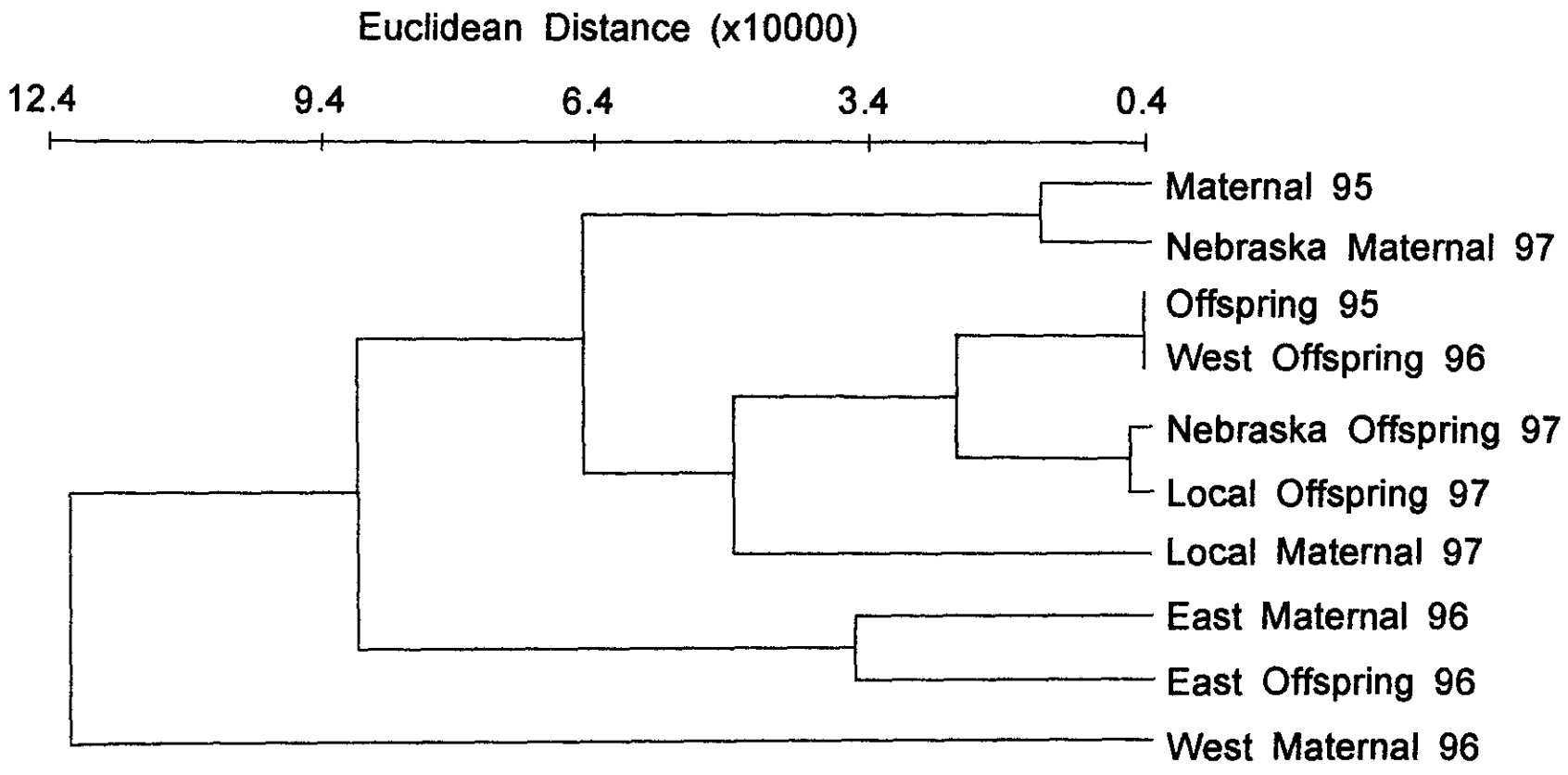


Table 3. Mean (1 SE) plant performance measures of big bluestem originally from Nebraska and local seed sources growing at Goose Lake Prairie.

Original Seed Source	n	Height (cm)	Culms	Inflorescences	Insect Damage	Green
Nebraska	44	176.0 (3.5) a *	1.7 (0.2) a	10.7 (1.2) a	0.23 (0.06) a	0.50 (0.08) a
Local	15	243.7 (3.3) b	11.2 (1.3) b	63.1 (11.8) b	0.00 (0.00) b	0.00 (0.00) b

* Different letters in the same column indicate significant differences ($p<0.05$) between groups.

Discussion

Populations established by seed, as are most restored prairies, are expected to consist of a large number of randomly distributed genotypes (Ellstrand & Roose 1987). Our results show that all three restored *Andropogon* populations were genetically diverse, with a decrease in genetic diversity between the parental and offspring generations. The oldest restored population (Site 95) was the most diverse, which is the opposite of what one would predict based on our parental and offspring diversity estimates. Several studies of grassland species have demonstrated an increase in viability associated with increased heterozygosity (Clegg & Allard 1973; Shaal & Levin 1976; Clegg et al. 1978; Ritland & Gander 1987; Dudash 1990; Fenster 1991). By removing the homozygous genotypes, selection would increase the proportion of heterozygous individuals as the cohort ages. This pattern of genotypic change has also been observed in forest tree species and was interpreted as selection against homozygous individuals (Mitton 1989 and citations therein).

Ennos and Clegg (1982) used computer simulations to show that if genotypes were clumped in space (genetically structured population) and pollen flow was limited, then outcrossing rates would be reduced relative to a non-structured population. In two previous studies conducted at Goose Lake Prairie, Dudash (1990) and Fenster (1991) demonstrated that progeny derived from within genetic neighborhood crosses were less fit then progeny from among neighborhood crosses. However, the only genetically structured population (Site 97) in this study did not have any appreciable decrease in outcrossing rates relative to the non-structure populations. The genetic structuring, in this case, was a result of the Nebraska and local *Andropogon* populations being planted in adjacent area and not genetic structuring as a nonrandom distribution of individuals related by decent.

Alternatively, Site 96-east had low outcrossing rates, low genetic diversity, low reproductive output, and was located 150 m east across the Open

Field with the wind predominately in a west to east direction. Wind dispersed pollen follows a leptokurtic distribution with most of the pollen being dispersed near the pollen donor (Nurminiemi et al. 1998). If the increased inbreeding was a result of limited pollen flow across the Open Field, then this would suggest a distance limitation to *Andropogon* pollen dispersal. Therefore, some physical distance between local and non-local *Andropogon* populations should be established in order to maintain the genetic integrity of the local genotype. Based on our results, a conservative distance of 150 m would be a reasonable starting point.

If a non-local population of *Andropogon* is growing in close proximity to a native population, the two can interbreed freely. The intraspecific hybrid progeny (seed cohort) will be a combination of local and non-local genes. What affect this would have on the native population will depend on the relative fitness of the hybrids, seedling recruitment, and competitive interactions (intra- and inter-specific) within the plant community. Established *Andropogon* populations are maintained almost exclusively by vegetative propagation, despite a seed rain of ca. 3000 seeds/m^2 in native populations (Foster & Gross 1997). Vegetative reproduction through rhizomes and tillering could explain why the Nebraska populations maintain the Nebraska genetic composition for more than 20 years. However, if this seed cohort was harvested and used in a restoration project (limiting the plant material to 'local' sources), then the unintentional introgression of non-local genes into the newly restored population would be instantaneous.

In a study comparing species composition of remnant and adjacent restored tallgrass prairies. Kindscher and Tieszen (1998) found that remnant prairies contained more species than the restored sites and species richness decreases as you moved farther from the remnant prairies. It appeared that early successional species were displaced, but the more conservative native prairie species were unable to become quickly established (Mlot 1990;

Schramm 1990; Sperry 1994). However, the restored prairies were established with commercial warm season grasses or a combination of commercial grasses and forb seed from the adjacent remnant prairie. Aarssen and Turkington (1985) suggested that increased persistence of species associations fosters competitive coevolution whereby competitive abilities become more balanced by reciprocal selection during succession. Hence, the introduction of non-local plants could transfer adaptive genes to remnant populations, with a resulting shift in competitive interactions in the community. This is especially dangerous with grass cultivars that have been selected for fecundity, vegetative vigor, or other traits that promote aggressiveness (Lesica & Allendorf 1999).

Literature Cited

Aarssen, L. W. R. Turkington. 1985. Biotic specialization between neighboring genotypes in *Lolium perenne* and *Trifolium repens* from a permanent pasture. Journal of Ecology 73: 605-614.

Clegg, M. T., and R. W. Allard. 1973. Viability versus fecundity selection in the slender wild oat, *Avena barbata* L. Science **181**:667-668.

Clegg, M.T., A. L. Kahler, and R. W. Allard. 1978. Estimation of life cycle components of selection in an experimental plant population. Genetics **89**:765-792.

Ellstrand, N. C., and M. L. Roose. 1987. Patterns of genotypic diversity in clonal plant species. American Journal of Botany **74**:123-131.

Ennos, R. A., and M. T. Clegg. 1982. Effect of population substructuring on estimates of outcrossing rate in plant populations. Heredity **48**:283-292.

Dudash, M. R. 1990. Relative fitness of selfed and outcrossed progeny in a self-compatible, protandrous species, *Sabatia angularis* L. (Gentianaceae): A comparison in three environments. Evolution **44**:1129-1139.

Fenster, C. B. 1991. Gene flow in *Chamaecrista fasciculata* (Leguminosae) II. Gene Establishment. Evolution **45**:410-422.

Foster, B. L., and K. L. Gross. 1997. Partitioning the effects of plant biomass and litter on *Andropogon gerardii* in old-field vegetation. Ecology **78**:2091-2104.

Gustafson, D. J., D. J. Gibson, and D. L. Nickrent. 1997. 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. Illinois Department of Natural Resources Technical Report.

Jackson, L., N. Lopoukhine, and D. Hillyard. 1995. Commentary Ecological Restoration: a definition and comments. Restoration Ecology **3**:71-75.

Jonsson, B. O., I. Jonsdottir, and N. Cronberg. 1996. Clonal diversity and allozyme variation in populations of the Arctic sedge *Carex bigelowii* (Cyperaceae). Journal of Ecology **84**:449-459.

Keeler, K. H. 1990. Distribution of polyploid variation in big bluestem (*Andropogon gerardii*, Poaceae) across the tallgrass prairie region. Genome **33**:95-100.

Keeler, K. H. 1992. Local polyploid variation in the native prairie grass *Andropogon gerardii*. American Journal of Botany **79**:1229-1232.

Keeler, K. H., G. A. Davis. 1999. Comparison of common cytotypes of *Andropogon gerardii* (Andropogoneae, Poaceae). American Journal of Botany **86**:974-979.

Kindscher, K., and L. L. Tieszen. 1998. Floristic and soil organic matter changes after five and thirty-five years of native tallgrass prairie restoration. Restoration Ecology **6**:181-196.

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

McKone, M.J., C. P. Lund, and J. M. O'Brien. 1998. Reproductive biology of two dominant prairie grasses (*Andropogon gerardii* and *Sorghastrum nutans*, Poaceae): male-biased sex allocation in wind-pollinated plants. American Journal of Botany **85**:776-783.

Mlot, C. 1990. Restoring the prairie. Bioscience **40**:804-809.

APPENDIX A: Site photographs from September 1998.

1. General Goose Lake Prairie State Natural Area prairie picture taken from the observation area atop the visitor center (facing South).

2. General Goose Lake Prairie State Natural Area prairie picture taken from the observation area atop the visitor center (facing East).

3. Site 95. Nebraska population established in the 1970's (facing North).

4. Site 95. Same location (facing West).

5. Site 96. West section with the Open Field on the right (facing North).

6. Site 96. West section looking out across the Open Field (facing East).

7. Site 96. East section looking at the restored plants planted in rows (facing North).

8. Site 96. East section notice the non-random spacing of the plants (facing North).

9. Site 97. Looking down the demarcation line with the Nebraska plant on the left and local plants on the right (facing East).

10. Site 97. Notice that the local *Andropogon* culms on the right were taller than the Nebraska culms (facing East).