Flowering Phenology and Genetic Similarity among Local and Recently Introduced Populations of *Andropogon gerardii* in Ohio

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Abstract

In Ohio and elsewhere, recent grassland plantings in the federal Conservation Reserve Program (CRP) have become much more extensive than native prairie remnants. The seed source for CRP grasslands in Ohio often comes from as far away as Missouri or Texas, which may be undesirable from the standpoint of conservation genetics. The goal of this study was to examine the potential for gene flow from large, recently introduced populations of Big bluestem (*Andropogon gerardii*, Poaceae) to small local populations of this outcrossing perennial species. We examined the potential for cross-pollination between three local populations and three introduced CRP populations by comparing flowering phenologies. Flowering times overlapped extensively, indicating that cross-pollination is possible where local and introduced genotypes co-occur. To compare genetic variation in local and CRP populations, we analyzed variation at 68 RAPD loci in six populations of each type. Somewhat surprisingly, we found no significant differences in the genetic diversity or composition between the two groups (local vs. CRP). In summary, we found that local and introduced populations of Big bluestem have the potential to interbreed, based on their flowering periods, but further research is needed to determine whether local genotypes harbor unique genetic variation that could be jeopardized by hybridization with introduced genotypes.

Key words: ***Andropogon gerardii*, Big bluestem, Conservation Reserve Program, gene flow, genetic diversity, flowering phenology, local genotypes.**

Introduction

In Ohio, tallgrass prairie originally occupied more than 260,000 ha before European settlement, but less than 1% of the original prairie remains, mostly as small fragmented remnants (Troutman 1979). Fortunately, many agencies and private landowners are now actively conserving remnant prairies. The popularity of native prairie species has spurred many restoration projects throughout the state, ranging from large-scale programs to small garden prairies on both public and privately owned land.

One program that has contributed to prairie plantings in Ohio and elsewhere is the United States Department of Agriculture's Conservation Reserve Program (CRP). The CRP was established under the Food Security Act of 1985 to encourage farmers to plant long-term cover to improve soil, water, and wildlife resources. As of 1999, 152,605 ha was allocated to the CRP in Ohio (Swanson et al. 1999). Farmers have a variety of choices of what to plant, but within the past several years there has been a push to plant warm-season native prairie grasses. It has been estimated that CRP grasslands are 50 times more abundant than native prairies in Ohio (Swanson et al. 1999). Seed for these CRP plantings are most often not from local seed sources, which are limited, but from states such as Missouri and Texas. Thus, nonlocal genotypes for CRP grasslands can occur in the vicinity of remnant prairies.

In general, remnant plant populations are thought to have a high genetic diversity and locally adapted genotypes as compared to artificially planted sites (e.g., Fenster & Dudash 1994; Reinartz 1995). When non-native genotypes are introduced, there is the potential for breaking up adapted gene complexes and loss of low-frequency alleles through "swamping" of native material (e.g., Knapp & Rice 1994; Ayres et al. 1999; review in Hufford & Mazer 2003). Although the use of mixed or nonlocal seed for reintroductions has typically been discouraged (IUCN 1995), local stocks may be rare and seed from outside sources must be used as a supplement, especially for large-scale projects. Because native prairie stands in Ohio are scarce, seed are often purchased from large seed companies based farther west.

Very little is known about the genetic composition of remnant populations of Ohio prairie species. Ohio lies on the eastern edge of the prairie peninsula, and its original prairies were described as "an extension of the heart of the prairie province" located in Kansas, Iowa, and Nebraska (Transeau 1935). Ohio prairies have always been disjunct

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from more extensive tallgrass prairie farther west, and this may affect their genetic composition. The purpose of our study was to (1) understand the potential for cross-pollination and subsequent gene flow between CRP and local genotypes of Big bluestem, which is a dominant species of tallgrass prairie and (2) investigate genetic similarities between these two population types using random amplified polymorphic DNA (RAPD) markers.

Materials and Methods

Study Species

Big bluestem (*Andropogon gerardii*) is a perennial, warm-season grass with short rhizomes that give rise to compact bunches of tillers. At maturity, the Big bluestem is 2–3 m tall, with the floral spikes in a characteristic "turkey-foot" pattern. Native to the tallgrass prairie, its range extends from northern Mexico to Canada and from the deciduous forests in the east to the Rocky Mountains in the west (Gleason & Cronquist 1964). Big bluestem is a self-incompatible, wind-pollinated species that typically flowers from mid-July through mid-August (Cochrane 1993). As a polyploid species, it has chromosome numbers ranging from 60 to 90 (Norman et al. 1997). Hexaploids (6x) are more common across its range (Keeler 1992), and 6x and 9x cytotypes are known to interbreed (Norman et al. 1997).

Big bluestem is a model plant in the study of prairies due to its widespread occurrence and the extensive amount of literature on the plant's biology (e.g., Jurik & Kliebenstein 2000; Schultz et al. 2001; Silletti & Knapp 2001). Genetic diversity studies of Big bluestem have been carried out using RAPD markers to examine variation within natural populations (Gustafson et al. 1999; Cavender 2001). This study is the first to use this technique to compare local prairie diversity with CRP grasslands and to examine the synchrony of their flowering times.

Study Sites

Our 13 study sites are concentrated in two formerly glaciated regions of Ohio: Erie Plains (or Firelands) in the north and Darby Plains in central Ohio. All the 13 sites were used for the genetic diversity study, whereas a subset of the populations in the Darby Plains was used in the phenology study (Table 1). Local remnant populations and CRP populations were identified within each study region (Fig. 1). In all the populations except one (Eastman), Big bluestem was very abundant, covering nearly 50% of the site. Other grass species included Indian grass (*Sorghastrum nutans*), Switchgrass (*Panicum virgatum*), and Little bluestem (*Schizachyrium scoparium*). When possible, we interviewed property owners and experts from state agencies to learn the sources of seeds for CRP plantings and the burning history of each site.

In the Erie Plains, we sampled three CRP sites ranging from 0.7 to 2.1 ha in size (Table 1). These sites were planted within the past 4 years with seed purchased from Missouri (Sharp Brothers Seed Co., Clinton, MO, U.S.A.). The two local populations in the Erie Plains include the largest known prairie remnant in Ohio (Resthaven's Castalia Prairie) and a small remnant population along a fencerow (Eastman; Table 1). The burning history at Eastman is unknown, whereas Resthaven has been burned every 2–3 years. These sites are located in Erie, Sandusky, and Ottawa counties and distances among them range from 15 to 43 km (Fig. 1).

In the Darby Plains, we sampled three CRP sites ranging from 2.3 to 2.6 ha in size (Table 1). All the CRP sites were planted within the past 4 years with seed purchased from Texas (Bamer Seed Company, Muleshoe, TX, U.S.A.) and are burned frequently (every 1–2 years). Biggert was burned in the spring of 2001, just before the study began. The local sites in the Darby Plains include four small remnants (Table 1). Two of the sites (Smith and Bigelow) are pioneer cemeteries protected by the state as

Table 1. Ohio study sites for DNA analysis and flowering phenology of Big bluestem.

	CRP	*Local*	*Restored*
Erie Plains	Witt Farm (Ottawa, 0.7 ha), source: Missouri	Resthaven Wildlife Area (Erie, 4.0 ha)	
	Kracer Farm (Ottawa, 2.1 ha), source: Missouri	Eastman hedgerow (Ottawa, 10 plants)	
	Ayres Farm (Sandusky, 0.8 ha), source: Missouri		
Darby Plains	Gardner Road* (Madison, 2.6 ha), source: Texas	Bigelow Cemetery SNP* (Madison, 0.2 ha), burned in the spring 2001	Battelle-Darby (Franklin, 0.7 ha)
	Betherd* (Franklin, 2.6 ha), source: Texas	Smith Cemetery SNP* (Madison, 0.2 ha), burned in the spring 2001	
	Biggert Road* (Franklin, 2.3 ha), burned in the spring 2001, source: Texas	Milford Center Prairie (Union, 0.2 ha)	
		Indian Ridge* (Franklin, 0.3 ha)	

Locations of sites (county and size) and seed sources for CRP plantings are noted. Unless otherwise indicated, $N = 26$ plants per site.
*Sites that were used in the study of flowering phenology; burning in 2001 is noted.

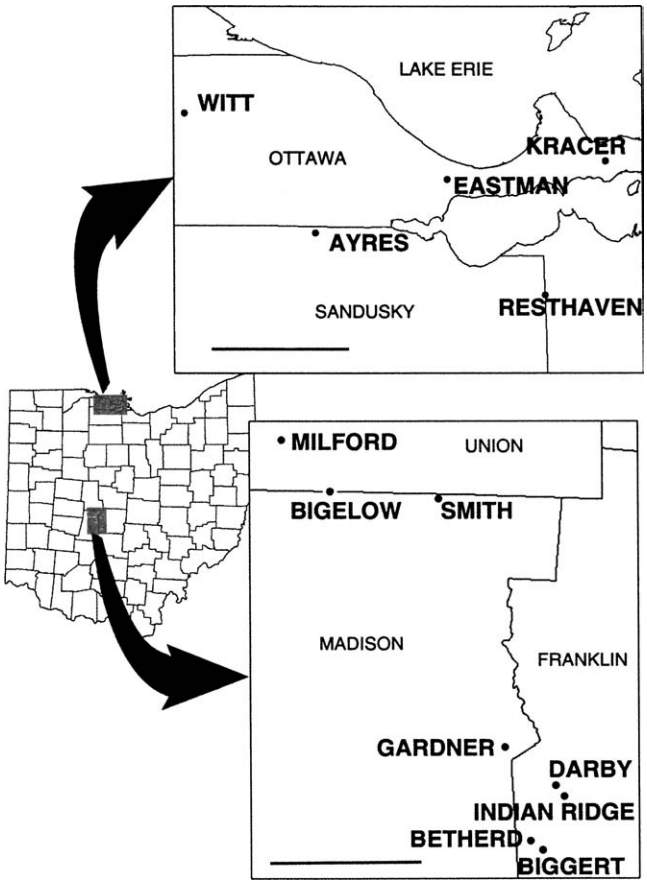

Figure 1. Study populations in the Erie Plains (top) and Darby Plains (bottom) of Ohio. Scale bar = 15 km; asterisks indicates CRP populations.

nature preserves. Milford lies below a powerline along former railroad tracks, and Indian Ridge is located in Battelle-Darby Metropark. All the four remnants are burned on a regular rotation, and Smith and Bigelow were burned in the spring of 2001. An additional site, Battelle-Darby, was planted with locally collected native seed in 1981 and was also used in the genetic analysis. This site is located at the Battelle-Darby Metropark and is burned every 2–3 years. The Darby Plains sites are 1–39 km apart (Fig. 1).

Although the sites were chosen in areas where the potential for gene flow exists, it seems highly unlikely that gene flow between populations of CRP and local prairie has been common. Most sites are separated by fragmented forested and urban areas that are not conducive to gene flow by pollen or seed dispersal among sites. The sites with remnant local populations are small (0.2–4 ha) and would not produce a substantial amount of pollen or seed for long-distance dispersal. Also, the CRP sites have been planted within the past 4 years and have not had an extended period of time to establish and cross-pollinate with local sites. We examined only adult plants and not newly produced seeds or seedlings. Thus, we assume that the genetic analysis at this early stage in the coexistence of CRP and local Big bluestem has not been affected by extensive gene flow among sites.

Flowering Phenology

The potential for cross-pollination between local Big bluestem and introduced genotypes was investigated in 2001 at six sites (three local and three CRP) on the Darby plains in central Ohio (Table 1; Fig. 1). At each site, 40 individuals were flagged and monitored weekly for evidence of stigma receptivity and shedding of pollen. Designated plants were at least 5 m apart to avoid resampling of clonal individuals. Clonal genotypes are fairly easy to distinguish as distinct bunches because Big bluestem does not spread laterally by stolons or extensive rhizomes. Flowering phenology was compared between CRP and local populations, and between unburned and recently burned populations.

Genetic Variation

Sampling and Polymerase Chain Reaction Conditions. Leaf tissue was sampled from a total of 322 individuals in six local populations, six CRP populations, and one restored population (Table 1; Fig. 1). Sampled plants were spaced at least 5 m apart to reduce the likelihood of sampling clonal individuals. DNA was extracted from 26 individuals per population, with the exception of Eastman, where all individuals were sampled ($N = 10$). A modification of the cetyl trimethylammonium bromide (CTAB) method was used to extract total genomic DNA (Doyle & Doyle 1987).

Genetic diversity was assessed using RAPD primers previously known to provide highly variable banding patterns in Big bluestem (Gustafson et al. 1999; Cavender 2001). We used two 10-mer primers (OPB-7 and OPB-12; Operon Biotechnologies, Inc., Huntsville, AL, U.S.A.) that produced 68 reproducible bands based on the presence of DNA fragments. Reactions were performed in 25 μL total volumes with the following conditions: 0.5 μL of DNA, 16.85 μL of ddH_20, 2.5 μL of 10× polymerase chain reaction (PCR) buffer (Gibco/BRL Gaithersburg, MD, U.S.A.), 1.5 μL of 50mM $MgCl_2$ (Gibco/BRL), 200 μM of each deoxynucleotide-triphosphate (dNTP)s (Gibco/BRL), 5 pmol of primer, and 1.2 μL of *Taq* DNA polymerase (Gibco/BRL). Minor dilutions were made to the concentrations of DNA for optimization of primers as needed. A negative control, including all ingredients except template DNA, was included with each set of reactions to detect contamination. Thermocycler conditions were as follows: initial denaturation at 94°C for 2 minutes, 45 cycles of denaturation at 94°C for 1 minute, annealing at 36°C for 1 minute, and extension at 72°C for 2 minutes, and a final extension at 72°C for 7 minutes; soak at 4°C. Total PCR products were run out on 1.2% Tris-acetate-EDTA (TAE) agarose gels and stained with ethidium bromide. Bands were visualized under UV light, and the images were captured digitally. Bands were scored as present or absent using the Kodak ID imaging software

(Eastman Kodak Co., Rochester, NY, U.S.A.). Homology assessments were made across gels based on a 1-kb DNA ladder (Invitrogen Corporation, Carlsbad, CA, U.S.A.). Bands of similar molecular weight and migration distance across individuals were assumed to be homologous (Adams & Rieseberg 1998). Duplicate gels were run for all individuals, and nonreplicated bands were eliminated from analyses.

Data Analysis. For each population and each group (local vs. CRP), genetic variation was quantified by calculating the percentage of polymorphic bands, the number of unique bands, and Shannon's information index, a measure of genetic diversity (POPGENE, version 1.31; Yeh et al. 1999). Shannon's index was calculated for each population as $H = -\sum p_i(\log p_i)$, where p_i is the frequency of the ith band (Lewontin 1972). Nei's (1978) unbiased genetic distance was calculated between all pairs of populations. The degree of relatedness among populations was assessed using genetic distance values of Reynolds et al. (1983) in a neighbor-joining tree in PHYLIP, version 3.57c (Felsenstein 1993). Mantel tests were used to test for congruence between genetic distances and corresponding geographic distances. Mantel (1967) tests were carried out in NTSYSpc, version 2.02, with 2,000 permutations to test the significance of correlations. The following matrices were analyzed: (1) all populations combined; (2) CRP populations; (3) local populations; (4) Erie Plains populations; and (5) Darby Plains populations.

Hierarchical structure within and among the populations was examined using an analysis of molecular variance (AMOVA; Excoffier et al. 1992). Based on genetic distances among individuals, total variance was partitioned into covariance components according to intraindividual, interindividual, and interpopulation differences (Excoffier et al. 1992). The resulting variance components were used to estimate variation among groups (CRP vs. local), among populations within groups, and within populations. An additional AMOVA was performed to compare variance difference among seed sources (local Ohio populations, CRP populations from Missouri seed sources, and CRP populations from Texas seed sources). AMOVA was performed using squared Euclidean distances from ARLEQUIN, version 2.0 (Schneider et al. 2000).

Results

Flowering Phenology

In the Darby Plains of central Ohio, Big bluestem flowered from 28 June until 29 September 2001. Flowers were protandrous, with a period of pollen release before stigma receptivity (Selbo 2002). Flowering started earliest in two of the three local populations but overlapped extensively with the CRP populations (Fig. 2). These results indicate the potential for cross-pollination between local and nonlocal genotypes of Big bluestem. The three populations that flowered earlier were also sites that were burned in the spring of 2001 (Fig. 2). Burning not only controls woody species and removes litter (dead grass) but also exposes the soil to the sun, and this warming may allow earlier plant growth and subsequent flowering.

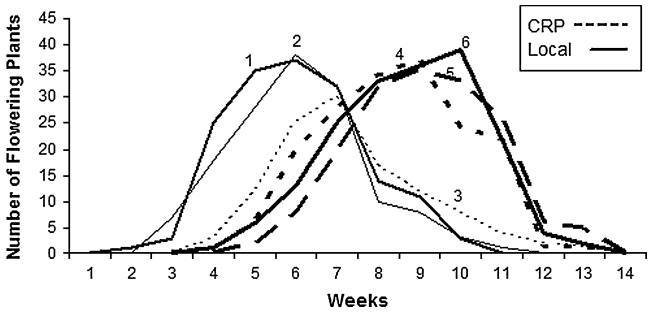

Figure 2. Number of Big bluestem individuals flowering each week throughout the summer at six sites (week 1 indicates 29 June 2001, week 14 indicates 28 September 2001). Forty individuals were monitored at each site (three CRP and three local). 1 = Bigelow, 2 = Smith, 3 = Biggert, 4 = Betherd, 5 = Gardner, 6 = Indian Ridge Remnant. Sites 1, 2, and 3 were burned in the spring of this study, 2001.

Genetic Variation

We scored a total of 68 RAPD fragments, which we refer to as loci, on 322 individuals from the 13 populations. The number of bands per population ranged from 38 to 52 (Table 2). CRP populations exhibited a total of 63 bands, and local populations exhibited a total of 66 bands. The percentage of bands that were polymorphic ranged from 63 to 75% among the CRP populations and 55 to 76% among the local populations (Table 2). No loci were monomorphic across the populations. Unique bands were found in very low proportions in two CRP populations and four local populations (Table 2).

Genetic variation within CRP and local populations was similar and relatively high. Genetic diversity within populations (measured as Shannon's information index) ranged from 0.22 to 0.27 in both CRP and local populations (Table 2). Additionally, the AMOVA results indicated no significant difference in genetic variation between CRP and local populations (Table 3) or between Texas versus Missouri seed sources for CRP populations (Selbo 2002). Most of the variation (approximately 84%) occurred within populations, whereas 16% of the variation was partitioned among populations within the two groups (CRP and local).

A neighbor-joining tree showed no distinct clustering among population types or geographic areas (Fig. 3). Genetic distance values ranged from 0.016 to 0.038 for local populations and 0.010 to 0.037 for CRP populations. An overall Mantel test failed to show significant correlation between geographic and genetic distance. Pairwise comparisons between CRP, local, Erie Plains, and Darby Plains populations also failed to show a correlation (Selbo 2002). Thus,

Table 2. Statistics generated from RAPD data comparing CRP and local populations of Big bluestem.

Populations	*No. of Loci Per Population*	*% Polymorphic Loci*	*No. of Unique Fragments*	*Genetic Diversity (H)*
CRP				
Witt	48	70.6	1	0.23
Ayres	43	63.2	1	0.22
Kracer	51	75.0	0	0.24
Gardner	44	64.7	0	0.23
Betherd	46	67.7	0	0.23
Biggert	50	73.5	0	0.27
All CRP	63	$\overline{X} = 69.1$	2	0.27
Local				
Eastman	38	55.9	0	0.24
Resthaven	45	66.2	1	0.22
Indian Ridge	51	75.0	2	0.23
Milford	52	76.5	2	0.26
Bigelow	51	75.0	0	0.25
Smith	45	66.2	0	0.22
All local	66	$\overline{X} = 69.1$ (71.7 w/o Eastman)	5	0.28
Restored				
Battelle-Darby	48	70.6	0	0.23

Genetic diversity (H) was calculated using Shannon's information index (Lewontin, 1972). $N = 26$ samples per population, except Eastman, where $N = 10$.

none of our analyses of genetic structure detected differences in the diversity or genetic composition of local versus CRP populations of Big bluestem.

Discussion

This study documents the potential for pollen-mediated gene flow between local and CRP populations that occur near each other. The six populations in the phenology study overlapped extensively in their flowering times. Two of the three local populations flowered earlier than two of the three CRP populations. However, spring burning could be the reason for these differences in peak flowering because the three populations that were burned that season flowered earlier than those that were not burned. In addition to pollen, seed dispersal could also move genes from larger CRP populations to smaller local populations of Big bluestem. Thus, it is clear that non-native genes from CRP populations could disperse into small remnant prairies when the two occur near each other. This leads to the question of whether gene flow from CRP populations could be harmful to local remnant populations of Big bluestem.

Table 3. AMOVA representing the partitioning of genetic variance in Big bluestem.

Source of Variation	*df*	*Sum of Squares*	*Percentage of Variation*	*p Value*
Among groups (CRP vs. Local)	1	39.42	−0.14	0.51
Among populations within groups	10	399.74	16.31	<0.001
Within populations	284	1967.65	83.83	<0.001

There is no significant difference between CRP and local populations.

Understanding the genetic composition of populations may aid in making restoration decisions that maintain natural evolutionary processes (Fenster & Dudash 1994; Moritz 1999). A major concern is the potential for non-local genotypes to disrupt local adaptation or degrade the genetic integrity of native populations through cross-pollination. In this regard, the management implications of our study are not clear because our genetic analyses revealed high and similar types of genetic diversity in local versus CRP populations. This raises several questions: (1) whether the RAPD loci in this study are representative of other selectively neutral markers; (2) whether local populations have already experienced gene flow from introduced genotypes of Big bluestem; and (3) whether evolutionarily important differences could be overlooked in studies that focus on neutral molecular markers rather than phenotypic variation (e.g., Reed and Frankham 2001). Each of these possibilities is discussed briefly below.

First, using 68 polymorphic RAPD loci, we found that 84% of the genetic variation in Big bluestem occurred within populations and 16% occurred among populations. Gustafson et al. (1999) reported similar patterns of genetic structure in remnant populations of Big bluestem in Arkansas' Grand Prairie, with 89% of the total genetic variation residing within populations and 11% residing among populations. Although we used only two primers and Gustafson et al. (1999) used six, our study generated 68 polymorphic loci compared to 37 polymorphic loci in their study. RAPD markers are presumed to be distributed arbitrarily throughout the genome (Nybom & Bartish 2000), so the lower number of primers coupled with more polymorphic bands per primer in our study

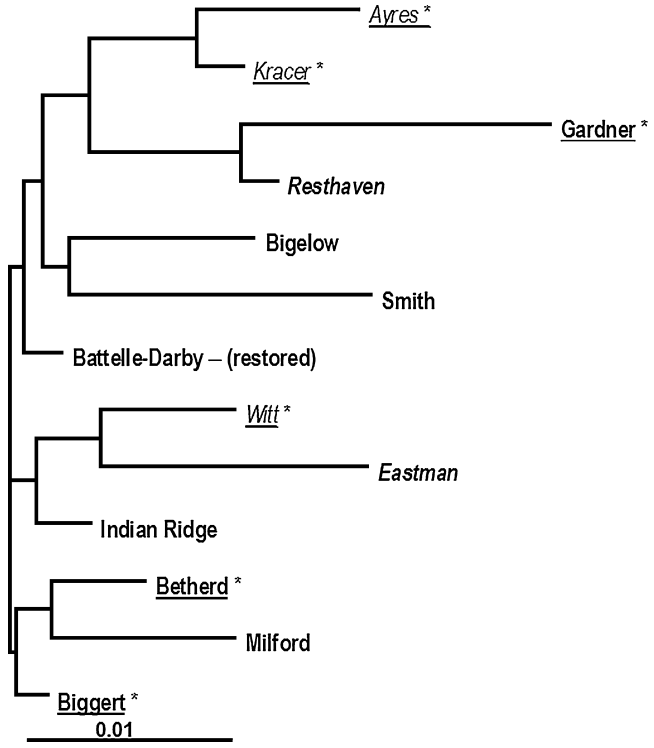

Figure 3. Unrooted neighbor-joining tree showing relationships between populations. CRP populations are followed by an asterisk, Darby Plains populations are bold, and Erie Plains populations are italicized. Branch length scale indicates the amount of divergence between clusters based on Reynolds genetic distance (Reynolds et al. 1983) (scale bar = 1% distance between two populations).

may not reflect a deficiency in sampling. In any case, it is noteworthy that these two studies found similar patterns of genetic structure in Big bluestem. It is possible that a much broader survey of molecular marker variation would detect differences between local and CRP populations in Ohio. However, given the widespread distribution of Big bluestem in prairies throughout North America, and extensive seed and pollen dispersal, it is possible that genetic and geographic distances are not highly correlated.

Another possible explanation for our results is that local populations have already experienced gene flow from western sources of Big bluestem genotypes in the past. This seems unlikely because Big bluestem has not been used in CRP plantings until very recently and local and CRP populations of Big bluestem are often at least 10 km apart (Materials and Methods). Nonetheless, Big bluestem may have been imported for pasture and forage in the past, providing a chance for nonlocal genotypes to mix with local ones. This possibility cannot be ruled out, but it seems doubtful based on reports from farmers and local botanists.

A third consideration is whether selectively neutral markers should be used to assess the evolutionary potential of local populations (e.g., Volis et al. 2001). Even though we observed no obvious differences between CRP and local Big bluestem using RAPD markers, it is possible that these groups differ in heritable phenotypic variation and local adaptation. A review by Reed and Frankham (2001) showed that significant correlations between molecular and heritable quantitative measures of genetic diversity are uncommon. Also, in a study comparing isozymes and quantitative traits in purple needlegrass, Knapp and Rice (1998) report regional but dissimilar differentiation between phenotypic diversity and molecular genetic diversity. Based on their results, the investigators cast doubt on the usefulness of isozymes for predicting translocation effects of purple needlegrass for restoration purposes (Knapp & Rice 1998). In a contrasting example, Waldmann and Andersson (1998) showed that allozymes and phenotypic characters were correlated in populations of *Scabiosa* spp. To gain a clearer insight into the importance of the assessed genetic variance of Big bluestem populations in Ohio, it would be useful to investigate phenotypic variation in addition to molecular variation.

In conclusion, both molecular and phenotypic variations should be considered when making recommendations about seed sources for restored grassland populations. Several issues are raised when nonlocal populations are introduced for restoration or conservation purposes. Montalvo and Ellstrand (2001) report a "disruption of local adaptation" when nonlocal *Lotus scoparius* was planted for restoration in combination with the local genotypes of the same species. They discourage the use of distant populations in restoration projects and suggest that local adaptation and the chance of outbreeding depression support this rationale. Although populations of nonlocal Big bluestem may not currently threaten the genetic variation of local populations in Ohio, an increase of CRP acreage may result in greater contact, and the evolutionary consequences of this process are not clear. Farmland placed into CRP has been increasing in Ohio since the inception of the program in 1985 (USDA 2005). The potential for a large amount of nonlocal seed to come in direct contact with remnants will likely increase as CRP and other restoration projects gain popularity. In order to maintain genetically diverse populations that may have locally adapted genotypes, it may be prudent to recommend the use of local seed sources when possible. In addition, using only local seed for prairie restoration purposes will help protect this vanishing ecosystem and preserve its authenticity.

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