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GENETIC DIVERSITY AND COMPETITIVE ABILITIES OF *DALEA PURPUREA* (FABACEAE) FROM REMNANT AND RESTORED GRASSLANDS

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Allozyme and randomly amplified polymorphic DNA (RAPD) analyses were used to characterize the genetic relationships of *Dalea purpurea* from remnant and restored Illinois tallgrass prairies and a large remnant tallgrass prairie in Kansas. The remnant Illinois populations were less genetically diverse than the restored Illinois populations and the Kansas population. These restored Illinois populations were established with at least two seed sources that were locally collected. There was little population divergence ($F_{ST} = 0.042$), which is consistent with other perennial forbs, while the genetic relationships among populations reflected geographic proximity. In a greenhouse competition experiment, differences in performance between seedlings was not related to the remnant or restored status of Illinois populations, but plants from Kansas were significantly smaller than Illinois plants. Genetic diversity and competitive ability were not associated with the size of the original source population. Our data indicate that using multiple local seed sources for restoration projects will maintain the local gene pool while enhancing the regional genetic diversity of this species.

Keywords: conservation genetics, local seed source, tallgrass prairie, restoration ecology.

Introduction

Fragmentation of continuous habitat into smaller and more isolated patches can potentially alter the spatial distribution of genetic diversity (Gilpin and Soulé 1986; Lande 1988; Barrett and Kohn 1991; Fenster and Dudash 1994; Fore and Guttman 1996, 1999). Models of the effect of habitat fragmentation on population genetic structure indicate that increased isolation of populations will decrease gene flow and increase genetic differentiation among populations (Templeton et al. 1990; Hanski 1991; Fore and Guttman 1992; Husband and Barrett 1996). Population genetic theory and empirical studies (Newman and Pilson 1997; Saccheri et al. 1998) indicate that long-term population viability is positively related to levels of genetic variation (Barrett and Kohn 1991; Dolan 1994; Knapp and Rice 1996; Linhart and Grant 1996; Knapp and Connors 1999).

Founder effects in newly established populations, accompanied by limited gene flow between populations, may further reduce the genetic variation within a population, thus constraining the adaptive flexibility of the population and potentially contributing to reduction in fitness (Williamson and Werth 1999). For restored populations, long-term fitness may be affected by the choice of local or nonlocal seed, single or multiple sources, native or cultivated varieties, and the resultant adaptability and competitiveness of the seedlings as they establish in the new environment. Crosses among genetically related individuals may result in loss of fitness because of inbreeding depression, while crosses among individuals from genetically divergent source populations may have reduced fitness because of outbreeding depression (Fenster and Dudash 1994; Gustafson et al. 2001). Furthermore, differences in intraspecific competitiveness among populations may determine the genetic identity of the establishing population (Aarssen and Turkington 1985; Turkington 1994). Thus, an ideal approach for guiding the choice of source populations for plant restoration projects would be to assess the performance of different seed sources in multiple environments across the range of the species. For example, Etterson and Shaw (2001) found that seed production of the native annual legume *Chamaecrista fasciculata* was dramatically reduced in nonnative populations relative to local populations. Reduced fecundity could influence the genetic variance, inbreeding, and demography of subsequent generations and hence population persistence (Etterson and Shaw 2001). In another study of an annual plant, Newman and Pilson (1997) showed a significant reduction in germination and survivorship rates in experimental populations with a low genetic effective population size (N_e) relative to populations with a high N_e. These studies link the effects of genetic diversity, evolutionary potential, and long-term fitness of annual species in experimentally established populations; however, it is unclear whether these same relationships are observed in perennial species. A better understanding of the interactions among fitness, genetic diversity and similarity, and competitiveness of a perennial plant species would benefit conservation biologists seeking to maintain existing and restore altered natural ecosystems.

The goals of this study were to estimate genetic diversity, genetic relationships, and intraspecific competitive abilities of *Dalea purpurea* from remnant and restored Illinois tallgrass prairies and Konza Prairie, Kansas. Konza Prairie was included to allow us to compare genetic diversity, genetic similarity, and competitive abilities of *D. purpurea* from a large (3487 ha)

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remnant Kansas prairie to the smaller (0.4–97 ha) Illinois prairies. *Dalea purpurea* Vent. (purple prairie clover, Fabaceae) was selected as the study species because it is a native perennial diploid (2n = 14) clover species that occurs in a variety of North American grasslands and has been shown to be a core species in the tallgrass prairie (Great Plains Flora Association 1986; Gibson 1989; Gleason and Cronquist 1991). Genetic diversity and genetic relationships were studied using two different molecular markers, protein (allozyme) electrophoresis and randomly amplified polymorphic DNA (RAPD), which differ in their ability to detect genetic variation. An intraspecific competition experiment was used to determine whether differences in plant performance exist among select populations and whether these differences were associated with genetic diversity estimates. Specifically, we wanted to know whether (1) there were differences in genetic diversity between remnant populations and restored Illinois populations established with multiple local seed sources, (2) the Illinois populations were genetically different from the Kansas population, and (3) intraspecific competitive abilities differed among sources.

Material and Methods

Sites

In the past 160 years, the tallgrass prairies of Illinois have been reduced from 8.9 million hectares to less than 1000 hectares (White 1978; Robertson and Schwartz 1994; Robertson et al. 1997; Robertson 2001). The remnant tallgrass prairies are typically small and isolated within agricultural and urban landscapes (Robertson and Schwartz 1994; Robertson 2001). Restored prairies were included in this study to provide baseline population genetic information on recently reestablished populations. Twenty-five of the 30 black soil (tallgrass) prairies listed in the Illinois Department of Natural Resources state database, which constituted >90% of the acreage of this prairie type in Illinois in 1995, were surveyed for *Dalea purpurea*, and it occurred in six sites (Gustafson 2000). In October 1995, seeds were collected from three remnant (Grant Creek Prairie, Gensburg-Markham Prairie, and Pellville Cemetery Prairie) and three restored (Black Hawk State Historical Site, Morton Arboretum, and Mason County State Nursery) prairies. A site was considered to be a remnant if it had never been plowed and if any species enrichment practices were conducted only with seeds originating from that site. The Gensburg-Markham Prairie (Gensburg-Markham, 41°36′N, 87°41′W, 38.5 ha), designated as a natural area in 1972, is the largest remnant tallgrass prairie in the Chicago area and is managed by Northeastern Illinois University (McFall 1991). The Grant Creek Prairie Nature Preserve (Grant Creek, 41°21′N, 88°11′W, 31.6 ha) was acquired by the State of Illinois in 1978 and had a history of cattle grazing before 1978 (McFall 1991). The Pellville Cemetery Prairie (Pellville, 40°27′N, 87°55′W, 0.4 ha) is the smallest of the remnant populations sampled and was managed with periodic mowing until 1989. All three Illinois remnant prairies are now managed with annual or biennial spring burning to suppress woody species encroachment and to encourage prairie species (W. Glass and W. McClain [site managers], personal communication).

The three restored Illinois prairies were each established with seed from remnant populations within 80 km and have been managed with periodic spring burning (site managers, personal communication). The Schulenberg Prairie at Morton Arboretum (Morton Arboretum, 41°49′N, 88°04′W, 40.5 ha) is one of the oldest and largest restored tallgrass prairies in Illinois. Restoration of this prairie began in 1962 (finished in 1972) on land that had originally been in row crop production for many years (education coordinator, personal communication). The Black Hawk State Historical Site (Black Hawk, 41°28′N, 90°35′W, 0.4 ha) population was established in 1985 (site manager, personal communication) and is the smallest restored population in our study. The Mason County State Nursery (Mason County, 40°19′N, 89°54′W, 97.2 ha) population was established in the mid-1970s. This population is different from the other restored populations because the primary focus of this site is to produce seed to be used by the Illinois Department of Transportation and the Illinois Heritage Program. Mason County management practices include irrigation and the application of fertilizer to promote seed production in *D. purpurea* and other native prairie forbs (site manager, personal communication).

To measure the genetic distinctiveness of the Illinois populations, *D. purpurea* seeds were also collected from one, 004B (55.1 ha), of several watersheds that contained *D. purpurea* at Konza Prairie Biological Station (Konza Prairie, 39°05′N, 96°35′W), Kansas. Since 1979, this ungrazed watershed has been burned every four years. Konza Prairie is a 3487-ha remnant tallgrass prairie in the Flint Hills of Kansas, ca. 750 km west of the Illinois populations (Knapp et al. 1998).

Plants

At each site, seeds were collected from 30 randomly selected individuals using the following sampling strategies. In large prairies (Gensburg-Markham, Grant Creek, Konza Prairie), a single transect traversing the population with 20 m between individual collections. Multiple parallel transects spaced 10 m apart and 10 m between each individual collection were used to sample the smaller populations (Morton Arboretum, Pellville, Black Hawk). A bulk seed collection from 100+ plants was provided by Mason County (site manager, personal communication). Bulk seed samples were collected from 50 to 100 individuals in each population and used in the competition experiment.

Seeds were removed from the parent plants, stored on ice, transported to Southern Illinois University, Carbondale, and dry cold stratified at 4°C for 4 mo. Seeds from the individual collections were scarified and germinated in petri dishes on moistened filter paper, and one randomly selected seedling from each maternal plant was potted in a 16.5-cm-diameter pot containing a commercial soilless media (Promix-HP, Hummert International, Earth City, Mo.) and grown in a greenhouse. Ambient lighting in the greenhouse was supplemented with standard grow lamps from 0700 to 1900 hours. Plants were watered as needed and fertilized with 350 mg m^{-2} of Peter's Fertilizer (N : P : K, 20 : 20 : 20) every 3 wk. Seeds from each bulk collection were scarified, thoroughly mixed, sown into flats, and grown in the greenhouse as described.

Allozyme Electrophoresis

Approximately 1.0 g of fresh leaf tissue from each 2-mo-old seedling was homogenized in the Tris-HCl extraction buffer of Wendel and Weeden (1989), placed in 1.5-mL microcentrifuge tubes, and centrifuged at 10,000 rpm (12,000 *g*) for 15 min. The supernatant was stored frozen (−80°C) until needed. Enzyme separation was accomplished using 13% w/v starch gel (Starch Art hydrolized potato starch, Smithville, Tex.). Five enzyme systems coding for nine putative loci were resolved and selected for use in this study. Enzymes were assayed using two gel/electrode buffer systems: (A) Tris EDTA borate pH 8.0 (Wendel and Weeden 1989) and (B) Ridgeway pH 8.1 (Ridgeway et al. 1970). Enzyme staining protocols were essentially as reported in Wendel and Weeden (1989). 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-1 and 2, 5.3.1.9, B), phosphoglucomutase (pgm-2, pgm-3, 2.7.5.1, B), 6-phosphogluconate dehydrogenase (6PGD-1 and 2, 1.1.1.44, A), and triose phosphate isomerase (TPI-1 and 2, 5.3.1.1, A). Genetic interpretation was based on electrophoretic patterns and known enzyme subunit structure and intercellular compartmentalization (Kephart 1990). Alleles with the greatest mobility were designated as *a*, the following ones *b*, *c*, etc. In addition to alphabetic genotypes (e.g., *aa*, *ab*, *bb*), the number of multilocus genotypes for each individual and allele frequencies for each locus and for all populations were calculated (table A1).

RAPD Profiling

To obtain genomic DNA, fresh leaf tissue (0.1 g) from 1-mo-old seedlings was ground to a powder in liquid nitrogen, 1.3 mL of 94°C CTAB buffer (100 mM Tris-HCl, 1.4 M NaCl, 30 mM EDTA, 2% (w/v) cetyltrimethylammonium bromide [CTAB]) was added, and grinding was continued. Three units of Protease K and 60 μL dithiotreitol (0.5 M) were added and mixed, and the extract transferred to a 2.0-mL microfuge tube and incubated for 30 min at 45°C. The sample was extracted with chloroform : isoamyl alcohol (24 : 1) and centrifuged at 10,000 rpm for 15 min at 4°C. The aqueous layer was transferred to a 1.5-mL microfuge tube, 0.5 mL of −20°C isopropanol was added and mixed, and the sample was stored in a −20°C freezer for 30 min. The DNA was pelleted, air-dried, and resuspended in 300 μL of TE buffer (1 mM Tris-HCl, 0.1 mM EDTA); 200 μL of 4 M ammonium acetate and 1.0 mL of −20°C 100% ethanol were added; and the sample was stored at −20°C for 60 min. The DNA was pelleted, air-dried, and dissolved in 100 μL of TE buffer with RNase (one unit). The DNA concentration of each sample was quantified by comparing to a standard of known concentration on agarose gels stained with ethidium bromide. The genomic DNA was diluted to a working concentration of ca. 5 ng μL^{-1}.

Thirty 10-base oligonucleotide primers (Operon Technologies) were surveyed, and six were selected that showed intense and reproducible bands. The following primers (with sequence and number of bands scored in parentheses) were used: A-04 (dAATCGGGCTG, 5), B-07 (dGGTGACGCAG, 3), B-12 (dCCTTGACGCA, 4), B-18 (dCCACAGCAGT, 7), N-13 (dAGCGTCACTC, 5), and L-12 (dGGGCGGTACT, 4). 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 $MgCl_2$, 0.2 mM of each dNTP (Pharmacia), 20 ng of primer, 0.2 μL Taq polymerase (Promega), and ca. 5 ng of template DNA. One negative control, containing all of the amplification reagents and no template DNA, was run with each reaction. Samples were overlaid with 30 μL of mineral oil. Amplifications were performed in a Stratagene Robocycler (model 400860, Stratagene, La Jolla, Calif.) 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) were electrophoresed in 1.0% TBE (0.089 M Tris, 0.089 M boric acid, 0.002 M EDTA) agarose gels. After the dye migrated 15 cm, gels were stained with ethidium bromide, scanned, and stored as digitized image files with a Gel-doc 1000 system (BioRad Laboratories, Hercules, Calif.). The Molecular Analyst (BioRad Laboratories) software package was used to adjust the contrast, thus enhancing the visualization of the DNA bands. A DNA molecular mass marker (Promega, G1741) was used to standardize band intensities and to estimate the size of the RAPD bands. If cross contamination was indicated, with the presence of amplification products in the negative control lane, the run was repeated. The presence or absence of 28 intense and reproducible bands constituted the genotype score for each individual.

Competition Experiment

A greenhouse competition experiment using a diallel design (Gibson et al. 1999) was used to test for differences in competitive abilities among pairs of seedlings from two remnant Illinois populations (Gensburg-Markham, Pellville), two restored Illinois populations (Black Hawk, Mason County), and the remnant Kansas (Konza Prairie) population. This experiment consisted of two plants per pot with all combinations of the five populations and six replicates per combination. One plant was designated as the target and the other the associate for the purpose of measurements and analyses. Five-week-old seedlings of approximately the same size were planted equal distances from each other and the side of a 16.5-cm-diameter pot containing commercial soilless media (Promix-HP) and watered as needed. A settling period of 14 d was allowed for the replacement of dead seedlings, with the experiment starting April 7, 1996. The plants were harvested after 99 d and dried at 80°C for 48 h, and then final biomass (total, shoot, root, inflorescences) was determined. Maximum plant height was measured at 7-d intervals throughout the experiment, but these data are not presented here because the final harvest data reflect the same relationships among populations as did the temporal height data. Nitrogen-fixing *Rhizobium* inoculum was not included in the experimental design; however, nodules were present, but not quantified, on each individual plant (Gustafson 2000).

A greenhouse intraspecific competition experiment lacks the realism of abiotic factors, the plant-soil community, and plant-herbivore interactions that could influence plant population dynamics under field conditions. If, however, a phenomenon is observed under the highly controlled greenhouse conditions, then it is likely to be important in natural populations (Gibson

et al. 1999; Gibson 2002). Seedling competition experiments with long-lived perennials may not reflect competitive interactions in established populations; however, seedling performance during one growing season can be informative considering that all the restored populations in this study were established with seed (Gustafson 2000).

Data Analysis

For each population, number of individual plants sampled (N), mean number of alleles per locus (A), percent polymorphic loci (P), mean observed heterozygosity (H_{obs}, direct-count estimate), mean Hardy-Weinberg expected heterozygosity (H_{exp}, unbiased estimate), and Wright's F statistics were computed using BIOSYS-1 (Swofford and Selander 1981). Chi-square analysis was used to test for Hardy-Weinberg equilibrium goodness of fit for the entire data set and each population using the computer program TFPGA (Miller 1997). An inbreeding coefficient representing the fractional reduction in heterozygosity relative to a random-mating population, with the same allele frequencies, was calculated as $F = (H_{exp} - H_{obs})/H_{exp}$ (Swofford and Selander 1981; Hartl and Clark 1997). Linear regression was used to model the relationship between the number of individuals sampled within a population and the inbreeding coefficient (F). For the RAPD analysis, three to seven intense and reproducible bands from six primers were scored for each individual. The percent polymorphic bands (PB) and the number of different multiband phenotypes (MBP) within a population were used as descriptive measures of diversity (Ellstrand and Roose 1987; Jonsson et al. 1996). Genetic variation (both allozyme and RAPD) was apportioned within and among populations using Shannon's diversity, $H' = -\Sigma p_i \log_2 p_i$, where p_i is the frequency of the band (Lewontin 1972; King and Schaal 1989; Gustafson et al. 1999). This measure is appropriate for qualitative data, can be decomposed into nested hierarchical levels, and is relatively insensitive to the dominant inheritance of the RAPD marker (Dawson et al. 1995).

Relationships among populations, based on allozyme and RAPD frequency data, were investigated using principal component analysis (PCA) and parallel analysis (PA) to establish which PCA axes were appropriate for interpretation (SAS Institute 1989). Parallel analysis was used to derive the 95th percentile eigenvalues for each successive PCA axis, based on Monte Carlo analysis of Longman et al.'s (1989) regression equations. Only axes with eigenvalues greater than the PA eigenvalues were retained for interpretation (Franklin et al. 1995). Mantel's test was used to test for an association between the Euclidean distance (allozyme and RAPD) and geographic distance (km) between populations, with comparisons among the Illinois populations and among all populations (McCune 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 for this test was that the degree of genetic similarity between pairs of populations and the distance between them were not related.

The populations used in the competition experiment were not randomly selected, and so the target and associate treatments (populations) were treated as fixed effects. Final biomass (total, shoot, root, inflorescences) data were analyzed with a two-way ANOVA. This experiment was not a full factorial design and therefore it was not possible to test for target by associate interactions. In the event of significant main effects, Tukey's honest significant difference means separation procedure was used to test for differences among means. When the data did not conform to normality or equal variances, logarithm and square root transformations were performed. Pearson's correlation analysis was used to test for associations between plant performance, genetic diversity measures, and the area of the site. All competition experiment analyses were performed using SYSTAT (Wilkinson 1996).

Results

Patterns of Genetic Diversity

There were no fixed allelic differences among the populations; however, Black Hawk (AAT-2, B allele, 8.3%), Grant Creek (PGM-3, C allele, 2.2%), and Konza Prairie (PGM-2, C allele, and PGM-3, B allele, both at 3.8%) populations had unique alleles at low frequencies (table A1). The mean number of allozyme alleles per locus ranged from 1.3 (Pellville and Gensburg-Markham) to 1.7 (Konza Prairie). Remnant and restored Illinois populations averaged fewer alleles per locus than the Konza Prairie (Kansas) population (table 1). Percent polymorphic loci ranged from 11.1% to 33.3%, with the restored populations (29.6%) averaging higher values than the remnant (18.5%) Illinois populations (table 1). Observed heterozygosity estimates ranged from 0.047 to 0.089, and two of the remnant populations had the lowest while two restored populations had the highest heterozygosity. Two loci (AAT-2, $\chi^2 = 61.6$, df = 1, $P < 0.001$; TPI-1, $\chi^2 = 140$, df = 1, $P < 0.001$) deviated significantly from Hardy-Weinberg equilibrium at the species level, while Black Hawk (AAT-2, $\chi^2 = 7.3$, df = 1, $P < 0.01$), Morton Arboretum (TPI-1, $\chi^2 = 20$, df = 1, $P < 0.0001$; TPI-2, $\chi^2 = 8.2$, df = 1, $P < 0.001$), and Gensburg-Markham (TPI-1, $\chi^2 = 20$, df = 1, $P < 0.0001$) had significant deviations within populations. Wright's F statistics indicate little population differentiation ($F_{ST} = 0.055$) and low levels of inbreeding ($F_{IS} = 0.120$) for the seven populations sampled. However, inbreeding estimates of the fractional reduction in heterozygosity relative to a random-mating population, with the same allele frequencies, indicate a 28% reduction in the remnant Gensburg-Markham and a 40% reduction in the restored Morton Arboretum populations (table 1). There was no linear relationship between inbreeding and sample size ($F = 0.50$–0.019 [sample size], $r^2 = 0.134$, $P = 0.42$, df = 1, 5). Shannon's diversity was used to partition genetic variation among populations. At the species level, 77% of the allozyme variation was partitioned within and 23% among *Dalea purpurea* populations. There was no difference in the distribution of genetic variation among the populations within the remnant and restored categories. There was no significant correlation between the allozyme diversity estimates and the area of the site.

All 140 individuals sampled in the allozyme analysis were also used in the RAPD study. The percent polymorphic bands (PB) ranged from 78.6% (Mason County) to 100% (Grant

Table 1

Estimates of Genetic Variation at Nine Allozyme Loci in Seven Populations of *Dalea purpurea* Sampled in 1995

Population	N	A	P	H_{obs}	H_{exp}	F	H'
Illinois:							
Remnant:							
Pellville	26	1.3 (0.2)	11.1	0.047 (0.042)	0.046 (0.042)	−0.022	0.12
Grant Creek	23	1.4 (0.2)	11.1	0.063 (0.048)	0.054 (0.039)	−0.165	0.15
Gensburg-Markham	20	1.3 (0.2)	33.3	0.056 (0.035)	0.078 (0.041)	0.282	0.19
Mean		1.3	18.5	0.055	0.059	0.032	0.15
Restored:							
Black Hawk	18	1.6 (0.2)	22.2	0.080 (0.060)	0.090 (0.059)	0.111	0.24
Mason County	20	1.4 (0.2)	33.3	0.089 (0.065)	0.091 (0.068)	0.022	0.23
Morton Arboretum	20	1.4 (0.2)	33.3	0.061 (0.055)	0.102 (0.057)	0.401	0.26
Mean		1.5	29.6	0.085	0.094	0.178	0.24
Kansas:							
Konza Prairie	13	1.7 (0.3)	11.1	0.068 (0.041)	0.081 (0.054)	0.160	0.23
Species mean		1.5	19.7	0.073	0.074	0.123	0.21

Note. N = sample size, A = mean number of alleles per loci, P = percent polymorphic loci, H_{obs} = observed heterzygosity, H_{exp} = expected heterozygosity, F = inbreeding coefficient, and H' = Shannon's diversity. Numbers in parentheses are standard errors.

Creek) (tables 2, A2). The remnant populations averaged 94.1% PB, while the average of the restored Illinois populations was lower at 88.0% PB. The Konza Prairie, at 86% PB, was lower than five of the six Illinois populations sampled (table 2). The multibanding phenotype (MBP) profiles were unique for every individual (table 2). The distribution of RAPD marker variation was found mostly within populations (86%) and less among (14%) populations. Unlike the allozyme data, there was a difference in the distribution of RAPD variation within the remnant and restored categories. For remnant populations, 91% of the variation was partitioned within and 9% among populations, while the restored populations possessed 86% of the variation within and 14% among populations. RAPD diversity estimates were not correlated with size of the site.

Population Relationships

Population relationships were investigated using PCA, with allele frequency or RAPD band frequency data. Parallel analysis of the allozyme and RAPD data indicated that the first two axes were statistically significant and appropriate for interpretation.

The first two axes of the allozyme PCA accounted for 63% of the variance, with the first axis accounting for 40% (eigenvalue = 6.4) and the second axis for 23% (eigenvalue = 3.9) of the variance in the correlation matrix. PCA of the allozyme data revealed predictable associations, with the six Illinois populations different from Konza Prairie (fig. 1*A*). Within Illinois, the remnant Gensburg-Markham population associated with the restored Morton Arboretum population. The remaining Illinois populations formed two associations, with two remnant (Pellville and Grant Creek) and two restored (Black Hawk and Mason County) populations grouping together (fig. 1*A*).

The first two axes of the RAPD PCA accounted for 59% of the variance with axis 1 (eigenvalue = 10.9) and axis 2 (eigenvalue = 5.7) accounting for 39% and 20% of the variance, respectively (fig. 1*B*). As with the allozyme PCA results, the Konza Prairie population was different from the six Illinois populations (fig. 1*B*). The relationships among populations within Illinois were not consistent with the allozyme analysis.

Mantel's test was used to test for significant associations between genetic similarity and geographic distance between populations. There was a significant association ($Z = 0.625$, $P < 0.05$) between Euclidean distance and geographical distance among the six Illinois populations using allozyme data, but no significant association ($Z = -0.001$, $P = 0.56$) with RAPD data. There was no significant association when the Konza Prairie population was included in either the allozyme or RAPD analyses.

Competitive Abilities

The competition experiment consisted of all pairwise combinations of two remnant (Pellville and Gensburg-Markham)

Table 2

RAPD Analysis of Seven *Dalea purpurea* Populations Sampled in 1995

Population	N	MBP	PB	H'
Illinois:				
Remnant:				
Pellville	26	26	89.3	1.05
Grant Creek	20	20	100.0	1.22
Gensburg-Markham	20	20	92.9	1.12
Total or mean	66	66	94.1	1.13
Restored:				
Black Hawk	23	23	89.0	1.07
Mason County	21	21	78.6	0.98
Morton Arboretum	21	21	96.4	1.21
Total or mean	65	65	88.0	1.09
Kansas:				
Konza Prairie	18	18	86.0	1.12
Species total or mean	149	149	89.4	1.11

Note. N = sample size, MBP = multibanding phenotypes, PB = percent polymorphic bands, and H' = Shannon's diversity.

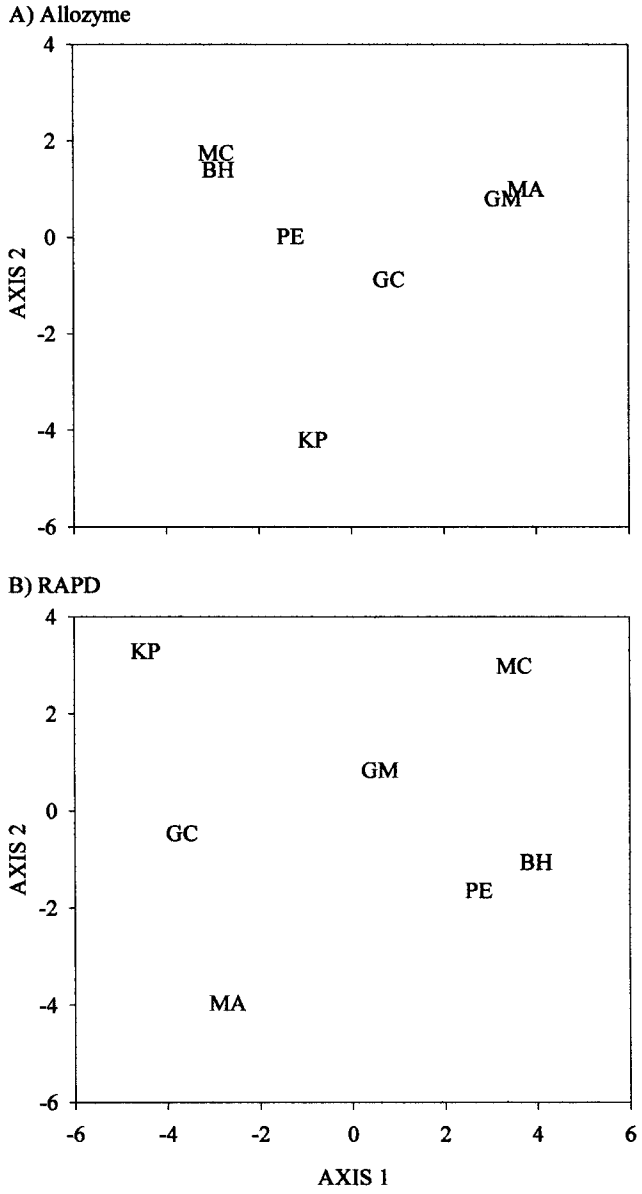

Fig. 1 Principal component analysis depicting the relationships among *Dalea purpurea* populations from three remnant (Pellville [*PE*], Grant Creek [*GC*], and Gensburg-Markham [*GM*]) and three restored (Black Hawk [*BH*], Mason County [*MC*], and Morton Arboretum [*MA*]) Illinois populations and Konza Prairie (*KP*), Kansas, sampled in 1995, based on (*A*) allozyme and (*B*) RAPD frequency data.

and two restored (Black Hawk and Mason County) Illinois populations and a Konza Prairie, Kansas, population. Analysis of the final dry biomass (total, shoot, root, inflorescences), height, and number of inflorescences indicated a significant ($P < 0.01$) target main effect, but no effect of the associate plant source population (table 3). Pellville plants were significantly taller than the Gensburg-Markham plants, with the plants from the two restored populations (Black Hawk and Mason County) intermediate (fig. 2). The Konza Prairie plants were shorter than plants from all four Illinois populations (fig. 2). Pellville and Black Hawk plants produced more inflorescences than the Konza plants, and Gensburg-Markham and Mason County plants produced an intermediate number. All four Illinois populations produced significantly larger amounts of mean dry biomass (total, shoot, root, and inflorescences) than the Konza Prairie plants (fig. 2). There was a significant negative correlation between the mean number of inflorescences produced ($r^2 = -0.876$, $P < 0.05$) and the geographic area of the source population. Plants from the two smallest source populations (Pellville and Black Hawk, both 0.4 ha) produced more inflorescences than the larger Mason County (97.2 ha) and Konza Prairie (55.1 ha). There were no associations ($P > 0.05$) among the genetic diversity measures and mean height, biomass (total, shoot, root, inflorescences), and number of inflorescences in the competition experiment.

Discussion

Patterns of Genetic Diversity

Dalea purpurea has lower levels of genetic diversity than reported for other members of the Fabaceae (Hamrick and Godt 1989, 1997) but comparable to those of the federally endangered cedar glade endemic *Dalea foliosa* (Gray) Barneby (Wiltshire 1991). The similarity in genetic diversity to the rare congener is surprising because *D. purpurea* is a widely distributed species (Gleason and Cronquist 1991) and a significant component of the tallgrass prairie community (Gibson 1989; Collins and Glenn 1991). The restored populations had 13%–38% higher allozyme diversity estimates relative to the remnant populations, which is likely a result of establishment with seed from multiple source populations. These results are consistent with allozyme studies of restored populations of other species (Gustafson 2000) and theoretical predictions (Knapp and Dryer 1997; Montalvo et al. 1997; Lesica and Allendorf 1999). Gensburg-Markham (remnant) and Morton Arboretum (restored) populations showed evidence of nonrandom mating, which is interesting because the Gensburg-Markham population is within the "local" area in which seeds were collected from remnant grasslands and used to establish the Morton Arboretum population. The relatively high diversity estimates in the small remnant population indicates that small isolated remnant populations can contribute to the overall diversity of the species and are potential seed sources for future restoration projects.

The remnant population from Konza Prairie (Kansas) had equal or higher diversity estimates than the remnant Illinois populations for four of the five diversity measures. The three Illinois remnants were small (0.4, 31.5, and 38.5 ha) and contained only one *D. purpurea* population at each site (D. J. Gustafson, personal observation), whereas the Konza Prairie 004B watershed is larger (55.1 ha) than the Illinois sites, and there are multiple watersheds containing *D. purpurea* populations within the 3487-ha area of Konza Prairie (Gibson 1989). The Illinois populations exist in a landscape of agricultural fields and urban development, making gene flow (via seed or pollen) among such widely spaced populations unlikely. At Konza Prairie, *D. purpurea* is an intermediate or core species, according to the core-satellite species hypothesis, with a frequency in 10-m^2 sample plots of 50%–100% depending

Table 3

Summary ANOVA Results of a *Dalea purpurea* Greenhouse Experiment (1996) Examining Competitive Differences in Mean Height, Number of Inflorescences, and Biomass (Total, Shoot, Root, Inflorescences) among Two Remnant and Two Restored Illinois Populations and One Kansas Population

				Dry biomass			
Source	df	Height	Inflorescences	Total	Shoot	Root	Inflorescences
Target	4	617*	34*	3116*	3244*	1677*	2916*
Associate	4	227	9	170	102	640	847
Error	70	104	7	390	379	464	380

Note. Values are mean squares.
* $P \leq 0.01$.

on the management treatment (Gibson 1989; Collins and Glenn 1991). Overall, plant abundance and genetic diversity estimates of *D. purpurea* at Konza Prairie are consistent with predictions for a landscape-scale metapopulation maintained through frequent local gene flow. Additional genetic and demographic studies of core, intermediate, and satellite species within native and disturbed landscapes are needed to comprehensively test such theoretical predictions of the metapopulation model (Hanski 1991; Husband and Barrett 1996; Fore and Guttman 1999).

The diversity estimates based on RAPD analysis, although using the same populations and 98% of the same individuals as sampled for allozymes, did not show the same pattern of diversity as the allozyme analysis. Remnant populations averaged higher PB (94.1%) than the restored populations (88.0%), as did Shannon's diversity measures. The difference between allozyme and RAPD diversity estimates may reflect differences in the two methods to detect fine-scale genetic differences. For example, allozyme markers identify 21 unique multilocus phenotypes (allozyme fingerprints) out of 140 individuals sampled, while the RAPD markers identified 149 unique multibanding phenotypes (RAPD fingerprints) out of the 149 individuals sampled.

The distribution of genetic variation was consistent with other outcrossing perennial herbaceous species with little population divergence ($F_{ST} = 0.042$). Shannon's diversity (H') was used to partition variation into nested hierarchical levels, for both allozyme and RAPD data. Most of the variation was retained within (allozyme = 77%, RAPD = 86%) and less among (allozyme = 23%, RAPD = 14%) populations. These results are consistent with other allozyme and RAPD studies of outcrossing perennial species (Hamrick 1989; Hamrick and Godt 1989, 1997; Dolan 1994; Huff et al. 1993, 1998; Huff 1997; Gustafson et al. 1999, 2001; Bingham and Ranker 2000; Lee et al. 2000; Fleishman et al. 2001; Wen and Hsiao 2001; Zawko et al. 2001).

Maze and Turkington (1996) showed a negative relationship between genetic variation of *Trifolium repens* and grassland age. They attributed this decrease in variation over time as the selective elimination of genotypes. In our study, testing for differences in genetic diversity among only three restored populations of different ages would lack statistical power. However, this study does provide baseline genetic diversity estimates for remnant and restored *D. purpurea* populations that could then be compared to future genetic sampling of these same populations. Understanding the distribution of genetic diversity in a temporal context will provide valuable insight into the evolutionary dynamics of plant populations and aid in the restoration and conservation of plant populations.

Population Relationships

Illinois populations were genetically different from Konza Prairie (Kansas) populations according to both allozyme and RAPD data, which is consistent with similar studies of the two dominant grasses (*Andropogon gerardii* and *Sorghastrum nutans*) of the tallgrass prairie (Gustafson et al. 1999; Gustafson 2000). Allozyme analysis was better able to resolve within-Illinois relationships than the RAPD analysis, with three paired associations reflecting geographic proximity. Genetic similarity among populations decreased as the geographic distance between populations increased. This result is important because it shows that the restored populations established with seed from sources within 80 km retained the local genotype. The continued use of locally collected seed for restoration projects will preserve the local genotype while maintaining the regional diversity.

Competitive Abilities

There was no discernible relationship between plant performance (height, biomass measures, number of inflorescences) and genetic diversity, although there was a significant seed source effect on competitiveness. The significant target plant effects and nonsignificant associate (neighbor) plant effects in the competition experiment indicate fundamental differences in competitiveness among source populations manifest regardless of the source of competing neighbors. Kansas plants, for example, were significantly shorter and produced less biomass (total, shoot, root, and inflorescences) than all four Illinois source populations regardless of which population the competing plant originated. This result is consistent with a similar study of a dominant grass species (*A. gerardii*) of the tallgrass prairie, in which competitive ability was related to the target plant population identity (Gustafson 2000). Despite limitations of the simple pairwise intraspecific competition experimental design (Gibson et al. 1999), this study identifies significant seed source (population) effects that could then be studied in more complex intra- and interspecific field competition experiments (Aarssen and Turkington 1985). Mixing of different source populations may occur through the introduction of nonlocal seed or through gene flow (seed or pollen dispersal) between adjacent but different populations (Gustafson et al 2001). Under such conditions, and following dis-

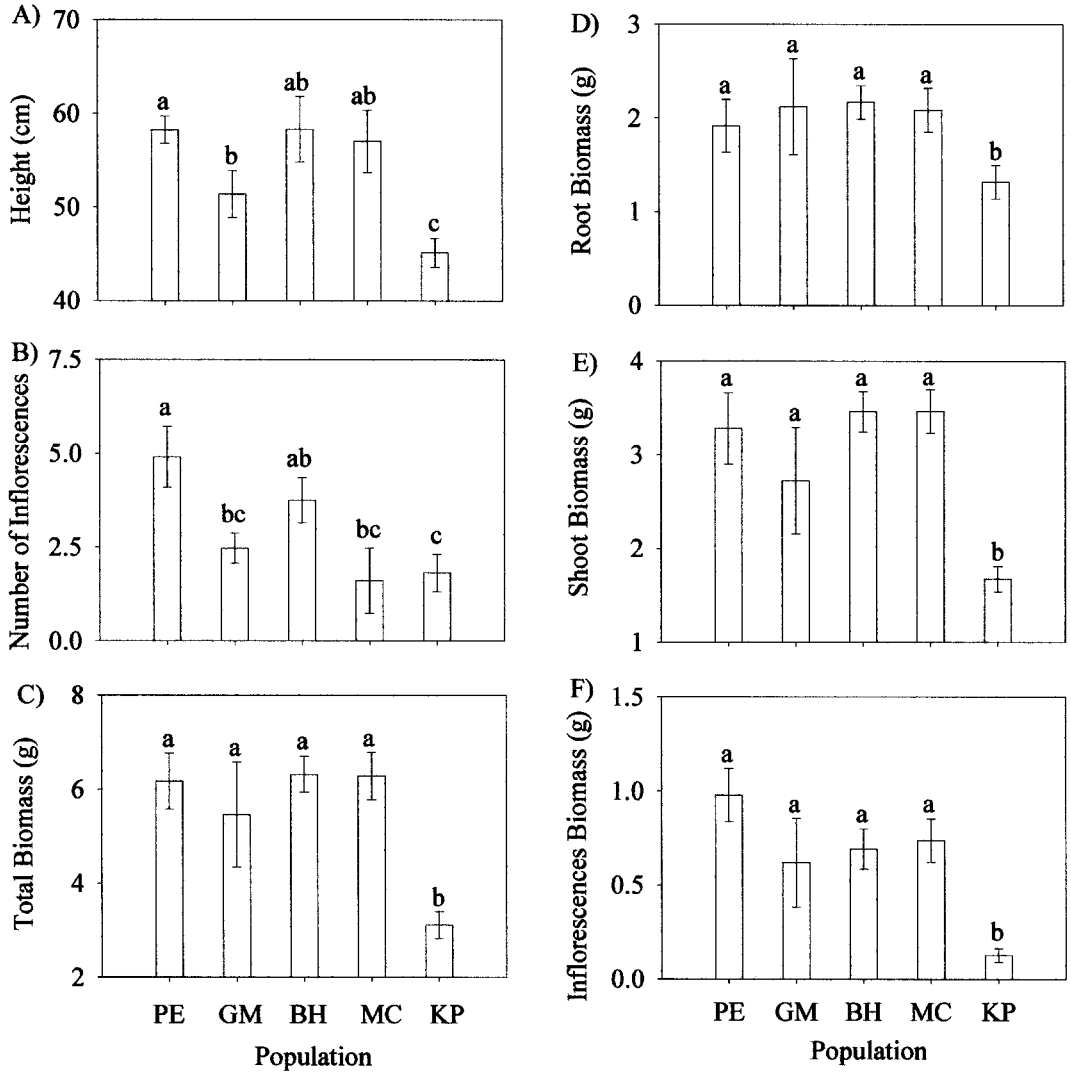

Fig. 2 Greenhouse diallel intraspecific competition experiment with *Dalea purpurea* from two remnant (Pellville [*PE*] and Gensburg-Markham [*GM*]) and two restored (Black Hawk [*BH*] and Mason County [*MC*]) Illinois populations and Konza Prairie (*KP*), Kansas. Values represent mean (±1 SE) for (*A*) height, (*B*) inflorescences, (*C*) total biomass, (*D*) root biomass, (*E*) shoot biomass, and (*F*) inflorescences biomass. Different letters above bars within the same graph represent significant differences ($P > 0.05$).

turbance-induced competitive release (Julita and Grace 2002), seedling competition is likely to be intense. Our study indicates that the outcome of seedling competition among different source populations under these conditions is likely to have significant ramifications for the genetic composition of these spatially structured populations at both the local and metapopulation level (*sensu* Freckleton and Watkinson 2002).

Restoration Implications

Most of the genetic variation was retained within populations, while genetic diversity was not related to size of the prairie. This suggests that small remnant populations can contribute much to the genetic diversity of restoration projects. The restored populations were established with seed from multiple sources within an 80-km collection distance of the new site. The goals of restorationists using locally adapted seeds have been to preserve the local genotype and to increase the genetic diversity in the restored populations. Several authors have suggested that using locally collected seed will preserve the local genotype (Handel et al. 1994; Linhart 1995; Clewell and Rieger 1997; Gordon and Rice 1998) and that multiple seed sources will decrease the probability of low genetic diversity due to founder effects (Knapp and Dryer 1997; Montalvo et al. 1997; Lesica and Allendorf 1999; Fischer et al. 2000); however, ours is one of the first studies to provide empirical support for these theoretical predictions.

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Appendix

Table A1

Isozyme Frequency Data of *Dalea purpurea* from Three Remnant and Three Restored Illinois Populations and Konza Prairie, Kansas, Collected in 1995

Locus and allele	Remnant			Restored			Konza Prairie, Kansas
	Pellville	Grant Creek	Gensburg-Markham	Black Hawk	Mason County	Morton Arboretum	
AAT-2:							
A	1.00	1.00	1.00	0.92[a]	1.00	1.00	1.00
B	0.00	0.00	0.00	0.08[b]	0.00	0.00	0.00
GPI-1:							
A	1.00	1.00	1.00	1.00	1.00	1.00	1.00
GPI-2:							
A	0.77	0.78	0.85	0.64	0.50	0.73	0.69
B	0.19	0.20	0.15	0.19	0.35	0.18	0.15
C	0.08	0.02	0.00	0.17	0.15	0.00	0.12
D	0.00	0.00	0.00	0.00	0.00	0.10	0.04
6-PGD-1:							
A	1.00	1.00	1.00	1.00	1.00	1.00	1.00
6-PGD-2:							
A	1.00	1.00	1.00	1.00	1.00	1.00	1.00
TPI-1:							
A	1.00	1.00	0.88[a]	1.00	1.00	0.850[a]	1.00
B	0.00	0.00	0.13	0.00	0.00	0.15	0.00
TPI-2:							
A	1.00	0.96	0.93	0.97	0.95	0.925[a]	0.96
B	0.00	0.04	0.08	0.03	0.05	0.08	0.04
PGM-2:							
A	0.98	1.00	1.00	0.97	0.95	1.00	0.96
B	0.02	0.00	0.00	0.03	0.05	0.00	0.00
C	0.00	0.00	0.00	0.00	0.00	0.00	0.04[b]
PGM-3:							
A	1.00	0.98	1.00	1.00	1.00	1.00	0.96
B	0.00	0.00	0.00	0.00	0.00	0.00	0.04[b]
C	0.00	0.02[b]	0.00	0.00	0.00	0.00	0.00

[a] Allele frequencies at this locus were significantly different from Hardy-Weinberg equilibrium.

[b] Allele that is unique to this population.

Table A2

RAPD Frequency Data of *Dalea purpurea* from Three Remnant and Three Restored Illinois Populations and Konza Prairie, Kansas, Collected in 1995

Primer and band	Remnant			Restored			Konza Prairie, Kansas
	Pellville	Grant Creek	Gensburg-Markham	Black Hawk	Mason County	Morton Arboretum	
A-04:							
1	0.154	0.050	0.050	0.174	0.286	0.000	0.000
2	0.923	0.600	0.650	0.739	0.619	0.714	0.200
3	0.077	0.100	0.150	0.261	0.286	0.000	0.000
4	1.000	0.850	1.000	1.000	1.000	0.810	1.000
5	0.885	0.300	0.800	0.957	1.000	0.095	0.133
B-07:							
1	0.080	0.333	0.263	0.045	0.000	0.350	0.385
2	0.760	0.917	0.158	0.727	0.278	0.850	0.692
3	0.680	0.083	0.316	0.636	0.556	0.150	0.154
B-12:							
1	0.615	0.353	0.471	0.762	0.600	0.429	0.167
2	0.615	0.706	0.765	0.714	0.867	0.571	0.750
3	0.654	0.647	0.706	0.762	0.800	0.857	0.750
4	0.500	0.706	0.529	0.524	0.867	0.524	0.333
B-18:							
1	1.000	0.842	1.000	1.000	1.000	0.889	0.750
2	0.192	0.421	0.526	0.143	0.286	0.500	0.167
3	0.538	0.474	0.316	0.333	0.286	0.167	0.250
4	0.962	0.842	0.947	0.952	1.000	0.833	0.583
5	0.385	0.368	0.316	0.190	0.381	0.333	0.333
6	0.192	0.211	0.263	0.381	0.143	0.389	0.250
7	0.154	0.316	0.105	0.143	0.000	0.278	0.083
L-12:							
1	0.538	0.900	0.650	0.261	0.850	0.563	1.000
2	1.000	0.600	0.800	1.000	0.900	0.750	0.722
3	0.654	0.900	0.850	0.609	0.600	0.563	0.722
4	0.077	0.150	0.050	0.043	0.050	0.125	0.000
N-13:							
1	0.269	0.300	0.050	0.261	0.238	0.050	0.250
2	0.269	0.450	0.400	0.348	0.476	0.250	0.750
3	0.577	0.250	0.800	0.391	0.333	0.300	0.313
4	0.308	0.100	0.450	0.652	0.571	0.350	0.188
5	0.808	0.250	0.250	0.522	0.286	0.550	0.250

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