

# Conservation genetics of two co-dominant grass species in an endangered grassland ecosystem

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## Summary

1. Global habitat fragmentation and loss of undisturbed grasslands has led to the use of non-local seed and cultivars in restoration. There is concern that these sources may be genetically depauperate and their introduction may lead to loss of unique local genotypes. Within this context we considered the issue with regard to the once widespread but now highly fragmented North American tallgrass prairie.

2. We characterized the genetic diversity and genetic relationships of the co-dominant species in this system, big bluestem *Andropogon gerardii* and Indian grass *Sorghastrum nutans*, from seven remnant and six restored local tallgrass prairies, a non-local remnant prairie, and five cultivated varieties.

3. Randomly amplified polymorphic DNA (RAPD) analysis of these grasses showed that genetic diversity was mostly retained within rather than among populations, and did not differ among restored or remnant populations or cultivars.

4. Genetic diversity estimates were not correlated with the area of the grassland, nor was there a clear association between diversity and species abundance. All of the restored grasslands in this study were established with seed from at least two local populations and were as genetically diverse as remnant sites.

5. Principal components analysis of RAPD band frequencies showed that the local remnant and restored populations were genetically different from the non-local remnant grasslands and were consistently different to the cultivars. The genetic relationships among local remnant and restored populations reflected biogeography and human activities.

6. *Synthesis and applications.* Restoration practitioners have often assumed that small populations are genetically depauperate and therefore the need for multiple seed sources to increase genetic diversity outweighs concerns over potential genetic differences among widespread species. Our research, however, indicates that genetic diversity is much less of an issue in these perennial outcrossing autopolyploid grasses than genetic differences among local and non-local or cultivar seed sources. Combining these results with our previous research, indicating differences in plant performance as a function of the source population, suggests that genetic differences and ecological performance among local and non-local seed sources are more of a concern than genetic diversity. Translocating non-local seed in order to increase diversity, or using cultivars, is likely to alter the genetic structure of remnant populations and potentially influence the associated community and affect ecosystem structure and function in unforeseen ways.

*Key-words:* *Andropogon gerardii*, grassland restoration, local seed, prairie conservation, *Sorghastrum nutans*

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## Introduction

Grasslands throughout the world are becoming increasingly degraded or lost due to anthropogenic influences (Handel, Robinson & Beattie 1994; Stevenson, Bullock & Ward 1995; Kindscher & Tieszen 1998). Maintenance of genetic diversity is a central theme in conservation and restoration biology, with the belief that genetic variation will increase the probability of population persistence (Montalvo *et al.* 1997; Palmer, Ambrose & Poff 1997; Lesica & Allendorf 1999; Etterson & Shaw 2001; Fleishman *et al.* 2001; Noy-Meir & Briske 2002). However, empirical studies have been equivocal, showing positive, negative and no relationships between genetic diversity and population size (McClenaghan & Beauchamp 1986; Sampson, Hopper & James 1988; van Treuren *et al.* 1991; Meffe & Carroll 1994; Prober & Brown 1994; Raijmann *et al.* 1994; Prober, Spindler & Brown 1998; Gustafson, Gibson & Nickrent 1999, 2001; Young, Brown & Zich 1999; Buza, Young & Thrall 2000; Fleishman *et al.* 2001; Arafeh *et al.* 2002), which suggests that the genetic responses to vegetation community reduction and fragmentation are species specific and more complex than initially thought (Young, Brown & Zich 1996).

It is important to characterize the component species in ecological conservation and restoration (Pywell *et al.* 2003). Conservation genetic studies tend to focus on rare and endangered species, which typically do not contribute significantly to the productivity of the ecosystem (Grime 1999). Conversely, genetic variation in populations of the dominant species may significantly regulate ecosystem function (Seliskar *et al.* 2002). Moreover, the loss of genetic diversity associated with increased inbreeding is generally assumed to present a stronger threat to formerly dominant species that have suffered recent reductions in distribution than to naturally rare species (Barrett & Kohn 1991; Fischer, van Kleunen & Schmidt 2000). Empirical and theoretical studies indicate that long-term population viability is positively related to levels of genetic variation (Barrett & Kohn 1991; Ellstrand & Elam 1993; Dolan 1994; Linhart & Grant 1996; Young, Brown & Zich 1996; Newman & Pilson 1997; Knapp & Rice 1998; Fischer, van Kleunen & Schmidt 2000; Etterson & Shaw 2001). If the conservation management unit is the vegetation community, then characterizing and managing genetic variability of dominant species may be more important than actively managing the minor components of that community.

This study focused on the tallgrass prairie in Illinois, USA; however, loss of native grasslands to agriculture and urbanization has occurred throughout the world. Historically, Illinois contained 25% (8.9 million ha) of the North American tallgrass prairie, yet today less than 0.01% of the original high-quality prairie remains. Approximately 210 of the 253 (83%) prairie remnants in the state are less than 4 ha in size (Robertson & Schwartz 1994; Robertson, Anderson & Schwartz

1997; Robertson 2001). Community dynamics in these prairies have been severely altered because they lack the full complement of natural processes, such as landscape fires and large migrating herbivores, and because gene flow patterns have probably been disrupted because of fragmentation (Robertson & Schwartz 1994; Robertson, Anderson & Schwartz 1997).

Big bluestem *Andropogon gerardii* Vitman and Indian grass *Sorghastrum nutans* (L.) Nash. are both dominant warm-season perennial grasses of the tallgrass prairie (Weaver & Fitzpatrick 1935; McMillan 1959; Risser *et al.* 1981). Both are self-incompatible polyploid species that have been used extensively in North American prairie restorations, as cover crops to reduce soil erosion and as a native forage crop for livestock (Keeler 1990; Schramm 1990; Diboll 1997; Norrmann, Quarin & Keeler 1997; Kindscher & Tieszen 1998; McKone, Lund & O'Brien 1998). In the Grand Prairie region of Arkansas, geographical proximity could not be used to predict genetic relatedness of populations (Gustafson, Gibson & Nickrent 1999). These remnant Arkansas populations were, however, genetically different from two cultivated varieties (cultivars) and two remnant *A. gerardii* populations from Illinois. Morphological and genetic differences have also been observed between restored *A. gerardii* populations in Illinois that were established with seed from Illinois and Nebraska (Gustafson, Gibson & Nickrent 2001). There was evidence of extensive mixing of the Illinois and Nebraska populations, although assessing hybrid fitness relative to non-hybrid crosses was not determined in this study. Because genetic introgression from distant provinces can reduce fitness of local genotypes (Keller, Kollmann & Edwards 2000) and alterations to population phenology can affect organisms whose reproductive cycles are timed to plant flowering (Jones, Hayes & Sackville Hamilton 2001), it is clear that seed provenances are an important issue in plant conservation and restoration ecology (Sackville Hamilton 2001; Wilkinson 2001).

The goals of this study were to assess the genetic diversity and genetic relationships among remnant and restored tallgrass prairies and select cultivars, using the co-dominant perennial grass species *A. gerardii* and *S. nutans*. Randomly amplified polymorphic DNA (RAPD) analysis has been successfully used to assess genetic relationships among populations of our target species (Gustafson, Gibson & Nickrent 1999) and species of conservation interest (Zawko *et al.* 2001; Arafeh *et al.* 2002), as well as to identify genetic differences between individuals with otherwise identical isozyme fingerprints (Gustafson 2000). Specifically, we wanted to know (i) if genetic diversity correlated with the area of the site; (ii) if remnant populations were less diverse than restored populations that were established with multiple seed sources; (iii) what the genetic relationships among remnant populations, restored populations and cultivars for each species were; and (iv) whether the overall patterns of genetic diversity and genetic relatedness were similar between these two co-dominant grass species.

## Materials and methods

### POLYPLOIDY

Both grasses are dominant perennial autopolyploidy grasses of the tallgrass prairie ecosystem, which affects the choice of molecular mark and data analyses. *Sorghastrum nutans* ( $2n = 4x = 40$ ) is a tetraploid outcrossing autopolyploid, while the ploidy level of *A. gerardii* is more complicated. *Andropogon gerardii* is a complex polyploidy with two polyploidy cytotype races (hexaploid  $2n = 6x = 60$  and enneaploid  $2n = 9x = 90$ ), with an increasing frequency of the enneaploids in the western range of the species (Keeler 1990; Norrmann, Quarin & Keeler 1997). Co-occurring hexaploid and enneaploid cytotypes at Konza Prairie, Kansas, have been shown to interbreed and produce fertile euploids and aneuploids, although the aneuploids are rarely observed in nature (Norrmann, Quarin & Keeler 1997; Keeler, Williams & Vescio 2002). The high polyploidy levels and the potential for interpolyploid hybridization in *A. gerardii* was another reason for selecting RAPD markers, because RAPDs are not affected by variation in ploidy levels as other DNA fingerprinting or protein methods are (Weising *et al.* 1995).

### PLANT MATERIAL

Seeds of both grasses were collected from 25 mesic black soil tallgrass prairies in Illinois, which constitute more than 80% of the sites and more than 90% of the tallgrass prairie area remaining in Illinois (Robertson 2001). Seeds of both species were collected from each site because seeds are what restoration ecologists/conservation biologists use to establish or augment populations (Diboll 1997). Only sites from which we obtained germinable seed from more than 10 individuals were used in this study, which resulted in the inclusion of 13 *A. gerardii* and five *S. nutans* Illinois populations. We defined a remnant population as one that had never been in cultivation. A restored population was defined as a grassland that had been planted with native tallgrass flora on a site that had historically been a tallgrass prairie. The restored populations were established with multiple seed collections from remnant populations within 20–150 km of the site (site managers, personal communications), with the exception of Goose Lake Prairie Natural Area, for which a Nebraska source was used (Gustafson, Gibson & Nickrent 2001). Seeds of both species representing a non-local remnant population were collected from watershed 004B at Konza Prairie Biological Station, Kansas, a 3487-ha tallgrass remnant approximately 750 km west of the Illinois sites (Knapp *et al.* 1998). This site was chosen because it is one of the largest and best remaining examples of a North American tallgrass prairie. In both the Illinois and Konza Prairie communities, the dominant native  $C_4$  grasses are *A. gerardii*, *S. nutans*, *Panicum virgatum* L. and *Schizachyrium scoparium* (Michx.) Nash. Three *A. gerardii* (Roundtree, Pawnee

and Kaw) and two *S. nutans* (Rumsey and Cheyenne) cultivars were purchased as bulk seed and included in this study (Table 1). All five cultivars were developed as forage crops with high yield, medium to late maturation, some resistance to lodging and fungal infections (<http://Plant-Materials.NRCS.USDA.GOV>) and have been used in restoration, reclamation and soil conservation projects (Harrington 1989; Frank 1994).

In October of 1995, seeds were collected from 30–33 randomly selected individuals at each remnant and restored site, with at least 10 m between sampled individuals. Seeds were removed from the maternal plant, stored on ice, transported to Southern Illinois University at Carbondale and cold-dry stratified at 4 °C for 3 months. Chaff was removed and the seeds germinated in Petri dishes on moistened filter paper. One randomly selected seedling from each maternal plant was grown under glasshouse conditions in a square pot (816 cm<sup>3</sup>) containing a commercial soil-less medium. Plants were watered as needed, fertilized with 350 mg m<sup>-2</sup> N20%–P20%–K20% fertilizer every 3 weeks, and ambient light supplemented with standard grow lamps from 07:00 to 19:00 h. Bulk seed collections provided by Mason County State Nursery (Havana, Illinois) and the cultivar accessions were sown in glasshouse flats containing the soil-less medium and grown under conditions described above.

### RAPD PROFILING

DNA extraction and RAPD methodologies followed Gustafson, Gibson & Nickrent (1999). Thirty 10-base oligonucleotide primers (Operon Technologies, Alameda, California) were surveyed for each species, with four to five primers selected based on consistent and readily interpretable banding patterns. Restriction Fragment Length Polymorphism (RFLP) analysis was used to confirm the homology of a subset of RAPD bands for both species. The following four primers (with sequence and number of bands scored in parentheses) were used in the *A. gerardii* analysis: B-01 (dGTTTCGCTCC, 11), B-07 (dGGTGACGCAG, 8), B-12 (dCCTTGACGCA, 9) and N-13 (dAGCGTCACTC, 9). RAPD characterization of *S. nutans* populations was based on following five primers: B-07 (dGGTGACGCAG, 8), B-15 (dGG-AGGGTGTT, 6), B-18 (dCCACAG-CAGT, 9), F-12 (dACGGTACCAG, 7) and L-19 (dGAGTGGTGAC, 9).

### GENETIC ANALYSES

Hierarchical genetic structure of both species was assessed using analysis of molecular variance (AMOVA) in ARLEQUIN version 2.0 (Schneider, Roessli & Excoffier 2000). The variance components were used to compute fixation indices. The significance of the fixation indices (among and within populations) was tested using a hierarchical, non-parametric permutation approach. Genetic diversity was also estimated using the number

**Table 1.** Letter designation, name, location, area and percentage cover of *Andropogon gerardii* and *Sorghastrum nutans* of 13 Illinois prairies, Konza Prairie (KS) and five cultivars. Remnant (A–G), restored (H–M), Kansas (N) and Cultivar (O–S)

| Letter | Population name                                       | Latitude (N)       | Longitude (W)      | Area (ha)         | <i>A. gerardii</i> (%) | <i>S. nutans</i> (%) |
|--------|---|--------------------|--------------------|-------------------|------------------------|----------------------|
| A      | Sunbury Railroad Prairie                              | 41°05'             | 88°37'             | 5                 | 14                     | 12                   |
| B      | Weston Cemetery Prairie                               | 40°45'             | 88°36'             | 2                 | 13                     | 7                    |
| C      | DeSoto Railroad Prairie <sup>1</sup>                  | 37°51'             | 89°14'             | 13.4              | 1                      | 19                   |
| D      | Wheelock Railroad Prairie <sup>1</sup>                | 41°40'             | 90°15'             | 16.2              | 1                      | 10                   |
| E      | Iroquois County Conservation Area (ICCA)              | 40°59'             | 87°34'             | 506 <sup>2</sup>  | 20                     | 35                   |
| F      | Tomlinson Pioneer Cemetery Prairie                    | 40°21'             | 87°56'             | 0.4               | 40                     | 16                   |
| G      | Grant Creek Prairie                                   | 41°21'             | 88°11'             | 31.6              | 7                      | 6                    |
| H      | Goose Lake Prairie (NE source)                        | 41°21'             | 88°18'             | 622 <sup>2</sup>  | 16                     | 46                   |
| I      | Fermi-Laboratory Tract No. 1                          | 41°50'             | 88°15'             | 156 <sup>3</sup>  | 43                     | 6                    |
| J      | Mason County State Nursery (MCSN)                     | 40°19'             | 89°54'             | 97.2              | N/A                    | N/A                  |
| K      | Meadowbrook Park <sup>1</sup>                         | 40°05'             | 88°14'             | 12.2              | 20                     | 24                   |
| L      | Weldon Springs State Park <sup>1</sup>                | 40°07'             | 88°55'             | 6.8               | 54                     | 34                   |
| M      | Black Hawk State Historical Site (BHSHS)              | 41°28'             | 90°35'             | 0.4               | 3                      | 39                   |
| N      | Konza Prairie Research Natural Area (KS) <sup>1</sup> | 39°05'             | 96°35'             | 3487 <sup>2</sup> | 57                     | 19                   |
|        | Cultivar name   | Species            | Original source    | Year              |                        |                      |
| O      | Roundtree   | <i>A. gerardii</i> | Moorhead, IA       | 1983 <sup>4</sup> |                        |                      |
| P      | Pawnee  | <i>A. gerardii</i> | Pawnee County, NE  | 1963 <sup>5</sup> |                        |                      |
| Q      | Kaw   | <i>A. gerardii</i> | Riley County, KS   | 1950 <sup>5</sup> |                        |                      |
| R      | Rumsey  | <i>S. nutans</i>   | Jackson County, IL | 1983 <sup>4</sup> |                        |                      |
| S      | Cheyenne  | <i>S. nutans</i>   | Supply, OK         | 1945 <sup>5</sup> |                        |                      |

1, Both species. 2, Total area of the site. 3, Area of the inner loop. 4, Elsberry, MO. 5, Manhattan, KS.

of polymorphic bands (PB) and genetic diversity (GD); the latter is the probability that two randomly selected bands are different averaged across all RAPD primers (Schneider, Roessli & Excoffier 2000). Spearman rank order correlations were used to test for an association between area of the site and genetic diversity. One-way analysis of variance on rank data was used to determine if our measures of genetic diversity differed between remnant (including the Kansas remnant), restored and cultivar accessions.

For each species, relationships among populations were investigated using RAPD band frequency data in a principal components analysis (PCA; PCOrd Version 4, MJM Software, Glenden Beach, OR), with parallel analysis (PA) to establish which PCA axes were appropriate for interpretation (SAS Institute Inc., Cary, NC). PA, a technique that decomposes the correlation matrix, was used to derive the 95th percentile eigenvalues for each component based on Monte Carlo analysis of the Longman *et al.* (1989) regression equations. Only axes with eigenvalues greater than the PA eigenvalues were considered significant ( $\alpha \leq$

0.05) and retained for interpretation (Franklin *et al.* 1995).

## Results

Most of the *A. gerardii* RAPD variation was retained within populations (87.5%), although there were significant differences among *A. gerardii* populations (Table 2). PB decreased from cultivar to restored to remnant populations but these differences were not significant (Table 3). The smallest restored *A. gerardii* population (Black Hawk) had the lowest GD (0.234) and one of the largest restored populations (Fermi) had the highest GD (0.319) (Table 3). The mean GD estimates were not different among remnant, restored and cultivar populations (Table 3). There was no correlation between *A. gerardii* PB ( $R = 0.1$ ,  $P > 0.05$ ) or GD ( $R = 0.4$ ,  $P > 0.05$ ) and the area of the site.

*Sorghastrum nutans* retained 87.8% of the genetic variation within populations, with significant differences among populations (Table 2). PB ranged from a low of 67.5% for Konza to a high of 87.1% for Wheelock, but

**Table 2.** AMOVA analyses of RAPD data from 17 *Andropogon gerardii* (a) and eight *Sorghastrum nutans* (b) populations. \*Estimates of population fixation ( $F_{ST}$ ) averaged over all loci indicated significant genetic differentiation among populations within a species ( $P < 0.001$  for both)

|     | Source | d.f. | SS     | Variance components | % variation | $F_{ST}$ |
|-----|--------|------|--------|---------------------|-------------|----------|
| (a) | Among  | 16   | 419.8  | 0.74                | 12.50       | 0.125*   |
|     | Within | 464  | 2418.3 | 5.21                | 87.50       |          |
|     | Total  | 480  | 2838.2 | 5.95                |             |          |
| (b) | Among  | 7    | 136.5  | 0.79                | 12.20       | 0.121*   |
|     | Within | 133  | 761.7  | 5.72                | 87.80       |          |
|     | Total  | 140  | 898.2  | 6.52                |             |          |

**Table 3.** Summary of *Andropogon gerardii* and *Sorghastrum nutans* RAPD genetic diversity estimates. Sample size (*n*), percentage polymorphic markers (PB), and average gene diversity (GD) over all RAPD primers. One-way ANOVA *F*-statistics testing for difference in genetic diversity among remnant, restored and cultivar groups

| Population       | <i>Andropogon gerardii</i> |      |       | <i>Sorghastrum nutans</i> |      |       |
|------------------|----------------------------|------|-------|---------------------------|------|-------|
|                  | <i>n</i>                   | PB   | GD    | <i>n</i>                  | PB   | GD    |
| Sunbury          | 32                         | 75.6 | 0.264 | –                         | –    | –     |
| Weston           | 20                         | 70.2 | 0.271 | –                         | –    | –     |
| DeSoto           | 33                         | 78.3 | 0.300 | 11                        | 74.3 | 0.303 |
| Wheelock         | 19                         | 70.2 | 0.250 | 13                        | 87.1 | 0.329 |
| ICCA             | 29                         | 75.6 | 0.300 | –                         | –    | –     |
| Tomlinson        | 32                         | 78.3 | 0.315 | –                         | –    | –     |
| Grant Creek      | 32                         | 75.6 | 0.289 | –                         | –    | –     |
| Mean             | 28                         | 74.8 | 0.284 | 12                        | 80.7 | 0.316 |
| Goose Lake       | 32                         | 78.3 | 0.278 | –                         | –    | –     |
| Fermi-Laboratory | 31                         | 78.3 | 0.319 | 14                        | 79.4 | 0.320 |
| MCSN             | 27                         | 78.3 | 0.277 | –                         | –    | –     |
| Meadowbrook      | 31                         | 78.3 | 0.285 | 26                        | 79.4 | 0.273 |
| Weldon Springs   | 31                         | 72.9 | 0.243 | 25                        | 74.3 | 0.271 |
| BHSHS            | 21                         | 70.2 | 0.234 | –                         | –    | –     |
| Mean             | 28.8                       | 76.2 | 0.273 | 21                        | 77.7 | 0.288 |
| Konza Prairie    | 18                         | 70.2 | 0.291 | 13                        | 67.5 | 0.218 |
| Roundtree        | 31                         | 75.6 | 0.267 | –                         | –    | –     |
| Pawnee           | 31                         | 78.3 | 0.285 | –                         | –    | –     |
| Kaw              | 31                         | 78.4 | 0.283 | –                         | –    | –     |
| Rumsey           | –                          | –    | –     | 14                        | 79.4 | 0.336 |
| Cheyenne         | –                          | –    | –     | 25                        | 82.0 | 0.314 |
| Mean             | 31                         | 77.4 | 0.278 | 19.5                      | 80.7 | 0.325 |
|                  | $F_{2,13}$                 | 1.72 | 0.34  | $F_{2,4}$                 | 0.99 | 7.78  |
|                  | <i>P</i>                   | 0.42 | 0.71  | <i>P</i>                  | 0.65 | 0.27  |

there were no differences among the mean remnant, restored or cultivar values (Table 3). GD estimates ranged from 0.336 for Rumsey (cultivar) to 0.218 for Konza (remnant) populations, with no difference in the mean GD among remnant, restored and cultivar populations (Table 3). There was no correlation between *S. nutans* PB ( $R = -0.1$ ,  $P > 0.05$ ) and GD ( $R = -0.02$ ,  $P > 0.05$ ) and the area of the site.

The first (eigenvalue = 5.82, percentage of variance = 20.1) and second (eigenvalue = 5.35, percentage of variance = 18.4) axes explained 38.5% of the variance in our PCA correlation matrix of relationships among *A. gerardii* populations, and were statistically significant and appropriate for interpretation.

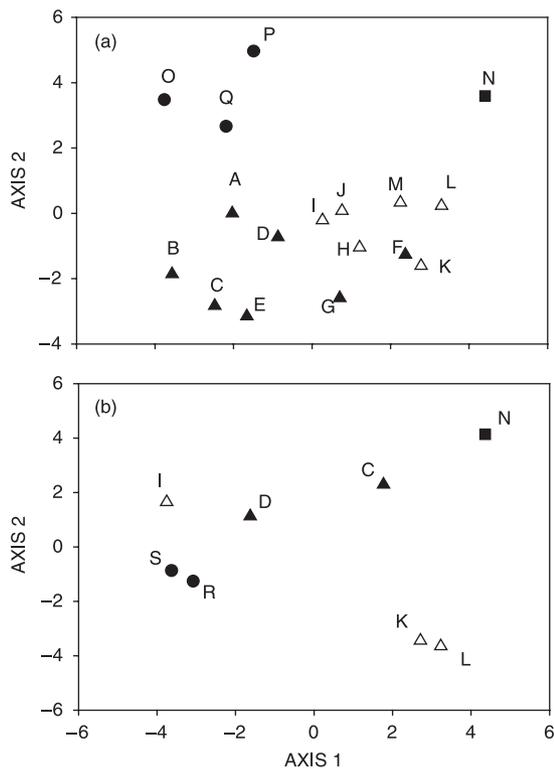
In general there appeared to be several associations that corresponded to cultivars, Kansas remnant (Konza) and Illinois populations (Fig. 1a). Most of the remnant and restored Illinois populations were separated by the first axis, with negative axis one scores for five of the seven remnant sites and positive axis one scores for all the restored populations. The second axis separated the cultivars and Kansas site from the Illinois populations.

As with the *A. gerardii* relationships, *S. nutans* cultivars and the Kansas Konza (remnant) population were genetically different from the Illinois populations (Fig. 1b). Axis one (eigenvalue = 9.92, percentage of variance = 27.5) and axis two (eigenvalue = 6.75, percentage of variance = 18.7) accounted for 46.2% of the variance in the *S. nutans* PCA correlation matrix, were statistically significant and were retained for interpretation.

Meadowbrook Park and Weldon Springs State Park are both restored tallgrass prairies located in central Illinois. These populations were close to each other on the ordination, indicating that they were more genetically similar to each other than to any other *S. nutans* population (Fig. 1b). Both sites were established by the same restoration practitioner. The Fermi restored tallgrass prairie is located in north-eastern Illinois and the ordination showed that the population of *S. nutans* was genetically different from the other two restored sites from central Illinois.

## Discussion

There was no association between genetic diversity estimates of *A. gerardii* and *S. nutans* and the area of the site, indicating that even an extremely small remnant population can possess a substantial amount of genetic variation. Maintenance of genetic diversity in both species may reflect similarities in several life-history traits (Dolan 1994; Godt & Hamrick 1998; Gordon & Rice 1998; Knapp & Rice 1998; Friar *et al.* 2001; Zawko *et al.* 2001). These co-dominant C<sub>4</sub> grasses of the tallgrass prairie typically propagate through clonal vegetative reproduction (rhizomatous growth) (Hartnett 1989); however, spatial genetic studies suggest that most clonal plants do not produce stands of a single genotype (Ellstrand & Roose 1987; Gustafson, Gibson & Nickrent 1999). Based on protein electrophoresis fingerprints and polyploidy levels, Keeler, Williams & Vescio (2002)



**Fig. 1.** PCA depicting the relationships among (a) seven remnant and six restored Illinois populations, one remnant Kansas site and three cultivars of *Andropogon gerardii*, and (b) two remnant and three restored Illinois populations, one remnant Kansas site and two cultivars of *Sorghastrum nutans*. Filled triangles, Illinois remnant; open triangles, Illinois restored; square, Kansas remnant; circles, cultivars. See Table 1 for site names.

found that *A. gerardii* clones averaged 2 m in diameter, with an average of 31.8 genetic individuals 100 m<sup>-2</sup>. In addition, both *A. gerardii* and *S. nutans* are autopolyploids that we have shown here partition most of their genetic variation within rather than among populations. Empirical and theoretical studies have suggested that the rate of decline in genetic diversity is slower in polyploidy species than diploid species (Bingham 1980; Stebbins 1985; Glendinning 1989; Bever & Felber 1992). Being rhizomatous, outcrossing and polyploid, perennial grasses probably contribute to the maintenance of genetic diversity in these small isolated remnant populations.

When establishing or restoring plant communities, the genetic diversity of the new population is influenced by the genetic composition of the source seed, species-specific life-history traits, and gene flow among adjacent conspecific populations. Restored tallgrass prairies in Illinois are not typically located within 1 km of remnant prairies, thus the genetic diversity in these restored populations probably reflects the use of multiple seed sources to establish these prairies. In this study of remnant and restored dominant grasses, the restored populations were as genetically diverse as the remnant populations. Without historical genetic data, however, we do not know how the current levels of genetic

diversity compare with the tallgrass prairie ecosystem prior to habitat reduction and fragmentation.

As a group, the Illinois populations of both *A. gerardii* and *S. nutans* were genetically more similar to one another than they were to the remnant Kansas population and the cultivars. Our results are consistent with previous genetic studies of prairie plants (Gustafson, Gibson & Nickrent 1999, 2001, 2002) and provide important genetic information for government and conservation organizations to use for effective management of these native species. Relationships between the remnant and restored populations within Illinois were complex, possibly reflecting a combination of spatial partitioning and effects of human activity. Restored *A. gerardii* populations tended to be more genetically similar to one another than they were to remnant *A. gerardii* populations that were located in close proximity. For example, Weldon Springs and Black Hawk (200 km apart) were restored by the same restoration practitioner and were genetically similar, despite the fact that Wheelock Railroad Prairie is a remnant tallgrass prairie located 40 km from Black Hawk and was presumably a local seed source for the Black Hawk restoration project. Other population relationships, such as between the Tomlinson Cemetery Prairie (remnant) and Meadowbrook Park (restored) *A. gerardii* populations, probably reflect geographical proximity and the restoration practitioners' practice of using local populations as seed sources. Analysis of the Illinois *S. nutans* restored populations gave similar results to those of the *A. gerardii* populations. The central Illinois restored populations (Meadowbrook Park and Weldon Springs) were genetically more similar to each other than either were to the restored tallgrass prairie in north-eastern Illinois (Fermi).

Natural resource managers, government agencies and the conservation communities recognize the importance of understanding the genetic consequences of their management decisions; however, most have not focused upon dominant members of such ecosystems. Dominant species have a disproportionately large influence on subordinate species and greatly influence ecosystem properties in remnant and restored ecosystems. This is the first conservation genetic study of two co-dominant species from remnant and restored populations of the endangered North American prairie ecosystem. The interpretation is similar for both *A. gerardii* and *S. nutans*. Genetic diversity estimates of plants from small remnant populations were as diverse as those from large populations, thus highlighting the value of such remnant prairies as important sources of genetic variation for the species. While this research does not specifically quantify the geographical range of a 'local' genotype, it is clear that *A. gerardii* and *S. nutans* from Konza Prairie, Kansas, are genetically different than the Illinois populations in this study. Taking the genetic information of this study in combination with differences in mycorrhizae dependency (Schultz *et al.* 2001) and population-specific differences

in competitive ability (Gustafson 2000), we support earlier studies suggesting ecotypic variation occurs in the dominant grasses of the tallgrass prairie (McMillan 1959). Cultivars of these grasses are not genetically less diverse than remnant populations, but we have shown that these cultivars are genetically different than remnant populations (Gustafson, Gibson & Nickrent 1999, 2001). Indeed, the original source of the Kaw cultivar of *A. gerardii* is from within 5 km of the Konza Prairie remnant population. Commercially available cultivars of native grasses are typically subjected to human selection and their relatively inexpensive seed is often purchased in large quantities for large-scale restoration projects (Baer *et al.* 2002). It is now necessary to determine how these cultivars compare over time to native ecotypes in terms of various fitness components and their effect on ecosystem structure and function (Sackville Hamilton 2001; Wilkinson 2001).

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### References

- Arafah, R.M.H., Sapir, Y., Shmida, A., Iraki, N., Fragman, O. & Comes, H.P. (2002) Patterns of genetic and phenotypic variation in *Iris haynei* and *I. atrofusca* (*Iris* sect. *Oncocyclus* = the royal irises) along an ecogeographical gradient in Israel and the West Bank. *Molecular Ecology*, **11**, 39–53.
- Baer, S.G., Kitchen, J., Blair, J.M. & Rice, C.W. (2002) Changes in ecosystem structure and function along a chronosequence of restored grasslands. *Ecological Applications*, **12**, 1688–1701.
- Barrett, S.C.H. & Kohn, J.R. (1991) Genetic and evolutionary consequences of small population size in plants: implications for conservation. *Genetics and Conservation of Rare Plants* (eds D.A. Falk & K.E. Holsinger), pp. 3–30. Oxford University Press, New York, NY.
- Bever, J.D. & Felber, F. (1992) The theoretical population genetics of autopolyploidy. *Oxford Surveys in Evolutionary Biology*, **8**, 185–217.
- Bingham, E.T. (1980) Maximizing heterozygosity in autopolyploids. *Polyploidy, Biological Relevance* (ed. W. Lewis), pp. 471–490. Plenum Press, New York, NY.
- Buza, L., Young, A. & Thrall, P. (2000) Genetic erosion, inbreeding and reduced fitness in fragmented populations of the endangered tetraploid pea *Swainsona recta*. *Biological Conservation*, **93**, 177–186.

- Diboll, N. (1997) Designing seed mixes. *The Tallgrass Restoration Handbook: For Prairies, Savannas and Woodlands* (eds S. Packard & C.F. Mutel), pp. 135–150. Island Press, Covelo.
- Dolan, R.W. (1994) Patterns of variation in relation to population size isolation and phylogeographic history in royal catchfly (*Silene regia*; Caryophyllaceae). *American Journal of Botany*, **81**, 965–972.
- Ellstrand, N.C. & Elam, D.R. (1993) Population genetic consequences of small population size: implications for plant conservation. *Annual Review of Ecology and Systematics*, **24**, 217–243.
- Ellstrand, N.C. & Roose, M.L. (1987) Patterns of genotypic diversity in clonal plant species. *American Journal of Botany*, **74**, 123–131.
- Etterson, J.R. & Shaw, R.G. (2001) Constraint to adaptive evolution in response to global warming. *Science*, **294**, 151–154.
- Fischer, M., van Kleunen, M. & Schmidt, B. (2000) Genetic allele effects on performance, plasticity and developmental stability in a clonal plant. *Ecology Letters*, **3**, 530–539.
- Fleishman, E., Launer, A.E., Switky, K.R., Yandell, U., Haywood, J. & Murphy, D.D. (2001) Rules and exceptions in conservation genetics: genetic assessment of the endangered plant *Cordylanthus palmatus* and its implications for management planning. *Biological Conservation*, **98**, 45–53.
- Frank, C.L. (1994) *Illinois Agronomy Handbook: 1995–1996*. Circular 1333. University of Illinois at Urbana-Champaign, College of Agriculture Cooperative Extension Service, Urbana, IL.
- Franklin, S.B., Gibson, D.J., Robertson, P.A., Pohlmann, H.T. & Fralish, J.T. (1995) Parallel analysis: a method for detecting significant principle components. *Journal of Vegetation Science*, **6**, 99–106.
- Friar, E.A., Boose, D.L., LaDous, T., Roalson, E.H. & Robichaus, R.H. (2001) Population structure in the endangered mauna loa silversword, *Argyroxiphium kauense* (Asteraceae), and its bearing on reintroduction. *Molecular Ecology*, **10**, 1657–1663.
- Glendinning, D. (1989) Some aspects of autotetraploid population dynamics. *Theoretical and Applied Genetics*, **78**, 233–242.
- Godt, M.J.W. & Hamrick, J.L. (1998) Isozyme diversity in the grasses. *Population Biology of Grasses* (ed. G.P. Cheplick), pp. 11–29. Cambridge University Press, Cambridge, UK.
- Gordon, D.R. & Rice, K.J. (1998) Patterns of differentiation in wiregrass (*Aristida beyrichiana*): implications for restoration efforts. *Restoration Ecology*, **6**, 166–174.
- Grime, J.P. (1999) Benefits of plant diversity to ecosystems: immediate, filter and founder effects. *Journal of Ecology*, **86**, 902–910.
- Gustafson, D.J. (2000) *Genetic and competition studies of Andropogon gerardii, Sorghastrum nutans, and Dalea purpurea from remnant and restored tallgrass prairies*. PhD thesis, Southern Illinois University, Carbondale, IL.
- Gustafson, D.J., Gibson, D.J. & Nickrent, D.L. (1999) Random amplified polymorphic DNA (RAPD) variation among remnant big bluestem [*Andropogon gerardii* Vitman] populations from Arkansas' Grand Prairie. *Molecular Ecology*, **8**, 1693–1701.
- Gustafson, D.J., Gibson, D.J. & Nickrent, D.L. (2001) Characterizing three restored *Andropogon gerardii* Vitman (big bluestem) populations established with Illinois and Nebraska seed: established plants and their offspring. *Seeds for the Future; Roots of the Past* (eds N. Bernstein & L.J. Ostrander), pp. 118–124. Proceedings of the 17th North American Prairie Conference, Northern Iowa Area. Community College, Mason City, IA.
- Gustafson, D.J., Gibson, D.J. & Nickrent, D.L. (2002) Genetic diversity and competitive abilities of *Dalea purpurea* (Fabaceae) from remnant and restored grasslands. *International Journal of Plant Sciences*, **163**, 979–990.

- Handel, S.N., Robinson, G.R. & Beattie, A.J. (1994) Biodiversity resources for restoration ecology. *Restoration Ecology*, **2**, 230–241.
- Harrington, J.A. (1989) Major prairie plantings on highway corridors to test methods, value of resulting vegetation. *Restoration and Management Notes*, **7**, 31–32.
- Hartnett, D.C. (1989) Density- and growth stage-dependent responses to defoliation in two rhizomatous grasses. *Oecologia*, **80**, 414–420.
- Jones, A.T., Hayes, M.J. & Sackville Hamilton, N.R. (2001) The effect of provenance on the performance of *Crataegus monogyna* in hedges. *Journal of Applied Ecology*, **38**, 952–962.
- Keeler, K.H. (1990) Distribution of polyploid variation in *Andropogon* (*Andropogon gerardii*, Poaceae) across the tallgrass prairie region. *Genome*, **33**, 95–100.
- Keeler, K.H., Williams, C.F. & Vescio, L.S. (2002) Clone size of *Andropogon gerardii* Vitman (big bluestem) at Konza Prairie, Kansas. *American Midland Naturalist*, **147**, 295–304.
- Keller, M., Kollmann, J. & Edwards, P.J. (2000) Genetic introgression from distant provenances reduces fitness in local weed populations. *Journal of Applied Ecology*, **37**, 647–659.
- Kindscher, K. & Tieszen, L.L. (1998) Floristic and soil organic matter changes after five and thirty-five years of native tallgrass prairie restoration. *Restoration Ecology*, **6**, 181–196.
- Knapp, A.K., Briggs, J.M., Hartnett, D.C. & Collins, S.L. (1998) *Grassland Dynamics: Long-Term Ecological Research in Tallgrass Prairie*. Oxford University Press, Oxford, UK.
- Knapp, E.E. & Rice, K.J. (1998) Comparison of isozyme and quantitative traits for evaluating patterns of genetic variation in purple needlegrass (*Nassella pulchra*). *Conservation Biology*, **12**, 1031–1041.
- Lesica, P. & Allendorf, F.W. (1999) Ecological genetics and the restoration of plant communities: mix or match. *Restoration Ecology*, **7**, 42–50.
- Linhart, Y.B. & Grant, M.C. (1996) Evolutionary significance of local genetic differentiation in plants. *Annual Review of Ecology and Systematics*, **27**, 237–277.
- Longman, R.S., Cota, A.A., Holden, R.R. & Fekken, G.C. (1989) A regression equation for the parallel analysis criterion in principle components analysis: mean and 95th percentile eigenvalues. *Multivariate Behavioral Research*, **24**, 59–69.
- McClenaghan, L. & Beauchamp, A. (1986) Low genetic differentiation among isolated populations of the California fan palm (*Washingtonia filifera*). *Evolution*, **40**, 315–322.
- McKone, M.J., Lund, C.P. & O'Brien, J.M. (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.
- McMillan, C. (1959) The role of ecotypic variation in the distribution of the central grassland of North America. *Ecological Monographs*, **29**, 285–308.
- Meffe, G.K. & Carroll, C.R. (1994) *Principles of Conservation Biology*. Sinauer, Sunderland, MA.
- Montalvo, A.M., Williams, S.L., Rice, K.J., Buchmann, S.L., Cory, C., Handel, S.N., Cabhan, G.P., Primack, R. & Robichaux, R.H. (1997) Restoration biology: a population biology perspective. *Restoration Ecology*, **5**, 277–290.
- Newman, D. & Pilson, D. (1997) Increased probability of extinction due to decreased genetic effective population size: experimental populations of *Clarkia pulchella*. *Evolution*, **51**, 354–362.
- Norrmann, G.A., Quarin, C.L. & Keeler, K.H. (1997) Evolutionary implications of meiotic chromosome behavior, reproductive biology, and hybridization in 6x and 9x cytotypes of *Andropogon gerardii* (Poaceae). *American Journal of Botany*, **84**, 201–207.
- Noy-Meir, I. & Briske, D.D. (2002) Response of wild wheat populations to grazing in Mediterranean grasslands: the relative influence of defoliation, competition, mulch and genotype. *Journal of Applied Ecology*, **39**, 259–278.
- Palmer, M.A., Ambrose, R.F. & Poff, N.L. (1997) Ecological theory and community restoration ecology. *Restoration Ecology*, **5**, 291–300.
- Prober, S. & Brown, A. (1994) Conservation of the grassy white box woodlands. I. Population genetics and fragmentation of *Eucalyptus albens* Benth. *Conservation Biology*, **8**, 1003–1013.
- Prober, S., Spindler, L.H. & Brown, A.H.D. (1998) Conservation of the grassy white woodlands: effects of remnant population size on genetic diversity in the allotetraploid herb *Microseris lanceolata*. *Conservation Biology*, **12**, 1279–1290.
- Pywell, R.F., Bullock, J.M., Roy, D.B., Warman, L.I.Z., Walker, K.J. & Rothery, P. (2003) Plant traits as predictors of performance in ecological restoration. *Journal of Applied Ecology*, **40**, 65–77.
- Raijmann, L., van Leeuwen, N., Kersten, R., Oostermeijer, J., Nijs, H. & Menken, S. (1994) Genetic variation and outcrossing rate in relation to population size in *Gentiana pneumonanthe* L. *Conservation Biology*, **8**, 1014–1026.
- Risser, P.G., Birney, E.C., Blocker, H.D., May, S.W., Parton, W.J. & Weins, J.A. (1981) *The True Prairie Ecosystem*. US/IBP Synthesis Series Number 16. Hutchinson, Stroudsburg.
- Robertson, K.R. (2001) Planning with plants in Illinois. *Bioconservation and Systematics* (eds J.B. Phipps & P.M. Catling), pp. 28–45. Proceedings of the Canadian Botanical Association Conference Symposium in London, Ontario, Canada.
- Robertson, K.R. & Schwartz, M.W. (1994) *Prairies, Changing Illinois Environment: Critical Trends*. Vol. 3. *Ecological Resources*. Illinois Department of Energy and Natural Resources, Natural History Survey Division, Champaign, IL.
- Robertson, K.R., Anderson, R.C. & Schwartz, M.W. (1997) The tallgrass prairie mosaic. *Conservation in Highly Fragmented Landscapes* (ed. M.W. Schwartz), pp. 55–87. Chapman & Hall, New York, NY.
- Sackville Hamilton, N.R. (2001) Is local provenance important in habitat creation? A reply. *Journal of Applied Ecology*, **38**, 1374–1376.
- Sampson, J., Hopper, S. & James, S. (1988) Genetic diversity and the conservation of *Eucalyptus crucic* Maiden. *Australian Journal of Botany*, **36**, 447–460.
- Schneider, S., Roessli, D. & Excoffier, L. (2000) *Arlequin, Version 2.0: A Software for Population Genetics Analysis*. Genetics and Biometry Laboratory, University of Geneva, Geneva, Switzerland.
- Schramm, P. (1990) Prairie restoration: a twenty-five year perspective on establishment and management. *Proceedings of the North American Prairie Conference* (eds D.D. Smith & C.A. Jacobs), pp. 169–177. Proceedings of the North American Prairie Conference, University of Northern Iowa, Cedar Rapids, IA.
- Schultz, P.A., Miller, R.M., Jastrow, J.D., Rivetta, C.V. & Bever, J.D. (2001) Evidence of mycorrhizal mechanism for the adaptation of *Andropogon gerardii* (Poaceae) to high- and low-nutrient prairies. *American Journal of Botany*, **88**, 1650–1656.
- Seliskar, D.M., Gallagher, J.L., Burdick, D.M. & Mutz, L.A. (2002) The regulation of ecosystem functions by ecotypic variation in the dominant plant: a *Spartina alterniflora* salt-marsh case study. *Journal of Ecology*, **90**, 1–11.
- Stebbins, G.L. (1985) Polyploidy, hybridization and the invasions of new habitats. *Annals of the Missouri Botanical Garden*, **72**, 824–832.
- Stevenson, M.J., Bullock, J.M. & Ward, L.K. (1995) Re-creating semi-natural communities: effect of sowing on establishment of calcareous grassland. *Restoration Ecology*, **3**, 279–289.

- van Treuren, R., Bijlsma, R., van Delden, W. & Ouborg, N. (1991) The significance of genetic erosion in the process of extinction. I. Genetic differentiation in *Salvia pratensis* and *Scabiosa columbaria* in relation to population size. *Heredity*, **66**, 181–189.
- Weaver, J.E. & Fitzpatrick, T.J. (1935) The prairie. *Ecological Monographs*, **4**, 109–295.
- Weising, K., Nybon, H., Wolff, K. & Meyer, W. (1995) *DNA Fingerprinting in Plants and Fungi*. CRC Press, Boca Raton, FL.
- Wilkinson, D.M. (2001) Is local provenance important in habitat creation? *Journal of Applied Ecology*, **38**, 1371–1373.
- Young, A., Boyle, T. & Brown, T. (1996) The population genetic consequences of habitat fragmentation for plants. *Trends in Ecology and Evolution*, **11**, 413–418.
- Young, A.G., Brown, A.H.D. & Zich, F.A. (1999) Genetic structure of fragmented populations of the endangered daisy *Rutidosis leptorrhynchoides*. *Conservation Biology*, **13**, 256–265.
- Zawko, G., Krauss, S.L., Dixon, K.W. & Sivasithamparam, K. (2001) Conservation genetics of the rare and endangered *Leucopogon obtectus* (Ericaceae). *Molecular Ecology*, **10**, 2389–2396.

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