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ENVIRONMENTAL CORRELATES OF CYTOTYPE DISTRIBUTION IN *ANDROPOGON GERARDII* (POACEAE)[1]

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- *Premise of the study:* Information about geographic distribution of cytotypes can provide insight into the origin and maintenance of autopolyploid complexes and builds a foundation for understanding cytotype differentiation and the dynamics of mixed-ploidy populations. Here, we investigate environmental correlates of the geographic distributions of $6x$ and $9x$ individuals in the ecologically dominant grass *Andropogon gerardii* to examine the role of climate in shaping patterns of cytotype distribution in this species.
- *Methods:* Flow cytometry was used to estimate ploidy level in 352 individuals from 32 populations across North America. Ecological differentiation of cytotypes was tested by relating BIOCLIM variables to cytotype distribution using principal components analysis and partial linear regression.
- *Key results:* Broad geographic sampling confirmed two primary cytotypes—$6x$ (hexaploid) and $9x$ (enneaploid)—and revealed that $9x$ plants are more common than previously thought. Enneaploids occur frequently in the southern portions of the range, with hexaploids dominating in northern regions. Mixed-ploidy populations were common (46.9%). Principal components analysis and partial linear regression indicated that reduced summer precipitation and increased variation in diurnal and seasonal temperature range were significant predictors of the frequency of $9x$ plants in a population.
- *Conclusions:* Results indicate that (1) geographic distribution of $6x$ and $9x$ individuals is nonrandom; (2) environmental variables are associated with cytotype distribution in *A. gerardii*; and (3) nearly half of populations surveyed include both $6x$ and $9x$ individuals. The persistence of mixed-ploidy populations may reflect a combination of recurrent polyploid formation and the prevalence of clonal reproduction.

Key words: *Andropogon gerardii*; autopolyploid; cytogeography; cytotype distribution; Poaceae; polyploidy; tallgrass prairie; whole genome duplication.

Polyploidization, or whole genome duplication, is widely recognized as a driving force in the evolution of plants (Stebbins, 1971; Soltis and Soltis, 1999; Otto and Whitton, 2000; Wood et al., 2009; Parisod et al., 2010), with the majority of angiosperms thought to be of polyploid origin (Cui et al., 2006). Evolutionary biologists generally recognize two categories of polyploids, allopolyploids, which result from genome duplication following hybridization of distinct plant lineages, and autopolyploids, which form following genome duplication within a single plant lineage, usually through the union of unreduced gametes or the union of a reduced and an unreduced gamete (Soltis and Soltis, 2000). Historically, autopolyploids were considered rare and may have gone unnoticed due to morphological similarities between cytotypes (Parisod et al., 2010). However, increased attention to and detection of autopolyploid complexes indicates that intraspecific genome duplication is far more common than previously thought (Rausch and Morgan, 2005; Soltis et al., 2007; Parisod et al., 2010), with multiple species now showing evidence of recurrent autopolyploidization (Soltis and Soltis, 1989; Segraves et al., 1999; Stuessy et al., 2004; Ramsey et al., 2008).

In autopolyploids, genome duplication within a plant lineage often results in a mosaic of different cytotypes across the range of a species (Soltis, 1984; Keeler, 1990; Segraves et al., 1999; Halverson et al., 2008; Baack, 2004; Suda et al., 2007b; Ståhlberg, 2009; Martin and Husband, 2013). Understanding the mechanisms underlying the origin(s) and maintenance of different cytotypes in autopolyploid lineages is a primary goal of current work. Differences in the geographic distribution of cytotypes of some polyploid complexes have been associated

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with environmental conditions (e.g., light levels [Lumaret et al., 1987], temperature [Hardy et al., 2000], precipitation [Levin, 2002; Johnson et al., 2003; Glennon et al., 2014]). However, cytogeographic patterns may reflect other factors, such as differential rates of colonization resulting from among-cytotype variation in seed viability and seedling establishment (Stebbins, 1985; Sonnleitner et al., 2010), genetic drift and founder effects associated with postglacial recolonization (Stebbins, 1985; Weber and Schmid, 1998; Sonnleitner et al., 2010), and challenges associated with frequency-dependent mating (Levin, 1975). It is worth noting that these explanations for cytotype distributions are not mutually exclusive.

An additional question in autopolypoid complexes addresses the mixture of cytotypes within populations. Are populations usually dominated by one cytotype, or are multiple cytotypes maintained within the same population? Although some polyploid species show little or no within-population cytotype variation (Thompson and Lumaret, 1992; Thompson et al., 1997; Raabová et al., 2008; Godsoe et al., 2013), mixed cytotype populations have been recorded for a range of taxa (Keeler, 1990; Husband and Schemske, 2000; Halverson et al., 2008; Mock et al., 2012). In some cases, mixed populations appear to be restricted to narrow contact zones between two or more cytotype ranges (Husband and Schemske, 2000; Baack, 2004; Stuessy et al., 2004). However, other taxa show evidence of mixed-ploidy populations across large sections of the geographic range of the species (Burton and Husband, 1999; Halverson et al., 2008; Mock et al., 2012).

The occurrence of mixed cytotype populations in numerous species has spurred an ongoing debate about whether these populations represent transient or stable states. One model, the minority cytotype exclusion hypothesis, posits that the transient mixed-ploidy state may result from mate limitation experienced by the rare cytotype(s) (Levin, 1975). This model predicts that the rare cytotype(s) will exhibit decreased fitness and eventual extinction because crosses between individuals with the same cytotype are necessary for the maintenance of that cytotype. An alternative explanation is that stable mixed cytotype populations could be maintained if recurrent formation of polyploids is occurring (e.g., Halverson et al. [2008]). This could happen through the continued production of unreduced gametes and subsequent fusion of unreduced and reduced gametes. This process would provide a dynamic, nearly limitless source of autopolyploid variation on which selection could act (Felber, 1991; Soltis and Soltis, 1995, 1999; Husband et al., 2013). An additional factor that may be contributing to the maintenance of mixed ploidy populations is asexual reproduction, particularly parthenogenesis and high rates of clonal reproduction, which facilitate the persistence of polyploid individuals over many years or decades (Joly and Bruneau, 2004; Yamauchi et al., 2004; Kao, 2007).

Despite accumulating evidence for the prevalence of cytotype variation within autopolyploid species, there are still unanswered questions about the drivers of patterns of cytotype diversity within and among populations across broad geographic scales. In widespread species that span a range of climates, are certain environmental variables consistently associated with cytogeographic patterns? In this study, we investigate patterns of cytotype distribution and environmental correlates of cytotype diversity in big bluestem (*Andropogon gerardii* Vitman; Poaceae), the dominant grass of the North American tallgrass prairie ecosystem (Knapp et al., 1998, 2002; Silletti et al., 2004; Nippert et al., 2009). *Andropogon gerardii* is a widespread, long-lived, outcrossing, highly clonal bunchgrass with multiple ploidy levels that occurs across an environmentally heterogeneous range spanning most of North America. The two most common cytotypes in *A. gerardii* are hexaploid ($2n = 6x = 60$) and enneaploid ($2n = 9x = 90$) (Keeler and Davis, 1999), and chromosome behavior of the *A. gerardii* $6x$ and $9x$ cytotypes at meiosis suggests an autopolyploid origin of the $9x$ cytotype. Previous work suggests that the $9x$ cytotype is derived from $6x$ cytotypes through the union of a reduced gamete with a nonreduced gamete (Norrmann et al., 1997). The cytotypes occur in single or mixed ploidy populations (Keeler, 1990) and are morphologically indistinguishable (Keeler and Davis, 1999, Keeler, 2004, C. McAllister, personal observation); however, the $9x$ cytotype shows significantly lower fitness (e.g., lower frequency of viable seed and lower seedling establishment) than the $6x$ cytotype (Keeler, 2004). The $6x$ and $9x$ cytotypes can interbreed, forming offspring with a range of chromosome numbers ($2n$ = 68–80). However, the result of intercytotype crossing, aneuploid individuals and intermediate euploids ($7x$ and $8x$), are rare relative to the common cytotypes, often comprising less than 5% of individuals in populations with multiple cytotypes (Keeler et al., 1987; Keeler, 1990, 1992).

Previous studies of cytogeographic patterns in *A. gerardii* suggest that the distributions of the two most common cytotypes are not random with respect to geography or environmental variables. Keeler (1990) surveyed 11 populations of *A. gerardii* across the Midwest. Her samples spanned a naturally occurring east–west precipitation gradient ranging from over 1200 mm rain per year in Ohio in the east to 400 mm per year in Kansas in the west (Borchert, 1950; Luaenroth et al., 1999). The frequency of the $9x$ cytotype increased westward as precipitation decreased along this gradient. Subsequent investigation of populations of *A. gerardii* around Boulder, Colorado (average precipitation: 486 mm/year) also showed high frequencies of $9x$ plants (Keeler and Davis, 1999; Keeler, 2004). These studies presented an interesting pattern, but sampled only a small subset of the geographic range of the species and did not include any explicit tests for associations between ploidy level and climatic variables.

Here, we extend Keeler's work by estimating ploidy levels in *A. gerardii* populations collected across the geographic and environmental range of the species and by incorporating geographic information system (GIS)-based climatic data to statistically assess the relationship between cytotype distribution and climate. The specific objectives of this study are to (1) determine the frequencies and geographic patterns of *A. gerardii* cytotypes on a range-wide scale and (2) test for associations between geographic distribution of the cytotypes and climatic variables.

MATERIALS AND METHODS

Plant material—Thirty-two populations of *A. gerardii* were sampled from across the range of the species during August 2011 and August 2012 (Table 1). Each population was separated from other sampled populations by at least 80 km. Whenever possible, samples were collected from populations in remnant, unplowed prairies with no known history of overseeding (seeding over established prairie as part of restoration efforts) to avoid possible inclusion of nonnative genotypes. Only seven of our sampling localities (21%) were roadside populations. Sampling localities were selected primarily in the historical extent of the tallgrass prairie (e.g., Minnesota to Texas, Kansas to Ohio; Fig. 1), where *A. gerardii* was the dominant plant species. In addition, several "outlier" populations were also chosen to represent more extreme edges of the range of the species (e.g., areas outside of the historical extent of the tallgrass prairie,

Table 1. Population locations of *Andropogon gerardii* and cytotypic composition. Voucher specimens from each locality were deposited at the Missouri Botanical Garden (MO).

				Cytotype proportions (%)		
Locality	Longitude	Latitude	*N*	6*x*	9*x*	Voucher specimens
Principia College Hill Prairies, Elsah, IL	−90.3163	38.93571	12	100	–	CAM698
Lindahl Hill Prairie, Rock Island, IL	−90.5574	41.4395	10	100	–	CAM696
Chesney Prairie Natural Area, Siloam Springs, AR	−94.4825	36.21904	9	56	44	CAM690, CAM691
Baker Prairie Natural Area, Harrison, AR	−93.1336	36.24348	11	73	27	CAM684, CAM685
Railroad Prairie Natural Area, Carlisle, AR	−91.7156	34.78203	10	80	20	CAM700, CAM701
Roadside population (Highway 165), Fenton, LA	−92.932	30.33925	10	60	40	CAM704, CAM705
University of Houston Coastal Center, Texas City, TX	−95.0421	29.3881	8	87.5	12.5	CAM706, CAM707
Pontotoc Ridge Preserve, Ada, OK	−96.6008	34.5113	9	11	89	CAM708, CAM709
Tallgrass Prairie Preserve, Pawhuska, OK	−96.4201	36.8458	10	60	40	CAM710, CAM711
Roadside population (State Route 52), Sublette, IL	−89.1702	41.59575	9	100	–	CAM718, CAM719
Avoca Prairie State Natural Area, Avoca, WI	−90.2912	43.1942	10	100	–	CAM720, CAM721
Trenton Bluff Prairie State Natural Area, Hager City, WI	−92.566	44.619	9	100	–	CAM716, CAM717
Roscoe Prairie Scientific and Natural Area, Roscoe, MN	−94.6812	45.4179	9	100	–	CAM722, CAM723
Foxhome Prairie Preserve, Foxhome, MN	−96.2833	46.3258	10	100	–	CAM724, CAM725
Roadside population (Route 18), Wyndmere, ND	−97.1343	46.3396	11	100	–	CAM726, CAM727
Sioux Prairie, Colman, SD	−96.7887	44.0295	10	90	10	CAM728, CAM729
Sioux City Prairie Preserve, Sioux City, IA	−96.4305	42.5268	10	70	30	CAM730, CAM731
Folsom Point Preserve, Council Bluffs, IA	−95.8017	41.1527	9	100	–	CAM732, CAM733
Compass Plant Prairie State Nature Preserve, Aid, OH	−82.4753	38.61632	12	100	–	CAM771
Drover's Prairie Conservation Area, Sedalia, MO	−93.2908	38.53181	12	100	–	CAM797
Anderson County Prairie Preserve, Welda, KS	−95.2748	38.16897	11	91	9	CAM802, CAM803
Konza Prairie Biological Station, Manhattan, KS	−96.5582	39.08581	11	27	73	CAM841
Roadside population (N. 120th Rd.), Minneapolis, KS	−97.706	39.14492	10	90	10	CAM844, CAM847
Roadside population (Hubbell Rd.) Hebron, NE	−97.5764	40.14152	10	100	–	CAM860
Pine Ridge, Ft. Collins, CO	−105.136	40.53429	12	58	42	CAM938
Jewel Mountain Habitat Conservation Area, Boulder, CO	−105.244	39.8771	10	30	70	CAM949, CAM963
Roadside population (Route 72), Raton, NM	−104.271	36.93788	12	–	100	CAM974, CAM975
Lincoln National Forest, Ruidoso, NM	−105.677	33.35288	12	100	–	CAM994, CAM998, CAM1000
Balcones Canyonland National Wildlife Refuge, Austin, TX	−98.0688	30.61866	13	–	100	CAM1037, CAM1038
Riesel Prairie, Riesel, TX	−96.858	31.46247	12	–	100	CAM1074, CAM1075
Roadside population (County Road 1140), Celeste, TX	−96.2428	33.30943	9	–	100	CAM1076, CAM1078
Rock Hill Blackjacks Heritage Preserve, Rock Hill, SC	−81.023	34.904	9	–	100	–

but still within the contemporary, natural range of *A. gerardii*, including sites in Colorado, New Mexico, South Carolina). Leaf material was collected from 10 plants per population and dried separately in silica gel. Because clones of *A. gerardii* can be up to 2 m in diameter (Keeler et al., 2002), leaves were collected from plants that were at least 5 m apart to avoid sampling from the same clone. Sampling was otherwise random. In populations with flowering individuals, samples were collected without regard for reproductive status. Voucher specimens from each locality were deposited at the Missouri Botanical Garden (MO).

DNA content measurement and ploidy assignment—DNA content estimates for all samples were made using flow cytometry (FCM), following the basic method of Galbraith et al. (1983). Ploidy determined on the basis of DNA content is often referred to as "DNA ploidy" (Suda et al., 2006); from here forward we use "ploidy" for convenience. Ploidy levels were assigned to individual plants by comparing their 2C nuclear DNA contents with published 2C values for plants of known ploidy; we used rye (*Secale cereale* L. 'Dankovské') as the DNA content standard (16.19 pg/2C; Doležel et al., 1998). We also compared our DNA content estimates with previously reported DNA content measurements from chromosome-counted 6*x* and 9*x* plants of *A. gerardii* (Norrmann et al., 1997). Preliminary testing demonstrated that high-quality dried leaf tissue from *A. gerardii* produced estimates of DNA content very close to those of fresh tissue. Consequently, for this study, approximately 2 cm^2 of dried leaf tissue with midveins removed and 1 cm^2 fresh leaf tissue from the DNA content standard (*S. cereale*) were chopped with a new razor blade in a petri dish with 1 mL ice-cold LB01 buffer (Doležel et al., 1989) containing 100 μg/mL propidium iodide and 50 μg/mL RNAse). After chopping for approximately 20 s to release nuclei into the buffer, the sample was filtered through a 30 μm Partec Celltrics filter. Samples were then centrifuged at 7600 × *g* for 10 s. After centrifugation, 300 μL of the supernatant was removed, and the pelleted nuclei were resuspended. The nuclei were stained for 30–60 min before testing.

Samples were analyzed using a BD FACSCalibur flow cytometer (BD Biosciences, San José, California, USA) located at the University of Guelph, Guelph, Ontario, Canada. Samples were run for up to 5 min to acquire at least 1000 nuclei per G1 phase for both the standard and the test plant. We used the FL2 detector (585/42 nm) to measure propidium iodide fluorescence and analyzed fluorescence area (integrated fluorescence) histograms using ModFit LT for Mac software (Vers. 3.3.11, 2011; Verity Software House, Topsham, Maine, USA). We measured peak means, coefficients of variation (CV), and number of nuclei per peak. Standard recommendations for FCM quality control with plant material include restricting histogram peak coefficient of variation (CV) values to <5% (e.g., Doležel et al., 2007; Greilhuber et al., 2007); however, CV values between 5 and 10% are acceptable when the objective is to determine ploidy level rather than genome size (Suda et al., 2007a). In this study, a natural break in the range of sample CV values occurred around 8%, with the majority (96%) of our samples' CV values falling below this threshold. Therefore, we selected 8% as a somewhat arbitrary cutoff for CV values, and samples with peak CVs > 8% were removed from the analysis. Estimated DNA content of the nuclei from the test plants was calculated as (peak mean of test plant)/(peak mean of standard) × (DNA content of standard). Norrmann et al. (1997) present 2C DNA content estimates from chromosome-counted 6*x* (5.93 pg ± 0.26 SD) and 9*x* (8.92 pg ± 0.24 SD) plants collected in Florida, Nebraska, and Kansas. It is common in flow cytometry studies specifically related to cytotype identification to use published reference values for a species as guides in determining ploidy (e.g., Schönswetter et al. [2007]; Hülber et al. [2009]). To further compare our DNA content estimates with published values from chromosome-counted plants, we sampled from the same site in Kansas (Konza Prairie Biological Station, Manhattan, KS) and from a site located within 130 km of Keeler's Nebraska sites (Hebron, NE). We determined that our DNA content

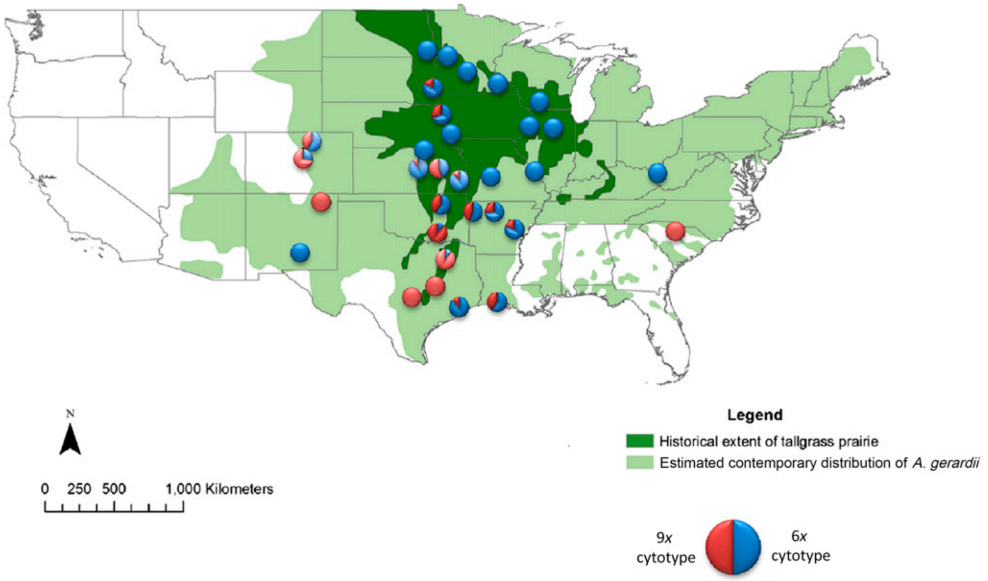

Fig. 1. Distribution of cytotypes of *Andropogon gerardii* across the United States. Pie charts represent population frequency of 6*x* (blue) and 9*x* (red) plants at each sampling locality. Historical distribution of tallgrass prairie modified from Anderson (2006) and Risser et al. (1981). Contemporary distribution of *A. gerardii* estimated from USDA/NRCS (2014) county-level distribution maps.

values generated for populations that were collected at or near the sites used by Keeler and colleagues to estimate DNA content, as well as for our other samples, overlapped with Keeler's previous estimates of DNA. Consequently, we felt comfortable assigning ploidy levels to all individual plants by comparing their 2C DNA content estimates to these reported values.

Geographic distribution of cytotypes—The frequency of 6*x* and 9*x* cytotypes was calculated for each of the 32 populations sampled in this study. To test for spatial autocorrelation of the frequency of the 9*x* plants, we used a Mantel test in the program PASSaGE, version 2.0.11.6 with 100 000 permutations (Rosenberg and Anderson, 2011). Relationships between latitude, longitude, and the frequency of higher order polyploids were investigated using linear regression (using XLSTAT v. 2013.3.05, Addinsoft, New York, New York, USA).

Statistical associations between cytotype distributions and climate—Climate data for the 32 localities were extracted from 19 BIOCLIM data layers at 2.5 arc min resolution (http://www.worldclim.org/bioclim; Hijmans et al., 2005) using the program DIVA-GIS version 7.5.0.0 (Hijmans et al., 2001). This level of resolution is appropriate for a study of this geographic scale. Although higher resolution BIOCLIM data layers are available (e.g., 30 s [~1 km^2] resolution), we chose 2.5 arc-min resolution because the area of several of our sampled populations of *A. gerardii* was larger than 1 km^2.

Many BIOCLIM variables are highly correlated with one another and reduction of multicollinearity is recommended before analysis or modeling (Robertson et al., 2001; Wiens and Graham, 2005). To assess correlations among variables, we extracted all 19 BIOCLIM variables for 1000 randomly generated points within the range of *A. gerardii*; then, following Rissler et al. (2006), we used a Pearson product correlation matrix to identify variables correlated at a level of ≥0.75. From this matrix, we first selected variables that were not strongly correlated with any other variables. From the remaining groups of correlated variables, we chose variables known to impact reproductive success or vegetative growth rates in *A. gerardii* (DeLucia et al., 1992; Knapp et al., 1998; Silletti and Knapp, 2002; Fay et al., 2003; Nippert et al., 2009). In total, seven BIOCLIM variables representing climatic variability and extremes of heat and precipitation during the growing season were selected: annual mean temperature, mean diurnal temperature range (mean of monthly (max temp-min temp), isothermality (mean diurnal range/annual range in temp × 100), max temperature of the warmest month, annual precipitation, precipitation seasonality (coefficient of variation), and precipitation of the warmest quarter.

Principal components analysis (PCA) was used to summarize variation in environmental variables associated with patterns of cytotype diversity in *A. gerardii* and to explore the relationship between ploidy level occurrence and environmental variables (XLSTAT v. 2013.3.05). Varimax rotation was applied to maximize the variances explained by each axis (Tabachnick and Fidell, 2013). Associations between climate and population-level frequency of 9*x* plants were examined using partial linear regression as a means of accounting for geographic distance among populations. The factor loadings from the PCA, as well as latitude and longitude of the sample localities, were used as predictors of population-level frequency of 9*x* plants in a partial linear regression analysis, conducted using the program SAM (Spatial Analysis in Macroecology, v. 4.0; Rangel et al., 2010). The frequency of 9*x* plants was arcsin square root transformed to homogenize variances.

RESULTS

Ploidy estimation—Ploidy was estimated for 352 plants from 32 populations. Twelve plants were removed from the data set because their CVs exceeded 8%, leaving 340 plants with an average peak CV for *A. gerardii* of 5.4% (range: 2.58–7.99%) and an average CV in the internal standard species, *S. cereale*, of 2.43% (range: 1.45–6.39%).

Our data showed two discrete, nonoverlapping DNA content clusters (Fig. 2) corresponding to the two common cytotypes (6*x* and 9*x*). The first cluster consists of individuals with estimated DNA contents between 5.39 and 6.93 pg/2C (mean: 6.44 ± 0.22 SD). The second cluster has estimated DNA contents between 8.67 and 10.84 pg/2C (mean: 9.52 ± 0.43 SD) (Fig. 2). These values are consistent with those previous FCM studies of chromosome-counted *A. gerardii* for both the 6*x* (5.93 pg/2C ± 0.26 SD) and 9*x* (8.92 pg/2C ± 0.24 SD) plants (Konza Prairie Biological Station, KS; Norrmann et al. [1997]) and samples from within ~130 km of two additional sites used to generate those reference values (Hebron, NE).

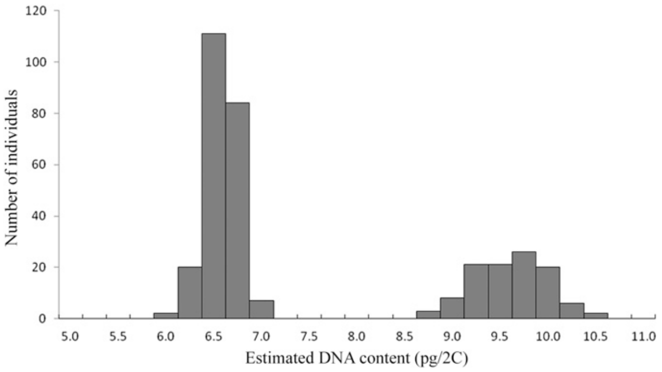

Fig. 2. Histogram of estimated DNA content (pg/2C) for all samples of *Andropogon gerardii*.

The presence of small numbers of individuals with estimates outside these clusters is consistent with previous studies that reported less than 5% of plants in natural populations with intermediate cytotypes or cytotypes <6*x* or >9*x* (Keeler, 1990, 1992; Keeler and Davis, 1999; Keeler, 2004). In the absence of chromosome counts for each of our 340 samples, we cannot determine whether the estimates correspond to rare cytotypes in our data (e.g., 7*x*, 8*x*, 10*x*) or if they represent variation around the 2C values of the two main clusters (6*x* and 9*x*, detailed above). To account for this, we took the conservative approach of excluding samples that deviated from the mean of a cluster by more than 10% from further analysis and classifying the remainder as 6*x* or 9*x*; this removed nine of 340 (2.6%) sampled plants, resulting in a final data set of 331 plants. Using this approach, 224/331 (67.7%) plants were classified as 6*x* and 107/331 (32.3%) as 9*x*.

Distribution of cytotypes—Clear geographic structuring of cytotypes in *A. gerardii* is evident (Fig. 1). A Mantel test showed significant relationship between geography and higher ploidy level ($r = 0.215$; $P = 0.0047$), indicating that populations in close proximity to one another have similar ploidy levels. Linear regression demonstrated a significant inverse relationship between latitude and relative frequency of 9*x* plants ($R^2 = 0.312$; $P < 0.001$), but not longitude ($R^2 = 0.04$; $P = 0.275$). Population-level frequency of 9*x* plants ranged from 0% in the northern portions of the sampled range to 100% in the southern portions of the range. The proportions of cytotypes within populations varied widely (Table 1). Of the 32 populations sampled, 53.1% contained only one cytotype, while 46.9% of populations included a mix of 6*x* and 9*x* cytotypes (Table 1). Most populations with single cytotypes included only 6*x* plants (76.5%); 23.5% of populations with single cytotypes included only 9*x* (Table 1).

Statistical associations between cytogeography and climate—After a correlation analysis reduced the 19 BIOCLIM variables to seven less-correlated variables, principal component analysis of the seven BIOCLIM variables recovered two principal components that together explained 80.9% of the variation (49.5% and 31.4%, respectively). Factor loadings indicated that principal component one represented a gradient from cooler, drier sites with high seasonal fluctuations in temperature to warmer, more mesic sites with lower seasonal fluctuations in temperature (Table 2). Principal component two represented a gradient from high annual and diurnal temperature ranges with low summer precipitation to sites with lower annual and diurnal temperature ranges with higher summer precipitation. Visual inspection of the distribution of 9*x* plants in Fig. 3 indicated no clear relationship between the frequency of 9*x* plants and PC axis 1, but did suggest a possible correlation between the frequency of 9*x* plants and PC axis 2. The majority of populations with high frequencies of 9*x* plants appear to be located in sites with high temperature fluctuation and low summer precipitation (Fig. 3).

To test these observations, partial linear regression was used to evaluate how well the two PC axes predicted frequency of higher order polyploids. However, because many environmental variables are influenced by geography, both longitude and latitude were included as partial predictors to account for the effects of spatial autocorrelation in the data. When these partial predictors were included, the correlation between PC2 and 9*x* frequency was significant, but the correlation between PC1 and 9*x* frequency was not (Table 3). These results demonstrate higher frequencies of 9*x* plants in drier areas that have higher variability in both diurnal and annual temperature ranges.

DISCUSSION

Flow cytometric screening of ploidy level in 331 plants from 32 populations confirms earlier reports of two main cytotypes in *A. gerardii*, 6*x* and 9*x* (Keeler, 1990, 1992, 2004; Keeler and Davis, 1999) and demonstrates that mixed ploidy populations containing both 6*x* and 9*x* cytotypes are more common than previously known. The differences in overall cytogeographic patterns between our study and Keeler's earlier work (Keeler, 1990, 1992, 2004; Keeler and Davis, 1999) (e.g., more mixed cytotype populations and more predominantly 9*x* populations) are likely explained by our larger sampling range. Notably, when we sampled close to previously tested sites, we found qualitatively similar results as previously published studies in terms of ploidy levels and cytotype mixtures (Keeler, 1990,

Table 2. Factor loadings from principal component analysis on climate variables associated with sampling localities for *Andropogon gerardii*.

Variable	PC 1	PC 2
Mean annual temperature (Bio 1)	0.963	0.092
Mean diurnal temperature range (Bio 2)	–0.376	0.847
Isothermality ((Bio2/temperature annual range) × 100) (Bio 3)	0.375	0.826
Maximum temperature of warmest month (Bio5)	0.820	–0.039
Annual precipitation (Bio12)	0.869	–0.411
Precipitation seasonality (coefficient of variation) (Bio15)	–0.867	0.157
Precipitation of warmest quarter (Bio18)	0.281	–0.772
% Variance explained	49.6%	31.4%

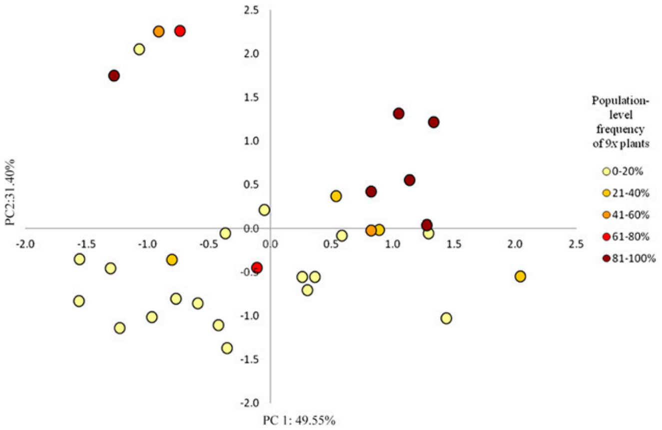

Fig. 3. Principal components analysis of environmental variables at sampling points for *Andropogon gerardii*. Points represent sampling localities; populations with higher frequencies of 9*x* plants are coded in dark red.

1992, 2004; Norrmann et al., 1997). However, whereas previous studies of *A. gerardii* suggest that populations containing predominantly or exclusively 9*x* cytotypes are rare or "nonexistent" (Norrmann and Keeler, 2003), our results reveal that high frequencies of 9*x* plants are common. Most of the predominantly 9*x* populations we detected were outside the range sampled in previously published studies. In this study, approximately one third of sampled plants were 9*x* plants, over 46% of populations had at least one 9*x* individual, and 25% of populations were dominated by 9*x* cytotypes (>50% 9*x* plants) (Table 1, Fig. 1). Next, we discuss the potential explanations for observed patterns of cytotype diversity in *A. gerardii* and also consider the significance of our novel finding of mixed ploidy populations for understanding the origins and evolution of this ecologically dominant species.

***Factors influencing nonrandom patterns of cytotype distributions in* A. gerardii**—There are several potential explanations for the observed nonrandom pattern of cytotype distribution for *A. gerardii* and the significant relationship between 9*x* plants and certain environmental variables. One explanation is that the different cytotypes have selective advantages in different environments. Another possibility is that observed cytotype distributions bear the signature of historical patterns of postglacial recolonization. A third possibility is that 9*x* individuals do not form with equal frequencies in all environments and that the production of unreduced gametes is associated with certain environments, making polyploid formation more common in these areas. Below, we discuss these three possible explanations for nonrandom distribution of 6*x* and 9*x* plants (adaptive significance of autopolyploids, historical processes, and differential formation of 9*x* cytotypes) in more detail. However, it is worth noting that it is likely a combination of these factors contributing to the maintenance of cytotype diversity and mixed-ploidy populations in *A. gerardii.*

TABLE 3. Results of partial linear regression between the frequency of higher DNA-ploidy levels and PC1 and PC2.

Variable	β	*t*	*P* value
Constant	0.589	0.177	0.861
PC1	0.246	1.165	0.255
PC2	**0.327**	**2.943**	**0.007**
Longitude	0.001	0.052	0.959
Latitude	0.001	0.024	0.981

Note: Frequency of high DNA vs. PC axes adj. $R^2 = 0.31$.

Adaptive significance of autopolyploidy—Is there a selective advantage to autopolyploidization? The fact that autopolyploidy occurs in a range of species suggests that genome duplication within lineages may offer an evolutionary advantage, at least in some taxa, in some areas. Parisod et al. (2010) posited that although studies show that autopolyploids do not experience the adaptive advantages brought about by heterosis (hybrid vigor) that are sometimes seen in allopolyploids, the polysomic inheritance and gene redundancy exhibited by autopolyploids could be advantageous, as they increase effective population size and relieve inbreeding depression, potentially resulting in a species' increased ability to adapt to novel environmental conditions. For example, Ramsey (2011) showed that autopolyploidization was associated with rapid intraspecific diversification leading to broad- and fine-scale ecological differentiation in the autopolyploid complex *Achillea millefolium* L. Although some polyploid complexes show little to no ecological differentiation between cytotypes (e.g., Johnson et al., 2003; Godsoe et al., 2013), ecological differentiation has been documented in other complexes. For example, in *A. millefolium* and in several other taxa [e.g., *Eragrostis cambessediana* (Kunth) Steud. (Levin, 2002), *Populus tremuloides* Michx. (Mock et al., 2012), several species of *Solanum* (Hijmans et al., 2007)], higher-level polyploids occupy more xeric habitats than do lower-level polyploids or their diploid progenitors.

The geographic distribution of cytotypes in *A. gerardii* is consistent with ecological differentiation and the possibility that autopolyploids enjoy a selective advantage in some climates. Extensive field observations revealed high frequencies of 9*x* individuals of *A. gerardii* occurring primarily in arid areas with thin or rocky soils, such as rocky mesa tops in the foothills of the Rocky Mountains in Colorado (~1600–1800 m a.s.l.), dry, sandy roadside populations in the Sangre de Cristo Mountains (~2400 m a.s.l.) in northeastern New Mexico, and in rocky ridge lines in southeast Oklahoma that are dominated by prickly pear cactus (*Opuntia* spp.) (C. McAllister, personal observations). Indeed, the only sites we noted in the southern or western portion of our sampled range that had low frequencies of 9*x* plants (or no 9*x* plants) were very near the Gulf Coast (Houston, Texas and Fenton, Louisiana), where temperature and precipitation are moderated by proximity to the sea, and at mesic, high elevation sites (e.g., a locality at ~2100 m a.s.l. in southeastern New Mexico, dominated by ponderosa pine (*Pinus ponderosa* Lawson & Lawson) and Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco)]. The high elevation site in New Mexico can be seen in a cluster in the top left quadrant of Fig. 3; these sites are all from Colorado and New Mexico, along the front range of the Rocky Mountains and the Sangre de Cristo mountains, where diurnal temperature fluctuations are likely much wider than at the sites of other populations in this study. Multivariate analysis of environmental variables corroborated our field observations and demonstrated that, after we accounted for spatial autocorrelation, the frequency of 9*x* plants was positively associated with high diurnal temperature range, high temperature seasonality, and negatively associated with high amounts of summer precipitation (Fig. 3).

The association of polyploidy with more xeric habitats has been observed broadly in the genus *Andropogon.* Stebbins (1975) noted that the polyploids *A. gerardii* and *A. hallii* Hack. (sister taxon to *A. gerardii*; Andropogoneae Phylogeny Group, unpublished manuscript) have broader geographic distributions in North America and occupy much drier habitats than do the diploid members of the genus, which are largely confined to the more mesic southeastern United States. Geographic distributions of cytotypes of *A. gerardii* documented in this study support and extend this observation; within *A. gerardii*, 9*x* cytotypes occupy more xeric habitats than the 6*x* cytotypes do [Fig. 1]).

While it is clear that 9*x* plants are distributed more frequently in drier areas, little is known about the physiological tolerances of the different cytotypes within *A. gerardii*. Keeler (1992) tested for patterns of microgeographic variation in the distribution of *A. gerardii* cytotypes within a series of small (40–120 m^2) plots in eastern Kansas, based on soil moisture, burn history, and grazing treatment but found no relationships. Direct tests for ecophysiological differences between the cytotypes are necessary to fully evaluate the possibility of ecological sorting between the cytotypes. Greenhouse studies investigating ecophysiological responses of *A. gerardii* cytotypes to abiotic stress are planned.

Cytotype distributions and historical processes—Differences in cytotype distribution could result from differential rates of colonization among cytotypes due to variation in seed viability and seedling establishment. Such differences could be particularly important in the tallgrass prairie ecosystem, which is less than 10 000 yr old in most places and developed through postglacial recolonization from Pleistocene refugia in the southern portions of North America (Anderson, 2006).

A developing body of work indicates that postglacial recolonization in autopolyploid complexes is not random with respect to ploidy level. For example, studies have shown that historically refugial areas harbor the highest frequencies of ancestral diploids or lower-ploid progenitors (Solís Neffa and Fernández, 2001; Trewick et al., 2002; Balao et al., 2009; Cosendai et al., 2013; but see Koch and Bernhardt, 2004), with polyploids occurring more frequently outside putative refugial areas. These data suggest that polyploids may migrate more effectively out of a refuge, that they form with greater frequency outside the refuge, and/or that they have a selective advantage in newly colonized areas (Brochmann et al., 2004; Godsoe et al., 2013). Interestingly, the geographic distribution of *A. gerardii* cytotypes does not conform to patterns of glacial recolonization exhibited by most other polyploid species with mixed cytotypes: in *A. gerardii*, 9*x* plants are more common near the proposed refugial areas (the southern United States), while 6*x* plants are more common in previously glaciated areas (central and northern United States).

The high frequency of 6*x* plants of *A. gerardii* documented away from putative glacial refuges in previously glaciated areas may reflect differences in fertility between cytotypes that led to differential colonization following glaciation (e.g., Sonnleitner et al., 2010). In *A. gerardii*, the 9*x* cytotype exhibits lower seed viability and lower rates of seedling establishment than the 6*x* cytotype (Keeler, 2004), which likely impacted the ability of 9*x* plants to successfully establish new populations during postglacial migration into novel habitats. While 6*x* individuals may be able colonize more effectively than 9*x* individuals, it seems unlikely that differential fertility is the primary factor determining cytotype distribution. If this were the case, one would expect that 6*x* individuals would outcompete 9*x* throughout the range. However, the widespread occurrence of 9*x* plants, populations with both 6*x* and 9*x* plants, and populations dominated by 9*x* plants suggests that this is not occurring.

Finally, stochastic processes taking place during the evolutionary history of a species may play a role in the geographic structuring of cytotype diversity. Movements of plants during and following postglacial migration may have resulted in founder effects in some populations (te Beest et al., 2012). Likewise, genetic drift may impact cytogeographic patterns in *A. gerardii*, resulting in the random fixation of 6*x* or 9*x* cytotypes in different populations. Some populations sampled for this study were located within small native prairie remnants or in isolated populations within the natural geographic range of *A. gerardii* but outside the historical distribution of the tallgrass prairie, such as a small (<10 acre) hillside population in Ohio and a tiny (<1 acre) remnant of the Piedmont prairie sampled in South Carolina (Table 1). These populations exhibited fixation of one cytotype or the other (Table 1), an observation consistent with neutral genetic processes like founder effects or drift.

Differential formation of 9x cytotypes—An important factor shaping geographic distributions of 6*x* and 9*x* cytotypes in *A. gerardii* is the way in which 9*x* individuals form and the frequency with which they form. The most likely mechanism underlying the formation of the 9*x* cytotype in *A. gerardii* is that it is derived from the 6*x* cytotype, formed by the union of one reduced and one unreduced gamete (Norrmann et al., 1997; Norrmann and Keeler, 2003). Cytogeographic patterns seen in *A. gerardii* may reflect differences in where, how, and when 9*x* individuals form. Have 9*x* individuals originated multiple times from 6*x* progenitors, and if so, are 9*x* plants more likely to form under certain environmental conditions? Several studies document recurrent formation of allopolyploids (Brochmann and Elven, 1992; Soltis and Soltis, 1999; Sharbel and Mitchell-Olds, 2001; Soltis et al., 2004), and a growing body of literature provide evidence for recurrent formation of autopolyploids (Soltis and Soltis, 1989; Segraves et al., 1999; Stuessy et al., 2004; Ramsey et al., 2008). The 9*x* cytotype in *A. gerardii* may have evolved more than once; ongoing population genomics analyses provide support for this phenomenon (C. McAllister et al. unpublished data).

A higher frequency of 9*x* plants in hotter, drier areas, as observed in this study, may be directly related to higher frequencies of unreduced gametes in these areas. If the formation of unreduced gametes in *A. gerardii* is linked to environmental stressors, then populations in areas that experience stressful abiotic or biotic conditions might produce higher numbers of unreduced gametes relative to areas with less stressful climatic or biotic conditions. Higher numbers of unreduced gametes increase the likelihood of the establishment of new polyploid plants. In many species, the formation of unreduced gametes is genetically controlled and is a heritable trait (Bretagnolle and Thompson, 1995). Previous studies demonstrate that there is an environmental component to the production of unreduced gametes, the formation of which can result from abiotic and biotic factors such as extreme temperature, fluctuation in temperature, water stress, plant nutrition, wounding/herbivory, and disease (Belling, 1925; De Mol, 1928; Kostoff, 1933; Grant, 1953; McHale, 1983; Ramsey and Schemske, 1998; Ramsey, 2007).

The link between low summer precipitation and increased frequency of 9*x* plants detected here could reflect patterns of historical drought stress linked to increased rates of unreduced gamete formation and subsequent 9*x* formation. A low but consistent level of formation of unreduced gametes could allow recurrent, in situ generation and establishment of higher-order polyploids, particularly in areas that have experienced recurrent abiotic stress. It is possible that recurrent polyploidization, coupled with clonal growth of long-lived individuals and low seed recruitment, could play a key role in shaping the cytogeographic patterns of *A. gerardii* (Glenn-Lewin et al., 1990; Keeler, 2004; Benson and Hartnett, 2006). Knowledge of the circumstances under which unreduced gametes form is currently lacking and explicit tests of the role of drought in the formation of unreduced gamete formation in *A. gerardii* are underway.

Intraspecific cytotype variation and the maintenance of mixed-ploidy populations—Recent advances in flow cytometry, including improvements in the processing of silica-dried samples, have led to an increase in large-scale field studies of cytotypic variation in plant species (Suda and Trávniček, 2006; Kron et al., 2007; Husband et al., 2013), many of which have revealed that intraspecific cytotype variation and mixed cytotype populations are more common than previously thought (Burton and Husband, 1999; Solís Neffa and Fernández, 2001; Halverson et al., 2008; Schönswetter et al., 2007; Suda et al., 2007b; Kao, 2008; Duchoslav et al., 2010; Fehlberg and Ferguson, 2012; McIntyre, 2012; Mock et al., 2012; Sabara et al., 2013). Likewise, in our study, nearly half of populations sampled showed a mixture of cytotypes.

Why do multiple ploidy levels persist within populations? The minority cytotype exclusion hypothesis posits that in the absence of reproductive isolation mechanisms between cytotypes, the minority cytotype will experience frequency-dependent selective forces, resulting in the decline and eventual extinction of that cytotype in the population (Levin, 1975). However, several aspects of plant reproductive biology do not promote minority cytotype exclusion, including differences in flowering time between cytotypes (Husband and Sabara, 2003), shifts from self-incompatibility to self-compatibility (Felber, 1991; Husband, 2004), recurrent polyploid formation, and high rates of clonal reproduction (Kao, 2007).

In *A. gerardii*, minority cytotype exclusion is likely being counteracted by some level of postzygotic isolation between the cytotypes in conjunction with vigorous clonal reproduction, and likely recurrent formation of 9*x* plants, resulting in stable, mixed-ploidy populations. While the 6*x* and 9*x* cytotypes are self-incompatible and interfertile, intercytotype crosses produce a limited numbers of seeds with an array of chromosome numbers (aneuploids; (Norrmann et al., 1997; Norrmann and Keeler, 2003). Seedling recruitment from these aneuploids appears largely unsuccessful, as established aneuploid individuals resulting from intercytotype crosses typically make up <5% of individuals within a population (Keeler, 1992, 2004).

Another possible factor contributing to the maintenance of stable, mixed-ploidy populations is that *A. gerardii* relies almost exclusively on asexual reproduction for recruitment and establishment (Glenn-Lewin et al., 1990; Keeler, 1995). Several studies have shown that seedling establishment of *A. gerardii* is extremely rare. Benson and Hartnett (2006) found that more than 99% of stems in study plots in eastern Kansas were from vegetatively reproduced ramets or tillers. Moreover, Abrams (1988) reported that although *A. gerardii* provided over 78% canopy coverage at study sites in a Kansas tallgrass prairie, an average of only 1.5 seedlings sprouted from soil samples collected from those sites. High rates of asexual reproduction have been shown to facilitate the formation and persistence of mixed ploidy populations by removing the density-dependent selective pressures faced by a minority cytotype (Yamauchi et al., 2004; Kao, 2007). Although the formation of 9*x* plants is likely relatively rare compared with formation of 6*x* plants, once formed, the 9*x* individuals can spread vegetatively. Furthermore, Keeler (2004) showed no differences between rates of vegetative growth between cytotypes. This, together with a long life span (50–100 yr) for genets in *A. gerardii* (Keeler, 2004) could play a significant role in the long-term maintenance of mixed ploidy populations.

Broad-scale analyses of geographic patterns and potential drivers of cytotype diversity are an important step in understanding the causes and consequences of whole genome duplication. The large-scale assessment of ploidy level diversity in *A. gerardii* presented here provides a more complete and nuanced view of cytogeographic patterns than previously existed for this ecologically dominant species and demonstrates a clear link between 9*x A. gerardii* and environmental variables. Future work should focus on understanding the roles of differential rates of unreduced gamete production across environments, adaptive significance of higher-order polyploids relative to their diploid progenitors under different climates, and the extent of recurrent polyploidization across the geographic range of the species.

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