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Local Adaptation, Genetic Divergence, and Experimental Selection in a Foundation Grass across the US Great Plains' Climate Gradient

Running head: Local adaptation across climate gradient

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ABSTRACT

Many prior studies have uncovered evidence for local adaptation using reciprocal transplant experiments. However, these studies are rarely conducted for a long enough time to observe succession and competitive dynamics in a community context, limiting inferences for long-lived species. Furthermore, the genetic basis of local adaptation and genetic associations with climate has rarely been identified. Here we report on a long-term (6-yr) experiment conducted under natural conditions focused on *Andropogon gerardii*, the dominant grass of the North American Great Plains tallgrass ecosystem. We focus on this foundation grass that comprises 80% of tallgrass prairie biomass and is widely used in 20,000 km^2 of restoration. Specifically, we asked 1) if ecotypes are locally adapted to regional climate in realistic ecological communities? 2) does adaptive genetic variation underpin divergent phenotypes across the climate gradient? 3) is there evidence of local adaptation if the plants are exposed to competition among ecotypes in mixed ecotype plots? Finally, 4) are local adaptation and genetic divergence related to climate? Reciprocal gardens were planted with 3 regional ecotypes (originating from dry, mesic, wet climate sources) of *Andropogon gerardii* across a precipitation gradient (500-1200 mm/yr) in the US Great Plains. We demonstrate local adaptation and differentiation of ecotypes in wet and dry environments. Surprisingly, the apparent generalist mesic ecotype performed comparably under all rainfall conditions. Ecotype performance was underpinned by differences in neutral diversity and candidate genes corroborating strong differences among ecotypes. Ecotype differentiation was related to climate, primarily rainfall. Without long-term studies, wrong conclusions would have been reached based on the first two years. Further, restoring prairies with climate-matched ecotypes is critical to future ecology, conservation and sustainability under climate change.

INTRODUCTION

Understanding climate driven selection within communities is needed to predict grassland response to warmer and drier summers in the North American Great Plains, and other grasslands. In the last 6 years, US grasslands have experienced severe drought, especially in 2012, the worst drought on record in ~50 years. Furthermore, one of the most important climatic changes

predicted for grasslands is alteration of amount and timing of precipitation events (IPCC 2013) and unprecedented "mega-droughts" (Cook *et al.* 2015). It is critical to assess if local adaptation limits a population's ability to adjust to changing climates, or if populations will have to migrate to match future climate conditions or be planted through restoration (Christmas *et al.* 2016; Nicotra *et al.* 2010). Ultimately, research needs to inform conservation and restoration managers to better identify the optimal ecotype (Broadhurst *et al.* 2008; Jones 2013; Bucharova *et al.*, 2017) on 20,000 km^2 of restored marginal land across the Great Plains, (Kettenring *et al.* 2014; Pickup *et al.* 2012) and to plant for forage supply in changing climates in an ecological foundation species (Gibson *et al.* 2016).

Habitats are often temporally and spatially variable especially with regard to climate, causing differential selection across climate gradients, genetic divergence among populations, and local adaptation (Linhart & Grant, 1996). A main goal of evolutionary biology is to understand factors that contribute to such population genetic divergence (Mayr 1963), formation of ecotypes (Clausen et al. 1940), and that ultimately lead to new species (Rundle & Nosil 2005). Yet, gaps exist in knowledge of local adaptation and ecotypic diversity among regionally distributed populations of most plant species (Falk *et al.* 2006), especially foundation species, growing in nature. Local adaptation is fundamental to evolution (Savolainen *et al.* 2013), and has implications for adaptation to global changes, conservation, and restoration (Hufford & Mazer, 2003; Nicotra *et al.*, 2010; Shaw & Etterson, 2012).

Intraspecific variation and local adaptation among plant populations have been widely studied, mostly in response to abiotic conditions, across large-scale climatic gradients (Clausen *et al.* 1940; McMillan 1959; Joshi *et al.* 2001; Bischoff *et al.* 2006; Ariza & Tielborger 2011; Munzbergova *et al.* 2017), altitude (Montesinos-Navarro *et al.* 2011), and finer scale environmental variation (Bradshaw 1984; Linhart & Grant 1996; Galloway & Fenster 2000; Montalvo & Ellstrand 2000; Etterson 2004; Knight *et al.* 2006; Lowry *et al.* 2009). However, little is known (Bischoff *et al.* 2006) about plant local adaptation in competitive settings. Consequently, intraspecific variation and local adaptation are rarely interpreted under realistic ecological (community) conditions under which it has evolved (Liancourt & Tielborger 2011; Liancourt *et al.* 2013; Grassein *et al.* 2014; Tomiolo *et al.* 2015; Lowe *et al.* 2017), which limits

the ability to predict the role and strength of local adaptation in natural communities. Several studies have demonstrated changes in interspecific plant interactions shaping local adaptation along stress gradients (Grassein *et al.* 2014; Tomiolo *et al.* 2015). Still, little empirical data exist for predicting species' adaptive response to natural, and now rapidly changing, selection pressures (Mimural *et al.* 2017). With increasing climate variability, it is crucial to understand local adaptation and species interactions in long-lived perennial plants in long-term studies (Metz & Tielborger 2016).

Here we investigate whether ecotypic variation in a dominant US Great Plains grass (*Andropogon gerardii,* common name big bluestem) is a result of local adaptation to climate using a reciprocal common garden platform established in 2009 across a precipitation gradient. This experiment focused on *A. gerardii* because it is an ecologically dominant grass that comprises up to 80% of biomass of tallgrass prairie (Weaver, 1932; Epstein *et al.* 1997; Knapp *et al.*, 1998). Within the Great Plains, *A. gerardii* occurs along a climate gradient in place for ~10,000 years (Axelrod 1985), allowing ample time for local adaptation to develop. Due to its wide distribution and dominance in the Great Plains (Epstein *et al.* 1997) and spatially varying climate, we expected extensive natural variation across this gradient among populations with formation of ecotypes (Johnson *et al.* 2015). Ecotypic variation among several grass species across a latitudinal gradient in the Great Plains was documented by the early seminal common garden studies of McMillan (1959). More recently, intraspecific variation in performance of switchgrass genotypes originating from different temperature and precipitation environments in a greenhouse common garden was examined by Aspinwall *et al.* (2013). They found that genotype largely explained functional trait variation as related to the climate of origin.

More specifically, this study aimed to assess genetically based local adaptation of *A. gerardii* ecotypes in realistic competitive settings across the Great Plains' precipitation gradient (500 to 1200 mm/yr precipitation across a ~1,000 km span from western Kansas to Illinois). We addressed the following questions: 1) Do ecotypes display local adaptation to regional climate when planted in realistic ecological communities? 2) Does adaptive genetic variation underlie divergent phenotypes? 3) Do we see evidence of local adaptation if the plants are exposed to competition among ecotypes of *A. gerardii* in mixed ecotype plots? 4) Is local adaptation related

to climate gradients? We hypothesized that locally adapted ecotypes would be more abundant in their home environment evidenced by outcompeting their non-local ecotypes in both single ecotype and mixed ecotype plots. If local adaptation was not strong, then we expected ecotypes to perform comparably across the climate gradient as mediated by plasticity. We expected genetic differences amongst ecotypes in terms of genetic divergence and outlier genetic loci that give rise to adaptive variation among ecotypes. Growing all ecotypes mixed together, allowing competition, was expected to be the most robust test for local adaptation by testing experimental selection in mixed ecotype plots. By identifying which ecotypes are "winning" in climatically varying sites, we can relate these differences to climate factors for local adaptation and genetic divergence. Finally, we expected the strong climate gradient of the Great Plains to drive both phenotypic and genetic variation.

This novel experiment assessed local adaptation in realistic ecological settings across a climate gradient including competitors, in a long-lived perennial grass. By contrast, most studies use monocultures in the absence of plant-plant competition, as is commonly done with single-spaced plants (Bischoff *et al.* 2006). Moreover, the long-term nature of the experiment (6 years) allowed community processes and climate to play out. However, most studies that vary phenotypes and genotypes in the field lasted 3 years or less (Franks *et al.*, 2014), and most studied annual plants (Franks *et al.* 2014). This study combined population genetics and identification of candidate genes with performance from long term experimental gardens, which is seldomly done (Villemereuill *et al.* 2016). The study assessed experimental selection by measuring outcome of competing *A. gerardii* ecotypes which, arguably, should be the most robust test for local adaptation across the climate gradients. This is rarely done with perennial plants and in long term studies (Ravenscroft *et al.* 2015). Finally, the study related both performance and genetic variation (Villemereuill *et al.* 2016) to climate and provided a strong test for environment in structuring adaptive variation (Schneider & Mazer 2016).

MATERIALS AND METHODS

We tested for local adaptation and ecotypic differentiation using several analyses, including 1) reciprocal garden experiments with *A. gerardii* ecotypes grown individually and in a mixture; 2)

tested the ability of genetic variation to predict ecotype; and 3) identified "outlier" single nucleotide polymorphisms (SNPs) and tested the degree to which their differentiation was explained by climate.

1. Plant materials and seed collection sites, climate of population source of origin

Andropogon gerardii is a perennial wind-pollinated that grows as a bunchgrass with tight tufts of culms produced from rhizomes. *A. gerardii* is an obligate outcrosser (Normann *et al.* 2003), with strong self-incompatibility. As with many other grasses, *A. gerardii* consists of a large polyploid genome (2 Gb). Seed of *A. gerardii* was collected by hand during autumn 2008, from three climatically distinct ecoregions along a precipitation gradient from Central Kansas (dry ecotype, mixed grass ecoregion, Kuchler 1964), Eastern Kansas (mesic ecotype, from the tall grass ecoregion Kuchler 1964), and Southern Illinois (wet ecotype) from the prairie savanna ecoregion Kuchler 1964) (Fig. 1, STable 1, SFig. 1 for photo of ecotypes). Prairies of Kansas are dominated by low stature grasses with few forbs (Knapp *et al.* 1998). Eastward, diversity and structure shifts from grass dominance to diverse communities of tall-stature forbs and shrubs (Kuchler 1964). Populations for seed collection were on original native prairies within an 80 km radius of the reciprocal garden planting site. Seeds from each population were collected on at least three dates and stored at 4 °C. All seed stocks were analyzed for seed filling, germination, and dormancy to determine percent live seed by Kansas Seed Crop Improvement Center (Manhattan, Kansas, USA).

2. Reciprocal garden design - Sown community plots

We used reciprocal gardens as the standard method to test the extent to which ecotypes are locally adapted to their home environment vs other locations. This experiment assessed local adaptation in realistic ecological settings across, which included competitors, in a long-lived perennial prairie community.

To do this, we reciprocally seeded each ecotype into plots at four sites: Western Kansas (Colby, Kansas, 500 mm MAP); Central Kansas (Hays, Kansas, 580 mm); Eastern Kansas (Manhattan,

Kansas, 871 mm); and Southern Illinois (Carbondale, Illinois, 1167 mm) (Fig. 1, Table 1, Fig. 2). The Western Kansas site in Colby, Kansas was included to test tolerance of ecotypes to more arid environments, as might be expected under future warming and drying. Big bluestem occurs in Western Kansas and Colorado, but only sporadically. This Western Kansas planting site was included to test the effects of increased drying beyond what is experienced by the species in its central distribution. All garden sites were under agricultural cultivation prior to reciprocal garden establishment. All soils were classified as loams (Table 1); specifically, the Eastern three sites were classified as silt loams, and Western Kansas (Colby, Kansas) as silt clay loam (Mendola *et al.* 2016). After accounting for percent live seed, seeds from four populations within each ecotype were mixed in equal quantities. Each ecotype and mixtures of ecotypes were reciprocally sown at each site in multi-species communities (Johnson *et al.* 2015). The experiment consisted of a randomized complete block design at each site with four blocks per site. Within a site, each block consisted of four plots (each 4 m x 8 m), 3 of which were seeded to a single regional ecotype (i.e., dry, mesic and wet) and the fourth plot with a mixture of all three regional ecotypes (i.e., mixed ecotype plot). Plots were separated by a 4–6 m buffer strip (Fig. 2). Plots were plowed within a week prior to garden establishment and sown to each regional ecotype in June 2009. Seeds were mixed with damp sand to aid in homogenous dispersal, hand-broadcast and hand-raked into soil. Shortly following seeding, 25 mm of supplemental irrigation was provided at the Central Kansas site to alleviate a severe deficit during establishment. This supplement increased precipitation to historical average for that time of year. Throughout the remaining experiment plots all sites received only natural rainfall without any supplemental water added. Seeding details are provided in Johnson *et al.* (2015). Species community composition of sown plots as well as seeding rate is typical for prairie restorations. We used 70:30 ratio of live C_4-grass to C_3-grass and forb seed (see Johnson *et al.* 2015). Total seed density for each plot was 580 seeds m^2, similar to that recommended for prairie restoration (Packard & Mutel 1997). *A. gerardii* was planted at a density of 270 live seeds m^2. Seeds of eight other species (*Sorgastrum nutans*, *Elymus canadensis*, *Ascelepias tuberosa*, *Chamaechrista fasiculata*, *Monarda fistulosa*, *Oligoneuron rigidum*, *Penstemon digitalis*, *Ruellia humilis*) were added to maintain characteristic functional group structure and competitive relationships of tallgrass prairie. Planted seeds of all species, except *Andropogon* and *Sorgastrum* were purchased from a commercial supplier (Ion Exchange Inc., Harpers Ferry, IA, USA) and sourced from across the

Great Plains. Additionally, plants of volunteer species (plants that came in on their own, not planted as part of the experiment) from regional seed sources also established in garden sites. Thus, the composition of the community at each garden site was a mix of mostly volunteers from regional species pool, and a few planted forb species (Wilson *et al.* 2016).

Reciprocal Garden of Single-Spaced Plants for Genotyping and Random Forest Training

In addition to the sown "community" plots described above we established plants in monoculture hereafter referred to as "single-spaced" plants. These reciprocal gardens comprised single-spaced plants for which we knew the ecotype identity and used these plants for 1) characterizing genetic differences among ecotypes, and their relation to climate and 2) predicting the ecotypes of plants in the mixed ecotype plots based on combinations of SNP markers unique to plants of known origin. We needed to predict ecotypes in the mixed plots because, although there are clear phenotype differences among ecotypes (SFig. 1), it is difficult to assign plants to the dry and mesic ecotypes because they are more phenotypically similar. We used the same seed sources described above in sown "communities" (Supplemental Table 1). These plantings were adjacent to the blocks of community plots. In winter 2009, a subset of seeds collected from each field-collected wild population was germinated and grown in 10 x 10 cm pots in a greenhouse, using standard greenhouse potting mix (Metro-Mix 510). In August 2009, 20 3-4 month old plants of 10 replicate blocks of 12 populations (3 climate regions x 4 populations per regional climate ecotype) were planted at each reciprocal garden site (Fig. 1, Table 1, STable 1). Plants were spaced 50 cm apart and water penetrable landscape cloth was placed around each plant to discourage growth of competing plants. The phenotypes have been described elsewhere (Olsen *et al.* 2013; Caudle *et al.* 2014; Mendola *et al.* 2016; Maricle *et al.* 2017).

3. Climate and Environment of the Reciprocal Garden Planting Sites

Data on daily precipitation were collected at each garden site (Table 1), all located at agricultural research stations. Rainfall (annual and growing season) for the years of the experiment in Table 1 and SFig.2. We used nearby NOAA weather stations for historical data on climate of source populations (STable 1).

4. Vegetative Cover as Estimate of Performance in Single Ecotype Plots

Measurements of vegetative cover of *A. gerardii* in single ecotype plots were made to assess plant performance of the different ecotypes planted across the climate gradient, and to assess the extent to which ecotypes are locally adapted to their home site.

Field Measurements 2010-2015

Vegetation cover was measured for six years in single ecotype plots from 2010-2015 within a week of each other across all sites. We focused on vegetative cover (as related to plant biomass) rather than seed production. To estimate percent cover, a 1.0 m^2 quadrat was used with one intersection every 10 cm for a total of 81 intersections. At every intersection, occurrence of *A. gerardii*, other grass, forb, or bare ground was recorded. We used four non-overlapping quadrats per plot for a total of 324 intersections per plot (324 per plot x 12 plots per site=3,888 intersections per site x 4 sites=15,552 intersections each year). Quadrats were randomly placed at least 50 cm from edge to minimize edge effect.

This study used cover as proxy for fitness rather than measuring seed production as vegetative cover is a good predictor of success in long-lived perennial plant (Dagleish & Hartnett 2006; Bensen & Hartnett 2006). Most growth, especially among dominant grasses, is clonal in these grassland communities (Knapp et al. 1998). Indeed, very little regeneration from seed occurs in prairies in general (Benson & Hartnett 2006; Lemoine *et al.* 2017; Dagleish & Hartnett 2006), including restored prairie (Willand *et al.* 2013) unless disturbed (Weaver 1932). Furthermore, seedlings are rarely observed in the extremely competitive environment of the prairie, nor did we observe seedlings or recruitment into our plots in the six years of the experiment. Thus, recruitment from seed into our plots is not likely to play a role in this system over the time frame of our experiment.

We have no estimate of growth belowground because that would have required destructive harvest of the plots. However, other studies focusing on mycorrhizal symbionts indicate that

local adaptation of *A. gerardii* may be explained in part on local mycorrhizal symbionts (Johnson *et al.* 2010). Mendola *et al.* (2016) demonstrate evidence for local adaptation measure by belowground production in the dry and wet ecotypes in the single-spaced plants in our experimental gardens.

Statistical Analyses of Vegetative Cover

A generalized linear mixed model with a logit link was fitted to a binomial response consisting of the number of intersection points at which *A. gerardii* was observed using a pre-defined grid with a total of 81 intersection points per quadrat. The linear predictors included the fixed effects of site, ecotype, year, and all 2- and 3-way interactions. Random effects in the linear predictor included block nested within site and also crossed with ecotype, to properly recognize experimental units for site and ecotype, as well as repeated measures over time. The random effect of block nested within site had to be removed from the model as its variance component estimate converged to zero; degrees of freedom for site were adjusted accordingly. In addition, random effects were included in the model to account for technical replication within each block (i.e., block (site) *ecotype * year) and overdispersion (i.e., block (site) *ecotype*year*rep) in the data.

Overdispersion was evaluated using the maximum-likelihood based fit statistic Pearson Chi-Square/DF. No evidence for overdispersion was apparent in the final model used for inference. The final statistical model used for inference was fitted using residual pseudo-likelihood. The model was fitted using the GLIMMIX procedure of SAS (Version 9.4, SAS Institute, Cary, NC) implemented using Newton-Raphson with ridging as the optimization technique. Kenward-Roger's procedure was used to estimate degrees of freedom and conduct corresponding adjustments on standard error estimates. Relevant pairwise comparisons were conducted using Bonferroni adjustments to avoid inflation of Type I error rate due to multiple comparisons.

In addition, we related plant cover by ecotype to rainfall from all the sites using regressions of cover vs rainfall for years 2014 and 2015. We used the two latest years of the experiment as it

allowed maximum time for community processes and successional dynamics to play out. The years 2014 and 2015 were average rainfall years.

5. Sample Collection for Genotyping

Single nucleotide polymorphisms (SNPs) from single-spaced plants of known population sources planted in reciprocal gardens were used for 1) characterizing population genetics of the source populations and relation to climate and 2) using ecotype-specific SNPs from known population sources to predict ecotypes of unknown plants in mixed plots using random forest models for classification.

Reciprocal Gardens-Single Spaced Plants for Genotyping

We used genotyping-by-sequencing (Poland and Rife 2012; Elshire *et al.* 2011; Lu *et al.* 2013) to identify the SNPs. Leaf samples were collected from individuals with known population origin from single-spaced plants from reciprocal gardens in Central Kansas (Hays, Kansas) and Eastern Kansas (Manhattan, Kansas) and Southern Illinois (Carbondale, Illinois). Number of plants genotyped from single-spaced plants resulted in 110 individuals from the dry ecotype, 106 from the mesic ecotype, and 98 from the wet ecotype. These plants (total 314 plants) were distributed amongst 12 populations. About 100 mg of leaf tissue was collected directly into 96-deep well matrix plates on ice then freeze dried, ground, and stored at -80°C until DNA isolation. *A. gerardii* is known to have different cytotypes (6x, 9x, base number of chromosomes=10) Norman and Keeler 2003), sometimes within the same population. For this reason, we analyzed all 480 plants in single-spaced plots for ploidy level using flow cytometry on a Becton Dickinson FACSCalibur and FACSVantage SE and results analyzed using MODFIT. We found ploidy level differences were very slight in our 3 ecotypes (12 populations total) (Galliart et al. unpublished) and that cytotype differences could not explain the sharp ecotype differences (Galliart et al. unpublished).

Predicting Ecotype Identity in Mixed Ecotype Plots

Samples from single-spaced plants were genotyped and used to develop a predictive random forest model to classify ecotype identity of individual plants from within the mixed plots based on SNPs. Leaf samples of individuals from mixed ecotype plots were collected every ~0.5 meters on diagonal transects in 2014 and 2015. Within each plot we collected a subset of plants from amongst hundreds of individual big bluestem in the plots. We collected a total of ~92 plants at each site (~23 plants per plot x 4 blocks) with 360 individuals analyzed in 2014 and 351 individuals analyzed in 2015 (total 711 plants). We felt confident that we did not sample an individual more than once as individuals were identified as a clearly delineated clump of bunchgrass with tight tufts with clear differentiation between individuals. Furthermore, SNP profiling and comparison of nucleotide differences among individuals in the same mixed plot did not show evidence of identical individuals as we would expect if the same plant was sampled twice (Galliart *et al.* unpublished).

Details on DNA isolation, library preparation, sequencing, and SNP identification are provided in supplemental methods.

6. Genetic Analyses

Ecotype Genetic Structure and Differentiation

We characterized ecotype genetic structure and differentiation to test how ecotypes are genetically distinguished and how genetics is structured by climate. To do this, we used single-spaced plants of known ecotype for analyses of genetic structure, differentiation and outlier analyses. For these analyses, we used all the SNPs in the data set. Population structure was assessed using *Structure* v2.3.4 (Falush *et al.* 2007). Run parameters included 20,000 burn-in and 500,000 MCMC chain length. Admixture was included and correlation between alleles was not assumed. Three separate iterations per K was performed. To identify optimal number of K genetic clusters, Evanno's delta K was calculated in *Structure Harvester* v0.6.94. K clustering and permutation were done in *CLUMPP* v1.1.2 and plot visualization in *DISTRUCT* v1.1. Genetic analysis for pairwise population F_{st} was implemented in *GenAlEx* v6.503 (Peakall and Smouse 2006; 2012) using twelve populations comprising the three regional ecotypes.

Importance of Climate vs Geography in Structuring Genetic Differentiation

Partial redundancy analyses (pRDA) was used to estimate the role of geographic differences (lat, long) vs climate in structuring neutral genetic variation. pRDA is an ordination technique (Oksanen *et al.* 2015) that partitions variation, in our case genetic variation, due to climate and geography (latitude and longitude) and joint contribution of climate and geography (Riordan *et al.* 2016). pRDA of genetic variation (Riordan *et al.* 2016, Laskey *et al.* 2012), "partials out" variance from geography while considering variance from climate, and separately "partials out" variance from climate while considering variance from geography. In this way, relative importance of climate vs geography in affecting genetic variation can be determined. Three models were run: The full model (Model 1) considered both climate variables and geography as explanatory variables, Model 2 was a partial model in which geography explained the genetic data conditioned on climate variables, and Model 3 was a partial model in which climate variables explained genetic data conditioned on geography. All precipitation variables were used in the model except for precipitation of the driest year and number of precipitation events >1.25 cm (Table 1) due to collinearity.

Outlier Genetic Analysis and Relation to Climate

Genetic "outliers" are those SNPs that show more differentiation compared to background levels of differentiation and are putatively under natural selection. We identified "outlier" SNPs in ecotypes and then related their differentiation to the climate of origin. First, *Bayenv2* (Guenther & Coop 2013) was used to identify "outlier" SNPs, a robust approach providing correction for population structure and demographic processes while controlling false positives (Guenther & Coop 2013; Lotterhos & Whitlock 2014). For *Bayenv2,* SNP data from single-spaced plants were used to generate a covariance matrix for populations to control for population structure. Four separate covariance matrices were generated running the MCMC chain to 10^6 iterations and visualized to ensure chain convergence. For all loci, population differentiation ranking statistic X^TX (Guenther & Coop 2013) was calculated. This statistic identifies loci that have greater differentiation than under neutral drift amongst populations. X^TX values were empirically ranked

and the top 1% of differentiated loci were conservatively retained as outliers (46 SNPs). *Bayenv2* was also implemented to relate SNPs to climate variables (Table 2). *BayeScan* v2.1 (Foll and Gaggiotti 2008) was used as a second method to identify consensus outliers (Lotterhos & Whitlock 2014). Parameters for *BayeScan* included 20 pilot runs of length 5K, 50K burn-in length, and a thinning interval of 10 with 5K final iterations. Prior odds for the neutral model was 10 and uniform prior on F_{is} had a lower bound of 0.0 and upper bound 1.0, with 1.0 representing complete inbreeding. Outlier loci were selected using q-values ≥ 0.5 for substantial evidence of selection.

7. Random Forest Model to Predict Ecotype Composition Based on SNPs Identified in the Mixed Ecotype Plots

Single-spaced plants were genotyped for ecotype-specific SNPs to classify ecotype identity of individual plants from within the mixed plots using a predictive random forest model. We needed to predict ecotypes in the mixed plots because, although there are clear phenotype differences among ecotypes (SFig. 1), it is difficult to assign plants to the dry and mesic ecotypes because they are more phenotypically similar. We used the random (decision) forest approach (Breiman 2001) as a powerful machine learning tool to classify individuals, in our case, into ecotype based on ecotype-specific SNPs. Random forest uses the ensemble method (Altman & Krzywinski 2017) for classification that operates by constructing many decision trees at training and taking a weighted vote from all of these trees for prediction. The ensemble method is preferred because it reduces the overall variance within the model and can help identify strong signals in noisy data, ultimately providing a robust method to generate a predictive model using large amounts of data such as found in genotype data. Using random forests to generate a predictive model first requires training the model using individuals with known ecotype classification. Once the model is validated for misclassification and accuracy with the training set, the training model can be used to predict unknown ecotypes based on SNPs. The model was used to predict the ecotype class, in our case ecotype based on SNPs with known classification from the single-spaced plants.

Random forest training and validation

The random forest dataset passed SNP quality control as described in supplemental methods. However, for the random forest model, we used only loci for which there were no missing data across all individuals, resulting in 522 SNPs. Using a random forest approach, we are able to generate a predictive model based on SNP profiles of individuals of known ecotype designation. SNPs from 314 individuals (110 from the dry ecotype, 106 from the mesic ecotype, and 98 from the wet ecotype were used to train and cross validate a random forest predictor model implemented in *randomForest* R package (Liaw & Wiener 2002). The random forest used SNPs as predictor variables at each split of decision trees (SFig. 3) and generated 500 trees for each forest. (After testing multiple values of predictor variables (SNPs), we used 22 SNP variables as optimum for training.) Ten unique groups of plants of known ecotype from single-spaced plants were generated to create ten validation sets to quantify overall misclassification rate. For each of the ten groups, nine groups were combined to train the random forest prediction model. The remaining one group was used for validating the accuracy of the model. Individuals in the validation sets had their known ecotype masked and used the training forests to predict to which ecotype the individual belonged. Individuals were classified to the ecotype bin based on greatest number of votes for that ecotype across all 500 trees (SFig. 3). Assignment of the masked individuals from the training model was compared to the true identity of plants to generate misclassification rates and provide a metric of how accurately we can predict ecotypes based on their genotype profile. This process was repeated with each of the ten unique ecotype groups to determine an overall misclassification rate.

Predicting Ecotype in Unknown Plants of Mixed Ecotype Plots

The next step was to predict ecotype identity of unknown plants growing in mixed ecotype plots using the trained random forest model. All 314 individuals from single-spaced plants were then combined to generate a random forest using the same model parameters described above with 22 predictor variables and 500 trees in each forest. Identity of genotyped plants from mixed ecotype plots from 2014 and 2015 (360, 351 individuals, respectively) were determined as the ecotype that received greatest number of votes across 500 trees in the final random forest. Analysis of

462 individuals from mixed plots across two years assesses annual variation in growth and
463 composition within long-term plots.
464
465 **RESULTS**
466
467 **Ecotypes Locally Adapted to Regional Climate in Realistic Ecological Communities**
468
469 When comparing ecotype differences by each garden site using a local vs foreign ecotype
470 comparison, (i.e., how an ecotype from that locality performs compared to foreign ecotypes
471 planted in the site), there was evidence of significant cover differences among ecotypes within a
472 site. In the Western Kansas reciprocal garden site (Colby, Kansas, (Table 1, Fig. 3), the driest
473 site, the dry ecotype cover (~20-40%) was significantly greater ($p < 0.046$) than the wet ecotype
474 (~5%), and in all years the dry ecotype was greater than mesic (~10-25%) but not significantly
475 different. A similar pattern was observed in the Central Kansas reciprocal garden (Hays, Kansas)
476 the next driest site, where in 5 out of 6 years, the dry ecotype cover (~25-40%) was significantly
477 greater ($p < 0.039$) than the wet ecotype (~5%). In all years at the Central Kansas reciprocal
478 garden (Hays, Kansas), the dry ecotype was greater than the mesic ecotype (~15-25%) but not
479 significantly different. Interestingly, in the Eastern Kansas reciprocal garden (Manhattan
480 Kansas), there were no significant differences among ecotypes across all years and cover ranged
481 from 20-35%, regardless of ecotype. In the Southern Illinois reciprocal garden (Carbondale,
482 Illinois), the wettest site, there were no significant differences among ecotypes during the first
483 two establishment years and all ecotypes maintained relatively low levels of cover (<10%). From
484 2012 onward, the dry ecotype continued to show significantly lower ($p < 0.018$) cover (<10%)
485 compared to the wet (25-40%) ecotype, but mesic (15-30%) and wet ecotypes (25-40%) were not
486 significantly different from each other.
487
488 Based on the same data, ecotypes showed signs of local adaptation when planted in their home
489 site compared to their away site (Table 1, Fig. 4). In all years, the dry ecotype (Fig. 4) had
490 significantly lower cover (cover <10% $p < 0.032$) than other ecotypes when planted in the
491 Southern Illinois reciprocal garden (Carbondale, Illinois, wettest site). For the wet ecotype (Fig.
492 4), in the first two years there were no significant differences between the reciprocal gardens in

493 western Kansas (Colby, Kansas), Central Kansas (Hays, Kansas) and Southern Illinois
494 (Carbondale, Illinois), that is driest, dry, and wettest, respectively (cover 10-20%) but was
495 significantly higher in Eastern Kansas (Manhattan Kansas) in 2010 ($p < 0.041$). Following the
496 establishment years, from 2013 onward, the wet ecotype had significantly increased cover (~25-
497 40%) in Eastern Kansas (Manhattan, Kansas) and Southern Illinois (Carbondale, Illinois ($p <$
498 0.049) but lower in western (Colby, Kansas) and Central Kansas (Hays, Kansas) sites, where the
499 cover of the wet ecotype was reduced to about 5% cover ($p< 0.003$). Interestingly, across all
500 years, there were no significant cover differences in the mesic ecotype among all four planting
501 sites (Fig. 4).
502
503 Regressions of cover by ecotype vs annual rainfall for combined years of 2014 and 2015, the
504 latest measurement years presumably when the vegetation was stabilized, showed that the dry
505 ecotype had highest cover with low rainfall, and decline in cover with increased rainfall as
506 occurs in the wettest site of Southern Illinois (Carbondale, Illinois, $p = 0.05$, $R^2 = 0.50$) (Fig. 5).
507 The wet ecotype showed the opposite pattern with low cover in Western and Central Kansas and
508 increase in cover with precipitation as occurs in Southern Illinois (Carbondale, Illinois, $p =$
509 0.007, $R^2=0.73$). Interestingly, cover of the mesic ecotype was only weakly related to rainfall (p
510 $= 0.26$, $R^2 = 0.21$, data not shown). This clearly shows cover of dry and wet ecotypes is related to
511 rainfall and corroborates their delineation. There were no significant correlations with other
512 variables (data not shown).
513
514 **Genetic Divergence Among Ecotypes Supports Phenotype Differences**
515
516 *Divergence and Diversity, Relation to Climate vs Geography*
517
518 *Structure* results indicate K=3 genetic clusters with two predominating, one occurring in dry and
519 mesic ecotypes and the other in wet ecotype (Fig. 6). Based on pairwise F_{st} (STable 3), only
520 slight neutral differentiation was observed between populations with F'_{st} (Meirmans *et al.* 2011)
521 of .028. In general, the wet ecotype showed greatest genetic distance with populations from
522 Kansas with F_{st} as high as 0.037. Populations from the dry and mesic ecotypes show lower
523 genetic distance as one might expect from geographic proximity, with F_{st} between 0.011-0.016.

We used pRDA analyses of genetic variation to quantify relative importance of climate vs geography in the full model (Model 1) that incorporates both climate and geography (STable 4). In the second model in which geography explained genetic variation conditioned on climate, total variance explained was 15%. In the third model in which climate variables explained genetic variation conditioned on geography, total variance explained was 74%. Thus climate structured genetic diversity more than geography (latitude and longitude). Total joint explained was 89% of total explained, leaving 11% unexplained by joint geography and climate variables. Bi-plot of the full model (1) (SFig. 4) showed that precipitation variables dominated loadings on pRDA1 and temperature variables explained loadings on pRDA2.

Outlier Analysis Related to Climate

For outlier analysis using *Bayenv2*, the top 1% of the X^TX values comprised 46 SNPs (STable 5). About half of the SNPs had annotations. Candidate genes function ranged from NAC transcription factors, peroxidases, glutamate synthetase, and GA1 (Sb01g021990.1) (STable 5), among others. Using *Bayenv2* to relate outlier SNPs to climatic variables, SNPs had more significant associations with temperature-related variables (mean annual temperature, seasonal diurnal temperature variation) followed to a lesser extent by variables related to precipitation (seasonal mean precipitation) (STable 6, SFig. 5). *BayeScan* v2.1 was used to provide a cross check of outliers between two methods to provide a list of consensus outliers. We identified 64 SNPs showing divergent selection, some of which were annotated (18 SNPs) and in common with *Bayenv* (15 SNPs) (STable 5, SFig 6). A SNP outlier near a gene of interest and identified in both *BayeScan* v2.1 and *Bayenv2* was GA1 and ranked as 14^{th} highest X^TX differentiated SNP (STable 5) from *Bayenv2* analysis. GA1 is a gene that codes for gibberellic acid, which is well known to be involved with controlling plant height and internode length (Milach *et al*. 2002). Across the climate gradient, the wet ecotype individuals show an increased frequency of the GA1 "tall" allele, while the dry ecotype is nearly fixed for the "short" allele (Fig. 7). GA1 was also identified in GWAS analyses using TASSEL, Galliart unpublished) and associated with height (Galliart et al. unpublished data).

Random Forest Training and Validation Using Plants of Known Ecotype

Individuals from the validation set from plants of known ecotype were assigned to one of three ecotypes (dry, mesic, wet) with accuracy of 79% (STables 7, 8) and overall misclassification rate of 21%. The highest rate of misclassification occurred with mesic individuals incorrectly called dry ecotype 26.4% (28/106 mesic plants). Of all ecotype pairs misclassified (21%, STable 7), 68% of those arose from mesic being called dry or vice versa. Importantly, misclassification of the wet ecotype was 4% of all wet ecotype individuals (4/98) and rarely misclassified (STables 7, 8). This is also shown in the training/validation triangle SFig. 7. Qualitatively, the training/validation triangle indicates excellent identification of wet ecotype individuals with somewhat less, but still good, discernment between dry and mesic ecotypes.

Evidence for Selection across the Climate Gradient: Ecotype Classification from Random Forest Model

We used random forest model training and validation of SNPs from plants of known ecotype to predict ecotype composition from unknown plants in mixed ecotype plots (Figure 8, STable 9, SFig 8). In mixed ecotype plots, in 2014, unknown individuals were predominantly predicted to be dry ecotype plants in Western Kansas (Colby, Kansas) Central Kansas (Hays, Kansas) (64 dry ecotype plants/88 total in Western Kansas (Colby, Kansas), 64 dry ecotype plants/90 total in Central Kansas (Hays, Kansas). A moderate number of mesic plants in mixed plots were predicted in Western Kansas (Colby, Kansas) and Central Kansas (Hays, Kansas) (22, 26, respectively). In Western Kansas (Colby, Kansas), only two plants were predicted as wet ecotype and no plants were predicted as wet ecotype in Central Kansas (Hays, Kansas). At the Eastern Kansas site (Manhattan, Kansas), mixed plots were predicted to be dominated by wet ecotype individuals (48 wet ecotype plants/85 total) with greater mixture of all ecotypes in Eastern Kansas (Manhattan, Kansas) (48 wet, 15 mesic, 22 dry ecotypes). At the Southern Illinois site (Carbondale, Illinois), wet ecotype dominates (65 wet ecotype plants/88 total) with 8 and 15 plants predicted for dry and mesic ecotypes, respectively. The percentage of predicted ecotype of individual plants is depicted in pie charts across sites (Fig. 8). We are potentially slightly underestimating role of mesic ecotypes in mixed plots across the range for 2014. However, in

spite of modest error rate of misclassification of mesic to and dry ecotypes, in Central Kansas (Hays, Kansas) and Western Kansas (Colby, Kansas), the dry ecotype still makes up the majority of ecotype identified. In the Eastern Kansas (Manhattan, Kansas) and Southern Illinois (Carbondale, Illinois) sites, in spite of the modest error rate of misclassification of mesic to dry ecotype, the wet ecotype is easily discernable from the others, and makes up the majority of the ecotype identified.

A similar pattern of ecotype composition was observed in 2015 (SFigs. 9,10, STable 10) and corroborates 2014 results. In dry Western Kansas (Colby, Kansas) and Central Kansas (Hays, Kansas), the dry ecotype again was predicted to dominate mixed plots with only one wet ecotype individual predicted in both sites. At the Eastern Kansas (Manhattan, Kansas) and Southern Illinois (Carbondale, Illinois) sites, ecotype composition showed the same trends as observed from 2014 sampling.

DISCUSSION

We found that one of the most dominant grasses of the North American Great Plains demonstrates local adaptation. Our study is unique in that it leverages a long-term data set (6 yr) and focuses on plants in realistic communities that allowed successional processes and climate variation to take place, thereby providing the most robust test for local adaptation. Supporting our findings, we find that local adaptation, candidate genes, and genetic variation were all related to climate. This study demonstrates clear ecotype differentiation in populations from the wettest (Southern Illinois) and driest (Western and Central Kansas) regions of the species' core distribution. Surprisingly, the apparent generalist mesic ecotype performs well at all sites and seems less affected by climate. Ecotype performance was explained by genetic differences in neutral diversity and candidate genes. Ecotype differentiation was related to climate, primarily rainfall, underscoring power of measuring genetic and phenotypic responses in common gardens (Lowe *et al.* 2017; Talbot *et al.* 2017; Villemereuil *et al.* 2016; De Kort *et al.* 2014) with experimental selection (Franks *et al.* 2016; Ravenscroft *et al.* 2015) under realistic conditions. Several other studies have demonstrated adaptation to climate starting with the early reciprocal transplant studies of Clausen *et al.* (1940) in the Sierra Nevada mountains using altitudinal

ecotypes of *Potentilla*. These seminal studies of Clausen, Keck, and Hiesey were followed up with McMillan's (1959) common garden studies of grass ecotypes in relation to the Great Plain's climate. More recently using a greenhouse approach, Munzbergova *et al.* (2017) showed that *Festuca rubra* populations originating from climates in Norway found that traits relating to foraging strategy varied with the climate of origin. Aspinwall *et al.* (2013) found that switchgrass genotype largely explained functional trait variation as related to the climate of origin. Largely writ, our results corroborate that ecotypic differentiation can occur across ecosystems spanning climatic gradients and that this local adaptation results in differential adaptive response to climate (e.g., Figs. 3,4,5). Uncovering and characterizing this local adaptation is essential to understanding responses to anticipated global change.

1. Local Adaptation in Perennial Grass Ecotypes in Long-term Single Ecotype Plots

Over the spatial climate gradient of the Great Plains, clear ecotype phenotypic differentiation of wet and dry ecotypes were observed in single ecotype plots. The wet ecotype outperformed others in Southern Illinois (Carbondale, Illinois) and the dry ecotype outperformed at Western Kansas (Colby, Kansas) and Central Kansas (Hays, Kansas). Several lines of evidence suggest that climate, especially precipitation, most strongly structured local adaptation, particularly at the dry end of the range margins. Furthermore, with a historic drought in 2012 in Kansas, the dry ecotype prevailed unaffected while the wet ecotype continued to decline. Interestingly, the mesic ecotype showed similar cover regardless of planting site and its performance was uncorrelated with rainfall at all sites, suggesting the mesic ecotype is a generalist that does moderately well over a range of rainfall conditions, potentially through plasticity. Interestingly, at the mesic Eastern Kansas (Manhattan, Kansas) planting site, all three ecotypes were not significantly different in cover, suggesting the mesic site can support all three ecotypes equally well, perhaps due to fluctuating drought and heavy rainfall.

Over the temporal gradient extending through 6 years, the trajectory for expression of local adaptation differed among sites and ecotypes. These patterns are only evident across longer times scales: a short-term, 2-yr study did not capture local adaptation at the Illinois (wet) site (Johnson *et al.* 2015). Only with longer periods of at least 4 years was this strong local adaptation

observed at the wettest site, while the dry ecotype performed well in dry regions from the start of experiment. The time-lag in response of the wet ecotype, especially at the wet site in Illinois, may be due to differences in competitive environments across the gradient. We surmise that local adaptation cannot be detected until early successional forbs are outcompeted by grasses (McCain et al. 2010). Thus, competition with forbs may have delayed expression of local adaptation of the wet ecotype in Illinois in the first few years, although further experimental studies are needed. Other researchers who have studied local adaptation in competitive environments have found that expression of local adaptation depends on biotic environment, including competition (Bischoff *et al.* 2006; Liancourt *et al.* 2015; Tomiolo *et al.* 2015) and facilitation (Johnson *et al.* 2010).

Differences in ecotype performance in singe ecotype plots corroborates sharp morphological differences among ecotypes observed in single-spaced plants (Caudle *et al.* 2014; Olsen *et al.* 2013; Mendola *et al.* 2016). The dry ecotype was dwarfed in size, short, having narrow leaves (SFig. 1) putatively to reduce evaporative loss (Johnson *et al.* 2015; Maricle *et al.* 2017) as an adaptation to drought. In contrast, the wet ecotype is tall, robust, and leafy (SFig. 1), presumably adapted to highly competitive environments where it grows amongst tall forbs and shrubs in wet prairies (Kuchler 1964). Interestingly, the dry ecotype flowers 3 weeks earlier than the wet ecotype, regardless of planting site, portending the beginning of reproductive isolation (Galliart *et al.* unpublished). This study and other several recent studies also highlight the importance of intraspecific variation, genetic (Malyshev *et al.* 2016; Poirier *et al* 2012) or phenotypic (Avolio *et al.* 2013; Des Roaches *et al.* 2017; Bolnik *et al.* 2011; Hamann *et al.* 2016), in ecological settings or in response to human-induced change (Mimural *et al.* 2017).

2. Genetic Analyses Support Differentiation of Wet and Dry Ecotypes

Genetically distinguished ecotypes support cover results across the precipitation gradient, similar to results observed by Gray *et al.* (2014) and Price *et al.* (2010). *STRUCTURE* plots show clear differentiation of dry and mesic from wet ecotypes, with admixture between adjacent dry and mesic ecotypes (Fig. 6). We have also shown that environmental factors, especially precipitation, explain more of genetic differences than does geographic location (SFig. 4, STable 4).

679

680 Ecotypes appeared functionally different (SFig. 1) suggesting adaptive variation in genetic
681 outliers. Ecotypes differ in terms of candidate genes such as NAC, glutamate synthetase,
682 peroxidase, and GA1. GA1, found in both Bayenv and Bayescan (STable 5) has high ecological
683 and functional significance. GA1 controls internode length and consequently height (Millach *et*
684 *al*. 2002). GA1 allele frequency varies clinally across the Great Plains; one form dominates in
685 the dry ecotype, characterized as short stature, or dwarfed (SFig. 1) while the alternate allele
686 dominates in the wet ecotype, characterized by a robust, tall form (SFig. 1). The association of
687 height and GA1 was also found in TASSEL analyses (Galliart unpub), corroborating observed
688 height differences between dry and wet ecotypes (with wet ecotypes growing 4.7x taller than the
689 dry ecotype). Height correlates with increased biomass, and greater competitiveness, as would be
690 advantageous in mesic prairies of the Eastern Great Plains which are dominated by tall forbs, and
691 shrubs (Kuchler 1964). Conversely, the dry ecotype from a xeric source of origin would be
692 advantaged by short stature to reduce evaporative loss as an adaptation to dry climates (Maricle
693 *et al.* 2017). These results provided powerful insight into candidate genes and genetic
694 mechanisms responsible for adaptive divergence.
695

696 Outlier SNPs identified in *Bayenv* showed a clear relationship with climate and associated with
697 temperature and precipitation variables (STable 6). Of the top 1% of outliers (46), 16 had a
698 significant association with annual mean temperature, 12 associated with seasonal diurnal
699 temperature variation, and 6 associated with growing season mean precipitation. Our study takes
700 similar approaches using outlier candidate genes across gradients, i.e., genome-environmental
701 associations as highlighted in recent excellent reviews. For example, Bragg *et al.* (2015) further
702 expanded on landscape genomics in non-model systems, especially foundation ecological
703 species; Rellstab *et al.* (2015) suggested a practical guide to studying the role of environment in
704 identifying adaptive loci; Sork *et al.* (2016) showed the importance of identifying underlying
705 candidate genes for phenotypes under climate selection with oaks as the focal species. Laskey *et*
706 *al.* (2018) suggest approaches to synthesize evidence from common gardens and genome-
707 environmental associations. Recent empirical studies have addressed various genome-
708 environmental associations. *Arabidopsis halleri* showed genomic footprints of selection to
709 altitude in the Alps (Fischer *et al.* 2013). Multiple species of oaks showed a signature of

selection in the same candidate genes amongst 71 populations in Switzerland (Rellstab *et al.* 2016). Laskey *et al.* (2012) used redundancy analyses to quantify the association between climate, geography and genomics in Eurasian *Arabidopsis* populations to discover that early spring temperature explained most of the variation. Pluess *et al.* (2016) related phenology candidate genes to climate, geographic and seasonality in European beeches. Finally, Exposito-Alonso *et al.* (2017) linked genetic variation to drought tolerance in *Arabidopsis* accessions from contrasting climates and highlighted the role of within species variation in the evolutionary response to climate.

3. Experimental Selection Studies Corroborate Wet and Dry Ecotypes

Letting the environment and biotic interactions impart selective pressures in local adaptation studies is a powerful approach to understand evolutionary processes. Indeed, this is the first time, to our knowledge, where ecotypes of the same species were grown together and allowed to compete over the long term. This should be the most robust test for local adaptation. Thus, by identifying which ecotypes are "winning" under spatially and temporally varying climate, we can relate these differences to identify climate drivers of local adaptation and intraspecific variation. Moreover, longer study periods are necessary to account for transient effects and allow competition and succession to have an effect.

We found that the dry ecotype, when grown with the other two ecotypes, outcompeted at the dry end of the gradient, as evidenced by its greatest proportion in mixed ecotype plots in Central and Western Kansas. Similarly, on the wet end of gradient, the wet ecotype exhibited local adaptation, as it occurred in greatest proportion in its wet home environment of Southern Illinois. If plant responses were due to phenotypic plasticity, we would have seen all three ecotypes equally represented in mixed plots across planting sites. These results mostly corroborate our findings in the single ecotype plots, but there was a surprising exception.

Although dry and wet ecotypes performed best in dry and wet environments, respectively, the mesic ecotype did not perform best in its home location of Eastern Kansas. This was also the case for single ecotype plots where no significant differences occurred in cover among ecotypes

741 in Eastern Kansas, where all ecotypes performed equally well. Further, the wet ecotype
742 outcompeted the mesic ecotype in the mixed plots located in Eastern Kansas. The years of mixed
743 ecotype plot collection had normal precipitation, so it is doubtful precipitation played a role.
744 Furthermore, this result was not due to lack of random forest discernment, as the wet ecotype is
745 easily distinguished from the others, and makes up the majority of the ecotype identified in
746 Eastern KS and Southern Illinois. So why did the mesic ecotype do comparatively poorly in its
747 home environment of Eastern Kansas, being outperformed by the wet ecotype? The wet ecotype
748 appears to be more competitive than the mesic ecotype in Eastern Kansas when the ecotypes
749 were planted together in the mixed ecotype plot compared to single ecotype plots. That is, the
750 wet ecotype wins inter-ecotype competition (between wet and mesic ecotypes) in the mixed
751 ecotype plots, but when grown among other wet ecotype plants in single ecotype plots, intra-
752 ecotype competition is stronger, resulting in overall low cover of wet ecotypes in single ecotype
753 plots. The wet ecotype putatively outcompetes the mesic ecotype in Eastern Kansas because it is
754 more vigorous due to its tall, robust stature (~3 times taller, ~2 times more biomass), thus
755 suppressing the shorter stature mesic ecotype, resulting in greater dominance of the wet ecotype
756 in Eastern Kansas. These results highlight the increased strength of biotic factors, especially
757 between-ecotype competition in the expression of local adaptation at the wetter end of the
758 gradient. At the dry end of the gradient, abiotic factors such as low precipitation are selective
759 pressures in local adaptation and the dry ecotype dominates in single and mixed ecotype plots.
760
761 Our results corroborate other studies (reviewed in Franks *et al.* 2014) showing selection over
762 time. Several studies show selection-induced treatment effects on phenotypes in intact
763 communities. The Buxton grassland studies of climate change treatments imposed over 15 years
764 shows adaptive selection and differentiation of phenotypes of species (Fridley *et al.* 2010), and
765 outliers sorting of genotypes (Ravenscroft *et al.* 2015) among treatments plots. Avolio & Smith
766 (2013) studied changes in phenotype in response to rainfall manipulation in intact grassland and
767 found *A. gerardii* phenotypic variation but no adaptive response to drought. Resurrection studies
768 in which phenotypes and genotypes from historical seed are compared with contemporary
769 progeny (Franks *et al.* 2018) have shown evidence for contemporary evolution. Franks *et al.*
770 (2016) showed rapid genome evolution in response to drought in *Brassica rapa*. Nevo *et al.*
771 (2012) found that cereal grasses in Israel collected as seed 28 years apart showed genetic and

phenotypic differentiation consistent with climate warming and drying. These studies show that with strong enough selection pressures, evolution is measurable in contemporary time.

4. Broader Implications for Climate Change, Conservation and Restoration

Several lines of evidence suggest that climate, especially seasonal precipitation and temperature variables, structures ecotypes and genetic divergence. First, cover of wet and dry ecotypes was correlated with precipitation, with wet ecotypes outperforming dry ecotypes in wet climates (Figs. 3, 4) and conversely, for dry ecotypes. Second, pRDA shows that climate, more than geographic location, structures neutral genetic variation. Third, outliers were related to both temperature and precipitation factors. Precipitation and temperature patterns for the last 10,000 years (Axelrod 1985) have been a selective pressure leading to adaptive variation. This has also been observed with experimental manipulation of rainfall and temperature (Avolio *et al.* 2013). The ability of species to tolerate extreme drought was demonstrated by Exposito-Alonso *et al.* (2018) in which they highlighted the role of within species variation in drought tolerance in *Arabidopsis* and its evolutionary response to climate. More broadly, the importance of precipitation as a selection force in plants and animal populations has been discovered through meta-analysis (Siepielski *et al.* 2016).

How climate structures *A. gerardii* genetics, form, and function is critical, as the foundation species of tallgrass prairie. Climate is predicted to change in the Great Plains (IPCC 2013), resulting in increased occurrence and severity of drought. We are currently manipulating rainfall with a rainout drought experiment in these same plots to address the role of drought. A recent phenotypic modeling study (Smith *et al.* 2017) predicted that, with climate change, populations of short-statured, dwarf forms of *A. gerardii* from dry parts of its range would be favored 600 km eastward, and result in 60% decrease in productivity and biomass. Evolutionary adaptation in *A. gerardii* may not be able to provide what ecology and future climate demands (Kokko *et al.* 2017). Reduction in productivity could have cascading effects on prairie function (Knapp *et al.* 1998), cattle forage production (Gibson *et al.* 2016), grassland restoration (Baer *et al.* 2018), and conservation. Furthermore, about 60% of agricultural production in Kansas (~$10 billion, NASS, 2014) was attributed to cattle production, with *A. gerardii* being the main forage grass for cattle.

Tallgrass prairie, one of the most diverse grasslands, is critically endangered with only 4% native prairie remaining (Samson and Knopf 1994) with *A. gerardii* being the iconic grass of prairies. Ultimately, this research will inform land managers which grass ecotypes are best suited for conservation and restoration for drier climates. Thus, knowing how to restore prairie with climate-matched ecotypes is critical to the future ecology, agricultural sustainability of critical grasslands.

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1197 **Tables**

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Reciprocal Garden Planting Site (Town, County) Soil Type	**Elev. (m)**	**Lat. (°N) Long (W)**	**Rainfall 6-year mean 2009-2016 (range) (cm)**	**Annual Number of Pcp Events >1.25 cm**	**Pcp Driest Year (cm)**	**Mean Annual rainfall (cm)**	**Growing Season Mean Rainfall (cm) (sum+sp)**	**Annual Diurnal Temp (°C)**	**Growing Seasonal Diurnal Temp (°C) (sum+sp)**	**Annual Mean Temp (°C)**	**Growing Season Mean Temp (°C) (sum+sp)**	**Temp Severity Index (# days over 95F)**
Western KS (Colby, KS Thomas, Co) KSU Ag Expt Station (Ulysses Silt Loam)	972	39.39 101.06	48.0 (29.4-66.8)	13.0	28.37 (1967)	52.5	39.44	-2.0	-2.0	10.9	16.7	21.3
Central KS (Hays KS Ellis Co) KSU Ag Expt Station (McCook Silt Loam)	603	38.85 99.34	54.6 (38.3-67.9)	15.4	36.27 (1988)	59.6	43.18	-3.2	-3.4	12.3	18.3	29.2
Eastern KS (Manhattan, KS	315	39.19 96.58	89.1 (61.5-	21.9	39.16 (1966)	90.5	63.47	-4.2	-4.3	12.8	18.9	23

Riley Co) USDA Plant Materials (Belvue Silt Loam)			110.2)									
Southern Illinois (Carbondale IL Jackson, Co) SIU Ag Research Station (Stoy Silt Loam)	127	37.73 89.17	125.6 (76.2- 125.6)	32.7	67.38 (1963)	119.8	64.51	-5.3	-5.1	13.5	19.0	6.3

Table 1. Historical Weather data (30-year normals) for planting site locations. Precipitation data for 6 years of the experiment are presented in SFig. 2.

Figure Caption

Fig 1. Location of reciprocal gardens planting and collections sites across the US Great Plains. White circle is reciprocal garden location. Black triangles are the collection prairie for the seeds. For prairie population acronyms, see STable 1. Western Kansas (Colby, Kansas) is the satellite reciprocal site to test the range of tolerance for big bluestem. Note that seeds were not collected in Colby.

Fig 2. Reciprocal garden transplant design for sown community plots. Single colors are single ecotype plots, checkerboard is mixed ecotype plot. At each planting site, there are 4 replicate plots. Ecotype plots at each site were randomized. Note that the Colby planting site had no local ecotype but was included to test the threshold of response to drier locations as might be experienced in the future.

Fig. 3. Vegetative cover (least square mean estimates with standard errors) by planting sites (Western Kansas (Colby, Kansas), Central Kansas (Hays, Kansas), Eastern Kansas (Manhattan, Kansas) and Southern Illinois (Carbondale, Illinois) for each ecotype in the single ecotype plots from years 2010-2015 across the Great Plains precipitation gradient. Letters indicate significant differences within years.

Fig 4. Vegetative cover (least square mean estimates with standard errors) by each ecotype in the single ecotype plots at planting sites from years 2010-2015 across the Great Plains precipitation gradient. Red=western KS, Orange=central KS, Green= Eastern KS, Blue = Southern Illinois. Letters indicate significant differences within a year.

Fig 5. Percent big bluestem dry (red) and wet (blue) ecotype cover versus the annual rainfall in the corresponding planting locations 2014 and 2015 combined.

Fig 6. STRUCTURE bar plot labeled by regional ecotype and by prairie. The most likely genetic grouping solution, K = 3, is shown. Each color indicates one genetic group, and each bar represents percentage membership to genetic group(s). Mixed membership indicates admixture.

Fig 7. Map indicating the allele frequencies for the GA1 outlier across the 12 populations focusing on the gradient in alleles across the climate gradient from Western Kansas to Southern Illinois. “Short” allele is in blue, alternative “tall” allele is in red.

Fig 8. Map showing the predicted ecotype composition of mixed ecotype plots across the reciprocal gardens in 2014. Dry ecotype denoted in red, Mesic ecotype denoted in green, and Wet ecotype denoted in blue.

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