Local adaptation and genetic divergence in the dominant grass *Andropogon gerardii* across the Great Plains' rainfall gradient

by

Matthew Galliart

B.S., Kansas State University, 2014

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Division of Biology
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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, most studies lack comprehensive investigation with only few responses or measurements included, and lack integrative studies combining both ecology and genomics. Here, we report on a long-term experiment focused on *Andropogon gerardii*, the dominant grass of the North American Great Plains tallgrass ecosystem. Our approach used a reciprocal garden platform in which four reciprocal garden sites were planted with three regional ecotypes of *Andropogon gerardii*, using dry, mesic, and wet ecotypes originating from western KS to Illinois that span 500–1,200 mm rainfall/year. We focus on this foundation grass that comprises 80% of tallgrass prairie biomass, is a major forage grass for cattle, and is widely used in restoration.

First, we aimed to assess genetically based local adaptation of *A. gerardii* ecotypes in realistic competitive settings**.** 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 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.

Second, we used the reciprocal gardens in plants growing singly without competition to detect genetic and environmental plasticity effects on phenotypic variation and combined with genetic analyses. The goal was to evaluate the extent to which *A. gerardii* ecotypes differ across spatially varying climatic regions, thus potentially signaling local specialization, i.e., genetic differentiation and adaptation of ecotypes to precipitation. Here we addressed the following questions: 1) Are *A. gerardii* ecotypes locally adapted to environment across the precipitation gradient? 2) What is the relative role of genetic constraints and plasticity in controlling phenotypic differences? 3) How will different ecotypes of *A. gerardii* respond under different climatic conditions, especially precipitation, when planted in home environment and reciprocally transplanted into foreign environments? 4) What are the underlying genetic bases for these traits? 5) How are genotypes and phenotypes structured by climate? Surprisingly, we did not detect consistent local adaptation. Rather, we detected co-gradient variation primarily for most vegetative responses. All ecotypes were stunted in western KS. Eastward, the wet ecotype was increasingly robust relative to other ecotypes. In contrast, fitness showed evidence for local adaptation in wet and dry ecotypes with wet and mesic ecotypes producing little seed in western KS. Earlier flowering time in the dry ecotype suggests adaptation to end of season drought. The wet ecotype was robust, tall with high biomass, and wide leaves putatively adapted for the highly competitive, light-limited Eastern Great Plains. We detected genetic differentiation and outlier genes associated with primarily precipitation. We identified candidate gene GA1 for which allele frequency associated with plant height. Sourcing of climate adapted ecotypes should be considered for restoration.

Finally, we aimed to determine to what extent this ecologically-important prairie grass, big bluestem, responds to environmental change, both ecologically and transcriptionally through expression of genes. Here we aimed to answer: 1) Do bluestem ecotypes growing across a longitudinal precipitation gradient transcribe a different suite of genes or show differential expression levels of the same genes in response to environment? 2) Is the local ecotype of big bluestem more transcriptionally responsive in its home environment? and 3) How does an ecologically important grass respond ecologically and at the level of its transcriptome to a drier or wetter environment at the margin of its range? Without long-term studies, wrong conclusions would have been reached based on insufficient data. Ultimately, restoring and conserving prairies with climate-matched ecotypes is critical to future ecology, conservation, and sustainability of these vital grasslands under climate change.

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Chapter 1 - Introduction and Overview

Local adaptation

Local adaptation is determined when plant performance of the local population in the native site is greater than plants of a foreign population when planted in the same environment (Kim & Donohue 2013; Linhart & Grant, 1996). Reciprocal gardens have been widely used to study local adaptation (Johnson *et al.* 2022). Many studies have focused on elevation gradients (Clausen *et al.* 1940; Knight *et al.* 2006; Byars *et al.* 2007; Montesinos-Navarro *et al.* 2011) Others have investigated local adaptation along precipitation gradients (Etterson 2004). Adaptation of grasses have been previously investigated across a north-south gradient by the seminal early work of McMillan (1959). Grassland species are known to become locally adapted across climatic gradients (Becker *et al.* 2006; Macel *et al.* 2007; Bischoff *et al.* 2007; Gray *et al.* 2014; Milano *et al.* 2016; Lowry *et al.*, 2019 Lovell *et al.*, 2021). In some cases, it has been found that local adaptation can be responsible for reproductive isolation (Zhang 2019). It is possible that in the face of climate change, foreign ecotypes could benefit and increase fitness in non-native sites (Anderson & Mitchell-Olds 2014).

Plant response to current and changing climate depends on the degree of adaptive potential within a species (Hufford and Mazer 2003; Etterson 2004; Nicotra *et al.* 2010; Shaw and Etterson 2012; Des Roach *et al.* 2017). Thus, more information is needed on adaptive potential to predict how species may respond to changes in climate, either through plasticity, adaptive genetic variation, or migration (Nicotra *et al.* 2010; Christmas *et al.* 2016). Most frequently, some combination of phenotypic plasticity and genetically based adaptive variation is observed in plant responses to environmental change (Crispo 2008; Lowry *et al.* 2019; Lovell *et al.* 2021). However, the extent to which plants respond via genetic adaptive response depends on the strength of local selection

pressures counteracting the homogenizing effects of gene flow among populations (Sambatti and Rice 2006; Gonzalo-Turpin and Hazard 2009).

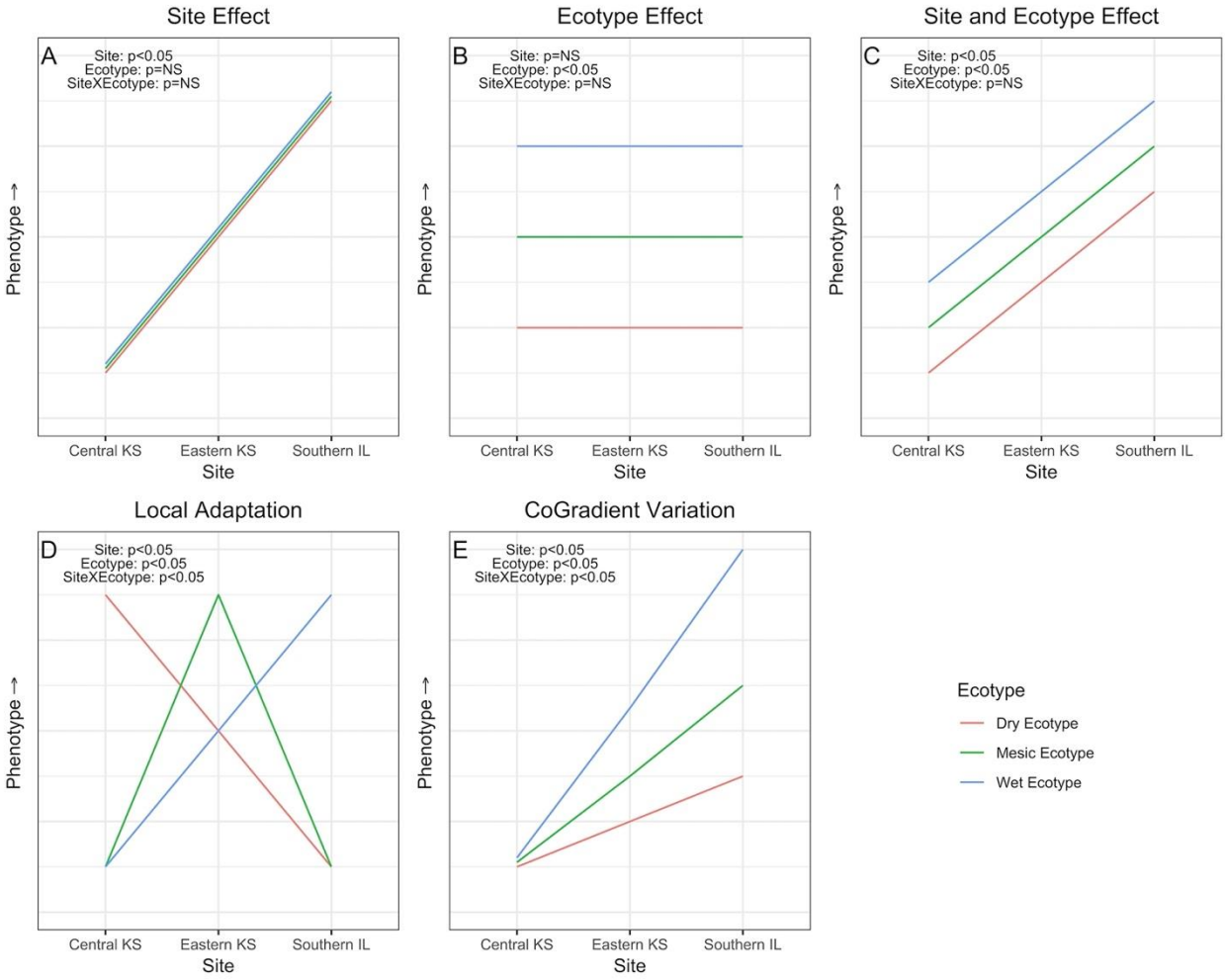

Figure 1.1. Model showing response of phenotypic variation of ecotypes across gradient. A) site effect, B) Ecotype Effect, C) Site and Ecotype Effects, D) Local adaptation, E) Co-Gradient Variation

Reciprocal gardens are the gold standard to detect genetic and plasticity effects on phenotypic variation (Johnson *et al.* 2022). Here, we used a reciprocal common garden approach (Clausen *et al.* 1940; McMillan 1959, 1965; Etterson 2004; Lowry *et al.* 2009; de Kort *et al.* 2014; Villemereuil *et al.* 2016; MacTavish & Anderson 2020; Lortie & Hierro 2022; Rushworth *et al.* 2022; VanWallendael *et al.* 2022). If genotype is the only main effect on phenotypic variation, phenotypes are then fixed by genetics (Fig. 1.1B across a gradient. If environment is the only

effect, then phenotypic variation is entirely plastic varying across sites (Fig. 1.1A. Both genotype and site may display main effects with no interaction (Fig. 1.1C). The interaction term G X E expresses the extent to which genotypes differ in their sensitivity to environment (Fig. 1.1D, 1.1E). Local adaptation occurs when populations perform best in their home environment compared to non-local populations in the same site (Fig. 1.1D). Other interactions in traits can also be detected such as co-gradient phenotypic variation (CoGV, Fig. 1.1E) and counter-gradient phenotypic variation (CnGV) (Conover and Schultz 1995; Eckhart *et al.* 2004; Crispo 2008; Conover *et al.* 2009; Anderson *et al.* 2015). Co-gradient variation occurs when positive covariance exists between environmental and genetic sources of variation, and it might be expected to evolve as adaptive phenotypic plasticity when populations exist in variable environments (Eckert *et al.* 2003).

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). Indeed, local adaptation appears as a common phenomenon in plant populations across broad climate scales. 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). This research addresses adaptive plant variation and local adaptation, especially in response to rainfall (Fay *et al.* 2003; Hajek & Knapp

2021), of an ecologically dominant and economically critical forage grass, *Andropogon gerardii Vitman,* big bluestem.

Reciprocal Garden Research Platform

We investigated ecotypic variation and local adaptation by using reciprocal gardens that have been in place since 2009 planted across a precipitation gradient in the central portion of the US Great Plains. The reciprocal garden takes two approaches: planting ecotypes in competitive community plots and in single-spaced ecotype plantings (Fig 1.2). Gardens were planted in Carbondale, IL (1200 mm), Manhattan, KS (870 mm), Hays KS (580 mm), and Colby, KS (500 mm) (Fig. 1.3, Table 1.1). No regional ecotype was collected from Colby, KS but we used it as a drier environment to test ecotype tolerance into even drier areas.

Figure 1.2. Aerial photograph of the planting sites. Left image shows the individually planted single spaced plants. Right image shows the sown community plots. Both images are from the Manhattan, KS planting site.

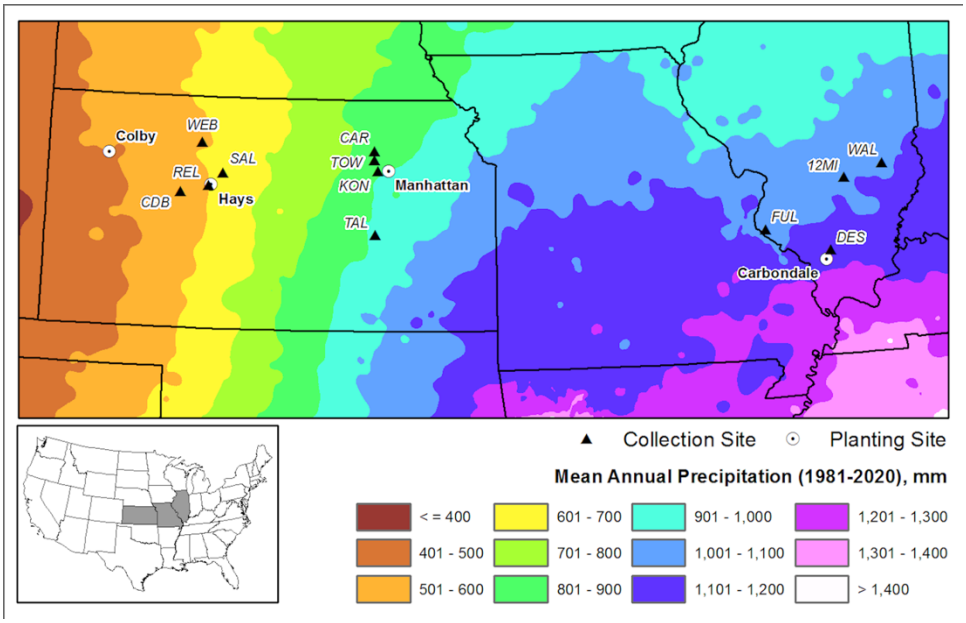

Figure 1.3. Map of planting sites and precipitation gradient. Circles indicate the planting sites of Colby, Hays, Manhattan, KS and Carbondale, IL. Triangles indicate source populations comprising the three regional ecotypes. Color overlay indicates the mean annual precipitation for the area.

For the seeded plots, a key innovation in our design is to test how these ecotypes perform in a multi-species, functional grassland community rather than using monocultures to test for adaptation (Fig. 1.2 Right panel) Seed of our focal species *A. gerardii* was collected from four native populations from three climatically distinct regions along a precipitation gradient, central KS (dry ecotype), eastern KS (mesic ecotype), and southern IL (wet ecotype), thus defining three regional ecotypes (each contains four populations) (Fig. 1.4, Table 1.2)

Table 1.1. . Historical Weather data (30 year normals) for planting site locations

Reciprocal Garden Planting Site	Elev. (m)	Lat. (°N)	Long. (°W)	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)
Colby, KS (Thomas, Co) KSU Ag Expt Station (Ulysses Silt Loam)	972	39.39	101.06	13.0	28.37 (1967)	52.5	39.44	-2.0	-2.0	10.9	16.7	21.3
Hays KS (Ellis Co) KSU Ag Expt Station (McCook Silt Loam)	603	38.85	99.34	15.4	36.27 (1988)	59.6	43.18	-3.2	-3.4	12.3	18.3	29.2
Manhattan, KS (Riley Co) USDA Plant Materials (Belvue Silt Loam)	315	39.19	96.58	21.9	39.16 (1966)	90.5	63.47	-4.2	-4.3	12.8	18.9	23
Carbondale IL (Jackson, Co) SIU Ag Research Station (Stoy Silt Loam)	127	37.73	89.17	32.7	67.38 (1963)	119.8	64.51	-5.3	-5.1	13.5	19.0	6.3

Table 1.2. Source location of 12 populations used with relevant location and site information. Four populations from each regional ecotype (Dry, Mesic, and Wet) are presented here.

Regional Ecotype	Prairie Name	Prairie Size (ha)	Prairie Abbreviation	County, State	Elev. (m)	Soil type	Latitude (°N)	Longitude (°W)
Dry	Webster Reservoir	356	WEB	Rooks, KS	606	Wakeeney-Harney Silt loam	39.41	99.50
Dry	Saline Experimental Range	880	SAL	Ellis, KS	641	Bogue-Armo Clay loam	39.02	99.14
Dry	Cedar Bluffs Reservoir	850	CDB	Trego, KS	688	Armo Clay loam	38.76	99.83
Dry	Relict Prairie	14	REL	Ellis, KS	640	Armo loam, Brownell gravelly loam	38.85	99.37
Mesic	Konza Prairie	1557	KON	Riley/Geary, KS	366	Benfield Florence silty clay loam	39.08	96.56
Mesic	Tallgrass National Park	4409	TAL	Chase, KS	392	Cline Sogen Silty clay loam	38.25	96.6
Mesic	Carnahan Cove	99	CAR	Pottawatomie, KS	389	Benfield Florence silty clay loam	39.34	96.62
Mesic	Top of the World Park	61	TOW	Riley, KS	379	Irwin silty clay loam	39.22	96.62
Wet	Desoto Prairie	0.4	DES	Jackson, IL	119	Orthents silty loam	37.85	89.14
Wet	Twelve Mile Prairie	28	TM	Effingham, Monroe, Fayette, IL	160	Cisne silty loam	38.78	88.83
Wet	Walters	5	WAL	Jasper, IL	150	Atlas silty clay loam	38.92	88.19
Wet	Fults	214	FUL	Monroe, IL	215	Menfro silty clay loam	38.17	90.19

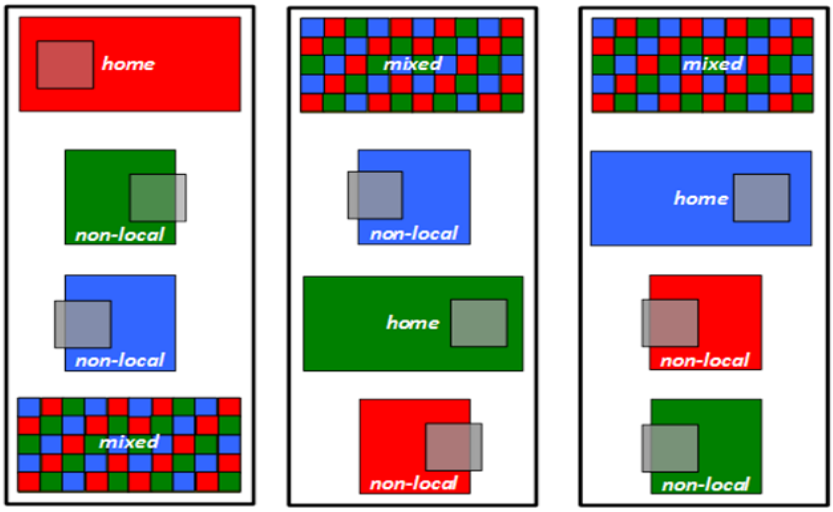

Figure 1.4. Experimental design of the sown community plots. Red indicates the dry ecotype, green indicates the mesic ecotype, and blue indicates the wet ecotype. Checkered boxes indicate plots in which all three ecotypes were sown together in the same plot.

The design of the experiment was that of a reciprocally transplanted, randomized complete block with four blocks per site, whereby each regional ecotype was represented by one plot (4 m x 8 m) in each block of every site including mixed ecotypes plots where all three ecotypes were planted together at equal seeding densities (Fig. 1.4). A 70:30 ratio of live C_4-grass to C_3-grass and forb seed was used to seed the community plots (Johnson *et al.* 2015). Each plot was seeded to a total density of 580 seeds m^2, which is similar to what is 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 the characteristic structure and competitive relationships of tallgrass prairie. Planted seeds of all species, except *Andropogon* and *Sorgastrum* (regionally collected), were purchased from a commercial supplier (Ion Exchange Inc., Harpers Ferry, IA, USA) and sourced

from across the Great Plains. All plots were sown by hand seeding in 2009 in accordance with typical prairie restoration guidelines (Baer *et al.* 2019).

For the reciprocal gardens comprised of single-spaced plants (Fig. 1.2. Left panel), the same three regional ecotypes were additionally planted in the four sites and have been similarly in place since 2009. Seeds from each of the 4 populations from the 3 ecotypes were germinated and growing in the greenhouse in 10 x 10 cm pots using Metro-Mix 510. In August 2009, 3 - 4 month-old plants form the 12 populations were then transplanted into the field 50 cm apart at each of the 4 planting sites (Colby, KS, Hays, KS, Manhattan, KS, and Carbondale, IL). The design of the experiment was that of a reciprocally transplanted, randomized complete block with 10 blocks per site. There is one individual from each of the four populations per ecotype in each block with 10 blocks (4 populations per ecotype x 3 ecotypes x 10 replicate blocks = totaling 120 plants or 40 per ecotype, 10 plants per population within an ecotype). Water-permeable landscape cloth was used to cover the planting areas to prevent volunteer growth of potential competitor plants.

Previous studies utilizing this reciprocal garden platform contribute greatly to knowledge on intraspecific variation (reproductive effort - Gibson *et al.* 2013, morphology - Olsen *et al.* 2013, Galliart *et al.* 2020, physiology - Caudle *et al.* 2014, genetics - Gray *et al.* 2014, local adaptation - Johnson *et al.* 2015, belowground processes - Mendola *et al.* 2015, genomics – Galliart *et al.* 2019, community processes - Wilson *et al.* 2016, Ren et al 2022, forage quality - Gibson *et al.* 2017, drought tolerance - Maricle *et al.* 2017, and phenotype distribution modeling - Smith *et al.* 2017., microbiome – Sakar *et al.* 2022).

In a recent review, Johnson *et al.* 2022 showed that reciprocal gardens where biotic factors are manipulated or even considered are few. In a literature survey (Johnson *et al.* 2022), they found only 17% studies had a biotic component. Furthermore, most reciprocal garden studies

investigating native grass are relatively short term and include only a few responses (Fig 1.5). This long term (> 10 year) study is a powerful platform to investigate natural selection and local adaptation in a dominant prairie grass. Additionally, here we include responses at multiple scales including morphology, physiology, genomics, and transcriptomics.

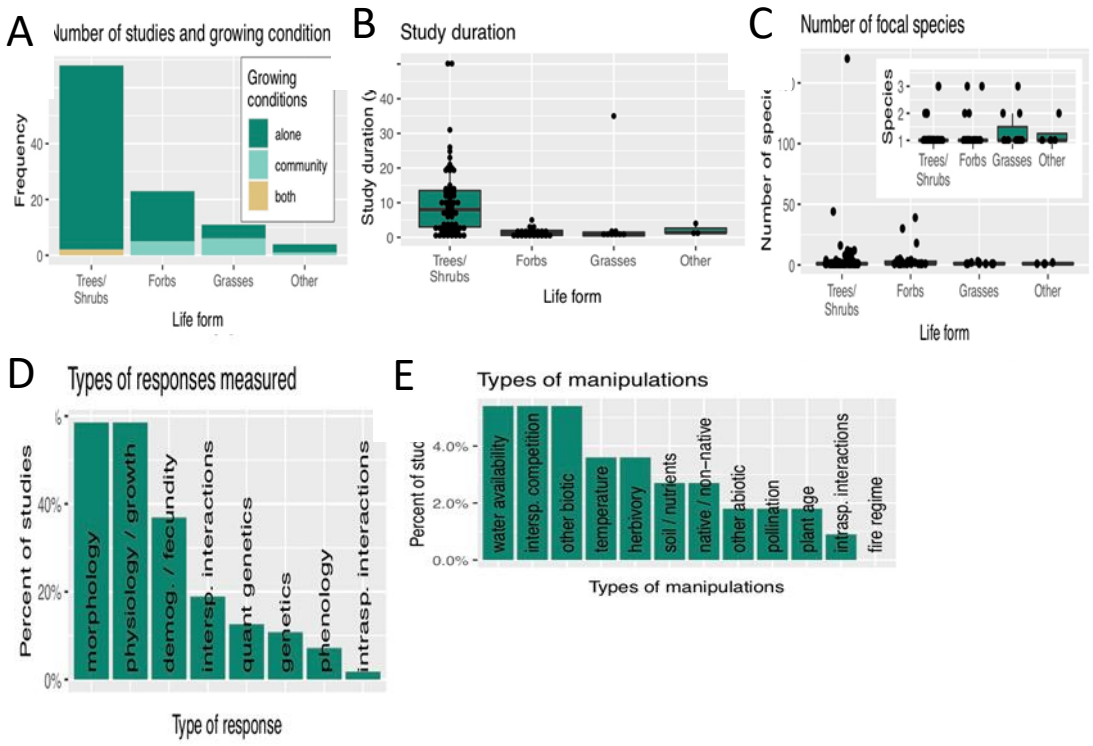

Figure 1.5. Charts outlining the number of studies taking various approaches from a literature review (Adapted from Johnson *et al.* 2022). A. Frequency of studies by organism type and planting type. B. Study duration by organism type. C. Number of focal species. D. Percent of studies measuring various response variables. E. Types of manipulations included in these reciprocal garden approaches.

Tests for natural selection in the field

Few studies have investigated natural selection in experimental field settings (Heschel *et al.* 2002; Reznick & Ghalambor 2005; Huang *et al.* 2010; Villemereuill *et al.* 2016). Natural selection in these field settings can exert strong selection forces on populations and even be strong enough to force out genotypes (Donohue *et al.* 2005). There is now plenty of empirical data for

predicting species evolutionary response to field natural selection in the face of climate change (Hamann *et al.* 2021). However, the genetic mechanisms of these responses is still not fully understood. (Hamann *et al.* 2021; Keagy *et al.* 2023). This makes it necessary to initiate these field-based experiments to understand and make predictions about selection under realistic field conditions. For studies that measure changes in phenotypes and genotypes in the field (i.e. contemporary evolution), 90% lasted only 3 years or less (Franks *et al.* 2014). The platform we will be utilizing has been in place for just under 10 years since 2009. With strong environmental changes, populations might not respond through genetic changes but rather with phenotypic plasticity (Reznick 2001). However, other studies have reported rapid evolution for populations in the face of strong environmental change (Stockwell *et al.* 2003). Natural selection can even cause evolutionary response in as little as a few generations (Franks *et al.* 2006). With strong selective forces, reproductive barriers can arise quickly in plant species (Rieseberg & Willis 2007). These barriers can ultimately lead to speciation.

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; Etterson 2004; Knight *et al.* 2006; Lowry *et al.* 2009; Esposito-Alonso *et al.* 2018; MacTavish & Anderson 2020). However, little is known (Mimura *et al.* 2017) 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, Christe *et al.* 2022). Still, little empirical data exist for predicting species' adaptive response to natural, and now rapidly changing, selection pressures (Mimura *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 (Ravenscroft *et al.* 2015; Metz & Tielborger 2016).

Taking genomics outdoors

Few studies focus on gene expression in wild plants under natural field conditions. With the advent of genomic technology going from the early days of micro arrays to RNA-Seq, there's been a linear increase in the number of gene expression papers. In a literature search, we detected 157 papers using "environment, nature, field, transcriptome, gene expression, RNA-Seq, plants" search terms for the period 2012 to 2023. Most gene expression studies have been conducted under controlled laboratory conditions and manipulate a single factor at a time such as water (12 papers), temperature (11 papers), salinity (4), or nutrients (10). Of those latest 104 studies done, surprisingly, only 21 are done in the field. While controlled studies are helpful in revealing mechanisms, the studies suffer from lack of ecological realism (Ungerer *et al.* 2008) and lack the dynamic nature of the environment in which they grow and have evolved. In nature, plants are responding to a multitude of factors, such as plant-plant competition (Ariza *et al.* 2011) or facilitation (Remke *et al.* 2021; Koziol *et al.* 2023), pathogen pressure (Bever *et al.* 2015) or their microbiome (Sarkar *et al.* 2022; Trivedi *et al.* 2022, Kazarina *et al.* 2023).

As indicated by a literature survey of 157 papers, few studies exist where transcriptomics

and gene expression embrace the variability of wild plants in the field. In fact, based on a non-exhaustive literature survey of transcriptomics studies as described above, only 15% were done with wild plants (not crop plant accessions), only 32% done in the field and even more surprising, only 4% were done with wild plants in the field). Furthermore, only about half of transcriptomics studies made ancillary measurements and few made manipulations in the field (mostly single manipulations). A few other studies have also been done in the field such as Plessis *et al.* (2015) (rice). Plessis *et al.* 2015 found that both climate and broad environmental differences had large impacts on transcriptional responses in rice. We have found that in spite of the variability of the "rough and tumble" real world, *A. gerardii* plants show a clear signal of the genetic background of each ecotype in addition to responding to all the other factors around them. Clearly, there is a sizeable knowledge gap and opportunity for new ecological and genomic insights by examining transcriptomics of wild plants growing in the field under the condition where they evolved and adapted (Plessis *et al.* 2015; Turner *et al.* 2020).

Study Plant Details- Big bluestem, *Andropogon gerardii*

Andropogon gerardii (big bluestem) is a warm-season and ecologically-dominant grass of the North American tallgrass prairie. It is a long-lived perennial that is an obligate outcrosser with a large polyploid genome (~2 Gb) occurring naturally as two main cytotypes of 6x and 9x (Norman *et al.* 2013). Previous work has been done to begin to investigate the genomic and transcriptomic response of *A. gerardii* across climate gradients (Gray *et al.* 2014; Avolio *et al. 2017*; Hoffman & Smith 2018; Galliart *et al.* 2019; Galliart *et al.* 2020). *Andropogon gerardii* has a wide distribution, spanning most of the United States, and is dominant in the Midwest grasslands (Fig 1.6). Approximately 70% of the biomass of the tallgrass prairie ecosystem is comprised of big bluestem

(Knapp *et al.*, 1998). With a distribution in the Great Plains that spans a wide climate gradient (Epstein *et al.*, 1997), this distribution suggests there is putative natural variation across the gradient. This natural variation allows for classification of big bluestem into regional ecotypes (Fig. 1.7).

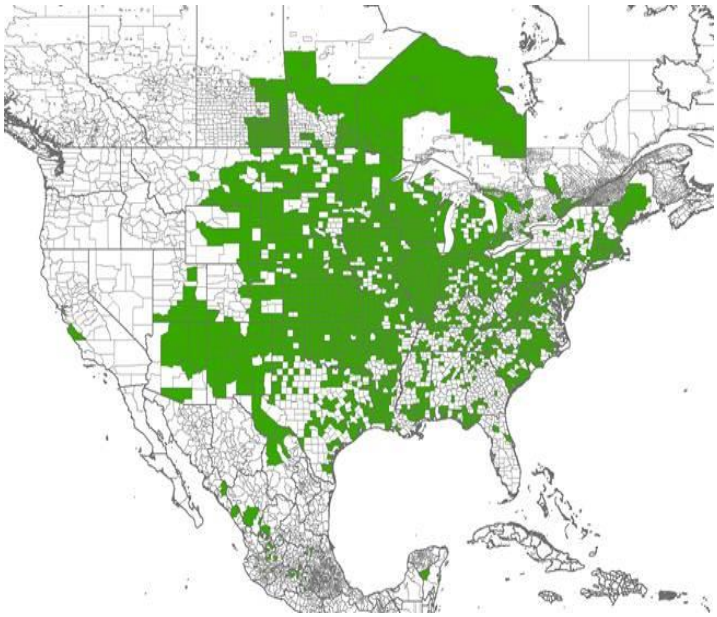

Figure 1.6. Map of central North America showing the distribution of *A.* gerardii (Smith *et al.* 2017).

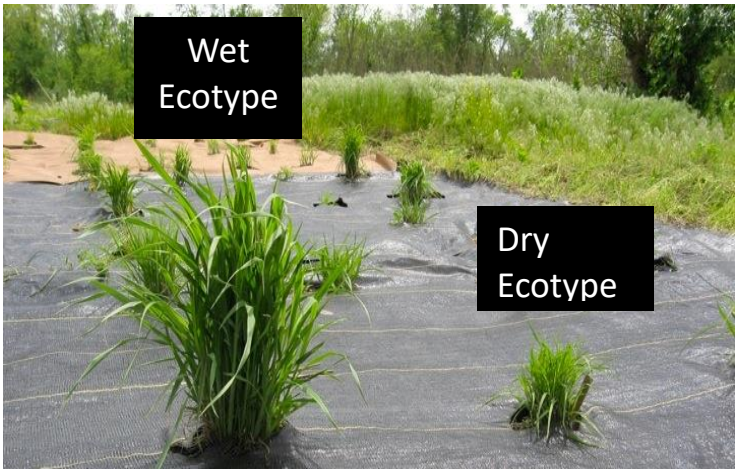

Figure 1.7. Regional ecotypes of *A. gerardii*. Left shows the Wet ecotype and right showing the Dry ecotype.

Within the Great Plains region, *A. gerardii* occurs along a sharp mean annual precipitation (MAP) gradient from western KS (500 mm MAP) to Illinois (1200 mm MAP) (Fig. 1.3), a climate gradient that has been in place for ~10,000 years since the last glaciation (Axelrod 1985), allowing ample time for natural selection to work in these populations. Grasslands are largely shaped, both structurally and functionally, by water availability (Fay *et al.* 2003). A vegetative shift from short grass in the arid west to tall grass prairie in the east is primarily explained by the presence of this sharp west-east precipitation gradient (Transeu 1935). Due to predicted future variablilty in climate for the Great Plains (IPCC, 2021) we can putatively expect future climate to favor xeric adapted dry ecotypes from central and western Kansas.

Andropogon gerardii has been extensively studied in response to climate change (Epstein *et al.*, 1998; Knapp *et al.*, 2001; Fay *et al.*, 2002; Fay *et al.*, 2003), management such as fire and grazing (Collins *et al.*, 1998; Veen *et al.*, 2008), morphology (Knapp *et al.* 2001; Olson *et al.*; Bachle & Nippert 2021) and physiological performance under varying resources (Silletti & Knapp, 2002; Swemmer *et al.*, 2006; Maricle *et al.* 2017; Caudle *et al.* 2014; Bachle & Nippert 2022). This work builds upon the previous work to characterize local adaptation at various levels of morphology, physiology, genetic, and transcriptional.

Significance for climate change and restoration

This work focuses on local adaptation to regional rainfall and drought. The Midwest US grasslands in characterized by periodic severe drought with the most recent severe drought in place since early 2022. Furthermore, one of the most important climatic changes predicted for the central grasslands of the U.S. is alteration in the amount and timing of precipitation events (IPCC 2020) (Fig. 1.8). Consequently, understanding the degree of natural variation in drought tolerance of

dominant grassland species, such as *A. gerardii*, is particularly urgent. Better understanding regional selection to drought is crucial to long-term conservation, mitigation against climate change, and agricultural sustainability.

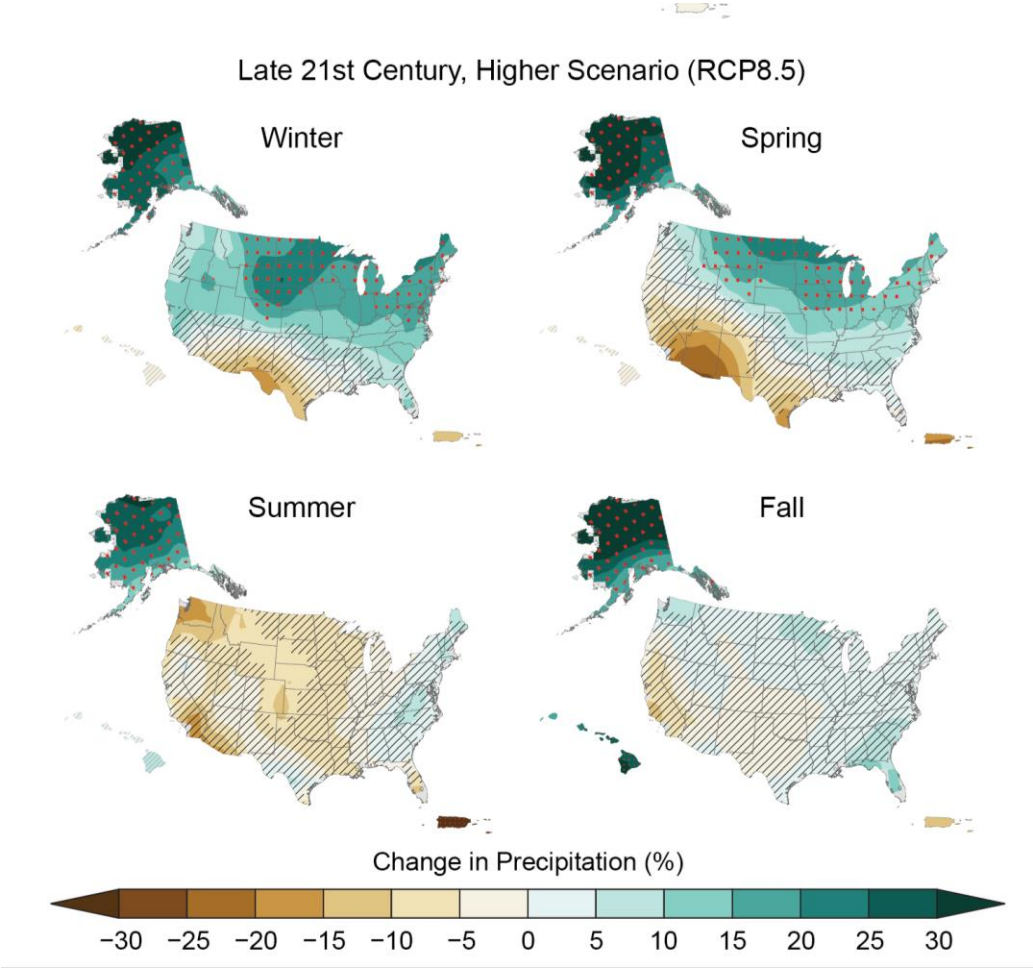

Figure 1.8. Projected changes in precipitation due to climate change across seasons. Brown colors indicate decreases in precipitation and blue colors indicate increases in precipitation. Hatched lines indicate significance across models. Adapted from 2020 NCA report.

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. US grasslands have experienced severe drought. Furthermore, one of the most important climatic changes predicted for grasslands is alteration of amount and timing of precipitation events (IPCC

2020, Leal Filho *et al.* 2021) 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 (Nicotra *et al.* 2010; Christmas *et al.* 2016). 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, (Pickup *et al.* 2012; Kettenring *et al.* 2014) and to plant for forage supply in changing climates in an ecological foundation species (Gibson *et al.* 2016).

Chapter 2 - Local Adaptation, Genetic Divergence, and Experimental Selection in a Foundation Grass across the US Great Plains' Climate Gradient

This chapter aims 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). **This chapter was published in the journal Global Change Biology (Galliart *et al.* 2019)** 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? ***Our rationale is that ecotypes competing against each other should be the most robust test of local adaptation.***

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 with the native ecotype out-performing non-native ecotypes in each ecotype's respective home planting site. 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.

For this chapter we use single ecotype seeded plots to determine percent cover of big bluestem from 2010-2015 across the reciprocal gardens for the three ecotypes. Additionally, to identify plant composition in the mixed ecotype plots we utilized SNP data from genotyping-by-sequencing of plants of known ecotype identity from the single-spaced plants. This data was used to train a random forest model to identify unknown ecotypes in the mixed ecotype community plots. Furthermore, SNP data was used to identify genetic variation and genetic outliers involved with ecotype differentiation.

Chapter 3 - Adaptive genetic potential and plasticity of trait variation in the ecological foundation prairie grass *Andropogon gerardii* across the US Great Plains' climate gradient: Implications for climate change and restoration

The overall goal of this chapter was to evaluate the extent to which *A. gerardii* ecotypes differ across spatially varying climatic regions, thus potentially signaling local specialization, i.e.,

genetic differentiation and adaptation of ecotypes to precipitation. **This was published in the journal Evolutionary Applications (Galliart *et al.* 2020).** We addressed the following questions: 1) Are *A. gerardii* ecotypes locally adapted to environment across the precipitation gradient? 2) What is the relative role of genetic constraints and plasticity in controlling phenotypic differences? 3) How will different ecotypes of *A. gerardii* respond under different climatic conditions, especially precipitation, when planted in home environment and reciprocally transplanted into foreign environments? 4) What are the underlying genetic bases for these traits? 5) How are genotypes and phenotypes structured by climate? ***Our rationale was that ecotypes grown individually without competition should allow for disentangling the relative roles of genotype and environment on plant phenotypes.***

We hypothesized if local adaptation is strongly enforced by precipitation in the dry region, a dry ecotype would outperform non-native ecotypes when planted in the dry end of the gradient, and wet ecotypes would also show a home-site advantage in the wet end of the gradient. For this chapter we utilized the single-spaced plant in monoculture across the reciprocal garden. In addition to planting ecotypes across their home and foreign sites, we also planted all three ecotypes of *A. gerardii* outside its core distribution in the Great Plains into an even drier region of its distribution (Western KS in Colby, KS) to test the extent to which ecotypes might respond to increasingly dry conditions as a surrogate for ecotypic response under future extreme dry conditions. Ultimately, this approach allows us to understand the relative roles of genotype (ecotype) and environment (planting site) on controlling morphology and phenotype and ultimately the roles of local adaptation and phenotypic plasticity in this dominant grass.

Chapter 4 – Ecological and transcriptional responses of wet and dry ecotypes of dominant prairie grass *Andropogon gerardii* across the Great Plains' climate gradient

Reciprocal gardens in the genomic era offer opportunities to link ecology and gene expression differences between wet and dry ecotypes growing reciprocally in wet and dry sites (Carbondale IL vs Hays KS). Transcriptome and ecological profiling of wet and dry ecotypes planted reciprocally allow us to test tolerance of ecotypes and assess the relative role of site and ecotype (genetic background). **This chapter is currently in preparation for submission to Molecular Ecology.** The objectives of this study are to determine to what extent an ecologically-important prairie grass, big bluestem, responds to environmental change, both ecologically and transcriptionally through expression of genes. Do bluestem ecotypes growing across a longitudinal precipitation gradient transcribe a different suite of genes or show differential expression levels of the same genes in response to environment? Is the local ecotype of big bluestem more transcriptionally responsive in its home environment? does an ecologically important grass respond ecologically and at the level of its transcriptome to a drier or wetter environment at the margin of its range? ***Our rationale is that gene expression analysis conducted under realistic conditions involving both abiotic and biotic stressors should provide a mechanistic basis for how A. gerardii responds to stressors.***

We hypothesized that the dry ecotype would show differential expression related to coping with low rainfall and drought in arid Central Kansas. In contrast, wet ecotypes should show differential expression related to carbon gain and optimizing growth in a light-limited, highly competitive shrub- and forb-rich environment typical of Illinois prairies. For purposes of this chapter, we are concerned with the subset of the populations of the dry ecotype (REL, SAL, CDB, WEB) and populations of the wet ecotype (DES, WAL, 12MI, FUL) planted reciprocally into sown community plots in Hays, KS (MAP 500 mm) and Carbondale Illinois (MAP 1200mm). We

chose wet and dry ecotypes and sites to focus on the extremes of the rainfall gradient to carry out the transcriptome and ecological profiling study.

Leaves for extraction of RNA and DNA were collected in the field from wet and dry sites during typical end of season drought and paired with measurements for gas exchange, and longer-term measures of *Andropogon gerardii* biomass and plant cover in ecotype plots in order to link gene expression with ecological responses. This study is an example of the power of reciprocal gardens combined with genomic tools and ecological responses to provide an integrative picture of how plants respond and adapt to the environment.

Overarching approach

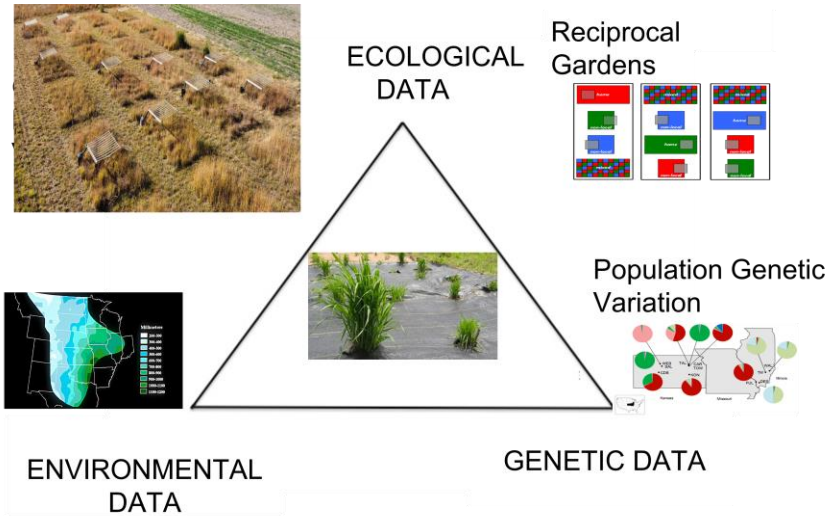

Figure 1.9. Conceptual framework for studying adaptive variation by combining ecological, environmental, and genetic data to understand mechanisms of variation and local adaptation.

This study leverages the integrative approach of ecological genomics which works to understand the genetic mechanisms underlying organismal adaptive responses to environments (Feder & Mitchell-Olds 2003; Ungerer *et al.* 2008). This is accomplished through integration of the fields of ecology, genetics, and climate. Broadly, this research integrates ecological

(morphology and physiological data from plants across a reciprocal garden platform), environmental (garden sites across a broad longitudinal climate gradient), and genetic (genomic and transcriptomic) data to study adaptive variation in A. gerardii (Fig. 1.9). The ultimate goal of this approach is to find the ecologically important genes in this species. That is, to seek to dissect and understand the genetic mechanisms underlying adaptive plant traits and behaviors and the mechanisms responsible for local adaptation in this dominant prairie grass.

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Chapter 2 - Local adaptation, genetic divergence, and experimental selection in a foundation grass across the US Great Plains' climate gradient

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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 (Mimura *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.

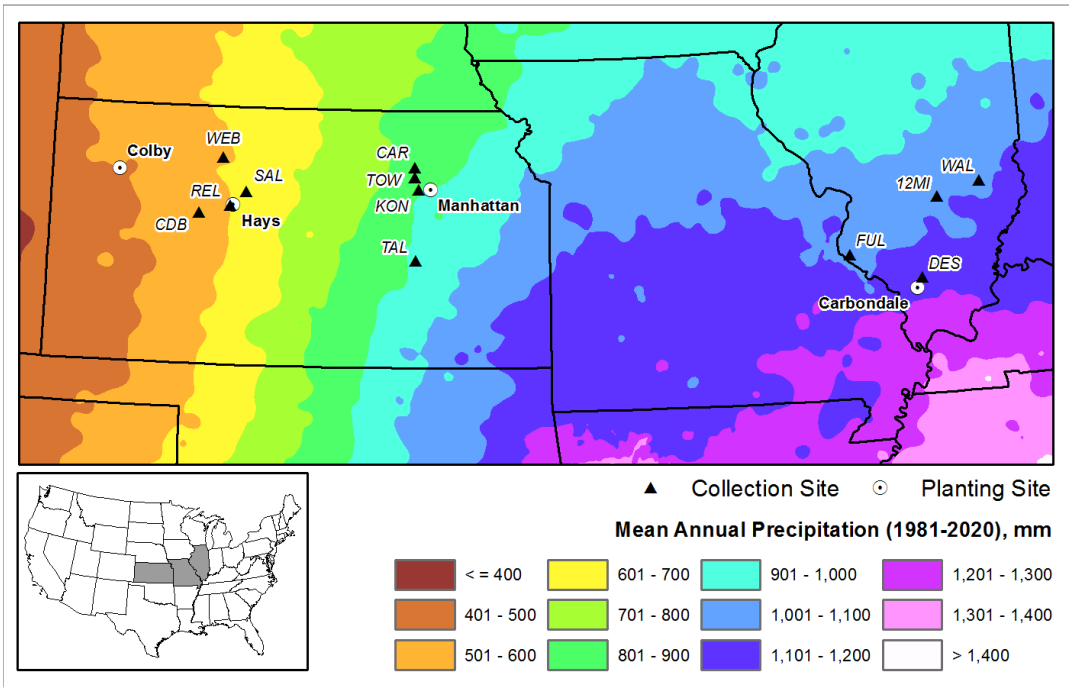

Figure 2.1. Location of reciprocal gardens planting and collections sites across the US Great Plains. White circle is reciprocal garden location. Black triangles are the collection prairie for the seeds. For prairie population acronyms, see Table 2.1. Western Kansas (Colby, Kansas) is the satellite reciprocal site to test the range of tolerance for big bluestem. Note that seeds were not collected in Colby.

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. 2.1, Table 2.1). 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. 2.1, Table 2.1, Fig. 2.2).

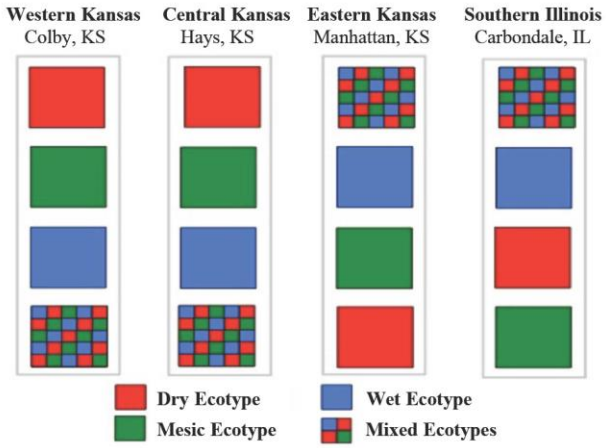

Figure 2.2. Reciprocal garden transplant design for sown community plots. Single colors are single ecotype plots, checkerboard is mixed ecotype plot. At each planting site, there are 4 replicate plots. Ecotype plots at each site were randomized. Note that the Colby planting site had no local ecotype but was included to test the threshold of response to drier locations as might be experienced in the future.

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 2.1); 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. 2.3). 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).

Table 2.1. Historical Weather data (30-year normals) for planting site locations.

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 Riley Co) USDA Plant Materials (Belvue Silt Loam)	315	39.19 96.58	89.1 (61.5-110.2)	21.9	39.16 (1966)	90.5	63.47	-4.2	-4.3	12.8	18.9	23
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

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 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. 2.1, Table 2.1, 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 2.1), all located at agricultural research stations. Rainfall (annual and growing season) for the years of the experiment

in Table 2.1 and Fig. 2.3 We used nearby NOAA weather stations for historical data on climate of source populations (STable 1).

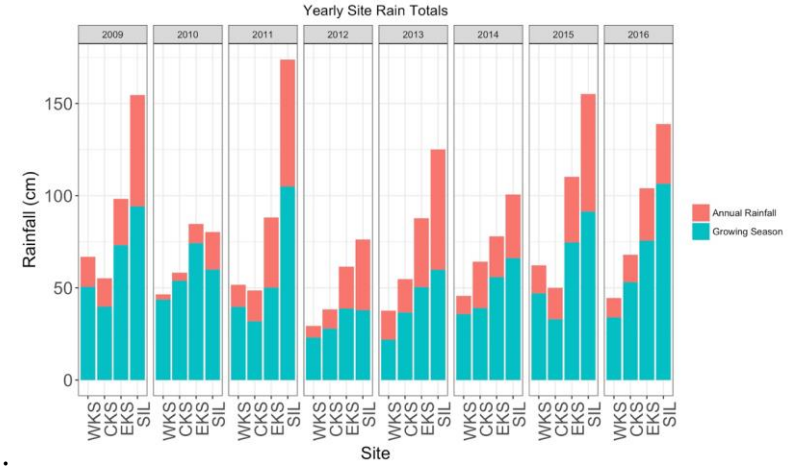

Figure 2.3. Chart showing the yearly rainfall totals for our four planting sites (WKS = Western Kansas (Colby, KS), CKS = Central Kansas (Hays, KS), EKS = Eastern Kansas (Manhattan, KS), SIL = Southern Illinois (Carbondale, IL) across the Great Plains within all years since the plots were established in 2009. Additionally, denoted by the blue bars is the total rainfall during the growing season of big bluestem. This was calculating by summing the amount of precipitation measured in the spring and in the summer. It is during this time, big bluestem conducts most of its growth.

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).

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 2.1) 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, 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

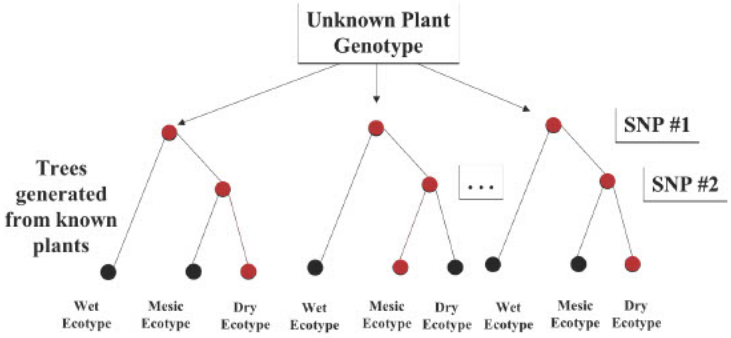

Figure 2.4. Conceptual diagram for random forest methodology. Genotypes of unknown plants are used in conjunction with the training random forest. Each node indicates a single loci where it is decided if the individual has one or the other allele. This process continues through additional SNPs until the individual is characterized and placed into one of our three bins (dry, mesic, or wet)

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 (Fig. 2.4 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 (Fig. 2.4). 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 individuals from mixed plots across two years assesses annual variation in growth and composition within long-term plots.

RESULTS

Ecotypes Locally Adapted to Regional Climate in Realistic Ecological Communities

When comparing ecotype differences by each garden site using a local vs foreign ecotype comparison, (i.e., how an ecotype from that locality performs compared to foreign ecotypes planted in the site), there was evidence of significant cover differences among ecotypes within a site. In the Western Kansas reciprocal garden site (Colby, Kansas, (Table 2.1, Fig. 2.5). In the driest site, the dry ecotype cover (~20-40%) was significantly greater ($p < 0.046$) than the wet

ecotype (~5%), and in all years the dry ecotype was greater than mesic (~10-25%) but not significantly different. A similar pattern was observed in the Central Kansas reciprocal garden (Hays, Kansas) the next driest site, where in 5 out of 6 years, the dry ecotype cover (~25-40%) was significantly greater ($p < 0.039$) than the wet ecotype (~5%). In all years at the Central Kansas reciprocal garden (Hays, Kansas), the dry ecotype was greater than the mesic ecotype (~15-25%) but not significantly different. Interestingly, in the Eastern Kansas reciprocal garden (Manhattan Kansas), there were no significant differences among ecotypes across all years and cover ranged from 20-35%, regardless of ecotype. In the Southern Illinois reciprocal garden (Carbondale, Illinois), the wettest site, there were no significant differences among ecotypes during the first two establishment years and all ecotypes maintained relatively low levels of cover (<10%). From 2012 onward, the dry ecotype continued to show significantly lower ($p < 0.018$) cover (<10%) compared to the wet (25-40%) ecotype, but mesic (15-30%) and wet ecotypes (25-40%) were not significantly different from each other.

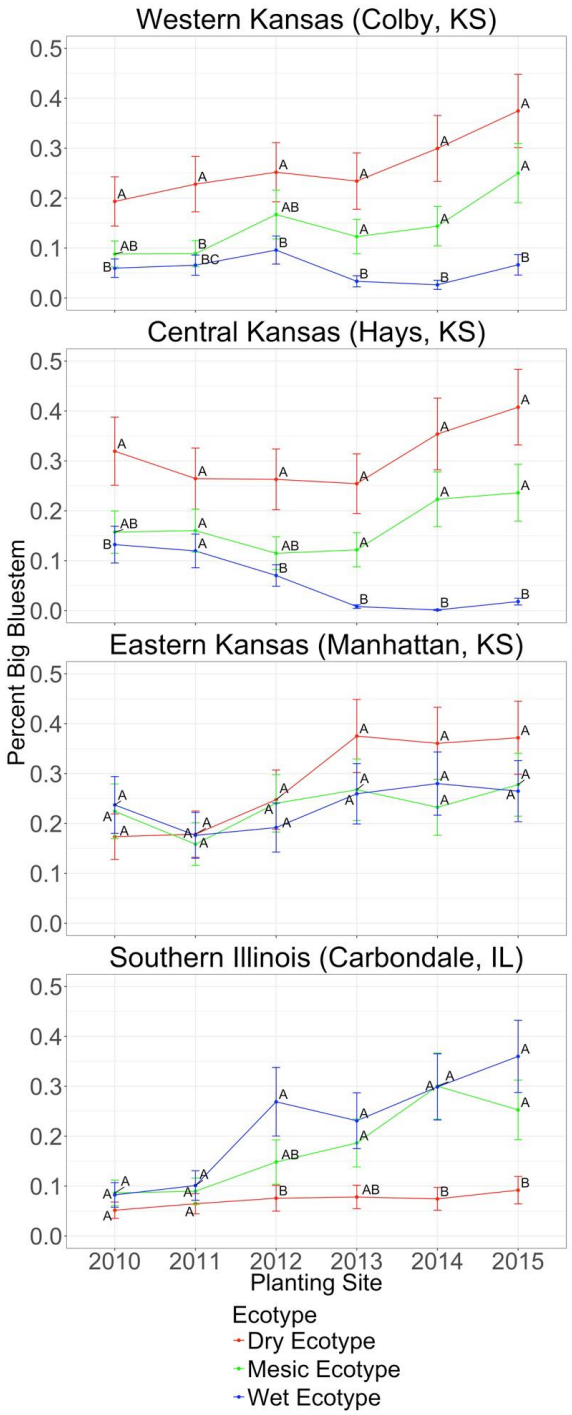

Figure 2.5. Vegetative cover (least square mean estimates with standard errors) by planting sites (Western Kansas (Colby, Kansas), Central Kansas (Hays, Kansas), Eastern Kansas (Manhattan, Kansas) and Southern Illinois (Carbondale, Illinois) for each ecotype in the single ecotype plots from years 2010-2015 across the Great Plains precipitation gradient. Letters indicate significant differences within years.

Based on the same data, ecotypes showed signs of local adaptation when planted in their home site compared to their away site (Table 2.1, Fig. 2.6). In all years, the dry ecotype (Fig. 2.6) had significantly lower cover (cover <10% $p < 0.032$) than other ecotypes when planted in the Southern Illinois reciprocal garden (Carbondale, Illinois, wettest site). For the wet ecotype (Fig. 2.6), in the first two years there were no significant differences between the reciprocal gardens in western Kansas (Colby, Kansas), Central Kansas (Hays, Kansas) and Southern Illinois (Carbondale, Illinois), that is driest, dry, and wettest, respectively (cover 10-20%) but was significantly higher in Eastern Kansas (Manhattan Kansas) in 2010 ($p < 0.041$). Following the establishment years, from 2013 onward, the wet ecotype had significantly increased cover (~25-40%) in Eastern Kansas (Manhattan, Kansas) and Southern Illinois (Carbondale, Illinois ($p < 0.049$) but lower in western (Colby, Kansas) and Central Kansas (Hays, Kansas) sites, where the cover of the wet ecotype was reduced to about 5% cover ($p< 0.003$). Interestingly, across all years, there were no significant cover differences in the mesic ecotype among all four planting sites (Fig. 2.6).

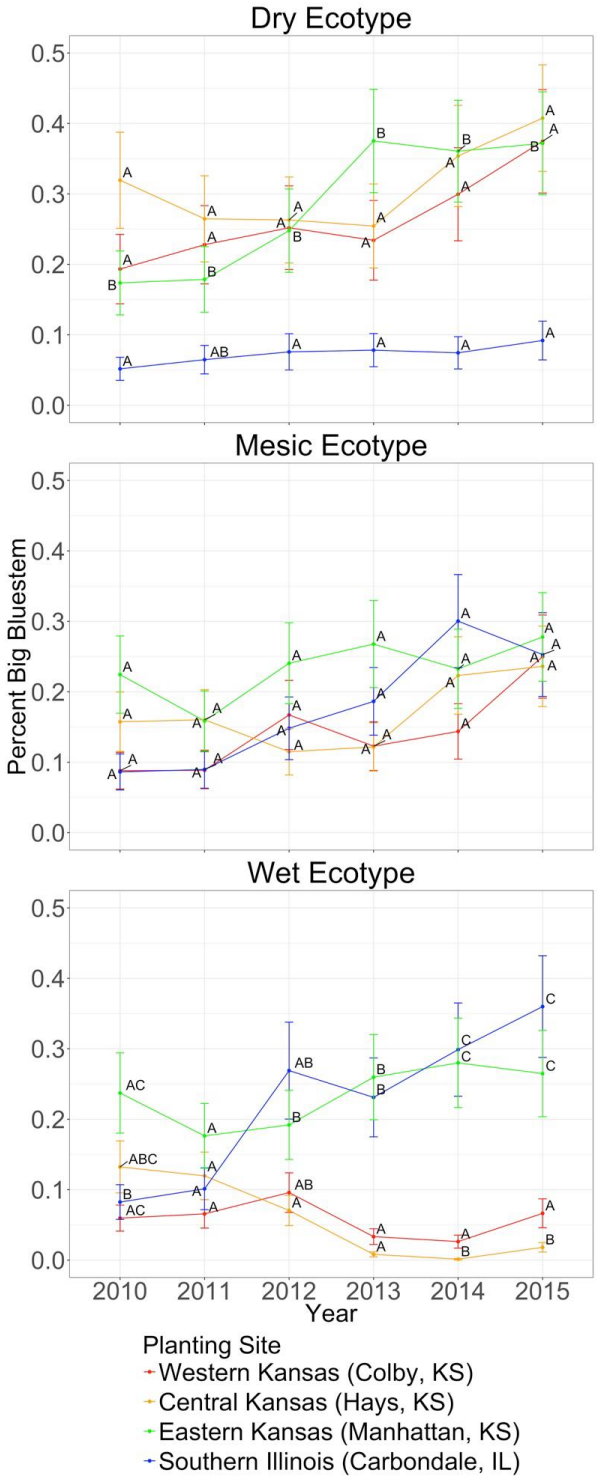

Figure 2.6. Vegetative cover (least square mean estimates with standard errors) by each ecotype in the single ecotype plots at planting sites from years 2010-2015 across the Great Plains precipitation gradient. Red=western KS, Orange=central KS, Green= Eastern KS, Blue = Southern Illinois. Letters indicate significant differences within a year.

Regressions of cover by ecotype vs annual rainfall for combined years of 2014 and 2015, the latest measurement years presumably when the vegetation was stabilized, showed that the dry ecotype had highest cover with low rainfall, and decline in cover with increased rainfall as occurs in the wettest site of Southern Illinois (Carbondale, Illinois, $p = 0.05$, $R^2 = 0.50$) (Fig. 2.7). The wet ecotype showed the opposite pattern with low cover in Western and Central Kansas and increase in cover with precipitation as occurs in Southern Illinois (Carbondale, Illinois, $p = 0.007$, $R^2=0.73$). Interestingly, cover of the mesic ecotype was only weakly related to rainfall ($p = 0.26$, $R^2 = 0.21$, data not shown). This clearly shows cover of dry and wet ecotypes is related to rainfall and corroborates their delineation. There were no significant correlations with other variables (data not shown).

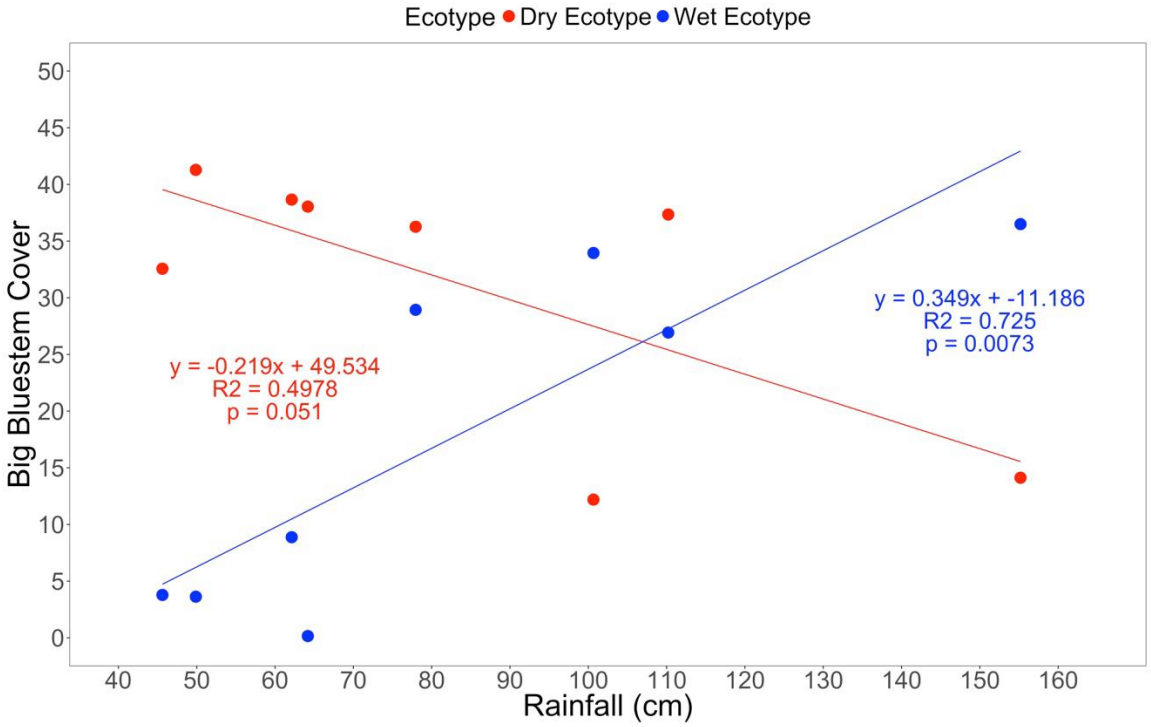

Figure 2.7. Percent big bluestem dry (red) and wet (blue) ecotype cover versus the annual rainfall in the corresponding planting locations 2014 and 2015 combined.

Genetic Divergence Among Ecotypes Supports Phenotype Differences

Divergence and Diversity, Relation to Climate vs Geography

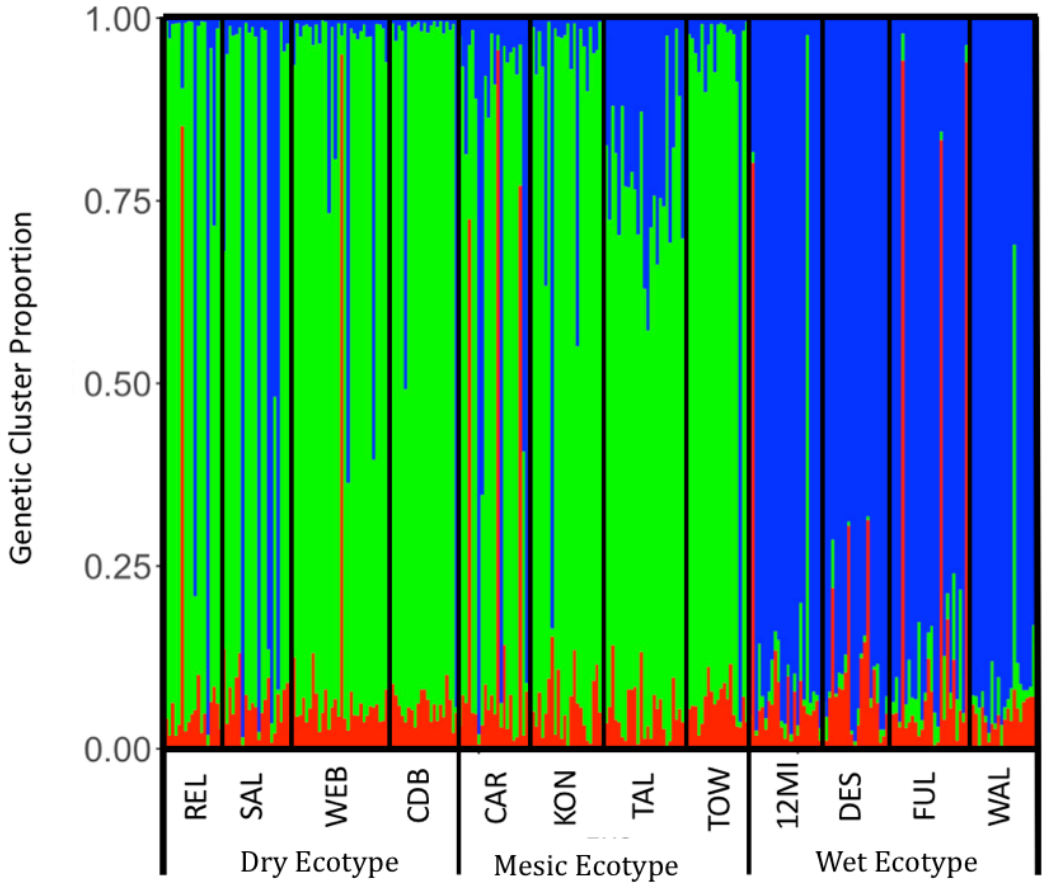

Figure 2.8. STRUCTURE bar plot labeled by regional ecotype and by prairie. The most likely genetic grouping solution, K = 3, is shown. Each color indicates one genetic group, and each bar represents percentage membership to genetic group(s). Mixed membership indicates admixture.

Structure results indicate K=3 genetic clusters with two predominating, one occurring in dry and mesic ecotypes and the other in wet ecotype (Fig. 2.8). Based on pairwise F_{st} (STable 3), only slight neutral differentiation was observed between populations with F'_{st} (Meirmans *et al.* 2011) of .028. In general, the wet ecotype showed greatest genetic distance with populations from

Kansas with F_{st} as high as 0.037. Populations from the dry and mesic ecotypes show lower genetic distance as one might expect from geographic proximity, with F_{st} between 0.011-0.016.

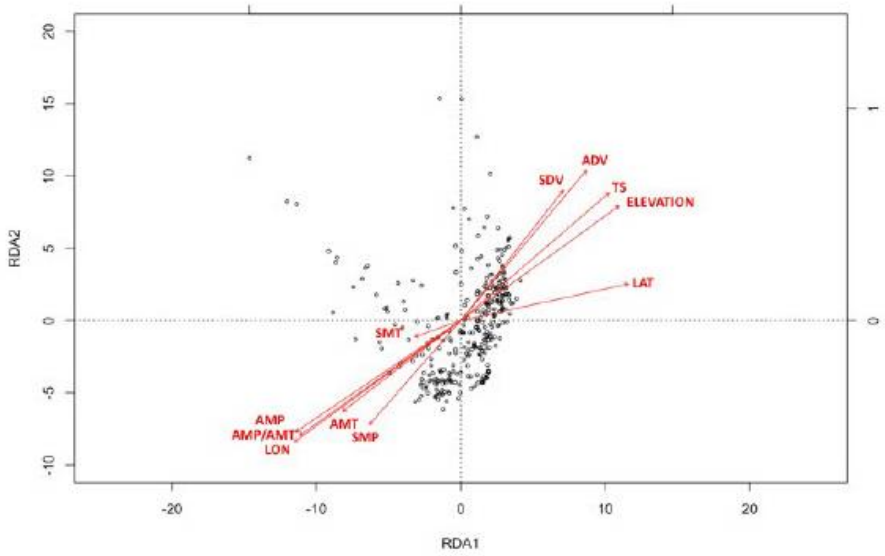

Figure 2.9. Bi-plot of the full RDA model showing points as individuals and loadings of climate variables and geography (latitude and longitude). For data see Table 2.1. AMP=mean annual precipitation, SMP=season mean precipitation, SMT=seasonal mean temperature, SDV=seasonal diurnal variation, ADV=annual diurnal variation, TS=temperature severity, AMP/AMT=aridity index, Lat=latitude, Long=longitude

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) (Fig. 2.9) 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, Fig. 2.10).

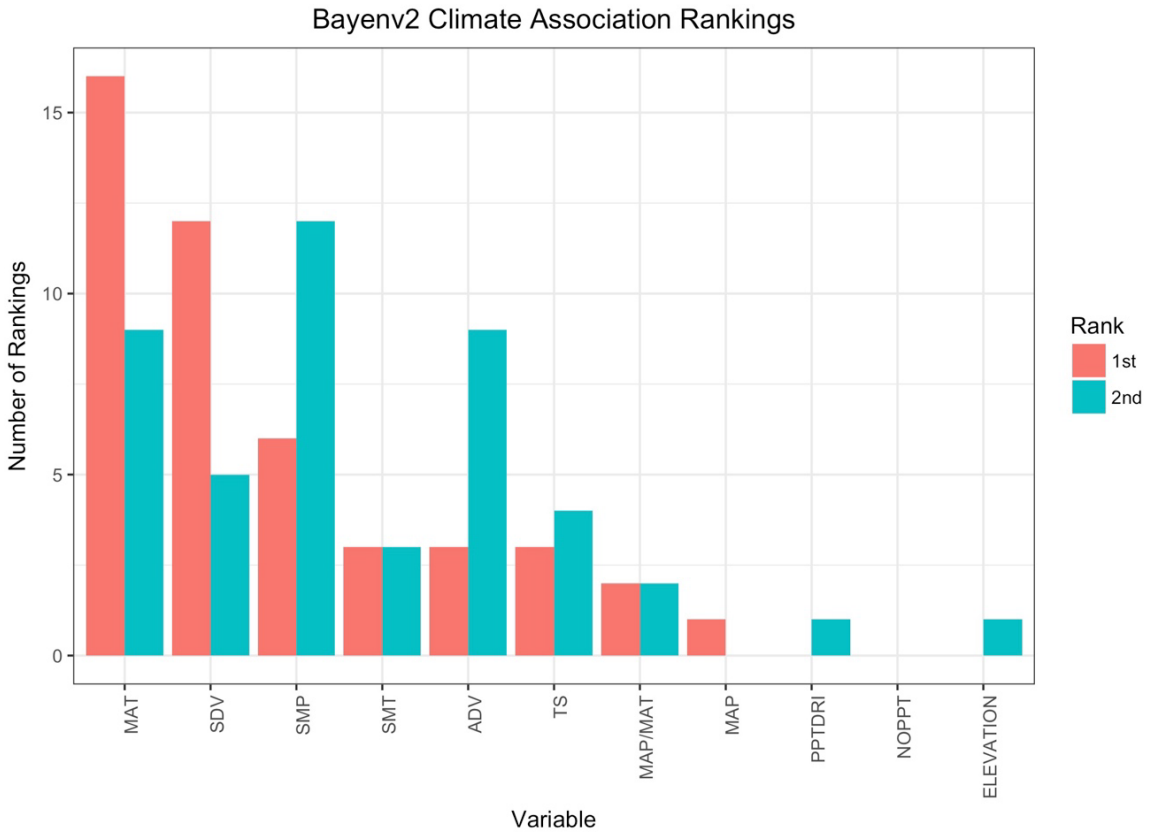

Figure 2.10. Number of SNPs significantly associated to climate variables using Bayenv2. MAT=mean annual temperature, SDV=seasonal diurnal variation, SMP=season mean precipitation, SMT=seasonal mean temperature, ADV=annual diurnal variation, TS=temperature severity, MAP/MAT=aridity index, MAP=mean annual precipitation, PPTDRI=precipitation of the driest year in last 30 years, NOPPT=number of precipitation events > 1.25 cm. Only the top two rankings were included in the figure.

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, Fig 2.11).

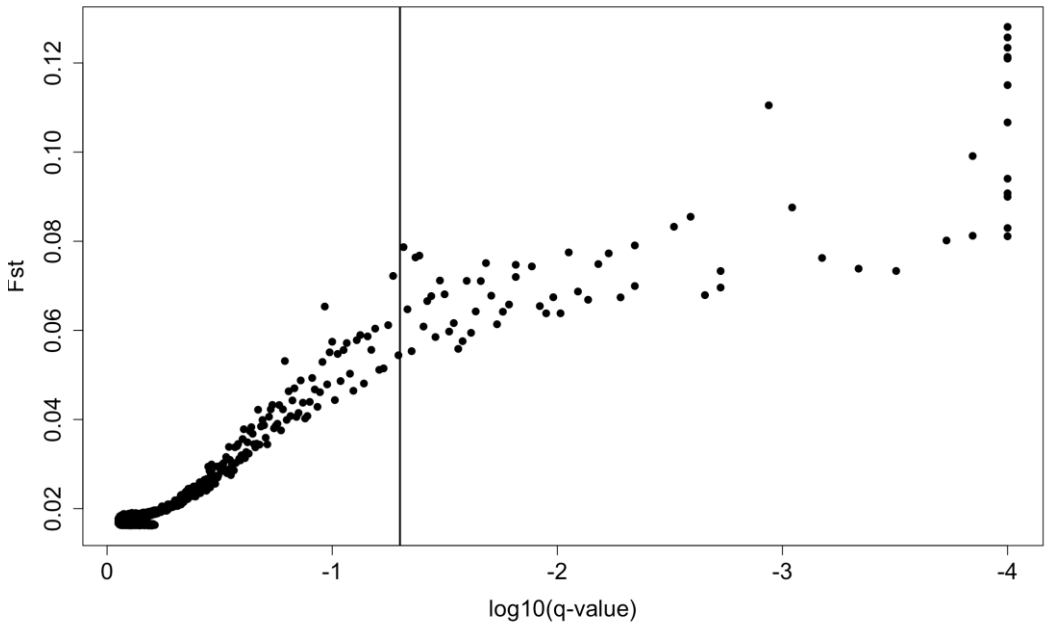

Figure 2.11. Plot showing the Fst and significance of markers as analyzed in Bayescan v2.1. Points to the right of the line indicates 64 markers with significant evidence of being under divergent selection having a q-value (p-value adjusted for FDR) lower than 0.05. The red circle indicates the significant SNPs.

A SNP outlier near a gene of interest and identified in both *BayeScan* v2.1 and *Bayenv2* was GA1 and ranked as 14th 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. 2.13). GA1 was also identified in GWAS analyses using TASSEL, Galliart unpublished) and associated with height (Galliart *et al.* unpublished data).

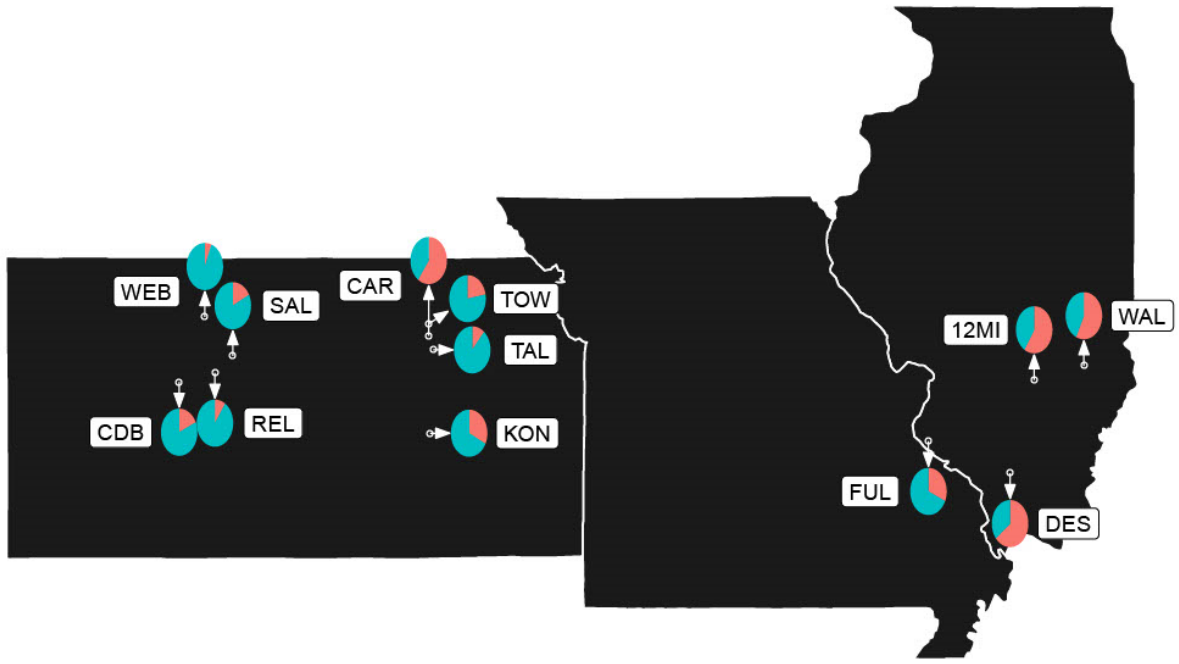

Figure 2.12. Map indicating the allele frequencies for the GA1 outlier across the 12 populations focusing on the gradient in alleles across the climate gradient from Western Kansas to Southern Illinois. "Short" allele is in blue, alternative "tall" allele is in red.

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 Fig. 2.13. Qualitatively, the training/validation triangle indicates excellent identification of wet ecotype individuals with somewhat less, but still good, discernment between dry and mesic ecotypes.

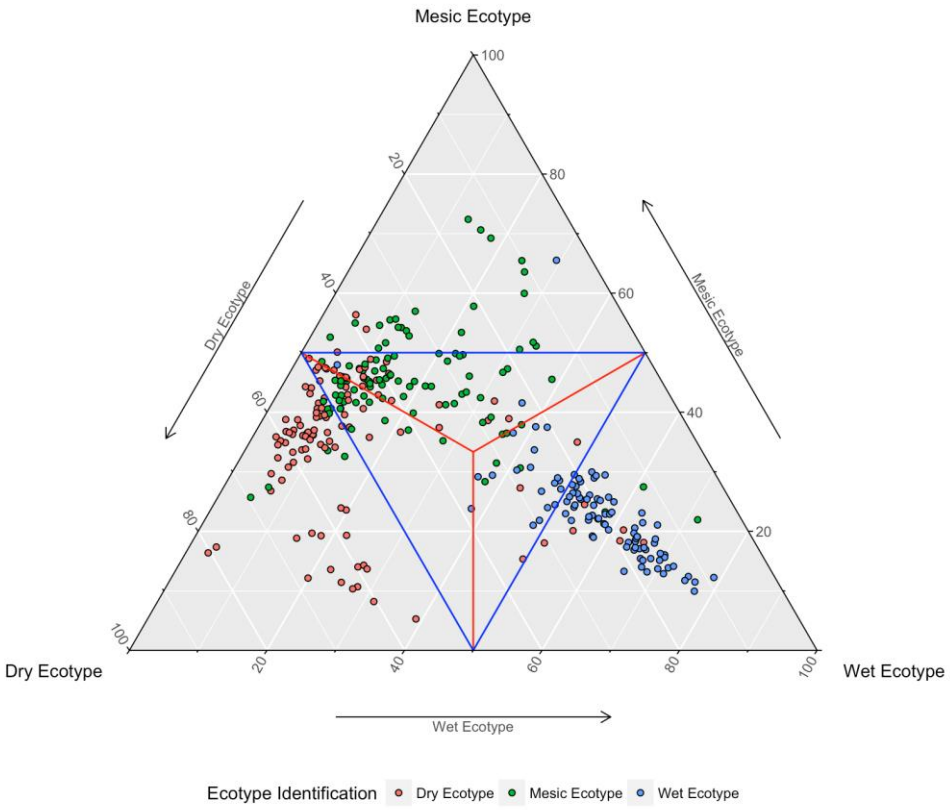

Figure 2.13. Plot showing training/validation triangle with percent votes of the individuals from the 10 fold-cross validation from random forest training and validation set. Dry ecotype denoted in red, mesic ecotype denoted in green, and wet ecotype denoted in blue. Individuals within the blue lines indicate individuals with poor discernment. Plants falling outside of the blue line are clearly discerned; that is, more than 50% of votes from the validation were for that ecotype. Plants that fall within the blue and red lines were predominantly assigned to an ecotype but had less than 50% of the votes for that ecotype.

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

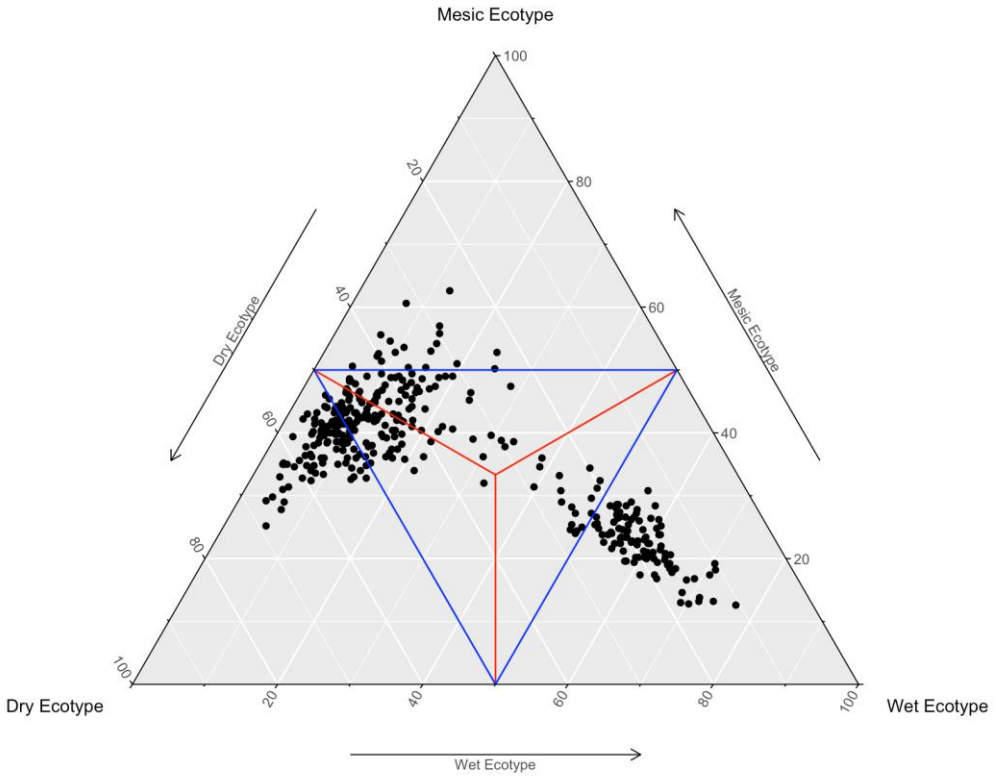

Figure 2.14. Plot showing mixed ecotype plot predictions based on percent votes of the individuals from mixed ecotype plots in 2014. Individuals within the blue lines indicate individuals with poor discernment. Plants falling outside of the blue line are clearly discerned; that is, more than 50% of votes from the validation were for that ecotype. Plants that fall within the blue and red lines were predominantly assigned to an ecotype but had less than 50% of the votes for that ecotype

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 (STable 9, Fig 2.15). 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. 2.16).

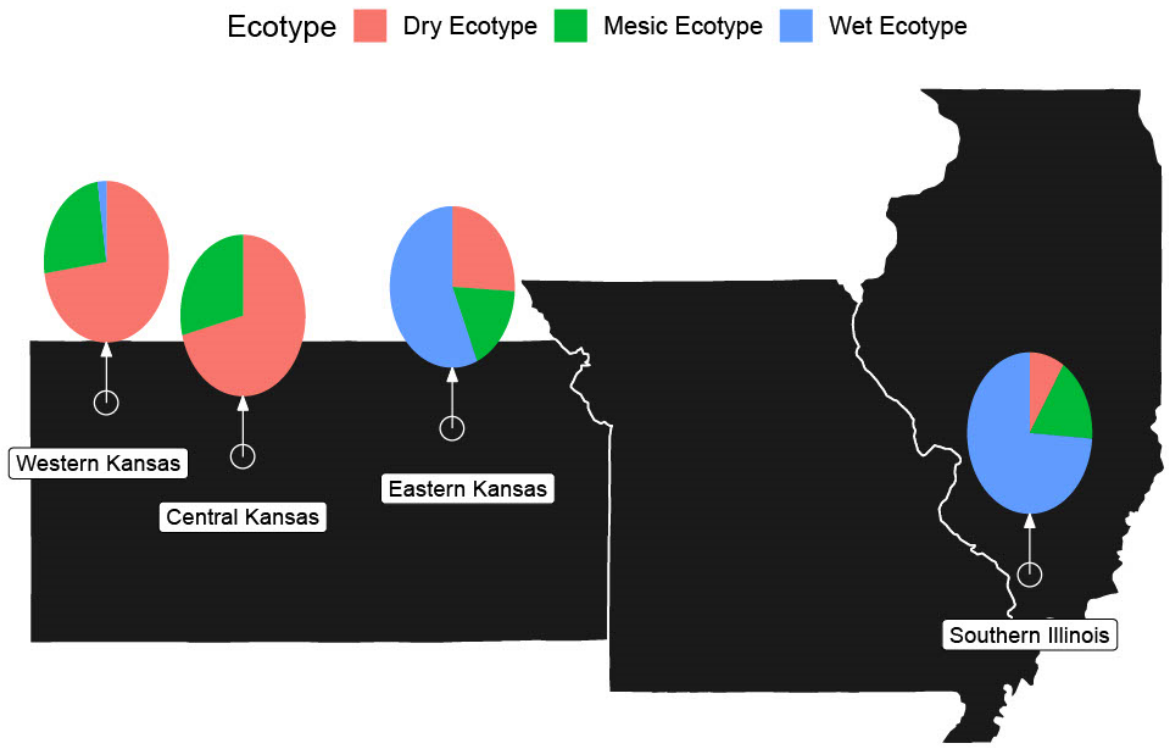

Figure 2.15. Map showing the predicted ecotype composition of mixed ecotype plots across the reciprocal gardens in 2014. Dry ecotype denoted in red, Mesic ecotype denoted in green, and Wet ecotype denoted in blue.

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.

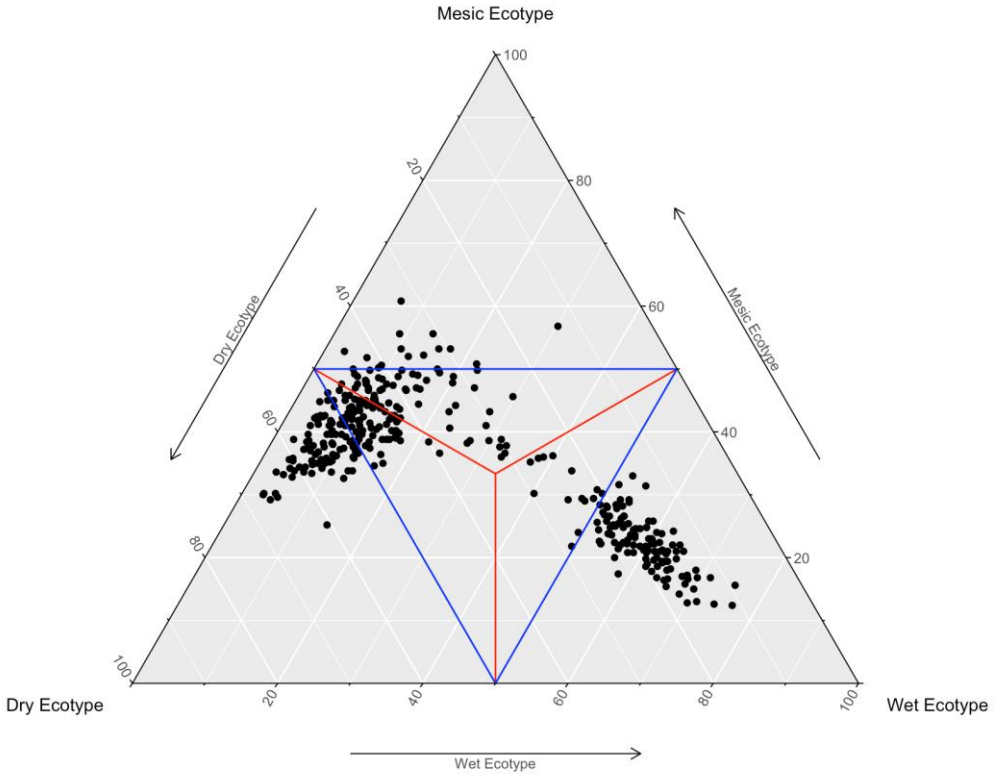

Figure 2.16. Plot showing mixed ecotype plot predictions based on percent votes of the individuals from mixed ecotype plots in 2015. Individuals within the blue lines indicate individuals with poor discernment. Plants falling outside of the blue line are clearly discerned; that is, more than 50% of votes from the validation were for that ecotype. Plants that fall within the blue and red lines were predominantly assigned to an ecotype but had less than 50% of the votes for that ecotype.

A similar pattern of ecotype composition was observed in 2015 (Figs. 2.16, 2.17, 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.

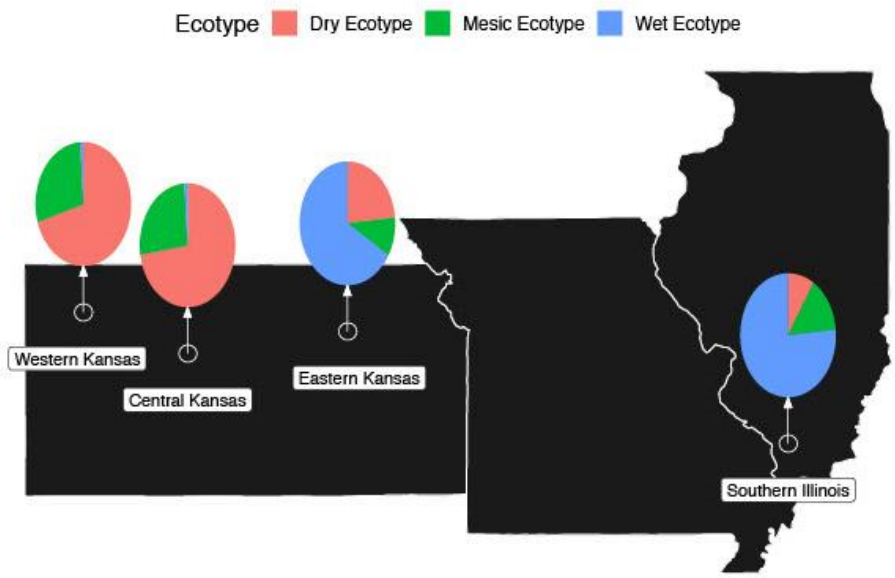

Figure 2.17. Map showing the predicted ecotype composition of mixed ecotype plots across the reciprocal gardens in 2015. Dry ecotype denoted in red, mesic ecotype denoted in green, and wet ecotype denoted in blue.

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. 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 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, 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 (Mimura *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. 2.8). We have also shown that environmental factors, especially precipitation, explain more of genetic differences than does geographic location (Fig. 2.9, STable 4).

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

Outlier SNPs identified in *Bayenv* showed a clear relationship with climate and associated with temperature and precipitation variables (STable 6). Of the top 1% of outliers (46), 16 had a significant association with annual mean temperature, 12 associated with seasonal diurnal temperature variation, and 6 associated with growing season mean precipitation. Our study takes similar approaches using outlier candidate genes across gradients, i.e., genome-environmental associations as highlighted in recent excellent reviews. For example, Bragg *et al.* (2015) further expanded on landscape genomics in non-model systems, especially foundation ecological species; Rellstab *et al.* (2015) suggested a practical guide to studying the role of environment in identifying adaptive loci; Sork *et al.* (2016) showed the importance of identifying underlying candidate genes

for phenotypes under climate selection with oaks as the focal species. Laskey *et al.* (2018) suggest approaches to synthesize evidence from common gardens and genome-environmental associations. Recent empirical studies have addressed various genome-environmental associations. *Arabidopsis halleri* showed genomic footprints of selection to 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 in Eastern Kansas, where all ecotypes performed equally well. Further, the wet ecotype outcompeted the mesic ecotype in the mixed plots located in Eastern Kansas. The years of mixed ecotype plot collection had normal precipitation, so it is doubtful precipitation played a role. Furthermore, this result was not due to lack of random forest discernment, as the wet ecotype is easily distinguished from other ecotypes, and makes up the majority of the ecotype identified in Eastern KS and Southern Illinois. So why did the mesic ecotype do comparatively poorly in its home environment of Eastern Kansas, being outperformed by the wet ecotype? The wet ecotype appears to be more competitive than the mesic ecotype in Eastern Kansas when the ecotypes were planted together in the mixed ecotype plot compared to single ecotype plots. That is, the wet ecotype wins inter-ecotype competition (between wet and mesic ecotypes) in the mixed ecotype plots, but when grown among other wet ecotype plants in single ecotype plots, intra-ecotype competition is stronger, resulting in overall low cover of wet ecotypes in single ecotype plots. The wet ecotype putatively outcompetes the mesic ecotype in Eastern Kansas because it is more

vigorous due to its tall, robust stature (~3 times taller, ~2 times more biomass), thus suppressing the shorter stature mesic ecotype, resulting in greater dominance of the wet ecotype in Eastern Kansas. These results highlight the increased strength of biotic factors, especially between-ecotype competition in the expression of local adaptation at the wetter end of the gradient. At the dry end of the gradient, abiotic factors such as low precipitation are selective pressures in local adaptation and the dry ecotype dominates in single and mixed ecotype plots.

Our results corroborate other studies (reviewed in Franks *et al.* 2014) showing selection over time. Several studies show selection-induced treatment effects on phenotypes in intact communities. The Buxton grassland studies of climate change treatments imposed over 15 years shows adaptive selection and differentiation of phenotypes of species (Fridley *et al.* 2010), and outliers sorting of genotypes (Ravenscroft *et al.* 2015) among treatments plots. Avolio & Smith (2013) studied changes in phenotype in response to rainfall manipulation in intact grassland and found *A. gerardii* phenotypic variation but no adaptive response to drought. Resurrection studies in which phenotypes and genotypes from historical seed are compared with contemporary progeny (Franks *et al.* 2018) have shown evidence for contemporary evolution. Franks *et al.* (2016) showed rapid genome evolution in response to drought in *Brassica rapa*. Nevo *et al.* (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. 2.7) 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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Chapter 2 Supplemental Tables

Ecotype, Region Elevation (m)	Prairie Name (abbrev, size ha)	Lat N Long W	County, State (Weather Station)	Number of Pcp Events >1.25 cm	Precip. Driest Year (cm)	Mean Annual rainfall (AMP) (cm)	Seasonal Mean Rainfall (SMP) (cm)	Annual Diurnal Temp (ADV) (°C)	Seasonal Diurnal Temp (SDV) (°C)	Annual Mean Temp (AMT) (°C)	Seasonal Mean Temp (°C)	Temp Severity Index (TS)	MAP (cm)/ MAT (°C)
Dry CKS (606m)	Webster Reservoir (WEB, 356)	39.41 99.50	Rooks, KS (Webster Dam)	17	25.96	58.70	39.35	15	15.4	12.4	19.8	0.100	4.72
Dry CKS (641)	Saline Experimental Range (SAL, 880)	39.02 99.14	Ellis, KS (Plainville)	16	36.32	61.7	31.0	14.6	15.5	11.8	20.7	.088	5.22
Dry CKS (688	Cedar Bluffs Reservoir (CDB, 850	38.76 99.83	Trego, KS (Cedar Bluffs Dam)	16	32.18	53.31	35.97	14.4	14.5	11.2	19.5	.091	4.72
Dry CKS 640	Relict Prairie (REL, 14)	38.85 99.37	Ellis, KS (Hays 1S)	16	32.61	58.0	37.7	14.6	15.5	12.0	20.6	.088	4.82
Mesic EKS 366	Konza Prairie KON, 1557	39.08 96.56	Riley/Geary, KS (Manhattan 6SW)	22	68.89	88.47	56.54	12.8	12.4	12.8	21.0	.048	6.92
Mesic EKS 392	Tallgrass National Park TAL, 4409	38.25 96.60	Chase, KS (Tallgrass Nat Park)	21	59.77	82.82	49.19	12.7	12.2	12.7	20.8	.066	6.54
Mesic EKS 389	Carnahan Cove CAR, 99	39.34 96.62	Pottawatomie, KS (Wamego)	23	52.35	87.20	53.34	13.0	13.1	13.0	21.4	.057	6.72
Mesic EKS 379	Top of the World Park TOW, 61	39.22 96.62	Riley, KS (Tuttle Dam)	21	45.11	81.12	50.70	13.1	13.2	11.7	20.3	.057	6.95
Wet SIL 119	Desoto Prairie DES, 0.4	37.85 89.14	Jackson, IL (Carbondale, Il)	33	67.41	115.92	53.53	12.3	12.6	13.2	21.1	.027	8.78
Wet SIL	Twelve Mile Prairie	38.78 88.83	Effingham, IL, (Monroe, Fayette,	25	70.01	107.57	51.83	11.7	12.4	12.3	18.7	.026	8.72

160	12MI, 28		Salem)										
Wet SIL 150	Walters	38.92 88.19	Jasper, IL (Newton/Charlesto)	27	69.18	104.04	50.80	10.8	11.7	13.4	21.8	.014	8.11
Wet SIL 215	Fults	38.17 90.19	Monroe, IL (Sparta)	31	69.38	111.27	55.14	11.9	12.6	13.2	21.0	.031	8.38

STable 1. Weather data for the source populations. Temperature severity index is number of days over 95F/total number of days. Number of precipitation events >1.25 cm per year. Soils were mostly loam as follows: Wakeeney-Harney Silt loam (WEB), Bogue-Armo Clay loam (SAL) ArmoClay loam (CDB) Armo loam and Brownell gravelly loam (REL), Benfield Florence silty clay loam (KON), Cline Sogen Silty clay loam (TAL), Benfield Florence silty clay loam (CAR), Irwin silty clay loam (TOW), Orthents silty loam (DES), Cisne silty loam (12MI), Atlas silty clay loam (WAL), Menfro silty clay loam (FUL).

Effect	Degrees of Freedom (Numerator)	Degrees of Freedom (Denominator)	F value	Pr > F
Planting Site	3	12	9.76	.0015
Ecotype	2	24	16.70	<.0001
Planting Site*Ecotype	6	24	14.43	<.0001
Year	5	176.9	8.34	<.0001
Planting Site*Year	15	164.8	7.65	<.0001
Ecotype*Year	10	167.9	5.76	<.0001
Ecotype*Planting Site*Year	30	156.5	3.37	<.0001

STable 2. Statistical analyses of the fixed effects of planting site, ecotype, and year and all possible two-way and three-way interactions on the response variable of big bluestem cover.

12MI (Wet)	DES (Wet)	FUL (Wet)	WAL (Wet)	KON (Mesic)	TAL (Mesic)	TOW (Mesic)	CAR (Mesic)	CDB (Dry)	REL (Dry)	SAL (Dry)	WEB (Dry)	
0.000												**12MI**
0.023	0.000											**DES**
0.020	0.022	0.000										**FUL**
0.018	0.021	0.020	0.000									**WAL**
0.029	0.034	0.026	0.028	0.000								**KON**
0.026	0.029	0.023	0.025	0.014	0.000							**TAL**
0.032	0.035	0.028	0.030	0.011	0.014	0.000						**TOW**
0.024	0.028	0.021	0.022	0.013	0.014	0.013	0.000					**CAR**
0.033	0.035	0.029	0.032	0.012	0.017	0.012	0.014	0.000				**CDB**
0.030	0.033	0.027	0.028	0.012	0.016	0.011	0.013	0.012	0.000			**REL**
0.024	0.028	0.021	0.023	0.012	0.014	0.012	0.011	0.013	0.011	0.000		**SAL**
0.033	0.037	0.029	0.033	0.012	0.016	0.011	0.014	0.011	0.012	0.013	0.000	**WEB**

STable 3. Pairwise F_{st} among pairs of populations.

Variation explained	**Model 1 (Full)** Joint Effect of Geography and Climate	**Model 2** Climate Conditioned by Geography	**Model 3** Geography conditioned by Climate
Constrained (total explained)	863	642 (74%)	129 (15%)
Total joint explained	771 (89%)		
Total unexplained	92 (11%)		
Total variation	18710	18710	18710

STable 4. RDA results for 9 climate variables (Elevation, Mean Annual Precipitation, Seasonal Mean Precipitation, Annual Diurnal Variation, Seasonal Diurnal Variation, Mean Annual Temperature, Temperature Severity Index, and Mean Annual Precipitation/Mean Annual Temperature) and geographic distance (Latitude and Longitude). Table shows the partitioning of variation between the full RDA (model 1) including the joint effects of both geography and climate, the partial RDA model 2 including the effects of climate conditioned by geography and the partial RDA model 3 including the effects of geography conditioned by climate.

Analysis	Markers	Chrom.	Position	Gene Id	Gene Information
Bayenv2	TP171997	7	11673324	Sb07g007530.1	PF01263 PTHR11122 KOG1594 AT5G57330.1 aldose 1-epimerase family protein
Bayenv2	TP4696	1	73079404	Sb01g050120.1	PTHR10343 AT3G52180.1 SEX4 SEX4 (STARCH-EXCESS 4); polysaccharide binding / protein tyrosine/serine/threonine phosphatase
Bayenv2	TP103404	3	52366325	Sb03g026040.1	PF00160 PTHR11071 KOG0885 5.2.1.8 K01802 AT4G33060.1 peptidyl-prolyl cis-trans isomerase cyclophilin-type family protein
Bayenv2	TP16994	9	51311839	Sb09g021780.1	PF02365 AT5G22380.1 anac090 anac090 (Arabidopsis NAC domain containing protein 90); transcription factor
Bayenv2	TP23740	2	67684318	Sb02g033050.1	AT5G42330.1 unknown protein
Bayenv2	TP63473	4	57503730	Sb04g027590.1	PF00191 PTHR10502 KOG0819 AT5G10230.1 ANNAT7 ANNAT7 (ANNEXIN ARABIDOPSIS 7); calcium ion binding / calcium-dependent phospholipid binding
Bayenv2	TP107489	1	47278328	Sb01g027420.1	PF05770 AT4G33770.1 inositol 1,3,4-trisphosphate 5/6-kinase family protein
Bayenv2	TP68168	9	188110	Sb09g000390.1	PF00023,PF07719,PF00515 PTHR18958 KOG0548 AT3G04710.1 ankyrin repeat family protein
Consensus	TP97659	1	26916072	Sb01g021990.1	PF01397,PF03936 5.5.1.13 K04120 AT4G02780.1 GA1 GA1 (GA REQUIRING 1); ent-copalyl diphosphate synthase/ magnesium ion binding
Consensus	TP22546	3	10907551	Sb03g010110.1	PF00097 AT1G75400.1 protein binding / zinc ion binding
Consensus	TP48670	7	18950899	Sb07g010450.1	PF00141 1.11.1.7 K00430 AT4G16270.1 peroxidase 40 (PER40) (P40)
Consensus	TP14132	3	59665754	Sb03g031310.1	PF00310,PF04898,PF01645,PF01493,PF07992,PF00070 PTHR11938 KOG0399 1.4.1.13 K00265 AT5G53460.1 GLT1 GLT1; glutamate synthase (NADH)
Bayescan	TP3814	2	56601981	Sb02g023140.1	PF07732,PF00394,PF07731 PTHR11709 KOG1263 AT4G39830.1 L-ascorbate oxidase, putative
Bayescan	TP23759	2	53236055	Sb02g021490.1	PF03055 PTHR10543 KOG1285 AT3G63520.1 CCD1 CCD1 (CAROTENOID CLEAVAGE DIOXYGENASE 1); 9-cis-epoxycarotenoid dioxygenase
Bayescan	TP27974	6	39909469	Sb06g014410.1	PF02416 AT5G52440.1 HCF106 HCF106; proton motive force dependent protein transmembrane transporter

Bayescan	TP63759	1	55492323	Sb01g032580.1	PF03195 AT1G67100.1 LBD40 LBD40 (LOB DOMAIN-CONTAINING PROTEIN 40)
Bayescan	TP69704	10	42173347	Sb10g019720.1	PF02453 PTHR10994 KOG1792 AT4G11220.1 BTI2 BTI2 (VIRB2-INTERACTING PROTEIN 2)
Bayescan	TP70880	9	47608900	Sb09g019060.1	PF02182,PF05033,PF00856 PTHR22884 KOG1082 AT5G13960.1 SUVH4 SUVH4 (SU(VAR)3-9 HOMOLOG 4); double-stranded methylated DNA binding / histone methyltransferase(H3-K9 specific) / methyl-CpG binding / methyl-CpNpG binding / methyl-CpNpN binding
Bayescan	TP79982	3	71975298	Sb03g044630.1	PF02362,PF06507 AT5G62000.1 ARF2 ARF2 (AUXIN RESPONSE FACTOR 2); protein binding / transcription factor
Bayescan	TP90370	3	6472172	Sb03g006365.1	PTHR23273 KOG0851 AT4G19130.1 DNA binding / nucleic acid binding / zinc ion binding
Bayescan	TP100780	5	5098115	Sb05g004185.1	PF03140 AT3G44710.1 unknown protein
Bayescan	TP105473	2	77853480	Sb02g044040.1	PF02178,PF00855 AT3G05430.1 PWWP domain-containing protein
Bayescan	TP110077	8	1046928	Sb08g001115.1	PF02493,PF01504 PTHR23086 KOG0229 2.7.1.68 K00889 AT3G07960.1 phosphatidylinositol-4-phosphate 5-kinase family protein
Bayescan	TP119018	1	60290323	Sb01g036670.1	PF00046 PTHR19418 AT3G03660.1 WOX11 WOX11 (WUSCHEL related homeobox 11); DNA binding / transcription factor
Bayescan	TP150845	4	61081684	Sb04g031080.1	PF03169 PTHR22601 KOG2262 AT5G64410.1 OPT4 OPT4 (OLIGOPEPTIDE TRANSPORTER 4); oligopeptide transporter
Bayescan	TP152852	2	77115416	Sb02g043300.1	PF00046 PTHR19418:SF56 AT2G33880.1 HB-3 HB-3; transcription factor

STable 5. Candidate gene annotations from the top 1% of Bayenv2 outlier analysis (46), Bayescan analysis (50), and the consensus markers (15) between the two analyses. Identification is from mapping big bluestem reads to the sorghum genome to find the position. Table shows identifies how the gene was found, using Bayenv or Bayescan or both (Analysis), big bluestem marker id (Markers), to which sorghum chromosome the marker maps (Chromosome), position on that chromosome (Position), gene identifier of the gene near the sorghum position (Gene Id), and the annotation information from that gene (Gene Information).

Footnote: Bayenv outliers lacking annotation included TP67155, TP505, TP125027, TP39152, TP168589, TP35977, TP61552, TP29318, TP119835, TP123355, TP69553, TP143151, TP171774, TP105122, TP147959, TP132908, TP103399, TP156046, TP168955, Both Bayenv and Bayescan TP1712, TP114252, TP118569, TP5219, TP62618, TP19463, TP52052, TP27663, TP104813, TP34353, TP47256; Bayescan TP24620, TP25391, TP27700, TP28582, TP38460, TP48595, TP49355, TP61449, TP77269, TP79145, TP91916, TP95678, TP97404, TP97704, TP129182 , TP124284, TP144242, TP161527, TP164302, TP172967, TP173308

Marker Name	Mean Annual Temperature (C)	Seasonal Mean Temperature (C)	Seasonal Mean Precipitation (cm)	Annual Diurnal Variation (C)	Seasonal Diurnal Variation (C)	Precipitation of the Driest Year (cm)	Number of Precipitation Events >1.25 cm	Temperature Severity Index	Mean Annual Precipitation (cm)	Mean Annual Precipitation / Mean Annual Temperature	Elevation (m)
TP1712	***9.70E+01***	1.43E+00	2.78E-01	**1.65E+00**	4.51E-01	3.14E-01	3.37E-01	2.85E-01	2.26E-01	1.68E-01	2.48E-01
TP171997	***9.39E-01***	1.47E-01	3.19E-01	3.53E-01	6.49E-01	1.52E-01	1.72E-01	**7.02E-01**	3.11E-01	3.02E-01	3.20E-01
TP67155	**3.16E+00**	8.93E-01	1.38E+00	4.79E-01	***6.45E+00***	1.69E-01	2.37E-01	5.90E-01	2.19E-01	1.41E-01	1.65E-01
TP114252	***1.35E+00***	4.45E-01	**5.83E-01**	5.03E-01	3.96E-01	3.25E-01	2.32E-01	2.64E-01	2.07E-01	1.85E-01	1.73E-01
TP505	3.44E-01	2.15E-01	3.54E-01	**3.66E-01**	***8.07E-01***	1.58E-01	1.62E-01	3.46E-01	2.27E-01	3.03E-01	2.18E-01
TP125027	**1.14E+00**	5.26E-01	***2.24E+00***	1.01E+00	4.02E-01	1.70E-01	1.41E-01	2.64E-01	1.46E-01	1.26E-01	1.35E-01
TP39152	3.99E-01	1.84E-01	**2.79E+00**	5.99E-01	***4.53E+00***	6.69E-01	1.91E-01	4.18E-01	2.63E-01	4.66E-01	3.19E-01
TP4696	4.02E-01	2.32E-01	**1.15E+00**	3.76E-01	***1.37E+00***	2.29E-01	1.80E-01	4.48E-01	2.28E-01	2.19E-01	1.96E-01
TP168589	4.83E-01	1.70E-01	3.85E-01	3.48E-01	***7.62E-01***	1.80E-01	1.84E-01	**5.08E-01**	2.63E-01	2.08E-01	1.93E-01
TP35977	4.30E-01	1.82E-01	**4.31E-01**	***4.73E-01***	3.69E-01	1.48E-01	1.46E-01	3.16E-01	1.72E-01	1.56E-01	1.58E-01
TP118569	4.17E-01	1.88E-01	**6.00E-01**	3.65E-01	***7.03E-01***	1.94E-01	1.62E-01	2.07E-01	1.55E-01	1.30E-01	1.33E-01
TP61552	**1.81E+00**	***1.85E+00***	3.72E-01	6.15E-01	3.55E-01	8.71E-01	9.27E-01	9.54E-01	8.08E-01	2.63E-01	7.37E-01
TP29318	***1.33E+00***	**1.06E+00**	4.12E-01	8.57E-01	5.06E-01	2.73E-01	2.27E-01	2.33E-01	2.37E-01	1.51E-01	3.76E-01
TP97659	6.05E-01	1.58E-01	**7.95E-01**	3.88E-01	***1.63E+00***	2.26E-01	1.63E-01	2.75E-01	1.65E-01	2.19E-01	1.59E-01
TP119835	3.69E-01	1.48E-01	**6.30E-01**	3.44E-01	***6.93E-01***	1.88E-01	1.55E-01	5.13E-01	1.73E-01	3.80E-01	1.76E-01
TP5219	7.60E-01	1.87E-01	6.37E-01	**9.10E-01**	***1.42E+00***	2.52E-01	1.93E-01	2.15E-01	1.74E-01	1.88E-01	1.52E-01
TP103404	***1.84E+00***	1.59E-01	3.36E-01	4.30E-01	3.05E-01	1.54E-01	1.74E-01	**6.03E-01**	2.07E-01	1.68E-01	1.47E-01
TP62618	***7.41E-01***	2.53E-01	**4.21E-01**	4.09E-01	3.89E-01	1.92E-01	1.85E-01	2.62E-01	2.21E-01	2.78E-01	1.91E-01
TP22546	***7.63E+00***	2.74E-01	4.12E-01	**5.23E-01**	4.28E-01	1.84E-01	1.70E-01	2.80E-01	2.04E-01	2.06E-01	1.65E-01
TP19463	***1.13E+00***	2.97E-01	4.01E-01	4.58E-01	**5.12E-01**	2.03E-01	2.38E-01	2.67E-01	2.09E-01	1.96E-01	1.87E-01
TP16994	3.73E-01	2.82E-01	***2.85E+00***	3.87E-01	6.82E-01	6.41E-01	2.97E-01	3.13E-01	3.64E-01	**9.67E-01**	6.05E-01
TP123355	***1.34E+00***	3.67E-01	3.53E-01	3.84E-01	**4.10E-01**	2.86E-01	2.00E-01	2.78E-01	1.93E-01	1.71E-01	1.60E-01
TP69553	4.71E-01	7.15E-01	3.45E-01	4.67E-01	5.37E-01	3.96E-01	7.10E-01	5.59E-01	***1.31E+00***	6.04E-01	**8.23E-01**

TP143151	4.52E-01	1.44E-01	***1.12E+00***	**4.57E-01**	3.38E-01	1.48E-01	1.37E-01	1.62E-01	1.55E-01	2.67E-01	1.57E-01
TP171774	**1.59E+00**	***1.05E+01***	4.62E-01	3.45E-01	4.07E-01	8.85E-01	5.04E-01	3.04E-01	3.76E-01	3.74E-01	3.53E-01
TP105122	3.65E-01	2.50E-01	8.93E-01	5.39E-01	**1.48E+00**	1.52E-01	1.84E-01	***2.91E+00***	2.81E-01	1.65E-01	2.29E-01
TP23740	***3.14E+00***	3.05E-01	5.37E-01	**1.25E+00**	3.66E-01	1.91E-01	1.94E-01	2.13E-01	1.70E-01	1.36E-01	1.57E-01
TP147959	***8.86E-01***	1.34E-01	2.71E-01	**5.68E-01**	3.30E-01	1.44E-01	1.29E-01	1.52E-01	1.44E-01	1.40E-01	1.17E-01
TP48670	8.16E-01	4.28E-01	***2.49E+00***	1.29E+00	1.81E+00	3.63E-01	1.88E-01	**2.49E+00**	1.81E-01	2.35E-01	3.09E-01
TP52052	***1.06E+00***	3.29E-01	**6.03E-01**	4.35E-01	3.86E-01	1.98E-01	1.91E-01	2.30E-01	1.68E-01	1.58E-01	1.56E-01
TP81246	3.27E-01	1.64E-01	**1.06E+00**	7.52E-01	***1.27E+00***	3.36E-01	3.14E-01	1.88E-01	2.43E-01	1.98E-01	3.16E-01
TP142486	1.29E+00	8.59E-01	3.10E-01	5.58E-01	4.09E-01	**1.73E+00**	3.93E-01	1.78E-01	5.40E-01	***4.59E+00***	5.31E-01
TP27663	***3.52E+00***	1.55E-01	7.97E-01	**1.49E+00**	4.90E-01	1.67E-01	1.98E-01	6.80E-01	2.68E-01	2.41E-01	1.67E-01
TP118455	**6.23E-01**	1.66E-01	3.32E-01	***1.21E+00***	3.70E-01	1.36E-01	1.35E-01	1.83E-01	1.38E-01	1.26E-01	1.21E-01
TP104813	**6.48E-01**	2.53E-01	***7.23E-01***	4.37E-01	4.20E-01	1.80E-01	2.03E-01	1.75E-01	2.06E-01	2.64E-01	1.92E-01
TP63473	**3.71E-01**	1.37E-01	2.63E-01	***4.08E-01***	3.53E-01	1.43E-01	1.34E-01	1.79E-01	1.64E-01	1.99E-01	1.70E-01
TP132908	***1.28E+00***	1.97E-01	3.41E-01	**8.94E-01**	3.34E-01	1.64E-01	1.56E-01	5.54E-01	1.70E-01	1.77E-01	1.42E-01
TP107489	4.02E-01	7.57E-01	4.30E-01	3.42E-01	***1.37E+00***	3.36E-01	2.55E-01	4.67E-01	4.84E-01	**8.29E-01**	6.46E-01
TP103399	4.33E-01	2.26E-01	4.41E-01	3.66E-01	**1.21E+00**	2.56E-01	1.66E-01	***8.37E+00***	1.77E-01	2.19E-01	1.67E-01
TP156046	**1.59E+00**	***2.35E+00***	5.79E-01	3.37E-01	3.75E-01	6.37E-01	2.51E-01	2.00E-01	3.02E-01	9.49E-01	4.53E-01
TP34353	5.55E-01	**2.65E+00**	1.64E+00	3.74E-01	1.57E+00	2.38E+00	6.67E-01	2.59E-01	5.82E-01	***3.42E+00***	1.99E+00
TP47256	***1.02E+00***	4.90E-01	7.36E-01	3.86E-01	**8.21E-01**	3.02E-01	2.74E-01	2.22E-01	2.20E-01	1.73E-01	1.80E-01
TP68168	5.74E-01	4.80E-01	**1.40E+00**	4.53E-01	***3.35E+00***	4.45E-01	2.19E-01	1.24E+00	2.18E-01	3.21E-01	2.96E-01
TP14132	**6.79E-01**	2.43E-01	***1.32E+00***	3.78E-01	3.95E-01	1.93E-01	2.04E-01	2.55E-01	1.92E-01	1.52E-01	1.76E-01
TP168955	3.69E-01	1.98E-01	**1.33E+00**	4.05E-01	1.12E+00	1.46E-01	1.94E-01	***5.06E+00***	2.78E-01	1.31E-01	1.89E-01
TP3231	***8.83E-01***	**5.98E-01**	4.26E-01	3.93E-01	3.92E-01	3.19E-01	1.88E-01	1.85E-01	1.75E-01	1.59E-01	1.49E-01

STable 6. Bayenv2 climate association with SNPs and 11 climate variables. For each marker, the environmental variable most strongly associated with the SNP is indicated with both bold and italics and the second strongest association is indicated with only bold.

Summed across 10 unique separate validation sets	Number individuals misclassified	Misclassification Rate
Mesic as Dry	28	8.9%
Mesic as Wet	8	2.5%
Dry as Mesic	17	5.4%
Dry as Wet	9	2.9%
Wet as Dry	1	0.3%
Wet as Mesic	3	0.1%
Misclassified total	66	21%
Correctly assigned	248	79%
Total number of individuals	314	-

STable 7. Table showing the misclassification rates of the 10-fold cross validation. Note that 79% are correctly assigned. Total of 314 individuals analyzed with 110 dry, 106 mesic, and 98 wet ecotype.

Ecotype Comparison	Number misclassified	Percent misclassified
Mesic - Dry	45	68%
Mesic - Wet	11	17%
Dry - Wet	10	15%
Total Misclassified	66	-

STable 8. Table showing number of plants misclassified between ecotype pairs out of the total individuals misclassified (66 out of 314 or 21%), indicating that most of the misclassification occurred between dry and mesic ecotypes.

2014 Mixed Plots	WKS Colby Kansas	CKS Hays Kansas	EKS Manhattan Kansas	SIL Carbondale Illinois
Dry ecotype	64 (0) 72%	64 (0) 72%	22 (0) 24%	8 (0) 9%
Mesic ecotype	22 (+4) 25%	26 (+4) 28%	15 (+4) 17%	15 (+4) 17%
Wet ecotype	2 (-4) 2%	0 (-4) 0%	48 (-4) 56%	65 (-4) 74%
Total Individuals	88	90	85	88

STable 9. Predicted numbers of plants of each ecotype in mixed plots across sites based on 2014. Table shows number of plants and percentages assigned to each of three ecotype classes across four planting sites in 2014. Plus and minus in parentheses indicate potentially how many plants were misidentified. For example for dry, 0 is no net error adjustment. For example mesic, as many as 4 mesic additional individuals potentially could occur in western KS (Colby). For wet ecotypes in western KS (Colby), as many as 4 fewer individuals could occur in Colby.

2015 Mixed Plots	WKS Colby Kansas	CKS Hays Kansas	EKS Manhattan Kansas	SIL Carbondale Illinois
Dry ecotype	64 (0) 70%	66 (0) 73%	21 (0) 24%	8 (0) 9%
Mesic ecotype	26 (+4) 29%	24 (+4) 26%	9 (+4) 10%	13 (+4) 15%
Wet ecotype	1 (-4) 1%	1 (-4) 1%	59 (-4) 66%	68 (-4) 76%
TOTAL	91	91	89	89

STable 10. Predicted numbers of plants of each ecotype in mixed plots across sites based on 2015. Table shows number of plants and percentages assigned to each of three ecotype classes across four planting sites in 2015. Plus and minus in parentheses indicate potentially how many plants were misidentified. For example for the dry ecotype, 0 is no net error adjustment. For example mesic ecotype, as many as 4 mesic additional individuals potentially could occur in western KS (Colby). For wet ecotypes in western KS (Colby), as many as 4 fewer individuals could occur in Colby.

Chapter 3 - Adaptive genetic potential and plasticity of trait variation in the ecological foundation prairie grass *Andropogon gerardii* across the US Great Plains' climate gradient: Implications for climate change and restoration

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INTRODUCTION

Plant response to current and changing climate depends on adaptive potential within species (Hufford and Mazer 2003; Etterson 2004; Nicotra *et al.* 2010; Shaw and Etterson 2012; Des Roches *et al.* 2017). More information is needed to predict how species respond to changes in climate, either through phenotypic plasticity, adaptive genetic variation or migration (Nicotra *et al.* 2010; Christmas *et al.* 2016). Most frequently, some combination of phenotypic plasticity and genetic variation is observed in plant responses to environmental change (Crispo 2008; Conover *et al.* 2009). Reciprocal gardens are a powerful approach to detect genetic variation vs phenotypic plasticity and sheds light on how species might cope with environmental change (Anderson and Gerzon 2015). If environment were the only effect on phenotype, then phenotypic variation would

be entirely environmentally plastic, varying across sites yet remaining unchanged among ecotypes within a site (Fig. 3.1A). If genotype (we refer to as ecotype, E) were the only driver of phenotypic

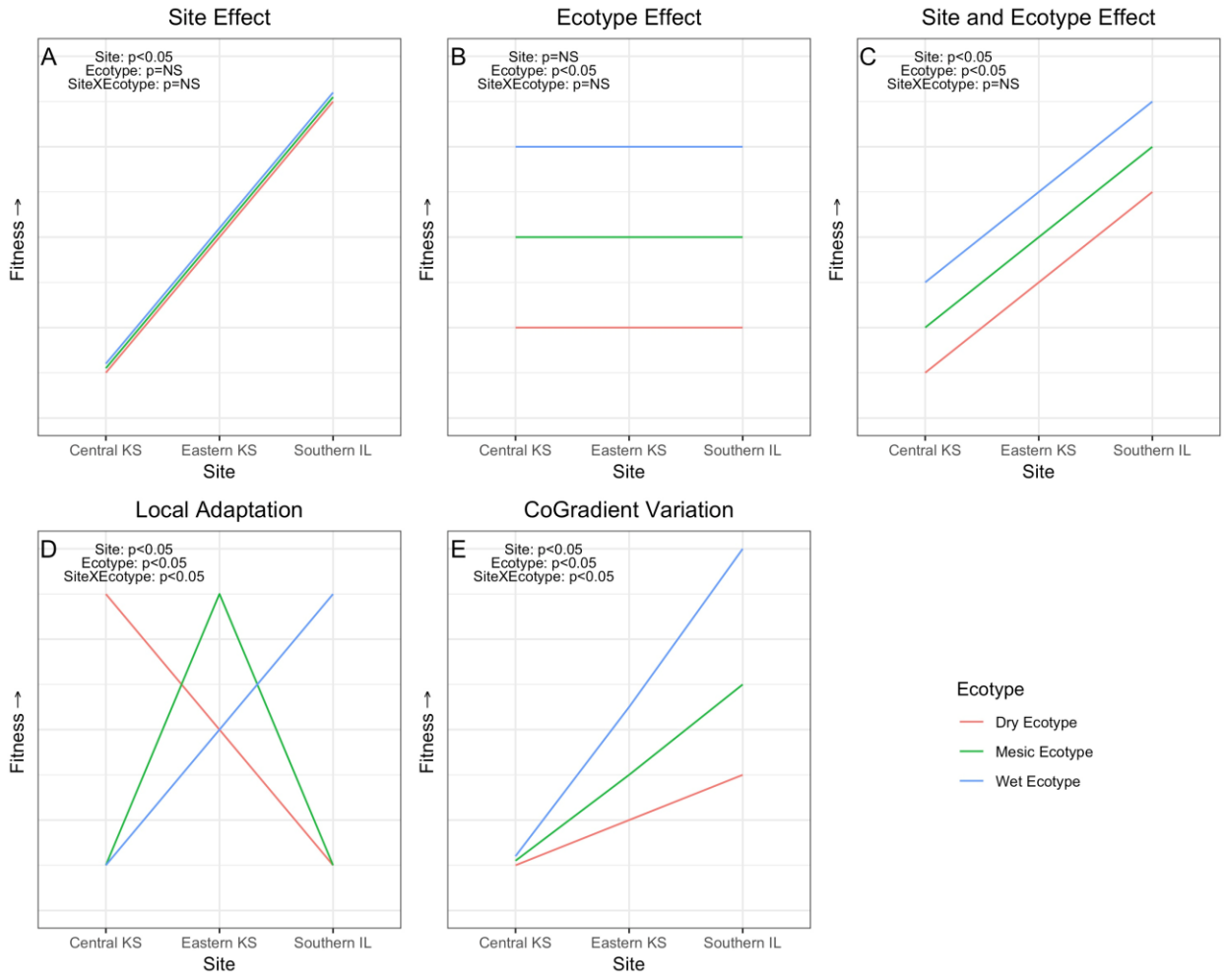

Figure 3.1. A) Main effect of site S only, , B) Main effect of ecotype E only, C) Main effects of site S and ecotype E, no interaction, D) Local adaptation S X E, E) Co-Gradient Variation. Illustration of plausible phenotypic patterns of S and E effects, represented by ecotypes across an environmental gradient.

variation, phenotypes for each ecotype would be fixed (Fig. 3.1B) across an environmental gradient, such that ecotype should be the same regardless of planting site. In our case, environment refers to the different planting sites (S). Another possibility is that ecotype and site both exert separate and independent main effects on phenotype (S, E i.e., no interaction, Fig. 3.1C). If effects

of ecotype and site interact, the interaction term S X E would express the extent to which ecotypes differed in their sensitivity to environment (Fig. 3.1D, 3.1E). One such interactive pattern is described by local adaptation, which occurs when ecotypes perform best in their home environment compared to non-local ecotypes in the same site (Fig. 3.1D). Finally, other forms of interaction include co-gradient (CoGV, Fig. 3.1E) and counter-gradient (CnGV) variation (Chapin *et al.* 1981; Conover and Schultz 1995; Eckhart *et al.* 2004; Anderson *et al.* 2015, Ensing and Eckert 2019.) whereby synergistic, positive effects (in case of CoGV) or inhibitory, negative effects (in case of CnGV) exist between environmental and genetic sources of variation. Other idiosyncratic interactions are possible as well.

In this study, we focus on phenotypic variation in ecotypes of big bluestem (*Andropogon gerardii* Vitman), a long-lived dominant perennial and clonal C_4 grass (Weaver and Fitzpatrick 1932; Epstein *et al.* 1997; Knapp *et al.* 1998), in response to a precipitation gradient. *Andropogon gerardii* comprises up to 80% of biomass in tall grass prairies (Knapp *et al.* 1998) and has wide natural distribution across the eastern U.S. (http://plants.usda.gov). This species is planted widely in the 3 million ha of Conservation Reserve grassland restoration throughout the Great Plains. Within the U.S. Great Plains, *A. gerardii* occurs along a 1050-km-long precipitation gradient from western KS (500 mm of mean annual precipitation [MAP]) to Illinois (1200 mm MAP). This precipitation gradient and these grasslands have been in place for the last 10,000 years since the last glaciation (Axelrod 1985). This allows us to test the extent of genetic variation and phenotypic plasticity to spatially varying climate, especially precipitation, and to use the climate gradient as a proxy for future climate changes.

Grassland once covered one third of continental North America (Bailey 1998) and 40% of Earth's surface (Gibson 2009), and remains a threatened biome (Hoekstra *et al.* 2005). Changes in

precipitation threaten many natural ecosystems (Weltsin *et al.* 2003; Knapp *et al.* 2008; Cook *et al.* 2015; IPCC 2017, 2018). Grasslands, however, are highly responsive to precipitation change (Axelrod 1985; Knapp *et al.* 2001). Worldwide, grasslands are characterized by frequent droughts (Knapp *et al.* 1998; Knapp and Smith 2001; Knapp *et al.* 2001; Craine *et al.* 2012), with predictions of more frequent drought for the U.S. Great Plains in the future (Cook *et al.* 2015; IPCC 2018). Yet, the degree of intraspecific variation is poorly known for most species (Des Roches *et al.* 2017). Furthermore, adaptive variation across environmental gradients is poorly characterized for most plants, especially for foundation plant species that largely control ecosystem processes (Whitham *et al.* 2006; Wymore *et al.* 2011). Consequently, understanding natural variation in genetic vs. phenotypic plasticity across the precipitation gradient of a dominant grassland species is particularly timely in the face of climate change. Ultimately, our results will assist conservation and restoration managers to better identify the optimal climate-matched ecotype for restorations (Pickup *et al.* 2012; Kettenring *et al.* 2014) and forage supply in changing climates for a major ecological foundation species (Aitken and Whitlock 2013; Gibson *et al.* 2016).

We used a reciprocal common garden approach to detect genetic and phenotypic plasticity effects on phenotypic variation of *A. gerardii* (Clausen *et al.* 1940; McMillan 1959, 1965; Etterson 2004; Byars *et al.* 2007; Lowry *et al.* 2009; Gonzalo-Turpin and Hazard 2009; de Kort *et al.* 2014; Villemereuil *et al.* 2016). Four reciprocal garden sites, planted with three regional ecotypes of *A. gerardii* (4 populations per ecotype), span a precipitation gradient. Specifically, this study aimed to answer the following questions. (1a) How will ecotypes of *A. gerardii* respond under different climatic conditions, especially precipitation, when reciprocally transplanted into its local vs non-local environments? (1b) More specifically, what is the relative role of genetic variation and phenotypic plasticity in controlling phenotypic differences? We hypothesized if local adaptation

is strongly enforced by precipitation in the dry region, a dry ecotype would outperform foreign ecotypes when planted in the dry end of the gradient, and wet ecotypes would also show a home-site advantage in the wet end of the gradient (Fig. 3.1D). Additionally, we planted ecotypes of *A. gerardii* outside its main distribution in the Great Plains into an even drier region of its distribution (Western KS in Colby, KS) to test the extent to which ecotypes might respond to increasingly dry conditions (Weltzin *et al.* 2003; Cook *et al.* 2015) as a surrogate for ecotypic response under future extreme dry conditions (Shaw and Etterson 2012; De Frenne *et al.* 2013). 2) How do ecotypes compare when planted in their home environment? Using a subset of the reciprocal garden data with ecotypes growing in their home site, we predicted an ecotype-specific suite of traits such that climate drivers, especially precipitation, were expected to control morphology and fitness of ecotypes in their home sites. As precipitation became more favorable moving eastward, plants may be expected to be more robust in vegetative traits (taller, greater canopy area, wider leaves) and show increased reproductive fitness. 3) What are the underlying genetic bases for ecotype differences in traits and how are genotypes and phenotypes structured by climate? We predicted phenotypic variation is influenced by genetic differentiation among ecotypes, with genetic outliers and candidate genes potentially associated with climate, especially precipitation. (4) What are the implications for climate change and restoration? To answer these questions and test hypotheses, we present results of vegetative performance and fitness measurements of *A. gerardii* in reciprocal gardens and in their home site garden. We further relate responses to genetic differentiation, candidate genes, and climate drivers. These results provide a comprehensive understanding of *A. gerardii* ecotype responses to climate, across the Great Plains and will allow us to predict responses of *A. gerardii* to current and changing climates and inform restoration.

Table 3.1. . Seed collection sites defining populations within an ecotype and associated environmental information of the site. Temperature severity index is number of days over 95F/total number of days. Number of precipitation events >1.25 cm per year. Soils were mostly loam as follows: Wakeeney-Harney Silt loam (WEB), Bogue-Armo Clay loam (SAL) ArmoClay loam (CDB) Armo loam and Brownell gravelly loam (REL), Benfield Florence silty clay loam (KON), Cline Sogen Silty clay loam (TAL), Benfield Florence silty clay loam (CAR), Irwin silty clay loam (TOW), Orthents silty loam (DES), Cisne silty loam (12MI), Atlas silty clay loam (WAL), Menfro silty clay loam (FUL).

Ecotype, Region Elevation (m)	**Prairie Name (abbrev, size ha)**	**Lat N Long W**	**County, State (Weather Station)**	**Number of Precipitation Events >1.25 cm (NOPPT)**	**Precipitation Driest Year (cm) (PPTDRY)**	**Mean Annual rainfall (AMP) (cm)**	**Seasonal Mean Rainfall (SMP) (cm)**	**Annual Diurnal Temp (ADV) (°C)**	**Seasonal Diurnal Temp (SDV) (°C)**	**Annual Mean Temp (AMT) (°C)**	**Seasonal Mean Temp (°C)**	**Temp Severity Index (TS)**	**MAP (cm)/ MAT (°C)**
Dry CKS (606)	Webster Reservoir (WEB, 356)	39.41 99.50	Rooks, KS (Webster Dam)	17	25.96	58.70	39.35	15	15.4	12.4	19.8	0.100	4.72
Dry CKS (641)	Saline Experimental Range (SAL, 880)	39.02 99.14	Ellis, KS (Plainville)	16	36.32	61.7	31.0	14.6	15.5	11.8	20.7	.088	5.22
Dry CKS (688)	Cedar Bluffs Reservoir (CDB, 850)	38.76 99.83	Trego, KS (Cedar Bluffs Dam)	16	32.18	53.31	35.97	14.4	14.5	11.2	19.5	.091	4.72
Dry CKS (640)	Relict Prairie (REL, 14)	38.85 99.37	Ellis, KS (Hays 1S)	16	32.61	58.0	37.7	14.6	15.5	12.0	20.6	.088	4.82
Mesic EKS (366)	Konza Prairie (KON, 1557)	39.08 96.56	Riley/Geary, KS (Manhattan 6SW)	22	68.89	88.47	56.54	12.8	12.4	12.8	21.0	.048	6.92
Mesic EKS (92)	Tallgrass National Park (TAL, 4409)	38.25 96.60	Chase, KS (Tallgrass Nat Park)	21	59.77	82.82	49.19	12.7	12.2	12.7	20.8	.066	6.54
Mesic EKS (389)	Carnahan Cove (CAR, 99)	39.34 96.62	Pottawatomie, KS (Wamego)	23	52.35	87.20	53.34	13.0	13.1	13.0	21.4	.057	6.72

Mesic EKS (379)	Top of the World Park (TOW, 61)	39.22 96.62	Riley, KS (Tuttle Dam)	21	45.11	81.12	50.70	13.1	13.2	11.7	20.3	.057	6.95
Wet SIL (119)	Desoto Prairie (DES, 0.4)	37.85 89.14	Jackson, IL (Carbondale, Il)	33	67.41	115.92	53.53	12.3	12.6	13.2	21.1	.027	8.78
Wet SIL (160)	Twelve Mile Prairie (12MI, 28)	38.78 88.83	Effingham, IL, (Monroe, Fayette, Salem)	25	70.01	107.57	51.83	11.7	12.4	12.3	18.7	.026	8.72
Wet SIL (150)	Walters Prairie (WAL, 5)	38.92 88.19	Jasper, IL (Newton/ Charleston)	27	69.18	104.04	50.80	10.8	11.7	13.4	21.8	.014	8.11
Wet SIL (215)	Fults Prairie (FUL, 214)	38.17 90.19	Monroe, IL (Sparta)	31	69.38	111.27	55.14	11.9	12.6	13.2	21.0	.031	8.38

METHODS

1. Plant materials and seed collection sites.

Seeds were collected by hand in autumn 2008 (3 separate dates), from three climatically distinct ecoregions (Kuchler 1964) along a precipitation gradient from central, eastern KS, and southern IL (Table 3.1, Fig. 3.2): mixed grass (dry ecotype from Central KS), tallgrass (mesic

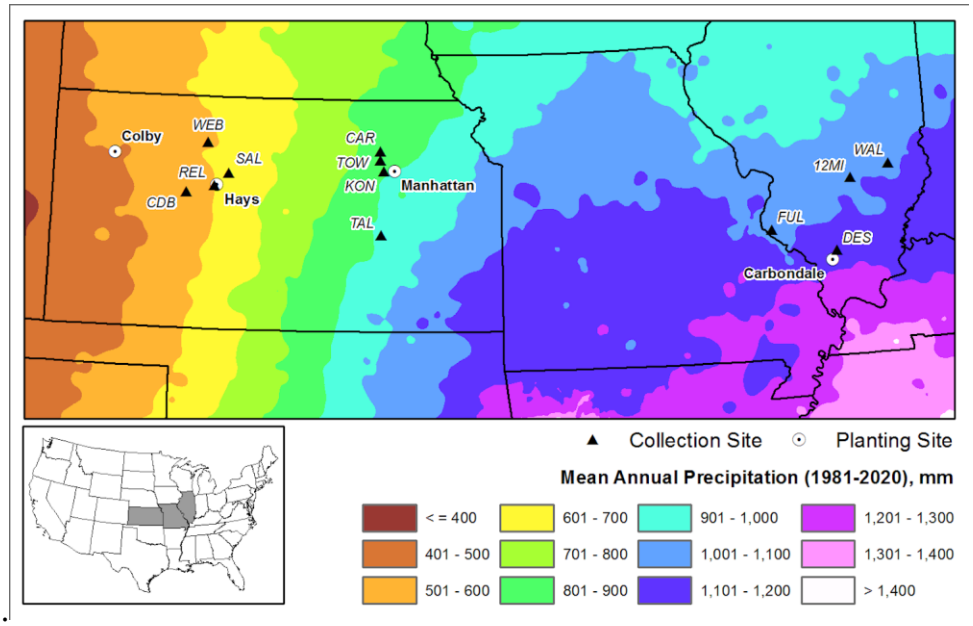

Figure 3.2 Regional map depicting the location of reciprocal gardens planting sites (white circles) and seed collections sites (black triangles) across the US Great Plains. For prairie population acronyms, see STable 1. The planting site in Western Kansas (Colby, Kansas) was the satellite reciprocal site to test the range of tolerance for big bluestem. Note that seeds were not collected in Colby.

ecotype from Eastern KS), and prairie savannah (wet ecotype from Illinois). Mixed and tallgrass prairies are open grassland, dominated by low stature grasses with few forbs (Knapp *et al.* 1998). In the prairie savannah ecoregion, diversity and structure shift to communities of tall stature, robust forbs and shrubs and scattered trees (Kuchler 1964). In each region, seeds were collected from four sites (Table 3.1, Fig. 3.2), each referred to as a population. Populations from the same region

jointly defined an ecotype. Populations originated from intact, never restored prairies generally within an 80 km radius of each reciprocal garden planting site (Table 3.1). Seeds were collected multiple times from each population with collections from different parents throughout each population. Several kilograms of seed were collected from each population site, so it is unlikely the plants in the plots are related. Thus, plants should be representing distinct maternal families. Seeds were stored in paper bags and kept dry at 4.4°C until germination the following spring.

2. Reciprocal garden planting sites.

Reciprocal garden plants were used to measure vegetative and reproductive morphology of ecotypes in relation to climate and to characterize genetic diversity, structure, and outlier loci. Seeds collected from native prairie were used in the reciprocal gardens. In winter 2009, a subset of seeds from each population was germinated and grown in greenhouse using potting mix (Metro-Mix 510). In August 2009, 3-4 month old plants of all 12 populations were planted at each of 4 garden sites, western KS in Colby, central KS in Hays, eastern KS in Manhattan and Illinois in Carbondale (Fig. 3.2, Table 3.2). Phenotypic measurements began two years after germination and planting in the gardens, thus minimizing maternal effects and transplant shock. At each planting site, the design consisted of 10 rows (blocks), each containing 12 plants corresponding to 4 four populations of each of the 3 ecotypes in random order (10 blocks X 12 plants (4 populations X 3 ecotypes) = 120 plants per site) (Table 3.1, Fig. 3.2). Although populations of *A. gerardii* can be found at the western range of distribution in western KS, by design, there was no western KS ecotype (from Colby). The goal for this study was to assess the ecotypes found within the core of *A. gerardii* distribution. However, the western KS planting site was incorporated into the reciprocal garden to test tolerance of ecotypes to even drier conditions, similar to what *A. gerardi*

Table 3.2. Geographical descriptors and summary of historical weather data (30-year normals) as descriptors of environmental conditions for the planting sites of the reciprocal garden platform.

Reciprocal Garden Planting Site (Town, County) Soil Type	Elev. (m)	Lat. (°N) Long (W)	Rainfall 6-year mean 2006-2009 (range) (cm)	Annual Number of Pcp Events >1.25 cm (NOPPT)	Pcp Driest Year (cm) (PPT DRY)	Mean Annual rainfall (cm) MAP	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 Riley Co) USDA Plant Materials (Belvue Silt Loam)	315	39.19 96.58	89.1 (61.5-110.2)	21.9	39.16 (1966)	90.5	63.47	-4.2	-4.3	12.8	18.9	23
Southern Illinois (Carbondale IL Jackson, Co) SIU Ag Research Station (Stoy Silt Loam)	127	37.73 89.17	125.6 (76.2-173.8)	32.7	67.38 (1963)	119.8	64.51	-5.3	-5.1	13.5	19.0	6.3

might experience predicted by climate change. These very dry western KS prairies are similar physiognomically to the other grasslands, with similar clay loam soils, and similar species composition. (Unfortunately, in all of these sites, we cannot control for any differences in biotic community such as herbivores and microbes). Within each row, plants were spaced 50 cm apart. The soil around each plant was covered with water-penetrable landscape cloth to discourage growth of competing plants. Nearly all plants of all ecotypes survived transplantation to all sites, even into western KS. Note that these reciprocal gardens with single-spaced plants of only *A. gerardii* are a separate research platform (described Olsen *et al.* 2013; Caudle *et al.* 2014; Mendola *et al.* 2015; Maricle *et al.* 2017; Kramer *et al.* 2018; Varvel *et al.* 2018) from reciprocal gardens with *A. gerardii* ecotypes planted with other prairie species in a seeded community (Johnson *et al.* 2015; Wilson *et al.* 2016; Maricle *et al.* 2017; Galliart *et al.* 2019).

3. Climate and soil.

The four garden planting sites (Table 3.2) were all under agricultural cultivation prior to reciprocal garden establishment, and were characterized as silt loam soils (Mendola *et al.* 2015). For each planting site, data on long-term average rainfall and temperature were collected from local agricultural research stations or nearby NOAA weather stations. Climate information from the population source of origin (where seeds were collected), was gathered from nearby NOAA stations (Table 3.1).

4. Phenotype response variables.

Phenotypic measurements began in 2010, two years after germination and planting in the gardens, thus minimizing maternal effects and transplant shock. In 2010, we made non-destructive

vegetative measurements of all plants in all sites. Measurements were made during the height of the growing season (maximum biomass, mid-summer) Once plants were firmly established by 2011, we made a destructive collection of biomass at the end of the growing season in September/October 2011. In 2012, we mainly measured reproductive responses during end (August/September) of the growing season. Some variables (canopy area, diameter, height) were measured in 2010 and 2014, two- and five-years post-transplant, to examine interannual variation. While we would have preferred to measure all traits in all years, this would have been very difficult as these sites are 1,000 km apart. Therefore, for those traits that we repeated in another year, we chose to measure traits that would reflect the overall plant status such as canopy area, height, and diameter. We did not harvest roots because of its destructive nature and harm to long-term plots.

4A. Vegetative response variables

Vegetative emergence: First emergence from the ground was recorded starting in March, and plants were observed once per week to detect first emergence. Time of emergence was expressed in Julian days and recorded as the difference from the earliest Julian day of emergence observed in the dataset.

Canopy area: Non-destructive estimates of canopy area were made using photographs of all plants at all garden sites during July in 2010 and 2014. Images were taken using a Nikon Coolpix camera from directly above each plant with white background. A ruler was placed next to the plant to set image scale. Images were imported into ImageJ v1.8.0 and converted to black and white to delineate plant from background. Canopy area was determined by pixel counts (Image J, Rasband 1997-2008, online resource-https://imagej.nih.gov/ij/) after selecting outline of plant and using

reference scale to provide area in cm^2. Measurement error was approximately 2% based on repeated measurements of the same ImageJ photos. On a separate set of non-study plants, we correlated ImageJ canopy area with actual leaf area measurements (the gold standard for leaf area) from plants using a leaf scanner ($r=0.95$ $p<0.0001$).

Plant canopy diameter: The images taken for canopy area were also used to determine plant canopy diameter, defined as two orthogonal measurements of plant width (cm); these measurements were subsequently averaged. Measurements were made in years 2010 and 2014.

Height: Plant height was measured in 2010 and 2014. Height was determined by extending leaves vertically and measured to the nearest cm. Measurement were taken from the ground to the highest point of extension. Reproductive stalks were not included.

Blade width: On each plant, two mature leaves were measured in mm at their broadest section (at approximately 2/3 from the tip of the leaf) and recorded to nearest full unit; measurements were then averaged for each plant. Blade width was measured in 2010.

4B. Vegetative and reproductive biomass

We harvested reproductive and vegetative biomass by cutting the plant at soil level, storing in bags for drying, and later separation and weighing. Vegetative biomass included all leaves, while reproductive biomass included flowering stalks and seeds. All samples were weighed on a Denvers Instruments balance DI-5K or Ohaus Precision standard balance. Results are presented as grams $plant^{-1}$.

4C. Reproductive characteristics

Anthesis: Anthesis was defined as occurring when anthers were first visible. Data are presented for 2012. In each site, plants were observed twice per week. For each plant, a binary flowering response was recorded (yes flowering=1; no flowering=0). When anthesis was observed, days to anthesis relative to emergence were also recorded.

Seed production: Seed and stalks were collected in 2012. Dates of collections occurred multiple times from September to November to ensure all seed was collected. For each plant, a binary seed production response was recorded (yes seed production=1; no seed production=0). For those plants producing seed, all seeds were stored in paper bags and air-dried, seed and stalk were then separated by hand and each was weighed in grams.

4 C. Statistical analyses of phenotype response variables

For phenotype responses with one measurement year (vegetative emergence, blade width, biomass, days to anthesis, probability of anthesis, seed production, and probability of seed production), statistical analyses were conducted using a generalized linear mixed model fitted to each variable with a probability distribution that recognized its continuous or discrete nature, accordingly. The linear predictor included fixed effects of planting site (western KS, central KS, eastern KS, and Illinois), ecotype (dry, mesic, wet) and their 2-way interaction. The random effects of block nested within planting site and population nested within ecotypes were fitted to recognize the experimental units for planting sites and ecotypes, respectively. By fitting a population effect,

the model explicitly incorporates variation between populations within an ecotype. Models for all traits were fitted using the GLMMIX procedure of SAS v9.3. For all traits the heterogeneous residual variances were fitted for each planting site to enhance model fit, as determined using maximum-likelihood based Bayesian Information Criteria. Multiple testing for all traits was subjected to Bonferroni adjustments to prevent inflation of Type I error. Least square mean estimates and estimated standard errors are presented. Special statistical considerations are described below.

For vegetative emergence, the model assumed a Poisson distribution of the response implemented with a log link function to account for the integer count nature of the response. The likelihood-based Pearson Chisq/df statistic did not indicate any evidence of overdispersion. Parameter estimation was conducted using residual pseudolikelihood with Newton-Raphson with ridging as the optimization technique. Kenward-Roger's procedure was used to estimate degrees of freedom and to make the corresponding adjustments in estimation of standard errors.

For anthesis, the statistical model contained missing cells, which corresponded to the wet and mesic ecotypes in the Colby planting site, as no data was available because plants of these ecotypes did not reach anthesis in Colby. Thus, inference is limited to the combinations of ecotype and site for which data was available. Model assumptions were checked using studentized residuals and were considered to be reasonably met. Kenward Roger's procedure was used to estimate degrees of freedom and adjust estimates of standard errors. For Probability of anthesis, overdispersion was evaluated using the maximum-likelihood based fit statistic Pearson Chi-Square/DF. No evidence for overdispersion was apparent. The final statistical model used for inference was fitted using residual Pseudo-Likelihood. For seed production, model assumptions were checked using studentized residuals and were considered to be appropriately met. Kenward

Roger's procedure was used to estimate degrees of freedom and adjust estimates of standard errors. Estimated least square means for levels of the fixed effects of interest are reported after backtransformation to the original data scale.

For traits measured in multiple years, in addition to the fixed effects of ecotype, site and ecotype*site, the fixed effect of time and their 3-way interaction was included to account for repeated measures across the two collection years. This 3-way interaction was included for all trait measured in multiple years (canopy area, diameter, and height). Model assumptions were checked using studentized residuals and were considered to be appropriately met. Models for all traits was fitted using the GLMMIX procedure of SAS v9.3. For all traits the heterogeneous residual variances were fitted for each planting site to enhance model fit, as determined using maximum-likelihood based Bayesian Information Criteria. Least square mean estimates and estimated standard errors are presented. In addition, for canopy area, a general linear mixed model was fitted to the response "canopy", expressed in the log scale. Kenward-Roger's procedure was used to estimate degrees of freedom and make the corresponding adjustments in estimated standard errors. Least square mean estimates expressed in the original scale after backtransformation are presented.

4.D. Differential canopy area response of ecotypes to rainfall

Comparisons of canopy area to difference between rainfall of population of origin and rainfall from planting site were conducted in SAS v9.3 using the GLMMIX procedure. For differential canopy area in response to rainfall, we used a quadratic function (R^2 = 0.97 for all ecotypes) because it fit the data better than a linear function (Dry - R^2 = 0.812, Mesic – 0.8373, Wet – 0.846). The model included the fixed effects of ecotype, difference in rainfall, and difference in rainfall^2 and the random effects of population nested within ecotype and blocks nested within

site. The slopes of ecotype response vs rainfall differential were compared using an ANCOVA to identify if the responses to rainfall differs between ecotypes i.e. do certain ecotypes exhibit a greater increase in canopy area with increasing rainfall.

5. Comparison of ecotypes in their home environment

In order to characterize whether each ecotype had a suite of phenotypic traits, we compared phenotype variables of each ecotype in their home site, namely dry ecotype in central Kansas, mesic ecotype in Eastern Kansas and wet ecotype in Illinois. (Note that we did not use western KS plants because we did not have a western KS home ecotype.) We used 106 plants comprising dry (34), mesic (35), and wet ecotypes (37). We also used random forest classification and PCA as complementary approaches to characterize ecotypes in home environment.

Random forests were used to test classification of ecotypes based on morphological traits in home site (Breiman 2001). Random forests use an ensemble method (Altman and Krzywinski 2017) for classification based on morphological traits and operates by constructing many decision trees at training and taking a weighted vote of predictions from these trees for final prediction, in our case, ecotype. Implementation of random forests and description of cross validation approach, and classification error are presented in Supplemental Methods.

For PCA, a data subset consisted of 7 traits (i.e. canopy area, height, blade width, diameter and seed production, days to emergence, and days to anthesis). Plants that did not flower were included with date of flowering one week past the last flowering date observed. Each variable was standardized to a zero mean and a variance = 1 due to differential scaling. Scree plots were evaluated to describe the proportion of the total variance described by each principal component. Data was analyzed using PCA as implemented in R v2.15.3. A stepwise model selection approach

was used to explore associations between the first 3 PC scores and (Table 3.1) environmental explanatory variables. The Schwarz Bayesian Information Criterion was used as criterion for model fit and selection. Stepwise selection was conducted using the GLMSELECT procedure of SAS v9.3.

6. Genotyping and genetic analyses

The objective was to characterize genetic diversity among ecotypes, identify genetic outliers and candidate genes among SNPs, relate genetic outliers to climate of population source of origin and relate genotype to phenotype in home site. We used genotyping-by-sequencing to identify SNPs (Elshire *et al.* 2013; Lu *et al.* 2013; Narum *et al.* 2013). Leaf samples were collected in 2014 from the same plants as used for phenotyping within the reciprocal gardens from the central KS, western KS, and southern IL planting sites. Total number of plants genotyped (314) included dry (110), mesic (106) and wet (98). DNA sample collection, preparation and SNP calling analyses can be found in Supplemental Methods.

A. Genetic differentiation.

Pairwise population differentiation F_{ST} was implemented in *GenAlEx* v6.503 (Peakall and Smouse 2006, 2012) using twelve populations comprising three regional ecotypes. PCoA of genetic distance was calculated from SNP markers performed in R package *Adgenet* (Jombart 2008). Scores for genetic distance Principal coordinates 1, 2 and 3 were each regressed on 11 environmental explanatory variables (Table 3.1) using stepwise model selection. For the PCoA stepwise regression using climate variables, we used Schwartz Bayesian Information Criterion to

identify which environmental variables from population of origin are most associated with genetic divergence. Stepwise regression was implemented using GLMSELECT procedure in SAS v9.3.

B. Outlier genetic analysis and association with climate variables.

We identified "outlier" SNPs as those show greater differentiation compared to background using on two independent methods, *Bayescan* and *Bayenv* and related their differentiation to population climate of origin. We used a Bayesian approach to estimate the posterior probability that a marker is under selection as implemented in *Bayescan v2.1* (Foll and Gaggiotti 2008) to identify SNP outliers (Lotterhos and Whitlock 2015). To relate climate variables of population climate of origin (Table 3.1), we evaluated strength of association between outlier F_{ST} and 11 environmental variables using *BayeScEnv* (Villemereuil and Gaggiotti 2015).

Second, we used *Bayenv2*, a robust approach that provides correction for population structure and demographic processes while controlling false positives (Guenther and Coop 2013; Lotterhos and Whitlock 2015). Population differentiation ranking statistic X^TX (Guenther and Coop 2013) was calculated for all loci to identify loci with greater differentiation than under neutral drift amongst populations. See Supplemental Methods.

Lastly, partial redundancy analyses (pRDA) was used to estimate the role of geographic differences (latitude and longitude) vs climate (Table 3.1) in structuring genetic variation. pRDA is an ordination technique (Oksanen *et al.* 2015) that partitions variation, in our case genetic variation, due to climate and geography and joint contribution of climate and geography (Riordan *et al.* 2016). See Supplemental Methods.

C. Relating genotype to phenotype.

We performed separate genome wide association (GWAS) of the 4,641 SNP markers with each of the phenotypic variables measured, namely emergence, canopy area, height, blade width, diameter, seed weight, and days to anthesis, using *TASSEL* v5.0 software (Bradbury *et al.* 2007). Association in *TASSEL* v5.0 was performed using a mixed linear model, including a kinship matrix calculated as genetic relationship to account for relatedness between individuals along with Q values from *Structure* v2.3.4 (Falush *et al.* 2007) to account for population structure. Run parameters for STRUCTURE 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 were 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. SNPs were individually associated with phenotype and Bonferroni multiple test correction was used to identify SNPs significantly associated with phenotypes. Data consisted of plants from their home planting site only (106 plants total, 34 dry ecotype in Central Kansas, 35 mesic ecotype in Eastern Kansas, and 37 wet ecotype in Illinois). Unfortunately, we cannot present Manhattan plots due to the lack of availability of a genome for *A. gerardii*.

RESULTS

1. Reciprocal gardens show pattern of phenotypic plasticity and ecotype genetic effects on phenotypic variation

A. Vegetative responses

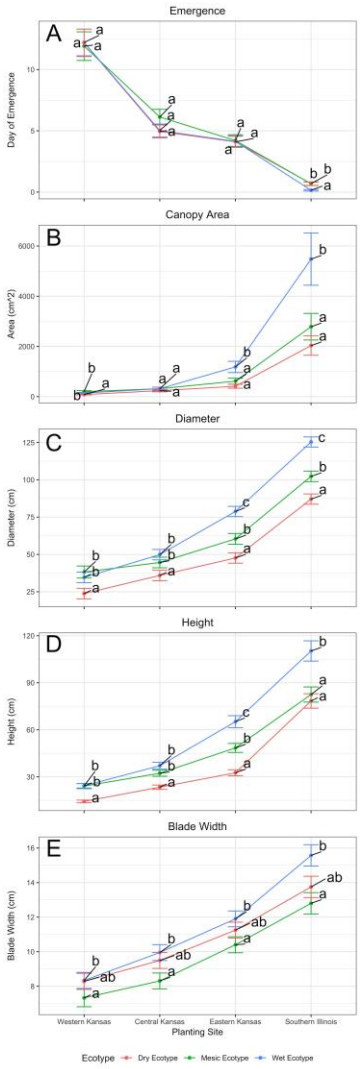

Figure 3.3. Least square mean estimates (± standard error) of vegetative morphological traits for ecotypes (dry, mesic, wet) across reciprocal garden planting sites in Western Ks (Colby KS), Central KS (Hays KS), Eastern KS (Manhattan KS), and Illinois (Carbondale Illinois). A) Days at emergence, B) Canopy area (cm2), C) Plant diameter (cm), D) Plant height (cm), and E) Blade width (mm). Sites with different letters indicate significant differences.

Date of vegetative emergence: Given significant interaction between site (S) and ecotype (E) (p=0.0107, Fig. 3.3A, Supplemental Tables 1, 2) , we focused inference on simple effects. That is, we conduct pairwise comparisons between sites for a given ecotype and second, between ecotypes

within a given site. Overall, the most striking pattern in time to emergence (relative to first day of emergence) was a general decrease in ranking of days to emergence WKS > CKS> EKS > Illinois with emergence occurring 12 days later in the westernmost sites relative to the easternmost site. Comparing ecotypes within a site, for all ecotypes, there was no evidence for differences in time to emergence for all KS sites. However, in Illinois, the wet ecotype emerged slightly earlier (half day) than other ecotypes.

Canopy area: Canopy area showed a significant 3-way interaction between S, E and Time (T) ($p = 0.0174$, S Supplemental Table 2). To explain this 3-way interaction, we conduct simple-effects analyses for each year (Fig. 3.3B and for 2014, Fig. 3.4A). Overall, the general pattern of canopy area for 2010 and 2014 seems to be consistent with CoGV (Fig 3.1E), with an ecotype-specific increase in canopy area from west to east. Specifically, at the dry end (i.e. western KS and central KS sites), all ecotypes showed small canopy area and only small differences between ecotypes were detected. Moving east toward more favorable climates, ecotypes showed increasing canopy area and maintained their relative rankings (i.e. dry < mesic < wet ecotypes) though differences between ecotypes increased towards the east. For example, the dry ecotype canopy area increased from western KS site (82.6 ± 15.8 cm^2) to Illinois (2,031.8 ± 286.0 cm^2) while in the same locations, the wet ecotype increased from 140.2 (± 26.9) cm^2 to 5,479.2 (± 1,040.8) cm^2. Furthermore, the wet ecotype showed a disproportionately larger canopy relative to dry and mesic ecotypes in the two wetter-most sites (i.e. eastern KS and Illinois). A similar general pattern holds for 2014 (Supp Table 1, Fig. 3.4A), except ecotype-specific canopy areas across sites were

substantially larger, probably due to the fact that plants were (bigger) in 2014 because they were more established.

Canopy diameter: We measured canopy diameter in 2010 and 2014. Canopy diameter showed a significant 3-way interaction between S, E and T ($p = 0.0002$, Supplemental Table 2). To explain this 3-way interaction, we conduct simple-effects analyses for each year. For brevity, we compare ecotypes within each site. Fig. 3.3C shows estimated mean canopy diameter (±SE) for ecotypes at each site in 2010 and 2014 is presented in supplementary materials (Fig 3.4B). The general pattern of canopy diameter for 2010 and 2014 appears consistent with CoGV (Fig. 3.1E), with an ecotype-specific increase in canopy diameter from west to more favorable climates of the eastern sites (Supplemental Table 1). Specifically, at the dry end (i.e. western KS and central KS sites), all ecotypes showed small canopy diameter with only small, but significant, differences between ecotypes detected. The dry ecotype had significantly smaller diameter than mesic and wet ecotypes at the dry end. Moving east on the gradient, ecotypes showed increasing canopy diameter, increasing by as much as 3.6 fold, while maintaining their relative rankings (i.e. wet > mesic > dry ecotype) though differences in diameter between ecotypes increased towards the east, similar to canopy area. For eastern KS and Illinois sites, all ecotypes were significantly different from each other within a site with ranking wet> mesic> dry with the wet ecotype increasing diameter disproportionately. A similar general pattern holds for 2014 (Supplemental Table 1, Fig. 3.4B), except ecotype-specific canopy diameter across sites was substantially greater in 2014.

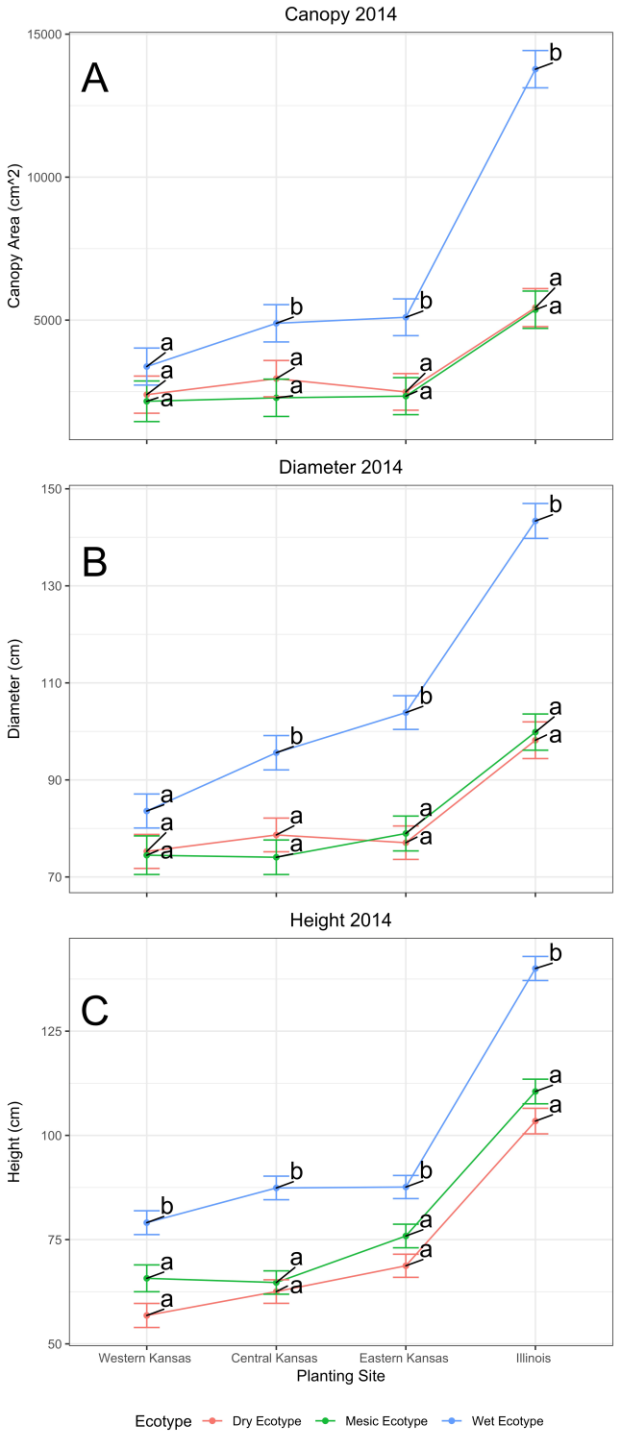

Figure 3.4. Sampling year 2014. Least square mean estimates (± standard error) of Vegetative morphological traits for ecotypes (dry, mesic, wet) across reciprocal garden planting sites for Western KS (Colby KS), Central KS (Hays KS), Eastern KS (Manhattan KS), and Illinois (Carbondale Illinois). A canopy area (cm2), b) diameter (cm), c) height (cm), d) blade width (mm). Sites with different letters indicate significant differences.

Height: Height showed a significant 3-way interaction between S, E and T ($p < 0.0001$, Supplemental Table 2). We conducted simple-effects analyses for each year to explain the 3-way interaction. Our main comparison is ecotypes within each site. Fig. 3.3D shows estimated mean height (±SE) for ecotypes at each site in year 2010 and 2014 is presented in Fig. 3.4C. Overall, the general pattern of canopy height for both 2010 and 2014 appears consistent with CoGV (Fig. 3.1E), with an ecotype-specific increase in canopy height from west to east (Supplemental Table 1). At the dry end (i.e. western KS and central KS), all ecotypes showed reduced height, with averages in the range of 14-37 cm high. However, for Western and central KS sites, the dry ecotype was significantly shorter than the mesic and wet ecotypes. No differences were detected between mesic and wet ecotypes. Moving east, ecotypes showed increasing canopy height, as much as 5.5 fold, and especially disproportionately for the much taller wet ecotype in the two wetter-most sites (i.e. eastern KS and Illinois). A similar general pattern holds for 2014 (Supp Table 1, Fig. 3.4C), except the wet ecotype was substantially taller in 2014.

Blade width: We focus on significant main effects of ecotype ($p=0.0264$) and site ($p<0.0001$) on blade width, given the non-significant interaction ($p=0.4162$) (Fig. 3.3E, Fig. 3.1C, Supplemental Table 2). Regardless of site, blade width differed among ecotypes such that wet ecotype leaves were wider (average 11.4 ± 0.38 mm) than mesic ecotype leaves (average 9.7 ± 0.38 mm) (Supplemental Table 1). There was no evidence for differences in blade width between dry (average 10.7 ± 0.38 mm) compared to wet and mesic ecotypes at any of the sites. Considering site differences, leaf width was observed to increase (Fig. 3.3E): western KS (8.0 ± 0.29 mm) < central KS (9.3 ± 0.28 mm) < eastern KS (11.2 ± 0.28 mm) < Illinois (14.0 ± 0.37 mm).

Differential canopy area response of ecotypes to rainfall gradient

Canopy area, as scaled by difference in precipitation compared to home site (Fig. 3.5), showed that the wet ecotype canopy area was disproportionately more responsive to increased rainfall based on comparison of quadratic slopes of ecotype. (We used a quadratic function because this function fit better than a linear one). The quadratic slope of wet ecotype response was estimated at 1.56 ± 0.22. That is, for every increase in one cm in precipitation, it corresponds to a 1.56 cm^2 exponential increase in area ($p<0.001$). The wet ecotype was more responsive to increased rainfall than mesic ecotype (0.91 cm^2 exponential increase in area (slope estimate 0.91 ± 0.11, $p<0.0001$) and dry ecotype (0.82 cm^2 exponential increase in area (slope estimate 0.82 ± 0.17, $p<0.0001$). The quadratic slopes of mesic and dry ecotypes in response to rainfall differential were significantly different from wet ecotype (both $p<0.0001$) but dry and mesic ecotypes were not significantly different from each other. This indicates the wet ecotype is more responsive to precipitation.

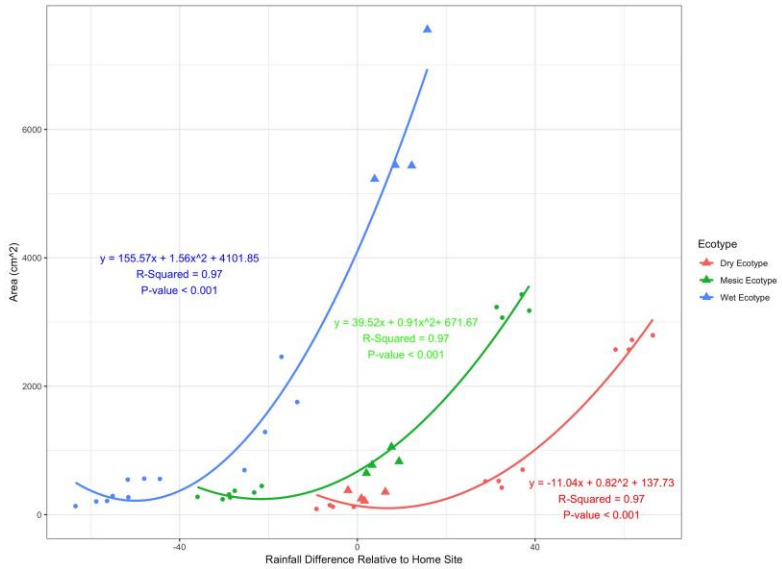

Figure 3.5. Fitted quadratic regression lines for canopy area (cm^2) relative to difference in rainfall at the home site for wet, mesic, and dry ecotypes, compared to rainfall at population source of origin. Note that the home site is depicted by triangles.

B. Biomass Harvest

Vegetative and reproductive biomass both showed a significant 2-way interaction between S and E (Supplemental Table 2, both $p < 0.0001$). To explain these 2-way interactions, we conduct simple-effects analyses for both vegetative and reproductive biomass. Supplemental Fig. 4 shows estimated mean vegetative (A) and reproductive biomass (B) (±SE) for ecotypes at each site in 2011. The general pattern of vegetative biomass appears consistent with CoGV (Fig. 3.1E) and similar to the pattern for canopy area (Fig. 3.3B), with an ecotype-specific increase in vegetative biomass from west to east (Fig. 3.6A). At the dry end of the gradient (i.e., western KS and central KS sites), all ecotypes showed small biomass and no evidence for differences between ecotypes (average 235.1 ± 58.28 g plant^{-1}). Moving east toward more favorable climates, the wet ecotype showed a disproportionately larger biomass relative to dry and mesic ecotypes in the two wetter-most sites (i.e. eastern KS and Illinois), and especially in Illinois (1,124.9 ± 55.2 g plant^{-1}).

Moving on to reproductive biomass, the most striking pattern is that in Western KS, very little reproductive biomass was produced with no evidence for differences among ecotypes (mean 25 g plant^{-1}, Fig 3.6B, Supplemental Table 1). Moving eastward in central KS, reproductive biomass was significantly greater for the dry ecotype (103.0 ± 14.7 g plant^{-1}, Supplemental Tables 1,2) while there was no evidence for differences between mesic and wet ecotypes (mean 46.3 ± 14.5 g plant^{-1}). From central KS to eastern KS, the pattern reverses and the wet ecotype is significantly greater (134.5 ± 14.5 g plant^{-1}) than dry and mesic ecotypes which are not different from each other (mean 72.9 ± 14.9 g plant^{-1}). At the wettest site, there is a disproportionate increase in reproductive biomass for the wet ecotype (307.7 ± 14.5 g plant^{-1}). In summary, the wet and dry

ecotypes show evidence of local adaptation (Fig. 3.1D, Fig. 3.6), but not the mesic ecotype, with highest biomass for the dry and wet ecotypes in their home environment.

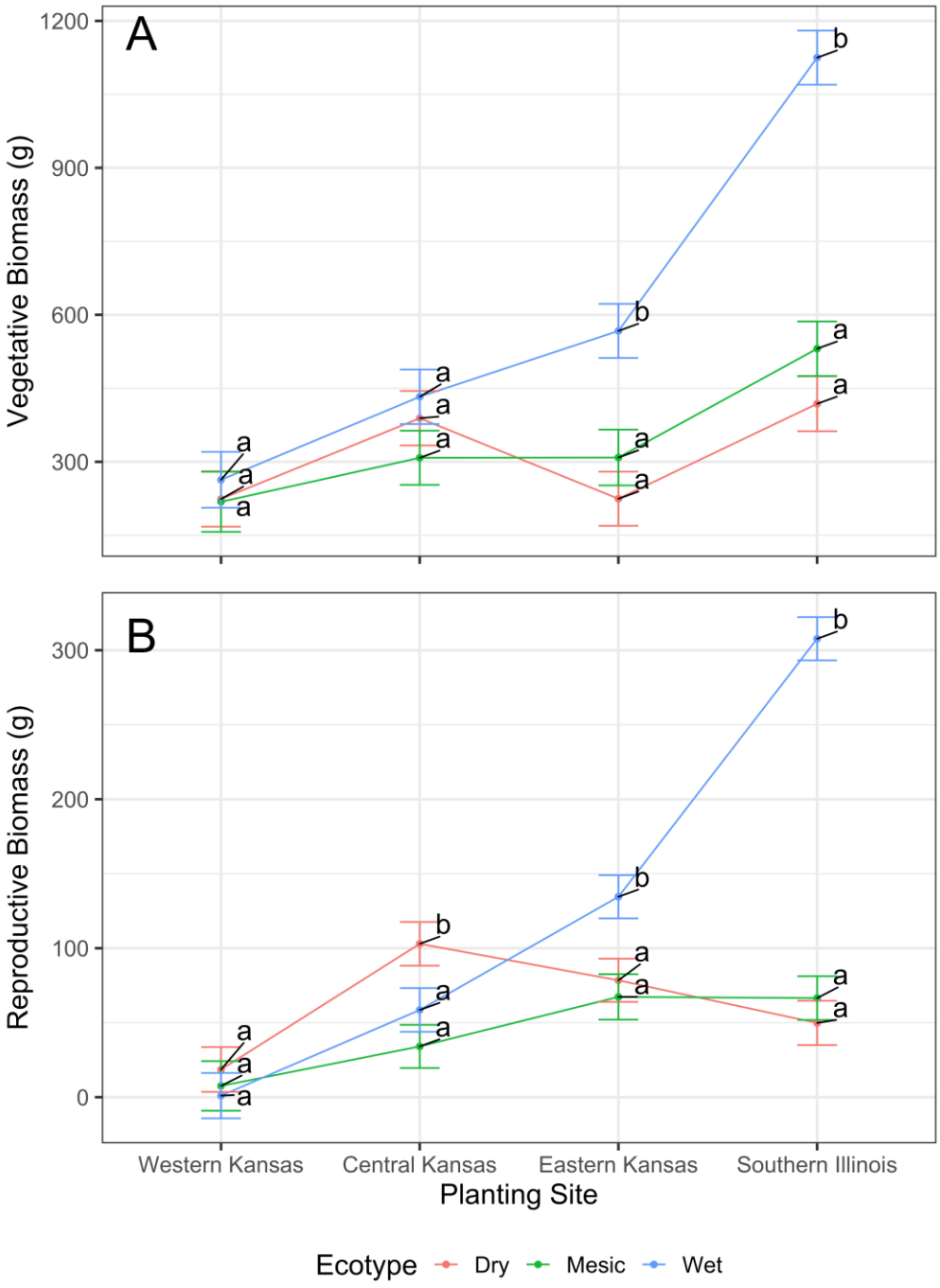

Figure 3.6. . Least square mean estimates (± standard error) for vegetative and reproductive biomass across reciprocal garden planting sites in Western Ks (Colby KS), Central KS (Hays KS), Eastern KS (Manhattan KS), and Illinois (Carbondale Illinois). A) vegetative and B) reproductive. Different letters indicate significant differences among home site ecotypes.

C. Reproductive responses

Probability of anthesis: Main effects of ecotype ($p = 0.004$) and site ($p < 0.0001$) were significant for the probability of anthesis (Fig. 3.7A, Supplemental Tables 1, 2) with no evidence for interaction ($p = 0.1649$). At the driest site, in western KS, probability of reaching anthesis was drastically reduced and close to 0 for both wet and mesic ecotypes (probability.053 ± 0.07 and 0.033 ± 0.03, respectively) compared to the dry ecotype (0.38 ± 0.08). Moving eastward to central KS, the wet (0.79 ± 0.07) and dry ecotypes (0.82 ± .06) were significantly more likely to reach anthesis than the mesic ecotype. In eastern KS and Illinois, probability of anthesis was maximized for all ecotypes (wet 0.93 ± 0.04, dry ecotype 0.95 ± .04 and mesic 0.89 ± 0.05). For all ecotypes, plants were significantly more likely to reach anthesis on the eastern-most sites relative to western-most planting sites.

Days to anthesis: For those plants that did reach anthesis, days to anthesis differed by ecotype ($p = 0.0019$) and site ($p < 0.0001$), but no evidence for any interaction was apparent ($p = 0.7543$) (Supplemental Table 2, Fig. 3.7B). Within a site, the dry ecotype flowered sooner than other ecotypes, with some site-specific differences. In western KS, the dry ecotype was the only ecotype to flower (by October 13) and reached anthesis at approximately 157 ± 5 Julian days. In central KS, there was no evidence for differences in days to anthesis among ecotypes (Supplemental Table 2) (wet 195 ± 13 days, mesic 180 ± 13 days, dry 162 ±7 days). In eastern KS and Illinois sites, the dry ecotype flowered significantly earlier than the mesic and wet ecotypes, but the latter two showed no evidence of differences in days to anthesis. In summary, the dry ecotype flowered sooner than wet and mesic ecotypes by about 20 days (Supplemental Table 1, Fig. 3.7B). In

comparing sites, all ecotypes flowered earlier going eastward by about 60 days (180 Julian days in western KS vs 120 days in Illinois) (Supplemental Table 1, Fig. 3.7B).

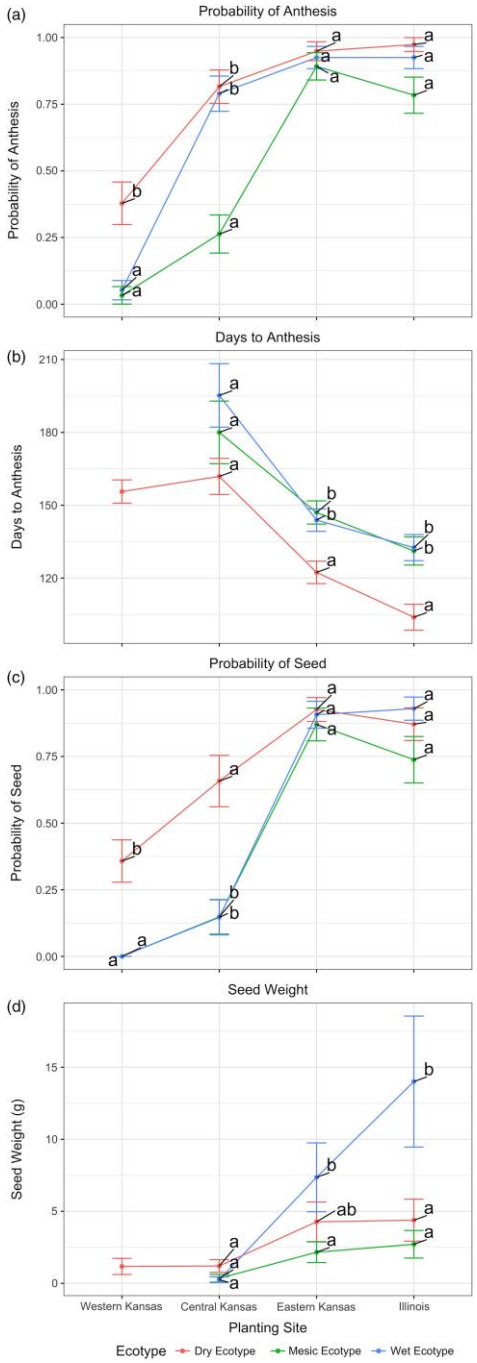

Figure 3.7. Least square mean estimates (±SE) of reproductive fitness traits for ecotypes (dry, mesic, wet) across reciprocal garden planting sites in Western KS (Colby KS), Central KS (Hays KS), Eastern KS (Manhattan KS), and Illinois (Carbondale Illinois). (a) Probability of anthesis, (b) days to anthesis, (c) probability of seed production, (d) seed mass. Sites with different letters indicate significant differences within a site.

Probability of producing seed: The site main effect ($p < 0.0001$) was confounded with a S X E interaction ($p = 0.0330$) (Supplemental Table 2) so we conducted simple-effects analyses with main comparison being ecotypes within each site. (Fig. 3.7C, Supplemental Table 1). The most striking pattern was observed in western KS, whereby the probability of producing seed was estimated at 0.36 ± 0.08 for the dry ecotype, but was negligible for other ecotypes, primarily because they never reached anthesis (Fig. 3.7B). In central KS, probability of seed production was not significantly different (Supplemental Table 2) between wet and mesic (0.15 ± 0.07) ecotypes, but probability was significantly less than dry ecotype in Central KS (0.66 ± 0.10). Going from central KS to eastern KS, probability of producing seed increased for all ecotypes (wet 0.91 ± 0.05, mesic 0.87 ± 0.06, dry 0.93 ± 0.05) but was not significantly different among ecotypes in Eastern KS. Going to the wettest site, there is no significant difference in probability compared to eastern KS and no significant difference among ecotypes there (wet 0.93 ± 0.04, mesic 0.74 ± 0.09, dry 0.87 ± 0.06), but the probability was high as conditions become mesic and wet.

Seed weight: Because site (S) main effect ($p < 0.0001$) was confounded with a S X E interaction ($p = 0.0081$) (Supplemental Table 2), we conducted simple-effects analyses with the main comparison being ecotypes within each site (Fig. 3.7D, Fig. 3.1E, Supplemental Table 1, CoGV). The most striking pattern is that in the western KS site, the dry ecotype was the only ecotype producing seed (1.17 ± .56 g), albeit a small amount, while the other ecotypes failed entirely to produce seed because they did not flower there. Moving eastward in central KS, seed weights were similarly as low and were not significantly different among ecotypes (Supplemental Table 1) (wet 0.25 ± 0.20 g, mesic 0.35 ± 0.26 g, and dry 1.20 ± 0.44 g). From Central KS to Eastern KS sites, seed weight increased for all ecotypes (wet 7.36 ± 2.39 g, mesic 2.16 ± 0.72 g, dry 4.27 ± 1.37 g)

but only mesic differed from wet ecotype. Finally, in the wettest site, there is no significant increase in seed weight compared to eastern KS site. However, the wet ecotype (14.01 ± 4.55 g) was significantly greater than dry (4.38 ± 1.46 g) and mesic ecotypes (2.71 ± 0.97 g), with no evidence for differences between dry and mesic ecotypes. Much of the pattern detected for seed weight mirrors that of reproductive biomass such that seed mass increased west to east. However, with reproductive biomass we see evidence of local adaptation (Fig 3.1D) of the dry and mesic ecotypes, not seen in seed weight in 2012.

2. Home site comparisons shows suite of ecotype-specific traits and climate controls-

a. Subset of home site response variables.

For this data set, we consider response variables measured on ecotypes in their home site (dry ecotype in central KS, mesic ecotype in eastern KS, and wet ecotype in Illinois). Starting with vegetative variables, for emergence, there was no evidence for difference in ecotype emergence (Fig. 3.8A) in dry and mesic home sites, but ecotype emergence in dry and mesic home sites were slightly but significantly later ($p<0.0001$) than the wet ecotype, which emerged 4 days sooner in its home site. For the remaining vegetative responses (Fig.3.8B-F), all showed increases going west to east as climate gets more favorable (central KS < eastern KS < Illinois) (Supplemental Table 1). Canopy area for dry ecotype (236 ± 44 cm^2) was significantly smaller (38%, $p=0.0007$) than mesic ecotype canopy area (615 ± 118 cm^2), which was significantly smaller (11%, $p<0.0001$) than wet ecotype canopy area (5,479 ± 1041 cm^2). Also, dry ecotype diameter in central KS (36 ± 3 cm), was significantly smaller ($p<0.0001$) than mesic ecotype diameter in eastern KS (60 ± 4 cm) which in turn was significantly smaller ($p<0.0001$) than wet ecotype diameter in Illinois (127 ± 4 cm). For height, dry ecotype (23 ± 1 cm) was significantly shorter ($p<0.0001$) than the mesic

ecotype (48 ± 3 cm), and significantly shorter ($p<0.0001$) than wet ecotype (110 ± 6 cm). For blade width, dry ecotype (9.49 ± 0.46 mm) was not significantly different than mesic ecotype blade width (10.40 ± 0.46 mm) but significantly narrower ($p<0.0001$) than blade width of wet ecotype (15.58 ± 0.62 mm). For both vegetative and reproductive biomass, there was no evidence of differences between the dry and mesic ecotypes and both were significantly smaller than the wet ecotype.

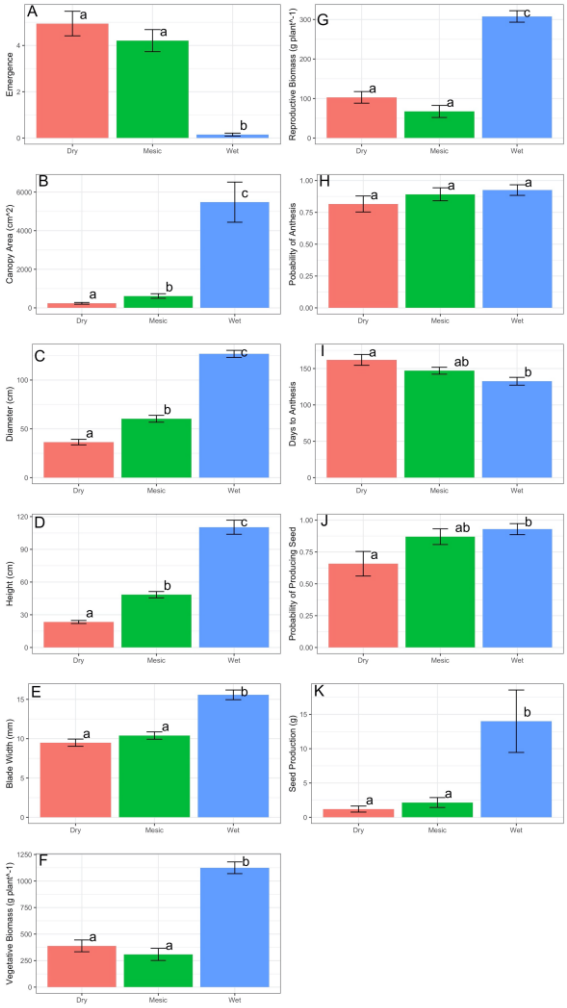

Figure 3.8. Least square mean estimates for vegetative morphological traits and reproductive fitness traits for ecotypes within their home site. Note that Colby was not included because there was no Eastern KS ecotype. A) canopy area, B) height, C) blade width, D) diameter, E) vegetative biomass, F) reproductive biomass, G) probability of anthesis, H) days to anthesis, I) probability of seed production, J) seed mass. Within a trait, Different letters indicate significant differences among home site ecotypes.

Looking at reproductive variables, for probability of anthesis, there were no significant differences among ecotypes in their respective home site (dry ecotype 0.82 ± 0.06, mesic: 0.89 ± 0.05; wet: 0.93 ± 0.04). For the remaining variables, they generally varied from west to east (Fig. 3.8 G-K, Supplemental Table 1). Days to anthesis was longer for the dry ecotype (estimated 162 ± 7 days) compared to the wet ecotype (132.5 ± 5 days). However, the mesic ecotype (147 ± 5 days) showed intermediate days to anthesis and no evidence for difference from the dry or wet ecotype in home sites (Supplemental Table 1). The dry ecotype had a 0.66 ± 0.09 probability of seed production but was significantly less (p=0.022) than probability of seed production for the wet ecotype (.93 ± 0.04). The mesic ecotype was intermediate (0.87 ± 0.06) and showed no evidence for differences from wet and dry ecotypes in their home sites. Fitness in terms of grams of seed per plant was greatest for the wet ecotype (14.01 ± 4.55 g) relative to the mesic ecotype (2.16g ± 0.72 g) and dry ecotype (1.20 ± 0.44 g).

b. Random forest modeling of ecotype traits in home site.

The random forest classification approach corroborated the traits of ecotype morphologies and reproductive fitness in the home site comparison. We assigned to one of three ecotypes (dry, mesic, wet) with accuracy of 94.3% (Supplemental Table 3, Fig. 3.9).

Highest rate of misclassification occurred with individuals of the mesic ecotype, with 4.7% (6 plants) incorrectly classified as dry ecotype and 1% (1 plant) of dry ecotype incorrectly classified as mesic ecotype. Importantly, the wet ecotype in its home environment was never misclassified (Supplemental Table 3). The classification performance of random forests appears to support distinct vegetative and reproductive morphologies, consistent with three ecotypes.

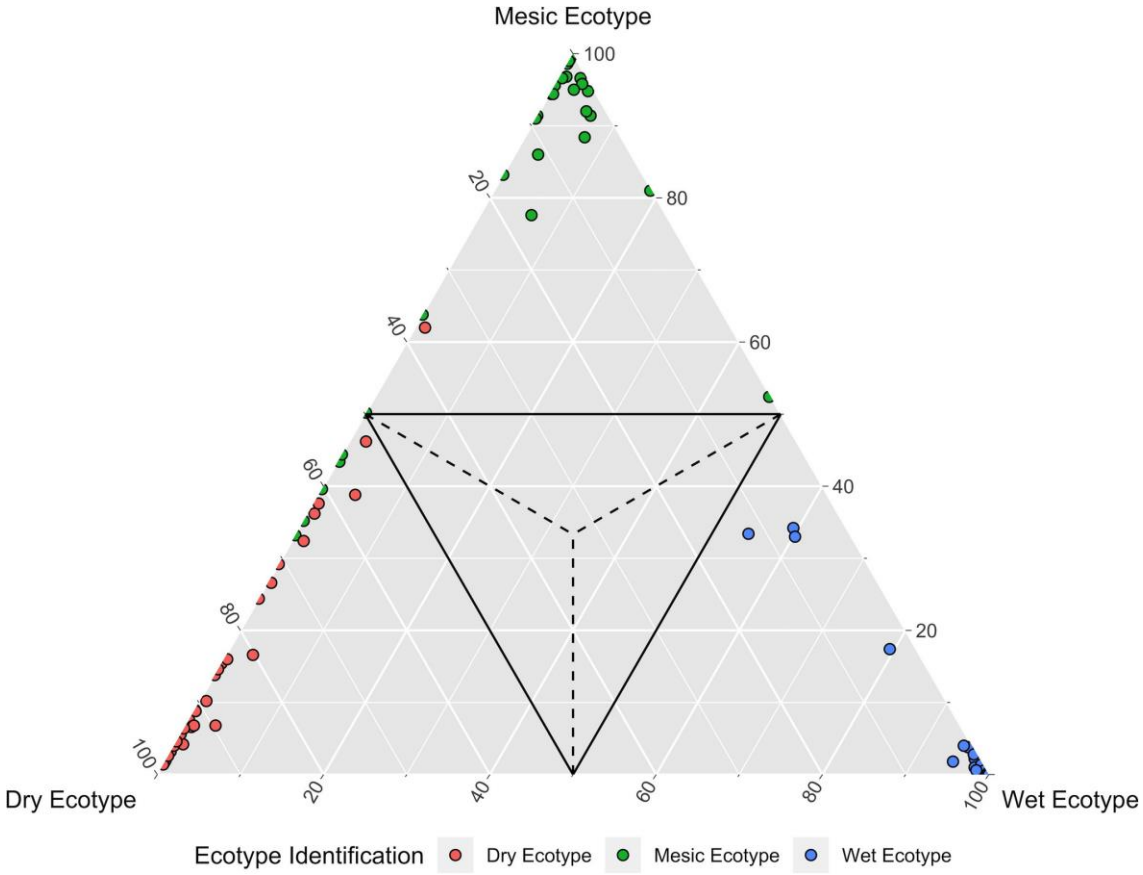

Figure 3.9. Classification plot obtained from the random forest analyses showing training/validation triangle with percent votes of the individuals from the 10 fold-cross validation from random forest training and validation set. Each point is an individual. Dry ecotype is denoted in red, mesic ecotype is denoted in green, and wet ecotype is denoted in blue. Individuals within the solid lines indicate individuals with poor discernment of the algorithm (<50% votes). Plants falling outside of the solid lines are clearly discerned; that is, more than 50% of votes from the validation were for that ecotype

c. PCA of traits from ecotypes grown in their home sites and associations with climate variables from population source of origin.

Using principal components analysis to also characterize the ecotypes, we identified ecotype-specific traits that characterized each ecotype in its home environment. Traits for the wet ecotype represent an especially distinct assemblage compared to dry and mesic ecotypes in their home environments. Scatterplot of PC1 and PC2 of vegetative and reproductive response variables

(Fig. 3.10, Supplemental Table 3) show main clustering of the wet ecotype with overlap between the dry and mesic ecotypes. Scree plots indicate over 60% of variation is explained by PC1 (Fig. 3.10), whereas the first three PC axes account for 80% of the variation in the data. PC1 seemed to be heavily influenced by all variables, as all loadings had an absolute value greater than 0.50. Specifically, in order of loadings, diameter (loading -0.95), height (loading -0.94), canopy area (loading -0.93), vegetative emergence (loading 0.83), blade width (loading -0.76), seed weight (loading -0.57) and finally, days to anthesis (loading 0.55).

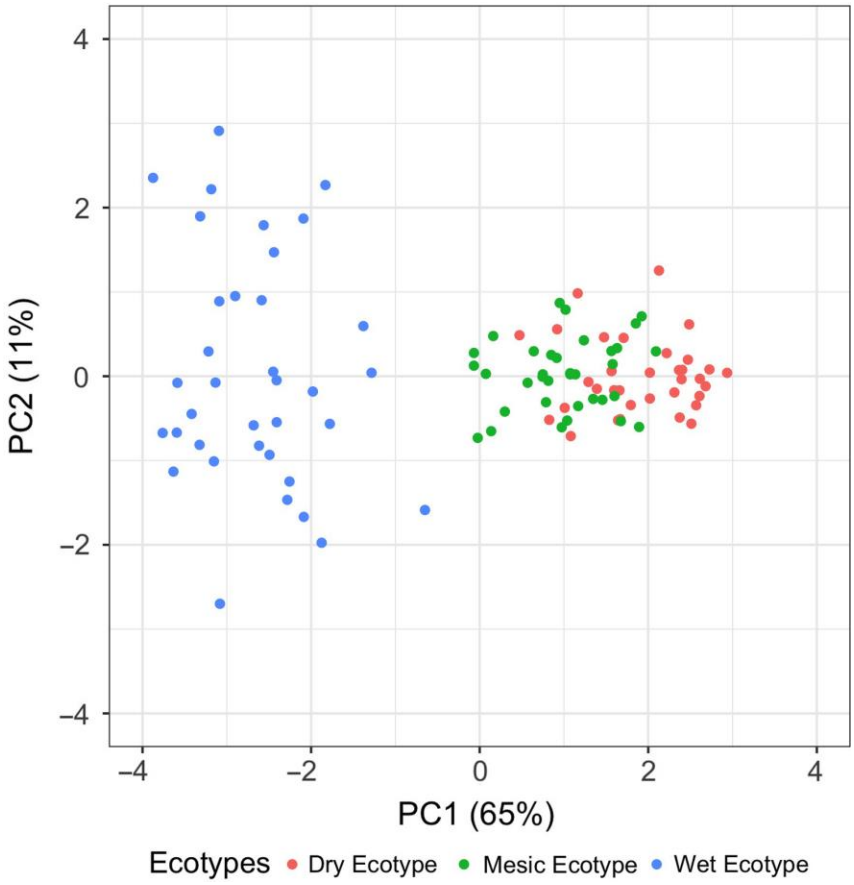

Figure 3.10. Scatter plot of the first two principal component scores for vegetative and reproductive traits corresponding to 106 plants corresponding to ecotypes growing in their homesite. Abbreviations and symbols correspond to regional ecotypes (Red = dry ecotype from Central Kansas; Green = mesic ecotype from Eastern Kansas; Blue = wet ecotype from Illinois). Mesic and dry ecotypes in their home environments were differentiated from the wet ecotype in Illinois prairies mostly along the first PCA axis

Next, we used stepwise regression to evaluate association between ecotypes from PCA scores to home-climate variables to determine which climate variables are important in explaining ecotypic variation. Scores for PC1 (Supplemental Table 4) were negatively associated with seasonal diurnal mean temperature variation (-0.65 ± 0.21) and number of precipitation events (-0.255 ± 0.022) and positively associated with seasonal mean precipitation (0.08 ± 0.02), seasonal mean temperature (0.40 ± 0.09), and annual diurnal temperature variation (1.61 ± 0.20). Scores corresponding to PC2 and PC3 were not significantly associated with any climatic variables (results not shown).

Genetic variation among ecotypes aligns with phenotypic variation

A. Divergence and diversity.

Mesic and dry ecotypes are genetically differentiated from wet ecotype, with reduced diversity in wet ecotype. Principle coordinates analysis (PCoA) of SNP allelic frequencies revealed sorting of wet ecotype as a distinct cluster with partial overlap between dry and mesic ecotype individuals (Fig. 3.11) as was the case for PCA of morphological phenotypes (Fig. 3.10). Scree plots indicate that 18.8% of variation was explained by PCo 1, whereas 4.4% and 4.1% were explained by PCo 2 and 3 respectively. In assessing the association between PCo scores 1 and 2 to environmental conditions, the stepwise selection approach showed that mean annual precipitation (-0.38 ± 0.02) and seasonal precipitation (0.50 ± 0.06) were significantly associated to PCo 1 (Supplemental Table 5) and annual mean temperature (-1.54 ± 0.04), mean annual

precipitation (-0.23 ± 0.02), number of precipitation events <1.25 cm (1.03 ± 0.10), and seasonal mean temperature (1.51 ± 0.24) with PCo 2.

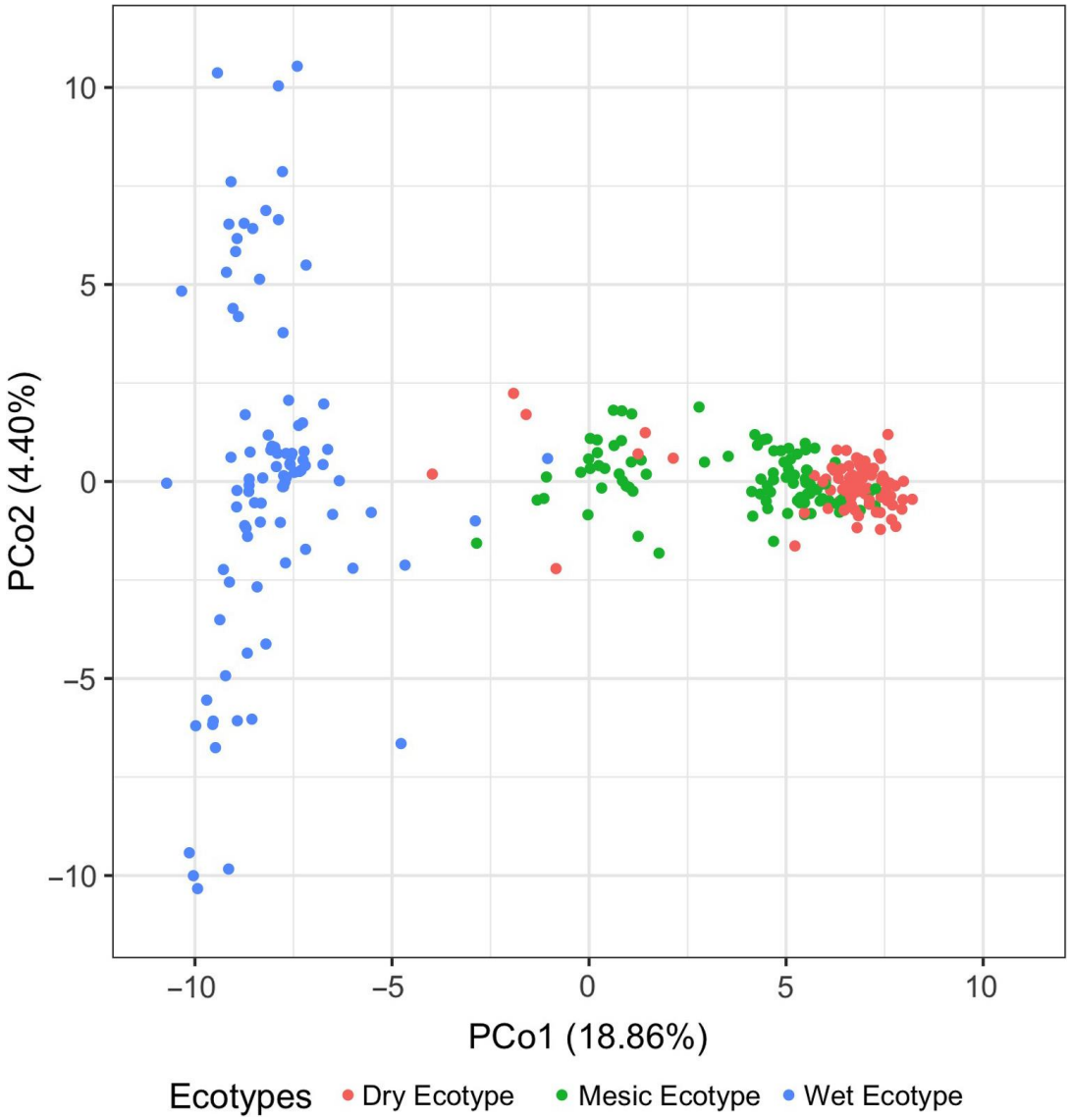

Figure 3.11. Scatter plot of the first two principal coordinates scores for allelic frequency of 4,641 SNP marker loci. There were 314 plants genotyped (110 dry, 106 mesic, and 98 wet ecotype). Abbreviations and symbols correspond to regional ecotypes (Red = dry ecotype from Central Kansas; Green = mesic ecotype from Eastern Kansas; Blue = wet ecotype from Illinois). Mesic and dry ecotypes in their home environments were differentiated from the wet ecotype in Illinois prairies mostly along the first axis

The extent of pairwise genetic differentiation among populations is characterized by F_{st} statistics (Supplemental Table 6). As expected, populations within a given ecotype show low

pairwise genetic distances. Comparing between ecotypes, pairwise F_{st} values 1) between populations of mesic and dry ecotypes F_{st} average is 0.013, 2) between populations of wet and mesic ecotypes F_{st} average is 0.028 and 3) between the wet and dry ecotypes F_{ST} average is 0.030. (F_{sr} estimates: Dry ecotype – 0.012, Mesic ecotype – 0.013, Wet ecotype – 0.021) The greatest genetic distance was apparent between populations from the wet ecotype relative to populations from mesic or dry ecotypes with F_{st} as high as 0.037. Pairs of populations with F_{st} values of 0.028 or below are considered to have undergone slight neutral differentiation (Miermans *et al.* 2011).

B. Genetic outlier analyses and associations with climate.

We identified 64 SNPs in Bayescan showing significant divergent selection across populations, 18 of which were annotated (See Supplemental Table 6). We also used *Bayen*v2 to identify outliers, and provided a list of consensus outliers between methods (Supplemental Table 7). Using two separate approaches allows us to obtain consensus outlier loci to strengthen inferences on selection. For outlier analysis using Bayenv2, the top 1% of the X^TX values comprised 46 SNPs, about half of which had annotations. Candidate gene functions included NAC transcription factors, peroxidases, glutamate synthase, and GA1 (Sb01g021990.1) (Supplemental Table 7), among others. One of the SNP outliers found within a gene of interest and identified in both BayeScan v2.1 and Bayenv2 was GA1, which ranked as 14th highest X^TX differentiated SNP. Gene GA1 codes for a gene ent-copalyl diphosphate synthase that is involved with the first step in the synthesis of gibberellic acid (Hedden and Thomas 2012).

We used *Bayescenv* to assess association between SNP allelic frequency to environmental conditions; out of total 4,641 SNP considered, a subset of 440 SNPs showed significant associations (q-value < 0 .05) with at least one environmental factor (Fig. 3.12, Supplemental Table

8). (Note that SNPs were often associated with more than one environmental factor). Of those 440 SNPs, the greatest number showed a significant association (q-value < 0.05) with seasonal rainfall (96 SNPs) and secondarily, with aspects of temperature (mean annual temperature 76 SNPs, seasonal diurnal variation 60 SNPs). Second, we took only those SNPs identified as outliers in Bayescan and *Bayenv* (110, Supplemental Table 7) and associated their occurrence to climate (Fig. 3.12). Of the SNPs identified as outliers from *Bayescan and Bayenv*, the greatest number of significant associations were significantly associated (q-value < 0.05) with seasonal mean precipitation (41 SNPs), seasonal diurnal variation (29 SNPs) and seasonal annual temperature (25 SNPs) (Supplemental Table 8, Fig. 3.12).

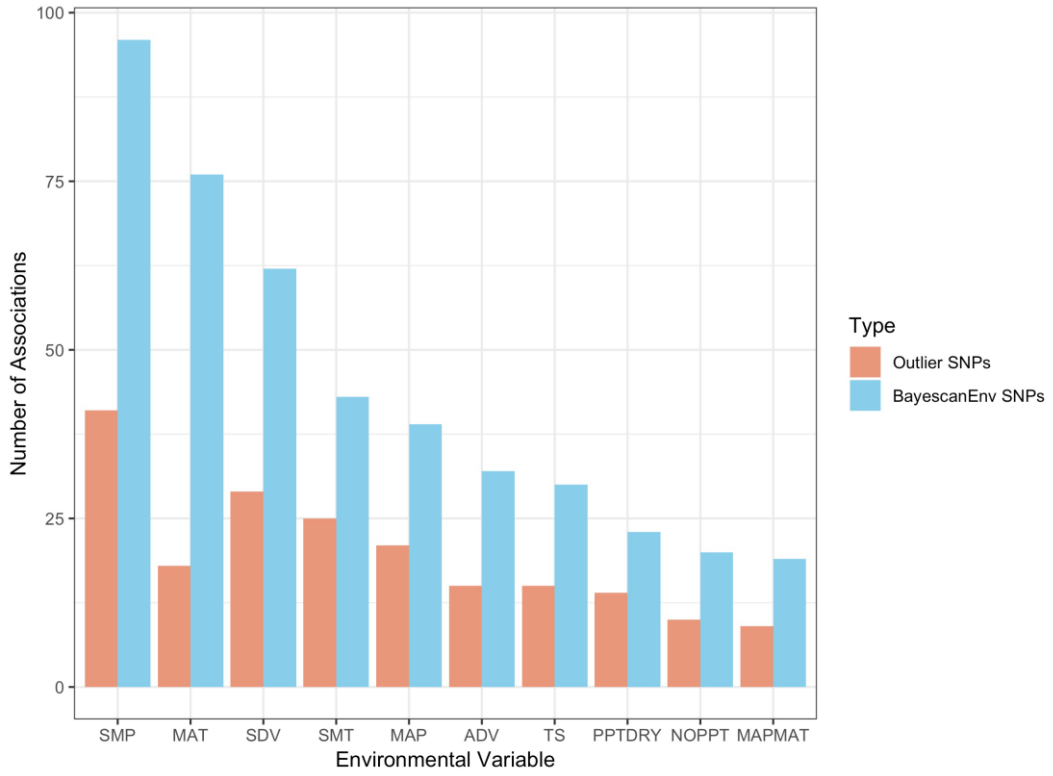

Figure 3.12. Histogram depicting the number of SNP markers associated with environmental conditions based on BayescanEnv analyses. 440 SNPs (out of a total of 4462) were identified in BayescanEnv as being significantly associated with climate. Approximately 10% (of the 4462) were significantly associated with environmental factors (as defined in table 1) and the number of SNPs occurrences for each environmental variable is denoted by blue bar. (Not that a single SNP can be associated with more than one variable) SNPs were most associated

with precipitation variables (20%) and secondarily, with aspects of temperature (mean annual temperature 76, seasonal diurnal variation 60). If one takes all the outliers SNPs identified in Bayescan and Bayenv, 197 SNPs were significantly associated with climate variables (defined in table 1) and the number of SNP occurrences for each environmental variable is denoted by red bar. Outlier SNPs (red bar) were most associated with precipitation variables (20%, or 41), then followed by seasonal mean precipitation followed to a lesser extent by variables related to temperature (seasonal diurnal variation 29, and seasonal annual temperature 25). Climate variable acronyms as in Table 1.

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 (Supplemental Table 9). 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. Geographic and environmental loadings of the full model (1) (Supplemental Table 10) showed that precipitation variables dominated loadings on pRDA1 and temperature variables explained loadings on pRDA2.

C. GWAS: Relating Genotype to Phenotype

Using *TASSLE* to associate genotype and phenotype, 163 SNPs were significantly associated to a morphological variable. Number of SNP associations were: height 87, emergence 38, canopy area 28, blade width 7, diameter 2, and anthesis 1. (Supplemental Table 11). Of the 163 significant SNPs, 33 were also identified as outliers (Supplemental Table 7), with canopy area having 9 SNP associations, height 11, anthesis 1, and emergence 12. Several of these SNP stand out as being functionally significant based on annotations, notably HB-3 transcription factor, GA-1 ent-copalyl diphosphate synthase/ magnesium ion binding, BAN (BANYULS), oxidoreductase

and WOX11 (WUSCHEL related homeobox 11) DNA binding / transcription factor (Supplemental Table 11).

Most notably, the SNP within the GA1 gene showed association with height in ecotypes (GWAS) and was also a genetic outlier in both Bayescan and Bayenv (Supplemental Table 7). We regressed plant height as a function of GA1 frequency and showed evidence for a linear relationship GA 1 SNP variation and height. Results showed the "tall allele" (arbitrarily, the allele that dominates in tall plants) (Fig. 3.13) had the greatest frequency in the wet ecotype, intermediate frequency in mesic ecotype and lowest frequency in dry ecotype, each in their home environments. The wet ecotype was about 5x taller than dry ecotype in their home sites (Fig. 3.3D, Fig. 3.4C).

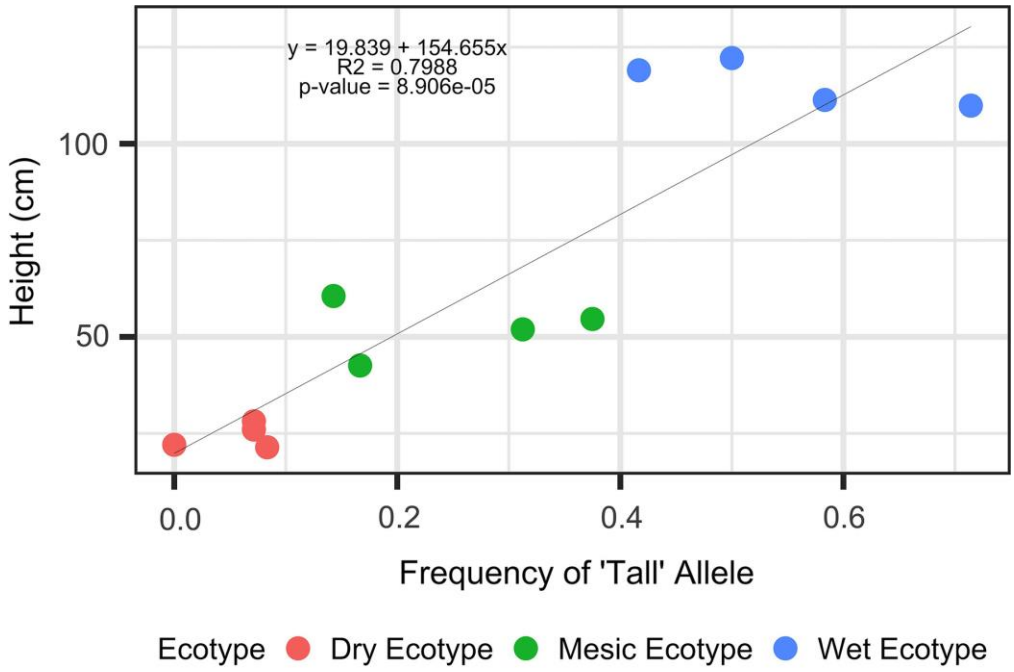

Figure 3.13. Scatter plot and fitted regression line depicting average population plant height as a function of allelic frequency for the "tall" allele of the GA1 outlier. Each population is color-coded by ecotype. Red = dry ecotype, green = mesic ecotype, blue = wet ecotype. The four points per ecotype represent the four source populations

DISCUSSION

Here, we document ecotypic variation in the dominant prairie grass *A. gerardii* across the spatially varying climatic gradient of the U.S. Great Plains. We found evidence for local adaptation only for reproductive biomass in wet and dry ecotypes. We observed strong CoGV in most vegetative traits (Conover and Schultz 1995; Crispo 2008; Conover *et al.* 2009). Further, home site comparisons clearly highlight the extent to which ecotypes have adaptively diverged in their home site. Finally, phenotypic differences among ecotypes are underpinned by genetic differences and outliers in traits such as height and stress response. We found a notable outlier SNP in GA1 whose allele frequency varies clinally with height of ecotypes across the Great Plains. Genetic outliers show climate associations, primarily with growing season precipitation and secondarily growing season temperature. Below we delve into reciprocal garden phenotypic responses, home site trait syndromes, genetic bases for traits, and finally implications for climate change and restoration.

1. Phenotypic variation in reciprocal gardens show role for plasticity and genetics.

Transplant experiments are ideal to investigate forms of phenotypic variation and to quantify the extent to which phenotypic differences across environmental gradients are caused by genetic variation and/or phenotypic plasticity (Clausen *et al.* 1940; McMillan 1959, 1965). A number of studies have investigated sources of variation of plant performance in environments with contrasting climate selection pressures (Clausen *et al.* 1940; Schmid 1985; Weber and Schmid 1998; Bell *et al.* 2000; Etterson 2004), all using reciprocal transplant experiments. Both plasticity and genetic variation have been widely observed in many settings, such with altitude (Clausen *et al.* 1940; Byars *et al.* 2007; Gonzalo-Turpin and Hazard 2009), precipitation gradients (Anderson

et al. 2015; Eckhart *et al.* 2004), and latitudinal clines (McMillan 1959, 1965; Chapin *et al.* 1981; McGraw *et al.* 2015).

We expected a balance between environmental phenotypic plasticity vs. genetic adaptive variation (Bradshaw 1984; Linhart and Grant 1996) due to differing strength of spatially varying selective forces such as precipitation. If selection from long-term climate were strong, especially due to strong spatial heterogeneity in rainfall (Axelrod 1985), local adaptation to climate could be expected (Galliart *et al.* 2019). Local adaptation is defined as an interaction between ecotype and site, with ecotypes from local populations outperforming non-local transplants in different climates (Linhart and Grant 1996) and depicted as S x E (Fig. 3.1). Interestingly, local adaptation was only observed in for reproductive biomass (Fig. 3.6B) in wet and dry ecotypes. However, we did observe complicated patterns of S, E and S X E interactions. Next we consider the types and strength of these effects and their patterns of interaction.

We detected environmentally relevant S X E interactions, especially for seed production (Fig. 3.7D). For example, only the dry ecotype produced seed in Western KS. Conversely, all ecotypes produced seed on the wet end of the gradient, though the wet ecotype produced significantly more seed compared to dry and mesic ecotypes under the favorable conditions of Illinois. This suggests that spatially varying climate may be imparting strong selection on reproductive fitness in these ecotypes and causing differentiation for this key fitness trait.

Ecotypes also differed in terms of flowering time. The dry ecotype flowered earlier (21-30 days) compared to the other ecotypes depending on site. This is a putative adaptation to speed up reproduction in response to end of season drought in central and western KS. Early flowering time in response to drought has also been observed in reciprocal garden studies of *Clarkia* (Eckhart *et al.* 2004) and in experimental evolution studies with *Mimulus* (Dickman *et al.* 2019) and *Brassica*

(Hamann *et al.* 2018). However, most examples of changes in flowering time deal with latitude and day length (McMillan 1959, 1965) or coastal-inland gradients (Lowry *et al.* 2009). Strong co-gradient plasticity of phenology across environmental gradients seen in *Rhinanthus minor* could provide a mechanism to buffer against variable climates (Ensing and Eckert 2019). In contrast, the flowering time differences observed here portends for beginnings of genetically based reproductive isolation (Nosil 2005) especially between wet and dry ecotypes, further reductions in gene flow, and ultimately speciation.

In contrast with reproductive traits, most vegetative response variables showed a pattern of CoGV (Fig. 3.1E). CoGV was observed such that at the dry end of the gradient in western KS site, several vegetative responses (canopy area, height, diameter) were similar in magnitude and did not show evidence for differences among ecotypes there. Presumably, harsh dry conditions minimized ecotype trait differences, even for the local ecotype. Small stature is expected to be favored as an adaptation to reduce water loss in dry and windy conditions of the western KS reciprocal gardens (Kramer *et al.* 2018) and in other studies (Eckhart *et al.* 2004; Byars *et al.* 2007). Moving eastward into more favorable, mesic environments, ecotype differences in vegetative traits became more apparent and showed clear signs of CoGV (Figs. 3.1E, 3.3B-D, Fig. 3.4). Nearly all vegetative phenotypes of the three ecotypes increased in magnitude under more favorable conditions of increasing rainfall, with the wet ecotype responding disproportionately greater than the other two ecotypes across the gradient (Figs. 3.3B-D, Fig. 3.4). Our results agree with a recent review (Conover *et al.* 2009) that documented that CoGV was often associated with morphological variables. This is a similar pattern observed along altitudinal clines (Clausen *et al.* 1940), such as *Potentilla glandulosa* in the Sierra Nevada Mountains, *Poa* in Australian mountain (Byars *et al.* 2007), and *Festuca* in the Pyrenees (Gonazalo-Turpin and Hazard 2009). In stressful

environments, such as high elevations or the dry, water-limited western Great Plains, plants putatively allocate more resources to growth and survival than flowering and seed production (Ogden and Harper 1970; Harper 1977; Bloom *et al.* 1985; Chapin 1991; Gonzalo-Turpin and Hazard 2009). In more favorable habitats, plants produce more seed. In the moister, favorable environment of Illinois, ecotypes showed greatly increased seed production, especially of the local ecotype.

Interestingly, our results do not agree with recent studies showing local adaptation of vegetative cover of wet and dry ecotypes in seeded community plots over 5 years (Galliart *et al.* 2019) where ecotypes grew with other species, as they would in nature. In the community plots, ecotypes compete with themselves and other species for resources such as light, nutrients and water. In contrast, the single plants of this reciprocal garden experiment grew without competition and rarely showed signs of local adaptation. Apparently, only in a realistic community do we observe local adaptation expressed in these ecotypes, showing the importance of biotic interactions in driving local adaptation (Bischoff *et al.* 2006; Grassein *et al.* 2014; Galliart *et al.* 2019), thus highlighting the need to put local adaptation in the context of the community.

2. Home site comparisons show suites of traits that distinguish ecotypes.

Home site comparisons highlight the extent to which these ecotypes have adaptively diverged. Home site comparisons are also aligned with random-forest-based classification and PCA scatterplots, both showing distinctive trait assemblages for each ecotype. Traits often respond to selection by climate (Chapin *et al.* 1991, Chapin *et al.* 1993) and are often correlated, resulting in adaptive trait syndromes (Ackerly *et al.* 2000; Aspinwall *et al.* 2013). Our results also agree with recent meta-analyses of plant form in grasses such that grasses of the wetter, limited

environments were characterized by broad leaves (Galleher *et al.* 2019). In contrast, grasses of open habitats were characterized by narrow, short leaves (Gallaher *et al.* 2019), putatively associated with water-limited open, arid environments.

In terms of adaptive trait syndromes, in the wettest site, plants of the wet ecotype were robust, tall, with large canopy, wide diameter, high vegetative biomass, and broad leaves. These traits may allow the wet ecotype to compete with the abundant tall forbs and shrubs in the highly competitive, species-dense, light-limited prairie peninsula of Illinois (Kuchler 1964). Indeed, height is a major determinant of a plant's ability to compete for light (Moles *et al.* 2010), based on global analyses. The wet ecotype in its climate characterized by adequate moisture and temperature also had a high probability of flowering and producing large quantities of seed. Similarly, at Konza Prairie, *A. gerardii* produced more flowering stalks in wetter years (LaPierre *et al.* 2011) and showed reduced flowering stalk production under rainout shelters (Swemmer *et al.* 2006). Similar to results from Eckhart *et al.* (2004), in our study, the wet ecotype took the shortest time to flower in its home environment (132.5 days) because it putatively experiences greater number of growing degree days sooner in the moderate climate of Illinois compared to other sites (3799 growing degree days in Hays, KS vs 4087 in Illinois; Johnson *et al.* 2015).

On the other hand, at the dry end of the gradient, plants were dwarfed, with small diameter, reduced canopy area (Kramer *et al.* 2018) and low vegetative biomass and narrow leaves, indicative of a water-limited environment. Yet, the dry ecotype flowered soonest out of the ecotypes, perhaps due to harsher conditions (Table 3.1), and shorter growing degree days there. Thus, growth form and fitness were limited by the harsh water-limited environment of central KS. Put in broader terms, the local ecotype must adjust growth to match its limited resources (Chapin *et al.* 1991; Chapin *et al.* 1993), putatively water in central KS and light in Illinois.

Extensive phenotypic variation of *A. gerardii* has been observed from this experimental reciprocal garden platform in anatomy (Olsen *et al.* 2013), chlorophyll absorbance (Caudle *et al.* 2014), root production (Mendola *et al.* 2016), photosynthesis (Maricle *et al.* 2017), stomates (Varvel *et al.* 2018) and morphology (Kramer *et al.* 2018). In terms of plant productivity across the Great Plains climate gradient, Epstein *et al.* (1996) reported an increase in productivity with increased rainfall along a longitudinal gradient for *A. gerardii*. Avolio and Smith (2013) also found phenotypic variation in *A. gerardii* in experimental rainfall manipulations. Looking at temperature, in a study of switchgrass genotypes from varying latitudes of origin, Aspinwall *et al.* (2013) found evidence for the role of temperature in a suite of adaptive traits related to morphology and physiology.

3. Genetic differences underlying phenotypes.

Phenotypes were structured, in part, by genetic differences among ecotypes (Gray *et al.* 2014; Price *et al.* 2012). The wet ecotype was sharply differentiated from the dry ecotype in terms of morphology and reproductive features. In spite of having low F_{st} and low levels of neutral genetic differentiation among populations and ecotypes, presumably selection pressures associated with mainly precipitation were strong enough to maintain differences in spite of gene flow. Other studies have found differentiation in spite of gene flow (Gonzalo-Turpin and Hazard 2009). Importantly, the strong differences in flowering time between wet and dry ecotypes portend the future reduction flow and reproductive isolation.

Genetic outliers were likely of adaptive significance and suggest divergent selection, presumably due to selection from spatially varying climate. Ecotypes differed in terms of candidate genes such as NAC transcription factor, glutamate synthase, peroxidase, and GA1. The SNP in

GA1 had an allele frequency that varied clinally across the Great Plains and has high ecological and functional significance with wet ecotypes growing 4.7 times taller than the dry ecotype, Fig. 3.13). Expression of GA1 controls internode length and consequently height (Millach *et al.* 2002). Height correlates with increased biomass, and putatively greater competitiveness (Moles *et al.*2010), as would be advantageous in wet, light-limited prairie peninsula dominated by tall forbs and shrubs (Kuchler 1964). Conversely, the dry ecotype would be advantaged by short stature to reduce evaporative loss as an adaptation to water-limited climates such as central and western KS (Maricle *et al.* 2017; Kramer *et al.* 2018). Other studies have also identified other candidate genes across altitudinal gradients (Rellstab *et al.* 2016; Pluess *et al.* 2016), latitudinal gradients (Hancock *et al.* 2011), and precipitation gradients (Exposito-Alonso *et al.* 2018). These studies provide powerful insight into candidate genes and genetic mechanisms responsible for adaptive divergence.

Outlier SNPs identified in *Bayscanenv* showed a clear relationship with climate especially precipitation and temperature variables. Furthermore, pRDA shows that climate, more than geography structures genetic variation. Our study took an approach using outlier candidate genes across gradients, i.e., genome-environmental associations as highlighted in recent reviews (Bragg *et al.* 2015; Rellstab *et al.* 2015; Sork *et al.* 2016; Laskey *et al.* 2018).

Recent empirical studies have detected genome-environmental associations. *Arabidopsis halleri* showed genomic footprints of selection to altitude in the Alps (Fischer *et al.* 2013). Laskey *et al.* (2012) used redundancy analyses to quantify the association between climate, geography and genomics in Eurasian *Arabidopsis* populations and discovered 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.* (2018) 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.

4. Implications for climate change.

Understanding how climate structures genetics, form, and function of a dominant grass species is critical to predicting grassland response to climate change. Several lines of evidence suggest that climate, especially seasonal precipitation and temperature variation, structures *A. gerardii* ecotype form and genetic variation. Other studies of *A. gerardii* using redundancy analyses corroborate that climate, more than geographic location (distance), structures neutral genetic variation (Galliart *et al.* 2019). Third, genetic outliers based on *Bayscanenv* were related to both seasonal precipitation and temperature variation. Precipitation and temperature patterns have been in place for the last 10,000 years (Axelrod 1985), leading to selective pressure and ultimately adaptive differentiation. Our study showed a complex mosaic of abiotic stressors, not just precipitation, across the Great Plains to which *A. gerardii* must adapt either genetically and/or plastically. Adaptation was observed with experimental manipulation of rainfall and temperature (Ravenscroft *et al.* 2015), showing some genotypes were preferred in different experimental treatments. Resurrection experiments show selection after drought (Dickman *et al.* 2019, Hamann *et al.* 2018). *Arabidopsis* ecotypes tolerated extreme drought (Exposito-Alonso *et al.* 2018) through within-species variation in drought tolerance and its evolutionary response to climate.

Such knowledge of intraspecific variation across precipitation gradients and the relative importance of plasticity vs. genetic adaptive variation are critical to predict and model grassland biome responses to climate change (Aspinwall *et al.* 2013; Smith *et al.* 2017; Yurkonis & Harris 2019). This knowledge is urgently needed to predict grassland response to warmer and drier

summers in the Great Plains. The year 2012 was the worst drought in 50 years with unprecedented "mega-droughts" predicted for the central U.S. (Cook *et al.* 2015). A recent phenotypic modeling study predicted that by 2070, with climate change, populations of short-statured, dwarf forms of *A. gerardii* from dry parts of its range would be favored ~800 km eastward and result in 60% decrease in biomass, if it can migrate there in time or be planted through restoration (Smith *et al.* 2017). Reduction in productivity could have cascading effects on prairie function (Knapp *et al.* 1998, Hoover *et al.* 2014), cattle forage production (Gibson *et al.* 2016), grassland restoration (Baer *et al.* 2019), and conservation.

5. Informing restoration.

Tallgrass prairie, one of the most diverse grasslands, is critically endangered (Hoekstra *et al.* 2005) with only 4% native prairie remaining (Samson and Knopf 1994). For example, less than 1,000 ha remain in eastern tallgrass prairie compared to the original 9 million ha (Samson and Knopf 1994). Our study informs land management and restoration strategies because knowledge of climate-matched ecotypes (Hufford and Mazer 2003; McKay *et al.* 2005; Nicotra *et al.* 2010) is critical to the future ecology and sustainability of grasslands. Of particular interest, we provide scientific foundation to land managers on ecotype suitability to climate, which is relevant to the USDA Conservation Reserve program (http://www.ks.nrcs.usda.gov/programs/crp/) that restores grasslands on marginal agricultural lands (SCS 1990). *Andropogon gerardii* is widely used in these conservation plantings throughout the North American central grassland, covering nearly 3 million ha (http://www.fsa.usda.gov). Furthermore, about 60% of total agricultural production in KS (~$10 billion, NASS 2018) was attributed to cattle production, and *A. gerardii* is the main forage grass for cattle in this region. Our experiment demonstrates that local adaptation may limit a

population's ability to adjust to changing climates. If populations cannot adjust to environmental change through phenotypic plasticity, populations will have to migrate to match their future climate conditions (Smith *et al.* 2017), or migration will need to be facilitated through human intervention, restoration (Nicotra *et al.* 2010; Christmas *et al.* 2016). Thus, knowledge of climate-matched ecotypes is urgently needed to prevent local extinction in changing climates.

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Chapter 3 Supplemental Tables

	Ecotype	Western Kansas	Central Kansas	Eastern Kansas	Southern Illinois
Emergence (units) 2010	**Dry**	12.1817 ± 1.1169	***4.9455 ± 0.5306***	4.1009 ± 0.4573	0.6913 ± 0.1437
	Mesic	11.9116 ± 1.1679	6.1284 ± 0.6271	***4.2081 ± 0.4766***	0.6732 ± 0.14
	Wet	12.2145 ± 1.0912	5.0276 ± 0.5322	4.1392 ± 0.4561	***0.1498 ± 0.06224***
Canopy (cm^2) 2010	**Dry**	81.57 ± 15.7679	***235.54 ± 44.9974***	411.4 ± 78.1480	2031.76 ± 385.95
	Mesic	195.93 ± 41.3724	314.95 ± 59.6531	***614.58 ± 118.148***	2785.08 ± 529.05
	Wet	140.2 ± 26.9490	314.03 ± 59.6531	1181.66 ± 224.47	***5479.18 ± 1040.8***
Canopy (cm^2) 2014	**Dry**	2393.83 ± 649.52	***2953.63 ± 634.6***	2490.33 ± 640.44	5437.11 ± 664.7
	Mesic	2162.06 ± 709.71	2283.75 ± 652.19	***2341.46 ± 647.35***	5363.91 ± 652.12
	Wet	3376.03 ± 647.33	4889.11 ± 652.11	5099.52 ± 642.88	***13775 ± 652.11***
Diameter (cm) 2010	**Dry**	24.3974 ± 2.7323	***36.4123* ± 2.8323**	48.1352 ± 3.4505	87.5931 ± 3.6794
	Mesic	38.1319 ± 2.9733	44.4393 ± 2.8325	***60.3327 ± 3.5115***	102.30 ± 3.6786
	Wet	35.1818 ± 2.6848	50.4293 ± 2.7820	80.2354 ± 3.3977	***126.76 ± 3.6285***
Diameter (cm) 2014	**Dry**	75.2644 ± 3.5022	***78.6601 ± 3.4727***	77.0613 ± 3.444	98.1805 ± 3.7798
	Mesic	74.4843 ± 3.9772	74.0586 ± 3.5592	***78.9596 ± 3.5917***	99.856 ± 3.7289
	Wet	83.5922 ± 3.5037	95.6035 ± 3.5397	103.88 ± 3.4747	***143.37 ± 3.6014***
Blade Width (mm) 2010	**Dry**	8.2821 ± 0.4661	***9.4908 ± 0.4607***	11.25 ± 0.458	13.75 ± 0.6169
	Mesic	7.3248 ± 0.5099	8.306 ± 0.4607	***10.4016 ± 0.4636***	12.8 ± 0.6169
	Wet	8.3354 ± 0.4635	9.95 ± 0.458	11.9 ± 0.458	***15.575 ± 0.6169***
Height (cm) 2010	**Dry**	14.301 ± 0.8569	***23.387 ±1.3809***	32.577 ± 1.9097	78.399 ± 4.5961
	Mesic	24.078 ± 1.5976	32.284 ± 1.9064	***48.434 ± 2.8822***	82.487 ± 4.8359
	Wet	24.312 ± 1.4467	37.074 ± 2.1735	65.175 ± 3.8209	***110.283 ± 6.4654***
Height (cm) 2014	**Dry**	56.8105 ± 2.8852	***62.5429 ± 2.8264***	68.725 ± 2.7721	103.43 ± 3.0608
	Mesic	65.7187 ± 3.2185	64.7111 ± 2.7989	***75.8707 ± 2.8272***	110.53 ± 2.9517
	Wet	79.066 ± 2.8571	87.3967 ± 2.8279	87.6125 ± 2.7721	***140.03 ± 2.8866***
Vegetative Biomass (g per plant)	**Dry**	223.98 ± 56.48	***388.94 ± 55.63***	224.53 ± 55.24	418.37 ± 56.04
	Mesic	218.27 ± 61.33	308.12 ± 55.24	***308.68 ± 56.94***	530.84 ± 55.63
	Wet	263.16 ± 57.03	432.72 ± 55.65	567.14 ± 55.24	***1124.94 ± 55.23***
Reproductive Biomass (g per plant)	**Dry**	18.5807 ± 15.0391	***102.97 ± 14.6761***	78.4569 ± 14.505	49.8466 ± 14.853
	Mesic	7.5699 ± 16.6620	34.0776 ± 14.505	***67.3245 ± 15.238***	66.5200 ± 14.676
	Wet	1.0163 ± 15.2386	58.5502 ± 14.678	134.54 ± 14.5051	***307.73 ± 14.5051***
Probability of Anthesis 2012	**Dry**	0.3784 ± 0.07973	***0.8158 ± 0.06289***	0.9487 ± 0.03532	0.9737 ± 0.02597
	Mesic	0.03333 ± 0.03277	0.2632 ± 0.07143	***0.8919 ± 0.05105***	0.7838 ± 0.06768
	Wet	0.05263 ± 0.03622	0.7895 ± 0.06613	0.9250 ± 0.04165	***0.9250 ± 0.04165***
Days to Anthesis Julian days 2012	**Dry**	155.62 ± 4.8113	***161.87 ± 7.4362***	122.35 ± 4.6211	103.89 ± 5.3609
	Mesic	-	180.00 ± 12.8923	***147.01 ± 4.7781***	131.16 ± 5.7908
	Wet	-	195.22 ± 13.0580	143.88 ± 4.6222	***132.54 ± 5.3620***

Probability of Seed Production 2012	**Dry**	0.3580 ± 0.07934	***0.6579 ± 0.09646***	0.9264 ± 0.04467	0.8713 ± 0.06134
	Mesic	8.117E-9 ± 0.000017	0.1485 ± 0.06487	***0.8703 ± 0.06161***	0.7380 ± 0.08700
	Wet	7.725E-9 ± 0.000014	0.1463 ± 0.06566	0.9068 ± 0.05035	***0.9296 ± 0.04340***
Seed Production (g per plant) 2012	**Dry**	1.1704 ± 0.5601	***1.2018 ± 0.4397***	4.2709 ± 1.3739	4.3822 ± 1.4588
	Mesic	-	0.3467 ± 0.2648	***2.1576 ± 0.7191***	2.7106 ± 0.9565
	Wet	-	0.2481 ± 0.1982	7.3587 ± 2.3897	***14.0057 ± 4.5491***

S Table 1. Estimated least square means and corresponding SE of vegetative, biomass, and reproductive response variables by ecotype for each planting site. Note: Home site in bold italics

	Site		Ecotype		Site x Ecotype		Time		Site x Time		Ecotype x Time		Site x Ecotype x Time	
Variable	$F_{Df\ n,\ Df\ d}$	P	$F_{Df\ n,\ Df\ d}$	P	$F_{Df\ n,\ Df\ d}$	P	$F_{Df\ n,\ Df\ d}$	P	$F_{Df\ n,\ Df\ d}$	P	$F_{Df\ n,\ Df\ d}$	P	$F_{Df\ n,\ Df\ d}$	P
Canopy Area (2010, 2014)	213.33 $_{3,\ 36.26}$	**<0.0001**	14.63 $_{2,\ 9.1}$	**0.0015**	5.33 $_{6,\ 805.6}$	**<0.0001**	1204.87 $_{1,\ 795.2}$	**<0.0001**	90.44 $_{3,\ 795.2}$	**<0.0001**	11.48 $_{2,\ 795.2}$	**<0.0001**	1.50 $_{6,\ 795.2}$	0.01738
Diameter (2010, 2014)	270.35 $_{3,\ 33.27}$	**<0.0001**	3.66 $_{2,\ 8.95}$	0.0688	4.96 $_{6,\ 803.5}$	**<0.0001**	426.70 $_{1,\ 793.4}$	**<0.0001**	36.65 $_{3,\ 793.4}$	**<0.0001**	18.04 $_{2,\ 793.4}$	**<0.0001**	4.38 $_{6,\ 793.4}$	**0.0002**
Height (2010, 2014)	108.68 $_{3,\ 37.53}$	**<0.0001**	24.00 $_{2,\ 8.1}$	**0.0004**	27.85 $_{6,\ 801.1}$	**<0.0001**	108.68 $_{1,\ 792.8}$	**<0.0001**	12.31 $_{3,\ 792.8}$	**<0.0001**	39.99 $_{2,\ 792.8}$	**<0.0001**	5.04 $_{6,\ 792.8}$	**<0.0001**

	Site		Ecotype		Site x Ecotype	
Emergence	218.84 $_{3,\ 28.73}$	**<0.0001**	3.80 $_{2,\ 22.3}$	**0.0380**	2.81 $_{6,\ 439}$	**0.0107**
Blade Width (2010)	94.16 $_{3,\ 37.1}$	**<0.0001**	5.33 $_{2,\ 10}$	**0.0264**	1.01 $_{6,\ 331}$	0.4162
Vegetative Biomass (2011)	13.31 $_{2,\ 9}$	**0.0020**	62.86 $_{3,\ 36}$	**<0.0001**	16.01 $_{6,\ 385}$	**<0.0001**
Reproductive Biomass (2011)	31.15 $_{2,\ 9}$	**<0.001**	36.70 $_{3,\ 36}$	**<0.0001**	27.61 $_{6,\ 385}$	**<0.0001**
Probability of Anthesis (2012)	29.82 $_{3,\ 27}$	**<0.0001**	10.56 $_{2,\ 9}$	**0.0044**	1.68 $_{6,\ 27}$	0.1649
Days to Anthesis (2012)	41.22 $_{3,\ 27}$	**<0.0001**	9.13 $_{2,\ 17.6}$	**0.0019**	0.47 $_{4,\ 27}$	0.7543
Probability of Seed Production (2012)	42.17 $_{2,\ 27}$	**<0.0001**	3.10 $_{2,\ 7.7}$	0.1028	3.07 $_{4,\ 27}$	**0.0330**
Seed Production (2012)	17.31 $_{3,\ 44.57}$	**<0.0001**	3.52 $_{2,\ 13.6}$	0.0587	3.53 $_{4,\ 223.9}$	**0.0081**

S Table 2. Statistical analyses of response variables from 2010, 2011, 2012 and 2014. Factors include planting site (S), Ecotype (E), and S X E interactions. Upper table; Variables with multiple years of measurements (Canopy Area, Diameter, and Height). Lower table Variables with only one year of measurements (Blade width, Vegetative biomass, Reproductive biomass, Probability of anthesis, Days to anthesis, Probability of seed production, and Seed production). For variables measures across two years (2010 and 2014 for canopy area, diameter, and height) the full three-way interaction with Time (T) is provided.

	Dry Ecotype	Mesic Ecotype	Wet Ecotype
Dry Ecotype	33 (31.1%)	1 (0.9%)	0
Mesic Ecotype	5 (4.7%)	30 (28.3%)	0
Wet Ecotype	0	0	37 (34.9%)

S Table 3. Table showing the classification rates of the 10-fold cross validation. Note that 94.3% are correctly assigned (data from along the diagonal). Total of 106 individuals analyzed with 34 dry, 35 mesic, and 37 wet ecotypes.

Parameter Estimates Principal Coordinate 1					
Parameter	DF	Estimate	Standard Error	t-value	p-value
Intercept	1	-18.83779	2.902155	-6.49	1.0000
Annual Diurnal Variation	1	1.614488	0.196429	8.22	0.0118
Number of Precipitation Events > 1.25 cm	1	-0.255112	0.025752	-9.91	0.0010
Seasonal Mean Precipitation	1	0.081677	0.021503	3.8	<.0001
Seasonal Mean Temperature	1	0.404559	0.086862	4.66	<.0001
Seasonal Diurnal Variation	1	-0.6459	0.213481	-3.03	0.0032

S Table 4. Final model obtained from stepwise regression depicting selected environmental conditions considered to be significantly associated with morphology PC score 1 for ecotypes grown in their home site

Parameter Estimates Principal Coordinate 1					
Parameter	DF	Estimate	Standard Error	t-value	p-value
Intercept	1	8.569889	1.465610	5.85	1.0000
Mean Annual Precipitation	1	-0.381848	0.022347	-17.09	<.0001
Seasonal Mean Precipitation	1	0.496011	0.055912	8.87	<.0001
Parameter Estimates Principal Coordinate 2					
Parameter	DF	Estimate	Standard Error	t-value	p-value
Intercept	1	-15.448569	3.852113	-4.01	1.0000
Annual Mean Temperature	1	-1.540529	0.435119	-3.54	0.0005
Mean Annual Precipitation	1	-0.231000	0.024784	-9.32	<.0001
Number of Precipitation Events > 1.25 cm	1	1.035900	0.095066	10.90	<.0001
Seasonal Mean Temperature	1	1.509246	0.240293	6.28	<.0001

S Table 5. Final model obtained from stepwise regression depicting selected environmental conditions considered to be significantly associated with genetic distance principle coordinates 1 and 2 for ecotypes grown in their home site. Adjusted R^2 PC1 = 0.5593 and Adjusted R^2 PC2 = 0.3936.

12MI (Wet)	DES (Wet)	FUL (Wet)	WAL (Wet)	KON (Mesic)	TAL (Mesic)	TOW (Mesic)	CAR (Mesic)	CDB (Dry)	REL (Dry)	SAL (Dry)	WEB (Dry)	
0.000												12MI
0.023	0.000											DES
0.020	0.022	0.000										FUL
0.018	0.021	0.020	0.000									WAL
0.029	0.034	0.026	0.028	0.000								KON
0.026	0.029	0.023	0.025	0.014	0.000							TAL
0.032	0.035	0.028	0.030	0.011	0.014	0.000						TOW
0.024	0.028	0.021	0.022	0.013	0.014	0.013	0.000					CAR
0.033	0.035	0.029	0.032	0.012	0.017	0.012	0.014	0.000				CDB
0.030	0.033	0.027	0.028	0.012	0.016	0.011	0.013	0.012	0.000			REL
0.024	0.028	0.021	0.023	0.012	0.014	0.012	0.011	0.013	0.011	0.000		SAL
0.033	0.037	0.029	0.033	0.012	0.016	0.011	0.014	0.011	0.012	0.013	0.000	WEB

S Table 6. Pairwise F_{st} among pairs of populations.

Footnote: Pairwise F_{st} between ecotypes: Wet-Mesic = 0.0275, Standard Deviation = 0.004; Wet-Dry = 0.0297, Standard Deviation = 0.004; and Mesic-Dry = 0.013, Standard Deviation = 0.002.

Analysis	Markers	Chr	Position	Gene Id	Gene Information
Bayescan	TP3814	2	56601981	Sb02g023140.1	PF07732,PF00394,PF07731 PTHR11709 KOG1263 AT4G39830.1 L-ascorbate oxidase, putative
Bayescan	TP23759	2	53236055	Sb02g021490.1	PF03055 PTHR10543 KOG1285 AT3G63520.1 CCD1 CCD1 (CAROTENOID CLEAVAGE DIOXYGENASE 1); 9-cis-epoxycarotenoid dioxygenase
Bayescan	TP27974	6	39909469	Sb06g014410.1	PF02416 AT5G52440.1 HCF106 HCF106; proton motive force dependent protein transmembrane transporter
Bayescan	TP63759	1	55492323	Sb01g032580.1	PF03195 AT1G67100.1 LBD40 LBD40 (LOB DOMAIN-CONTAINING PROTEIN 40)
Bayescan	TP69704	10	42173347	Sb10g019720.1	PF02453 PTHR10994 KOG1792 AT4G11220.1 BTI2 BTI2 (VIRB2-INTERACTING PROTEIN 2)
Bayescan	TP70880	9	47608900	Sb09g019060.1	PF02182,PF05033,PF00856 PTHR22884 KOG1082 AT5G13960.1 SUVH4 SUVH4 (SU(VAR)3-9 HOMOLOG 4); double-stranded methylated DNA binding / histone methyltransferase(H3-K9 specific) / methyl-CpG binding / methyl-CpNpG binding / methyl-CpNpN binding
Bayescan	TP79982	3	71975298	Sb03g044630.1	PF02362,PF06507 AT5G62000.1 ARF2 ARF2 (AUXIN RESPONSE FACTOR 2); protein binding / transcription factor
Bayescan	TP90370	3	6472172	Sb03g006365.1	PTHR23273 KOG0851 AT4G19130.1 DNA binding / nucleic acid binding / zinc ion binding
Bayescan	TP100780	5	5098115	Sb05g004185.1	PF03140 AT3G44710.1 unknown protein
Bayescan	TP105473	2	77853480	Sb02g044040.1	PF02178,PF00855 AT3G05430.1 PWWP domain-containing protein
Bayescan	TP110077	8	1046928	Sb08g001115.1	PF02493,PF01504 PTHR23086 KOG0229 2.7.1.68 K00889 AT3G07960.1 phosphatidylinositol-4-phosphate 5-kinase family protein
Bayescan	TP119018	1	60290323	Sb01g036670.1	PF00046 PTHR19418 AT3G03660.1 WOX11 WOX11 (WUSCHEL related homeobox 11); DNA binding / transcription factor
Bayescan	TP150845	4	61081684	Sb04g031080.1	PF03169 PTHR22601 KOG2262 AT5G64410.1 OPT4 OPT4 (OLIGOPEPTIDE TRANSPORTER 4); oligopeptide transporter
Bayescan	TP152852	2	77115416	Sb02g043300.1	PF00046 PTHR19418:SF56 AT2G33880.1 HB-3 HB-3; transcription factor
Consensus	TP97659	1	26916072	Sb01g021990.1	PF01397,PF03936 5.5.1.13 K04120 AT4G02780.1 GA1 GA1 (GA REQUIRING 1); ent-copalyl diphosphate synthase/ magnesium ion binding
Consensus	TP22546	3	10907551	Sb03g010110.1	PF00097 AT1G75400.1 protein binding / zinc ion binding
Consensus	TP48670	7	18950899	Sb07g010450.1	PF00141 1.11.1.7 K00430 AT4G16270.1 peroxidase 40 (PER40) (P40)
Consensus	TP14132	3	59665754	Sb03g031310.1	PF00310,PF04898,PF01645,PF01493,PF07992,PF00070 PTHR11938 KOG0399 1.4.1.13 K00265 AT5G53460.1 GLT1 GLT1; glutamate synthase (NADH)
Bayenv2	TP171997	7	11673324	Sb07g007530.1	PF01263 PTHR11122 KOG1594 AT5G57330.1 aldose 1-epimerase family protein
Bayenv2	TP4696	1	73079404	Sb01g050120.1	PTHR10343 AT3G52180.1 SEX4 SEX4 (STARCH-EXCESS 4); polysaccharide binding / protein tyrosine/serine/threonine phosphatase
Bayenv2	TP103404	3	52366325	Sb03g026040.1	PF00160 PTHR11071 KOG0885 5.2.1.8 K01802 AT4G33060.1 peptidyl-prolyl cis-trans isomerase cyclophilin-type family protein

Bayenv2	TP16994	9	51311839	Sb09g021780.1	PF02365 AT5G22380.1 anac090 anac090 (Arabidopsis NAC domain containing protein 90); transcription factor
Bayenv2	TP23740	2	67684318	Sb02g033050.1	AT5G42330.1 unknown protein
Bayenv2	TP63473	4	57503730	Sb04g027590.1	PF00191 PTHR10502 KOG0819 AT5G10230.1 ANNAT7 ANNAT7 (ANNEXIN ARABIDOPSIS 7); calcium ion binding / calcium-dependent phospholipid binding
Bayenv2	TP107489	1	47278328	Sb01g027420.1	PF05770 AT4G33770.1 inositol 1,3,4-trisphosphate 5/6-kinase family protein
Bayenv2	TP68168	9	188110	Sb09g000390.1	PF00023,PF07719,PF00515 PTHR18958 KOG0548 AT3G04710.1 ankyrin repeat family protein

S Table 7. Candidate gene annotations from Bayescan analysis (50), the top 1% of Bayenv2 outlier analysis (46), and the consensus markers (15) between the two analyses. Identification is from mapping big bluestem reads to the sorghum genome to find the position. Table shows identifies how the gene was found, using Bayenv or Bayescan or both (Analysis), big bluestem marker id (Markers), to which sorghum chromosome the marker maps (Chr) position on that chromosome (Position), gene identifier of the gene near the sorghum position (Gene Id), and the annotation information from that gene (Gene Information).

Footnote: Bayenv outliers lacking annotation included TP67155, TP505, TP125027, TP39152, TP168589, TP35977, TP61552, TP29318, TP119835, TP123355, TP69553, TP143151, TP171774, TP105122, TP147959, TP132908, TP103399, TP156046, TP168955, Both Bayenv and Bayescan TP1712, TP114252, TP118569, TP5219, TP62618, TP19463, TP52052, TP27663, TP104813, TP34353, TP47256; Bayescan TP24620, TP25391, TP27700, TP28582, TP38460, TP48595, TP49355, TP61449, TP77269, TP79145, TP91916, TP95678, TP97404, TP97704, TP129182 , TP124284, TP144242, TP161527, TP164302, TP172967, TP173308

Marker Number	SMP	MAT	SDV	SMT	MAP	ADV	TS	PPTDRI	NOPPT	MAP /MAT
142	-	-	0.0010002	-	-	-	-	-	-	-
392	-	-	0.019604	-	0.017604	0.013203	-	-	-	-
429	-	-	-	-	-	-	-	-	-	-
478	0.005201	-	0.015403	0.014003	-	-	-	-	-	-
561	-	-	0.0056011	-	-	-	-	-	-	-
730	0.00040008	-	0.026605	-	-	-	-	-	-	-
752	0.0026005	-	-	0.010802	-	-	-	-	-	-
789	0.026205	-	0.005001	0.014403	-	-	-	-	-	-
919	0.014803	-	-	-	-	-	-	-	-	-
929	0.0018004	-	-	0.0054011	0.013003		-	-	-	-
969	0.00080016	-	0.003800 8	0.016603	0.0030006	0.0044009	0.0044009	0.011602	0.008401 7	0.008601 7
999	0.00080016	0.00080016	0.002000 4	***0.027806***	0.0066013	0.0086017	0.013603	0.016203	0.015803	***0.021204***
1124	0.00020004	-	-	-	-	-	-	-	-	-
1186	-	-	0.002600 5	-	0.0018004	0.0040008	0.0012002	0.0016003	**0.023405**	0.011202
1219	0.0028006	-	-	-	-	**0.021404**	-	-	-	-
1227	-	0.00080016	0.021604	0.00040008	0.0020004	-	0.014203	-	***0.023805***	0.013603
1252	0.00020004	-	-	0.0028006	0.00080016	0.00020004	0.0012002	0.00080016	0.001800 4	0.000600 12
1272	0.0094019	-	-	-	-	-	-	-	-	-
1341	0.00040008	0.00080016	0.001200 2	0.0030006	0.020604	-	-	**0.020804**	-	-
1573	-	-	-	-	-	-	-	-	-	-
1620	0.0024005	-	0.002200 4	-	0.011602	0.0074015	0.0034007	0.010802	-	-
1764	0.012803	-	-	-	-	-	-	-	-	-
1781	-	0.00040008	0.011202	0.011202	-	-	-	-	-	-
1857	***0.030206***	-	-	-	-	-	-	-	-	-
2065	0.023605	-	-	-	-	-	-	-	-	-
2072	0.016003	-	-	-	-	-	-	-	-	-
2205	0.0072014	-	-	0.0026005	***0.024805***	-	-	-	-	-
2354	-	-	-	-	-	-	-	-	-	-
2438	-	-	-	-	-	-	-	-	-	-
2522	-	-	0.011802	-	-	-	-	-	-	-
2598	-	-	-	-	-	-	-	-	-	-
2754	0.010402	-	0.002200 4	-	-	-	-	-	-	-
2788	0.014203	-	0.006001 2	0.0060012	-	-	-	-	-	-
2792	-	-	0.012402	-	-	-	-	-	-	-
2912	-	0.00060012	0.000200 04	0.013803	-	-	0.0030006	0.0036007	0.001200 2	0.002400 5

2921	-	-	-	-	-	0.00060012	-	-	-	-
3060	0.010802	0.0046009	-	-	-	-	-	-	-	-
3146	-	-	-	-	-	-	-	-	-	-
3167	0.0016003	0.0030006	0.021204	0.0074015	0.022204		-	-	-	-
3302	-	-	-	0.012402	0.00040008	0.0022004	0.0044009	0.0054011	-	0.012002
3305	-	-	-	-	-	-	-	-	-	-
3402	0.0036007	-	-	0.005201	0.015203	-	-	-	-	-
3435	0.0036007	0.00060012	-	-	-	-	-	-	-	-
3438	0.0074015	-	-	0.0022004	-	-	-	-	0.013403	-
3482	0.0012002	0.00020004	***0.028606***	**0.025405**	0.0076015	-	-	-	-	-
3514	-	0.0030006	0.00200004	-	0.00040008	0.0028006	0.00020004	0.0024005	0.00380008	0.00120002
3536	0.024405	-	-	-	-	-	-	-	-	-
3567	-	0.00020004	-	-	0.020804	-	-	-	-	-
3587	-	0.0024005	0.00060012	0.0028006	0.013403	0.004801	0.013603	0.018204	-	-
3781	0.0022004	0.0020004	0.022605	-	0.0034007	-	0.010002	-	0.0058012	**0.013803**
3806	0.00080016	-	-	-	-	-	-	-	-	-
3935	0.0020004	0.0026005	-	-	-	-	-	-	-	-
4008	0.0054011	-	0.025405	-	**0.023805**	-	-	-	-	-
4057	0.00080016	-	0.0034007	0.010802	-	-	-	0.016203	-	-
4189	0.0038008	-	-	0.00060012	-	-	-	-	-	-
4268	-	0.0022004	0.0090018	0.0014003	0.013803	0.004801	**0.016203**	***0.021604***	-	-
4349	0.0062012	***0.014803***	-	0.0014003	-	-	-	-	-	-
4371	0.0018004	**0.0056011**	0.024205	0.00020004	-	***0.022204***	-	-	-	-
4451	0.00040008	0.00040008	-	0.0032006	-	-	-	-	-	-
4464	-	-	-	-	-	-	-	-	-	-
4470	0.0020004	0.0024005	**0.028006**	-	0.0062012	-	0.012402	-	0.0068014	-
4513	0.0040008	-	0.00040008	-	-	0.011402	0.014003	0.0046009	-	-
4560	0.019404	-	-	-	-	0.016603	***0.019604***	0.011202	-	-
4573	**0.030206**	-	-	-	-	-	-	-	-	-

S Table 8. *Bayscenv* climate association with SNPs and 11 climate variables. For each environmental variable (column), the most strongly associated SNP is indicated with bold italics and underlined, and the second strongest association is indicated with only bold. Only significant associations between outliers and environmental variables are presented here (q-value < 0.05), values indicate estimates of the strength of selection for a given loci with each climate variable (dashes indicate nonsignificant associations)

.

Variable	Markers	Chromosome #	Position	Gene Id	Gene Information
Height	TP14132	Chromosome_3	59665754	Sb03g031310.1	PF00310,PF04898,PF01645,PF01493,PF07992,PF00070 PTHR11938 KOG0399 1.4.1.13 K00265 AT5G53460.1 GLT1 GLT1; glutamate synthase (NADH)
Height	TP152852	Chromosome_2	77115416	Sb02g043300.1	PF00046 PTHR19418:SF56 AT2G33880.1 HB-3 HB-3; transcription factor
Height	TP22546	Chromosome_3	10907551	Sb03g031310.1	PF00097 AT1G75400.1 protein binding / zinc ion binding
Height	TP48670	Chromosome_7	18950899	Sb07g010450.1	PF00141 1.11.1.7 K00430 AT4G16270.1 peroxidase 40 (PER40) (P40)
Height	TP70880	Chromosome_9	47608900	Sb09g019060.1	PF02182,PF05033,PF00856 PTHR22884 KOG1082 AT5G13960.1 SUVH4 SUVH4 (SU(VAR)3-9 HOMOLOG 4); double-stranded methylated DNA binding / histone methyltransferase(H3-K9 specific) / methyl-CpG binding / methyl-CpNpG binding / methyl-CpNpN binding
Height	TP97659	Chromosome_1	26916072	Sb01g021990.1	PF01397,PF03936 5.5.1.13 K04120 AT4G02780.1 GA1 GA1 (GA REQUIRING 1); ent-copalyl diphosphate synthase/ magnesium ion binding
Canopy Area	TP63759	Chromosome_1	55492323	Sb01g032580.1	PTHR10887 KOG1801 AT1G65810.1 tRNA-splicing endonuclease positive effector-related
Canopy Area	TP70880	Chromosome_9	47608900	Sb09g019060.1	PF02182,PF05033,PF00856 PTHR22884 KOG1082 AT5G13960.1 SUVH4 SUVH4 (SU(VAR)3-9 HOMOLOG 4); double-stranded methylated DNA binding / histone methyltransferase(H3-K9 specific) / methyl-CpG binding / methyl-CpNpG binding / methyl-CpNpN binding
Emergence	TP119018	Chromosome_1	60290323	Sb01g036670.1	PF00046 PTHR19418 AT3G03660.1 WOX11 WOX11 (WUSCHEL related homeobox 11); DNA binding / transcription factor
Emergence	TP70880	Chromosome_9	47608900	Sb09g019060.1	PF02182,PF05033,PF00856 PTHR22884 KOG1082 AT5G13960.1 SUVH4 SUVH4 (SU(VAR)3-9 HOMOLOG 4); double-stranded methylated DNA binding / histone methyltransferase(H3-K9 specific) / methyl-CpG binding / methyl-CpNpG binding / methyl-CpNpN binding
Blade Width	TP171997	Chromosome_7	11673324	Sb07g007530.1	PF01263 PTHR11122 KOG1594 AT5G57330.1 aldose 1-epimerase family protein
Blade Width	TP26923	Chromosome_3	73373694	Sb03g046210.1	PF00036 PTHR10891 KOG0027 AT1G76640.1 calmodulin-related protein, putative
Blade Width	TP124709	Chromosome_6	58175486	Sb06g029600.1	PF01370 PTHR10366 KOG1502 1.3.1.77 K08695 AT1G61720.1 BAN BAN (BANYULS); oxidoreductase
Diameter	TP144464	Chromosome_6	44415397	Sb06g016010.1	PF06258 AT5G22350.1 ELM1 ELM1 (ELONGATED MITOCHONDRIA 1)
Diameter	TP69676	Chromosome_2	27812706	Sb02g014480.1	PF07719,PF00515,PF00564 PTHR22904 KOG4151 AT2G25290.1 binding

STable 9. SNPs associated with response variables based on *TASSLE* analyses. Total number of SNPs associated = 173 (anthesis 11, height 87, canopy area 28, blade width 7, emergence 38, diameter 2). Of those SNPS that were associated in *TASSLE* and also *Bayescan* outliers, there were a total of 33 (anthesis 1, height 11, canopy area 9, blade width 0, emergence 12, and diameter 0). Only those associations with annotated SNPs are shown here. Gray shading indicates the GA1 SNP.

Markers that were associated, but not annotated: TP118569, TP19463, TP129182, TP14132, TP164302, TP19463, TP25391, TP27700, TP34353, TP47256, TP48595, TP52052, TP61449, TP62618, TP79145, TP91916, TP40577, TP53225, TP64928, TP82066, TP93930, TP106232, TP107688, TP124841, TP125449, TP126048, TP147316, TP153041, TP174440, TP177326, TP15153, TP70053,, TP7498, TP106075, TP140561, TP68489, TP135366, TP169807, TP12076, TP162226, TP44305, TP100504, TP49032, TP101481, TP135939, TP123320, TP18961, TP163294, TP138332, TP143687, TP505, TP180468, TP160634, TP156386, TP151095, TP164220, TP29647, TP117156, TP97430, TP62307, TP86868, TP106592, TP100082, TP144170, TP24765, TP102312, TP113188, TP71544,

TP148121, TP34326, TP131010, TP129945, TP80136, TP160911, TP125027, TP93235, TP132908, TP104813, TP114252, TP19463, TP34353, TP52052, TP62618, TP79145, TP53225, TP93930, TP169807, TP82066, TP103399, TP124841, TP135366, TP107688, TP174440, TP138332, TP147316, TP106232, TP177326, TP7498, TP106075, TP162226, TP173892, TP92195, TP104813, TP114252, TP161527, TP19463, TP34353, TP47256, TP52052, TP61449, TP62618, TP79145, TP82066, TP124841, TP177326, TP87444, TP53225, TP70053, TP68489, TP107688, TP93930, TP156386, TP174440, TP147316, TP113188, TP40577, TP44305, TP7498, TP106232, TP18961, TP160372, TP2227, TP30673, TP140561, TP97430, TP124841, TP22543, TP160634, TP174440.

Chapter 4 - Bringing genomics outdoors: Ecological and transcriptional responses of wet and dry ecotypes of a dominant prairie grass *Andropogon gerardii* across the Great Plains' climate gradient

This chapter is currently in preparation for submission planned for *Molecular Ecology*.

INTRODUCTION

Ecologists and evolutionary biologists have long been intrigued by the role of environment vs genetic background in controlling expression of phenotypes (Clausen *et al.*, 1940; Clausen *et al.* 1948; Turesson, 1922; VanWallendael et al, 2022; MacTavish & Anderson 2020) adaptation, fitness and ultimately formation of new species (Rundle & Nosil, 2005). Early seminal studies of Turreson (1922) and Clausen *et al.* (1948) introduced the concept of environment (E) vs genotype (G) and G x E interactions. These studies often used reciprocal gardens, i.e., cross-transplanting ecotypes of the same species into different environments, as the gold standard to assess environmental and genetic controls (VanWallendael *et al.*, 2022; Johnson *et al.*, 2021; Lortie & Hierro, 2021). Early studies were followed by seminal work by McMillan (1965) in Great Plains grasslands and later other studies in model organisms such as *Arabidopsis* (Exposito-Alonso *et al.*, 2018; Agren & Schemske, 2012), *Mimulus* (Selby & Willis, 2018; Popovic & Lowry, 2020), and *Panicum* switchgrass (Lovell *et al.*, 2021; Lowry *et al.*, 2019; Milano *et al.* 2016). Now, fast forward, into the genomic era where we can routinely measure differences in nucleotide variation in DNA (Feder & Mitchell-Olds, 2003) and gene expression in non-model organisms to find the genes that matter in nature. Thus, the next grand biological challenge is to take genomics outdoors

to understand organismal response in the natural environment (Feder & Mitchell-Olds, 2003; Narum *et al.*, 2013; Savolainen *et al.*, 2013; Ungerer *et al.*, 2008). Genomic technology has ushered in the new era of ecological genomics (Narum *et al.*, 2013; Savolainen *et al.*, 2013) allowing us to drill down to DNA and RNA variation to probe ecological responses of organisms to their changing environment. With this approach, we have discovered plant response to environment such as heavy metals at the level of SNP in metal transporters in *Mimulus* (Selby & Willis, 2018), drought tolerance in grasses *Panicum hallii* (Lovell *et al.*, 2016; Lovell *et al.*, 2021), and *Boechera* (MacTavish & Anderson, 2020).

Ecotypic variation has been widely studied across plant species for over a century (Johnson *et al.* 2021; MacTavish & Anderson 2020; VanWallendael et al, 2022; Rushworth *et al.* 2022). Previous work has uncovered differences in physical characteristics such as drought tolerance (Exposito-Alonso *et al.*, 2018), flowering time (Fournier-Level *et al.*, 2022) and phenology across ecotypes (Taylor *et al.* 2019, Smith *et al.* 2017; McMillan, 1969). These studies take advantage of natural variation across environmental gradients such as elevation (Montesinos-Navarro *et al.* 2011), temperature or latitude (Schemske, 2009), or rainfall (Chen & Schemske, 2015). Ultimately, these have proven useful to several fields including agriculture, especially the use of natural variation of landraces in plant breeding (Gaurav *et al.*, 2022; Reynolds *et al.* 2020), conservation and restoration ecology (Hufford & Mazer, 2003; A. Gibson *et al.* 2016, Baer *et al.* 2019), as well as broadening basic understanding of evolutionary adaptation to the environment (MacTavish & Anderson, 2020; Lovell *et al.*, 2021; Lowry *et al.*, 2019; Milano *et al.* 2016; Exposito-Alonso *et al.*, 2018). However, only in the last decade has a multidisciplinary approach been possible which merges traditional ecological studies with modern molecular techniques to study ecotypic variation across scales (Ungerer *et al.*, 2008; Feder & Mitchell-Olds, 2003)). In this

paper, we utilize the strong rainfall gradients across the Great Plains of the US to reveal adaptation in plant form and functional traits (Etterson, 2004; Lovell *et al.*, 2021; Lowry *et al.*, 2019; Milano *et al.* 2016; McMillan, 1969) and gene expression. As an example of how much we have advanced, ecotypes of a dominant grass, *Andropogon gerardii*, along a latitudinal temperature gradient of the Great Plains, were originally described over fifty years ago (McMillan, 1959) but have not been characterized at the genetic level until recently (Galliart *et al.* 2019, 2020; Gray *et al.*, 2014; Hoffman & Smith, 2017; Price *et al.*, 2012). Surprisingly, rarely has grass variation been studied in response to precipitation which is arguably the single most important environmental factor driving intraspecific variation (Siepielski *et al.*, 2017).

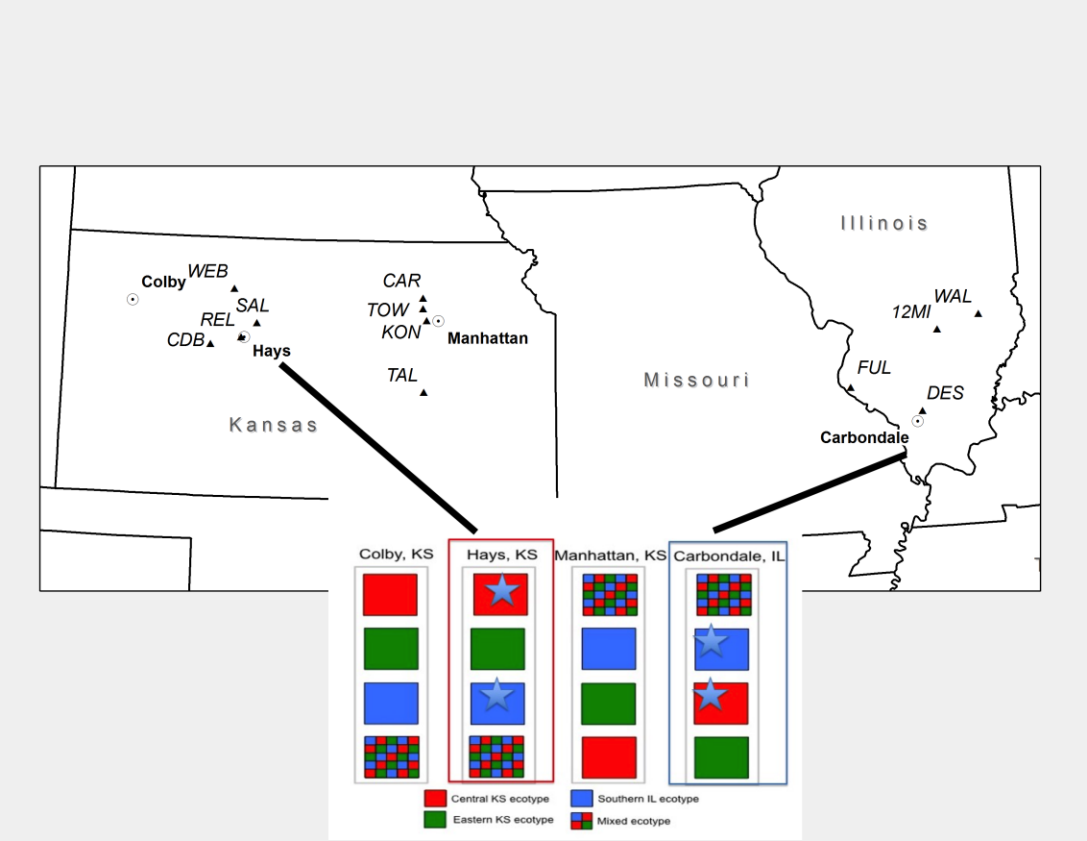

Figure 4.1. Location Map. Plot map showing the reciprocal garden platform across the US Great Plains. For this study, only the Dry Ecotype (from Central Kansas) and Wet Ecotype (from Illinois) planted in the Hays, KS (Central Kansas) and Carbondale, IL (Southern Illinois) were used. Lower insert stars the seeded community plots used from the two planting sites (Dry Ecotype in red and Wet Ecotype in blue)

The distribution of *A. gerardii* across such a sharp precipitation gradient, ranging from 1260 MAP mm rainfall/year in southern IL to 580 mm MAP rainfall/year in parts of western, KS (Figure 4.1) portends that ecotypic differentiation may be widespread in *A. gerardii* populations (Galliart *et al.*, 2019, 2020; Gray *et al.*, 2014; Johnson *et al.*, 2015; Mendola *et al.*, 2015, Caudle *et al.*, 2014, Maricle *et al.*, 2017; Varvel *et al.* 2018, Kramer *et al.*, 2018, Smith *et al.* 2017; Olsen *et al.* 2013; Gibson *et al.* 2016; Gibson *et al.* 2013). Here, we probe gene expression and genetic differentiation coupled with ecological and physiological differences in response to rainfall in ecotypes of *A. gerardii* (Vitman) to understand ecological processes of adaptation to regional rainfall. *Andropogon gerardii* big bluestem is a dominant, perennial C_4 grass (Weaver & Fitzpatrick, 1932) in tallgrass prairie and comprises approximately 70% of prairie biomass (Knapp *et al.* 2002). It is an economically vital rangeland forage grass (Rogler, 1944; Risser *et al.*, 1981), supporting an $8.34 billion cattle industry (USDA NASS 2020) in just KS and bioenergy crop in marginal dry lands (Gan *et al.*, 2012; Zhang *et al.*, 2015). Grasslands are also important for carbon sequestration (Seastedt & Knapp, 1993; Yang *et al.*, 2019; Zhou *et al.*, 2018) and support high levels of biological diversity (Hoekstra *et al.*, 2005).

Andropogon. gerardii has been extensively studied in terms of climate (Fay *et al.* 2002; Fay *et al.* 2003), community structure (Collins *et al.*, 1998; Knapp *et al.*, 1999; Silletti & Knapp, 2002), physiological (Bachle & Nippert, 2022; Maricle *et al.* 2017; Caudle *et al.*, 2014) and morphological responses (Bachle & Nippert 2021; Knapp *et al.* 2001; Olsen *et al.* 2013; Epstein *et al.*, 1998), and restoration (Willand *et al.*, 2013, Baer *et al.* 2005). While *A. gerardii* is an excellent ecological model, it lacks many of the genomic tools that are well-developed for model organisms. Consequently, studies probing the genetic basis of ecological responses and adaptation to climate of this dominant grass are lacking until recently (Galliart *et al.*, 2019, 2020; Gray *et al.*,

2014; Hoffman & Smith, 2017) Now RNA-seq technology is readily available for probing response to the environment (Hoffman & Smith 2018).

Here we probe the transcriptome of wet and dry ecotypes of *A. gerardii* Vitman cross-transplanted in reciprocal gardens across the Great Plains' rainfall gradient to reveal genetic underpinnings of their ecological differences. These grasslands are largely shaped, both structurally and functionally, by water availability (Fay *et al.*, 2003; Hajek & Knapp, 2021) and rainfall (Sala *et al.*, 1988; Luo *et al.*, 2021) which is the focus of our study. Additionally, the current grassland biome has formed about 10,000 years, since the last glaciation (Axelrod, 1985), allowing adequate time for natural selection to take place over the sharp rainfall gradient.

We focus on rainfall because it's likely a dominant driver in selection because across the Great Plains, it limits plant productivity (Knapp *et al.*, 2001), affects plant community composition (Wilson *et al.* 2016) and sequestration of carbon (Luo *et al.*, 2021). Indeed, one of the most important climatic changes predicted for the central grasslands of the US is alteration in the amount and timing of precipitation events (IPCC 2021) as well as megadroughts (Cook *et al.* 2015). Thus, changes in precipitation are expected to pose a serious threat to the sustainability of tallgrass prairie (Knapp *et al.* 2002; Hajek & Knapp, 2021). Thus, these grasslands are threatened by changes in climate, especially precipitation. We can take advantage of genomics to help us understand plant response to changes in precipitation and results may guide us on matching ecotypes to climate for conservation and restoration (Hufford and Mazer 2003; Mckay *et al.*, 2005; Baer *et al.* 2019).

Our reciprocal garden platform where wet and dry ecotypes are cross-transplanted across the rainfall gradient (Galliart *et al.*, 2019, 2020; Gray *et al.*, 2014; Johnson *et al.*, 2015; Mendola *et al.*, 2015, Caudle *et al.*, 2014, Maricle *et al.*, 2017; Varvel *et al.* 2018, Kramer *et al.*, 2018, Smith *et al.* 2017; Olsen *et al.* 2013; Gibson *et al.* 2016; Gibson *et al.* 2013) offers unprecedented

opportunities in the genomic era to link ecology and gene expression in the field (Johnson *et al.*, 2021). This approach allows us to test tolerance between wet and dry ecotypes growing reciprocally across a precipitation gradient (1260 MAP in Carbondale IL vs 580 MAP in Hays KS) and to assess the relative role of site (the local environment and regional climate) and ecotype (genetic background) on ecological and genetic responses. The objectives of this study are to determine to what extent this foundation prairie grass *A. gerardii* differs ecologically, genetically, and transcriptionally. Our research is motivated by the following questions: Do *A. gerardii* ecotypes growing across a precipitation gradient show differential gene expression in response to their regional rainfall? What are the genes involved that allow these ecotypes to be adapted to their respective environment and how does this relate to ecological response? At the same time, how does this dominant foundation grass respond ecologically when cross-transplanted from its home to a drier or wetter environment?

We hypothesized that regional climate of the site and genetic background or ecotype and their interaction would be strong determinants of ecological and genetic responses. Further, we also predicted that the local ecotype would perform better than the foreign ecotype in its home environment, as evidenced by increased canopy cover and biomass, increased photosynthetic rates and more favorable gas exchange compared to growing in the foreign environment. Additionally, we expected that plants regardless of ecotype grown in the Illinois site with have higher biomass and gas exchange due to more favorable moisture availability when compared to the central KS site due to water limitations. For genetic responses, we expected that the dry ecotype would show differential expression in genes such as heat shock proteins, aquaporins, and other osmotic stress related genes that allow these plants to cope with low rainfall in water-limited Central Kansas (Kuchler 1964). While in contrast, wet ecotypes should show differential expression in genes that

allow for enhanced carbon and nitrogen gain and optimized growth in the light-limited, highly competitive shrub- and forb-rich eastern Great Plains prairies (Kuchler 1964).

This study harnesses the power of reciprocal gardens (Lortie and Hierro 2021, Johnson *et al.* 2021) combined with genomic tools and ecological responses to provide an integrative picture of how plants respond and adapt to the environment (Johnson *et al.*, 2021, VanWallendael *et al.* 2022). It also highlights the importance of doing gene expression studies in the field with non-model wild plants to detect gene expression responses in nature with relevance to the ecological conditions under which these plants have evolved (Turner *et al.* 2020).

MATERIALS AND METHODS

To link ecology and genomics, we combined ecological and transcriptome measurements. Leaves for extraction of RNA and DNA were collected and flash frozen with liquid N in the field during the typical end of season drought and paired with concurrent short-term measurements of gas exchange, and long-term measures of *A. gerardii* biomass and plant cover in reciprocal garden ecotype plots at wet and dry sites to link gene expression with ecological responses.

For purposes of this paper, we are focusing on the dry and wet ecotypes planted reciprocally into sown community plots (see details below and Table 4.1, Table 4.2) in the dry (MAP 580 mm in Hays KS) and wet (MAP 1,260 mm in Carbondale Illinois) reciprocal garden sites. (We did not study the mesic ecotype in the intermediate rainfall site). See Johnson et. al. 2015, Galliart *et al.* (2019, 2020) for a complete design of the entire reciprocal garden platform for this study. We chose to focus on wet and dry ecotypes and sites at the extremes of the rainfall gradient to carry out the transcriptome study and ecological responses as they demonstrate extremes in phenotype based on other studies on these plots (Galliart *et al.*, 2019, 2020) and are likely sites for range

extension. (Sexton *et al.* 2009, Moran *et al.* 2022).

Table 4.1. Source location of 8 populations used with relevant location and site information. Four populations from each geographic region (CKS and SIL) are presented here.

Region	Ecotype	Prairie Name	Prairie Size (ha)	Prairie Abbreviation	County, State	Elev. (m)	Soil type	Longitude	Latitude
CKS	Dry	Webster Reservoir	356	WEB	Rooks, KS	606	Wakeeney-Harney Silt loam	39.41	99.50
CKS	Dry	Saline Experimental Range	880	SAL	Ellis, KS	641	Bogue-Armo Clay loam	39.02	99.14
CKS	Dry	Cedar Bluffs Reservoir	850	CDB	Trego, KS	688	Armo Clay loam	38.76	99.83
CKS	Dry	Relict Prairie	14	REL	Ellis, KS	640	Armo loam, Brownell gravelly loam	38.85	99.37
SIL	Wet	Desoto Prairie	0.4	DES	Jackson, IL	119	Orthents silty loam	37.85	89.14
SIL	Wet	Twelve Mile Prairie	28	TM	Effingham, Monroe, Fayette, IL	160	Cisne silty loam	38.78	88.83
SIL	Wet	Walters	5	WAL	Jasper, IL	150	Atlas silty clay loam	38.92	88.19
SIL	Wet	Fults	214	FUL	Monroe, IL	215	Menfro silty clay loam	38.17	90.19

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

For the subset of the reciprocal garden experiment, specifically dry and wet ecotypes presented here, seed of *Andropogon gerardii* was collected by hand during autumn, from dry and wet precipitation regions across the central Great Plains from central KS and southern IL (Table 4.1, Figure 4.1). These regions roughly correspond to the ecoregions (Kuchler, 1964) of Great Plains Steppe (central KS) and Prairie Parkland Temperate (southern IL). Overall, the prairies of central KS are primarily comprised of low stature grasses with relatively few woody forbs (Knapp *et al.*, 1998). Moving eastward towards Illinois, the prairie communities increase in diversity comprising of tall stature forbs, grasses, and shrubs (Kuchler, 1964). Seeds were collected from four

populations within each region. These four populations from each region are combined and jointly defined a regional ecotype (referred to as dry ecotype for central KS and wet ecotype for southern IL). We have previously characterized the ecotypic variation of the source populations to their respective regional ecotype (Galliart *et al.*, 2020, Johnson *et al.* 2015; Caudle *et al.* 2014; Maricle *et al.* 2017) and thus confirmed the regional nature of the ecotypes. The dry ecotype was comprised of REL, SAL, CDB, and WEB populations and wet ecotype comprised of DES, WAL, 12MI, and FUL populations (Table 4.1). Populations (Table 4.1) were mixed in equal proportions and sown for plot establishment.

Table 4.2. Geographical descriptors and summary of historical weather data (30-year normals) as descriptors of environmental conditions for the planting sites of Hays, Kansas and Carbondale, Illinois.

Reciprocal Garden Planting Site (Town, County) Soil Type	Elev. (m)	Lat. (°N) Long (W)	Rainfall 6-year mean 2006-2009 (range) (cm)	Annual Number of Pcp Events >1.25 cm (NOPPT)	Pcp Driest Year (cm) (PPT DRY)	Mean Annual rainfall (cm) MAP	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)
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
Southern Illinois (Carbondale IL Jackson, Co) SIU Ag Research Station (Stoy Silt Loam)	127	37.73 89.17	125.6 (76.2-173.8)	32.7	67.38 (1963)	119.8	64.51	-5.3	-5.1	13.5	19.0	6.3

2. Reciprocal garden design- Sown community plots

For the purposes of the wet and dry ecotypes focused on here, these two ecotypes were reciprocally seeded into plots at two garden sites: Hays, KS at the KSU agricultural station and Carbondale, IL at the Agricultural Research Farm (Table 4.2, Figure 4.1). Each of the ecotypes were reciprocally sown at each site in multi-species communities (Johnson *et al.*, 2015). A key innovation of this novel research platform is that it provides an opportunity to test for the

expression of local adaptation of ecotypes in multi-species communities, not single-spaced plants growing in monoculture, allowing full interactions of ecotypes with the surrounding biotic community, especially neighboring plant competition. Soil at both planting sites were classified as silt loam (Goad, 2012).

The experimental randomized complete block design contained four blocks per site. Within a site, for the purposes of this paper, each block contained plots (4m x 4m) with separate wet and dry regional ecotypes (See subset of plots in Fig 4.1). See more details about plot establishment and seeding in Johnson *et al.*, (2015).

Four 20 cm long, EC-20 soil moisture probes (Decagon Devices, Inc., Pullman, WA) were used to monitor soil moisture in representative plots arranged diagonally across each site, with one probe per block (Figure S2). Probes were installed in early May at the beginning of the growing seasons and were allowed to settle and percent soil moisture was subsequently measured twice weekly.

3. Ecological Measurements

Here we report measurements on dry and wet ecotypes planted reciprocally in Hays, KS and Carbondale, IL. Gas exchange measurements were made at time of RNA collection, and biomass and cover were long-term measurements extending subsequently over several years.

Canopy Cover

Measurements of vegetative cover of *A. gerardii* in wet and dry ecotype plots were made to assess plant performance of the different ecotypes planted across the rainfall gradient, and to assess the extent to which ecotypes can tolerate stress outside of their local environment. We focused on

vegetative cover (as related to plant biomass). To estimate percent cover, a 1.0 m^2 quadrat was used with one intersection every 10 cm for a total of 81 intersections per quadrat. 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 4 replicate plots per site x 2 ecotypes x 2 sites (Hays and Carbondale sites) for a total of 5184 intersections. Quadrats were randomly placed at least 50 cm from edge to minimize edge effect.

End-of-Season Biomass

For purposes of this paper, we present end of season biomass for only *A. gerardii*, the focal species in wet and dry sites. Biomass was measured at peak season in September of 2010-2014. Four 20 cm x 50 cm (0.1 m^2) frames were clipped in each plot at each site; in 2011, three 0.1 m^2 frames were clipped in each plot. All sites were clipped within a one-week period to minimize temporal variation in biomass. All A. gerardii biomass was dried at 55˚C to a constant mass and weighed to estimate biomass (Briggs & Knapp, 1991). Community biomass will be presented elsewhere.

Physiological gas exchange measurements

Photosynthetic rates were taken on each individual plant randomly flagged at least a half hour prior to starting the leaf collection and on same plants for RNA. All measurements were made within a two-hour time frame in mid-afternoon. Gas exchange measures are intended to represent instantaneous measures of plant performance. Gas exchange was measured once during leaf collection. We measured photosynthesis (A, *u*mol CO_2 m^{-2} s^{-1}(micromoles CO_2 per square meter per second), stomatal conductance (gs, mol H_2O m^{-2} s^{-1}), conductance (moles water per square meter per second), transpiration (E, mmol H_2O m^{-2} s^{-1} (Trmmol), WUE, and internal carbon

dioxide (Ci) from ~ 4 plants per block were randomly selected from each block. We used a LI-6400 (Li-Cor Inc., Lincoln, NE, USA) for gas exchange measurements. Gas exchange was measured on a young, fully expanded leaf with CO_2 levels at 385 ppm, humidity and temperature at ambient levels, and photosynthetically active radiation (PAR) at 1500 *u*mol photons m^{-2} s^{-1}. Measurements were made on sunny days between 10:00 and 15:00 h to minimize adjustment time to leaf chamber conditions. Measurements were made when photosynthesis and stomatal conductance had stabilized (<3% coefficient of variation). For purposes of this paper, we reported here photosynthesis measurements on dry and wet ecotype planted reciprocally in Hays, KS and Carbondale, IL. Other papers (Johnson *et al.* 2015, Maricle *et al.* 2017, Varvel *et al.* 2018) provided more detail on gas exchange from across the whole gradient and all four sites, but lack the genetic component provided here.

4. Genetic Analyses

DNA Genotyping and genetic analyses. The main objective was to characterize genetic diversity between wet and dry ecotypes. We used genotyping-by-sequencing to identify SNPs (Elshire *et al.*, 2011; Galliart *et al.*, 2020; Lu *et al.*, 2013; Narum *et al.*, 2013). Leaf samples for DNA analysis were collected from the plants from the original populations used to originally seed the plots (REL, SAL, CDB, WEB for dry populations and WAL, 12MI, DES, and FUL for wet populations). Total number of plants genotyped (218) included 110 from the dry ecotype, and 98 from the wet ecotype. Note that for the DNA analyses we could separate populations within ecotypes, but for the leaf collection for RNA, we could not separate populations within an ecotype because seeds from the populations were mixed during plot establishment in the field plots. DNA sample collection, preparation and SNP calling analyses can be found in Supplemental Methods. PCoA of genetic

distance was calculated from SNP markers performed in R package *Adgenet* (Jombart 2008). Pairwise population differentiation F_{ST} was implemented in *GenAlEx* v6.503 (Peakall & Smouse, 2012) using eight populations comprising two regional ecotypes.

RNA and gene expression

Leaf tissue was collected at the end of the first full year of growth 2010 in August in Hays, Kansas and Carbondale, Illinois. Roots were not collected due to the destructive nature of root sampling in long-term plots. At the date of collection and gas exchange measures, soil moisture averaged ~5% in Hays and ~25% in Carbondale. Within each of the four replicated plots per ecotype, three plants were randomly flagged, for a total of twelve plants sampled per ecotype. For each plant collected, 2-3 mature leaves were taken. All collections were performed within a two-hour time frame in mid-afternoon. Leaves were flash frozen and put in liquid N in the field and transferred to -80C freezer at the laboratory**.** At the same time, separate leaves were measured for gas exchange. See supplemental methods for Illumina protocol and sample preparation for Illumina sequencing.

De Novo Transcriptome Assembly, Gene Expression, and Functional Annotation

The next phase in the gene expression analysis was transcriptome assembly and mapping of reads (see general pipeline in Figure S3). Quality of reads was assessed using *FastQC v0.11.9 (*Andrews, 2010). *De novo* transcriptome assembly was conducted using *Trinity v2.8.5* (Grabherr *et al.*, 2011). Using Trimmomatic *(*Bolger *et al.,* 2014), extra Illumina adapters were removed, and reads were trimmed using a sliding window of 4 bases having removing reads with a phred score below 20. The assembly included reads from three individuals per ecotype per site sequenced on Illumina

HiSeq and with one of those individuals also sequenced on Illumina MiSeq to allow for longer read lengths to be incorporated for an improved *de novo* assembly.

After transcriptome assembly, statistics were assessed using the built-in perl script available with *Trinity* (Table 3). Contigs from the *de novo* assembly were clustered using default similarity thresholds CD HIT EST v4.8.1 (Li & Godzik, 2006). Following clustering, TransDecoder v5.5.0 (https://github.com/TransDecoder*)* was used to identify and predict coding regions from the clustered sequences. Finally, BUSCO v4.0.2 *(*Felipe *et al.*, 2015*)* was used to identify orthologs in the assembly. For alignment and mapping of transcripts, we used STAR v2.7.5 (Dobin *et al.*, 2013). The clustered and filter transcriptome was used for alignment and mapping. First, the transcriptome was indexed using STAR v2.7.5. Each of the 12 individual samples was mapped to the indexed reference transcriptome. Next, gene counts were obtained using the *rsem-calculate-expression* procedure as implemented in STAR v2.7.5.

The final phase of the gene expression study was to quantify differences in gene expression between site and ecotype and ecotypes in their home site, conduct annotations and functional analysis of the significantly expressed genes, and enrichment analyses. The ultimate goal was the identification of candidate genes related to functional differences between ecotypes and sites and in their homesites. To identify differentially expressed genes, we used DESeq2 v3.112 (Love *et al.*, 2014). Data was analyzed using three separate approaches by 1) combining different ecotypes within a site to identify expression differences between sites, 2) combining the same ecotype from different sites to identify expression differences between ecotypes and 3) ecotypes in their home site, the wet ecotype in Carbondale Illinois and the dry ecotype in Hays, KS. Differentially expressed genes were then identified using BlastX. BLAST2GO (Götz *et al.*, 2008) was implemented to determine gene annotations and GO terms. GO terms were analyzed in REVIGO

(Supek *et al.*, 2011) for enrichment analysis.

RESULTS

1. Ecological Response

Canopy

Local adaptation became evident based on the cover data in Hays and Carbondale from 2010-2015 (Figure 4.2). Even as early as the first year 2010, in the dry site of Hays, KS (Figure 4.2a), the dry ecotype showed significantly greater cover ($p<0.0001$) than the wet ecotype. Thereafter, the dry ecotype cover continued to increase, even after the extreme drought of 2012. On the other hand, the poor performance of the wet ecotype in the dry site, especially after the extreme drought of 2012, became detectable ($p=0.0345$). Furthermore, the wet ecotype failed to recover to its pre-drought cover ($p<0.0001$). Moving on to the wet site of Carbondale Illinois (Figure 4.2b), for the first few years (2010, 2011), there were no significant differences between ecotypes (p = NS) However, by 2012, cover between the ecotypes began to diverge with the wet ecotype having as much as 3x the cover of the dry ecotype ($p=0.0108$), while the dry ecotype cover remained low for the entire 2010-2015 period ($p <0.0001$).When comparing ecotypes in their home sites the dry ecotype grown in its homesite (Hays, KS) maintained a similar moderate cover across all years (~ 0.25 – 0.4) (Fig 4.2a). For the wet ecotype grown in its homesite (Carbondale, IL) cover was initially low (~0.1) for the first two years of the planting with moderate cover (~ 0.25 – 0.35) for the subsequent years (Fig 4.2b).

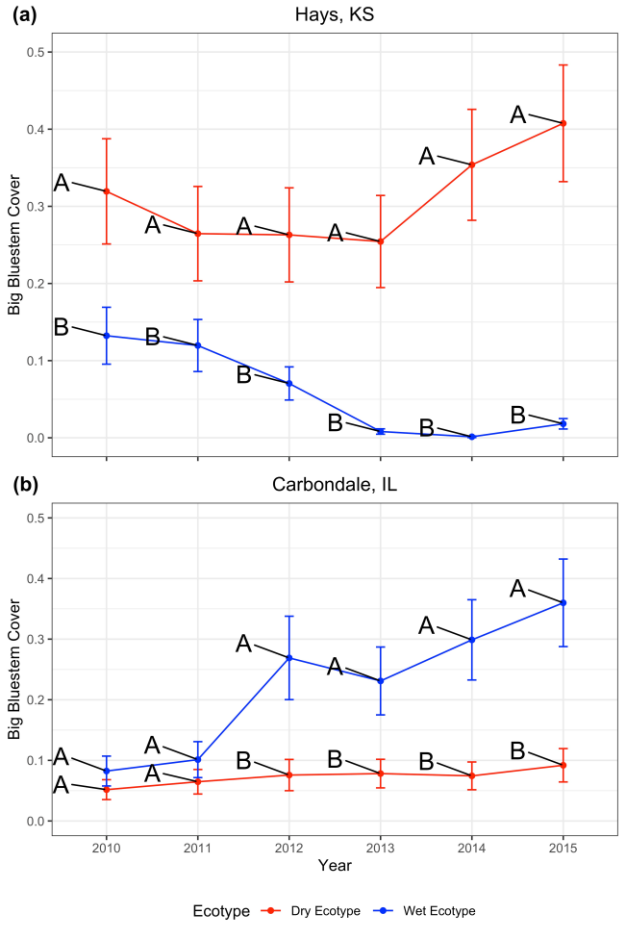

Figure 4.2. Cover difference between the Dry Ecotype (red) and Wet Ecotype (blue) in Central Kansas (Top - a) and Southern Illinois (Bottom - b) from 2010-2015 based on four 1m x 1m quadrats per plot. Figure indicates the proportion of the community plots are big bluestem. Differing letters indicate significate differences.

Plant Biomass

A similar pattern of local adaptation was evident based on biomass harvest at the end of the season (Figure 4.3). For biomass, there was a significant S effect ($p = 0.0114$), E effect ($p = 0.0123$), and S x E effect ($p < 0.0001$). In the dry site of Hays KS (Figure 4.3a), there were no significant differences observed between wet and dry ecotypes (Figure 4.3a). Moving on to the wet site of Carbondale Illinois, by 2013, biomass of the wet ecotype far surpassed the dry ecotype (Figure

4.3b, p=0.0039). By 2014, the wet ecotype biomass was as much as 4-5 times greater than the dry ecotype ($p<0.0001$). Similar to the pattern in the cover data, the biomass produced by the dry ecotype in Illinois remained very low for the entire period 2010-2014 (66.3 $\pm$ 64.5 g m^{-2}). When comparing ecotypes in their home sites for the dry ecotype grown in its homesite (Hays, KS) the dry ecotype has moderate biomass across years (189.3 $\pm$ 64.5 g m^{-2}) (Figure 4.3a). For the wet ecotype grown in its homesite (Carbondale, IL) biomass is initially quite low with a rapid increase to 1303.3 $\pm$ 150.5 grams in the most recent year of 2014 (Figure 4.3b). In summary, the biomass data reflects similar patterns when compared to the cover data.

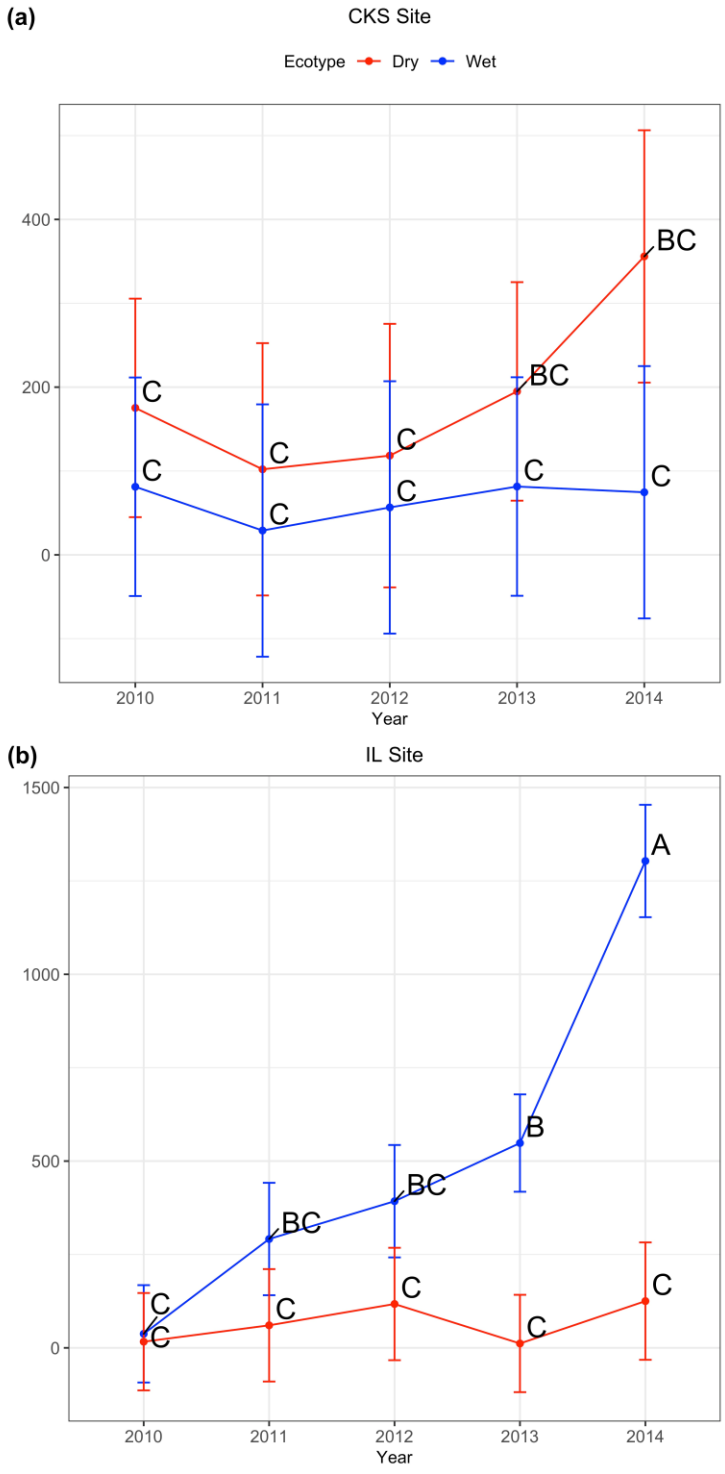

Figure 4.3. Annual net primary production (measured in grams) for the Dry Ecotype (red) and Wet Ecotype (blue) in Central Kansas (Top – a) and Southern Illinois (Bottom – b) from 2010-2014. Differing letters indicate significant differences.

Physiological Gas Exchange Measurements

Site (S) and ecotype (E) effects were detected in the gas exchange measurements during the time of leaf collection for RNA (Figure 4). We found significant differences in photosynthesis between S ($p=0.022$) and E ($p=0.034$), with no interaction (Figure 4.4a). Interestingly, the wet and dry ecotypes in the dry site appeared to have a higher photosynthetic rate but that rate decreased in the wet site ($p=0.0222$). This was contrary to expectation that the higher soil moisture conditions in the wet site should result in higher photosynthetic rates. In terms of stomatal conductance (Figure 4.4b), the stomates were more open in the dry site compared to the wet site (S, $p<0.0001$) and not affected by E or S x E. Again, this result was also contrary to expectation that higher soil moisture conditions in the wet site would result in greater stomatal conductance. Moving on to transpiration (Figure 4.4c), the dry site showed higher transpiration compared to the wet site (S, $p=<0.0001$) as well as a smaller but significant ecotype effect (E, $p=0.01$) but no S x E (p=NS). In terms of WUE (Figure 4.4d), the dry site had a lower WUE ($p=0.0139$). Finally, the dry site had higher internal C concentration ($p=0.0527$) Figure 4.4e), as it seemed these plants were more photosynthetically active in the dry site compared to the wet site, but with no E x S ($p=0.77583$) or E effects($p=0.67274$). These data follow if the dry site has higher photosynthetic rates leading to higher transpiration and increased stomatal conductance. Taken together, these results were unexpected as we presumed that low water availability in the dry site would limit gas exchange, and the wet site, with a more favorable water balance, would show more favorable gas exchange, provided there are no other limitations, such as light competition from forbs and shrubs due to shading. When comparing ecotypes in their home sites, the dry ecotype grown in its homesite (in dry Central KS) had a significantly higher photosynthetic rate ($p=0019$, Figure 4a), stomatal conductance ($p<0.0001$)Figure 4d), and transpiration rate ($p<0.0001$, (Figure 4.4b when compared

to the wet ecotype grown in its homesite (in wet Southern IL).There were no significant differences between the ecotypes grown in their respective homesites for intrinsic water use efficiency (Figure 4.4c) and internal CO_2 concentrations (Figure 4.4e).

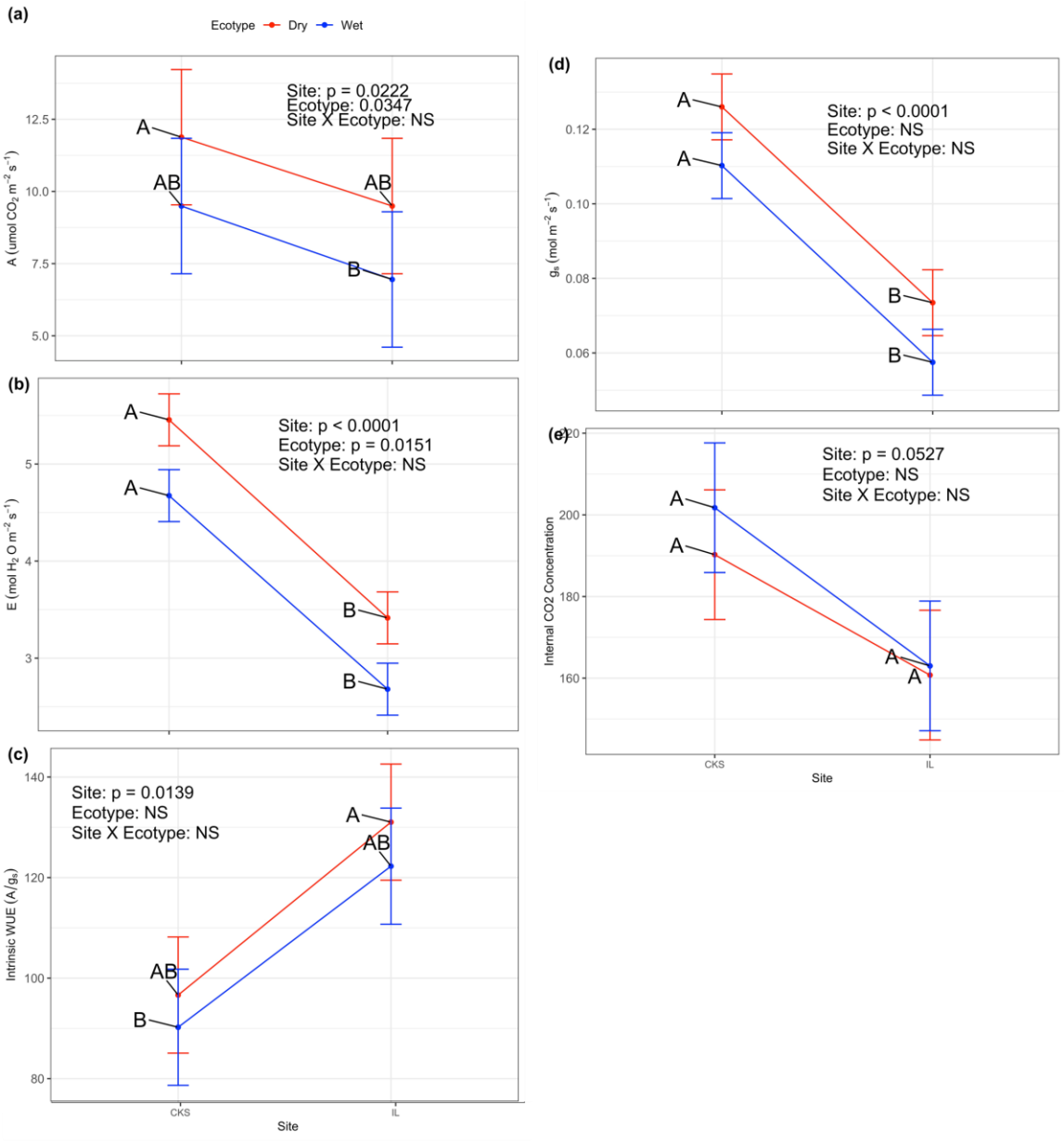

Figure 4.4 Physiology measurements for the Dry Ecotype (red) and Wet Ecotype (blue) in 2010. Planting location across the x-axis with Central Kansas (left) Southern Illinois (right). a) Photosynthetic rates measured in A (umol CO2 m-2s-1). b) Conductance measure in E (mol H2O m-2s-1). c) Intrinsic water use efficiency measured as A/gs d) Stomatal conductance measured as gs (mol m-2s-1) e) Internal CO2 concentration measured as umol CO2. Differing letters indicate significant differences.

2. Genotype differences between wet and dry ecotypes

We identified 4,641 SNPs using the UNEAK pipeline and visualized genetic differences among ecotypes using PCoA (Figure 4.5). Principle coordinate 1 explained 7.39% of the variation with individuals from the dry ecotype were clearly genetically differentiated from the wet ecotype along that axis, whereas principle coordinate 2 explained 1.70% of the variation with no ecotype discernment along the axis. Population level genetic differences were also supported by pair-wise F_{st} analyses (Table S1). The pairwise comparison of populations had a range of F_{st} from 0.011 to 0.014 (average 0.012 $\pm$0.002) for pairwise comparison between dry populations and .018 to 0.023 (average 0.021$\pm$ 0.005) pairwise comparison between wet populations. Comparing between wet and dry populations, it ranged from .022 to .039 (average 0.030 $\pm$0.006 indicating greater genetic differentiation between populations from wet and dry climatic regions. When considering F_{st} at the ecotype level, the F_{st} for wet ecotype-dry ecotype pairwise comparison was 0.030 (SE $\pm$ 0.006).

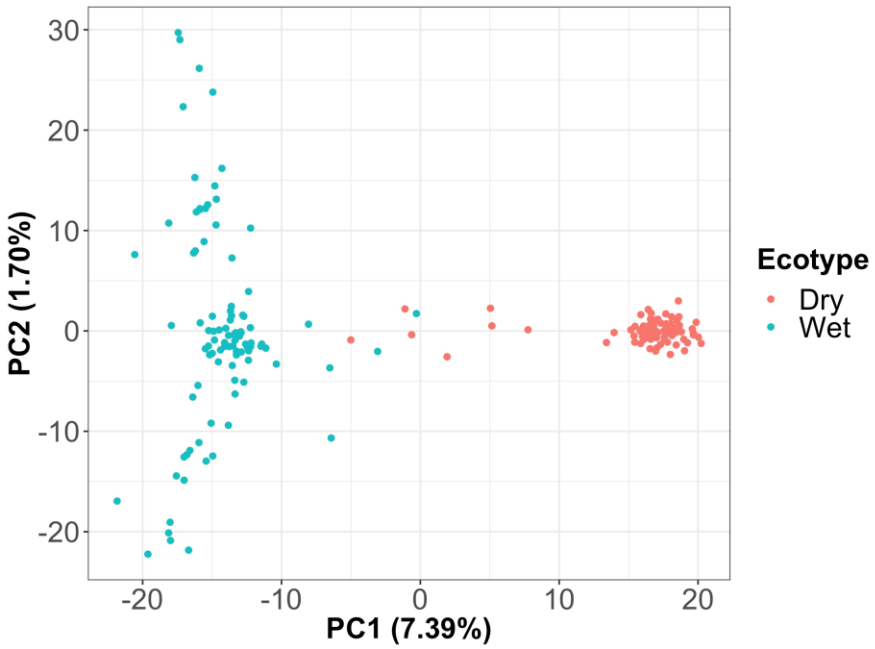

Figure 4.5. PCoA showing genetic differences between the Wet (blue) and Dry (red) Ecotypes based on 4,641 SNPs. Principal Coordinate 1 explains 7.39% of variation and Principal Coordinate 2 explains 1.70% of variation.

3. Differential gene expression between ecotypes and sites and ecotypes in home sites

Assembly. Assembly statistics and metrics demonstrated how well the transcriptome assembled (Table 3). Following assembly from Trinity the transcriptome contained 1,130,406 contigs. Subsequently, after clustering with CDHIT (Fu *et al.* 2012) to reduce the number of redundant contigs and Transdecoder (Haas *et al.* 2013) to identify transcripts that could be translated, the total transcriptome was reduced to 777,470 and 618,168 contigs respectively for each step. We found efficient assembly resulting in a final assembly with 618,168 contigs. The transcriptome was then compared to near-universal single-copy orthologs using BUSCO (Manni *et al.* 2021). Of 425 plant orthologs contained in the transcriptome, 93.6% were complete BUSCO matches, 5.9% fragmented matches, and only 0.5% missing matches indicating a relatively complete transcriptome.

The reciprocal garden design allowed us to test the relative importance of site (S) vs ecotype (E). For the differential gene expression, we considered expressed genes in response to site (with ecotypes combined) and in response to ecotypes (with sites combined) and those that showed S x E. Thus, we could determine sets of genes differentially regulated in wet and dry sites, regardless of ecotype and sets of genes differentially expressed in wet and dry ecotypes, regardless of site, as well as those ecotypes uniquely expressed in particular sites (S x E). Finally, we investigated differential expression of wet and dry ecotypes in their home sites in order to compare ecotypes in the sites where these ecotypes have evolved.

Volcano plots These plots demonstrated the important site and ecotype differences in gene expression (Figure 4.6). When ecotypes were considered with sites combined, there were 264 genes significantly differentially upregulated (based on A = 0.1 cutoff) in the wet ecotype (Figure

4.6a, positive log fold change) and corresponding 396 genes differentially upregulated (A < 0.1) in the dry ecotype (Figure 6a, negative log fold change). An even stronger pattern for the site effect was observed when sites are considered with ecotypes combined (Figure 4.6b) with 948 genes differentially upregulated (A < 0.1) in the wet site (Figure 4.6b, positive log fold change) and 591 genes differentially upregulated (A < 0.1) in the dry site (Figure 6b, negative log fold change). This showed that both site and ecotype are important determinants of gene expression, but with site having ~2.5 x more differentially regulated genes (1539 total) compared to ecotypes (660 total). We also compared differential expression of wet and dry ecotypes growing in their respective home sites and found a total of 378 differentially expressed (161 upregulated in the dry ecotype in Hays KS and 217 upregulated in the wet ecotype. Volcano plots of home site comparisons are shown in supplemental Figure S4.

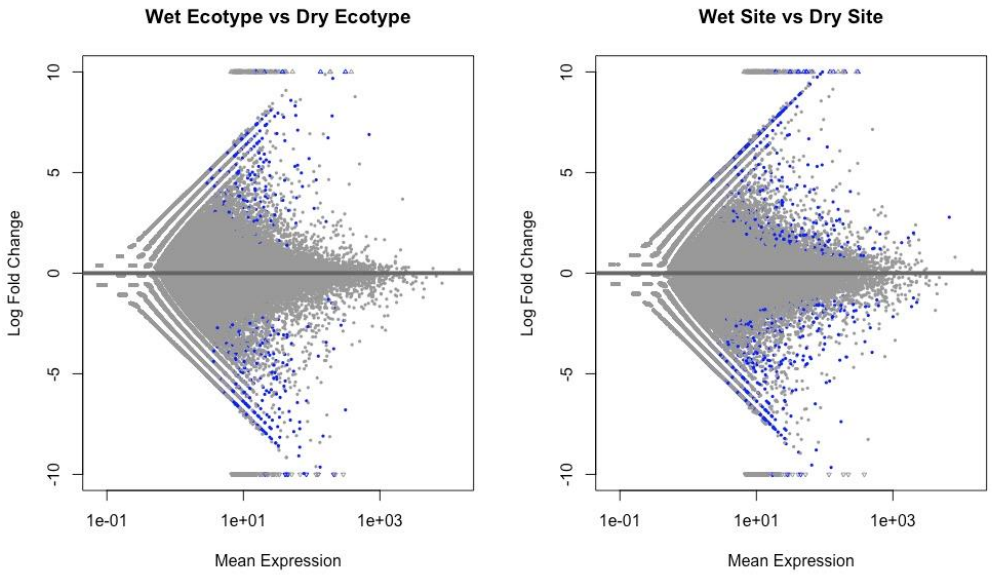

Figure 4.6. a. Ecotype b. Site. Volcano plots from DeSeq2 differential gene expression analysis. Panel a (left) shows differentially expressed genes between the Wet and Dry ecotypes (above 0 = upregulated in the wet ecotype, below 0 = upregulated in the dry ecotype). Panel b (right) shows differentially expressed genes between the Illinois and Central Kansas sites (above 0 = upregulated in the Illinois site, below 0 = upregulated in the Central Kansas site). Blue dots indicate significant differentially expressed genes based on an Alpha of 0.1.

Heat maps. The heat maps illustrate how diametrically opposed gene expression is between sites and ecotypes (Figure 4.7) across replicate samples. Comparing heat maps for the wet vs dry ecotype based on differentially expressed genes (with sites combined), clusters clearly showed upregulated (yellow) and down-regulated (blue) genes in the dry ecotype (Figure 4.7a, left panel) and an almost diametrically opposed pattern in the wet ecotype (right panel). In Figure 4.7b, where wet and dry sites are compared based on differentially expressed genes, the dry site (left panel) showed the pattern of clusters of downregulated (blue) and upregulated genes (yellow). Similarly, this pattern of gene clusters is diametrically opposed in the wet site (Figure 4.7b, right panel). In Figure S5, the heat maps for each ecotype in their respective home sites show genes that are differentially expressed within each ecotype in their home site.

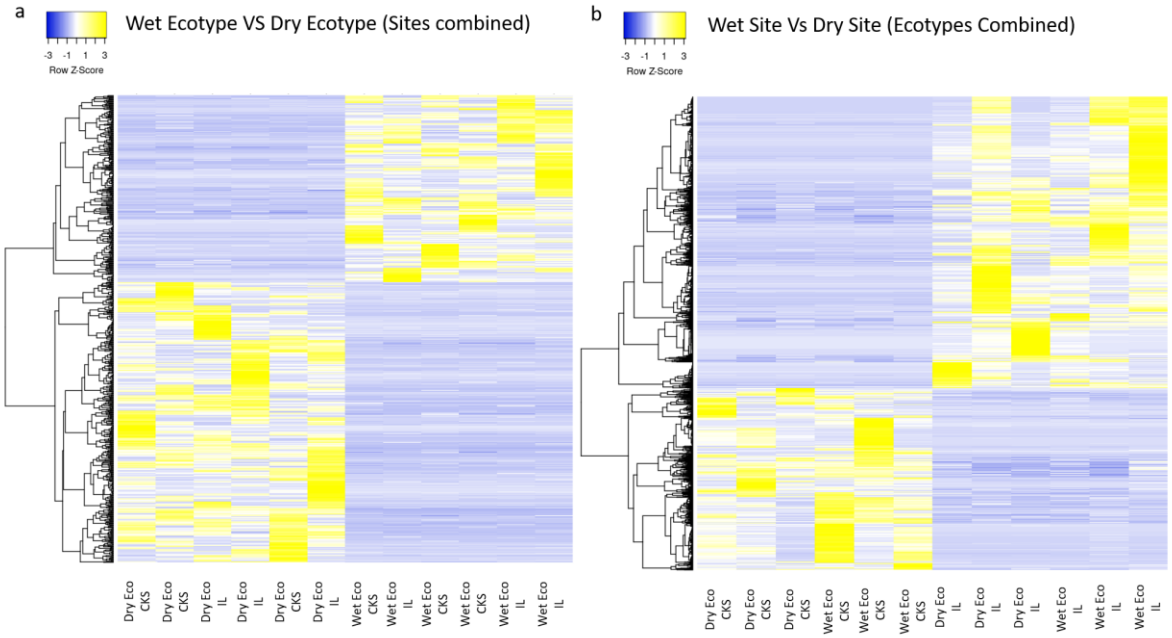

Figure 4.7. a. Ecotype b. Site. Heat maps showing all differentially expressed genes. Plots are clustered on both ecotype/site and gene. Yellow indicates higher expression and blue indicates lower expression. Panel a (left) shows all differentially expressed genes between the Wet and Dry ecotype. Panel b (right) shows all differentially expressed genes between the Central Kansas and Illinois planting sites.

Differentially expressed genes and functional annotation

***Site versus ecotype*.** Based on functional analysis of differentially expressed genes using BLAST2GO, we found contrasting expression of genes putatively related to site and ecotype differences with selected ecologically relevant candidate genes For the wet ecotype (sites combined), genes involved with transcription factors such as MYB, and MADS box and several light response genes (light induced protein and chlorophyll a-b binding protein) were notably upregulated (Table 4). In contrast, in the dry ecotype (sites combined) genes involved with abiotic factors such as heat stress (heat shock protein), drought stress (heat shock transcription factor), and aquaporins were upregulated. When comparing between sites (ecotypes combined), for the wet site, genes involved with metabolism such as lipids (glycolipid transfer protein), nitrogen (ammonium transporter) and carbon (duf636 domain protein), WRKY, and oxidation-reduction (mitochondrial succinate dehydrogenase and stearoyl-CoA desaturase) were significantly upregulated (Table 4). In contrast, for the dry site, genes involved with heat (heat shock protein) cuticle biosynthesis (ECERIFERUM 1), and drought stress (and myb-related protein 3R-1), high light induced protein and aquaporins were upregulated.

Plots from REVIGO functional gene enrichment analyses (Supek *et al.* 2011) showed enriched GO terms of differentially regulated genes between wet and dry ecotypes (Figure 4.8a.) and wet and dry sites (Figure 4.8b). Overall, there were more enriched terms (313) for differentially expressed genes for the ecotype comparison than the site comparison (147). First, we report ecotype comparisons, then site comparisons for molecular function and biological process. For the ecotype comparison (sites combined, Fig. 4.8a), upregulated molecular function terms for the wet ecotype contained genes prominently associated with DNA and RNA binding, transcription factors, and calcium binding factors and for biological processes, genes were involved with light

response, transport, and ubiquitin-dependent processes. In contrast, for the dry ecotype, molecular function terms contained genes for nucleotide binding and catalytic activity. and biological processes in the dry ecotype terms were involved with defense and stress response. When comparing across sites (ecotypes combined,) for the wet site, terms involved with ion binding and DNA and RNA binding were detected for molecular function and for biological processes they were RNA modification and translocation and varied stress responses. In contrast for the dry site, important molecular function terms were involved with transferase activity and other enzyme activities and for biological processes, environmental stimulus and stress, cell wall, and lipid metabolic processes.

Figure 4.8. Left panel shows plots for differentially expressed genes between the Dry (Top) and Wet (Bottom) Ecotypes. Right panel shows plots for differentially expressed genes between the Central Kansas (Top) and Illinois (Bottom) sites.

Ecotypes in their respective home site. Notable genes with differential expression of ecotypes in their respective home sites were detected based on Deseq 2 analyses and B2Go annotations (Table 4). For the dry ecotype at its home site, upregulated genes were involved with cuticle biosynthesis (3-ketoacyl-CoA synthase 6-like and ECERIFERUM 1), ABA (MYB relate protein 1), stress (Calmodulin-binding transcription activator 4 and disease resistance protein RGA2) and flowering responses (TGA4). (In contrast, for the wet ecotype at its home site, oxidative stress responses (peroxidase 65-like and superoxide dismutase) and nitrogen assimilation (glutamine synthetase) were upregulated.

Based on the REVIGO functional analyses, we next compared the ecotypes growing within their homesites For the dry ecotype in the dry site, for molecular function, main terms were involved with ion binding, catalase activity and kinase activity and for biological processes terms were involved with nitrogen metabolism, homeostasis, and biosynthesis processes. When considering molecular function for the wet ecotype in the wet site, terms were associated with enzymatic activity and ion binding and for biological processes, terms involved carbohydrate transport and metabolic processes.

DISCUSSION

This reciprocal garden platform over the climate gradient of the Great Plains allowed us to disentangle the relative importance of site and ecotype at the ecological and now at the transcriptome level. Contrasts in gene expression highlight and corroborate the observed functional differences in form and function of wet and dry ecotypes growing in light-limited and water-limited prairies, respectively. Our study further showed that the different genetic and transcriptomic signatures of each ecotype forecasted the ecological differences and local

adaptation to rainfall that played out in subsequent years based on ecological data. Doing these reciprocal garden studies outside embraced natural variation of field conditions such as herbivory, competition, and other biotic interactions rather than trying to control variation as in a greenhouse experiment or in planted monocultures. The plant response in the "rough and tumble" real world under which these plants evolved, in response to both abiotic (Hamann *et al.* 2021; Chaudhry & Sidhu, 2022) and biotic (Turner *et al.*, 2020) factors resulting in adaptation provides a new window into genetic and ecological mechanisms enabling response to climate, especially as precipitation regimes change in the future (Exposito-Alonso *et al.* 2018). Few reciprocal garden studies have taken the opportunity to probe genomic response (Zhang *et al.* 2022, Serba *et al.* 2016). However, we expect more ecological genomics studies in the future as it becomes easier to conduct transcriptome and genomic profiling on non-model species. (Hoffman & Smith 2018)

Relative role of site and ecotype in ecological response. Both site and ecotype play important roles in controlling form and function of *A. gerardii*. Cover and biomass were strongly affected by ecotype and site and their interaction of ecotype x site thus supporting our original hypothesis that the local ecotype would perform optimally in its home environment compared to growing in the foreign environment, a hallmark of local adaptation. Only the wet ecotype performed better in terms of biomass and cover in the home site. Interestingly, local adaptation was only observed after several years in the wet site, presumably as community succession processes played out in the surrounding ecological community. Notably, if one had stopped taking measurements of cover or biomass a few years into the experiment, the very strong local adaptation of the wet ecotype would have been missed! This highlights the importance of long-term measurements of adaptation, especially in long-lived perennial plants. Most measurements of adaptation are of duration less

than 3 years (Franks *et al.*, 2014, Johnson *et al.* 2021).

Surprisingly, physiological results were quite the opposite of our original expectations. We had originally presumed that low water availability in the dry site would limit gas exchange, and that the wet site would show more favorable gas exchange (provided there are no other limitations, see below). However, to the contrary, the dry ecotype in the dry site showed higher photosynthetic rates leading to higher transpiration, increased stomatal conductance and higher internal CO_2. This is also in agreement with Caudle *et al.* 2014 who found higher SPAD (chlorophyll absorbance, as a proxy for photosynthesis) and Maricle *et al.* 2017 who found higher photosynthetic rates in dry sites. In contrast, the wet ecotype in the wet site, in spite of more favorable moisture had lower photosynthetic rates, stomatal closure (reduced conductance) and lower internal CO_2 suggesting other non-stomatal limitations to gas exchange. We speculate that light limitation caused by shading from forbs and shrubs in the wet site limits gas exchange in *A. gerardii* more than water availability. For example, based on PAR measurements (Systema unpublished), light reaching the soil surface was only 5% in the wet site in Carbondale, but ranged from 19-53% light penetration to the soil surface in central and western KS, respectively. This suggests that the stomatal closure (due to low light) in the wet site limits photosynthesis presumably due to shading from neighboring plants, especially forbs and shrubs. The closed stomates then became limiting for photosynthesis and subsequently, internal CO_2 decreased, and photosynthesis declined.

The physiological data suggesting light limitation in the wet site agrees with other morphological and plant architecture studies as well as differential expression of candidate genes (described below). Considering ecotype traits in home site, Galliart *et al.* 2020 found the wet ecotype was robust, tall with high biomass and wide leaves putatively adapted for the highly competitive, light-limited eastern Great Plains. In contrast, the dry ecotype was characterized by

reduced canopy area and diameter, dwarfed stature, and low vegetative biomass--all traits putatively adapted for water limitation and to reduce water loss. Furthermore, the dry ecotype flowered almost three weeks earlier than the wet ecotype, putatively as an adaptation to producing seed before the end of season drought (Shavrukov *et al.* 2017). Interestingly, genome association studies identified candidate gene GA1 for which allele frequency was associated with plant height (Galliart *et al.* 2019). The ecotypes were genetically differentiated and outlier genes were associated with primarily precipitation. Taken together, the dry and wet ecotypes are distinct and adapted to the regional environments of dry Central KS and light-limited Illinois prairies.

Genomics in reciprocal gardens. Combining reciprocal gardens with genomics opens up a window of opportunity to reveal adaptation (Lortie et al 2021) and the genetic underpinnings for the observed ecological responses between ecotypes (VanWallendael *et al.* 2022). In a recent review (Johnson *et al.* 2021), a literature survey showed that only 11% of reciprocal gardens included a genetic component (either DNA, RNA), indicating a missed opportunity to use genomics to shed light on ecology. Even fewer studies identified candidate genes involved in response. The current studies and others (Hoban *et al.* 2016, Lovell *et al.*, 2016, Zhang *et al.* 2022, Serba *et al.* 2016) demonstrate new insights from genomics that would not be revealed by examining ecological responses alone. For example, the transcriptome component of this experiment proved to be a sensitive indicator of plant response and portended later ecological response of ecotypes, especially adaptation. In some ways, the transcriptome showed more clearly than the early ecological measurements that wet and dry ecotypes were genetically and transcriptionally distinct. For example, already in the first year of the study, the transcriptome showed clear ecotype effects while the ecological response and local adaptation of the ecotypes

was still emerging and developing in the wet site. While gene expression measurements were made in only the first year, expression differences as related to ecotype foretell the strong local adaptation that became pronounced in later years. Genetic differences apparent in the first year, later become even more apparent ecologically. In some ways, one could consider the transcriptome as being a dynamic organismal sensor revealing what the plant is experiencing.

Plants are constantly bombarded by both abiotic and biotic elements of their natural setting in the ecological community (VanWallendael *et al.* 2022; Gull *et al.* 2019). The relay of stress information from environment to plant involves stress recognition followed by a cascade of events at the cellular and molecular level that leads to gene expression and changes in physiology or metabolism (Khan et al 2018). Overall, transcription factors are important in regulating gene expression in all living organisms and are important in plants response to their environment. (Gonzalez 2016). Some of important transcription factors including, WRKY, MYB, NAC and AP2/ERF are important in regulating plant responses to varied abiotic and biotic stressors (Javed *et al.* 2020). Our results suggest that different levels expression of key transcription factors, among other genes, are involved in the different adaptive responses of the ecotypes (see details below).

We learned from volcano plots (Figure 4.6) and heat maps (Figure 4.7), that large clusters of genes are diametrically opposed in their expression patterns between sites and ecotypes. From the Revigo analyses (Figure 4.8), we saw clear functional differences in differentially expressed genes between wet vs dry ecotypes and wet vs dry sites. Our initial prediction about gene expression differences between ecotypes was seen with the dry ecotypes showing differential expression related to coping with drought in arid western KS, while wet ecotypes showing differential expression related to carbon gain, nitrogen assimilation, and optimizing growth in a light-limited, highly competitive shrub- and forb-rich environment typical of wet Illinois prairies.

Going into the details of the functional differences between ecotypes, by comparing wet and dry ecotypes (sites combined), we found functional differences between ecotypes in the Revigo analyses (Figure 4.8). For example, differentially expressed genes of molecular function related to transcription factor activity and ion binding were notable. Particularly noteworthy in the dry ecotype were several transcription factors related to coping with abiotic stress (NAC1 (You *et al.* 2014), heat stress transcription factors (heat stress transcription factors) (Guo *et al.* 2016), Myb related protein (Ambawat *et al.* 2013) and growth and development (FAR1 (Ma & Li 2018)), ion binding (calmodulin (Yang & Poova 2003), cytochrome p450 (Pandian *et al.* 2020) and aquaporins (Hachez *et al.* 2014). In contrast, the wet ecotype showed differentially expressed genes in response to light (chlorophyll a binding (van Amerongen *et al.* 1994), light-induced protein (Hayami *et al.* 2015) and various metabolic processes (especially lipids) as well as different transcription factors (MADS box transcription factor (Teo *et al.* 2019), auxin response factor (Li *et al.* 2016)) and aminopeptidase activity and root hair defective (Wang *et al.* 1997). Interestingly, while we also saw upregulation and disproportional enrichment of transcription factors in the wet ecotype, we detected upregulation of completely different transcription factors distinguishing wet from dry ecotypes (Table 4) such as MYB and MADs box transcription factors (Teo *et al.* 2019) in the wet ecotype and heat shock transcription factor (Guo *et al.* 2016) in the dry ecotype. Taken together, these results show that differential expression of different transcription factors likely plays an important role responsible for different form and function of wet and dry ecotypes and their response to regional climate.

In a separate analysis supporting these results, Galliart *et al.* (2019) found a SNP in the gene glutamine synthetase related to nitrogen uptake between wet and dry ecotypes with one form of the SNP being associated with higher N concentration in leaves of the dry ecotype and resulted

in higher SPAD in the dry ecotype (Galliart *et al.* 2019). Similarly, a polymorphism in the GA1 gene, responsible for internode elongation and plant height, was associated with 4.5 times taller plants of the wet ecotype compared to the dwarfed dry ecotype (Galliart *et al.* 2019). Height is one of the most important features allowing competitive advantage in response to light limitation These contrasts in gene expression and DNA polymorphism highlight and corroborate the observed functional differences in form and function of wet and dry ecotypes growing in light-limited and water-limited prairies, respectively.

Even stronger differences exist between wet and dry sites when ecotypes are combined with nearly double the number of genes upregulated in response to site. These site-related genes attest to differing abiotic and biotic constraints operating in these contrasting prairies. There was predominance of stress transcription factors expressed in the dry site, such as DREB (Agarwal *et al.* 2006, Khan 2011), ERF (Xu *et al.* 2008), AP2/EREBP (Kizis *et al.* 2001), WRKY (Chen *et al.* 2017), as well as genes conferring adaptation to osmotic stress (dessication-induced 1VOC) presumably as adaptations to water limitation (Mulako 2008), high light (high light-induced protein), extreme temperature (heat shock proteins, cytochrome p450) and early flowering (flowering locus t) and senescence (constans heading date 1) (Kim *et al.* 2008). Dehydration-responsive element binding (DREB) transcription factors (Agarwal *et al.* 2006, Khan 2011) have been documented to be differentially expressed in response to stress. These transcription factors, especially DREB have been found in past drought expression data of big bluestem using microarray technology (Travers *et al.* 2010). Other studies under drought stress found that in *A. gerardii*, little changes in heats shock proteins and other major stress response genes with the primary changes observed in osmotic stress related genes (Smith *et al.* 2016). Furthermore, when

compared to other species, *A. gerardii*, appears to have changes in gene expression related to stress alleviation rather than complete prevention. (Hoffman & Smith 2018).

In contrast, the wet site showed another suite of genes reflecting the competitive biotic environment of the eastern Great Plains, with extreme competition for light with forbs and shrubs, and general overrepresentation of genes enhancing metabolism in lipid, nitrogen assimilation and carbon gain and oxidation-reduction (glutamine synthetase and transferase).

Taken together, gene expression data supports and corroborates the observed strong ecotype and site differences and sheds light on the value of integrating ecology and genomics.

Bringing genomics outdoors. This paper is one of few that focuses on gene expression in wild plants under natural field conditions. With the advent of genomic technology going from the early days of micro arrays (Travers *et al.* 2010) in the early 2000's to RNA-Seq around 2010, there's been a linear increase in the number of gene expression papers from 2012-2021. In a literature search, we detected 104 papers using "environment, nature, field, transcriptome, gene expression, RNA-Seq, plants" search terms (see search deposited in dryad). Most gene expression studies have been conducted under controlled laboratory conditions to manipulate a single factor such as water (9 papers), temperature (8 papers), salinity (4), or nutrients (9). Of these, surprisingly, only 14 are done in the field. While controlled studies are helpful in revealing mechanisms, the studies suffer from lack of ecological realism and lack the dynamic nature of the environment in which plants grow and have evolved (Ungerer *et al.* 2008, Feder and Mitchell-Olds 2003). In nature, plants are responding to a multitude of factors, such as plant-plant competition (Ariza *et al.* 2011), facilitation such as mycohrizzae (Remke *et al.* 2021, Koziol *et al.* 2023)), herbivory, pathogens (Bever *et al.* 2015) and collectively, their microbes known as their microbiome (Sarkar *et al.* 2022, Trivedi *et*

al. 2022,). In a recent review, Johnson *et al.* 2021 showed that reciprocal gardens where biotic factors are manipulated or even considered are few. In this survey (Johnson *et al.* 2021), they found only 17% studies had a biotic component.

As indicated by our literature survey (104 papers, see dryad), our study is also one of few studies where transcriptomics and gene expression are done embracing the variability of wild plants in the field. Of the 104 papers surveyed, only 13% were done with wild plants (not crop plant accessions), only 35% done in the field and even more surprising, only 3% were done with wild plants in the field). Furthermore, only about half of transcriptomics studies made ancillary measurements and few made manipulations in the field (mostly single manipulations). A few other studies have also been done in the field such as Plessis *et al.* (2015) (rice). Plessis *et al.* 2015 found that both climate and broad environmental differences had large impacts on transcriptional responses in rice. We have found that in spite of the variability and "rough and tumble" real world, these *A. gerardii* plants show a clear signal of the genetic background of each ecotype in addition to responding to all the other factors around them. Clearly, there is a sizeable knowledge gap and opportunity for new ecological and genomic insights by examining transcriptomics of wild plants growing in the field under the condition where they evolved and adapted. (Turner *et al.* 2020, Plesis *et al.* 2015)

Ecological genomics informs response to climate change and climate matched ecotypes for restoration.

The Great Plains is predicted to experience unprecedented drought in coming years (Cook *et al.*, 2015, IPCC 2021). This study provides an integrative picture about the adaptability and constraints of plant response to climate. How populations and ecotypes respond is lodged in their genome. By

taking an integrative approach from genes to whole plant response, we can start to uncover genetic mechanisms and pathways, and how selection from the strong precipitation gradient has promoted diverging populations into distinct wet and dry ecotypes, each uniquely adapted to their regional climate (Lortie and Hierro 2021; Anderson & Song, 2020).

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Chapter 5 - Conclusions, significance, and future directions

We found that one of the most dominant grasses of the North American Great Plains demonstrates local adaptation of ecotypes to rainfall. These studies focus on taking multiple approaches using a well-established, long term reciprocal garden platform. This includes using realistic communities that allowed successional processes and climate variation to take place combined with data from a platform of plants that are individually grown in non-competitive settings. Broadly supporting our findings, we find that local adaptation, candidate genes, and genetic variation were all related to climate, primarily rainfall.

We demonstrated 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. Taken together, our results corroborate ecotypic differentiation across ecosystems spanning climatic gradients and that this local adaptation results in differential adaptive response to climate. Uncovering and characterizing this local adaptation is essential to understanding responses to anticipated global change.

One novel approach we took was to grow ecotypes in mixed ecotype plots to assess competitive success of the ecotypes. Letting the environment and biotic interactions impart selective pressures in local adaptation studies is a powerful approach to understand evolutionary

processes. 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.

As previously mentioned, it is crucial in long lived perennials to undertake experiments under natural conditions across broad time scales. For long-lived perennial plants, it is important to allow adequate time for establishment and natural processes to unfold. One striking example is highlighted by measurements of cover from the single ecotype community plots. As shown in chapter 2, particularly in the wet and dry site, had measurements only been taken for two years there would have been very different interpretation of results. In the Carbondale, IL site it took at least three years for the community plots to become established enough for the signals of local adaptation to be apparent. These interpretations along with recent reviews (Johnson *et al.* 2022) highlight the value in longer temporal ranges of experimental studies. As these communities continue to be exposed to varied climates across the longitudinal range of these study sites along with year-to-year variation within planting sites, it is vital to continue to make measurements on changes over time. These further data points would include community composition and cover, morphology, biomass, reproductive traits, and as well as a future gene expression study to determine whether important genes are similarly regulated over broader time scales. Overall, including this overarching approach will provide the data necessary to produce the most robust predictions on how this valuable grass will respond to future changing climates.

One striking result found was that ecotypes differed in terms of flowering time. The dry ecotype flowered earlier (21-30 days) compared to the other ecotypes depending on site. This is a putative adaptation to speed up reproduction in response to end of season drought in central and

western KS. Early flowering time in response to drought has also been observed in reciprocal garden studies of *Clarkia* (Eckhart *et al.* 2004) and in experimental evolution studies with *Mimulus* (Dickman *et al.* 2019) and *Brassica* (Hamann *et al.* 2018). Strong co-gradient plasticity of phenology across environmental gradients seen in *Rhinanthus minor* could provide a mechanism to buffer against variable climates (Ensing and Eckert 2019). In contrast, the flowering time differences observed here portends for beginnings of genetically based reproductive isolation (Nosil 2005) especially between wet and dry ecotypes, further reductions in gene flow, and ultimately speciation.

Traits often respond to selection by climate (Chapin *et al.* 1991, Chapin *et al.* 1993) and are often correlated, resulting in adaptive trait syndromes (Ackerly *et al.* 2000; Aspinwall *et al.* 2013). Our results also agree with recent meta-analyses of plant form in grasses such that grasses of the wetter, light-limited environments were characterized by broad leaves (Galleher *et al.* 2019). In contrast, grasses of open habitats were characterized by narrow, short leaves (Gallaher *et al.* 2019), putatively associated with water-limited open, arid environments. We observe ecotype differentiation based on selection on resource limitations, with water limitation selecting for the narrow, short statured dry ecotype in xeric areas of western KS, and with light competition selecting for the broad, tall statured wet ecotype in mesic areas of Illinois.

We found genetic outliers that were likely of adaptive significance and suggest divergent selection, presumably due to selection from spatially varying climate. Additionally, ecotypes differed in terms of candidate genes such as NAC transcription factor, glutamate synthase, peroxidase, and GA1. One of the strongest candidate genes found was in a SNP in GA1 had an allele frequency that varied clinally across the Great Plains and has high ecological and functional significance with wet ecotypes growing 4.7 times taller than the dry ecotype. Expression of GA1

controls internode length and consequently height (Millach *et al.* 2002). Height correlates with increased biomass, and putatively greater competitiveness (Moles *et al.* 2010), as would be advantageous in wet, light-limited prairie peninsula dominated by tall forbs and shrubs (Kuchler 1964). Conversely, the dry ecotype would be advantaged by short stature to reduce evaporative loss as an adaptation to water-limited climates such as central and western KS (Maricle *et al.* 2017; Kramer *et al.* 2018). Other studies have also identified other candidate genes across altitudinal gradients (Rellstab *et al.* 2016; Pluess *et al.* 2016), latitudinal gradients (Hancock *et al.* 2011), and precipitation gradients (Exposito-Alonso *et al.* 2018). These studies provide powerful insight into candidate genes, genetic mechanisms, and their relationship to phenotype and physiology responsible for adaptive divergence.

Broader impacts and significance

Tallgrass prairie, one of the most diverse grasslands, is critically endangered (Hoekstra *et al.* 2005; Hofer *et al.* 2016) with only 4% native prairie remaining (Samson and Knopf 1994). For example, less than 1,000 ha remain in eastern tallgrass prairie compared to the original 9 million ha (Samson and Knopf 1994). Our study informs land management and restoration strategies because knowledge of climate-matched ecotypes (Hufford and Mazer 2003; McKay *et al.* 2005; Nicotra *et al.* 2010) is critical to the future ecology and sustainability of grasslands. Of particular interest, we provide scientific foundation to land managers on ecotype suitability to climate, which is relevant to the USDA Conservation Reserve program (http://www.ks.nrcs.usda.gov/programs/crp/) that restores grasslands on marginal agricultural lands (SCS 1990). *Andropogon gerardii* is widely used in these conservation plantings throughout the North American central grassland, covering nearly 3 million ha (http://www.fsa.usda.gov). Furthermore, about 60% of total agricultural

production in KS (~$10 billion, NASS 2020) was attributed to cattle production, and *A. gerardii* is the main forage grass for cattle in this region. Our experiment demonstrates that local adaptation may limit a population's ability to adjust to changing climates. If populations cannot adjust to environmental change through phenotypic plasticity, populations will have to migrate to match their future climate conditions (Smith *et al.* 2017), or migration will need to be facilitated through human intervention, restoration (Nicotra *et al.* 2010; Christmas *et al.* 2016). Thus, knowledge of climate-matched ecotypes is urgently needed to prevent local extinction in changing climates.

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 2020), 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. 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.

Understanding how climate structures genetics, form, and function of a dominant grass species is critical to predicting grassland response to climate change. Several lines of evidence

suggest that climate, especially seasonal precipitation and temperature variation, structures *A. gerardii* ecotype form and genetic variation. Other studies of *A. gerardii* using redundancy analyses corroborate that climate, more than geographic location (distance), structures neutral genetic variation (Galliart *et al.* 2019). Third, genetic outliers based on *Bayscanenv* were related to both seasonal precipitation and temperature variation. Precipitation and temperature patterns have been in place for the last 10,000 years (Axelrod 1985), leading to selective pressure and ultimately adaptive differentiation. Our study showed a complex mosaic of abiotic stressors, not just precipitation, across the Great Plains to which *A. gerardii* must adapt either genetically and/or plastically. Adaptation was observed with experimental manipulation of rainfall and temperature (Ravenscroft *et al.* 2015), showing some genotypes were preferred in different experimental treatments. Resurrection experiments show selection after drought (Dickman *et al.* 2019, Hamann *et al.* 2018). *Arabidopsis* ecotypes tolerated extreme drought (Exposito-Alonso *et al.* 2018) through within-species variation in drought tolerance and its evolutionary response to climate.

Such knowledge of intraspecific variation across precipitation gradients and the relative importance of plasticity vs. genetic adaptive variation are critical to predict and model grassland biome responses to climate change (Aspinwall *et al.* 2013; Smith *et al.* 2017; Yurkonis & Harris 2019). This knowledge is urgently needed to predict grassland response to warmer and drier summers in the Great Plains. The year 2012 was the worst drought in 50 years with unprecedented "mega-droughts" predicted for the central U.S. (Cook *et al.* 2015). A recent phenotypic modeling study predicted that by 2070, with climate change, populations of short-statured, dwarf forms of *A. gerardii* from dry parts of its range would be favored ~800 km eastward and result in 60% decrease in biomass, if it can migrate there in time or be planted through restoration (Smith *et al.* 2017). Reduction in productivity could have cascading effects on prairie function (Knapp *et al.*

1998, Hoover *et al.* 2014), cattle forage production (Gibson *et al.* 2016), grassland restoration (Baer *et al.* 2019), and conservation.

Future directions

This work provides a springboard for future work and investigations. Utilizing reciprocal gardens across large time and spatial scales provide a powerful tool for understanding local adaptation. These platforms are further improved when including a variety of measures and responses (Johnson *et al.* 2022) including ecological, genetic, and transcriptomics.

Striking morphological differences among ecotypes of *A. gerardii* are described in chapters 2 and 3. A gene involved with gibberellic acid metabolism was found to be strongly associated with height with allele frequencies varying across ecotypes native to sites east to west. With the known differences between the ecotypes (particularly between the wet and dry ecotypes) this could prove to be a strong candidate gene underlying adaptation in this species. Two future experiments would be fruitful in probing the mechanisms of GA1 and its role in controlling height in these ecotypes. First, the experiment here that identified this gene of interest was conducted with GBS. It would be worthwhile to further investigate this by targeting the specific genomic region and gene. This would allow us to directly compare the genomic sequences of the three ecotypes and identify functional differences at a fine scale that could help explain the differences in phenotype based on genotype. Second, a greenhouse experiment in which the ecotypes are grown, and a foliar application of gibberellic acid applied to the plants. This would further elucidate the mechanisms underlying these differences by investigating whether simple addition of GA would be enough to "restore" the wet phenotype in the dry plants.

Finally, as outlined in chapter 2, we have robust method for easy identification of these ecotypes at the genetic level. It would be valuable to further utilize a genetic approach in the single

ecotype plots in which only a single regional ecotype was seeded into a community with other grasses and forbs. After seeding in 2009, no further seed input has been made to these plots beyond annual prescribed burns to remove aboveground biomass in early springs. Utilizing our ecotype genetic identification approach in the single ecotype plots would allow for confirmation of whether, after this time a decade, whether the ecotypes within their respective plot have remained "pure" to the identity that was used originally seeded. With these grasses being openly wind pollinated there is additional possibility of hybridization and outcrossing between the ecotypes. This would also give insight into the relative role of further establishment by seed since the initial seeding at the beginning of the study. Notably, based on phenotypes, ecotypes appear to be "faithful" to the plots in which they were originally seeded.

Furthermore, due to the strong selective pressures and strength of local adaptation, particularly in the wet and dry ecotypes as show by cover in the community plots. If in the future we were to see an increase in cover of the wet ecotype in western KS and increase of the dry ecotype in eastern KS, being able to quickly confirm ecotype identity would be valuable. Most likely changes in composition as suggested here would be due to migration of the home ecotype into adjacent plots and becoming established. We currently are not seeing any evidence of this, but by looking at the plot's ecotype "purity" as outlined in the previous paragraph would give a metric of the likelihood of an event like this to occur in the coming future.

Summary

Overall, this study exemplifies ecological genomics and identifying the mechanisms underlying organismal responses to environments. Through the integration of morphology, physiology, genomics, and transcriptomics in long term reciprocal garden plots across a natural

environmental gradient we have a better understanding of how *A. gerardii* would be expected to respond to future changing climates. We have identified signals of local adaptation primarily in ecotypes at the dry and wet ends of their localized range in the central Great Plains core of its distribution. Furthermore, we are beginning to identify important candidate genes underlying the diverse morphologies and variation seen in this important species of the tallgrass prairies. Continued work and innovation is needed to continue to fully characterize and understand the important genes of adaptation.

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