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

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

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12

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30 **Keywords:** *Andropogon gerardii*, ecotypes, climate change, genetic variation, intraspecific
31 variation, experimental selection

32 **ABSTRACT**

33
34 Many prior studies have uncovered evidence for local adaptation using reciprocal transplant
35 experiments. However, these studies are rarely conducted for a long enough time to observe
36 succession and competitive dynamics in a community context, limiting inferences for long-lived
37 species. Furthermore, the genetic basis of local adaptation and genetic associations with climate
38 has rarely been identified. Here we report on a long-term (6-yr) experiment conducted under
39 natural conditions focused on *Andropogon gerardii*, the dominant grass of the North American
40 Great Plains tallgrass ecosystem. We focus on this foundation grass that comprises 80% of
41 tallgrass prairie biomass and is widely used in 20,000 km² of restoration. Specifically, we asked
42 1) if ecotypes are locally adapted to regional climate in realistic ecological communities? 2) does
43 adaptive genetic variation underpin divergent phenotypes across the climate gradient? 3) is there
44 evidence of local adaptation if the plants are exposed to competition among ecotypes in mixed
45 ecotype plots? Finally, 4) are local adaptation and genetic divergence related to climate?
46 Reciprocal gardens were planted with 3 regional ecotypes (originating from dry, mesic, wet
47 climate sources) of *Andropogon gerardii* across a precipitation gradient (500-1200 mm/yr) in the
48 US Great Plains. We demonstrate local adaptation and differentiation of ecotypes in wet and dry
49 environments. Surprisingly, the apparent generalist mesic ecotype performed comparably under
50 all rainfall conditions. Ecotype performance was underpinned by differences in neutral diversity
51 and candidate genes corroborating strong differences among ecotypes. Ecotype differentiation
52 was related to climate, primarily rainfall. Without long-term studies, wrong conclusions would
53 have been reached based on the first two years. Further, restoring prairies with climate-matched
54 ecotypes is critical to future ecology, conservation and sustainability under climate change.

55 **INTRODUCTION**

56
57 Understanding climate driven selection within communities is needed to predict grassland
58 response to warmer and drier summers in the North American Great Plains, and other grasslands.
59 In the last 6 years, US grasslands have experienced severe drought, especially in 2012, the worst
60 drought on record in ~50 years. Furthermore, one of the most important climatic changes

61 predicted for grasslands is alteration of amount and timing of precipitation events (IPCC 2013)
62 and unprecedented “mega-droughts” (Cook *et al.* 2015). It is critical to assess if local adaptation
63 limits a population’s ability to adjust to changing climates, or if populations will have to migrate
64 to match future climate conditions or be planted through restoration (Christmas *et al.* 2016;
65 Nicotra *et al.* 2010). Ultimately, research needs to inform conservation and restoration managers
66 to better identify the optimal ecotype (Broadhurst *et al.* 2008; Jones 2013; Bucharova *et al.*,
67 2017) on 20,000 km² of restored marginal land across the Great Plains, (Kettenring *et al.* 2014;
68 Pickup *et al.* 2012) and to plant for forage supply in changing climates in an ecological
69 foundation species (Gibson *et al.* 2016).

70
71 Habitats are often temporally and spatially variable especially with regard to climate, causing
72 differential selection across climate gradients, genetic divergence among populations, and local
73 adaptation (Linhart & Grant, 1996). A main goal of evolutionary biology is to understand factors
74 that contribute to such population genetic divergence (Mayr 1963), formation of ecotypes
75 (Clausen *et al.* 1940), and that ultimately lead to new species (Rundle & Nosil 2005). Yet, gaps
76 exist in knowledge of local adaptation and ecotypic diversity among regionally distributed
77 populations of most plant species (Falk *et al.* 2006), especially foundation species, growing in
78 nature. Local adaptation is fundamental to evolution (Savolainen *et al.* 2013), and has
79 implications for adaptation to global changes, conservation, and restoration (Hufford & Mazer,
80 2003; Nicotra *et al.*, 2010; Shaw & Etterson, 2012).

81
82 Intraspecific variation and local adaptation among plant populations have been widely studied,
83 mostly in response to abiotic conditions, across large-scale climatic gradients (Clausen *et al.*
84 1940; McMillan 1959; Joshi *et al.* 2001; Bischoff *et al.* 2006; Ariza & Tielborger 2011;
85 Munzbergova *et al.* 2017), altitude (Montesinos-Navarro *et al.* 2011), and finer scale
86 environmental variation (Bradshaw 1984; Linhart & Grant 1996; Galloway & Fenster 2000;
87 Montalvo & Ellstrand 2000; Etterson 2004; Knight *et al.* 2006; Lowry *et al.* 2009). However,
88 little is known (Bischoff *et al.* 2006) about plant local adaptation in competitive settings.
89 Consequently, intraspecific variation and local adaptation are rarely interpreted under realistic
90 ecological (community) conditions under which it has evolved (Liancourt & Tielborger 2011;
91 Liancourt *et al.* 2013; Grassein *et al.* 2014; Tomiolo *et al.* 2015; Lowe *et al.* 2017), which limits

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

99
100 Here we investigate whether ecotypic variation in a dominant US Great Plains grass
101 (*Andropogon gerardii*, common name big bluestem) is a result of local adaptation to climate
102 using a reciprocal common garden platform established in 2009 across a precipitation gradient.
103 This experiment focused on *A. gerardii* because it is an ecologically dominant grass that
104 comprises up to 80% of biomass of tallgrass prairie (Weaver, 1932; Epstein *et al.* 1997; Knapp *et*
105 *al.*, 1998). Within the Great Plains, *A. gerardii* occurs along a climate gradient in place for
106 ~10,000 years (Axelrod 1985), allowing ample time for local adaptation to develop. Due to its
107 wide distribution and dominance in the Great Plains (Epstein *et al.* 1997) and spatially varying
108 climate, we expected extensive natural variation across this gradient among populations with
109 formation of ecotypes (Johnson *et al.* 2015). Ecotypic variation among several grass species
110 across a latitudinal gradient in the Great Plains was documented by the early seminal common
111 garden studies of McMillan (1959). More recently, intraspecific variation in performance of
112 switchgrass genotypes originating from different temperature and precipitation environments in a
113 greenhouse common garden was examined by Aspinwall *et al.* (2013). They found that genotype
114 largely explained functional trait variation as related to the climate of origin.

115
116 More specifically, this study aimed to assess genetically based local adaptation of *A. gerardii*
117 ecotypes in realistic competitive settings across the Great Plains' precipitation gradient (500 to
118 1200 mm/yr precipitation across a ~1,000 km span from western Kansas to Illinois). We
119 addressed the following questions: 1) Do ecotypes display local adaptation to regional climate
120 when planted in realistic ecological communities? 2) Does adaptive genetic variation underlie
121 divergent phenotypes? 3) Do we see evidence of local adaptation if the plants are exposed to
122 competition among ecotypes of *A. gerardii* in mixed ecotype plots? 4) Is local adaptation related

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

134
135 This novel experiment assessed local adaptation in realistic ecological settings across a climate
136 gradient including competitors, in a long-lived perennial grass. By contrast, most studies use
137 monocultures in the absence of plant-plant competition, as is commonly done with single-spaced
138 plants (Bischoff *et al.* 2006). Moreover, the long-term nature of the experiment (6 years) allowed
139 community processes and climate to play out. However, most studies that vary phenotypes and
140 genotypes in the field lasted 3 years or less (Franks *et al.*, 2014), and most studied annual plants
141 (Franks *et al.* 2014). This study combined population genetics and identification of candidate
142 genes with performance from long term experimental gardens, which is seldomly done
143 (Villemereuill *et al.* 2016). The study assessed experimental selection by measuring outcome of
144 competing *A. gerardii* ecotypes which, arguably, should be the most robust test for local
145 adaptation across the climate gradients. This is rarely done with perennial plants and in long term
146 studies (Ravenscroft *et al.* 2015). Finally, the study related both performance and genetic
147 variation (Villemereuill *et al.* 2016) to climate and provided a strong test for environment in
148 structuring adaptive variation (Schneider & Mazer 2016).

149
150 **MATERIALS AND METHODS**
151
152 We tested for local adaptation and ecotypic differentiation using several analyses, including 1)
153 reciprocal garden experiments with *A. gerardii* ecotypes grown individually and in a mixture; 2)

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

157

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

159

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

175

176 **2. Reciprocal garden design - Sown community plots**

177

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

182

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

185 Kansas, 871 mm); and Southern Illinois (Carbondale, Illinois, 1167 mm) (Fig. 1, Table 1, Fig. 2).
186 The Western Kansas site in Colby, Kansas was included to test tolerance of ecotypes to more
187 arid environments, as might be expected under future warming and drying. Big bluestem occurs
188 in Western Kansas and Colorado, but only sporadically. This Western Kansas planting site was
189 included to test the effects of increased drying beyond what is experienced by the species in its
190 central distribution. All garden sites were under agricultural cultivation prior to reciprocal garden
191 establishment. All soils were classified as loams (Table 1); specifically, the Eastern three sites
192 were classified as silt loams, and Western Kansas (Colby, Kansas) as silt clay loam (Mendola *et*
193 *al.* 2016). After accounting for percent live seed, seeds from four populations within each
194 ecotype were mixed in equal quantities. Each ecotype and mixtures of ecotypes were reciprocally
195 sown at each site in multi-species communities (Johnson *et al.* 2015). The experiment consisted
196 of a randomized complete block design at each site with four blocks per site. Within a site, each
197 block consisted of four plots (each 4 m x 8 m), 3 of which were seeded to a single regional
198 ecotype (i.e., dry, mesic and wet) and the fourth plot with a mixture of all three regional ecotypes
199 (i.e., mixed ecotype plot). Plots were separated by a 4–6 m buffer strip (Fig. 2). Plots were
200 plowed within a week prior to garden establishment and sown to each regional ecotype in June
201 2009. Seeds were mixed with damp sand to aid in homogenous dispersal, hand-broadcast and
202 hand-raked into soil. Shortly following seeding, 25 mm of supplemental irrigation was provided
203 at the Central Kansas site to alleviate a severe deficit during establishment. This supplement
204 increased precipitation to historical average for that time of year. Throughout the remaining
205 experiment plots all sites received only natural rainfall without any supplemental water added.
206 Seeding details are provided in Johnson *et al.* (2015). Species community composition of sown
207 plots as well as seeding rate is typical for prairie restorations. We used 70:30 ratio of live C₄-
208 grass to C₃-grass and forb seed (see Johnson *et al.* 2015). Total seed density for each plot was
209 580 seeds m², similar to that recommended for prairie restoration (Packard & Mutel 1997). *A.*
210 *gerardii* was planted at a density of 270 live seeds m². Seeds of eight other species (*Sorghastrum*
211 *nutans*, *Elymus canadensis*, *Asclepias tuberosa*, *Chamaechrista fasciculata*, *Monarda fistulosa*,
212 *Oligoneuron rigidum*, *Penstemon digitalis*, *Ruellia humilis*) were added to maintain
213 characteristic functional group structure and competitive relationships of tallgrass prairie.
214 Planted seeds of all species, except *Andropogon* and *Sorghastrum* were purchased from a
215 commercial supplier (Ion Exchange Inc., Harpers Ferry, IA, USA) and sourced from across the

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

220

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

222

223 In addition to the sown “community” plots described above we established plants in monoculture
224 hereafter referred to as “single-spaced” plants. These reciprocal gardens comprised single-
225 spaced plants for which we knew the ecotype identity and used these plants for 1) characterizing
226 genetic differences among ecotypes, and their relation to climate and 2) predicting the ecotypes
227 of plants in the mixed ecotype plots based on combinations of SNP markers unique to plants of
228 known origin. We needed to predict ecotypes in the mixed plots because, although there are clear
229 phenotype differences among ecotypes (SFig. 1), it is difficult to assign plants to the dry and
230 mesic ecotypes because they are more phenotypically similar. We used the same seed sources
231 described above in sown “communities” (Supplemental Table 1). These plantings were adjacent
232 to the blocks of community plots. In winter 2009, a subset of seeds collected from each field-
233 collected wild population was germinated and grown in 10 x 10 cm pots in a greenhouse, using
234 standard greenhouse potting mix (Metro-Mix 510). In August 2009, 20 3-4 month old plants of
235 10 replicate blocks of 12 populations (3 climate regions x 4 populations per regional climate
236 ecotype) were planted at each reciprocal garden site (Fig. 1, Table 1, STable 1). Plants were
237 spaced 50 cm apart and water penetrable landscape cloth was placed around each plant to
238 discourage growth of competing plants. The phenotypes have been described elsewhere (Olsen *et*
239 *al.* 2013; Caudle *et al.* 2014; Mendola *et al.* 2016; Maricle *et al.* 2017).

240

241 **3. Climate and Environment of the Reciprocal Garden Planting Sites**

242

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

247

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

249

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

253

254 *Field Measurements 2010-2015*

255

256 Vegetation cover was measured for six years in single ecotype plots from 2010-2015 within a
257 week of each other across all sites. We focused on vegetative cover (as related to plant biomass)
258 rather than seed production. To estimate percent cover, a 1.0 m² quadrat was used with one
259 intersection every 10 cm for a total of 81 intersections. At every intersection, occurrence of *A.*
260 *gerardii*, other grass, forb, or bare ground was recorded. We used four non-overlapping quadrats
261 per plot for a total of 324 intersections per plot (324 per plot x 12 plots per site=3,888
262 intersections per site x 4 sites=15,552 intersections each year). Quadrats were randomly placed at
263 least 50 cm from edge to minimize edge effect.

264

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

275

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

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

282

283 *Statistical Analyses of Vegetative Cover*

284

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

296

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

305

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

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

310

311 **5. Sample Collection for Genotyping**

312

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

318

319 *Reciprocal Gardens-Single Spaced Plants for Genotyping*

320

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

336

337 *Predicting Ecotype Identity in Mixed Ecotype Plots*

338

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

351

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

354

355 **6. Genetic Analyses**

356

357 *Ecotype Genetic Structure and Differentiation*

358

359 We characterized ecotype genetic structure and differentiation to test how ecotypes are
360 genetically distinguished and how genetics is structured by climate. To do this, we used single-
361 spaced plants of known ecotype for analyses of genetic structure, differentiation and outlier
362 analyses. For these analyses, we used all the SNPs in the data set. Population structure was
363 assessed using *Structure* v2.3.4 (Falush *et al.* 2007). Run parameters included 20,000 burn-in and
364 500,000 MCMC chain length. Admixture was included and correlation between alleles was not
365 assumed. Three separate iterations per K was performed. To identify optimal number of K
366 genetic clusters, Evanno's delta K was calculated in *Structure Harvester* v0.6.94. K clustering
367 and permutation were done in *CLUMPP* v1.1.2 and plot visualization in *DISTRUCT* v1.1.
368 Genetic analysis for pairwise population F_{st} was implemented in *GenAlEx* v6.503 (Peakall and
369 Smouse 2006; 2012) using twelve populations comprising the three regional ecotypes.

370

371 *Importance of Climate vs Geography in Structuring Genetic Differentiation*

372

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

387

388 *Outlier Genetic Analysis and Relation to Climate*

389

390 Genetic “outliers” are those SNPs that show more differentiation compared to background levels
391 of differentiation and are putatively under natural selection. We identified “outlier” SNPs in
392 ecotypes and then related their differentiation to the climate of origin. First, *Bayenv2* (Guenther
393 & Coop 2013) was used to identify “outlier” SNPs, a robust approach providing correction for
394 population structure and demographic processes while controlling false positives (Guenther &
395 Coop 2013; Lotterhos & Whitlock 2014). For *Bayenv2*, SNP data from single-spaced plants were
396 used to generate a covariance matrix for populations to control for population structure. Four
397 separate covariance matrices were generated running the MCMC chain to 10^6 iterations and
398 visualized to ensure chain convergence. For all loci, population differentiation ranking statistic
399 $X^T X$ (Guenther & Coop 2013) was calculated. This statistic identifies loci that have greater
400 differentiation than under neutral drift amongst populations. $X^T X$ values were empirically ranked

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

409

410 **7. Random Forest Model to Predict Ecotype Composition Based on SNPs Identified in the** 411 **Mixed Ecotype Plots**

412

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

430

431 *Random forest training and validation*

432

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

453

454 *Predicting Ecotype in Unknown Plants of Mixed Ecotype Plots*

455

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

462 individuals from mixed plots across two years assesses annual variation in growth and
463 composition within long-term plots.

464

465 **RESULTS**

466

467 **Ecotypes Locally Adapted to Regional Climate in Realistic Ecological Communities**

468

469 When comparing ecotype differences by each garden site using a local vs foreign ecotype
470 comparison, (i.e., how an ecotype from that locality performs compared to foreign ecotypes
471 planted in the site), there was evidence of significant cover differences among ecotypes within a
472 site. In the Western Kansas reciprocal garden site (Colby, Kansas, (Table 1, Fig. 3), the driest
473 site, the dry ecotype cover (~20-40%) was significantly greater ($p < 0.046$) than the wet ecotype
474 (~5%), and in all years the dry ecotype was greater than mesic (~10-25%) but not significantly
475 different. A similar pattern was observed in the Central Kansas reciprocal garden (Hays, Kansas)
476 the next driest site, where in 5 out of 6 years, the dry ecotype cover (~25-40%) was significantly
477 greater ($p < 0.039$) than the wet ecotype (~5%). In all years at the Central Kansas reciprocal
478 garden (Hays, Kansas), the dry ecotype was greater than the mesic ecotype (~15-25%) but not
479 significantly different. Interestingly, in the Eastern Kansas reciprocal garden (Manhattan
480 Kansas), there were no significant differences among ecotypes across all years and cover ranged
481 from 20-35%, regardless of ecotype. In the Southern Illinois reciprocal garden (Carbondale,
482 Illinois), the wettest site, there were no significant differences among ecotypes during the first
483 two establishment years and all ecotypes maintained relatively low levels of cover (<10%). From
484 2012 onward, the dry ecotype continued to show significantly lower ($p < 0.018$) cover (<10%)
485 compared to the wet (25-40%) ecotype, but mesic (15-30%) and wet ecotypes (25-40%) were not
486 significantly different from each other.

487

488 Based on the same data, ecotypes showed signs of local adaptation when planted in their home
489 site compared to their away site (Table 1, Fig. 4). In all years, the dry ecotype (Fig. 4) had
490 significantly lower cover (cover <10% $p < 0.032$) than other ecotypes when planted in the
491 Southern Illinois reciprocal garden (Carbondale, Illinois, wettest site). For the wet ecotype (Fig.
492 4), in the first two years there were no significant differences between the reciprocal gardens in

493 western Kansas (Colby, Kansas), Central Kansas (Hays, Kansas) and Southern Illinois
494 (Carbondale, Illinois), that is driest, dry, and wettest, respectively (cover 10-20%) but was
495 significantly higher in Eastern Kansas (Manhattan Kansas) in 2010 ($p < 0.041$). Following the
496 establishment years, from 2013 onward, the wet ecotype had significantly increased cover (~25-
497 40%) in Eastern Kansas (Manhattan, Kansas) and Southern Illinois (Carbondale, Illinois ($p <$
498 0.049) but lower in western (Colby, Kansas) and Central Kansas (Hays, Kansas) sites, where the
499 cover of the wet ecotype was reduced to about 5% cover ($p < 0.003$). Interestingly, across all
500 years, there were no significant cover differences in the mesic ecotype among all four planting
501 sites (Fig. 4).

502
503 Regressions of cover by ecotype vs annual rainfall for combined years of 2014 and 2015, the
504 latest measurement years presumably when the vegetation was stabilized, showed that the dry
505 ecotype had highest cover with low rainfall, and decline in cover with increased rainfall as
506 occurs in the wettest site of Southern Illinois (Carbondale, Illinois, $p = 0.05$, $R^2 = 0.50$) (Fig. 5).
507 The wet ecotype showed the opposite pattern with low cover in Western and Central Kansas and
508 increase in cover with precipitation as occurs in Southern Illinois (Carbondale, Illinois, $p =$
509 0.007 , $R^2 = 0.73$). Interestingly, cover of the mesic ecotype was only weakly related to rainfall (p
510 $= 0.26$, $R^2 = 0.21$, data not shown). This clearly shows cover of dry and wet ecotypes is related to
511 rainfall and corroborates their delineation. There were no significant correlations with other
512 variables (data not shown).

513

514 **Genetic Divergence Among Ecotypes Supports Phenotype Differences**

515

516 *Divergence and Diversity, Relation to Climate vs Geography*

517

518 *Structure* results indicate $K=3$ genetic clusters with two predominating, one occurring in dry and
519 mesic ecotypes and the other in wet ecotype (Fig. 6). Based on pairwise F_{st} (STable 3), only
520 slight neutral differentiation was observed between populations with F'_{st} (Meirmans *et al.* 2011)
521 of .028. In general, the wet ecotype showed greatest genetic distance with populations from
522 Kansas with F_{st} as high as 0.037. Populations from the dry and mesic ecotypes show lower
523 genetic distance as one might expect from geographic proximity, with F_{st} between 0.011-0.016.

524

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

534

535 *Outlier Analysis Related to Climate*

536

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

554

555 *Random Forest Training and Validation Using Plants of Known Ecotype*

556

557 Individuals from the validation set from plants of known ecotype were assigned to one of three
558 ecotypes (dry, mesic, wet) with accuracy of 79% (STables 7, 8) and overall misclassification rate
559 of 21%. The highest rate of misclassification occurred with mesic individuals incorrectly called
560 dry ecotype 26.4% (28/106 mesic plants). Of all ecotype pairs misclassified (21%, STable 7),
561 68% of those arose from mesic being called dry or vice versa. Importantly, misclassification of
562 the wet ecotype was 4% of all wet ecotype individuals (4/98) and rarely misclassified (STables 7,
563 8). This is also shown in the training/validation triangle SFig. 7. Qualitatively, the
564 training/validation triangle indicates excellent identification of wet ecotype individuals with
565 somewhat less, but still good, discernment between dry and mesic ecotypes.

566

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

569

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

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

592
593 A similar pattern of ecotype composition was observed in 2015 (SFigs. 9,10, STable 10) and
594 corroborates 2014 results. In dry Western Kansas (Colby, Kansas) and Central Kansas (Hays,
595 Kansas), the dry ecotype again was predicted to dominate mixed plots with only one wet ecotype
596 individual predicted in both sites. At the Eastern Kansas (Manhattan, Kansas) and Southern
597 Illinois (Carbondale, Illinois) sites, ecotype composition showed the same trends as observed
598 from 2014 sampling.

599

600 **DISCUSSION**

601

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

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

627

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

629

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

643

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

648 observed at the wettest site, while the dry ecotype performed well in dry regions from the start of
649 experiment. The time-lag in response of the wet ecotype, especially at the wet site in Illinois,
650 may be due to differences in competitive environments across the gradient. We surmise that local
651 adaptation cannot be detected until early successional forbs are outcompeted by grasses (McCain
652 et al. 2010). Thus, competition with forbs may have delayed expression of local adaptation of the
653 wet ecotype in Illinois in the first few years, although further experimental studies are needed.
654 Other researchers who have studied local adaptation in competitive environments have found
655 that expression of local adaptation depends on biotic environment, including competition
656 (Bischoff *et al.* 2006; Liancourt *et al.* 2015; Tomiolo *et al.* 2015) and facilitation (Johnson *et al.*
657 2010).

658
659 Differences in ecotype performance in single ecotype plots corroborates sharp morphological
660 differences among ecotypes observed in single-spaced plants (Caudle *et al.* 2014; Olsen *et al.*
661 2013; Mendola *et al.* 2016). The dry ecotype was dwarfed in size, short, having narrow leaves
662 (SFig. 1) putatively to reduce evaporative loss (Johnson *et al.* 2015; Maricle *et al.* 2017) as an
663 adaptation to drought. In contrast, the wet ecotype is tall, robust, and leafy (SFig. 1), presumably
664 adapted to highly competitive environments where it grows amongst tall forbs and shrubs in wet
665 prairies (Kuchler 1964). Interestingly, the dry ecotype flowers 3 weeks earlier than the wet
666 ecotype, regardless of planting site, portending the beginning of reproductive isolation (Gallart
667 *et al.* unpublished). This study and other several recent studies also highlight the importance of
668 intraspecific variation, genetic (Malyshev *et al.* 2016; Poirier *et al.* 2012) or phenotypic (Avolio
669 *et al.* 2013; Des Roaches *et al.* 2017; Bolnik *et al.* 2011; Hamann *et al.* 2016), in ecological
670 settings or in response to human-induced change (Mimural *et al.* 2017).

671 672 **2. Genetic Analyses Support Differentiation of Wet and Dry Ecotypes**

673
674 Genetically distinguished ecotypes support cover results across the precipitation gradient, similar
675 to results observed by Gray *et al.* (2014) and Price *et al.* (2010). *STRUCTURE* plots show clear
676 differentiation of dry and mesic from wet ecotypes, with admixture between adjacent dry and
677 mesic ecotypes (Fig. 6). We have also shown that environmental factors, especially precipitation,
678 explain more of genetic differences than does geographic location (SFig. 4, STable 4).

679

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

695

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

710 selection in the same candidate genes amongst 71 populations in Switzerland (Rellstab *et al.*
711 2016). Laskey *et al.* (2012) used redundancy analyses to quantify the association between
712 climate, geography and genomics in Eurasian *Arabidopsis* populations to discover that early
713 spring temperature explained most of the variation. Pluess *et al.* (2016) related phenology
714 candidate genes to climate, geographic and seasonality in European beeches. Finally, Exposito-
715 Alonso *et al.* (2017) linked genetic variation to drought tolerance in *Arabidopsis* accessions from
716 contrasting climates and highlighted the role of within species variation in the evolutionary
717 response to climate.

718

719 **3. Experimental Selection Studies Corroborate Wet and Dry Ecotypes**

720

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

729

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

737

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

741 in Eastern Kansas, where all ecotypes performed equally well. Further, the wet ecotype
742 outcompeted the mesic ecotype in the mixed plots located in Eastern Kansas. The years of mixed
743 ecotype plot collection had normal precipitation, so it is doubtful precipitation played a role.
744 Furthermore, this result was not due to lack of random forest discernment, as the wet ecotype is
745 easily distinguished from the others, and makes up the majority of the ecotype identified in
746 Eastern KS and Southern Illinois. So why did the mesic ecotype do comparatively poorly in its
747 home environment of Eastern Kansas, being outperformed by the wet ecotype? The wet ecotype
748 appears to be more competitive than the mesic ecotype in Eastern Kansas when the ecotypes
749 were planted together in the mixed ecotype plot compared to single ecotype plots. That is, the
750 wet ecotype wins inter-ecotype competition (between wet and mesic ecotypes) in the mixed
751 ecotype plots, but when grown among other wet ecotype plants in single ecotype plots, intra-
752 ecotype competition is stronger, resulting in overall low cover of wet ecotypes in single ecotype
753 plots. The wet ecotype putatively outcompetes the mesic ecotype in Eastern Kansas because it is
754 more vigorous due to its tall, robust stature (~3 times taller, ~2 times more biomass), thus
755 suppressing the shorter stature mesic ecotype, resulting in greater dominance of the wet ecotype
756 in Eastern Kansas. These results highlight the increased strength of biotic factors, especially
757 between-ecotype competition in the expression of local adaptation at the wetter end of the
758 gradient. At the dry end of the gradient, abiotic factors such as low precipitation are selective
759 pressures in local adaptation and the dry ecotype dominates in single and mixed ecotype plots.

760
761 Our results corroborate other studies (reviewed in Franks *et al.* 2014) showing selection over
762 time. Several studies show selection-induced treatment effects on phenotypes in intact
763 communities. The Buxton grassland studies of climate change treatments imposed over 15 years
764 shows adaptive selection and differentiation of phenotypes of species (Fridley *et al.* 2010), and
765 outliers sorting of genotypes (Ravenscroft *et al.* 2015) among treatments plots. Avolio & Smith
766 (2013) studied changes in phenotype in response to rainfall manipulation in intact grassland and
767 found *A. gerardii* phenotypic variation but no adaptive response to drought. Resurrection studies
768 in which phenotypes and genotypes from historical seed are compared with contemporary
769 progeny (Franks *et al.* 2018) have shown evidence for contemporary evolution. Franks *et al.*
770 (2016) showed rapid genome evolution in response to drought in *Brassica rapa*. Nevo *et al.*
771 (2012) found that cereal grasses in Israel collected as seed 28 years apart showed genetic and

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

774

775 **4. Broader Implications for Climate Change, Conservation and Restoration**

776

777 Several lines of evidence suggest that climate, especially seasonal precipitation and temperature
778 variables, structures ecotypes and genetic divergence. First, cover of wet and dry ecotypes was
779 correlated with precipitation, with wet ecotypes outperforming dry ecotypes in wet climates
780 (Figs. 3, 4) and conversely, for dry ecotypes. Second, pRDA shows that climate, more than
781 geographic location, structures neutral genetic variation. Third, outliers were related to both
782 temperature and precipitation factors. Precipitation and temperature patterns for the last 10,000
783 years (Axelrod 1985) have been a selective pressure leading to adaptive variation. This has also
784 been observed with experimental manipulation of rainfall and temperature (Avolio *et al.* 2013).
785 The ability of species to tolerate extreme drought was demonstrated by Exposito-Alonso *et al.*
786 (2018) in which they highlighted the role of within species variation in drought tolerance in
787 *Arabidopsis* and its evolutionary response to climate. More broadly, the importance of
788 precipitation as a selection force in plants and animal populations has been discovered through
789 meta-analysis (Siepielski *et al.* 2016).

790

791 How climate structures *A. gerardii* genetics, form, and function is critical, as the foundation
792 species of tallgrass prairie. Climate is predicted to change in the Great Plains (IPCC 2013),
793 resulting in increased occurrence and severity of drought. We are currently manipulating rainfall
794 with a rainout drought experiment in these same plots to address the role of drought. A recent
795 phenotypic modeling study (Smith *et al.* 2017) predicted that, with climate change, populations
796 of short-statured, dwarf forms of *A. gerardii* from dry parts of its range would be favored 600 km
797 eastward, and result in 60% decrease in productivity and biomass. Evolutionary adaptation in *A.*
798 *gerardii* may not be able to provide what ecology and future climate demands (Kokko *et al.*
799 2017). Reduction in productivity could have cascading effects on prairie function (Knapp *et al.*
800 1998), cattle forage production (Gibson *et al.* 2016), grassland restoration (Baer *et al.* 2018), and
801 conservation. Furthermore, about 60% of agricultural production in Kansas (~\$10 billion, NASS,
802 2014) was attributed to cattle production, with *A. gerardii* being the main forage grass for cattle.

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

809

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811

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816 **REFERENCES**

817

818 Altman, N., & Krzywinski, M. (2017). Points of Significance: Ensemble methods: bagging and
819 random forests. *Nature Methods*, 14, 933–934.

820

821 Ariza, C., Tielborger, K. (2011). An evolutionary approach to studying the relative importance of
822 plant–plant interactions along environmental gradients. *Functional Ecology*, 25, 932–942.

823

824 Aspinwall, M. J., Lowry, D. B., Taylor, S. H., Juenger, T. E., Hawkes, C. V., Johnson, M. V. V.,
825 Kiniry, J. R. & Fay, P. A. (2013). Genotypic variation in traits linked to climate and
826 aboveground productivity in a widespread C4 grass: evidence for a functional trait syndrome.
827 *New Phytologist*, 199(4), 966-980.

828

829 Avolio, M. L., and Smith M. D. (2013). Mechanisms of selection: Phenotypic differences among
830 genotypes explain patterns of selection in a dominant species. *Ecology*, 94, 953-965.

831

832 Axelrod, D. I. (1985). Rise of the grassland biome, Central North America. *The Botanical*
833 *Review*, 51(2), 163-201.

834
835 Baer, S. G., D. J. Gibson, and L. C. Johnson. (2018). In press. Restoring grassland in the context
836 of climate change. Chapter 18. Grasslands and Climate Change, D. J. Gibson and J. Newman,
837 editors. Cambridge University Press, UK.
838
839 Bensen, E. and Hartnett, D. (2006). The role of seed and vegetative reproduction in plant
840 recruitment and demography in tallgrass prairie. *Plant Ecology*, 187, 163-177.
841
842 Bischoff, A., Crémieux, L., Smilauerova, M., Lawson, C., Mortimer, S.R., Dolezal, J., Lanta, V.,
843 Edwards, A.R., Brook, A.J., Macel, M. (2006). Detecting local adaptation in widespread
844 grassland species – the importance of scale and local plant community. *Journal of Ecology*, 94,
845 1130–1142.
846
847 Bolnick, D.I., Amarasekare, P., Araujo, M. S., Burger, R., Levine, J. M., Novak, M., Rudolf, V.
848 H.W., Schreiber, S.J., Urban, M.C., Vasseur, D.A. (2011). Why intraspecific trait variation
849 matters in community ecology *Trends in Ecology and Evolution*, 26(4), 183-192
850
851 Bradshaw, A.D. (1984). Ecological significance of genetic variation between populations. In:
852 Dirzo R, Sarukhan J, eds. *Perspectives on Plant Population Biology*. Sunderland: Sinauer
853 Associates, 213-228.
854
855 Bragg G., Supple, M.A., Andrew, R. L. and Borevitz, J.O. (2015). Genomic variation across
856 landscapes: insights and applications. *New Phytologist*, 207, 953–967
857
858 Breiman, L. (2001). Random forests. *Machine learning*, 45(1), 5-32.
859
860 Broadhurst, L. M., Lowe, A., Coates, D. J., Cunningham, S. A., McDonald, M., Vesk, P. A., and
861 Yates, C. (2008). Seed supply for broadscale restoration: maximizing evolutionary potential.
862 *Evolutionary Applications*, 1(4), 587-597.
863

864 Bucharova, A., Michalski, S., Hermann, J. M., Heveling, K., Durka, W., Hölzel, N., and
865 Bossdorf, O. (2017). Genetic differentiation and regional adaptation among seed origins used for
866 grassland restoration: lessons from a multispecies transplant experiment. *Journal of Applied*
867 *Ecology*, 54, 127–136.

868

869 Caudle, K. L., Johnson, L. C., Baer, S. G., and Maricle, B. R. (2014). A comparison of seasonal
870 foliar chlorophyll change among ecotypes and cultivars of *Andropogon gerardii* (Poaceae) by
871 using nondestructive and destructive methods. *Photosynthetica*, 52(4), 511-518.

872

873 Clausen, J., Keck, D.D., Hiesey, W.M. (1940). Experimental studies on the nature of species. I.
874 Effect of varied environments on western North American plants. *Carnegie Institution of*
875 *Washington*, 520.

876

877 Cook, B. I., Ault, T. R., and Smerdon, J. E. (2015). Unprecedented 21st century drought risk in
878 the American Southwest and Central Plains. *Science Advances*, 1(1), e1400082.

879

880 Dalgleish, H.J., Ott, J.P., Setshogo, M.P., Hartnett, D.C. (2012). Inter-specific variation in bud
881 banks and flowering effort among semi-arid African savanna grasses. *South African Journal of*
882 *Botany*, 83, 127–133.

883

884 De Kort, H., Vandepitte, K., Bruun, H.H., Kopp, D.C., Honnay, O. and Mergeay, J. (2014).
885 Landscape genomics and a common garden trial reveal adaptive differentiation to temperature
886 across Europe in the tree species *Alnus glutinosa*. *Molecular Ecology*, 23, 4709–4721

887

888 Des Roches, S., Post, D. M., Turley, N. E., Bailey, J. K., Hendry, A. P., Kinnison, M. T.,
889 Schweitzer, J. A. and Palkovacs, E. P. (2017). The ecological importance of intraspecific
890 variation. *Nature Ecology and Evolution*, <https://doi.org/10.1038/s41559-017-0402-5>.

891

892 Doyle, J.J., Doyle, J.L. (1987). A rapid DNA isolation procedure for small quantities of fresh
893 leaf tissue. *Phytochemical Bulletin*, 19, 11–15.

894

895 Elshire, R. J., Glaubitz, J. C., Sun, Q., Poland, J. A., Kawamoto, K., Buckler, E. S., and Mitchell,
896 S. E. (2011). A robust, simple genotyping-by-sequencing (GBS) approach for high diversity
897 species. *PLoS one*, 6(5), e19379.

898

899 Epstein, H.E., Lauenroth, W.K., Burke, I.C., Coffin, D.P. (1997). Productivity patterns of C₃ and
900 C₄ functional types in the U.S. Great Plains. *Ecology*, 78, 722-731.

901

902 Etterson, J.R. (2004). Evolutionary potential of *Chamaecrista fasciculata* in relation to climate
903 change. I. Clinal patterns of selection along an environmental gradient in the Great Plains.
904 *Evolution*, 58, 1446-1456.

905

906 Exposito-Alonso, M., Vasseur, F., Ding, W., Wang, G., Burbano, H. A., Weigel, D. (2017).
907 Genomic basis and evolutionary potential for extreme drought adaptation in *Arabidopsis*
908 *thaliana*. *Nature Ecology and Evolution* 2, 352–358.

909

910 Falk, D., Palmer, M.A., Zedler, J.A. (2006). Foundations of Restoration Ecology. Editors.
911 *Society for Ecological Restoration*. 364.

912

913 Falush, D., Stephens, M., & Pritchard, J. K. (2007). Inference of population structure using
914 multilocus genotype data: dominant markers and null alleles. *Molecular Ecology Resources*,
915 7(4), 574-578.

916

917 Fischer, M. C., Rellstab, C., Tedde, A., Zoller, S., Gugerli, F., Shimizu, K. K., Holderegger, R.
918 and Widmer, A. (2013). Population genomic footprints of selection and associations with climate
919 in natural populations of *Arabidopsis halleri* from the Alps. *Molecular Ecology*, 22, 5594–5607.

920

921 Foll, M. & Gaggiotti, O.E. (2008). A genome-scan method to identify selected loci appropriate
922 for both dominant and codominant markers: A Bayesian perspective. *Genetics*, 180, 977–993.

923

924 Franks, S. J., Weber, J. J., and Aitken, S. N. (2014). Evolutionary and plastic responses to
925 climate change in terrestrial plant populations. *Evolutionary Applications*, 7(1), 123-139.

926
927 Franks, S. J., Kane, N. C., O'Hara, N. B., Tittes, S. and Rest, J. S. (2016). Rapid genome-wide
928 evolution in *Brassica rapa* populations following drought revealed by sequencing of ancestral
929 and descendant gene pools. *Molecular Ecology*, 25, 3622–3631
930
931 Frank, S.J., Hamann, E., Weis, A.E. (2018) Using the resurrection approach to understand
932 contemporary evolution in changing environments. *Evolutionary Applications*, 11(1), 17–28.
933
934 Fridley, J. D. & Grime, J. P. (2010). Community and ecosystem effects of intraspecific genetic
935 diversity in grassland microcosms of varying species diversity. *Ecology*, 91(8), 2272–2283
936
937 Galloway, L.F. & Fenster, C.B. (2000). Population differentiation in an annual legume: local
938 adaptation. *Evolution*, 54, 1173–1181.
939
940 Gibson, A. L., Espeland, E. K., Wagner, V. and Nelson, C. R. (2016). Can local adaptation
941 research in plants inform selection of native plant materials? An analysis of experimental
942 methodologies. *Evolutionary Applications* (10), 1219–1228.
943
944 Grassein, F., Lavorel, S., and Till- Bottraud, I. (2014). The importance of biotic interactions and
945 local adaptation for plant response to environmental changes: field evidence along an elevational
946 gradient. *Global change biology*, 20(5), 1452-1460.
947
948 Gray, M. M., St Amand, P., Bello, N. M., Galliard, M. B., Knapp, M., Garrett, K. A., Morgan, T.
949 J., Baer, S. G., Maricle, B. R., Akhunov, E. D., and Johnson, L. C. (2014). Ecotypes of an
950 ecologically dominant prairie grass (*Andropogon gerardii*) exhibit genetic divergence across the
951 US Midwest grasslands' environmental gradient. *Molecular ecology*, 23(24), 6011-6028.
952
953 Guenther, T. & Coop, G. (2013). Robust identification of local adaptation from allele
954 frequencies. *Genetics*, 195, 205–220.
955
956 Hamann, E., Kesselring, H., Armbruster, G. F. J., Scheepens, J. F. and Stocklin, J. (2016).

957 Evidence of local adaptation to fine- and coarse grained environmental variability in *Poa alpina*
958 in the Swiss Alps. *Journal of Ecology*, 104, 1627–1637.

959

960 Hancock, A.M., Brachi, B., Faure, N., Horton, M.W., Jarymowycz, L.B., Sperone, F. G.,
961 Toomajian, C., Roux, F., Bergelson, J. (2011). Adaptation to Climate Across the *Arabidopsis*
962 *thaliana* Genome. *Science*, 334, 83-86.

963

964 Hufford, K.M. & Mazer, S.J. (2003). Plant ecotypes: genetic differentiation in the age of
965 ecological restoration. *Trends in Ecology and Evolution*, 18, 147-155.

966

967 IPCC Climate change 2013: Physical Science Basis. Report of the IPCC.

968

969 Johnson, N. C., Wilson, G. W. T., Bowkera, M. A., Wilson, J. A., and Miller, R.M. (2010).
970 Resource limitation is a driver of local adaptation in mycorrhizal symbioses. *Proceedings of the*
971 *National Academy of Sciences of the United States of America*, 107, 2093–2098.

972

973 Johnson, L. C., Olsen, J. T., Tetreault, H., DeLaCruz, A., Bryant, J., Morgan, T. J., Knapp, M.,
974 Bello, N. M., Baer, S. G., and Maricle, B. R. (2015). Intraspecific variation of a dominant grass
975 and local adaptation in reciprocal garden communities along a US Great Plains' precipitation
976 gradient: implications for grassland restoration with climate change. *Evolutionary Applications*,
977 8(7), 705-723.

978

979 Joshi, J., Schmid, B., Caldeira, M.C., Dimitrakopoulos, P.G., Good, J., Harris, R., Hector, A.,
980 Huss-Danell, K., Jumpponen, A., Minns, A. (2001). Local adaptation enhances performance of
981 common plant species. *Ecology Letters*, 4, 536-544.

982

983 Jones, T. A. (2013). Ecologically appropriate plant materials for restoration. *BioScience*, 63,
984 211–219.

985

986 Kettenring, K., Mercer, K. L., Adams, C.R. and Hines, J. (2014). Application of genetic
987 diversity–ecosystem function research to ecological restoration. *Journal of Applied Ecology*, 51,

988 339–348.

989

990 Knapp, A.K., Briggs, J.M., Harnett, D.C., Collins, S.L. (1998). Patterns and controls of
991 aboveground net primary productivity in tallgrass prairie. *Grassland Dynamics: Long-Term*
992 *Ecological Research in Tallgrass Prairie*. New York: Oxford University Press, 193-221.

993

994 Knight, C.A., Vogel, H., Kroymann, J., Shumate, A., Witsenboer, H., Mitchell-Olds, T. (2006).
995 Expression profiling and local adaptation of *Boechera holboellii* populations for water use
996 efficiency across a naturally occurring water stress gradient. *Molecular Ecology*, 15, 1229-1237.

997

998 Kokko, H., Anurag, C., Croll, D., Fischer, M.C., Guillaume, F., Karrenberg, S., Kerr, B.,
999 Rolshausen, G., and Stapley, J. (2017). Can Evolution Supply What Ecology Demands? *Trends*
1000 *in Ecology and Evolution*, 32(3), 187

1001

1002 Kuchler, A.W. (1964). Potential Natural Vegetation of the Conterminous United States,
1003 *American Geographical Society, Special Publication*, 36.

1004

1005 Laskey, J.R., Des Marais, D.L., Mckay, J.K., Richards, J.H., Juenger, T.E. and Keitt, T. H.
1006 (2012). Characterizing genomic variation of *Arabidopsis thaliana*: the roles of geography and
1007 climate. *Molecular Ecology*, 21, 5512–5529.

1008

1009 Lasky, J.R., Forester, B.R., Reimherr, M. (2018). Coherent synthesis of genomic associations
1010 with phenotypes and home environments. *Molecular Ecology Resources*, 18(1), 91-106.

1011

1012 Lemoine, N.P., Dietrich, D. and Smith, M.D. (2017). Precipitation and environmental constraints
1013 on three aspects of flowering in three dominant tallgrass species. *Functional Ecology*, 31, 1894–
1014 1902.

1015

1016 Li, H. & Durbin, R. (2009). Fast and accurate short read alignment with Burrows-Wheeler
1017 Transform. *Bioinformatics*, 25, 1754-60.

1018

1019 Liancourt, P., Spence, L. A., Song, D. S., Lkhagva, A., Sharkhuu, A., Boldgiv, B., ... & Casper,
1020 B. B. (2013). Plant response to climate change varies with topography, interactions with
1021 neighbors, and ecotype. *Ecology*, 94(2), 444-453.
1022
1023 Liancourt, P., & Tielbörger, K. (2009). Competition and a short growing season lead to ecotypic
1024 differentiation at the two extremes of the ecological range. *Functional Ecology*, 23(2), 397-404.
1025
1026 Liaw, A., & Wiener, M. (2002). Classification and regression by randomForest. *R news*, 2(3),
1027 18-22.
1028
1029 Linhart, Y. B., & Grant, M. C. (1996). Evolutionary significance of local genetic differentiation
1030 in plants. *Annual review of ecology and systematics*, 27(1), 237-277.
1031
1032 Lotterhos, K. E., & Whitlock, M. C. (2015). The relative power of genome scans to detect local
1033 adaptation depends on sampling design and statistical method. *Molecular ecology*, 24(5), 1031-
1034 1046.
1035
1036 Lowe, W. H., Kovach, R. P., & Allendorf, F. W. (2017). Population genetics and demography
1037 unite ecology and evolution. *Trends in ecology & evolution*, 32(2), 141-152.
1038
1039 Lowry, D. B., Hall, M. C., Salt, D. E., & Willis, J. H. (2009). Genetic and physiological basis of
1040 adaptive salt tolerance divergence between coastal and inland *Mimulus guttatus*. *New*
1041 *Phytologist*, 183(3), 776-788.
1042 Lu, F., Lipka, A. E., Glaubitz, J., Elshire, R., Cherney, J. H., Casler, M. D., Buckler, E. S.,
1043 Costich, D. E. (2013). Switchgrass Genomic Diversity, Ploidy, and Evolution: Novel Insights
1044 from a Network-Based SNP Discovery Protocol. *PLoS Genetics*, 9(1), e1003215.
1045
1046 Malyshev, A. Khan M , Beierkuhnlein C, Steinbauer M, Henry H Jentsch A, Dengler J, Willner,
1047 E, Kreyling J. (2016). Plant responses to climatic extremes: within-species variation equals
1048 among-species variation. *Global Change Biology* 22, 449–464, doi: 10.1111/gcb.13114
1049

1050 Maricle, B. R., Caudle, K. L., Lindsey, K. J., Baer, S. G., & Johnson, L. C. (2017). Effects of
1051 extreme drought on photosynthesis and water potential of *Andropogon gerardii* (big bluestem)
1052 ecotypes in common gardens across Kansas. *Transactions of the Kansas Academy of Science*,
1053 120(1–2), 1-16.

1054

1055 Mayr, E. (1963). *Animal species and evolution*. *Harvard Press*, 797.

1056

1057 McCain, K. N. S., Baer, S. G., Blair, J. M., and Wilson, G. W. T. (2010). Dominant Grasses
1058 Suppress Local Diversity in Restored Tallgrass Prairie. *Restoration Ecology*, 18, 40-49.

1059

1060 McMillan C. (1959). The role of ecotypic variation in the distribution of the Central grassland of
1061 North America. *Ecological Monographs*, 29, 286-308.

1062

1063 Mendola, M. L., Baer, S. G., Johnson, L. C., and Maricle, B. R. (2015). The role of ecotypic
1064 variation and the environment on biomass and nitrogen in a dominant prairie grass. *Ecology*,
1065 96(9), 2433-2445.

1066

1067 Metz, J., and Tielbörger, K. (2016). Spatial and temporal aridity gradients provide poor proxies
1068 for plant–plant interactions under climate change: a large- scale experiment. *Functional*
1069 *Ecology*, 30(1), 20-29.

1070

1071 Milach, S. C. K., Rines, H. W., & Phillips, R. L. (2002). Plant height components and gibberellic
1072 acid response of oat dwarf lines. *Crop science*, 42(4), 1147-1154.

1073

1074 Mimura, M., Yahara, T., Faith, D. P., VázquezDomínguez, E., Colautti, R. I., Araki, H., ... &
1075 Hollingsworth, P. M. (2017). Understanding and monitoring the consequences of human impacts
1076 on intraspecific variation. *Evolutionary applications*, 10(2), 121-139.

1077

1078 Montalvo, A.M., Ellstrand, N.C. (2000). Transplantation of the subshrub *Lotus scoparius*: testing
1079 the home-site advantage hypothesis. *Conservation Biology*, 14, 1034–1045.

1080

1081 Montesinos-Navarro, A., Wig, J., Pico, F. X., & Tonsor, S. J. (2011). *Arabidopsis thaliana*
1082 populations show clinal variation in a climatic gradient associated with altitude. *New Phytologist*,
1083 189(1), 282-294.

1084

1085 Münzbergová, Z., Hadincová, V., Skálová, H., & Vandvik, V. (2017). Genetic differentiation
1086 and plasticity interact along temperature and precipitation gradients to determine plant
1087 performance under climate change. *Journal of Ecology*, 105(5), 1358-1373.

1088

1089 NASS 2014. National Agricultural Statistics Service.

1090

1091 Nicotra, A. B., Atkin, O. K., Bonser, S. P., Davidson, A. M., Finnegan, E. J., Mathesius, U., ... &
1092 van Kleunen, M. (2010). Plant phenotypic plasticity in a changing climate. *Trends in plant*
1093 *science*, 15(12), 684-692.

1094

1095 Nevo, E., Fu, Y. B., Pavlicek, T., Khalifa, S., Tavasi, M., & Beiles, A. (2012). Evolution of wild
1096 cereals during 28 years of global warming in Israel. *Proceedings of the National Academy of*
1097 *Sciences*, 109(9), 3412-3415.

1098

1099 Normann, G.A. & Keeler, K.H. (2003). Cytotypes of *Andropogon gerardii* Vitman (Poaceae):
1100 fertility and reproduction of aneuploids. *Botanical Journal of the Linnean Society*, 141, 95- 103.

1101

1102 Oksanen, J., F. G. Blanchet, R. Kindt, P. Legendre, P. R. Minchin, R. B. O'Hara, G. L.
1103 Simpson. (2015). *vegan*: Community ecology package, version 2.2-1. Website [http://CRAN.R-](http://CRAN.R-project.org/package=vegan)
1104 [project.org/package=vegan](http://CRAN.R-project.org/package=vegan) .

1105

1106 Olsen, J. T., Caudle, K. L., Johnson, L. C., Baer, S. G., and Maricle, B. R. (2013). Environmental
1107 and genetic variation in leaf anatomy among populations of *Andropogon gerardii* (Poaceae)
1108 along a precipitation gradient. *American Journal of Botany*, 100(10), 1957-1968.

1109

1110 Peakall, R. and Smouse, P.E. (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic
1111 software for teaching and research—an update. *Bioinformatics*, 28, 2537-2539

1112
1113 Pickup, M., Field, D. L., Rowell, D. M., & Young, A. G. (2012). Predicting local adaptation in
1114 fragmented plant populations: implications for restoration genetics. *Evolutionary Applications*,
1115 5(8), 913-924.
1116
1117 Pluess, A. R., Frank, A., Heiri, C., Lalagüe, H., Vendramin, G. G., & Orellana, S.
1118 (2016). Genome-environment association study suggests local adaptation to climate at the
1119 regional scale in *Fagus sylvatica*. *New Phytologist*, 210(2), 589-601.
1120
1121 Poland, J. A., & Rife, T. W. (2012). Genotyping-by-sequencing for plant breeding and
1122 genetics. *The Plant Genome*, 5(3), 92-102.
1123
1124 Poirier, M., Durand, J. L., & Volaire, F. (2012). Persistence and production of perennial grasses
1125 under water deficits and extreme temperatures: importance of intraspecific vs. interspecific
1126 variability. *Global Change Biology*, 18(12), 3632-3646.
1127
1128 Price, D., Salon, P., & Casler, M.D. (2012). Big bluestem gene pools in the Central and
1129 NorthEastern United States. *Crop Science*, 52, 189–200.
1130
1131 Ravenscroft, C.H., Whitlock, R. & Fridley, J.D. (2015). Rapid genetic divergence in response to
1132 15 years of simulated climate change. *Global Change Biology*, 21, 4165–4176.
1133
1134 Rellstab, C., Gugerli, F., Eckert, A. J., Hancock, A. M., & Holderegger, R. (2015). A practical
1135 guide to environmental association analysis in landscape genomics. *Molecular Ecology*, 24(17),
1136 4348-4370.
1137
1138 Rellstab, C., Zoller, S., Walthert, L., Lesur, I., Pluess, A. R., Graf, R., ... & Gugerli, F. (2016).
1139 Signatures of local adaptation in candidate genes of oaks (*Quercus* spp.) with respect to present
1140 and future climatic conditions. *Molecular ecology*, 25(23), 5907-5924.
1141

1142 Riordan, E. C., Gugger, P. F., Ortego, J., Smith, C., Gaddis, K., Thompson, P., & Sork, V. L.
1143 (2016). Association of genetic and phenotypic variability with geography and climate in three
1144 southern California oaks. *American journal of botany*, 103(1), 73-85.
1145
1146 Rundle, H. D., & Nosil, P. (2005). Ecological speciation. *Ecology Letters*, 8(3), 336-352.
1147
1148 SAS Version 9.4, SAS Institute, Cary, NC
1149
1150 Savolainen, O., Lascoux, M. & Meril, J. (2013). Ecological genomics of local adaptation. *Nature*
1151 *Reviews Genetics*, 14, 807.
1152
1153 Samson, F., & F. Knopf. (1994). Prairie Conservation in North America. *Bioscience*, 44, 418-
1154 421.
1155
1156 Shaw, R.G. & Etterson, J.R. (2012). Rapid climate change and the rate of adaptation: insight
1157 from experimental quantitative genetics. *New Phytologist*, 195, 752–765.
1158
1159 Siepielski, A. M., Morrissey, M. B., Buoro, M., Carlson, S. M., Caruso, C. M., Clegg, S. M., ...
1160 & Hereford, J. (2017). Precipitation drives global variation in natural selection. *Science*,
1161 355(6328), 959-962.
1162
1163 Smith, A.B., Alsdurf, J., Knapp, M. and Johnson, L.C. (2017). Phenotypic distribution models
1164 corroborate species distribution models: A shift in the role and prevalence of a dominant prairie
1165 grass in response to climate change. *Global Change Biology*, 23, 4365–4375.
1166
1167 Sork, V. L. (2016). Gene flow and natural selection shape spatial patterns of genes in tree
1168 populations: implications for evolutionary processes and applications. *Evolutionary applications*,
1169 9(1), 291-310.
1170

1171 Talbot, B., Chen, T. W., Zimmerman, S., Joost, S., Eckert, A. J., Crow, T. M., ... & Manel, S.
1172 (2016). Combining genotype, phenotype, and environment to infer potential candidate genes.
1173 *Journal of Heredity*, 108(2), 207-216.
1174
1175 Tomiolo S, VanDer Putten, and Tielborger K. (2015). Separating the role of biotic interactions
1176 and climate in determining adaptive response of plants to climate change *Ecology*, 96(5):1298–
1177 1308
1178
1179 Villemereuil, P., Gaggiotti, O. E., Mouterde, M., & Till-Bottraud, I. (2016). Common garden
1180 experiments in the genomic era: new perspectives and opportunities. *Heredity*, 116(3), 249.
1181
1182 Weaver, J. E., & Fitzpatrick, T. J. (1932). Ecology and relative importance of the dominants of
1183 tall-grass prairie. *Botanical Gazette*, 93(2), 113-150.
1184
1185 Wilson, L. R., Gibson, D. J., Baer, S. G., & Johnson, L. C. (2016). Plant community response to
1186 regional sources of dominant grasses in grasslands restored across a longitudinal gradient.
1187 *Ecosphere*, 7(4), e01329.
1188
1189 Willand, J. E., S. G. Baer, D. J. Gibson, and R. P. Klopff. (2013). Temporal dynamics of
1190 propagule sources for community regeneration during grassland establishment. *Plant*
1191 *Ecology*, 214, 1169-1180.
1192
1193 Zheng, X., Levine, D., Shen, J., Gogarten, S. M., Laurie, C., & Weir, B. S. (2012). A high-
1194 performance computing toolset for relatedness and principal component analysis of SNP data.
1195 *Bioinformatics*, 28(24), 3326-3328.
1196

1197 **Tables**

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

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

1200

1201 Table 1. Historical Weather data (30-year normals) for planting site locations. Precipitation data for 6 years of the experiment are
 1202 presented in SFig. 2.

1203 **Figure Caption**

1204

1205

1206 Fig 1. Location of reciprocal gardens planting and collections sites across the US Great Plains.
1207 White circle is reciprocal garden location. Black triangles are the collection prairie for the seeds.
1208 For prairie population acronyms, see STable 1. Western Kansas (Colby, Kansas) is the satellite
1209 reciprocal site to test the range of tolerance for big bluestem. Note that seeds were not collected
1210 in Colby.

1211

1212 Fig 2. Reciprocal garden transplant design for sown community plots. Single colors are single
1213 ecotype plots, checkerboard is mixed ecotype plot. At each planting site, there are 4 replicate
1214 plots. Ecotype plots at each site were randomized. Note that the Colby planting site had no local
1215 ecotype but was included to test the threshold of response to drier locations as might be
1216 experienced in the future.

1217

1218 Fig. 3. Vegetative cover (least square mean estimates with standard errors) by planting sites
1219 (Western Kansas (Colby, Kansas), Central Kansas (Hays, Kansas), Eastern Kansas (Manhattan,
1220 Kansas) and Southern Illinois (Carbondale, Illinois) for each ecotype in the single ecotype plots
1221 from years 2010-2015 across the Great Plains precipitation gradient. Letters indicate significant
1222 differences within years.

1223

1224 Fig 4. Vegetative cover (least square mean estimates with standard errors) by each ecotype in the
1225 single ecotype plots at planting sites from years 2010-2015 across the Great Plains precipitation
1226 gradient. Red=western KS, Orange=central KS, Green= Eastern KS, Blue = Southern Illinois.
1227 Letters indicate significant differences within a year.

1228

1229 Fig 5. Percent big bluestem dry (red) and wet (blue) ecotype cover versus the annual rainfall in
1230 the corresponding planting locations 2014 and 2015 combined.

1231

1232 Fig 6. STRUCTURE bar plot labeled by regional ecotype and by prairie. The most likely genetic
1233 grouping solution, $K = 3$, is shown. Each color indicates one genetic group, and each bar
1234 represents percentage membership to genetic group(s). Mixed membership indicates admixture.

1235

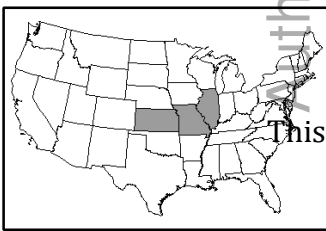
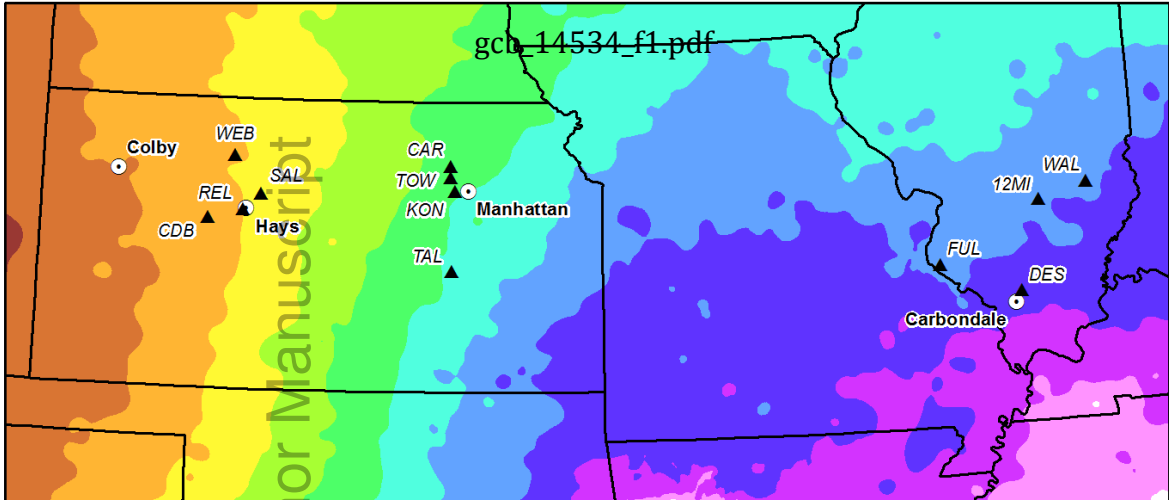
1236 Fig 7. Map indicating the allele frequencies for the GA1 outlier across the 12 populations
1237 focusing on the gradient in alleles across the climate gradient from Western Kansas to Southern
1238 Illinois. “Short” allele is in blue, alternative “tall” allele is in red.

1239

1240 Fig 8. Map showing the predicted ecotype composition of mixed ecotype plots across the
1241 reciprocal gardens in 2014. Dry ecotype denoted in red, Mesic ecotype denoted in green, and
1242 Wet ecotype denoted in blue.

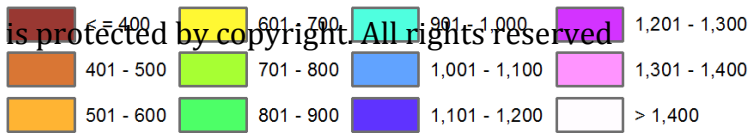
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▲ Collection Site ○ Planting Site

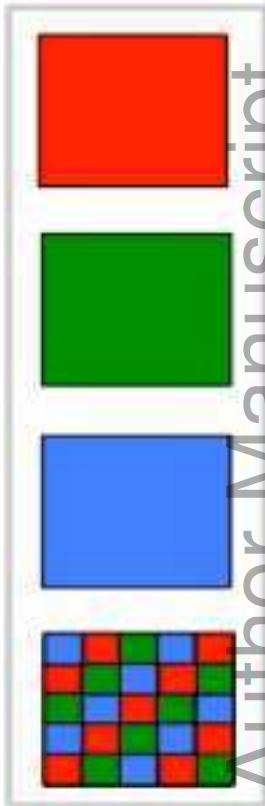
Mean Annual Precipitation (1981-2020), mm



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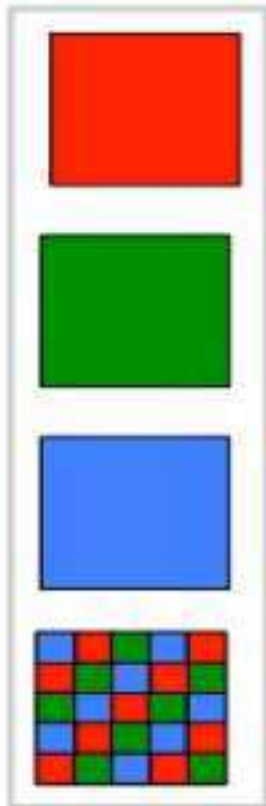
Western Kansas

Colby, KS



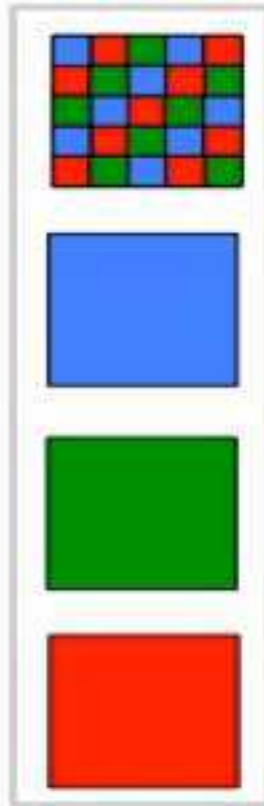
Central Kansas

Hays, KS



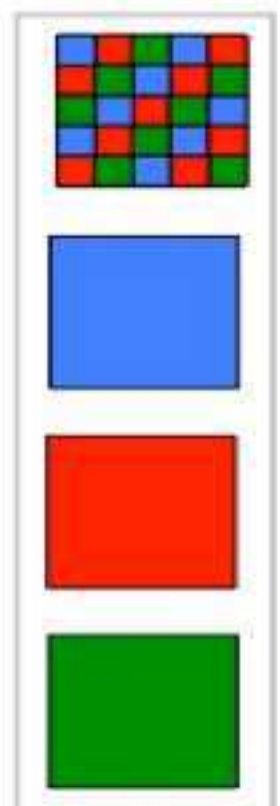
Eastern Kansas

Manhattan, KS



Southern Illinois

Carbondale, IL



Dry Ecotype

Mesic Ecotype



Wet Ecotype

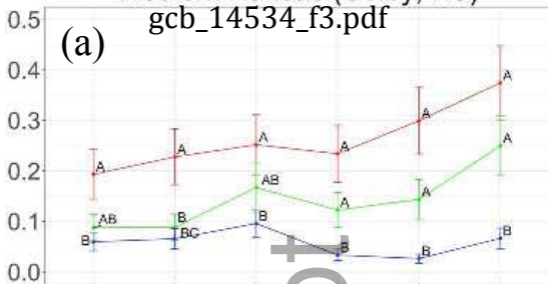
Mixed Ecotypes

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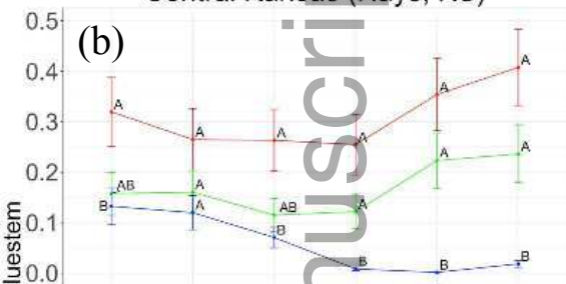
Western Kansas (Colby, KS)
gcb_14534_f3.pdf

(a)



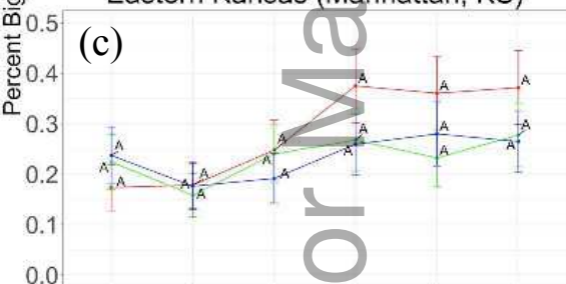
Central Kansas (Hays, KS)

(b)



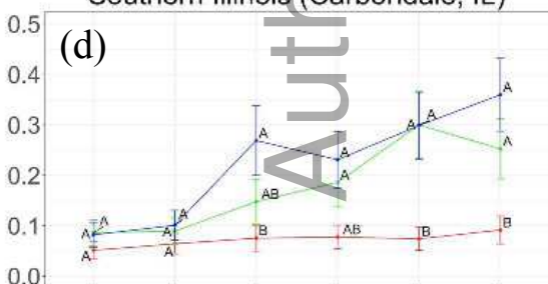
Eastern Kansas (Manhattan, KS)

(c)



Southern Illinois (Carbondale, IL)

(d)



2010 2011 2012 2013 2014 2015

Planting Site

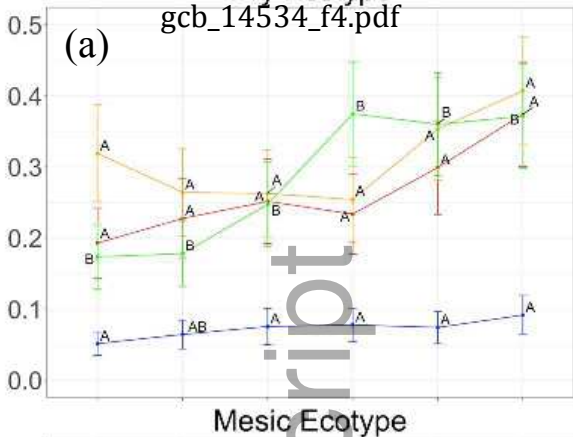
Ecotype

— Dry Ecotype

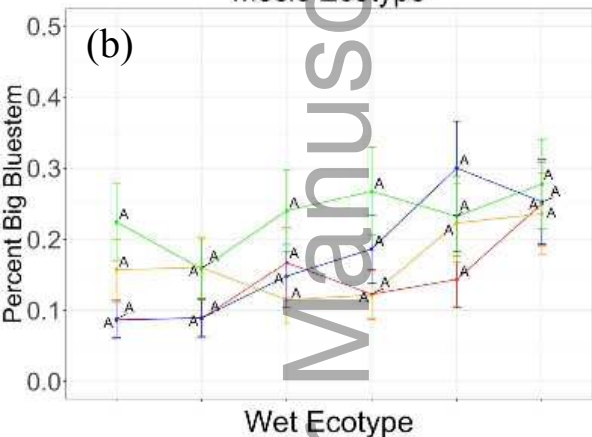
— Mesic Ecotype

— Wet Ecotype

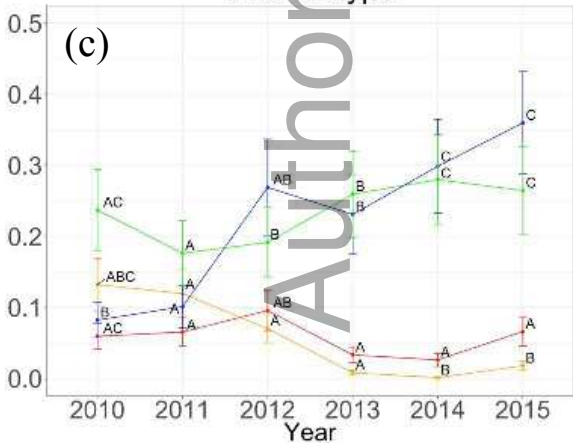
(a)



(b)



(c)

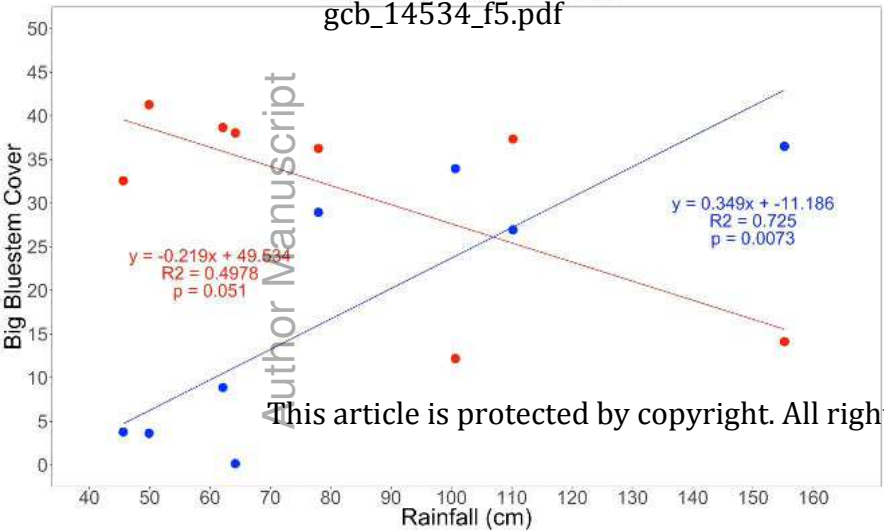


Planting Site

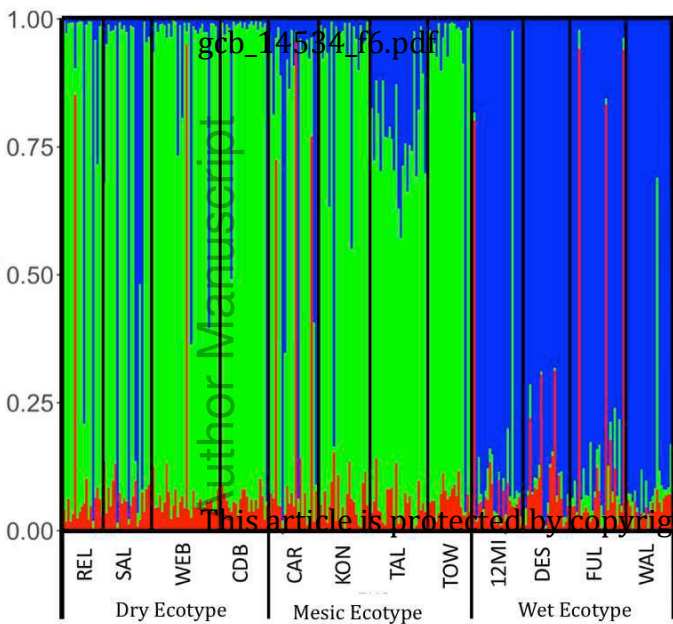
- Western Kansas (Cuba, KS)
- Central Kansas (Hays, KS)
- Eastern Kansas (Manhattan, KS)
- Southern Illinois (Carbondale, IL)

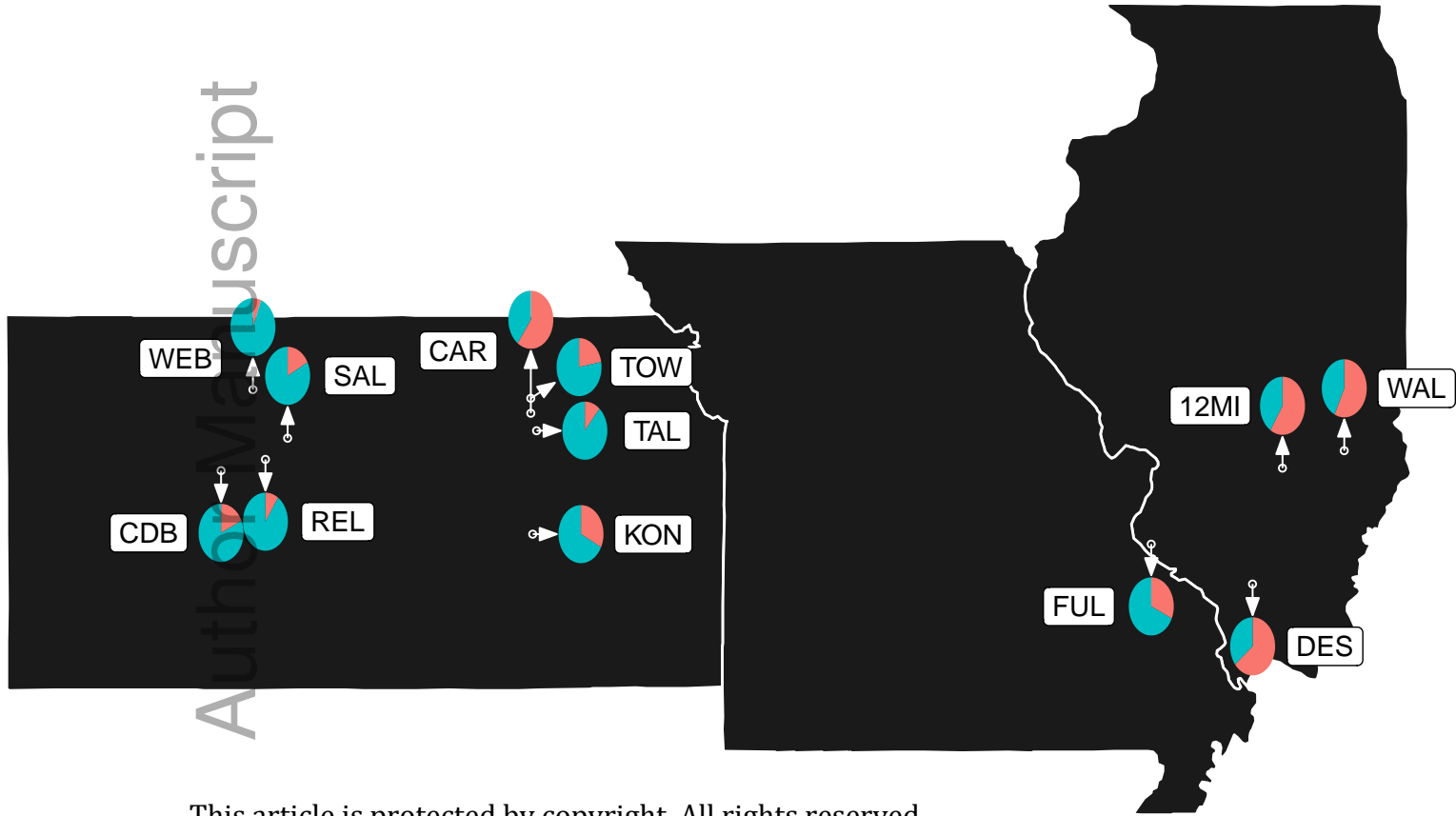
Ecotype ● Dry Ecotype ● Wet Ecotype

gcb_14534_f5.pdf

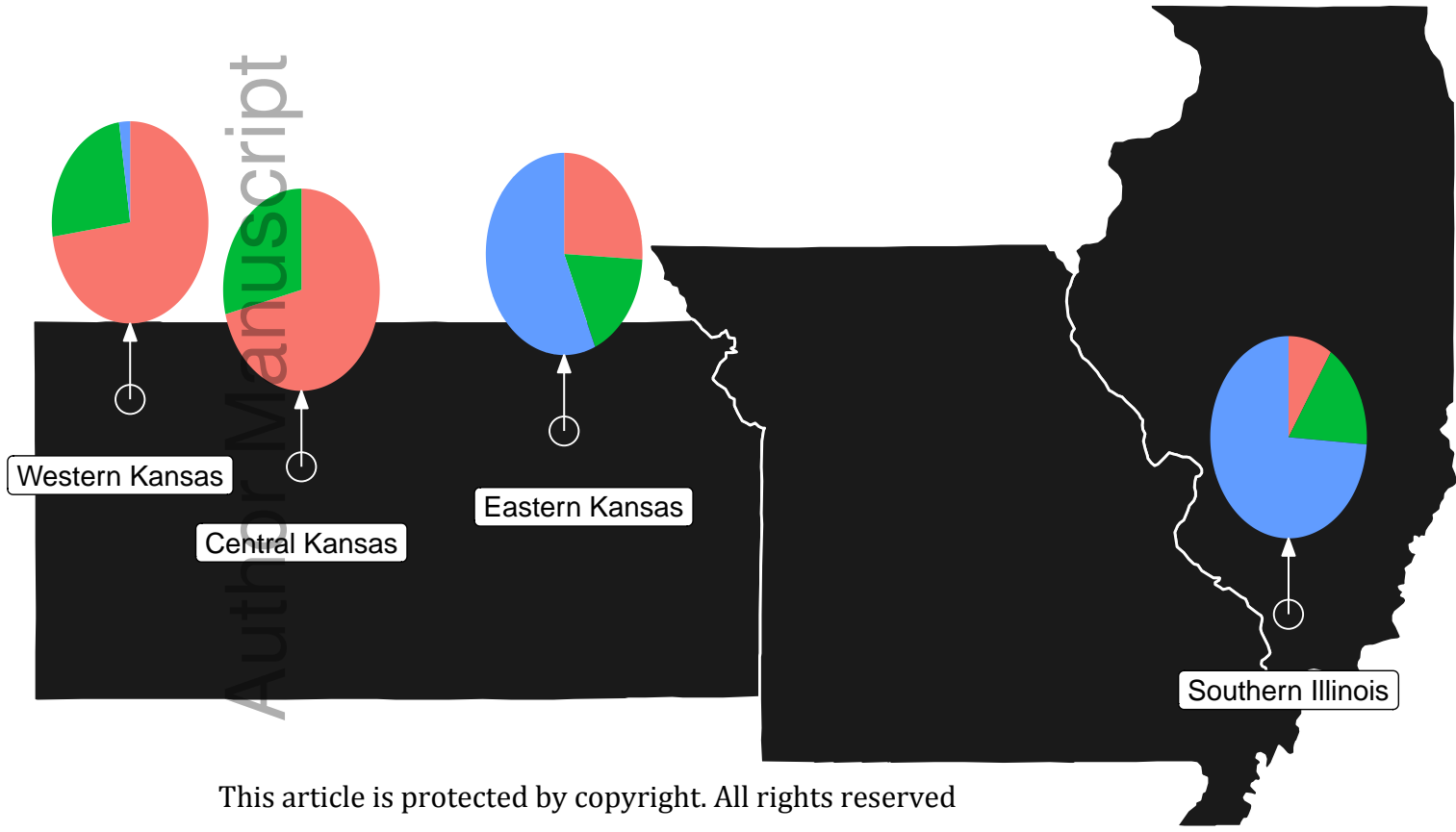


Genetic Cluster Proportion





Ecotype ■ Dry Ecotype ■ Mesic Ecotype ■ Wet Ecotype



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