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A POPULATION GENETIC TRANSECT OF *PANICUM HALLII* (POACEAE)[1]

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- *Premise of study:* Understanding the relationship between climate, adaptation, and population structure is of fundamental importance to botanists because these factors are crucial for the evolution of biodiversity and the response of species to future climate change. *Panicum hallii* is an emerging model system for perennial grass and bioenergy research, yet very little is known about the relationship between climate and population structure in this system.
- *Methods:* We analyzed geographic population differentiation across 39 populations of *P. hallii* along a longitudinal transect from the savannas of central Texas through the deserts of Arizona and New Mexico. A combination of morphological and genetic (microsatellite) analysis was used to explore patterns of population structure.
- *Key results:* We found strong differentiation between high elevation western desert populations and lower elevation eastern populations of *P. hallii*, with a pronounced break in structure occurring in western Texas. In addition, we confirmed that there are high levels of morphological and genetic structure between previous recognized varieties (var. *hallii* and var. *filipes*) within this species.
- *Conclusions:* The results of this study suggest that patterns of population structure within *P. hallii* may be driven by climatic variation over space. Overall, this study lays the groundwork for future studies on the genetics of local adaptation and reproductive isolation in this system.

Key words: climate; cline; ecotype; local adaptation; *Panicum hallii*; Poaceae; population structure.

Climate is one of the most important factors driving the evolution of biodiversity in plant species (Cowling and Lombard, 2002; Pennington et al., 2004; McElwain et al., 2011). Determining how species adapt and evolve in response to heterogeneity in climate across the natural landscape is thus of fundamental importance to botanists (Turesson, 1922; Clausen et al., 1940; Grant, 1981; Kawecki and Ebert, 2004; Hereford, 2009; Anderson et al., 2011; Lowry 2012a, b). Understanding the relationship between climate, adaptation, and population structure within a species is also crucial for predicting the response of populations and species to global climate change (Jump and Peñuelas, 2005; Visser, 2008; Hoffmann and Sgrò, 2011; McElwain et al., 2011).

One of the most exciting recent developments in adaptation research is the emerging synthesis of landscape ecological methods (e.g., global information system [GIS] layering) with studies of local adaptation (Manel et al., 2003; Joost et al., 2007; Schwartz et al., 2010; Lowry, 2010; Sork and Waits, 2010; Savolainen, 2011). In the last few years, major progress has been made in linking genetic and phenotypic variation to the multiple environmental variables that comprise climate (Coop et al., 2010; Fournier-Level et al., 2011; Hancock et al., 2011; Jones et al., 2012; Lasky et al., 2012). A crucial first step to understanding the processes of local adaptation and phenotypic evolution in any system is to characterize morphological and genetic population structure across space (Nordborg et al., 2005; Lowry et al., 2008; Song et al., 2009; Zalapa et al., 2011).

The recent expansion of genomic technologies has made the development of new model plant systems targeted to fundamental questions of adaptation and applied benefit possible (Town, 2006; International Brachypodium Initiative, 2010). New model grass systems, such as the annual species *Brachypodium distachyon* (International Brachypodium Initiative, 2010), are particularly desirable given the huge importance of the grass species to agriculture: rice, wheat, and corn alone account for almost 60% of all calories consumed by humans (Cassman et al., 2003). However, recent interest in perennial agriculture for both food and bioenergy means that model perennial grass systems need to be developed (DeHaan et al., 2005; Schmer et al., 2008; Heaton et al., 2008). Such systems would enhance our understanding of how plants with a perennial life history respond to abiotic stress and adapt to different environmental conditions (Thomas et al., 2000; Volaire, 2003; Roux et al., 2006; Rohde and Bhalerao, 2007; Albani and Coupland, 2010).

A rapidly emerging model system for the genomics perennial grasses is Hall's panicgrass, *Panicum hallii* Vasey (Lowry et al., 2012; Meyer et al., 2012). Until recently, very little research had been conducted with *P. hallii*, although it is has been shown to be a valuable food resource for native birds (Campbell-Kissock et al., 1985) and domesticated goats (Ramírez et al., 2004).

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Interest in *P. hallii* has increased due to its close evolutionary relationship (Zhang et al., 2011; Anderson et al., 2011) to the bioenergy crop switchgrass, *Panicum virgatum*, which occurs primarily as a tetraploid or octaploid (McMillan and Weiler, 1959; Costich et al., 2010; Triplett et al., 2012). As a diploid ($2n = 2x = 18$; Gould, 1958), *P. hallii* affords researchers the opportunity to carry out applicable genetic research without the challenges of working directly with the large, long-generation, obligately outbred, and genetically intractable polyploid *P. virgatum*. *P. hallii* has the advantage of a short generation time, small physical size, self-compatibility, relatively small genome size (~550 Mbp), and production of thousands of seeds per plant (Lowry et al., 2012; Meyer et al., 2012).

There are two major varieties of *P. hallii*, the widespread *P. hallii* var. *hallii* and the more restricted *P. hallii* var. *filipes* (Scribn.) Waller. *Panicum hallii* var. *hallii* is typically found in shallow dry rocky calcareous soils and has a range that spans from western Arizona to eastern Texas, north to Colorado and south into Mexico (Smeins et al., 1976; Waller, 1976; Hatch et al., 2003). *Panicum hallii* var. *filipes* is most commonly found on clay soils and mesic depressions along the Gulf Coast and Rio Grande Valley of Texas and Mexico (Gould, 1975; Waller, 1976; Hatch et al., 2003). Outside of these regions, *P. hallii* var. *filipes* is less common, but has been reported in arid area of the Rio Grande Plain and other parts of Texas (Gould, 1975; Waller, 1976). Both varieties are often found in disturbed areas following fire, grazing, and road construction (Campbell, 1929; Box and White, 1969; Gould, 1975; Hatch et al., 2003). In a recent study, we showed that both varieties are highly inbred, which suggests that outcrossing events are rare and that most individuals are the product of several generations of self-fertilization (Lowry et al., 2012). While it is unknown whether the two varieties hybridize in nature, we have successfully crossed them in the laboratory (D. Lowry, unpublished data).

Very little is known about patterns of genetic variation across *P. hallii* (Waller, 1976). Therefore, our main goal was to establish patterns of population divergence within *P. hallii* var. *hallii* (hereafter var. *hallii*) as well as divergence from *P. hallii* var. *filipes* (hereafter var. *filipes*). The three major aims of our study were to (1) determine the distribution of genetically based morphological variation across the state of Texas within *P. hallii*; (2) establish patterns of neutral genetic variation and population structure across Texas, Oklahoma, and the southwestern United States; and (3) explore associations between geography and climatic variables with morphological and genetic population structure. The results of this study highlight major patterns of divergence in *P. hallii* and lay the groundwork for our future genetic mapping studies to understand the mechanisms of morphological differentiation and local adaptation between geographic regions as well as between var. *hallii* and var. *filipes*.

MATERIALS AND METHODS

***Population collections*—**Most of the plant material used in this study (Fig. 1; Table 1) was derived from collections made during the summers of 2010 and 2011. Plants were haphazardly collected across populations from the field during the peak of flowering and brought back to the University of Texas greenhouses for propagation. Leaf tissue was collected from these plants for population genetic analysis and stored in a −80°C freezer. We also collected open-pollinated self-fertilized seeds to create population accessions. In the fall of 2010, seeds of population accessions were scarified with sand paper and germinated on paper towels. Once seedlings were ~1 cm tall, they were transplanted to 3.5-inch square pots filled with a 60:40 mix of Promix (Premier Tech Horticulture, Rivièredu-Loup, Quebec) to Turface (Profile, Buffalo Grove, IL). These plants were grown in the University of Texas greenhouse with supplementary lighting with a 16-h day length, 33°C peak midday temperature, and 22°C nights. Open-pollinated seeds were collected from these plants. An additional set of seeds were collected from lines that had been derived from seeds supplied by the USDA Kika de la Garza Plant Materials Center (Kingsville, TX) and grown in the same greenhouses: FIL, DUV, and UVL. These seeds were then used for the common garden greenhouse experiment described below. Additional sets of plants were collected from other regions of Oklahoma, Texas, New Mexico, and Arizona in the summer of 2011 that were used solely for population genetic analysis.

***Common garden greenhouse experiment*—**To assess the structure of morphological variation across *P. hallii* in Texas, we conducted a common garden experiment with 31 seed families derived from 16 populations. The following populations had more than one seed family in the experiment: DOF, DRB, HAL, HUE, KNT, MCR, PFL, PIS, SAN, WBW (range: 2–5 seed families per population). There was a large range (4–28) in the number of individuals per population due to differential germination success and seed availability. Seeds were scarified with sandpaper and placed onto a wet paper towel in square Petri plates on 25 February 2011. Seeds were stratified at 4°C in the dark for 10 d, until 7 March 2011 to promote coordinated germination. The seeds were then removed from the cold and placed into a growth chamber for germination set to 70% humidity with 16-h days at 23°C at a light level of 200 $\mu E \cdot m^{-2} \cdot s^{-1}$, with 8-h nights at 18°C. Germination date was recorded daily. Seedlings were transplanted when they were ~1 cm tall to the same mixture of soil as above into 3.5-inch square pots. Seedlings were initially misted from above once a day and then switched to gentle stream watering. To minimize environmental effects of different locations in the greenhouse, we fully randomized plants across flats and haphazardly rotated the position of the flats every other week beginning on 25 March 2011.

For each plant, we recorded the day of first flower and used this date to calculate flowering time as the difference from the germination date. We also measured five traits at the time of flowering. We counted the number of tillers and measured the following dimensions of the flowering tiller with a calipers and ruler: tiller height from soil to tip of the opening inflorescence, thickness of the internode between the first and second true leaves, width of the flag leaf, and length of the flag leaf. Once each plant had set seeds, we counted the number of spikelets on the first flowering inflorescence and collected seeds. We weighed a set of 10 seeds from each plant to calculate an average seed mass. To quantify the degree of erect vs. prostrate plant architecture, we counted the number of tillers that were above and below a 45° angle.

In mid-June, we harvested plants for biomass. During the harvest, plants were cut to their base, leaving ~1 cm of stem, and collected into paper lunch bags. Biomass was dried for 1 wk in a drying oven set to 65°C. To determine the regrowth potential of plants following the harvest, we allowed harvested plants to regrow for 3 wk. At that point, we scored plants for survival and quantified dried regrowth biomass for the plants that were still alive.

***Morphological population structure of* P. hallii *var.* hallii—**To determine whether there were regional differences within var. *hallii*, we assigned the populations from the common garden experiment to five regions of Texas: southern Texas (DUV, PIS), northeastern Edwards Plateau (HAL, HUE, PAJ, PFL, WBW), southwestern Edwards Plateau (COM, DOF, DRB, SEM, UVL), Stockton Plateau (SAN), and the Davis Mountain region (MCR, KNT). These groupings are based on geographic location with close correspondence to well-established ecoregions (Shaw et al., 2011).

To determine whether there was overall trait divergence between the five regions of Texas for var. *hallii,* we conducted a MANOVA analysis on the sum of responses of 10 traits in the program JMP 9.0.0 (SAS Institute, Cary, North Carolina, USA). To visually assess the degree of divergence between regions, we conducted a principal components (PC) analysis on trait correlations in JMP ($N = 10$ traits) and plotted the first two PCs against each other. Regrowth traits were not included in the PC analysis due to missing data for plants that did not regrow. To determine how quantitative trait variation is partitioned among regions, among populations within regions, among lines within populations, and among individuals (error) within populations, we fit univariate linear models to the nonregrowth morphological data using the nlme module (Pinheiro et al., 2012) of the statistical package R 2.12.0 (http://www.r-project.org/). Variance components were extracted from a model where population and region were fit as random effects. To determine whether population, region, or line contributed significantly to this model, we fit two simpler models with just one factor removed at a time. The whole model was then compared individually to the two simpler models with likelihood ratio tests.

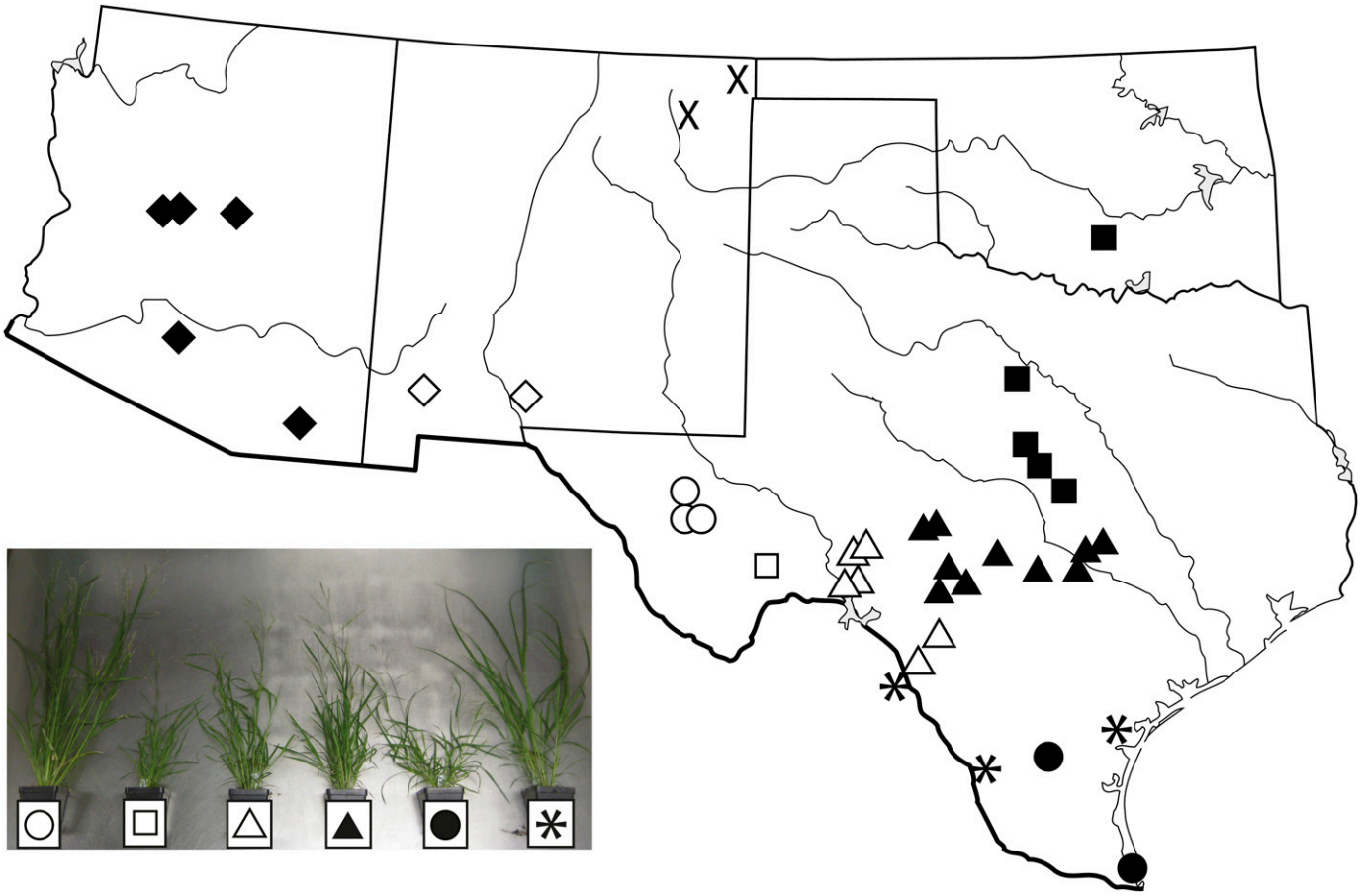

Fig. 1. Map of populations of *Panicum hallii* from which accessions were derived for this study. Populations are marked by region: New Mexico Chihuahuan (◇), New Mexico tablelands (X), Arizona (◆), Davis Mountains (○), Stockton Plateau (□), Oklahoma/Texas plains (■), Southern Texas (•), southwestern Edwards Plateau (△), northeastern Edwards Plateau (▲), and var. *filipes* (✱). Photograph depicts morphological divergence of plants from across Texas grown in a common garden (populations from left to right: MCR, SAN, DOF, HAL, PIS, and var. *filipes* [FIL]).

***Morphological divergence between var.* hallii *and var.* filipes—**To assess divergence between var. *hallii* and var. *filipes*, we conducted PC analysis as just described but with the addition of individuals from var. *filipes* (FIL) lines. We then plotted the first two PCs against each other. To determine the divergence in quantitative trait variation between the two varieties, we conducted two-way ANOVAs for the morphological traits and the first two PCs.

Since we only had one population of var. *filipes* available at the time of the experiment, we compared the results of our study to the findings of Waller (1976). In that study, morphological traits from 20 plants per population from 19 populations of var. *hallii* and 10 populations of var. *filipes* were measured on plants collected directly from the field.

***Genetic population structure*—**Population genetic analysis was conducted with a more geographically widespread set of populations than the morphological analysis as many population accessions were acquired after the common garden study had been conducted. The number of individuals varied widely between population samples, as different collectors were involved. Some of the accessions were derived from herbarium collections, for which only a single individual line was available. DNA was extracted from the tissue in 96-well plates using a modified CTAB protocol (Kelly and Willis, 1998). We screened DNA for 15 microsatellite loci. The development of these markers and the PCR conditions were described by Lowry et al. (2012). To reduce PCR stutter of fragments, we appended a Pig-tail (GTTTCTT) to the 5′ end of reverse primer following the methods of Brownstein et al. (1996). PCR products were mixed with 10 μL of HiDi and 0.5 μL of GeneScan 500 ROX size standard (Applied Biosystems, Foster City, California, USA) and were run on an ABI 3730 DNA Analyzer (Applied Biosystems) at the Georgia Genomics Facility. Sizes of PCR products were scored by eye using GeneMarker (SoftGenetics, State College, Pennsylvania, USA). Individuals with missing data at more than five loci were excluded from population genetic analyses.

Population summary statistics were calculated for populations containing three or more genotyped accessions (mean = 11.44 individuals per population). We calculated per population expected and observed heterozygosity with the program GenALEx v6.3 (Peakall and Smouse, 2006), while F_{IS} was calculated in the program FSTAT 2.9.3.2 (Goudet, 2001). Pairwise codominant genotypic distances (Smouse and Peakall, 1999) were calculated between populations in GenALEx. We then assessed isolation by distance (IBD) between the log-transformed genetic distance and geographic distance with a Mantel test in the program IBDWS 3.22 (Jensen et al., 2005). For the IBD analyses, geographic distances were calculated with the Geographic Distance Matrix Generator version 1.2.3 (Ersts, 2012).

To understand the pattern of population structure across collections of *P. hallii* var. *hallii* and var. *filipes*, we calculated pairwise codominant genotypic distances for all genotyped individuals. This pairwise matrix was then used to conduct principal coordinate analysis (PCA) in GenALEx. We used PCA as our primary means of understanding population structure, instead of model-based approaches (e.g., STRUCTURE; Pritchard et al., 2000) because of the high degree of self-fertilization in our system. The PCA also allowed us to assess the relationship of climate with genetic and morphological structure using the same methodology.

***Associations of climate and geography with population structure*—**Population structure is often associated with features of the natural landscape. To assess potential associations between climate and structure, we extracted WorldClim (Hijmans et al., 2005) climate variables at 30″ resolution for each sampled population using the program ArcGIS (Esri, Redlands, California,

TABLE 1. Populations locations and summary statistics: number of samples (N), average number of alleles per locus (N_a), observed (H_o) and expected (H_e) heterozygosity, and inbreeding coefficient (F_{IS}).

Pop ID	State	County/ Municipality	Region/Variety	Altitude (m a.s.l.)	Latitude	Longitude	N	N_a	H_o	H_e	F_{IS}
ARB	Oklahoma	Murray	Plains (OK/TX)	305	34.43	−97.15	8	1.267	0.000	0.056	1.000
ARR	Arizona	Santa Cruz	Arizona	1464	31.61	−110.51	1	NA	NA	NA	NA
BFL	Texas	Travis	NE Edwards	167	30.29	−97.78	11	2.800	0.020	0.416	0.956
CKR	Arizona	Maricopa	Arizona	1817	34.23	−112.32	9	1.467	0.000	0.138	1.000
CLA	New Mexico	Union	Tablelands (NM)	1463	36.64	−103.04	1	NA	NA	NA	NA
COM	Texas	Val Verde	SW Edwards	495	29.70	−101.21	7	3.067	0.019	0.460	0.965
COR	Arizona	Maricopa	Arizona	1110	34.29	−112.18	15	1.533	0.039	0.106	0.655
DOF	Texas	Val Verde	SW Edwards	408	29.89	−100.99	15	3.200	0.045	0.398	0.895
DRB	Texas	Val Verde	SW Edwards	456	29.94	−100.97	16	4.333	0.047	0.561	0.922
DUV	Texas	Duval	S Texas	211	27.75	−98.57	1	NA	NA	NA	NA
EPS	Texas	Maverick	var. *filipes*	250	28.46	−100.29	1	NA	NA	NA	NA
FIL	Texas	Cameron	var. *filipes*	5	27.65	−97.40	1	NA	NA	NA	NA
GEO	New Mexico	Harding	Tablelands (NM)	1780	36.04	−104.31	1	NA	NA	NA	NA
GIL	New Mexico	Grant	Chihuahuan (NM)	2101	32.83	−108.06	16	1.867	0.004	0.175	0.978
GRA	Texas	Kerr	NE Edwards	623	29.91	−99.24	3	1.600	0.178	0.224	0.407
HUE	Texas	Gillespie	NE Edwards	544	30.23	−99.02	5	1.933	0.000	0.290	1.000
JHC	Texas	Blanco	NE Edwards	343	30.21	−98.31	8	1.600	0.011	0.150	0.937
KNT	Texas	Jeff Davis	Davis	1328	31.05	−104.21	11	2.733	0.006	0.381	0.986
KWM	Texas	Kerr	NE Edwards	638	30.07	−99.51	3	1.533	0.000	0.222	1.000
HAL	Texas	Travis	NE Edwards	252	30.19	−97.87	13	2.267	0.059	0.274	0.800
LAR	Tamaulipas	Nuevo Laredo	var. filipes	200	27.47	−99.72	1	NA	NA	NA	NA
LMP	Texas	Bandera	NE Edwards	679	29.83	−99.59	13	3.267	0.039	0.477	0.926
MAV	Texas	Maverick	SW Edwards	264	28.74	−100.35	1	NA	NA	NA	NA
MCR	Texas	Jeff Davis	Davis	1757	30.68	−104.13	19	2.800	0.035	0.375	0.913
NMX	New Mexico	Dona Ana	Chihuahuan (NM)	1663	32.43	−106.58	2	NA	NA	NA	NA
PAJ	Texas	Kimble	NE Edwards	518	30.49	−99.74	13	2.867	0.051	0.380	0.878
PFL	Texas	Travis	NE Edwards	226	30.45	−97.64	15	3.267	0.095	0.369	0.760
PIN	Arizona	Gila	Arizona	1642	34.32	−111.40	2	NA	NA	NA	NA
PIS	Texas	Cameron	S Texas	5	25.99	−97.33	11	3.200	0.013	0.390	0.971
SAN	Texas	Terrel	Stockton	969	30.13	−102.56	4	1.333	0.000	0.113	1.000
SEM	Texas	Val Verde	SW Edwards	404	29.68	−101.31	3	1.667	0.000	0.307	1.000
SLS	Texas	Burnet	NE Edwards	390	30.99	−98.10	18	1.933	0.012	0.156	0.925
SMF	Texas	Mills	NE Edwards	467	31.31	−98.44	1	NA	NA	NA	NA
SMT	Texas	Mills	NE Edwards	488	31.56	−98.60	1	NA	NA	NA	NA
SPR	Texas	Jeff Davis	Davis	1761	30.67	−104.13	12	2.467	0.027	0.354	0.931
STW	Texas	Palo Pinto	Plains (OK/TX)	337	32.61	−98.49	23	2.933	0.015	0.427	0.966
TTM	Arizona	Pinal	Arizona	1311	32.75	−112.12	1	NA	NA	NA	NA
UVL	Texas	Ulvade	SW Edwards	277	29.21	−99.79	1	NA	NA	NA	NA
WBW	Texas	Kimble	NE Edwards	597	30.43	−99.80	15	3.067	0.093	0.354	0.753
Grand means for populations with $N > 2$ individuals							286	2.400	0.032	0.302	0.895

USA). The WorldClim data set included 19 BioClim variables derived from mean monthly precipitation as well as mean minimum, average (mean), and maximum monthly temperatures. We calculated the principal components of these 19 climate variables using JMP (on correlations) and extracted the first four PCs, all of which had eigenvalues greater than 1 and cumulatively explained 94.3% of the climate variation (Appendix 1).

To assess relationships between morphology, neutral genetic structure, climate, and geography, we explored correlations between principal components of the data. Overall, we assessed pairwise Pearson correlations between latitude, longitude, altitude, the first three genetic PCs, the first three morphological PCs, and the first four BioClim PCs in JMP. A Bonferroni correction for multiple comparisons was used as a conservative false discovery rate (FDR).

RESULTS

***Morphological population structure of* P. hallii *among regions*—**Overall, there was significant trait variation among regions of Texas (MANOVA, $F_{4,177} = 35.92$, $P < 0.0001$; Tables 2, 3). Principal component analysis revealed differentiation between regions of Texas (Fig. 2A). We extracted the first three PCs because their eigenvalues were greater than 1 and collectively explained 66.19% of the phenotypic variation (Appendix 1). Tiller thickness, tiller width, mean seed mass, and dry biomass were most heavily loaded onto PC1. Tiller height, spikelet number, and leaf length were most loaded onto PC2. Dry biomass, number of tillers, and tiller angle were most loaded onto PC3. In the plot of the first two PCs, there was no overlap of individuals from the Davis Mountains and Southern Texas, and there was differentiation between the southwestern and northeastern regions of the Edwards Plateau (Fig. 2A). The SAN population from the Stockton Plateau clustered with the northeastern Edwards and Southern Texas regions.

In linear modeling of trait variation, region significantly explained a portion of the genetic variation for five traits: tiller height, tiller thickness, leaf width, tiller angle, and mean seed mass (Table 3). Overall, region accounted for 71.63% of the variation in PC1, 39.44% of the variation in PC2, and none of the variation in PC3. In addition, there were significant line effects within populations for the first two PCs and six of the individual traits (Table 3).

We observed significant differences in survival after harvest ($\chi^2_{4,233} = 13.31$, $P = 0.0098$) and regrowth biomass ($F_{4,195} = 3.72$, $P = 0.0061$) among regions. The Davis Mountain's plants

TABLE 2. Texas regional means (standard error) for traits measured in the common garden greenhouse experiment.

Trait	Southern Texas	Northeastern Edwards	Southwestern Edwards	Stockton (SAN)	Davis Mountains	*P. hallii* var. *filipes*
Tiller height (mm)	37.46 (0.80)	38.97 (0.63)	49.52 (0.61)	37.43 (1.13)	47.47 (1.56)	78.22 (1.72)
Number of tillers	23.31 (1.53)	19.06 (0.58)	18.54 (0.51)	20.00 (1.24)	15.25 (0.45)	26.20 (0.74)
Tiller thickness (mm)	1.28 (0.05)	1.54 (0.04)	1.53 (0.04)	1.32 (0.13)	2.27 (0.06)	2.38 (0.03)
Leaf length (mm)	15.87 (0.55)	16.15 (0.35)	17.93 (0.34)	13.61 (0.68)	20.44 (0.65)	34.16 (1.37)
Leaf width (mm)	4.07 (0.11)	4.68 (0.08)	4.43 (0.08)	3.77 (0.16)	6.38 (0.13)	10.76 (0.22)
Flowering time (days)	56.86 (1.25)	54.01 (0.64)	55.75 (0.71)	53.72 (1.35)	49.12 (0.86)	81.65 (1.48)
Number of spikelets	80.89 (4.98)	84.25 (3.12)	139.72 (6.66)	50.67 (4.10)	72.49 (2.56)	692.50 (33.98)
% Tillers below 45°	43.29 (1.86)	10.61 (1.66)	10.10 (1.37)	7.07 (1.68)	3.69 (0.69)	0 (0.00)
Seed mass (mg)	0.77 (0.02)	1.34 (0.03)	0.93 (0.02)	1.16 (0.01)	1.73 (0.04)	0.60 (0.03)
Biomass (g)	5.03 (0.40)	6.05 (0.22)	5.98 (0.27)	5.45 (0.39)	9.79 (0.51)	23.76 (1.27)
Survival after harvest (%)	92.86	90.00	77.94	76.92	97.73	100.00
Regrowth mass (g)	0.84 (0.12)	0.83 (0.06)	0.68 (0.06)	0.40 (0.08)	0.99 (0.10)	1.10 (0.08)

had the greatest regrowth success, while the nearby Stockton Plateau (SAN) population was the least successful.

***Morphological divergence between var.* hallii *and var.* filipes—**There was a high level of morphological divergence between all of the var. *hallii* population samples and var. *filipes* (Table 2). In general, var. *filipes* had more tillers, greater height, greater biomass, and smaller seeds than var. *hallii*. These results were consistent with the findings of the multipopulation study by Waller (1976) in that var. *filipes* is generally larger than var. *hallii* (Appendix 2). The exception is seed size, for which var. *filipes* seeds are smaller. In contrast to var. *hallii*, all var. *filipes* plants survived harvesting. Regrowth biomass was also significantly greater for var. *filipes* ($F_{1,219}$ = 6.19, P = 0.0136).

The first two PCs of variation across the varieties had eigenvalues greater than 1 and collectively explained 72.92% in the 10 traits. Spikelet number, leaf width, dry biomass, and tiller height were most heavily loaded onto PC1, while mean seed mass, tiller thickness, and tiller angle were most heavily loaded onto PC2. The plot of the first two PCs showed a wide gap between the two varieties (Fig. 3).

***Genetic population structure across* P. hallii—**Overall, we successfully genotyped 302 individuals from 39 populations, including the three var. *filipes* accessions. Across populations with three or more genotyped individuals (N = 286 individuals, 25 populations), the overall mean (SE) F_{ST} = 0.600 (0.025), F_{IS} = 0.895 (0.019), and F_{IT} = 0.958 (0.008). On average, populations contained a moderate amount of variation (mean [SE] H_e = 0.302 [0.014]). However, individuals within populations were consistently homozygous across most of the 15 loci (mean [SE] H_o = 0.032 [0.004]). Among populations, we detected weak but significant isolation by distance (N = 39 populations; Z = 3265.7431; r = 0.1471; P = 0.0180).

Principal coordinate analysis revealed geographic structuring of populations within *P. hallii*. The first three PCs collectively accounted for 64.67% of the genetic variation. In general, the Texas populations east of the Davis Mountains region had genetic PC2 values greater than 0, while individuals from the Davis Mountains westward had values less than 0 (Figs. 2B, 2D, 4D). Populations were further divided geographically in the western range along genetic PC3, where Davis Mountain's individuals had values less than 0 and individuals from Arizona and New Mexico had values greater than 0.

All three accessions of var. *filipes* (EPS, FIL, LAR) clustered with central Texas populations in the PC plots (Fig. 2B–D). Even so, there was clear divergence from var. *hallii* across most of the 15 loci, with 19 of 37 (51.35%) of the alleles found in the three accessions being private to var. *filipes*.

***Associations of climate and geography with population structure—**Geographic variables (latitude, longitude, and altitude) were highly correlated (Table 4) because populations

TABLE 3. Partitioning of phenotypic variance components among regions, populations, and lines within populations.

Trait	N	Regions (N = 5)	Regions (%)	Pops (N = 15)	Pop (%)	Lines (N = 29)	Lines (%)	Individuals (Error)	Individuals (%)
PC1	182	2.3943**	71.63	0.1839	5.50	0.1977**	5.91	0.5667	16.95
PC2	182	0.6857**	39.44	0.047	2.70	0.2447**	14.07	0.7612	43.78
PC3	182	<0.0001	0.00	0.4978*	32.95	0.1265	8.37	0.8866	58.68
Tiller height (mm)	231	21.7833**	33.38	6.9439*	10.64	2.0655	3.16	34.4754	52.82
Number of tillers	231	3.8036	12.43	1.6447	5.37	3.3000*	10.78	21.8549	71.41
Tiller thickness (mm)	231	0.1002*	44.99	0.0084	3.77	0.0278***	12.48	0.0863	38.75
Leaf length (mm)	231	2.4457	16.60	<0.0001	0.00	3.7561***	25.49	8.5352	57.92
Leaf width (mm)	231	0.7275**	55.76	0.04253	3.26	0.1067**	8.18	0.428	32.80
Flowering time (days)	229	2.8894	7.46	1.2109	3.13	3.7061	9.57	30.9353	79.85
Number of spikelets	233	565.78	28.08	367.64	18.25	335.95***	16.67	745.45	37.00
Percentage tillers below 45°	233	0.0206*	57.70	0.0058**	16.25	0.0006	1.68	0.0087	24.37
Seed mass (mg)	206	0.0876**	65.47	0.0210**	15.70	0.0048*	3.59	0.0204	15.25
Biomass (g)	228	1.7933	23.74	0.7765	10.28	0.5055	6.69	4.4772	59.28
Regrowth mass (g)	200	0.0052	1.79	<0.0001	0.00	0.0259	8.93	0.259	89.28

Notes: Significance levels *** P < 0.001, ** P < 0.01, * P < 0.05

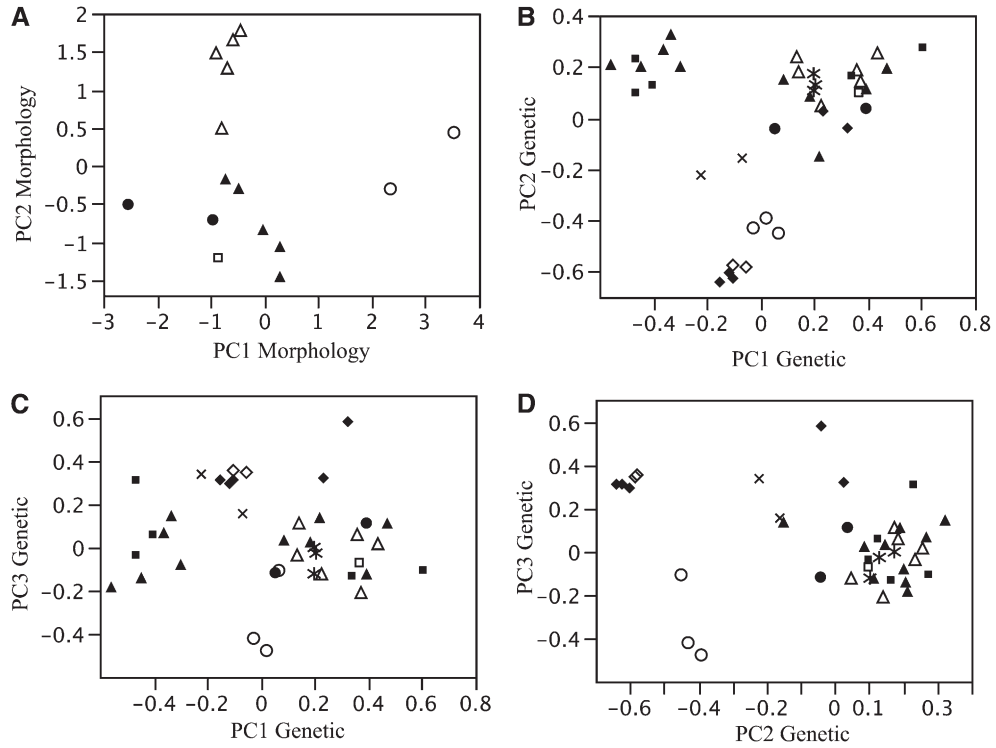

Fig. 2. Genetic and morphological population structure across *Panicum hallii.* (A) Principal component plot for the population means of the first two morphological PCs. Principal coordinate plots of the first three neutral genetic PCs plotted against each other (B, C, D). Key: • = Southern Texas, ○ = Davis Mountains, ▲ = northeastern Edwards Plateau, △ = southwestern Edwards Plateau, □ = Stockton plateau, ■ = OK/TX plains, ◇ = New Mexico Chihuahuan, ◆ = Arizona, × = New Mexico tablelands, ✱ = var. *filipes*.

were collected along a diagonal axis from low elevation along the Gulf Coast in the southeastern end of the transect to high elevation regions of the Davis Mountains, New Mexico, and Arizona at the northwestern end of the transect. These geographic variables were most strongly correlated with genetic PC2, and morphology PC1. Climatic factors were also strongly associated with neutral genetic structure: all of the first four principal components of climate (BioClim) were each correlated with a single component of neutral genetic variation. Significant correlations with individual BioClim factors are provided in Appendix 3.

While much of the neutral genetic and morphological population structure of *P. hallii* var. *hallii* appeared to be autocorrelated along our geographic transect, we also found evidence for a break in population structure in western Texas. Both altitude (Fig. 4A) and BioClim PC1 (Fig. 4B) showed a smooth linear relationship with longitude across Texas and New Mexico. However, both morphology PC1 (Fig. 4C) and genetic PC2 (Fig. 4D) biplots had a split between the Stockton Plateau and the Davis Mountains (102.5°–104° West longitude).

DISCUSSION

Our study is the first to examine trait and genetic population structure in *Panicum hallii*, an emerging model system for perennial grass biology and bioenergy biomass production (Lowry et al., 2012; Meyer et al., 2012). Overall, we found that populations were highly structured over our transect and that there is strong divergence between *P. hallii* var. *hallii* and *P. hallii* var. *filipes.* The major split in population structure in var. *hallii* occurs in western Texas, and future studies should focus on the reasons for this divergence. The results of this study lay the groundwork for understanding the relationship between climate, adaptation, and population structure across the southwestern United States.

***The structure of* Panicum hallii—**Most *P. hallii* individuals occur in nature as nearly homozygous inbred lines. Despite the mating system of *P. hallii*, its populations harbor an appreciable amount of morphological, physiological, and neutral genetic variation. Our sampling across Texas, Oklahoma, New Mexico, and Arizona revealed patterns of population structure at several different spatial scales. The most densely sampled region of our study was across the moisture gradient of the Edwards Plateau of central Texas. This region is primarily live oak savanna (*Quercus fusiformis*) and juniper woodland (*Juniperus ashei*), while gradually becoming drier moving west across the Plateau, with Chihuahuan desert vegetation starting to occur around the Devils River Area (Milstead, 1960; Fowler and Dunlap, 1986). We did not detect any obvious divergence in neutral genetic structure across the Edwards Plateau. However, our northwestern and southeastern Plateau collections were divergent along the second morphological PC, suggesting possible functional regional divergence due to local selective pressures. Future studies will be needed to determine whether this divergence is adaptive and is maintained despite gene flow (Slatkin, 1987; Lenormand, 2002).

The most striking pattern of population structure along the var. *hallii* transect was in western Texas. Individuals from the

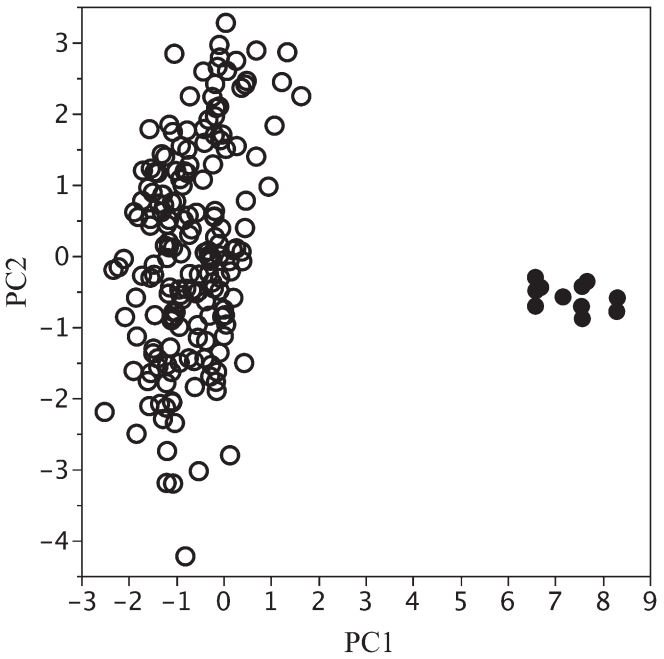

Fig. 3. Principal component (PC) plot of first two morphological PCs for all individuals of *Panicum hallii* var. *hallii* (○) and the FIL line of *P. hallii* var. *filipes* (•).

SAN population (Stockton Plateau) were among the smallest in our common garden experiment. In contrast, MCR (high elevation Davis Mountain meadow) and KNT (desert foothills of Davis Mountains) were morphologically the largest plants among var. *hallii* population accessions. The distance between MCR and SAN (163 km) is less than half of the distance of the spread of our sampled populations across Edwards Plateau (364 km), but the magnitude of phenotypic divergence is considerably greater. Likewise, neutral genetic structure appears to be split between the southwestern United States/western Texas and populations from the rest of Texas. This divergence may reflect the fact that this region of Texas is a transition point geographically, from the desert plains of the Stockton Plateau to higher elevation and topographically and biologically diverse region of the Davis Mountains (Poulos et al., 2007; Poulos and Camp, 2010). While New Mexico and Arizona populations were not available at the time of our common garden experiment, we have observed that they are morphologically similar to plants from the Davis Mountains in that they are often among the tallest var. *hallii* plants in our greenhouse collections. Future studies should examine the transition zone in western Texas with more detailed sampling and transplant experiments to determine whether the phenotypic divergence is due to local adaptation.

Hot dry desert valleys interspersed with many high elevation moderate climate "sky island" mountain ranges like the Davis Mountains region characterize much of Arizona, New Mexico, and Western Texas. Elevation has a huge impact on the composition of plant communities in the transition from desert valleys to sky islands (Whittaker and Niering, 1965, 1975; Van Devender and Spaulding, 1979; Poulos et al., 2007; Poulos and Camp, 2010). The sky islands are thought to be biodiversity hotspots, yet very little research has been conducted on the causes of diversity in these regions (Poulos and Camp, 2010). The steep gradients between desert valleys and sky islands could also lead to the formation of high and low elevation ecotypes within species; analogous to coastal, inland, and alpine ecotypes that have been shown to exist in many other plants (Turesson, 1922; Clausen, 1951; Grant, 1981; Lowry, 2012a). *Panicum hallii* may prove to be an excellent model for understanding the formation and maintenance of biological diversity in sky islands.

One exception to the overall pattern of population structure in our study was the COR and TTM populations, which were the only populations from Arizona and New Mexico that did not cluster in the tight group in the genetic PC plot (Fig. 2B). The COR population was collected in a dry Arizona desert valley region adjacent to the Bradshaw Mountains, where we collected the high elevation CKR population. COR is at a lower elevation (1110 m a.s.l.) than any of the other populations collected west of the Texas Stockton Plateau and thus suggests the possibility of population differentiation between high and low elevation of this region. TTM was the second lowest elevation population behind COR from Arizona and New Mexico. In the future, we plan to conduct an analysis of paired populations (Lowry et al., 2008; Jones et al., 2012) from sky islands and desert valleys to better understand the genetic basis of adaptations to elevation across the Chihuahuan and Sonoran deserts.

***The divergence of var.* hallii *and var.* filipes *as a model for switchgrass*—**The most striking divergence in our study was between the var. *hallii* populations and the FIL accession of var. *filipes*. The larger overall size and late flowering of var. *filipes* may reflect adaptation to the more mesic habitat of this variety (Waller, 1976). The pattern of large size and late flowering in mesic varieties and smaller size with early flowering in xeric varieties is often found both within and among plant species (Clausen and Hiesey, 1958; Roux et al., 2006; Lowry, 2012a).

A similar phenotypic pattern occurs within the bioenergy crop switchgrass (*P. virgatum*). The upland ecotype of switchgrass is found in areas away from sources of water and is typically smaller overall than the lowland ecotype, which occurs most often in riparian areas (Porter, 1966; Zalapa et al., 2011; Zhang et al., 2011). Understanding the genetic basis of divergence between upland and lowland switchgrass will be useful for directing breeding programs to maximize yields when both ecotypes are incorporated into breeding programs. Given the much greater ease of working with *P. hallii*, it may serve as a model for understanding the major phenotypic transition between upland and lowland ecotypes in switchgrass. To understand the genetic architecture of divergence between the two *P. hallii* varieties, we are currently in the process of genotyping an F2 population of a cross between the varieties with a new restriction site associated DNA (RAD) mapping method (Wang et al., 2012).

***Conclusions*—**This study lays the foundation for a research program to understand the mechanisms of phenotypic and genetic divergence within *P. hallii*. We have now identified major patterns of population structure within this species. *Panicum hallii* is a highly self-fertilizing species, which has resulted in high levels of among population structure. Across the species, a major division in structure occurs between western desert and eastern grassland/savanna regions. There is also considerable morphological and genetic divergence between var. *hallii* and var. *filipes*. More collections of var. *filipes* will be necessary to clarify the relationship of the varieties.

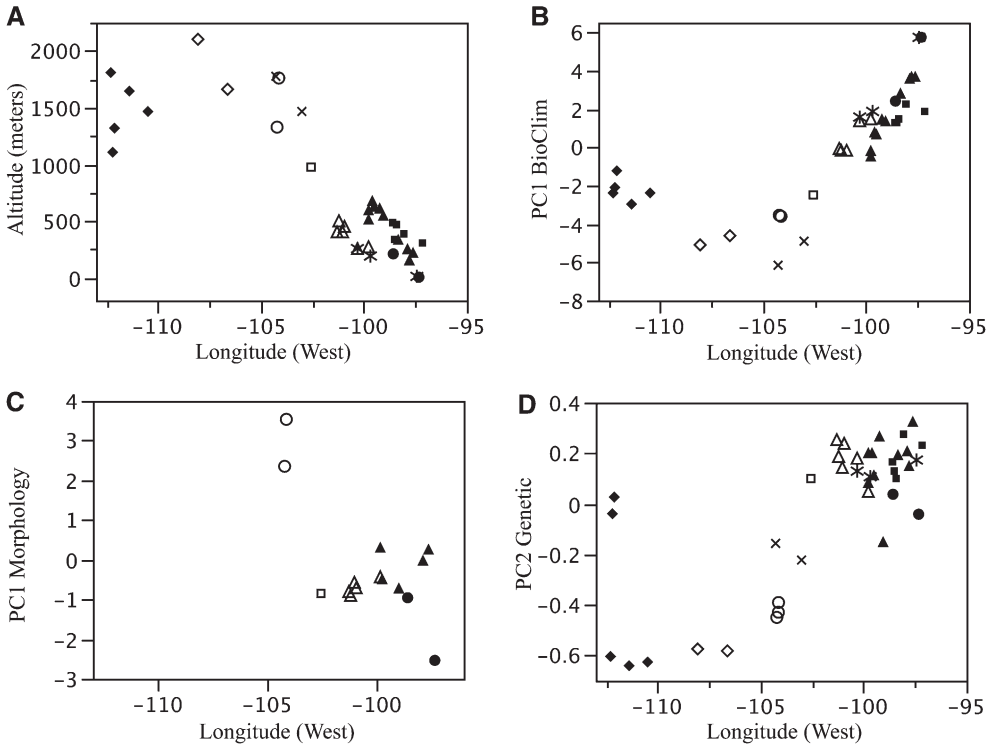

Fig. 4. Patterns of variation in *Panicum hallii* along a longitudinal transect: (A) Altitude, (B) BioClim PC1, (C) Morphology PC1, and (D) Genetic PC1. Key: • = Southern Texas, ○ = Davis Mountains, ▲ = northeastern Edwards Plateau, △ = southwestern Edwards Plateau, □ = Stockton plateau, ■ = OK/TX plains, ◇ = New Mexico Chihuahuan, ◆ = Arizona, × = New Mexico tablelands, ✱ = var. *filipes*.

We plan to follow up this study with future targeted collections and whole genome sequencing of accessions across the range of *P. hallii*. We are currently working with the Joint Genome Institute (DOE) to sequence the genomes of accessions from the populations examined in this study. Those sequenced lines will allow us to better examine geographic patterns of structure within the species and help to identify locally adaptive genes through linkage and association mapping (Anderson et al., 2011; Fournier-Level et al., 2011; Hancock et al., 2011; Jones et al., 2012).

TABLE 4. Strong correlations ($P < 0.005$) between geographic, climatic, genetic, and morphological variation.

Variable1	Variable2	*N*	Correlation
Longitude	Latitude	39	–0.5657*
Longitude	Altitude	39	–0.8177*
Longitude	PC2 genetic	39	0.7443*
Longitude	PC3 genetic	39	–0.5211
Longitude	PC1 BioClim	39	0.7281*
Longitude	PC4 BioClim	39	–0.5725*
Latitude	Altitude	39	0.6677*
Latitude	PC3 genetic	39	0.5753*
Latitude	PC2 BioClim	39	0.4736
Latitude	PC1 BioClim	39	–0.6780*
Altitude	PC2 genetic	39	–0.8475*
Altitude	PC1 BioClim	39	–0.9131*
Altitude	PC1 morphology	15	0.8453*
PC1 BioClim	PC2 genetic	39	0.7046*
PC2 BioClim	PC1 genetic	39	–0.508
PC3 Bioclim	PC2 genetic	39	0.5268*
PC4 BioClim	PC3 genetic	39	0.5412*

Notes: * Significance after Bonferroni correction for multiple comparisons at $P = 0.05$. *N* = number of populations included in each comparison.

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APPENDIX 1. Eigenvalues and percentage of variance explained for BioClim, morphological, and genetic PCs.

Principal component	Eigenvalue	% Variance	Cumulative %
Bioclim PC1	8.7039	45.81	45.81
Bioclim PC2	5.5421	29.169	74.979
Bioclim PC3	2.5404	13.37	88.35
Bioclim PC4	1.1325	5.96	94.31
Morphological PC1	3.5186	35.186	35.186
Morphological PC2	1.7547	17.547	52.733
Morphological PC3	1.3459	13.459	66.192
Genetic PC1	34.905	26.54	26.54
Genetic PC2	29.298	22.28	48.82
Genetic PC3	20.738	15.77	64.59

APPENDIX 2. Divergence of *Panicum hallii* var. *hallii* and *P. hallii* var. *filipes* in morphological traits as reported by Waller (1976).

	var. *hallii*		var. *filipes*	
Trait	Mean	Range	Mean	Range
Spiklet length	3.4	2.9–4.2	2.5	2.2–2.9
First glume length	2.1	1.6–2.6	1.5	1.1–1.7
Panicle length	150	80–280	203	90–300
Longest panicle branch length	70	30–150	120	60–200
Number of primary branches	9.5	6–16	17.3	10–29
Uppermost blade length	108	50–220	139	70–240
Width of widest branch	3	1.5–6.0	3.9	2.0–6.2

APPENDIX 3. Significant Pearson correlation coefficients after Bonferroni correction ($P = 0.05$) for individual BioClim climatic variables with the morphological and genetic principal components.

Variable	Morphology PC1	Genetic PC2
BioClim 1 (annual mean temperature)	–0.8809	0.7266
BioClim 2 (mean diurnal range)		–0.6278
BioClim 3 (isothermality)		–0.7839
BioClim 5 (maximum temperature of warmest month)		0.7045
Bioclim 6 (minimum temperature of coldest month)		0.5590
BioClim 10 (mean temperature of warmest quarter)	–0.8401	0.8084
BioClim 11 (mean temperature of coldest quarter)	–0.8062	0.5985
BioClim 14 (precipitation of driest month)		0.6062
BioClim 15 (precipitation seasonality)		–0.6680
BioClim 17 (precipitation of driest quarter)		0.5684