New Phytologist

Genotypic variation in traits linked to climate and aboveground productivity in a widespread C_4 grass: evidence for a functional trait syndrome

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Received: *5 February 2013*
Accepted: *19 April 2013*

New Phytologist (2013) **199:** 966–980
doi: 10.1111/nph.12341

Key words: C_4, climate change, evolution, genecology, *Panicum virgatum* (switchgrass), physiology, polyploidy.

Summary

- Examining intraspecific variation in growth and function in relation to climate may provide insight into physiological evolution and adaptation, and is important for predicting species responses to climate change.
- Under common garden conditions, we grew nine genotypes of the C_4 species *Panicum virgatum* originating from different temperature and precipitation environments. We hypothesized that genotype productivity, morphology and physiological traits would be correlated with climate of origin, and a suite of adaptive traits would show high broad-sense heritability (H^2).
- Genotype productivity and flowering time increased and decreased, respectively, with home-climate temperature, and home-climate temperature was correlated with genotypic differences in a syndrome of morphological and physiological traits. Genotype leaf and tiller size, leaf lamina thickness, leaf mass per area (LMA) and C : N ratios increased with home-climate temperature, whereas leaf nitrogen per unit mass (N_m) and chlorophyll (Chl) decreased with home-climate temperature. Trait variation was largely explained by genotypic differences ($H^2 = 0.33–0.85$).
- Our results provide new insight into the role of climate in driving functional trait coordination, local adaptation and genetic divergence within species. These results emphasize the importance of considering intraspecific variation in future climate change scenarios.

Introduction

A basic precept of evolutionary biology is that climate is a principal driver of species distributions and local adaptation of populations (Turesson, 1922; Clausen *et al.*, 1940; Rehfeldt *et al.*, 1999). Even so, we still lack a fundamental understanding of climate-driven trait coordination and climate-related physiological divergence among populations (Ackerly *et al.*, 2000; Campitelli & Simonsen, 2012; Wright & Sutton-Grier, 2012). It is also often unclear whether intraspecific patterns are caused by plastic responses to environmental variation or genetically based differences (Sultan, 2000; Etterson & Shaw, 2001; Chown *et al.*, 2010). Therefore, examining intraspecific patterns of growth, morphology and function in relation to climate will provide insight into physiological evolution and adaptation to climate, and is essential for predicting species responses to future climate (Arntz & Delph, 2001; Jump & Peñuelas, 2005; Albert *et al.*, 2010).

Previous studies, across a range of species, have demonstrated intraspecific physiological variation associated with climate. In common garden studies population variation in net photosynthetic rates (A_{CO2}), stomatal conductance (g_s) and leaf nitrogen (N) have all been associated with climate of origin (e.g. temperature, vapour pressure deficit, precipitation) (Oleksyn *et al.*, 1998; Benowicz *et al.*, 2000; Christman *et al.*, 2008; Marchin *et al.*, 2008). Intraspecific variation in intrinsic water-use efficiency (A_{CO2}/g_s or iWUE) has also been demonstrated and may reflect population adaptation to arid conditions because high iWUE may allow for carbon (C) fixation during water limitation (Comstock & Ehleringer, 1992; Voltas *et al.*, 2008).

Climate may also drive functional trait divergence via indirect selection on correlated traits, resulting in genetic divergence of entire trait syndromes (Falconer & Mackay, 1996; Lynch & Walsh, 1998; Ackerly *et al.*, 2000; Geber & Griffen, 2003). Genetic variation in leaf mass per area (LMA), leaf carbon (C)

and leaf N content, for instance, may co-vary with physiological traits, reflecting physiological trade-offs and adaptation to local environments (Chapin *et al.*, 1993; Reich *et al.*, 2003). For example, low LMA (thin) leaves can exhibit high A_{CO2} and may be more responsive to resource heterogeneity (Reich *et al.*, 2003; Poorter *et al.*, 2009), yet require greater N investment, have higher respiration rates, and exhibit shorter life spans (Reich *et al.*, 1998). Conversely, long-lived high LMA (thick) leaves often exhibit lower N concentrations and A_{CO2}, but demonstrate greater photosynthetic resource-use efficiency and stress resistance (Chapin *et al.*, 1993; Kikuzawa, 1995). While general patterns of trait coordination have been demonstrated across a wide range of plant species (Reich *et al.*, 2003; Wright *et al.*, 2004), intraspecific patterns are typically less evident (Albert *et al.*, 2011). Still, examining functional trait covariation among populations or genotypes, in relation to their climate of origin, provides insight into the factors that drive physiological divergence and local adaptation (Geber & Griffen, 2003; Albert *et al.*, 2011).

Despite the adaptive importance of various functional traits, heritability estimates can vary widely, owing to environmental sensitivity, developmental effects and trait co-variation (Donovan & Ehleringer, 1994; Geber & Dawson, 1997; Maherali *et al.*, 2008). Yet, heritability estimates indicate how a trait may respond to selection pressures such as climate, and because traits are often genetically correlated, a change in one strongly inherited adaptive trait can result in broad changes to an entire trait syndrome (Chapin *et al.*, 1993; Ackerly *et al.*, 2000). Therefore, heritability estimates for an array of functional traits, measured among genotypes representing populations originating from different climates may elucidate the environmental factors driving trait covariation and genetic divergence (Arntz & Delph, 2001).

Panicum virgatum (switchgrass) is an ideally suited plant species for examining climate-driven functional trait coordination and genetic differentiation. This C_4 NAD-malic enzyme (NAD-ME) type perennial bunchgrass is a key component of the tallgrass prairies of North America, and its geographic range spans substantial variation in temperature, precipitation, photoperiod and soil type. The species also has multiple uses including forage, soil conservation, and bioenergy production (Parrish & Fike, 2005; Schmer *et al.*, 2008). *Panicum virgatum* has shown strong evidence of local adaptation; genetic differentiation in productivity, flowering time and morphology have been primarily attributed to temperature (McMillan & Weiler, 1959; McMillan, 1965; Madakadze *et al.*, 1998; Casler *et al.*, 2004; Berdahl *et al.*, 2005). *Panicum virgatum* populations have also been generally classified in terms of habitat differentiation (Porter, 1966), with morphologically larger 'lowland' populations originating primarily from the southern US, while 'upland' populations largely occupy drier sites in the northern US. Ploidy also varies among populations of *P. virgatum* across its geographic range (Casler *et al.*, 2011). Increased ploidy may confer an altered phenotype capable of exploiting new ecological niches (Otto & Whitton, 2000; Maherali *et al.*, 2009). Thus, *P. virgatum* is well suited for studying not only climate-mediated functional trait coordination and genetic differentiation, but also physiological differentiation among ploidy types.

To understand how climate drives functional trait coordination and genetic differentiation of populations, we grew nine *P. virgatum* genotypes in a common garden in central Texas and measured growth, morphological development, leaf traits and aboveground net primary productivity (ANPP). The nine genotypes originated from a broad range of temperature and precipitation environments in the Great Plains. We used broad-sense heritability (H^2) estimates to determine the degree of genetic differentiation in all traits, compared morphology, leaf traits and ANPP between ploidy types, and used multivariate analyses to examine how genotype morphology, leaf traits and ANPP co-vary relative to the genotype's home climate. We hypothesized that genetic differentiation in morphology, leaf traits and ANPP would exhibit trends consistent with climate of origin; and whole-plant growth and ANPP would show higher H^2 than individual leaf traits, although a syndrome of key functional traits likely to vary with climate (e.g. LMA, iWUE, leaf N) would also show high H^2.

Materials and Methods

Experimental facility

This study was conducted at a rainout shelter facility located near Temple, Texas, USA (31°3′25.7″N, 97°20′50.9″W). Site elevation is *c.* 199 m above sea level. The mean maximum temperature in July–August is *c.* 35.0°C, and the mean minimum temperature in December is *c.* 3.0°C. Soils are Austin silty clay (fine-silty, carbonatic, Udorthentic Haplustol), and are well-drained, with medium to rapid runoff, and moderate to low permeability.

The facility consists of an 18.3 m × 73.0 m steel-framed rainout shelter (Windjammer Cold Frame, International Greenhouse Company, Danville, IL, USA) covered by a clear 240 μm polyethylene roof that transmits 90% of photosynthetically active radiation (PAR). The 2.1 m high walls on the side of the shelter are open, and the eaves on the open ends of the shelter are 4.2 m high, maximizing air movement and heat dissipation.

The rainout shelter excludes natural rainfall from 24 5 m × 5 m plots arranged in four blocks. Within each block, plots are spaced 0.25 m apart, and blocks are spaced 2.76 m apart. Each plot is bounded by a vertical barrier of 1.84 mm thick pond liner (ethylene propylene diene monomer, Firestone Specialty Products, Indianapolis, IN, USA) buried to a depth of 1.2 m below the soil surface, which prevents horizontal sub-soil water movement and root penetration from outside the plot. The barrier extends 10 cm above the soil surface and is supported by a 5 cm × 10 cm wood frame that prevents surface water flow into the plots from outside the shelter. Care was taken during construction to limit soil disturbance and compaction.

The plots were irrigated using 90° sprinklers (Hunter HP2000, Hunter Industries Inc., San Marcos, CA, USA) attached to 1 m risers on the corner of each treatment plot. The sprinklers were operated by a programmable controller (LEIT XRC Series Ambient Powered Irrigation Controller, DIG Corporation, Vista, CA, USA).

Genotypes

The study included nine different *Panicum virgatum* L. 'genotypes' representing different climatic origins and ploidy types (Table 1). We use the term 'genotype' to signify that these individuals originated from vegetative propagation of a single plant and are representatives of the gene pool (population) present in their locale. The use of these genotypes allows us to focus on broader patterns of genetic variation in relation to climate, rather than within-population variation. Four of the genotypes (WBC, WIL, WWF and ENC) were propagated from wild collections. Two genotypes, KAN and VS16, were derived from cv Kanlow and cv Summer, respectively. NAS is an upland genotype from northern Texas which has been used for land reclamation in the dry west. NOC is a USDA selection and while the lineage and exact collection location are unknown, it is an upland octoploid from the northern Great Plains. Genotype AP13 is an accession of cv Alamo, a lowland ecotype, which is being sequenced by the Joint Genome Institute (JGI-DOE) as part of the switchgrass genome project. VS16 is the primary upland ecotype being sequenced by JGI. Genotypes KAN, VS16, NAS, NOC and AP13 all originated from prairie-remnant populations. Although some genotypes are cvs, minimal domestication has left these cvs genetically similar to the wild prairie remnant populations from which they originated (Casler *et al.*, 2007).

Climate

The genotypes originated from 27 to 41°N latitude, representing a 11–22°C range in mean annual temperature (MAT; $r = 0.99$ with latitude) and a 650–1110 mm range in mean annual precipitation (MAP; not significantly correlated with latitude, $P = 0.25$) (Table 1). We derived several more detailed measures describing the climatic origin of the genotypes (Table 1). These measures described temperature extremes and seasonality, and most were highly correlated with latitude ($r = 0.99$) except for mean summer (May–September) precipitation (MSP; $r = 0.63$, $P = 0.10$). In addition, we calculated the annual heat–moisture ratio (AHM; ratio of mean annual temperature to mean annual precipitation) and the summer heat-moisture index (SHM; ratio of the mean temperature in the warmest month to summer precipitation). The AHM and SHM index the amount of precipitation available for plant growth (relative to atmospheric demand) (Rehfeldt *et al.*, 1999); higher ratios indicate greater potential for water deficit. Both AHM and SHM decreased with latitude ($r = -0.87$ and $r = -0.71$, respectively). These parameters indicate that the climate for genotypes from northern latitudes was cooler and less arid than for genotypes from southern latitudes (Table 1).

Propagation and establishment

Clonal replicates of each genotype were propagated via division and multiplication of rhizomes originating from a single plant. All replicates were grown outdoors in separate 3.79 l pots under the same conditions and were transplanted into the plots on 3 March 2011 (day of year, DOY 62). All previous year's tillers were cut at 10 cm above the growth media surface and all transplants were dormant (i.e. no green tissue was visible) at planting. The previous year's tillers were counted and tested as a covariate in the growth analysis. Within each plot, two replicates of each genotype were planted at 1 m × 1 m spacing. Genotypes were assigned in a stratified random manner to positions within each plot, with one of each replicate in the east and west halves of the plots, and with replicates never adjacent to each other. All plots received identical irrigation so that expression of genotypic differences could be explicitly studied. Plots were initially well watered to facilitate establishment (March–May, 45–52 mm wk^{-1}). Irrigation amounts then approximated the typical seasonal rainfall pattern for the study site; June, July, August, and September irrigation amounts

Table 1 Ploidy, geographic origin and historical climate data for the nine *Panicum virgatum* (switchgrass) genotypes included in this study

Variable[1]	VS16	NOC	KAN	NAS	WBC	WIL	AP13	WWF	ENC
Ploidy	4×	8×	4×	8×	4×	4×	4×	8×	8×
Lat. (°N)	40.7	–	35.1	33.1	30.1	29.1	28.3	28.1	26.9
Long. (°W)	95.9	–	95.4	96.1	98.0	98.2	98.1	97.4	98.1
MAP (mm)	861	–	1045	1110	855	701	850	903	646
MSP (mm)	518	–	503	468	397	345	408	410	315
MAT (°C)	10.8	–	15.5	17.2	20.3	20.6	21.2	21.2	22.3
MTCM (°C)	−4.9	–	2.5	5.4	10.1	10.3	12.1	12.3	13.2
MTWM (°C)	24.8	–	27.4	28.2	29.0	29.2	28.8	28.6	29.5
TD (°C)	29.7	–	24.9	22.8	18.9	18.9	16.7	16.3	16.3
DMT	138	–	70	58	17	30	13	9	10
SHM (°C m^{-1})	48.0	–	54.4	61.0	71.3	84.1	64.1	56.9	78.5
AHM (°C m^{-1})	12.5	–	14.8	15.5	23.7	29.4	24.9	23.5	34.5

Climate data (1971–2000) is from the National Oceanic and Atmospheric Administration (NOAA) weather station closest to the genotype's geographic origin.
[1]MAP, mean annual precipitation; MSP, mean summer precipitation (May–September); MAT, mean annual temperature; MTCM, mean temperature of the coldest month; MTWM, mean temperature of the warmest month; TD, temperature differential (MTWM – MTCM); DMT, days with minimum temperature < 0°C; SHM, summer heat: moisture index (MTWM : MSP); AHM, annual heat: moisture index (MAT : MAP).

were 37.5, 30.0, 15.0 and 15.0 mm wk^{-1}, respectively, for an annual total of 945 mm.

Ploidy determination

For genotypes whose ploidy was unknown (NOC, NAS, WBC, WIL, WWF, ENC), we determined ploidy by flow cytometry. Approximately 300–500 mg of young healthy leaf tissue was combined in a small Petri dish with 1 ml Partec Nuclei Extraction buffer mixed with 1 µl beta-mercaptoethanol (CyStain PI Absolute P reagent kit, Partec, Münster, Germany). The tissue was chopped manually (5–7 min) with a razor blade while in the extraction buffer and allowed to sit for several minutes.

The extractant was passed through a Partec 30 µm Cell Trics disposable strainer placed over a 5 ml Falcon test tube. Staining solution (2 ml staining buffer, 12 µl propidium iodide (PI) stock, and 6 µl RNase, all from the CyStain PI Absolute P reagent kit) was then added to the extractant and the tube placed on ice in a dark cooler. The stained sample was analyzed within 30 min on a flow cytometer (FACSCalibur with CellQuest Pro software, BD Biosciences, Franklin Lakes, NJ, USA). By comparing the flow cytometry of each genotype with an AP13 tetraploid standard, we established the ploidy status of each genotype.

Growth and morphology

Genotype growth was assessed by recording the tiller number, canopy height (cm), and basal area (cm^2) on all plants ($n = 432$) beginning at planting. Basal area was calculated assuming the area of an ellipse based on the diameter of each transplant measured in two perpendicular directions. Growth was measured every 8–9 d between DOY 70 and 144, and monthly thereafter. The presence of newly emerged panicles was recorded weekly following initial emergence. Between DOY 175 and 178, leaf area index (LAI, m^2 m^{-2}) for each plant was estimated from ceptometer (AccuPAR model LP-80, Decagon Devices, Inc., Pullman, WA, USA) measurements at 10 cm height, taken in two perpendicular directions through the center of the sward.

Tiller and leaf morphology was assessed at midseason (DOY 209–216) by measuring leaf length (cm), leaf width (mm), tiller internode length (cm) and internode diameter (mm). Measurements were made on three representative tillers per plant on all plants in one randomly chosen plot per block ($n = 72$), for a total of 24 tillers per genotype. Leaf length and width (midway along leaf) were measured on the second mature fully expanded (clearly defined ligule) leaf from the tiller apex. Internode length and diameter were measured between the first and second leaf at the base of each tiller. Internode diameter was measured with a digital caliper.

In mid-November (DOY *c.* 318), tillers were counted on all plants; each plant was harvested at 10 cm above the soil surface, dried at 65°C to a constant mass, and weighed to determine ANPP (g m^{-2}). Average tiller mass (g per tiller) for each plant was calculated as ANPP divided by the number of tillers at harvest.

Leaf functional traits

Leaf gas-exchange and chlorophyll fluorescence were measured monthly (between DOY 136 and 293) on all plants within the same four plots sampled for morphological characterization ($n = 72$). Leaf net CO_2 assimilation (A_{CO2}, µmol m^{-2} s^{-1}), stomatal conductance to water vapor (g_s, mmol m^{-2} s^{-1}), intrinsic water-use efficiency (A_{CO2}/g_s or iWUE; µmol $mmol^{-1}$), photochemical quenching of photosystem II (PSII) (q*P*, dimensionless) and efficiency of PSII (Φ_{PSII}) were measured on one or two fully expanded, mature upper canopy leaves using a LI-6400 portable photosynthesis system equipped with a modulated chlorophyll fluorometer (6400-40) integrated into the cuvette lid (Li-Cor, Inc., Lincoln, NE, USA). The order in which plots and genotypes were sampled was randomized. Measurements were taken between 10:00 and 14:00 h approximating midday conditions: ambient photosynthetic photon flux density during this period was at least *c.* 1500 µmol m^{-2} s^{-1}, which was the average midday PAR. Within the cuvette, PAR was maintained at 1500 µmol m^{-2} s^{-1} using an actinic light source. The chamber CO_2 supply was controlled at 405 µmol mol^{-1}, and the sample [CO_2] averaged 390 ± 6.7 µmol mol^{-1}. At the beginning of each sampling period the cuvette block temperature was set at ambient temperature. Leaf temperature was measured directly using the LI-6400 leaf thermocouple wire. Water vapor inside the chamber was not scrubbed so that relative humidity conditions inside the chamber tracked ambient conditions. Data were recorded when A_{CO2}, g_s, intercellular [CO_2] (C_i) and light adapted fluorescence had stabilized. Fluorescence parameters were calculated according to the built-in functions of the LI-6400 system.

The leaves sampled for gas exchange and chlorophyll fluorescence were immediately removed with scissors and leaf lamina thickness (µm) was measured on the base of each leaf using digital calipers, carefully avoiding the leaf midrib. A small section (50 mg fresh mass) at the base of the cut leaf was removed for total chlorophyll (Chl) content (mg g^{-1} dry mass) determination following Wellburn (1994). Leaf area of the remaining leaf sample was measured using a LI-3000A Portable Leaf Area Meter (Li-Cor). The sample was dried to a constant mass at 65°C, and leaf mass per unit leaf area (LMA; g m^{-2}) was calculated as leaf dry mass divided by leaf area. Dried leaf samples were ground into a fine powder using a ball mill (SPEX SamplePrep 8000D Mixer/Mill, SPEX SamplePrep LLC, Metuchen, NJ, USA), and stored under desiccation. Leaf C and N contents were measured using a combustion elemental analyzer (Flash 2000 Organic Elemental NC Analyzer, Waltham, MA, USA). Nitrogen per unit leaf area (N_l; mmol [N] m^{-2}) was obtained as the product of N per unit leaf mass (N_m; g kg^{-1}) and LMA, multiplied by the atomic mass of N. Photosynthetic nitrogen-use efficiency (PNUE; µmol mol^{-1} s^{-1}) was calculated as the ratio of A_{CO2} and N_l.

Data analysis

All statistical analyses were carried out in SAS 9.2 (SAS Institute, Inc., 2002). Genetic variation in whole-plant growth over time was examined using PROC MIXED with a repeated measures mixed model in the form:

$$Y_{ijkl} = \mu + T_i + B_j + P_k + G_l(P_k) + T_iB_j + T_iP_k + T_iG_l(P_k) + B_jP_k + B_jG_l(P_k) + R_{ijkl} + \varepsilon_{ijkl} \quad \text{Eqn 1}$$

where Y_{ijkl} represents the response variable (tiller number, canopy height, basal area), T_i represents the ith day of the year, B_j represents the jth block, P_k represents the kth ploidy level, G_l represents the lth genotype from within the kth ploidy level. All other terms represent the respective interactions and R_{ijkl} and ε_{ijkl} represent the repeated measures term and residual, respectively. The number of tillers in the previous year was tested as a covariate. Effects were tested using Type III sums of squares. Leaf trait differences among genotypes were tested using the model:

$$Y_{ijkl} = \mu + T_i + Z_j + P_k + G_l(P_k) + T_iZ_j + T_iP_k + T_iG_l(P_k) + Z_jP_k + Z_jG_l(P_k) + R_{ijkl} + \varepsilon_{ijkl} \quad \text{Eqn 2}$$

where Y_{ijkl} represents the response variable (A_{CO2}, g_s, iWUE, etc.) and all other parameters are the same as previously noted with the exception of Z_j, which is the effect of the jth plot. Lastly, genotype differences in leaf and tiller morphology were tested using the model:

$$Y_{jkl} = \mu + Z_j + P_k + G_l(P_k) + Z_jP_k + Z_lG_l(P_k) + \varepsilon_{jkl} \quad \text{Eqn 3}$$

where Y_{jkl} represents the response variable (leaf length and width; internode diameter and length) and the remaining effects in the model are the same as stated previously. When genotype or time × genotype effects were significant ($P \leq 0.05$), Tukey's adjustment was used for pairwise comparison of genotype means. In all models, measurement date, ploidy, genotype, and their respective interactions were considered fixed effects. The block or plot effect and interactions with block or plot were considered random effects.

To quantify the portion of growth, morphology and leaf trait (mid-summer only) variation attributable to genetic variation, broad-sense heritabilities (H^2) were calculated as:

$$H^2 = \sigma^2_G/(\sigma^2_G + \sigma^2_B + \sigma^2_{GB} + \sigma^2_\varepsilon) \quad \text{Eqn 4}$$

where σ^2_G, σ^2_B, σ^2_{GB}, and σ^2_ε are the genotype, block, interaction and residual variances. All mixed models and H^2 estimates were calculated using PROC MIXED.

Pearson correlation coefficients were calculated to determine the association between genotype growth and leaf trait means and the climatic conditions from which the genotypes originated. Correlation analyses were conducted in PROC CORR. All analyses were conducted at $P \leq 0.05$ significance level.

We used principal components analysis (PCA) (PROC FACTOR) with orthogonal (varimax) factor rotation to reduce the traits tested to a subset of variables that explained the majority of the observed variation, thereby reducing the influence of inter-trait correlations. We conducted the PCA using data collected during July (DOY 201) when the plants had reached peak morphological and physiological development. Derived variables or those calculated as ratios, such as iWUE, PNUE and N_l, were omitted to limit cross-confounding of factors. Parallel analysis was used to determine the number of significant principal components (PC) to retain (Franklin *et al.*, 1995; Peres-Neto *et al.*, 2005). Multiple regression (PROC REG) was performed to determine the climate variables that best explained the variation in genotype PC scores. Multicollinearity among the climate variables was tested using variance inflation factors (VIF). Climate variables with VIF > 10 were excluded from the model (Neter *et al.*, 1996). Model selection was based on Akaike Information Criterion (AIC) where lower AIC values indicate a more parsimonious model.

Results

Genotypic patterns of growth, morphology, ANPP and leaf traits

Over the entire growing season, there was a significant time × genotype effect on tiller number, basal area and canopy height, indicating that growth rates varied among genotypes (Table 2). The number of tillers in the previous year was a significant covariate for tiller growth ($P < 0.0001$) and basal area ($P = 0.02$), but not canopy height ($P > 0.05$). Genotypes originating from more southern locations (e.g. ENC, WWF, WIL) showed the earliest tiller production (DOY 69) and continued producing tillers until the end of the growing season. Tiller production began later (DOY 88–94) and ended earlier for genotypes from more northern locations (KAN, NOC, VS16; Fig. 1a).

End of season canopy height was significantly different among the genotypes (Fig. 1b). These differences were positively correlated with the genotypes' home temperature conditions, and negatively correlated with TD and DMT, indicating that genotypes from warmer, less seasonal climates grew tallest (Table 3). Although end of season tiller production and basal area were significantly different among genotypes (Table 2; Fig. 1a,c) these differences were generally uncorrelated with climate (Table 3).

Panicle emergence of early flowering genotypes (AP13, VS16, NAS, NOC) was *c.* 2 months ahead of late flowering genotypes (ENC, WWF; Fig. 2). Genotype mid-summer (DOY 178) panicle emergence was not associated with climate (all $P > 0.06$). Given that AP13, a genotype representing a warm-climate population, showed earlier panicle emergence than expected, we excluded AP13 from the analysis and found that, in this case, genotype mid-summer (DOY 178) panicle emergence showed

Table 2 Degrees of freedom and *F* values for ANOVA of *Panicum virgatum* (switchgrass) whole-plant growth, morphology and leaf traits

	Time		Ploidy		Genotype (ploidy)		Time × ploidy		Time × genotype (ploidy)	
Variable	df	*F*	df	*F*	df	*F*	df	*F*	df	*F*
Productivity										
Tillers	11,5045	**1300.3**	1,3	**12.5**	7,21	**9.9**	11,5045	**48.1**	77,5045	**36.2**
Basal area	6,2934	**828.3**	1,3	**202.6**	7,21	**18.0**	6,2934	**74.7**	42,2934	**10.6**
Canopy height	6,2930	**4495.6**	1,3	2.4	7,21	**204.3**	6,2930	**31.9**	42,2930	**61.8**
ANPP[1]			1,3	2.8	7,21	**47.9**				
Tiller mass[1]			1,3	**59.2**	7,21	**219.0**				
LAI[2]			1,3	9.1	7,21	**23.0**				
Morphology[2]										
Leaf length			1,3	0.2	7,21	**28.4**				
Leaf width			1,3	2.1	7,21	**32.5**				
Internode length			1,3	**21.5**	7,21	**23.2**				
Internode diameter			1,3	9.1	7,21	**10.7**				
Leaf traits										
A_{CO2}	5,15	**74.6**	1,3	2.1	7,21	**5.7**	5,317	**3.4**	34,317	**3.0**
g_s	5,15	**48.7**	1,3	2.3	7,21	**5.1**	5,317	**3.9**	34,317	**2.9**
iWUE	5,15	**3.2**	1,3	1.0	7,21	**13.2**	5,317	1.8	34,317	**1.9**
PNUE	5,15	**31.2**	1,3	**13.6**	7,21	**7.1**	5,317	**2.8**	34,317	**2.5**
Φ_{PSII}	5,15	**196.7**	1,3	2.3	7,21	**7.1**	5,317	**2.8**	34,317	**3.4**
q*P*	5,15	**263.0**	1,3	1.2	7,21	**7.9**	5,317	**2.3**	34,317	**3.8**
LMA	5,15	**78.9**	1,3	**83.3**	7,21	**20.5**	5,317	0.8	34,317	**1.6**
Leaf thickness	5,15	**12.7**	1,3	6.1	7,21	**23.1**	5,317	1.8	34,317	1.1
N_l	5,15	**119.4**	1,3	**42.7**	7,21	**8.8**	5,317	1.4	34,317	**1.7**
N_m	5,15	**214.2**	1,3	0.7	7,21	**33.7**	5,317	1.9	34,317	**2.5**
C : N	5,15	**102.9**	1,3	0.0	7,21	**27.8**	5,317	**2.4**	34,317	**5.0**
Chl	5,15	**15.8**	1,3	1.1	7,21	**21.8**	5,317	1.7	34,317	**2.2**

F-values in bold are significant at $P \leq 0.05$.

[1]ANPP and tiller mass were measured at the end of the growing season.

[2]LAI and morphological variables were measured at one time point (mid-summer).

Leaf traits: A_{CO2}, net photosynthetic rate; g_s, stomatal conductance to water vapour; iWUE, intrinsic water-use efficiency; PNUE, photosynthetic nitrogen-use efficiency, Φ_{PSII}, efficiency of PSII; q*P*, photochemical quenching of PSII; LMA, leaf mass area; N_l, nitrogen per unit leaf area; N_m, nitrogen per unit leaf mass; C : N, leaf carbon : nitrogen ratio; and Chl, total leaf chlorophyll content.

negative correlations with MAT, MTCM and AHM (defined in Table 1), and positive correlations with TD and DMT (Table 3), indicating a tendency for cooler climate genotypes to begin flowering earlier.

Genotypes also showed significant morphological variation, with leaf and internode dimensions generally decreasing in more northerly genotypes (Table 2; Fig. 3a,b). Genotype leaf and internode length decreased with MSP, and increased with MTWM and SHM (Table 3). Internode length increased with all three mean temperature variables and leaf width increased with MTWM (Table 3). These correlations suggested larger leaf and internode dimensions for genotypes originating from environments with warmer, drier summers.

Differences in ANPP among genotypes were highly significant (Table 2; Fig. 3c). Genotypes ENC, WWF, AP13, WIL and WBC were most productive (1195–1834 g m^{-2}), followed by NAS and KAN (474 and 547 g m^{-2}), and NOC and VS16 (130 and 125 g m^{-2}; Fig. 3c). Genotypic productivity differences were closely associated with climate of origin. Genotype ANPP and tiller mass increased with MAT, MTCM, MTWM, SHM and AHM ($r = 0.75$–0.92; Table 3), and decreased with TD and DMT ($r = -0.81$ to -0.93) indicating that genotypes from warmer, less seasonal climates, were most productive (Table 3). The number of tillers in the previous year was a significant covariate for ANPP ($P < 0.0001$) but not tiller mass ($P = 0.54$).

Leaf trait differences among genotypes varied over time for all traits except leaf thickness (Table 2; Supporting Information Figs S1, S2). With the exception of iWUE, gas-exchange and chlorophyll fluorescence parameters peaked between DOY 166 and 201 (Fig. S1). Physiological differences among genotypes were most pronounced during this period. In comparison, genotype N_m, N_l and Chl gradually decreased over time while LMA and C : N gradually increased over time (Fig. S2).

Genotypes ENC and WWF showed the highest leaf thickness (301 and 265 μm, respectively), followed by WBC, AP13 and WIL (249–252 μm), NOC, KAN and NAS (177–233 μm) and VS16 (169 μm) (Table S1). When averaged over the entire season, genotype iWUE, Φ_{PSII} and C : N increased with genotype

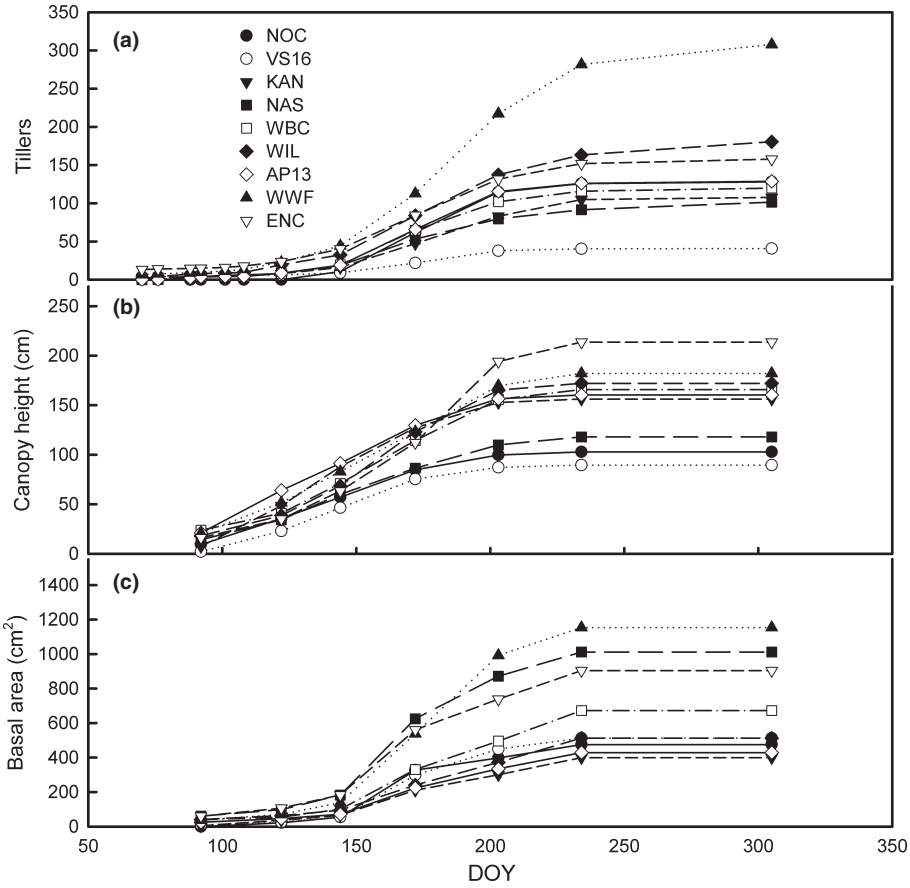

Fig. 1 Seasonal patterns of tiller production, height and basal area growth among *Panicum virgatum* (switchgrass) genotypes from different climatic origins. Each symbol represents one of the genotypes identified in Table 1. Standard error bars are not included in order to display genotype differences. DOY, day of year.

leaf thickness while genotype N_m and Chl decreased with genotype leaf thickness (Fig. 4).

Genotype leaf trait means showed strong correlations with temperature-related variables (Table 3), and none were correlated with MAP or MSP. Since temperature-related correlations were generally consistent over time, with the exception of May (data not shown), we only report correlations based on genotype means pooled over time (Table 3). Genotype iWUE, Φ_{PSII}, LMA, leaf thickness and C:N increased with MAT, MTWM and MTCM, and decreased with TD and DMT (Table 3). Moreover, genotype iWUE and leaf thickness increased with both heat–moisture indices, SHM and AHM. Genotype N_m decreased with increased MAT and MTCM (Table 3). Therefore, a suite of leaf traits co-varied with the genotype's home-climate temperature conditions.

Ploidy

Octoploids produced more tillers and were larger in basal area (174 ± 10 tillers and 888.3 ± 34 cm^2, respectively) than tetraploids (115 ± 10 tillers and 503.7 ± 33 cm^2, respectively) (Table 2). However, tetraploids produced larger tillers (6.9 ± 0.2 g per tiller) with longer internodes (17.8 ± 1.0 cm) than octoploids (5.7 ± 0.2 g per tiller and 15.4 ± 1.0 cm, respectively) (Table 2). Overall, ploidy had no effect on height growth or ANPP (Table 2).

Most photosynthetic traits showed a significant time × ploidy interaction, indicating that ploidy effects on leaf physiology were not consistent over time (Table 2). Even so, ploidy had a consistent significant effect on LMA and N_l (Table 2) with octoploids showing higher LMA and N_l (84.2 ± 1.2 g m^{-2} and 110.3 ± 1.8 mmol [N] m^{-2}) than tetraploids (76.6 ± 0.9 g m^{-2} and 98.0 ± 1.4 mmol [N] m^{-2}).

Broad-sense heritability

As expected, H^2 estimates were higher for whole-plant growth and morphological traits (0.38–0.85) than for leaf traits (Table 4). Broad-sense heritabilities for photosynthetic traits were moderate and ranged from 0.25 to 0.34 for g_s and PNUE, respectively (Table 4). Other leaf traits tended to show higher H^2 values and leaf thickness showed the highest H^2 (0.60) (Table 4).

Principal components analysis and trait associations with climate

Two PCs were identified which, cumulatively, explained 54.0% of the total variation in growth, morphology and leaf traits (Table 5). PC1, which represented a trait syndrome encompassing several growth, morphological and leaf functional traits, accounted for 33.6% of the total phenotypic variation. PC1 was

Table 3 Correlations between *Panicum virgatum* (switchgrass) genotype productivity, morphology and functional trait means, pooled over time, and genotype home-climate conditions ($n = 8$)

Variable	MAP	MSP	MAT	MTCM	MTWM	TD	DMT	SHM	AHM
Productivity									
Tillers	−0.23	−0.14	0.69	**0.71**	0.58	**−0.73**	−0.70	0.27	0.52
Basal area	0.10	0.04	0.35	0.37	0.31	−0.38	−0.36	0.03	0.19
Canopy height	−0.57	−0.58	**0.87**	**0.86**	**0.83**	**−0.85**	**−0.86**	0.67	**0.87**
ANPP	−0.59	−0.54	**0.92**	**0.92**	**0.82**	**−0.93**	**−0.90**	0.63	**0.86**
Tiller mass	−0.57	**−0.72**	**0.84**	**0.83**	**0.83**	**−0.81**	**−0.83**	**0.75**	**0.83**
LAI	−0.62	**−0.78**	**0.94**	**0.92**	**0.92**	**−0.91**	**−0.90**	**0.85**	**0.92**
Mid-summer panicle emergence[1]	0.60	0.38	**−0.82**	**−0.84**	−0.66	**0.88**	**0.81**	−0.46	**−0.80**
Morphology									
Leaf length	−0.41	**−0.86**	0.67	0.64	**0.79**	−0.58	−0.64	**0.85**	0.68
Leaf width	−0.20	−0.64	0.64	0.61	**0.77**	−0.55	−0.67	0.67	0.54
Internode length	−0.38	**−0.77**	**0.77**	**0.75**	**0.86**	−0.70	**−0.78**	**0.81**	0.69
Internode width	−0.19	−0.54	0.58	0.56	0.67	−0.51	−0.62	0.55	0.48
Leaf traits									
A_{CO2}	−0.34	−0.42	0.53	0.52	0.51	−0.51	−0.56	0.41	0.48
g_s	−0.06	0.04	−0.24	−0.25	−0.27	0.24	0.22	−0.14	−0.16
iWUE	−0.41	−0.60	**0.91**	**0.90**	**0.91**	**−0.87**	**−0.89**	**0.71**	**0.81**
PNUE	−0.05	−0.27	0.25	0.24	0.29	−0.21	−0.32	0.24	0.12
q*P*	−0.67	−0.35	0.24	0.22	0.15	−0.23	−0.23	0.33	0.46
Φ_{PSII}	−0.37	−0.46	**0.72**	**0.72**	0.68	**−0.72**	**−0.76**	0.49	0.60
LMA	−0.48	−0.62	**0.85**	**0.84**	**0.82**	**−0.83**	**−0.79**	0.69	**0.83**
Leaf thickness	−0.50	−0.64	**0.95**	**0.94**	**0.92**	**−0.93**	**−0.92**	**0.72**	**0.89**
N_m	0.37	0.54	**−0.74**	**−0.74**	−0.69	**0.75**	0.70	−0.59	−0.62
N_l	−0.43	−0.45	0.65	0.65	0.63	−0.64	−0.59	0.51	0.73
C : N	−0.40	−0.59	**0.93**	**0.93**	**0.88**	**−0.93**	**−0.91**	0.67	**0.77**
Chl	0.50	0.53	−0.66	−0.66	−0.59	0.67	0.60	−0.56	−0.68

Significant correlations are in bold.
[1]Excluding genotype 'AP13'. MAP, mean annual precipitation; MSP, mean summer precipitation (May–September); MAT, mean annual temperature; MTCM, mean temperature of the coldest month; MTWM, mean temperature of the warmest month; TD, temperature differential (MTWM − MTCM); DMT, days with minimum temperature < 0°C; SHM, summer heat: moisture index (MTWM : MSP); AHM, annual heat: moisture index (MAT : MAP). Leaf traits: A_{CO2}, net photosynthetic rate; g_s, stomatal conductance to water vapour; iWUE, intrinsic water-use efficiency; PNUE, photosynthetic nitrogen-use efficiency, Φ_{PSII}, efficiency of PSII; q*P*, photochemical quenching of PSII; LMA, leaf mass area; N_l, nitrogen per unit leaf area; N_m, nitrogen per unit leaf mass; C : N, leaf carbon: nitrogen ratio; and Chl, total leaf chlorophyll content.

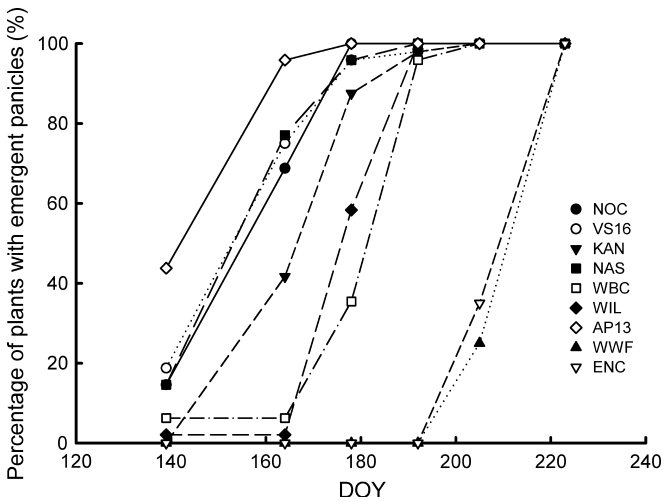

Fig. 2 Variation in panicle emergence over time among *Panicum virgatum* (switchgrass) genotypes from different climatic origins. Each symbol represents one of the genotypes identified in Table 1. DOY, day of year.

positively correlated with canopy height, leaf and tiller size, LMA, leaf thickness and C : N, and negatively correlated with N_m and Chl (Table 5; Fig. S3). PC2, which accounted for 19.9% of the total variation, was positively correlated with A_{CO2}, g_s, Φ_{PSII} and q*P* (Table 5; Fig S3).

Multiple regression analyses resulted in one climate variable, MTWM, that best explained the variation in genotype PC1 scores ($r^2 = 0.93$) (Fig. 5). Genotype PC1 scores increased as home-climate MTWM increased. Moreover, there was a positive relationship between genotype PC1 and genotype ANPP ($r^2 = 0.71$, $P < 0.01$) indicating that genotypes originating from climates with the warmest summers were the most productive and shared a common suite of functional and morphological traits. By contrast, no combination of climate variables was effective in explaining genotypic variation in PC2 scores (all $P > 0.05$), and genotype PC2 scores were not associated with genotype ANPP ($P = 0.19$).

Discussion

Our results indicate that genetic divergence in growth, morphology and function among these *P. virgatum* genotypes is strongly associated with the temperature conditions in which the genotypes originated. Moreover, a syndrome of several functionally adaptive traits with moderate to high heritabilities was correlated

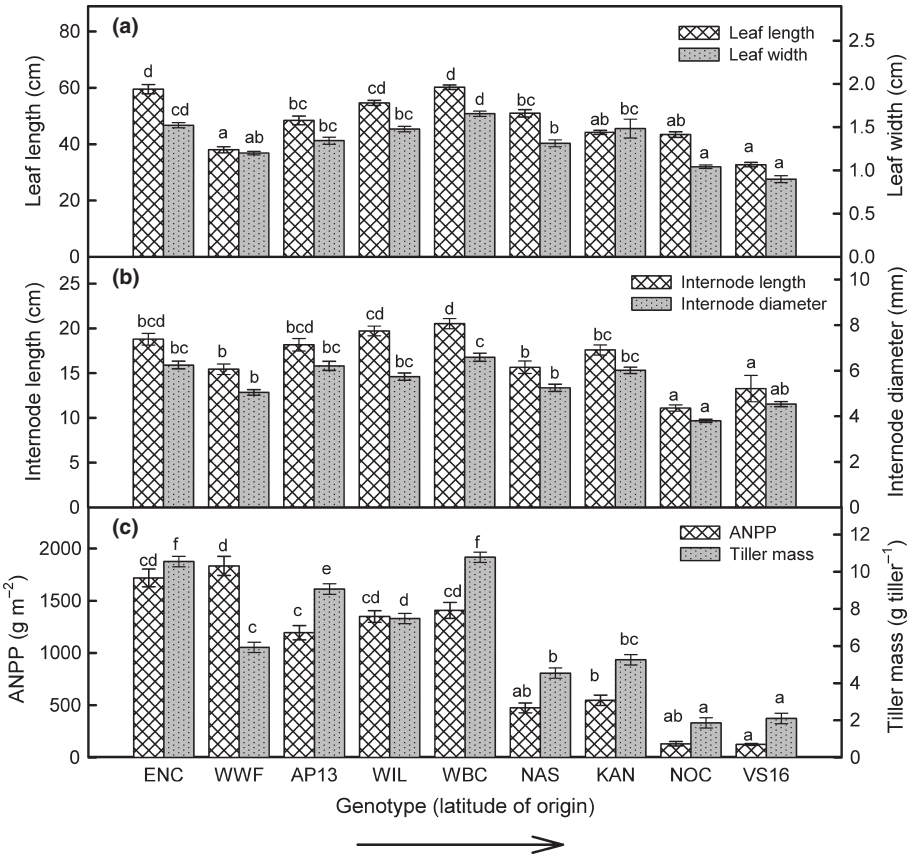

Fig. 3 Mean (± SE) aboveground net primary productivity (ANPP) and morphological trait values for *Panicum virgatum* (switchgrass) genotypes originating from different climatic origins where genotypes with the same letter are not significantly different at $P \leq 0.05$. See Table 1 for details of the genotypes.

with aboveground net primary productivity (ANPP). Overall, genotypic variation associated with climate, rather than ploidy, was the primary determinant of growth and functional strategies, and the trait syndrome we identified likely represents a continuum of traits reflecting adaptation to local climate, and would likely be important in determining the response of *P. virgatum* to climate change.

Genotypic trait variation aligns with climate

The results supported our hypothesis that variation in genotype productivity, morphology, and functional traits would be correlated with climate of origin. Consistent with previous findings, *P. virgatum* genotypes from warmer climates began growth earlier, flowered later, and were more productive than genotypes from cooler locations. Thus, genotypes originating from warmer habitats were more productive primarily due to a longer growing season, brought about by warmer temperatures (Casler *et al.*, 2007). Variation in vegetative and reproductive development in *P. virgatum* has long been associated with temperature (McMillan & Weiler, 1959; McMillan, 1965; Casler *et al.*, 2004, 2007; Berdahl *et al.*, 2005). However, warm climate genotypes originated from a climate similar to our site which may have bolstered their productivity. Nonetheless, we conclude that the observed developmental differences among genotypes were primarily related to the temperature conditions in which the genotypes originated. The observed pattern of genetic variation in growth, development and productivity may indicate that genotypes may be better described in terms of their position along a functional continuum, rather than as distinct ecotypes (Porter, 1966; Casler *et al.*, 2004).

It is possible that genotypic differences in flowering time and ANPP may be associated with photoperiod sensitivity linked to the genotype's geographic origin (Sanderson *et al.*, 1999; Casler *et al.*, 2004). Flower initiation in some C_4 grasses such as *P. virgatum* is dependent upon day length (Quinby, 1972), and moving genotypes from northerly latitudes with long summer day lengths, to southerly latitudes with shorter summer day lengths, may induce flowering, thereby reducing the vegetative growth phase and ANPP (Van Esbroeck *et al.*, 2003). We think this mechanism is unlikely to be a confounding effect because of the strong correlations between genotype growth and development and climate of origin which reinforce the idea that climate of origin is the principal determinant of these traits in *P. virgatum*. Even so, genetic variation in photoperiod sensitivity warrants further study.

As with phenology and productivity, the associations between temperature and genotype leaf and tiller morphology likely reflect adaptation to local climate. Often, genotypes adapted to arid conditions produce smaller leaves to reduce heat loads and conserve water (Cunningham *et al.*, 1999; Niinemets, 2001). However, larger leaves have greater resistance to heat and mass transfer through the leaf boundary layer, and differences in boundary layer depth may influence iWUE (Parkhurst & Loucks, 1972;

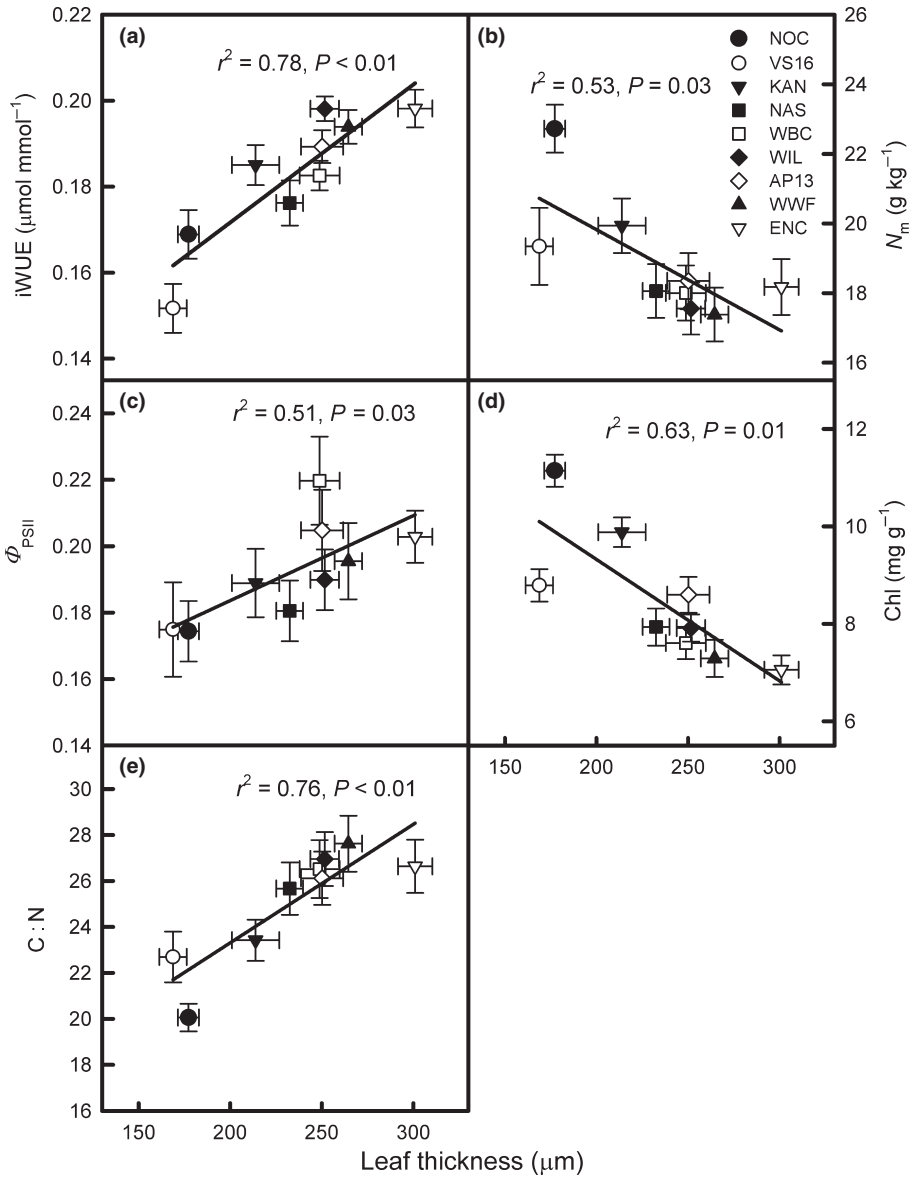

Fig. 4 Relationship between *Panicum virgatum* (switchgrass) genotype mean (± SE) leaf lamina thickness and genotype mean (± SE) (a) intrinsic water-use efficiency (iWUE), (b) nitrogen per unit leaf mass (N_m), (c) efficiency of PSII (Φ_{PSII}), (d) leaf chlorophyll (Chl), and (e) leaf carbon: nitrogen ratio (C : N).

Leigh *et al.*, 2012). In particular, larger leaf size in warm-climate genotypes may be an adaptation that optimizes iWUE (Parkhurst & Loucks, 1972). Larger leaf size may also be associated with chemical and structural modifications required for growth and function under warmer temperatures or longer growing seasons (Niinemets *et al.*, 2007). On the other hand, larger leaves require vasculature of greater size and strength, which may explain why large-leaved genotypes produced larger tillers. Overall, the observed morphological patterns are likely the outcome of trade-offs associated with temperature selection on structural and physiological traits (Parkhurst & Loucks, 1972).

Although we observed significant time × genotype interactions for many leaf traits, most likely the result of phenological differences, source-sink relationships, and environmental sensitivity (Long *et al.*, 2006), we found evidence that genotypic patterns of leaf trait variation are driven by climate, particularly temperature. Genotypes from the warmest environments showed a pattern of leaf trait values known to confer higher tissue investment costs, yet enhanced resource-use efficiency and stress tolerance (Grime, 1977; Chapin *et al.*, 1993). By contrast, genotypes from cooler climates showed a pattern of leaf trait values typical of plants with shorter growing seasons, higher physiological process rates and more rapid tissue turnover (Chapin *et al.*, 1993; Reich *et al.*, 2003). These patterns align with broader patterns observed among species (Reich *et al.*, 2003; Wright *et al.*, 2004), possibly indicating that climate may influence interspecific and intraspecific functional trait divergence in a similar way (Albert *et al.*, 2010).

One trait in particular, leaf thickness, showed consistent differentiation among genotypes (Fig. 4). Greater leaf thickness has been interpreted as an adaptation to arid conditions (Wright *et al.*, 2004; Poorter *et al.*, 2009). Thicker leaves may dampen peak leaf temperatures, thereby reducing negative effects on photosynthetic efficiency (Leigh *et al.*, 2012). Indeed, genotype leaf

New Phytologist (2013) 199: 966–980
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Table 4 Genotype means (± SE) and broad-sense heritability (H^2) estimates for productivity metrics, morphological traits and leaf functional traits among nine different *Panicum virgatum* (switchgrass) genotypes

Trait	NOC	VS16	KAN	NAS	WBC	WIL	AP13	WWF	ENC	H^2
Productivity										
Tillers	128 (7.3)	41 (2.5)	101 (6.2)	102 (7.6)	127 (5.3)	181 (6.7)	129 (5.2)	308 (14.7)	158 (6.1)	0.64
Basal area (cm^2)	475.5 (20.0)	512.7 (43.9)	399.6 (27.2)	1012.0 (66.0)	672.5 (31.1)	513.1 (24.5)	428.6 (21.3)	1153.7 (43.8)	903.8 (34.1)	0.52
Height (cm)	102.8 (2.0)	89.5 (1.7)	156.2 (3.4)	117.9 (3.5)	165.7 (1.9)	172.0 (1.9)	160.3 (1.8)	181.5 (2.2)	213.7 (2.7)	0.85
ANPP ($g\ m^{-2}$)	130.3 (20.8)	124.8 (8.3)	546.6 (49.9)	474.4 (47.2)	1408.0 (76.8)	1349.7 (55.4)	1194.9 (68.3)	1833.4 (91.7)	1719.4 (87.5)	0.66
Tiller mass (g per tiller)	1.86 (0.11)	2.10 (0.12)	5.26 (0.24)	4.54 (0.36)	10.78 (0.24)	7.48 (0.21)	9.07 (0.26)	5.93 (0.15)	10.55 (0.21)	0.81
Mid-season LAI ($m^2\ m^{-2}$)	1.48 (0.08)	0.65 (0.05)	1.20 (0.08)	1.38 (0.12)	2.02 (0.13)	2.32 (0.11)	1.69 (0.08)	2.07 (0.11)	2.37 (0.12)	0.38
Morphology[1]										
Leaf length (cm)	43.3 (1.6)	33.1 (1.3)	43.9 (1.0)	50.1 (1.4)	61.2 (2.6)	54.6 (1.5)	49.2 (2.6)	38.1 (1.6)	59.9 (2.7)	0.74
Leaf width (mm)	10.1 (0.4)	8.8 (0.5)	13.8 (0.4)	12.9 (0.9)	16.8 (0.5)	14.5 (0.5)	13.3 (0.7)	12.0 (0.4)	15.5 (0.4)	0.72
Internode length (cm)	11.0 (0.4)	11.4 (0.9)	17.6 (1.0)	15.7 (1.3)	21.1 (0.9)	20.4 (0.8)	18.4 (1.3)	16.0 (0.9)	18.9 (0.9)	0.63
Internode diameter (mm)	3.8 (0.1)	4.6 (0.2)	6.0 (0.2)	5.3 (0.3)	6.5 (0.3)	5.6 (0.2)	6.0 (0.3)	5.0 (0.2)	6.1 (0.3)	0.56
Leaf traits[1]										
A_{CO2} ($\mu mol\ m^{-2}\ s^{-1}$)	28.3 (2.1)	25.9 (2.5)	30.4 (1.9)	26.3 (1.5)	37.5 (2.8)	26.2 (2.6)	36.3 (1.8)	30.5 (1.6)	31.5 (0.8)	0.30
g_s ($mmol\ m^{-2}\ s^{-1}$)	203.7 (20.4)	203.4 (15.4)	167.2 (14.5)	173.0 (18.0)	255.1 (27.8)	138.3 (15.2)	205.7 (20.1)	162.4 (10.5)	205.1 (16.2)	0.25
iWUE ($\mu mol\ mmol^{-1}$)	0.143 (0.01)	0.131 (0.01)	0.186 (0.01)	0.163 (0.02)	0.156 (0.01)	0.191 (0.01)	0.181 (0.01)	0.189 (0.01)	0.161 (0.01)	0.27
PNUE ($\mu mol\ mol^{-1}\ s^{-1}$)	206.7 (16.1)	247.9 (22.8)	284.6 (25.0)	214.1 (21.3)	357.7 (34.6)	228.7 (18.8)	326.1 (25.4)	266.0 (12.3)	259.2 (15.5)	0.34
Φ_{PSII}	0.215 (0.02)	0.211 (0.02)	0.258 (0.01)	0.218 (0.01)	0.299 (0.02)	0.232 (0.02)	0.299 (0.01)	0.261 (0.01)	0.247 (0.01)	0.40
q*P*	0.65 (0.04)	0.62 (0.05)	0.76 (0.02)	0.62 (0.02)	0.78 (0.04)	0.68 (0.03)	0.80 (0.02)	0.74 (0.02)	0.68 (0.03)	0.32
LMA ($g\ m^{-2}$)	78.1 (1.3)	69.0 (3.0)	72.1 (3.0)	97.5 (10.3)	83.4 (3.5)	98.1 (3.5)	84.3 (1.3)	88.7 (1.7)	99.1 (4.8)	0.39
Leaf thickness (μm)	192.8 (10.8)	174.3 (7.9)	186.9 (12.0)	235.0 (13.7)	260.0 (10.2)	251.3 (15.2)	249.1 (8.9)	252.5 (12.6)	304.4 (6.8)	0.60
N_l (mmol [N] m^{-2})	137.1 (3.1)	104.2 (6.2)	109.0 (4.6)	129.4 (12.9)	107.0 (4.3)	113.8 (5.4)	113.4 (4.8)	114.9 (4.1)	124.2 (6.8)	0.19
N_m ($g\ kg^{-1}$)	24.7 (0.8)	21.2 (1.1)	21.3 (0.9)	18.8 (0.9)	18.2 (1.0)	16.5 (1.2)	18.9 (0.8)	18.2 (0.9)	17.7 (1.0)	0.43
C : N	17.8 (0.5)	20.3 (0.8)	20.9 (1.1)	23.0 (1.2)	24.1 (1.5)	27.0 (2.0)	23.3 (1.0)	23.9 (1.1)	25.3 (1.5)	0.33
Chl ($mg\ g^{-1}$)	11.7 (0.4)	9.4 (0.6)	9.7 (0.8)	7.4 (0.6)	6.3 (0.5)	6.6 (0.5)	7.9 (0.4)	6.3 (1.1)	7.1 (0.4)	0.52

[1]Means and heritability estimates are for measurements taken during July only. Leaf traits: A_{CO2}, net photosynthetic rate; g_s, stomatal conductance to water vapour; iWUE, intrinsic water-use efficiency; PNUE, photosynthetic nitrogen-use efficiency, Φ_{PSII}, efficiency of PSII; q*P*, photochemical quenching of PSII; LMA, leaf mass area; N_l, nitrogen per unit leaf area; N_m, nitrogen per unit leaf mass; C : N, leaf carbon: nitrogen ratio; and Chl, total leaf chlorophyll content.

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Table 5 Principal component factor loadings for productivity metrics, morphological traits and leaf traits in *Panicum virgatum* (switchgrass)

	PC 1	PC 2
Eigenvalue	5.71	3.38
% variance explained	33.6	19.9
Productivity and morphology		
Height	**0.78**	0.22
Tillers	0.13	0.15
Basal area	0.17	−0.10
LAI	0.48	0.20
Leaf length	**0.68**	0.12
Leaf width	**0.68**	0.38
Internode length	**0.69**	0.21
Internode diameter	**0.60**	0.33
Leaf traits		
A_{CO2}	0.06	**0.89**
g_s	−0.17	**0.73**
Φ_{PSII}	0.18	**0.90**
q*P*	0.07	**0.81**
LMA	**0.62**	−0.40
Leaf thickness	**0.77**	0.04
N_m	**−0.83**	0.22
C : N	**0.80**	−0.25
Chl	**−0.75**	−0.05

Only data collected during July were used in PCA. Values in bold are those with eigen scores > 50. Leaf traits: A_{CO2}, net photosynthetic rate; g_s, stomatal conductance to water vapour; iWUE, intrinsic water-use efficiency; PNUE, photosynthetic nitrogen-use efficiency, Φ_{PSII}, efficiency of PSII; q*P*, photochemical quenching of PSII; LMA, leaf mass area; N_l, nitrogen per unit leaf area; N_m, nitrogen per unit leaf mass; C : N, leaf carbon: nitrogen ratio; and Chl, total leaf chlorophyll content.

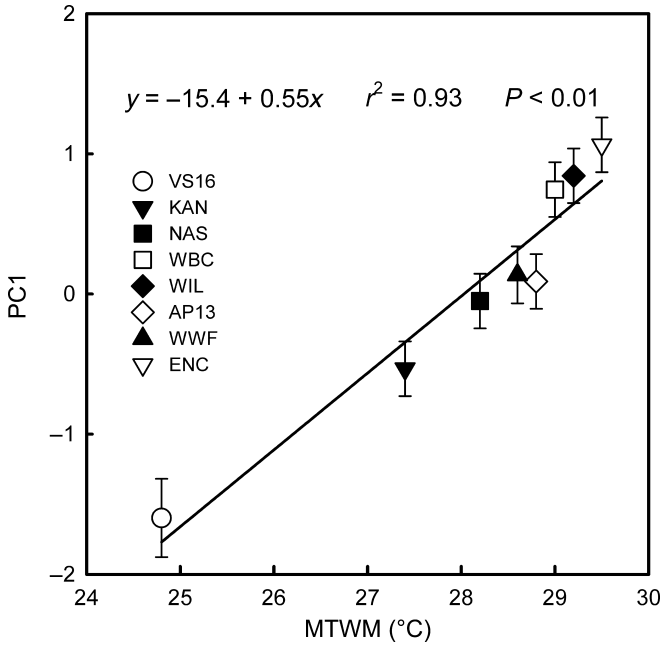

Fig. 5 Relationships between *Panicum virgatum* (switchgrass) genotype mean PC1 scores (± SE) and genotype's home-climate mean temperature of the warmest month (MTWM). Each symbol represents one of the genotypes identified in Table 1.

thickness, iWUE and Φ_{PSII} all increased with home climate temperature, suggesting that genotypes originating from habitats with warmer and longer growing seasons possess multiple traits associated with photosynthetic resource-use efficiency (Geber & Dawson, 1990, 1997; Donovan *et al.*, 2007). In comparison, Hartman *et al.* (2012) found no differences in A_{CO2} per unit transpiration among three latitudinally separated *P. virgatum* populations, yet significantly higher photochemical efficiency in southern populations.

The negative association between N_m and temperature, a common trend found within and among species, is thought to be driven by temperature-related effects on plant stoichiometry, litter decomposition and N mineralization (Oleksyn *et al.*, 1998; Reich & Oleksyn, 2004). Higher N_m in genotypes from cooler climates is considered an adaptation that allows for continued metabolic activity and growth under low temperatures (Reich & Oleksyn, 2004). The negative relationship between leaf thickness and N_m is consistent with the global leaf economics spectrum (Reich *et al.*, 2003; Wright *et al.*, 2004) which predicts that thicker, high LMA leaves may require more upfront resource investment, but are longer-lived and use resources more efficiently (Kikuzawa, 1995). This prediction matches the data for warm-climate genotypes that showed early growth initiation, late panicle emergence, thicker leaves, high LMA and high iWUE.

While climate of origin had a clear effect on genotype ANPP, morphology and leaf traits, ploidy effects were less consistent. Octoploids did show higher N_l which may reflect higher activities of N-rich photosynthetic enzymes (Warner *et al.*, 1987). Yet, higher N_l in octoploids did not translate into higher photosynthetic rates, and tetraploids and octoploids showed significant variation in leaf trait values over time. Although the reason for higher N_l in octoploids is unclear, high N_l is sometimes found in plants from dry habitats and may be associated with higher iWUE (Wright *et al.*, 2001), or an adaptation allowing for maximum light utilization (Cunningham *et al.*, 1999; Niinemets, 2001). In agreement with previous results in *P. virgatum*, productivity and physiological differences among these genotypes are most strongly linked to their climate of origin, rather than their ploidy (Nielsen, 1947; Wullschleger *et al.*, 1996; Casler *et al.*, 2004, 2007; O'Keefe *et al.*, 2013).

Important functional traits co-varied and were highly heritable

The PCA indicated that LMA, C : N, N_m and Chl co-varied (PC1) (Table 5; Fig. S3), and although iWUE was not included in the PCA, iWUE was correlated with the suite of leaf traits in PC1 (Fig. 4). Similar relationships between leaf thickness, LMA, iWUE, and leaf C and N to those we observed have been found for other C_4 plants (Brown & Byrd, 1997; Arntz *et al.*, 2000) as well as for more restricted studies of *P. virgatum* (Byrd & May, 2000). High LMA leaves tend to have lower concentrations of N-based proteins but higher concentrations of C-rich components such as lignin and phenolics (Poorter *et al.*, 2009), which may explain why genotype leaf thickness was positively correlated with leaf C : N ratio. Interestingly, the PCA revealed that leaf and

tiller morphology fall along the same axis as these key leaf traits, indicating a potential convergence of traits across levels of plant anatomical organization. These trait correlations could be the result of pleiotropic effects of individual genetic loci on multiple traits (McKay *et al.*, 2003; Remington & Purugganan, 2003). Alternatively, population structuring of alleles at different loci could each individually contribute to a component of the trait syndrome.

This entire suite of traits was individually and collectively (in PC1) strongly correlated with aspects of the genotype's home temperature conditions, most strongly the mean temperature of the warmest month (MTWM). The same trait syndrome was also associated with ANPP. Furthermore, the moderate to high H^2 of traits in PC1 provided evidence that home-climate temperature is the major selective factor driving genetic divergence of coordinated traits in *P. virgatum*. Importantly, these H^2 estimates may indicate that when combined, coordinated sets of physiological and morphological traits may show significant responses to future climate thereby influencing the nature of any adaptive responses in *P. virgatum*. Although genotypic expression may vary across environments (Campbell & Sorensen, 1978; Weinig *et al.*, 2003), our intensive study under common garden conditions provides a strong basis for understanding how climate may drive physiological divergence and local adaptation within species.

In broader terms, our study builds on classic genecology work (Turesson, 1922; Clausen *et al.*, 1940; Campbell, 1979; Rehfeldt *et al.*, 1999) and provides a more in-depth physiologically based understanding of genetic divergence within species. Recent genecology studies have also demonstrated broad associations between coordinated sets of traits and climate (Johnson *et al.*, 2010; Grady *et al.*, 2013). With climate change altering species phenology and distributions (Cleland *et al.*, 2006; Parmesan, 2006) and threatening species persistence (Jump & Peñuelas, 2005; Peñuelas *et al.*, 2013), studies such as ours may be important for predicting species range shifts and defining seed transfer zones based on physiological associations with climate (O'Neill & Aitken, 2004; Potter & Hargrove, 2012).

Importantly, our results support the concept of an adaptive trait syndrome reflecting genotypic functional strategies determined by climatic origin (Chapin *et al.*, 1993), where longer growing seasons necessitate conservation of resources, which reduces tissue turnover rates, reduces the demand for additional resources and increases stress resistance (Grime, 1977; Chapin *et al.*, 1993; Reich *et al.*, 2003). With extended periods of high temperature and net declines in soil moisture expected in many regions (IPCC, 2007; Karl *et al.*, 2009), functional traits associated with resource-use efficiency and stress tolerance may be key determinants of species growth and survival under novel conditions, as well as the sustainability of managed ecosystems (King *et al.*, 2013).

On the whole, our results provide a basic understanding of the role of climate in determining functional trait coordination and genetic differentiation. We propose that variation in growing season length (i.e. phenology) among genotypes, which is principally determined by the genotypes' home-climate temperature conditions, is the primary determinant of *P. virgatum* ANPP. Functional traits associated with ANPP are not drivers of productivity *per se*, rather they are traits having developed to match the requirements of a longer or shorter growing season. The syndrome we define is likely to be a key determinant of *P. virgatum* response to environmental change because of its high degree of genetic differentiation and the importance of its composite traits in resource uptake, use and turnover. These results provide new insight into the way in which climate drives functional trait coordination, physiological evolution and local adaptation. Anthropogenic climate change has the potential to alter linkages between environmental cues such as day length, temperature and growing conditions (e.g. frequency or occurrence of drought). These changes represent a clear challenge to the evolution of such functional trait syndromes.

Acknowledgements

We thank T. Quedensley, K. Tiner, A. Naranjo, A. Gibson, L. Crosby, C. Steele, K. Baker, N. Johnson, and Y. Sorokin for technical support, and several anonymous reviewers for their helpful comments. M.A. thanks M. Tjoelker and P. Reich for support during the writing of this manuscript. USDA is an equal opportunity provider and employer. This material is based upon work supported by the National Science Foundation (NSF) (IOS-0922457). Additional funding for D.B.L. was provided by USDA NIFA postdoctoral fellowship (2011-67012-30696). P.A.F. acknowledges support from USDA-NIFA (2010-65615-20632).

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Seasonal variation in physiological traits among *P. virgatum* genotypes from different climatic origins.

Fig. S2 Seasonal variation in leaf functional traits among different *P. virgatum* genotypes from different climatic origins.

Fig. S3 Results from principal components analysis (PCA) of phenotypic traits in *P. virgatum*.

Table S1 Mean leaf lamina thickness of *P. virgatum* genotypes originating from different climatic origins

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