Climate and stomatal traits drive covariation in nighttime stomatal conductance and daytime gas exchange rates in a widespread C_4 grass

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Summary

- Nighttime stomatal conductance (g_{sn}) varies among plant functional types and species, but factors shaping the evolution of g_{sn} remain unclear. Examinations of intraspecific variation in g_{sn} as a function of climate and co-varying leaf traits may provide new insight into the evolution of g_{sn} and its adaptive significance.
- We grew 11 genotypes of *Panicum virgatum* (switchgrass) representing differing home-climates in a common garden experiment and measured nighttime and daytime leaf gas exchange, as well as stomatal density (SD) and size during early-, mid-, and late-summer. We used piecewise structural equation modelling to determine direct and indirect relationships between home-climate, gas exchange, and stomatal traits.
- We found no direct relationship between home-climate and g_{sn}. However, genotypes from hotter climates possessed higher SD, which resulted in higher g_{sn}. Across genotypes, higher g_{sn} was associated with higher daytime stomatal conductance and net photosynthesis.
- Our results indicate that higher g_{sn} may arise in genotypes from hotter climates via increased SD. High SD may provide benefits to genotypes from hotter climates through enhanced daytime transpirational cooling or by permitting maximal gas exchange when conditions are suitable. These results highlight the role of climate and trait coordination in shaping genetic differentiation in g_{sn}.

Introduction

An increasing number of studies indicate that many plants maintain nontrivial rates of nighttime stomatal conductance to water vapor (g_{sn}) without the benefit of carbon (C) fixation (Caird *et al.*, 2007; Ogle *et al.*, 2012; Resco de Dios *et al.*, 2019). Variation in g_{sn} among plant functional types and species is considerable (Yu *et al.*, 2019). Several hypotheses have been proposed for why nighttime water loss occurs in plants, and why species vary in g_{sn}, yet the factors or mechanisms that contribute to variation in g_{sn} remain uncertain (Sadok, 2016). New studies investigating intraspecific variation in g_{sn} in relation to climate and morpho-physiological traits may elucidate the adaptive significance of g_{sn} (Arntz & Delph, 2001; Albert *et al.*, 2010; Resco de Dios *et al.*, 2019).

Nighttime water loss contradicts optimal stomatal theories which propose that plants should close stomata at night to avoid water losses in the absence of C fixation (Cowan & Farquhar, 1977). Based on this theory, avoidance of g_{sn} should be essential in arid environments, where water is the primary limitation on productivity. Yet, evidence suggests g_{sn} occurs in many species from arid habitats (Ogle *et al.*, 2012; Zeppel *et al.*, 2012), and the proportion of water lost at night relative to day (g_{sn}: g_s) may be higher in plants from water-limited environments compared to those from mesic environments (Yu *et al.*, 2019). A potential explanation of this observation is that maintaining water movement through the soil–plant–atmosphere continuum at night might maintain a hydraulic gradient in the soil near the roots, resulting in reduced soil moisture for neighboring species (Neumann *et al.*, 2014; Huang *et al.*, 2017; Yu *et al.*, 2018). Alternative explanations for high g_{sn} include potential improvement of nutrient acquisition via bulk flow; however, evidence supporting this is equivocal (Howard & Donovan, 2007; Christman *et al.*, 2009a; Kupper *et al.*, 2012; Hoshika *et al.*, 2019). Similarly, it has been proposed that bulk flow may redistribute nutrients to distal plant parts (Scholz *et al.*, 2007; Rohula *et al.*, 2014) or relieve excess leaf turgor in species that accumulate solutes/ions in arid or saline environments (Donovan *et al.*, 2001). While few

studies have tested these explanations, a recent review examining 176 species world-wide found minimal evidence to support them (Resco de Dios *et al.*, 2019).

Aligning with the hydraulic-based hypotheses, Yu *et al.* (2019) found higher maximum g_{sn} (measured at predawn) in species from arid environments compared to species from more mesic environments. Resco de Dios *et al.* (2019) found that species from sites with higher mean annual temperatures tend to lose more water at night relative to daytime (i.e. higher $g_{sn} : g_s$), while g_{sn} and mean annual precipitation were not associated across species; however, the inclusion of sclerophyllous species, which exhibit low g_{sn} because of their anatomy, likely affected the precipitation–g_{sn} relationship. This highlights a potential limitation of using across-species patterns of leaf trait variation to explore questions about evolutionary adaptation. Instead, common garden experiments which explore intraspecific variation in g_{sn} in relation to home-climate and covarying leaf traits could provide new insight into the functional or evolutionary role of g_{sn} (Rehfeldt *et al.*, 2002; Geber & Griffen, 2003; Etterson, 2004; Voltas *et al.*, 2008).

High predawn levels of g_{sn} are thought to prime stomata for photosynthesis (*A*) in the early daylight, before temperatures rise and evaporative demand increases (referred to as the 'priming hypothesis' or 'anticipation hypothesis') (Mansfield & Heath, 1961; Tobiessen, 1982; Resco de Dios *et al.*, 2016, 2019). Few studies have examined intraspecific variation in nighttime water loss; however, these studies have observed considerable variation in g_{sn} among genotypes of Arabidopsis (C_4 forb), *Distichlis spicata* (C_4 grass), and *Eucalyptus camaldulensis* (C_3 tree) (Christman *et al.*, 2008, 2009b; Resco de Dios *et al.*, 2016, respectively). Thus, while the early hypothesis that rates of water loss at night were the result of 'leaky' stomata (Barbour *et al.*, 2005), recent evidence of intraspecific variation of g_{sn} and interspecific differences across climatic gradients suggests it may be an adaptive trait.

Aside from prevailing daytime conditions (e.g. vapor pressure deficit, temperature, light), daytime rates of stomatal conductance (g_s) are influenced by stomatal anatomy (e.g. density and size), guard cell properties (shape, elasticity), and regulation of ion channels between guard cells and adjacent epidermal cells (Lawson & Vialet-Chabrand, 2019). Stomatal density has been proposed as a potential contributor to observed rates of g_{sn}, with abaxial stomata observed to remain open at night while adaxial stomata close in some species (Sharpe, 1973; Aben *et al.*, 1989; Caird *et al.*, 2007). Stomatal density has also been shown to co-vary with environmental gradients and is known to increase with growing season temperature and aridity (Gindel, 1969; Quarrie & Jones, 1977; Yang *et al.*, 2014); however, these stomata–climate relationships are not consistent across experiments (Clay & Quinn, 1978; Skelton *et al.*, 2012; Murphy *et al.*, 2014).

Across a wide range of species, a trade-off exists between stomatal density and size, where species that form leaves with higher stomatal density tend to have smaller stomata (Franks & Farquhar, 2001; Franks & Beerling, 2009; Franks *et al.*, 2009). Smaller stomata should require smaller changes in solute concentrations to drive stomatal movement than larger stomata, and

thus can adjust aperture more quickly (Raven, 2014), which allows species with a high density of small stomata to achieve higher rates of g_s compared to species with fewer, larger stomata (Hetherington & Woodward, 2003). Resco de Dios *et al.* (2016) found that *E. camaldulensis* genotypes which possessed stomata with a rapid response to light (i.e. 'fast' stomata) had greater predawn g_{sn}. This suggests that genotypes which possess a high density of small stomata will exhibit greater rates of g_{sn} compared to those with fewer, larger stomata, which has been observed within genera (Drake *et al.*, 2013) and across species (Kardiman & Ræbild, 2018). Alternatively, maintaining even minimal stomatal opening when possessing a high density of large stomata should increase g_s, regardless of the time of day (Nobel, 1999).

Panicum virgatum (switchgrass) is an ideal species for exploring factors shaping genetic differentiation in g_{sn}. This perennial C_4 rhizomatous grass species is a key component of the tallgrass prairies of North America, with a geographic range extending across substantial gradients of temperature, precipitation, photoperiod, and soil type. Its range extends over a large portion of North America, from central Mexico to southern Canada (Zhang *et al.* 2011a,b; Triplett *et al.*, 2012). The species has many uses including forage, soil conservation, and bioenergy production (Parrish & Fike, 2005; Schmer *et al.*, 2008). C_4 grasses, including *P. virgatum*, have generally shown higher rates of g_{sn} compared to trees and forbs (O'Keefe & Nippert, 2018; Yu *et al.*, 2019). Importantly, *P. virgatum* genotypes from different habitats and climates often show considerable differences in phenology, productivity, and leaf and stem morphology (McMillan, 1965; Casler *et al.*, 2004; Lowry *et al.*, 2014). There is also evidence that climatic adaptation results in covariation of leaf-scale physiology and morphological traits across genotypes of *P. virgatum* (Aspinwall *et al.*, 2013).

To understand how climate drives genetic differentiation in g_{sn} and covarying leaf traits, we sourced 11 genotypes of *P. virgatum* from individual sites in Texas/Mexico ($n = 6$ genotypes) and Florida ($n = 5$ genotypes) varying in average precipitation and temperature and grew them in a common garden experiment. We measured daytime and nighttime gas exchange parameters and stomatal traits during early-, mid-, and late-summer and addressed three questions: (1) Is genotypic variation an important source of variation in g_{sn} and $g_{sn} : g_s$ compared to other gas exchange and stomatal traits in *P. virgatum*? (2) Is genotypic variation in g_{sn} and $g_{sn} : g_s$ associated with average climate conditions at the genotype geographic origin? And (3) to what extent do nighttime and daytime gas exchange parameters co-vary with stomatal traits across genotypes of *P. virgatum*? For question 1, assuming genotypes are locally adapted to their source habitat and nighttime and daytime gas exchange and stomatal traits contribute equally to adaptation, we expect that genotypic variation is an equally important source of variation in g_{sn}, $g_{sn} : g_s$, and all traits. For question 2, we hypothesized that g_{sn} and $g_{sn} : g_s$ would increase with decreasing average precipitation at the genotype climate of origin. While climate–g_{sn} relationships have not been explored within species, there is some evidence that species from drier and warmer environments exhibit higher g_{sn} and $g_{sn} : g_s$ ratios than species from wetter and cooler environments (Yu

et al., 2019; Resco de Dios et al., 2019). For question 3, we hypothesized that g_{sn} and daytime gas exchange would covary across genotypes due to genotypic variation in stomatal density. Possessing leaves with higher stomatal density would mean any degree of stomatal opening would result in higher rates of stomatal conductance regardless of time of day (Nobel, 1999; Hetherington & Woodward, 2003; Franks & Beerling, 2009). Given evidence of higher rates of g_{sn} in plants from warmer, drier climates, we anticipate that stomatal traits will mediate the relationship between home-climate and genotypic variation in nighttime and daytime leaf gas exchange.

Materials and Methods

Plant material and growth conditions

This study included 11 different *Panicum virgatum* L. 'genotypes' from two general regions: Florida (FL) and Texas (TX), USA (with an additional genotype from Coahuila, Mexico (MX)). Genotypes originated from unique geographic and climatic origins (Table 1, Supporting Information Table S1 for weather station details). Across all genotypes, growing season precipitation (GSP; March through August) at the genotype's geographic origin varied substantially, ranging from 401 to 1147 mm and was higher among FL genotypes (mean = 942 mm) than TX/ MX genotypes (mean = 484 mm). Rainfall in the TX/MX region is primarily in May and September, with dry winter and summer periods (Modala et al., 2017). In Florida, the rainy season occurs in the summer months (May–August), while the remainder of the year is relatively dry (Ewel & Myers, 1990). Across all genotypes the mean growing season temperature maximum (GST_{max}) and mean annual maximum temperature (T_{max}) varied by 4.5 and 3.5°C, respectively. While the temperature variation here is lower than in studies that examine interspecific trends, others have found that home temperature conditions largely explain morphological and physiological differences among *P. virgatum* genotypes (Aspinwall et al., 2013; Lowry et al., 2014). Mean growing season maximum vapor pressure deficit (VPD) varied by 1.90 kPa (Table 1). Overall, there were numerous significant relationships (collinearity) between home-climate parameters, including: mean annual precipitation, growing season (March–August), annual aridity, potential evapotranspiration yr^{-1}, the ratio of evaporation to precipitation, growing season vapor pressure deficit, mean annual temperature, mean annual maximum temperature, and mean growing season maximum temperature (Fig. S1).

All genotypes were propagated from wild collections and none were domesticated cultivars. Ploidy of each genotype was determined as in Aspinwall et al. (2013). Nine genotypes were tetraploid (4×) and two were octoploid (8×). *Panicum virgatum* genotypes are often described as upland or lowland ecotypes. Lowland ecotypes are often found in riparian habitats throughout the southern portion of the species range and are tall with thick tillers and wide leaves. Upland ecotypes typically occur on drier sites and are more common throughout the central and northern portions of the species range. Upland ecotypes possess a distinctive morphology; they are short with thin tillers and leaves (Lowry et al., 2014; Casler et al., 2015). Our genotypes were morphologically variable, but none were distinctively upland. Thus, we considered all genotypes to be lowland ecotypes. We also found minimal evidence to suggest regional (TX vs FL) or ploidy-type differences in morphological and physiological traits (details on these measurements are discussed in the 'Leaf physiology' and 'Stomatal measurement' sections of the methods) (Tables S2–S4). We use the term 'genotype' to signify that these individuals originated from vegetative propagation of a single plant and are representatives of the local gene pool (population) at each location. The use of these genotypes allows us to focus on broader patterns of intraspecific variation in relation to climate, at the expense of exploring within-population variation.

Clonal replicates of each genotype were propagated via division and multiplication of rhizomes originating from a single plant collected in spring 2018. Entire plants (aboveground and belowground components) of each genotype were dug from the field and transported to the University of Texas at Austin. Rhizomes of these plants were subsequently divided and repotted into 3.8 l pots. Three to four ramets of each genotype were created from the initial collection. Ramets were transported to the University of North Florida (UNF) campus (Jacksonville, FL, USA) in November 2018. In February 2019, rhizomes of each genotype underwent another round of division to increase the number of replicates of each genotype (Table 1). Rhizome clusters were of similar size and were transplanted into 18.9 l plastic pots filled with a media composed of c. 50% sand and c. 50% garden soil (organic matter) mixed with slow-release fertilizer (Sta-Green Flower and Vegetable Soil, Rowlett, TX, USA). Replicates of each genotype were grown in an outdoor location on the UNF campus and were watered every 1–3 d before and during the experiment to ensure minimal water limitation.

Leaf physiology

Nighttime and daytime measurements of leaf gas exchange were conducted on all replicates of all genotypes at three sampling points (mid-June, mid-July, mid-August) during summer 2019 using three cross-calibrated portable infrared gas-analyzers (IRGAs); one LI-6400XT and two LI-6800 systems (LI-6800 or LI-6400XT; Li-Cor Inc., Lincoln, NE, USA). The LI-6400XT was fitted with a 2×3 cm^2 cuvette head while the LI-6800 was fitted with a 3×3 cm^2 cuvette head; both cuvettes were fitted with a red and blue LED light source. At each time point, measurements were made on recently mature, fully expanded, upper canopy leaves and were randomized across IRGAs and replicates of each genotype.

Leaves selected for nighttime (dark early morning) measurements were tagged and marked so that daytime measurements could be taken at a maximum of 8 h later on the same leaf sections. Nighttime measurements occurred between 03:45 and 06:00 h local time (i.e. predawn). Previous work has demonstrated circadian regulation of g_{sn}, with maximal values occurring before dawn (Caird et al., 2007; Ogle et al., 2012; Resco de Dios

Table 1 Ploidy, geographic origin, and average home-climate conditions of the 11 *Panicum virgatum* (switchgrass) genotypes included in this study, in order of longitude.

Reference genotype	Ploidy	Sample size (n)	State	County	Latitude	Longitude	GSP (mm)[1]	VPD (kPa)[2]	GST$_{max}$ (°C)[1]	T$_{max}$ (°C)[1]
1	4×	6	Florida	Martin	27°11′51.2″N	80°15′10.1″W	917	2.83	30.8	28.3
2	4×	5	Florida	Volusia	29°05′42.0″N	80°58′14.9″W	828	2.67	30.3	26.7
3	4×	6	Florida	Duval	30°26′13.0″N	81°24′47.9″W	775	2.57	29.5	25.7
4	4×	6	Florida	Hernando	28°34′12.0″N	82°22′48.0″W	1147	2.78	31.5	27.8
5	4×	4	Florida	Levy	29°16′17.0″N	82°26′34.1″W	1045	2.87	31.9	28.7
6	4×	5	Texas	Victoria	28°48′19.0″N	97°00′13.0″W	592	2.69	32.2	26.9
7	8×	4	Texas	San Patricio	28°07′48.0″N	97°24′02.9″W	531	2.75	32.3	27.5
8	8×	6	Texas	Kenedy	26°52′11.0″N	97°41′53.5″W	401	2.92	33.9	29.2
9	4×	3	Texas	Travis	30°19′48.0″N	97°55′12.0″W	502	2.59	32.9	25.9
10	4×	6	Texas	Bexar	29°35′24.0″N	98°32′24.0″W	538	2.68	32.5	26.8
11	4×	6	Coahuila, Mexico		29°19′28.9″N	101°21′54.0″W	340	2.77	33.9	27.7
Range							807 mm	1.90 kPa	4.5°C	3.5°C

Data include mean growing season (March–August) precipitation (GSP), mean growing season maximum vapor pressure deficit (VPD), mean growing season maximum temperature (GST$_{max}$), and mean annual maximum temperature (T$_{max}$).
Climate information for genotypes was obtained from the [1]Western Regional Climate Center SOD USA Climate Archive (wrcc.dri.edu/sod/sodusaarch.html) and [2]Prism Climate Group database (https://prism.oregonstate.edu/).

et al., 2016; Tamang *et al.*, 2019). Thus, predawn measurements provide a standardized approach for comparing maximum g_{sn} among genotypes. Daytime measurements occurred between 11:00 and 14:00 h.

All measurements were made at the prevailing ambient temperature conditions, which varied across time points (Figs S2, S3). Leaf temperature (T_{leaf}) was measured with the built-in Li-Cor leaf temperature thermocouple. Relative humidity conditions in the chamber were controlled so that they remained close to the ambient external conditions, but also varied depending upon water vapor fluxes from the leaf. Thus, the leaf to atmosphere vapor pressure deficit (VPD_{leaf}) in the chamber was permitted to vary over time; however, there were minimal changes in VPD_{leaf} across time points. Predawn VPD_{leaf} was modestly higher in August (mean ± standard error, 1.1 ± 0.03 kPa) than June or July (1.0 ± 0.02 and 0.77 ± 0.01 kPa, respectively) while daytime VPD_{leaf} was similar across all time points (1.7 ± 0.02 kPa, Fig. S3). Predawn T_{leaf} was similar in June (24.8 ± 0.02°C) and July (24.4 ± 0.01°C) and modestly higher in August (26.4 ± 0.01°C). Daytime T_{leaf} was slightly higher in August (33.8 ± 0.14°C) compared to June (31.7 ± 0.19°C) and July (32.1 ± 0.15°C). Flow rate in the cuvette was held constant at 500 μmol s^{-1}. The reference CO_2 supply was controlled at 410 μmol mol^{-1}. Predawn measurements were taken in darkness (0 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD)) while all daytime measurements were made at a saturating, fixed light intensity (1800 μmol m^{-2} s^{-1} PPFD) using the Li-Cor red-blue light source. We note that prevailing light conditions under which the genotypes were grown could affect leaf gas exchange among other physiological traits; however, we did not have access to a sensor to continuously monitor light and were not able to assess the potential impacts of prevailing light conditions (Granda *et al.*, 2020). Daytime measurements included steady state measurements of light-saturated net photosynthesis (A_{sat}, μmol m^{-2} s^{-1}), daytime stomatal conductance to water

vapor (g_s, mol m^{-2} s^{-1}), and the ratio $A_{sat}:g_s$ (i.e. intrinsic water use efficiency (iWUE, μmol mol^{-1})), while at nighttime g_{sn} (mol m^{-2} s^{-1}) was measured. Marked sections of leaves were later used for determination of stomatal density and size (see the 'Stomatal measurements' section in the Methods).

One leaf from a similar canopy position was collected from each plant immediately following predawn leaf gas exchange measurements (before sunrise) and was placed in a sealed plastic bag with moist paper. Leaves were taken to the lab and predawn leaf water potential (Ψ_{pd}, MPa) was measured using a Scholander-type pressure bomb (PMS Instruments, Corvallis, OR, USA). Volumetric soil water content (VWC, %) of the growth media was monitored on each sampling day using a time domain reflectometer probe (HydroSense II; Campbell Scientific, Logan, UT, USA). During the experiment, individual Ψ_{pd} measurements ranged from −0.02 to −0.7 MPa with a mean and standard deviation of −0.2 ± −0.01 MPa, indicating that plants were well-hydrated throughout the experiment (Fig. S3e). Similarly, VWC ranged from 31.8% to 49.2% (mean and standard error of 42.8 ± 0.30%), indicating that plants did not experience water limitation (Fig. S3).

Stomatal measurements

Marked leaf sections were clipped (June and August only), quickly sealed in a plastic bag with a moist paper towel to avoid curling and transported to the laboratory. Both the abaxial and adaxial surfaces of each leaf (*c.* 3 cm long sections at the midpoint of the fully expanded leaf) were covered with topcoat nail polish and allowed to dry (Taylor *et al.*, 2012). Once dried, the nail polish was removed and mounted on a standard microscope slide using a cover slip. Images were captured at ×400 magnification using a compound microscope (Olympus BX60F5, Tokyo, Japan) mounted with a camera (Spot Imaging, 7.2 Color Mosaic; Sterling Heights, MI, USA) using SPOT IMAGING software (v.5.1).

Images were analyzed using IMAGEJ (NIH; Bethesda, MD, USA) with three images captured per slide. We included all stomata in the field of view, including those on the periphery where the image edge cut through both guard cells. Data were subsequently averaged by slide. We obtained stomatal density (number of stomata mm^{-2}), stomatal size (the guard cell length multiplied by the guard cell pair width in µm^2, following Franks & Beerling (2009)), and the ratio of stomata on the abaxial to the adaxial surface (SR). Stomatal density was calculated for both the abaxial and adaxial leaf surfaces (SD$_{ab}$ and SD$_{ad}$, respectively) as was stomatal size (SS$_{ab}$ and SS$_{ad}$, respectively).

Data analyses

Analysis of variance was used to test the effects of sampling point (i.e. date), genotype, and date × genotype effects on nighttime and daytime gas exchange parameters and stomatal traits. All tests of statistical significance were conducted using RSTUDIO (R v.3.6.1, RSTUDIO v.1.2.1335; R Core Team, 2013) at α = 0.05. Next, we determined the trait (e.g. g_{sn}, g_{sn} : g_s, g_s, A_{sat}, SS$_{ab}$, SS$_{ad}$, SD$_{ab}$, SS$_{ad}$, SR) variance attributable to variation among sampling dates ('Date'; June, July, August), genotype ('Genotype'), and their interactive effects, as well as the variance not explained by these factors ('Residual') using LMER (Bates, 2010), where each parameter was treated as a random effect (i.e. no fixed effects, Aspinwall *et al.*, 2013). For each trait, variance attributed to date, genotype, their interaction, and the unexplained/residual portion was calculated by dividing individual variances by the sum of all trait variance. Data were log or square root transformed to improve homogeneity of variance.

Analysis of covariance (ANCOVA) was used to determine whether genotypic differences in gas exchange and stomatal traits were associated with home-climate at the genotype's geographic origin. Growing season precipitation, VPD, GST$_{max}$, and T_{max} were used as predictors of leaf gas exchange and stomatal traits. With respect to gas exchange measurements, we measured plants at three time points and used the average value for each genotype at each time point. Using the mean trait value for each genotype allows us to test for genetic correlations (Christman *et al.*, 2008). Regressions included 'Date' as a factor to determine if genetically-based relationships between home-climate, leaf gas exchange, and stomatal traits were time-dependent. For thoroughness, we also examined genetic correlations between traits and home-climate within each sampling month. To address how traits co-varied across genotypes, we used ANCOVA analyses while also accounting for potential temporal (i.e. 'Date') effects. While others have observed that daytime gas exchange affects nighttime gas exchange (Easlon & Richards, 2009), we used nighttime measurements as the independent variable in regression analysis as they were measured before daytime measurements. Similarly, we tested whether stomatal traits were significant predictors of genotypic variation in g_{sn}, but used stomatal traits as the independent variables given their general influence over gas exchange.

Finally, we used piecewise structural equation modelling (pSEM) to synthesize the genotypic covariation among gas exchange, stomatal traits, and home-climate in order to determine their direct and indirect relationships. A series of linear mixed models are used to estimate each path in pSEM, which accommodates smaller datasets than standard SEM approaches. It also assumes independence between initial (climate) and final variables (A_{sat}), and that the relationship between initial and final variables is significantly improved using intermediate variables (in this case, stomata, g_{sn} and g_s) (Duffy *et al.*, 2016; Oliveira *et al.*, 2016; Chieppa *et al.*, 2019). Furthermore, pSEM tests the hypothesized paths (in this case, home-climate → stomatal trait → g_{sn} → g_s → A_{sat}) but ensures direct separation of all the components within the model (i.e. home-climate → g_{sn}, home-climate → g_s, stomatal traits → g_s, etc.). These assumptions were tested using a chi-square test (α = 0.05) and Fischer's C statistic (Shipley, 2000). Linear mixed model equations were undertaken using the NLME package (Pinheiro *et al.*, 2013; Lefcheck, 2016), which included 'Date' as a random effect. Gas exchange data from July were excluded from the analysis because stomatal traits were only obtained in June and August. Path coefficients were standardized from −1 to 1 to indicate a strong negative or positive relationship, respectively.

Results

Genotypic variation in leaf gas exchange and stomatal traits

Average rates of g_{sn} and g_{sn} : g_s differed among genotypes, but these differences were dependent upon sampling date (i.e. date × genotype effect; Fig. 1; Table S5). Some genotypes showed rates of g_{sn} and g_{sn} : g_s that were consistently low or high over time, while others showed large changes over time (Fig. 1). Neither predawn T_{leaf} nor predawn VPD_{leaf} were significantly related to observed rates of g_{sn} (Fig. S4a,b); however, there was a significant inverse relationship between g_{sn} : g_s and predawn T_{leaf} ($R^2 = 0.24$, Fig. S4c) and daytime T_{leaf} ($R^2 = 0.16$; Fig. S4e). Predawn and midday VPD$_{leaf}$ were not associated with g_{sn} : g_s (Fig. S4d,f) Average daytime gas exchange rates (g_s, A_{sat}, iWUE) differed among genotypes, and these differences were relatively consistent over time (no date × genotype interaction). Over time, average rates of g_s differed by 43.6% among genotypes (0.172 mol m^{-2} s^{-1} to 0.268 mol m^{-2} s^{-1}) while average rates of A_{sat} differed by 18.1% among genotypes (25.1 µmol m^{-2} s^{-1} to 30.1 µmol m^{-2} s^{-1}). Comparatively, g_{sn} differed by 111.1% among genotypes (0.008 mol m^{-2} s^{-1} to 0.028 mol m^{-2} s^{-1}).

On average, SD$_{ab}$ and SD$_{ad}$ declined by 83.4% and 86.8% between June and August, respectively. Genotypes differed in SD$_{ab}$ and SD$_{ad}$ and these differences were consistent over time (no date × genotype interaction; Fig. 2; Table S5). Over time, average SD$_{ab}$ differed by 60.2% among genotypes (75.3 to 140.2 mm^{-2}) and SD$_{ad}$ differed by 56.5% among genotypes (81.4 to 145.5 mm^{-2}). Genotypes also differed in SS (both sides of the leaf), yet these differences were strongly dependent upon time (date × genotype interaction, Fig. 2a,b). Averaged across genotypes, SS declined modestly from June to August, but many genotypes increased or decreased SS over time (Fig. 2c,d). The
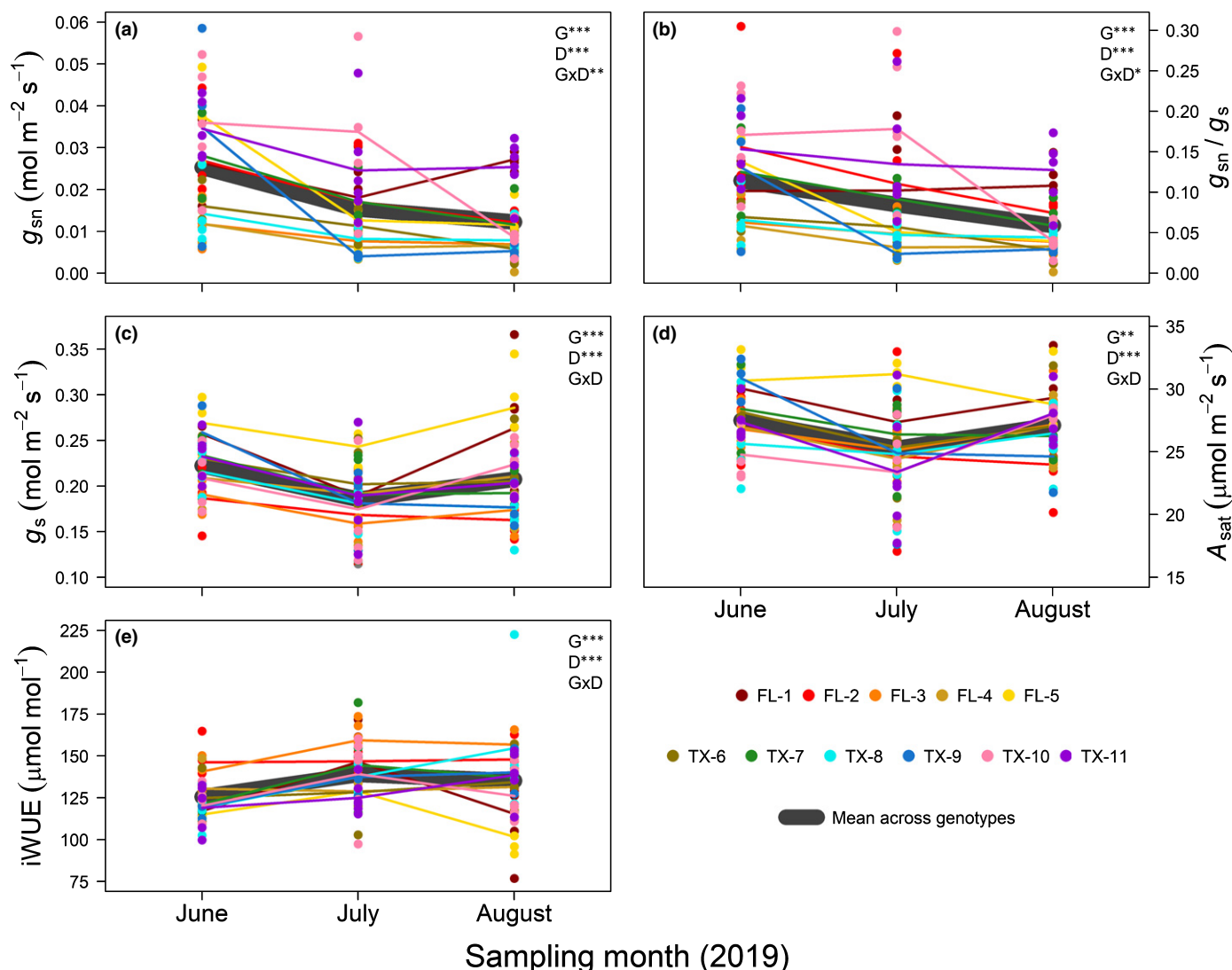
Fig. 1 Rates of nighttime (measured at predawn) stomatal conductance (g_{sn}) (a), the ratio of nighttime to daytime conductance ($g_{sn} : g_s$) (b), daytime stomatal conductance (g_s) (c), light-saturated net photosynthesis (A_{sat}) (d), and intrinsic water-use efficiency (iWUE) (e) for 11 genotypes of *Panicum virgatum* (switchgrass) measured at three time points. Colored lines represent average values for each genotype. The thick dark line is the overall mean value across genotypes. The effects of genotype (G), sampling date (D), and their interaction (G × D) on gas exchange parameters are indicated within each panel. Significance is denoted by asterisks: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Sample size for each genotype can be found in Table 1.

value of SR did not vary over time or among genotypes and averaged 1.18 ± 0.03 (Fig. 2e).

We expected that genotypic variation would account for an equally large percentage of the variation in all nighttime and daytime gas exchange traits and stomatal traits. Aside from residual variance, we found that date alone explained the most variance in g_{sn} (21.6%) and $g_{sn} : g_s$ (25.6%, Fig. S5). Genotype alone explained 14.6 and 13.7% of variance in g_{sn} and $g_{sn} : g_s$, respectively, while accounting for 10.0–29.1% of variance in daytime gas exchange parameters. In comparison, the interaction of date × genotype explained *c.* 20% of nighttime gas exchange while only accounting for 5.0–17.1% of daytime gas exchange, highlighting the importance of the date × genotype interaction in nighttime gas exchange. Genotype alone had little explanatory power for stomatal traits (range of 1.6–9.5%) while date was particularly important for SD (*c.* 50% compared to 0% and 3.9%

for SS_{ab} and SS_{ad}, respectively). Similar to g_{sn} and $g_{sn} : g_s$, the date × genotype interaction explained a significant portion of variance for SD_{ab} and SD_{ad} (28.4% and 29.0%, respectively).

Trait–climate relationships

Of the 20 relationships between home-climate (GSP, VPD, GST_{max}, T_{max}) and leaf gas exchange (g_{sn}, $g_{sn} : g_s$, g_s, A_{sat}, iWUE) we tested, we observed three significant relationships (Fig. S6; Table S6). Across genotypes and time points, average rates of g_s increased with T_{max} ($g_s = 0.01 T_{max} - 0.141$, $R^2 = 0.17$, $P = 0.017$). We also observed a decrease in iWUE across genotypes from warmer environments (iWUE $= -3.5 GST_{max} + 244.6$, $R^2 = 0.12$, $P = 0.046$; iWUE $= -5.0 T_{max} + 270.6 = R^2\ 0.16$, $P = 0.021$). However, we found no relationships between the genotypes' home-climate and g_{sn} or $g_{sn} : g_s$ (Table S6).
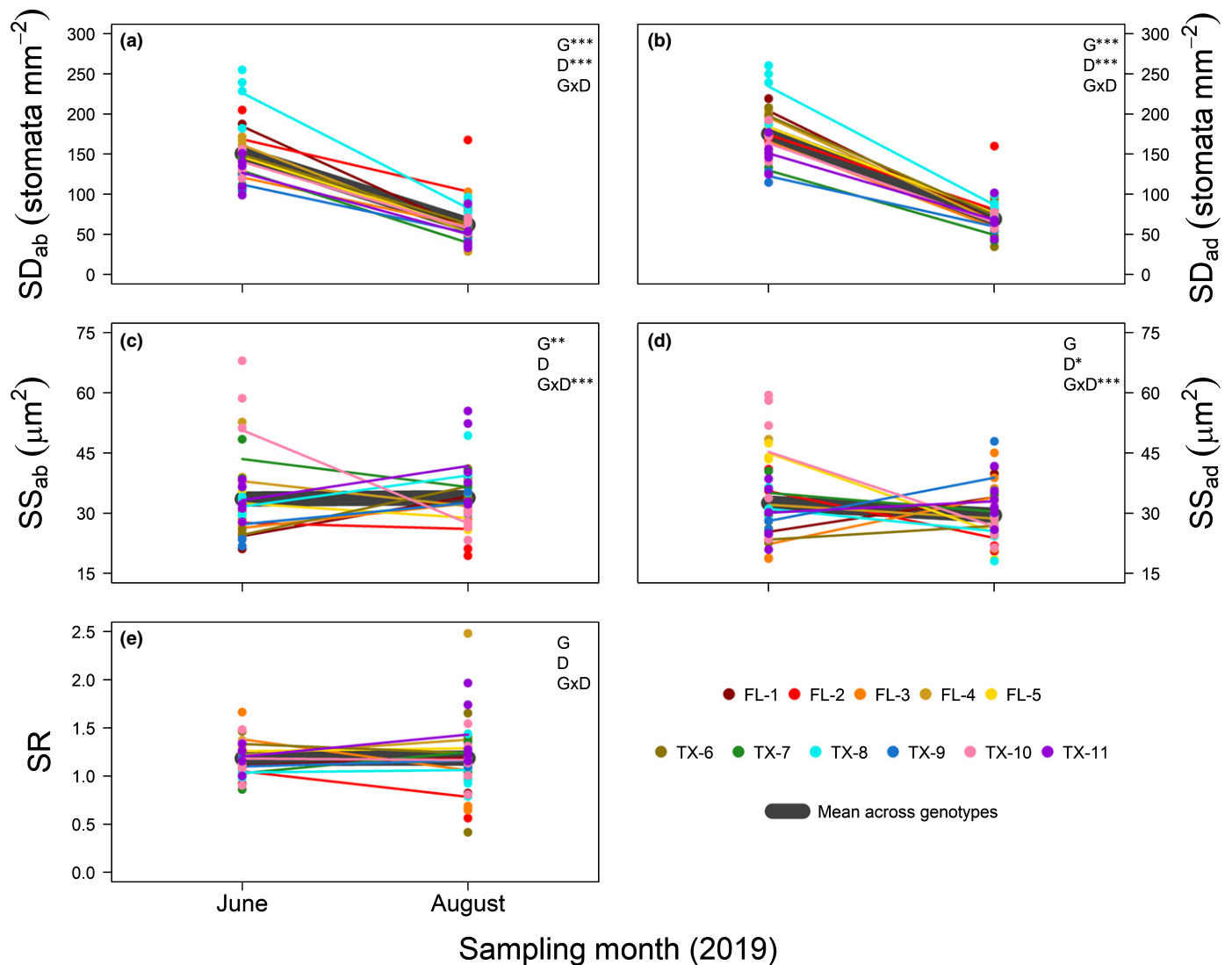
Fig. 2 Abaxial stomatal density (SD_{ab}) (a), adaxial stomatal density (SD_{ab}) (b), average abaxial stomatal size (SS_{ab}) (c), average adaxial stomatal size (SS_{ad}) (d), and the ratio of stomata on the adaxial to abaxial leaf surface (SR) (e) for 11 genotypes of switchgrass (*Panicum virgatum*), measured at two time points. Colored lines represent average values for each genotype. The thick dark line is the overall mean value across genotypes. The effects of genotype (G), sampling date (D), and their interaction (G × D) on stomatal traits are indicated within each panel. Sample size for each genotype can be found in Table 1.

Stomatal density tended to increase across genotypes from sites with greater T_{max} on both the abaxial (Fig. 3d) and adaxial leaf surface (Fig. 3h); however, the former relationship was more prevalent during the June sampling than in August (i.e. significant home-climate × date interaction, Table S7). Across genotypes, no relationships between home-climate and abaxial and adaxial SS or SR were observed (Table S7).

Relationships between nighttime gas exchange, daytime gas exchange, and stomatal traits

Across genotypes, we found significant relationships between average rates of nighttime and daytime gas exchange, which in all cases were not temporally dependent (i.e. no significant interactions with date, Table S8). Genotypes which exhibited higher rates of g_{sn} had higher rates of g_s and lower rates of iWUE ($g_s = 1.4g_{sn} + 0.2$, $R^2 = 0.16$, $P = 0.008$, iWUE $= -553.6g_{sn} + 142.3$, $R^2 = 0.16$, $P = 0.014$, Fig. 4c), although average rates of g_{sn} were not associated with average rates of A_{sat} across genotypes (Fig. 4a).

Overall, three of the five measured stomatal traits showed a significant relationship with g_{sn} (Fig. 5; Table S9). These relationships were not temporally dependent (i.e. no effect of date, Table S6). In general, greater values for SD (both sides) and SS (adaxial only) were positively correlated with greater rates of g_{sn} across genotypes (Fig. 5). We observed no significant genetic correlations between SS and SD on either leaf surface, nor across leaf surfaces ($P = 0.223$, data not shown). Particularly with SD_{ad}, we observed a shift between time points where high SD_{ad} was associated with greater g_{sn} during June, with a concomitant shift down in August (Fig. 5b).
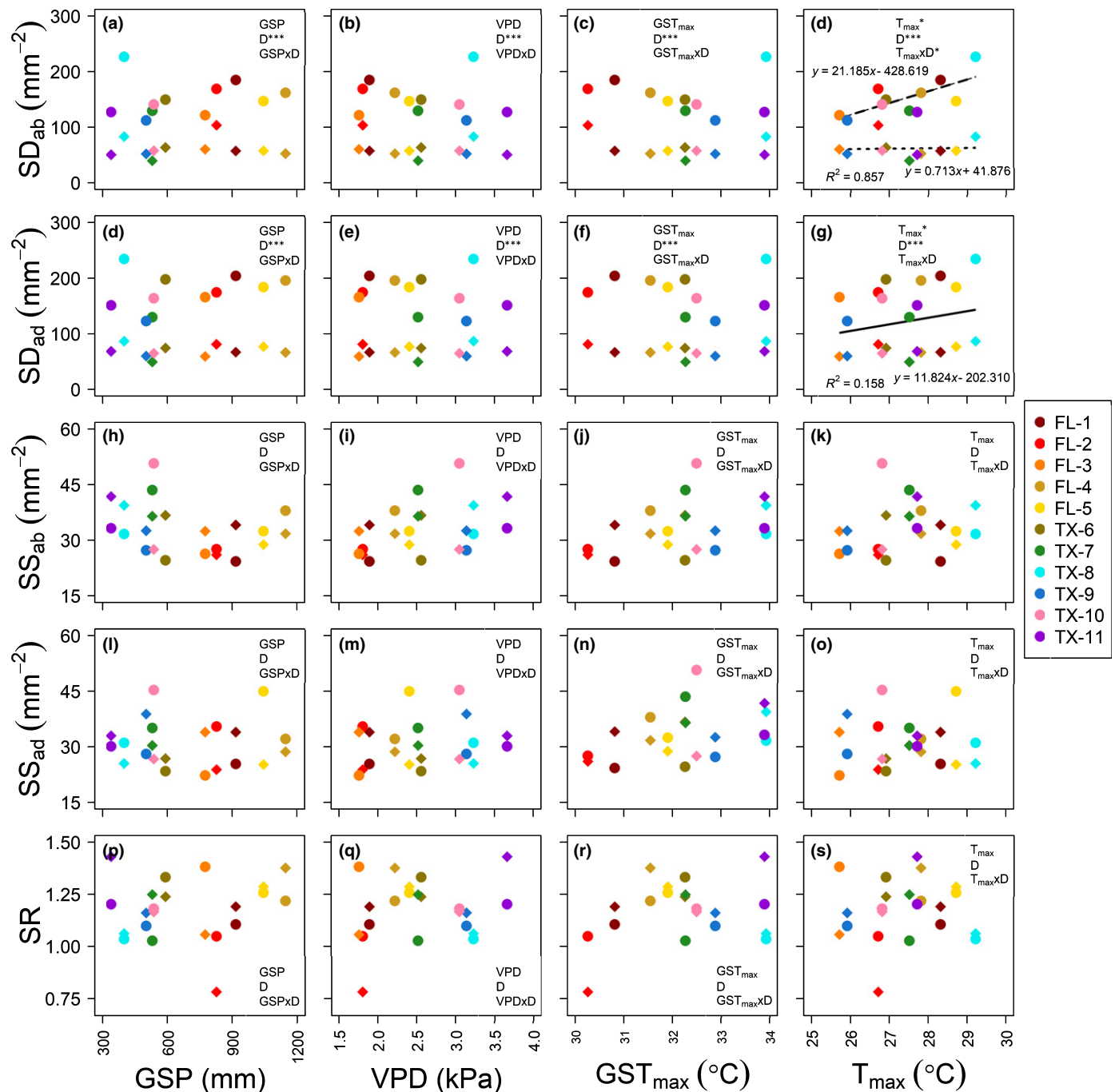
Fig. 3 Variation of stomatal traits with home-climate characteristics of mean annual precipitation (MAP), mean annual average temperature (T_{avg}), and mean annual maximum temperature (T_{max}) for 11 genotypes of switchgrass (*Panicum virgatum*) grown in common garden. Data for each genotype (colors) were averaged from data collected in June (circles) and August (diamonds). Overall significant relationships are denoted by solid black lines. Long dashed lines indicate significant relationship for data collected in June while short dashed lines indicate significant relationship for data collected in August (i.e. significant climate × date interaction). Traits are: abaxial stomatal density (SD_{ab} mm^{-2}), adaxial stomatal density (SD_{ad} mm^{-2}), average abaxial stomatal size (SS_{ab} µm^2), average adaxial stomatal size (SS_{ad} µm^2), and the ratio of stomata on the adaxial to abaxial leaf surface (SR). Significance is denoted by asterisks: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. For each panel, $n = 33$ (3 time points × 11 genotype mean values).

Linkages between climate, stomatal traits, and nighttime and daytime gas exchange

Piecewise structural equation models investigating genetic correlations between home-climate, stomatal traits, and g_{sn}, and in turn, g_s and A_{sat} (Fig. 6) aligned with the ANCOVA models (Figs 4, 5). We found genotypes from hotter sites (T_{max}) possessed higher SD which directly contributed to higher rates of g_{sn} (Fig. 6). In turn, genotypes which exhibited greater rates of g_{sn} had higher daytime g_s and A_{sat}. In the two significant pSEM models,
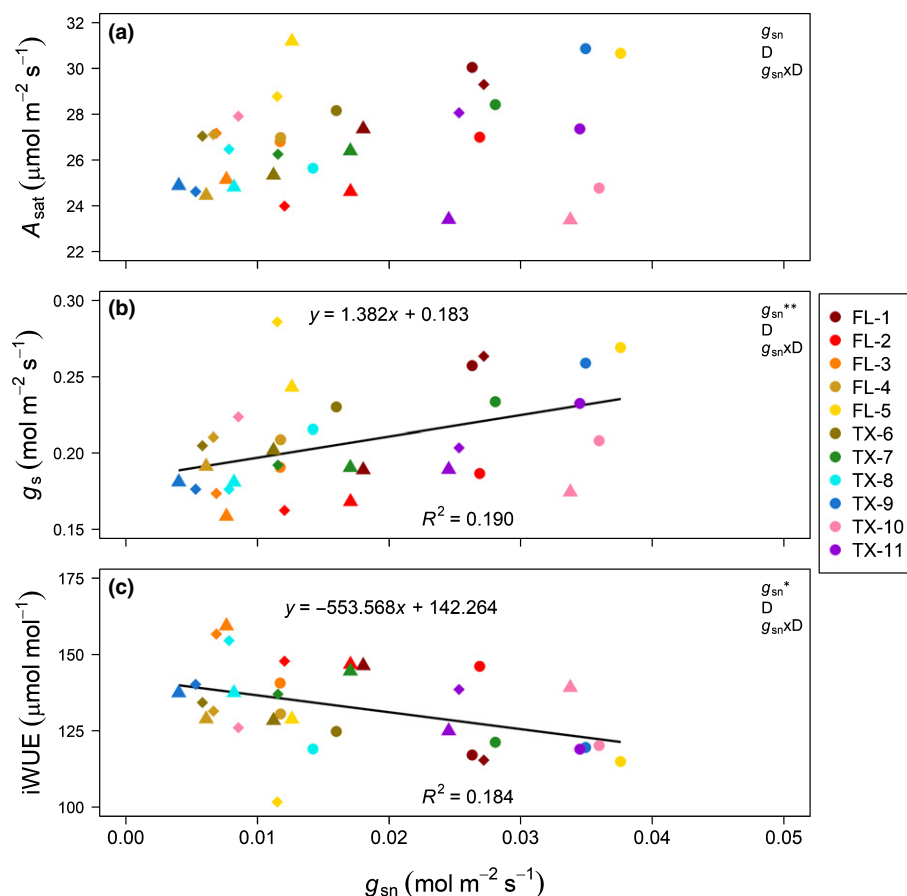
Fig. 4 Correlation of nighttime stomatal conductance (g_{sn} mol m^{-2} s^{-1}) with saturated photosynthesis (A_{sat} µmol m^{-2} s^{-1}) (a), daytime stomatal conductance (g_s mol m^{-2} s^{-1}) (b) and intrinsic water-use efficiency (iWUE µmol mol^{-1}) (c) for 11 genotypes of switchgrass (*Panicum virgatum*) grown in common garden. Data for each genotype (colors) were averaged from data collected in June (filled circles), July (filled triangles), and August (filled diamonds). The significance of the effects of g_{sn}, date (D) and their interaction are denoted by asterisks: *, $P < 0.05$; **, $P < 0.01$. For each panel, $n = 33$ (3 time points × 11 genotype mean values).

we found no other significant pathways between variables, highlighting the importance of SD in mediating the relationship between home-climate and g_{sn}, and subsequently daytime gas exchange. While all direct pathways were significant, the R^2 values of mixed effects models (which comprise each part of the pSEM in Fig. 6) that account for the random effect of 'Date' (R_C^2) were substantially improved compared to the R^2 values which only account for marginal effects (R_M^2). No other models that included any combination of the remaining stomatal traits or climatic variables were significant, corroborating the results of the ANCOVA analyses.

Discussion

We explored patterns of genotypic variation in g_{sn} in *P. virgatum* and determined relationships between home-climate and genotypic variation in nighttime and daytime leaf gas exchange and stomatal traits. We hypothesized that genotypic variation would be equally important in explaining variation of gas exchange and stomatal traits; our results partially supported this hypothesis. Genotypic differences explained a comparable amount of variation in g_{sn} and A_{sat}, yet genotypic differences explained a fraction of the variation in g_s and iWUE. Still, the interaction between date and genotype explained a substantial amount of variation of g_{sn} and $g_{sn} : g_s$, compared to daytime gas exchange parameters. We also hypothesized that genotypes from drier and warmer sites

would show higher rates of g_{sn} compared to those from wetter and cooler sites, which has been observed across species (Resco de Dios *et al.*, 2019; Yu *et al.*, 2019). We found no direct relationship between home-climate and g_{sn}, yet across genotypes we observed few relationships between average daytime gas exchange rates and home-climate. Upon further investigation, we found genotypes from warmer climates had higher SD on both sides of the leaf, which in turn resulted in greater g_{sn}. Thus, our hypothesis was partially supported, highlighting the role of stomatal traits in mediating the relationship between climate and g_{sn} in *P. virgatum*. We also found that higher g_{sn}, a result of possessing high SD, was genetically correlated with higher g_s and A_{sat}. Our results highlight the role of climate in determining genetic differentiation in g_{sn} and covarying leaf gas exchange and stomatal traits.

Across multiple studies, g_{sn} in grasses ranges from 0.011 to 0.207 mol H_2O m^{-2} s^{-1} (mean of 0.067 mol m^{-2} s^{-1}, 19-fold variation; Resco de Dios *et al.*, 2019) and from *c.* 0.005 to *c.* 0.065 mol H_2O m^{-2} s^{-1} (i.e. 13-fold variation, Yu *et al.*, 2019). Among the genotypes in our study, average g_{sn} ranged from 0.004 to 0.038 mol m^{-2} s^{-1} (9.5-fold variation), indicating that intraspecific variation in g_{sn} in *P. virgatum* may be nearly as large as variation in g_{sn} observed in other studies. Yu *et al.* (2019) included *P. virgatum* in their study, but the source of the plant material was not reported. Another study which examined genotypic variation in a desert C$_4$ grass (*D. spicata*) observed that g_{sn}
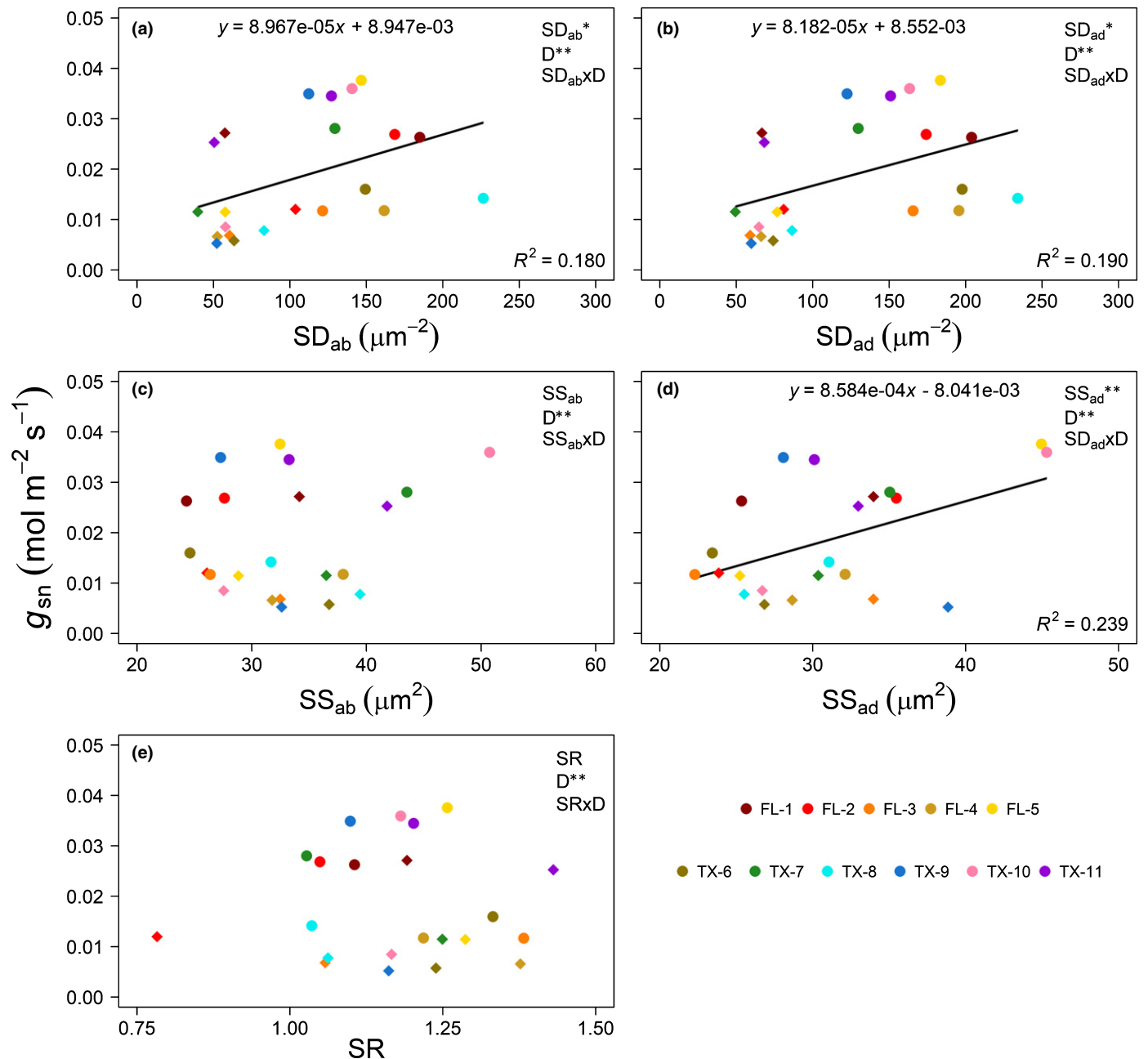
Fig. 5 Correlation of stomatal traits and nighttime stomatal conductance (g_{sn} mol m^{-2} s^{-1}) for 11 genotypes of switchgrass (*Panicum virgatum*) grown in common garden. Stomatal traits include abaxial stomatal density (SD$_{ab}$ µm^{-2}) (a), adaxial stomatal density (SD$_{ad}$ µm^{-2}) (b), average abaxial stomatal size (SS$_{ab}$ µm^2) (c), average adaxial stomatal size (SS$_{ad}$ µm^2) (d), and the ratio of stomata on the adaxial to abaxial leaf surface (SR) (e). Data for each genotype (colors) were averaged from data collected in June (circles) and August (diamonds). The significance of the effects of stomatal traits, date ('D'), and their interactions on g_{sn} are denoted by asterisks: *, $P < 0.05$; **, $P < 0.01$. For each panel, $n = 22$ (3 time points × 11 genotype mean values).

was between 5–20% of g_s (Christman *et al.*, 2009b) while Ogle *et al.* (2012) reported that g_{sn} was *c.* 40–75% of g_s in North American desert species. Among the genotypes in our study, which all originated from climates with GSP> 340 mm (i.e. no desert habitats), g_{sn} was 2.2–19.3% of daytime g_s.

Several studies have examined temporal changes in nighttime water loss for timespans longer than several d/wk (Donovan *et al.*, 2003; Grulke *et al.*, 2004; Phillips *et al.*, 2010; Montoro *et al.*, 2020). In congruence with those studies, we found that g_{sn}

differed strongly across sampling dates, despite relatively similar nighttime conditions (Fig. S3). In those studies and ours, g_{sn} generally declined over the course of the growing season. While the temporal dynamics of g_{sn} deserve further examination, the observed changes in g_{sn} over time may be due to changes in stomatal traits, which are known to vary with environmental conditions when leafing-out occurs (i.e. new leaves produced throughout year can vary in stomatal traits) (Bertolino *et al.*, 2019; McKown & Bergmann, 2020). Phenological changes
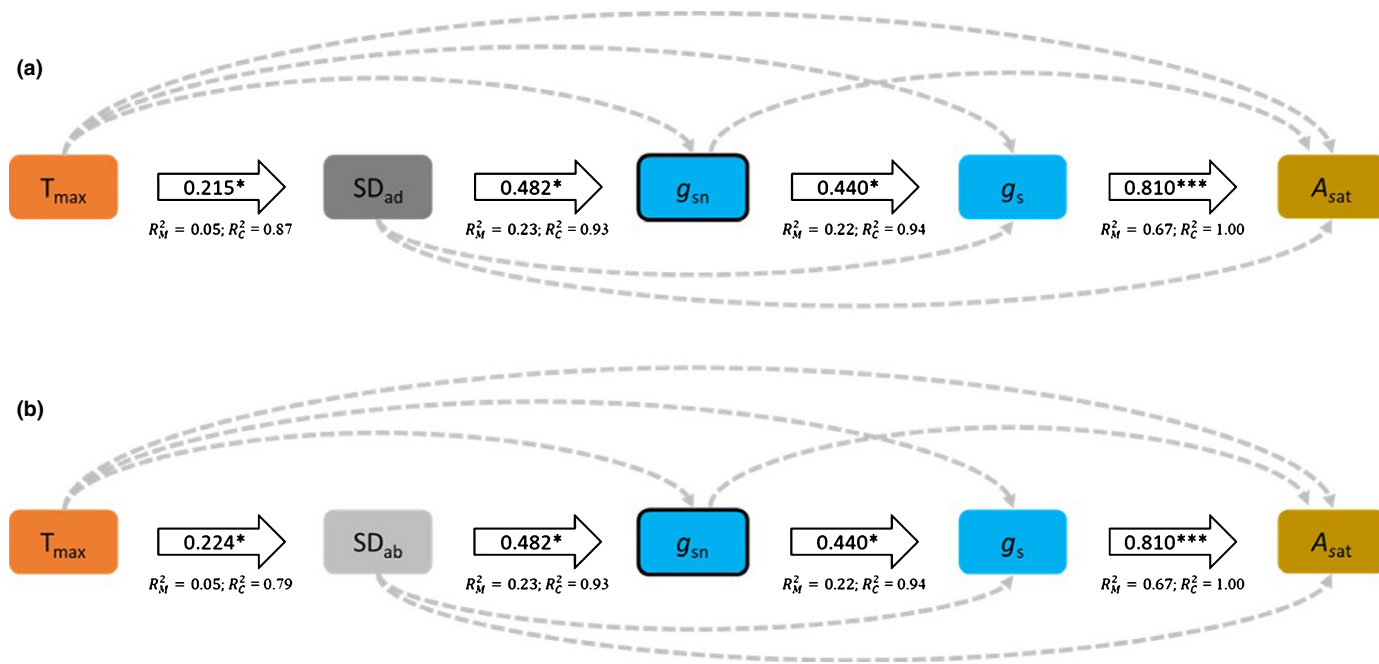
Fig. 6 Overall visualization of significant pathways from piecewise structural equation models indicating how home-climate and stomatal traits affect nighttime stomatal conductance (g_{sn}) in 11 genotypes of *Panicum virgatum* which integrate with daytime gas exchange parameters. Genotype's mean annual maximum temperature (T_{max}) was positively correlated with (a) SD_{ad} and (b) SD_{ab}. In turn, these stomatal traits had significant positive relationships with g_{sn} and daytime stomatal conductance (g_s) and photosynthesis (A_{sat}). Significant pathways (boxed arrows) show strength (−1 to 1) and directionality (all > 0, indicating positive relationships). Significance of these pathways are denoted by asterisks: *, $P < 0.05$; ***, $P < 0.001$. R_M^2 represents R^2 for fixed/marginal effects while R_C^2 is combined with R^2 for fixed and random effects ('Date'). Nonsignificant pathways, which test for direct separation between all variables included in the model, are indicated by grey arrows (i.e. there are no direct significant effects of these pathways). Fisher's C is an indication of the overall fit (greater is better) while overall P-values > 0.05 indicate overall independence between home-climate parameters and A_{sat}.

could also explain concomitant changes in SD and g_{sn}. In *P. virgatum*, peak growth and gas exchange rates typically occur in early summer (i.e. June) before the transition to flowering and seed set (Hartman *et al.*, 2012; Aspinwall *et al.*, 2013). Thus, the coordinated reductions in SD and g_{sn} between June and August may represent a developmental shift. Results from our pSEM indicated the underlying role of stomata and home-climate in nighttime and daytime gas exchange covariation; however, the robustness of the relationships between physiological components was primarily dependent on the inclusion of date as a random effect. This further highlights the need for studies to integrate seasonal sampling of g_{sn} into future experiments to further elucidate the drivers and adaptive significance of nighttime water loss.

Multiple lines of evidence suggest observed rates of g_{sn} are not a result of leaky stomata. Instead, g_{sn} may be an adaptive trait, given that genotypes within species may show considerable differences in g_{sn} (Resco de Dios *et al.*, 2019 and citations therein). We determined the proportion of variation in g_{sn} attributable to differences among genotypes. We found that genetics alone explained c. 50% less of the variation in g_{sn} and g_{sn} : g_s compared to daytime g_s; however, overall variation in g_{sn} and g_{sn} : g_s explained by genotypic differences was comparable to other gas exchange measurements. Importantly, the interaction of genotype and sampling date explained more variation in g_{sn} and g_{sn} : g_s compared to all other gas exchange traits. Our study would benefit from a large number of replicate plants (e.g. genotype TX-9) and genotypes; however, variation in stomatal data were

quite uniform across genotypes with different sample sizes (Table S3). Still, the proportion of variation in g_s explained by genotype alone was nearly double that of g_s, indicating that climate-mediated selection on daytime g_s (i.e. higher g_s in genotypes from hot climates) may indirectly result in genetic differentiation in g_{sn}.

In two large studies which examined interspecific relationships between home-climate and g_{sn}, the authors found that species from sites with higher mean annual precipitation and mean annual temperature exhibited higher g_{sn} (Yu *et al.*, 2019) and g_{sn} : g_s (Resco de Dios *et al.*, 2019). By contrast, Christman *et al.* (2008) found that Arabidopsis ascensions from sites characterized by greater annual *VPD* exhibited lower g_{sn}, while Sadok & Tamang (2019) found barley genotypes from more arid sites exhibited lower nighttime whole canopy transpiration rates compared to those from wetter sites. In our study, we observed no direct relationship between GSP or temperature conditions at the geographic origin of different *P. virgatum* genotypes and mean g_{sn}, lending little support for our hypothesis that genotypic variation in g_{sn} or g_{sn} : g_s would be explained by the climatic origin of the genotypes. Instead, average values of SD increased as home-temperature conditions at the genotypes' origins increased, which in turn drove greater rates of g_{sn}. This shift in SD, driving changes in g_{sn}, may be a result of phenological changes in southern *P. virgatum* genotypes, where high SD early in the year contributes to high gas exchange rates and rapid growth (Aspinwall *et al.*, 2013). We were unable to include genotypes from more northern sites due to poor growth at our site. Evidence suggests

genotypes from cooler sites have poor survival rates when transplanted further south (McMillan, 1969), likely contributing to our difficulty in getting adequate growth from more northern genotypes. However, more northern populations may not express similar patterns in stomatal traits as southern populations, given the comparative decrease in evaporative demand at northern locations and the strong relationship between VPD and SD (Carins Murphy *et al.*, 2014).

To our knowledge, the only other study to report a significant relationship between SD and g_{sn} found a negative relationship in grapevine – the opposite of the results of our experiment (Rogiers & Clarke, 2013). Others have observed no relationship between SD and g_{sn} (Ceulemans *et al.*, 1988; Lasceve *et al.*, 1997); however, none of these studies included grasses. A potential explanation for these opposing results is that *P. virgatum*, and grasses generally, possess highly efficient dumb-bell shaped stomata (fast, higher iWUE) compared to most species, which possess kidney-shaped stomata (Hetherington & Woodward, 2003). Perhaps grasses, particularly C_4 species, exhibit lower rates of stomatal closure at night since water availability is less crucial compared to species with less efficient photosynthetic pathways (O'Keefe & Nippert, 2018). We observed in our pSEM analysis that genotypes which exhibited higher g_{sn} associated with increased SD, also showed higher g_s and A_{sat} during the day (under well-watered, high light conditions), indicating a potential advantage of possessing higher SD; however, our interpretation may be limited to species which possess highly efficient stomata. Also, higher daytime gas exchange may require higher SD, which may indirectly result in higher g_{sn} (Christman *et al.*, 2008). In other words, hotter temperatures may drive selection for higher daytime g_s to meet demands for leaf cooling, which should require higher SD and indirectly cause g_{sn} to increase with any degree of stomatal opening.

Across several *P. virgatum* genotypes, we observed a strong genetic correlation between g_{sn} and daytime gas exchange, as observed in both the ANCOVAs and pSEM analyses. Previous work has provided mixed support for the idea that nighttime conductance influences daytime gas exchange or vice-versa (Ogle *et al.*, 2012; Rogiers & Clarke, 2013; Zeppel *et al.*, 2014; Tamang & Sadok, 2018); however, we found daytime and nighttime covariation to exist across *P. virgatum* genotypes. Furthermore, we were able to potentially implicate the importance of stomatal features in mediating the relationship between home-climate and g_{sn}: a relationship which has been observed by others (Christman *et al.*, 2008; Yu *et al.*, 2019; Resco de Dios *et al.*, 2019). An interesting result is that T_{max} (annual maximum), rather than GST_{max} (March–August), was a primary driver of genetic variation in SD. In southern genotypes of *P. virgatum*, the growing season can be > 300 d (McMillan, 1967), so annual climate parameters may be better predictors of stomatal traits than seasonal climate parameters. In our genotypes, the relationship between the T_{max} and GST_{max} was not significant (Fig. S1) suggesting that using annual parameters, which perhaps better describe the long growing season in southern switchgrass populations, may be more suitable in understanding climate–trait relationships compared to seasonal parameters.

While we did not measure early morning gas exchange to test the 'anticipation hypothesis' directly, we found that genotypes that demonstrated higher g_{sn} also showed higher rates of g_s and A_{sat} under well-watered, high light conditions. If conditions were less than ideal, we predict that possessing high SD would permit high rates of gas exchange early in the day. This would be particularly important in genotypes from more arid climates, potentially explaining why genotypes from warmer sites possessed higher SD. Still, home-VPD, which varied from 1.6 to 3.7 kPa, showed no relationship with stomatal trait or gas exchange data across genotypes in this study. While others have shown VPD to have a strong effect on SD (Carins Murphy *et al.*, 2014; Devi & Reddy, 2018; Du *et al.*, 2020), precipitation and VPD may be less important for lowland ecotypes compared to upland ecotypes of *P. virgatum* (no upland ecotypes were included in our study) since lowland plants typically have water access, making transpirational cooling possible under high temperatures. This could explain why temperature was more important than precipitation or VPD in this study. Furthermore, the sharp decline in SD from June to August across all genotypes could indicate that high potential gas exchange rates and transpirational cooling are more important earlier in the year, before plants are acclimated to persistently warm summer temperatures.

The decline in SD from June to August observed in our study (*c.* 80% on either leaf surface) was larger than expected, but not unprecedented. Zhang *et al* (2019) observed a 48% change in SD in *Robinia pseudoacacia* as new leaves formed during a 60-d soil moisture reduction. Carins Murphy *et al.* (2014) observed a 33% decline of SD in *Toona ciliata* trees grown at 0.6–1.0 kPa VPD compared to trees grown at 2.5–2.7 kPa. In our study, the 10-d mean daytime VPD preceding measurements of SD was 1.2 and 1.9 kPa (June and August, respectively; Fig. S2b). Thus, changes in SD could be attributed to environmental effects. Yet, with two time points we are unable to speculate about the potential influence of environmental conditions on SD. In the future, a larger number of stomatal images could be used to reduce the variation in SD estimates (Table S3).

Given the observed covariation of stomatal traits with gas exchange in *P. virgatum*, the relationship with climate-of-origin, and the known relationship between stomatal traits and potential gas exchange rates, our results indicate that g_{sn} may play a role in environmental adaptation via genetic correlations with SD and daytime g_s. We hypothesize that possessing higher densities of stomata at hotter sites could increase the capacity for daytime g_s and thus the ability to modulate leaf temperatures under hot conditions, while also contributing to sustained C fixation during periods (diurnally or seasonally) that would otherwise limit photosynthesis and C available for growth and metabolism (Mott *et al.*, 1982; Pandey *et al.*, 2007; Hill *et al.*, 2015). Thus, genetic differentiation in g_{sn} may arise indirectly through selection on daytime g_s and associated changes in SD. However, g_{sn} may play a more direct role in adaptation. Our pSEM approach revealed that g_{sn} was a key trait linking SD and daytime gas exchange, and if SD is a trait that facilitates high g_{sn} for the purposes of daytime C fixation, it would provide partial support for linking g_{sn} to adaptation under the anticipation hypothesis (Mansfield &

Heath, 1961; Tobiessen, 1982; Resco de Dios *et al.*, 2016, 2019). This study provides new insight into the factors shaping evolution and genetic differentiation of g_{sn}. Further work regarding the relationship between stomatal traits and g_{sn} is warranted, particularly in the context of other wide-ranged species which exhibit a high degree of genetic differentiation and local adaptation.

Author contributions

MJA, TB, VRdD, TEJ and DTT designed the experiment. MJA, TB, PG and JC collected data. JC and MJA analyzed the data. JC wrote the manuscript with input from MJA. TEJ provided plant material. All authors contributed to editing and revising the manuscript.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Bivariate relationships between home-climate parameters of the 11 *Panicum virgatum* genotypes used in this study.

Fig. S2 Air temperature and maximum daily vapor pressure deficit for summer of 2019 at University of North Florida's campus.

Fig. S3 Recorded measures of leaf temperature, vapor pressure deficit, predawn water potential and soil volumetric water content observed at either predawn or in daytime in 11 genotypes of *Panicum virgatum*.

Fig. S4 Average rates of nighttime stomatal conductance and the ratio of nighttime to daytime conductance in 11 *Panicum*

virgatum genotypes in relation to prevailing predawn and daytime leaf temperatures and leaf vapor pressure deficits.

Fig. S5 Proportion of trait variance explained by sampling month, genotype, and their interaction in 11 genotypes of *Panicum virgatum* (switchgrass) grown in common garden.

Fig. S6 Variation of nighttime stomatal conductance, the ratio of nighttime to daytime conductance, midday stomatal conductance, light saturated photosynthesis, and intrinsic water-use efficiency with home-climate characteristics for 11 genotypes of *Panicum virgatum* grown in common garden.

Table S1 Locations and identification names/numbers for weather stations used to obtain climate data for the 11 genotypes of *Panicum virgatum*, including the dates on which stations were recording.

Table S2 Mean values and standard errors for morphological traits for 11 genotypes of *Panicum virgatum*.

Table S3 Mean values and standard errors for stomatal traits for 11 genotypes of *Panicum virgatum*.

Table S4 Mean values and standard errors for physiological traits for 11 genotypes of *Panicum virgatum*.

Table S5 ANOVA table describing the effects of genotype, date, and their interaction on daytime and nighttime gas exchange, and stomatal traits for 11 genotypes of switchgrass (*Panicum virgatum*) grown in common garden.

Table S6 Relationship between daytime and nighttime gas exchange traits with climate of origin for 11 genotypes of switchgrass (*Panicum virgatum*) grown in common garden.

Table S7 Relationship between stomatal traits with climate of origin for 11 genotypes of switchgrass (*Panicum virgatum*) grown in common garden.

Table S8 Analysis of covariance of daytime and nighttime gas exchange parameters for the 11 genotypes of *Panicum virgatum* used in this study.

Table S9 Analysis of covariation of nighttime stomatal conductance and stomatal traits for the 11 genotypes of *Panicum virgatum* grown in common garden.

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