

Phylogenetic niche conservatism in C₄ grasses

Hui Liu · Erika J. Edwards · Robert P. Freckleton ·
Colin P. Osborne

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Abstract Photosynthetic pathway is used widely to discriminate plant functional types in studies of global change. However, independent evolutionary lineages of C₄ grasses with different variants of C₄ photosynthesis show different biogeographical relationships with mean annual precipitation, suggesting phylogenetic niche conservatism (PNC). To investigate how phylogeny and photosynthetic type differentiate C₄ grasses, we compiled a dataset of morphological and habitat information of 185 genera belonging to two monophyletic subfamilies, Chloridoideae and Panicoideae, which together account for 90 % of the world's C₄ grass species. We evaluated evolutionary variance and covariance of morphological and habitat traits. Strong phylogenetic signals were found in both morphological and habitat traits, arising mainly from the divergence of the two subfamilies. Genera in Chloridoideae had significantly smaller culm heights, leaf widths, 1,000-seed weights and stomata; they also appeared more in dry, open or saline habitats than those of Panicoideae. Controlling for phylogenetic structure showed significant covariation among morphological traits, supporting the hypothesis of phylogenetically

independent scaling effects. However, associations between morphological and habitat traits showed limited phylogenetic covariance. Subfamily was a better explanation than photosynthetic type for the variance in most morphological traits. Morphology, habitat water availability, shading, and productivity are therefore all involved in the PNC of C₄ grass lineages. This study emphasized the importance of phylogenetic history in the ecology and biogeography of C₄ grasses, suggesting that divergent lineages need to be considered to fully understand the impacts of global change on plant distributions.

Keywords Phylogenetic niche conservatism · C₃/C₄ photosynthesis · Poaceae · Morphology · Habitat

Introduction

Phylogenetic niche conservatism (PNC) is the tendency of closely related species to share broadly similar niches to their ancestors and, at the same time, similarities in their morphology, physiology, and life history (Wiens and Graham 2005). The hypothesis that species tend to retain ancestral ecological characteristics has been supported at scales ranging from the community and region (Silvertown et al. 2006; Losos 2008) to the globe (Crisp et al. 2009). However, while clearly important for understanding the functional interactions of species with the environment, PNC has been little explored in the context of global change biology (Wiens et al. 2010; Cooper et al. 2011). In this field, generalizations about species are usually made within functional rather than phylogenetic groupings (Edwards et al. 2007).

Photosynthetic pathway is used widely to discriminate plant functional groups in studies of global change impacts

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H. Liu · R. P. Freckleton · C. P. Osborne (✉)
Department of Animal and Plant Sciences,
University of Sheffield, Sheffield S10 2TN, UK
e-mail: c.p.osborne@sheffield.ac.uk

E. J. Edwards
Department of Ecology and Evolutionary Biology,
Brown University, Providence, RI 02912, USA

(e.g., Still et al. 2003; Sitch et al. 2008), since C_3 and C_4 photosynthesis show contrasting responses to CO_2 and temperature (Ehleringer et al. 1978), and have differing effects on plant water relations (Taylor et al. 2011). The C_4 pathway is especially prevalent in grasses, which dominate tropical and subtropical habitats, especially in savanna and prairie grassland ecosystems (Edwards et al. 2010). C_4 photosynthesis has evolved independently in multiple grass lineages (Christin et al. 2008; Vicentini et al. 2008). These lineages vary significantly in their physiology and anatomy (Christin et al. 2010), and this variation is grouped within three functional categories for the purposes of generalization.

Comparative studies among the three biochemical subtypes of C_4 photosynthesis [the NADP-malic enzyme (NADP-me), NAD-malic enzyme (NAD-me) and phosphoenolpyruvate carboxykinase (PCK) subtypes] have suggested that they occupy contrasting positions along environmental gradients. For example, in Australia, the percentage of NADP-me species in a grass flora increases with annual rainfall, while the equivalent relationship for the NAD-me subtype is negative (Hattersley 1983). Evidence from other regions like Namibia (Ellis et al. 1980) and Argentina (Cabido et al. 2008) has confirmed this pattern. However, this functional categorization is largely confounded with phylogeny (Hattersley 1983), and partial correlation analysis of the biogeographic occurrences of C_4 grasses in North America showed stronger predictive effects of subfamily than C_4 subtype (Taub 2000). Recent global scale phylogenetic analyses have also demonstrated the generality of these patterns, showing that several of the major grass clades inhabit strikingly different climatic regions, with C_4 lineages differentiated from their C_3 relatives primarily by precipitation (Edwards and Smith 2010). Good evidence has therefore accumulated for the differential ecological sorting of C_4 grass lineages. However, the extent to which this is underpinned by adaptive variation in anatomy and physiology of the C_4 photosynthetic pathway still remains unclear.

Our understanding of why different C_4 grass lineages occupy contrasting ecological niches also remains extremely limited. A crucial unanswered question about the environmental niche of C_4 grasses is whether geographical distributions are limited directly by water availability per se, or a correlated environmental factor such as tree cover. Previous work on the evolution of environmental niche in grasses has already shown that habitat water preference depends strongly upon phylogeny, but this work stopped short of directly contrasting habitat occupancy among the major grass clades (Osborne and Freckleton 2009). However, rainfall gradients in the tropical and subtropical regions occupied by C_4 grasses also cause increases in max-

imum woody plant cover (Sankaran et al. 2008), and recent work suggests that ecological transitions from shaded to open habitats have driven the evolutionary origins and diversification of C_4 species (Osborne and Freckleton 2009; Edwards and Smith 2010).

A simple approach to understand PNC would be to measure the degree to which traits correlate with phylogeny using a measure of phylogenetic signal. However, it is not straightforward to equate the degree of phylogenetic signal in data with an underlying model of evolution (Revell et al. 2008). In the case of PNC, there are various conceptual models, all of which yield different outcomes (Cooper et al. 2010). Three contrasting examples are: first, it could be that PNC arises because species inherit their niches from ancestors, but then slowly diverge as they move into new habitats. This would yield a Brownian model of trait evolution (Harvey and Pagel 1991). A second possibility is that the niche of a group of species is constrained, so that stabilizing selection prevents species moving too far from the niche optimum (Donoghue 2008). This would yield an Ornstein–Uhlenbeck model of trait evolution, in which phylogenetic dependence is *weaker* than predicted by a Brownian model. A third possibility is that species inherit their niches from their ancestors and, as a consequence of niche-filling, ancestors and descendants retain increasing amounts of similarity as evolution proceeds (Price 1997). This predicts that phylogenetic dependence is *stronger* than predicted by the Brownian model.

As pointed out by Cooper et al. (2010), care has to be taken in interpreting phylogenetic comparative data with an aim of understanding niche conservatism, as PNC can be equated with several evolutionary models. In this paper, we measure the degree of phylogenetic dependence in a suite of morphological traits and habitat preferences. The main objective in doing so is to measure the degree to which key traits are conserved within phylogenetic lineages, or to see whether they vary more independently than predicted by phylogeny. Thus, we interpret strong phylogenetic dependence as evidence of strong conservatism.

In this study, we focused on two major monophyletic subfamilies, Chloridoideae and Panicoideae, which together account for over 90 % of C_4 grass species. Using phylogenetic tests, we investigated at the genus level how morphological traits and habitat types vary or co-vary among different evolutionary groups. Our objectives were: (1) to measure the phylogenetic signal in the morphological and habitat traits of these C_4 grasses; (2) to investigate the extent to which morphological traits show correlated evolution and adaptation to habitat type across different C_4 lineages; and (3) to evaluate the extent to which C_4 photosynthetic subtype explains trait variation against this phylogenetic background.

Materials and methods

Genus sampling

All C_4 grasses belong to the PACMAD clade of the Poaceae. Chloridoideae and Panicoideae are the two largest subfamilies of this clade that together encompass C_3 and all three subtypes of C_4 species. Chloridoideae has three main tribes: Cynodonteae, Eragrostideae, and Zoysieae, which are mainly distributed in Africa and Australia, and tend to be particularly prevalent in drought-prone and temperate climate regions (Hartley and Slater 1960; Cross 1980). Panicoideae also has four main tribes: Andropogoneae (a tropical tribe with centres of diversity in Africa and India), Paniceae (a tropical tribe centered in East Africa), Paspaleae, and Arundinelleae (Cross 1980; Grass Phylogeny Working Group II 2012). In a previous phylogeny, Paniceae was paraphyletic (Giussani et al. 2001) and divided into two monophyletic groups based on the chromosome base number ($x = 9$ or 10); however, a recent treatment named $x = 10$ Paniceae as Paspaleae (Grass Phylogeny Working Group II 2012).

Data collection

A genus-level dataset was compiled from different sources (Online Resource 1 and 2). Initially, we listed all of the 363 C_4 grass genera described by Sage et al. (1999) where information about C_4 subtype was provided. This list was then filtered down to ~ 200 by the availability of nucleotide sequences for any species of each genus in the NCBI public archives, which we used to reconstruct the phylogenetic relationships of our study taxa. To simplify the comparison, we focused on the subfamilies Chloridoideae and Panicoideae, which further limited the dataset to 185 genera (Table 1). Four kinds of data were then compiled for each genus.

1. Categorical information: subfamily and tribe were based mainly on Watson and Dallwitz (1992 onwards) and Peterson et al. (2010). The photosynthetic type (including C_4 subtype) identification followed Sage et al. (1999).
2. Quantitative parameters: numbers of species in each genus (divided into annual or perennial), culm height (i.e., height of the stem, estimated as the mean of maximum and minimum values), leaf width and stomatal guard cell length were compiled from Watson and Dallwitz (1992 onwards) and Clayton et al. (2006 onwards), 1,000-seed weight values were from Royal Botanic Gardens, Kew (2009). Data for the extremely large seeds of domesticated maize were excluded from the analyses.

Table 1 Structure of the dataset, including the genus count for different life histories and photosynthetic types, and the total species number of the Chloridoideae and Panicoideae subfamilies

	Chloridoideae	Panicoideae	Total
Number of genera	84	101 (25)	185
Genera containing annual species	44	64 (15)	108
Genera containing perennial species	70	90 (20)	160
C_3 genera	0	25	
NAD-me genera	42	0	
NADP-me genera	2	62	
PCK genera	8	5	
Genera with unclear or mixed types	32	9	
Number of species	1,252	2,961 (434)	4,213

Data for the C_3 genera of the Panicoideae are given in parentheses

3. Qualitative descriptions: plant and leaf morphological traits were recorded from Watson and Dallwitz (1992 onwards). These included traits considered important for plant water relations, including “broad” and/or “narrow” leaf and “flat” and/or “rolled/folded” leaf.
4. Habitat data: information on water requirement (e.g., hydrophyte, xerophyte), tolerance of saline environments (halophyte, glycophyte) and habitat shading (shade, open) was recorded (Watson and Dallwitz 1992 onwards). A numerical score of water requirement was then assigned to each of the habitat types describing water availability, giving equal weighting to the extremes (Hydrophyte = 5, Helophyte = 4, Mesophyte = 3, Xerophyte = 1), and resulting in a continuous sequence of values for each genus. The sequence of these four values for each genus was summarized as a mean “water score” and the range as “water range”, following Osborne and Freckleton (2009).

Phylogenetic analyses

We reconstructed phylogenetic relationships among our taxa using molecular sequences available from the NCBI. We focused on six commonly sequenced gene regions for grasses: nuclear markers *phyB* and *nrITS* and chloroplast regions *ndhF*, *rbcL*, *trnK/matK*, and *trnLF*. Each genus in our dataset was represented by the one exemplar in the archive that had been sequenced for the greatest percentage of these loci, allowing us to build as complete a data matrix as possible. The final alignment contained 189 taxa and 7,350 characters. We built a phylogeny using Bayesian inference, as implemented in Mr. Bayes v3.1.2 and allowing

each gene region an independently optimized model of evolution. Briefly, we ran three independent runs of 10,000,000 generations each, sampling every 1,000th generation to build a posterior distribution of trees. The first 4,000,000 generations of each run were discarded as ‘burn-in’ to ensure stationarity. The remaining sampled trees were pooled from the three independent runs to produce a consensus phylogeny (Online Resource 4). Many areas of the tree lacked strong statistical support. To account for this phylogenetic uncertainty, 300 alternative phylogenies were randomly sampled from the posterior distribution, and all phylogenetic tests were run across all 300 trees.

We used Pagel’s λ to estimate the degree to which the residual variation of one trait shows “niche similarity” depending on phylogeny, according to the prediction of a simple Brownian model of trait evolution (Pagel 1999); i.e., it measures the extent to which traits evolve by random drift from their value in a common ancestor, and takes values between zero and one. A value of $\lambda = 0$ implies that there is no phylogenetic dependence, whereas $\lambda = 1$ indicates perfect phylogenetic dependence. Based on the approach of Freckleton et al. (2002), we used this test to detect phylogenetic signals in single traits, to build models of the association between pairs of traits whilst accounting for variable levels of phylogenetic signal, and to construct models for the dependence of plant traits on habitat traits. Furthermore, to compare how phylogenetic patterns arose for the relationships between morphological traits and habitat, we also carried out λ tests separately for the two subfamilies Chloridoideae and Panicoideae.

We used two methods to explore whether subfamily and photosynthetic type explain variance in morphological and habitat traits. First, we added subfamily as a factor into the phylogenetic models for single traits. This allowed us to evaluate the extent to which the divergence between Panicoideae and Chloridoideae explained phylogenetic structure in the data. Second, we added both subfamily and photosynthetic type (PT) into models, comparing the variance in traits explained by each factor. Two factors were transposed in two models as: M1, $y \sim$ subfamily + PT; M2: $y \sim$ PT + subfamily, where y was the trait of interest, and the terms tested using sequential (Type I) sums of squares. This allowed the importance of each variable to be assessed by testing whether each explained a significant proportion of variance once the other had been accounted for. In these models, only genera containing species with a single C_4 photosynthetic subtype were used to avoid the influence of mixed photosynthetic types, resulting in a smaller sample of 140 genera. We could not directly test interaction effects due to the highly biased distribution of photosynthetic type between subfamilies.

Results

Phylogenetic distribution of traits

Our phylogenetic tree showed that two subfamilies, Chloridoideae and Panicoideae, were each strongly supported as monophyletic (Fig. 1). Genera in Chloridoideae were clustered into one small (Triphideae) and three large (Zoysieae, Cynodonteae and Eragrostideae) lineages, matching the tribes defined by taxonomists and previous phylogenetic groupings. Meanwhile in Panicoideae, Paspaleae was closer to tribe Andropogoneae than to Paniceae. The representatives of the Arundinelleae appeared to be paraphyletic, one was sister to the Andropogoneae, and the other was an outgroup of Panicoideae. The colour codes

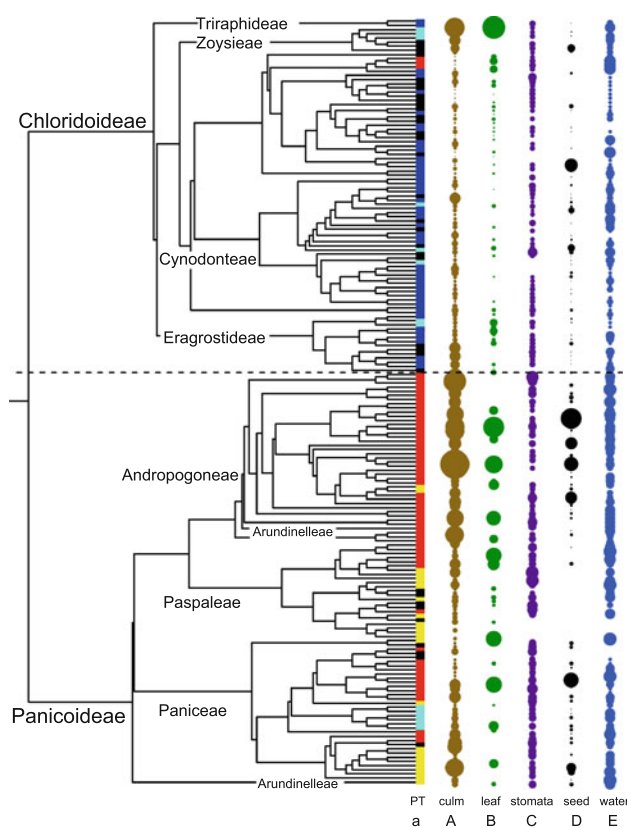


Fig. 1 Values of *A* (brown) culm height, *B* (green) leaf width, *C* (purple) guard cell length, *D* (black) 1,000-seed weight, and *E* (blue) water score mapped across the phylogenetic tree. This tree was selected among the 300 simulated phylogenetic trees of the 185 genera in the dataset, with the highest frequency in the histograms of 300 lambda values of quantitative indices (Online Resource 3). A full list of genus names in the consensus tree is in Online Resource 4. Dot sizes are proportionally scaled to fit the figure. Subfamilies (Chloridoideae and Panicoideae) and tribes are labeled. Photosynthetic types (PT) are shown in the branch tips (a) as coloured squares C_3 , yellow C_4 NAD-me, blue C_4 NADP-me, red C_4 PCK, cyan and mixed PT in one genus, black. Note that the seed weight for domesticated maize is shown in this figure but was excluded from the analysis

Table 2 Pagel's λ based on 300 phylogenetic trees in two models (A) single trait only and (B) subfamily effect against the phylogenetic background

Index (y)	n	(A) $y \sim 1$			(B) $y \sim$ subfamily			
		λ	$P_{(\lambda=0)}$	$P_{(\lambda=1)}$	F^P	λ	$P_{(\lambda=0)}$	$P_{(\lambda=1)}$
Annuals per genus	179	0.22	ns	ns	1.48 ^{ns}	0.21	ns	ns
Perennials per genus	179	0.02	ns	ns	2.83 ^{ns}	0.02	ns	*
Perennials per genus (%)	179	0.00	ns	***	0.14 ^{ns}	0.00	ns	***
Culm height (cm) ^a	181	0.55	***	***	1.70 ^{ns}	0.51	***	***
Leaf width (mm) ^a	66	0.59	***	*	7.37 ^{ns}	0.27	ns	***
Guard cell length (μm) ^a	138	0.58	***	***	14.12 ^{***}	0.25	ns	***
1,000-seed weight (g) ^a	101	0.30	***	***	19.84 ^{***}	0.00	ns	***
Narrow leaf	185	0.29	***	***	16.25 ^{***}	0.00	ns	***
Broad leaf	185	0.30	***	***	40.97 ^{***}	0.00	ns	***
Roll/fold leaf	185	0.27	***	***	20.52 ^{***}	0.00	ns	***
Water range	156	0.12	*	***	14.18 ^{***}	0.00	ns	***
Water score ^a	156	0.61	***	***	3.47 ^{ns}	0.49	ns	***
Shade habitat	185	0.30	***	***	2.52 ^{ns}	0.21	ns	***
Saline habitat	185	0.09	ns	***	8.35 ^{***}	0.00	ns	***

Sample size (n) deviated from 185 for missing values of some genera. All standard errors for the 300 lambda values were less than 0.01 except "annuals per genus" (0.02). P values for both $\lambda = 0$ and 1 were based on the tree of highest frequency among the 300 lambda values (Online Resource 3). F and P values for factors in the model are reported

To test the influences of trait variance, phylogenetic tests on means and maximum/minimum values of the four morphological traits were also compared. These did not change the qualitative patterns in results, therefore only results from means were reported

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns not significant

^a Data of traits were natural logged before tests

showed how photosynthetic types were confounded with subfamily, i.e., all of the NAD-me genera in the dataset belonged to Chloridoideae, while nearly all of the NADP-me genera existed in Panicoideae (Table 1; Fig. 1). Most of the genera with "uncertain photosynthetic type" were in lineages within the Chloridoideae, with both "NAD-me" and "PCK" species in one genus.

Mapping the five main traits across the tree showed not only clear distinctions in trait values between Chloridoideae and Panicoideae but also differences among smaller lineages (Fig. 1). Average values of the morphological traits of Chloridoideae were around half those of Panicoideae, and water score was generally smaller in Chloridoideae, indicating more genera living in drier habitats (all differences were statistically significant between subfamilies, data not shown). Several lineages notably contributed to the pattern. For example, the lowest values of culm height, leaf width and guard cell length occurred in the Cynodonteae, while the greatest culm height and leaf width values were from the Andropogoneae, and guard cell length was greatest in the Paspaleae. The 1,000-seed weight was polarized by several genera with extremely large seeds. One genus standing out with large plant size in Chloridoideae was *Neyraudia* from the small, early diverging lineage Triraphideae. For the water score, there were no clear contrasts within lineages (Fig. 1).

Phylogenetic signal in single traits

Morphological traits including culm height, leaf width, guard cell length, 1,000-seed weight and binomial indices of narrow/broad and rolled/folded leaves all showed phylogenetic dependence (all $P < 0.01$ for $\lambda = 0$), with values of λ varying from 0.27 to 0.59 (Table 2A). In contrast, occurrence of annual/perennial life history showed no clear phylogenetic signal, with λ values not distinguishable from either 1 or 0. The percentage of perennial species showed phylogenetic independence (P ns for $\lambda = 0$; <0.001 for $\lambda = 1$). For habitat traits, water range had a λ of 0.12, whereas the value of water score was 0.61 ($P < 0.05$ for $\lambda = 0$ in both cases). Furthermore, the occurrence of a genus in shade habitats was significantly related to phylogeny, whereas occurrence in saline habitats was independent of phylogeny (Table 2A). Across all tests, the estimated λ values for all the 300 phylogenetic trees showed that the phylogenetic uncertainty in λ was relatively small for all morphological and habitat traits (Online Resource 3). Overall, the values of λ for single traits were significantly different from zero or one except those traits related to species number (Table 2A).

The addition of subfamily as a factor into each statistical model reduced the value of λ in all single trait tests (Table 2B). Almost all the λ values for morphological traits

Table 3 F and λ values for phylogenetic linear models testing the relationships between two morphological traits based on 300 phylogenetic trees

	n	F^P	λ	$P_{(\lambda=0)}$	$P_{(\lambda=1)}$
Leaf width ~ culm height	65	64.97***	0.02	ns	***
Guard cell length ~ culm height	137	4.92*	0.11	ns	***
1,000-seed weight ~ culm height	100	6.77*	0.23	ns	***
Guard cell length ~ leaf width	46	0.03 ^{ns}	0.25	***	***

All data were natural logged before tests. Standard errors of 300 lambda values are not reported. The definitions of n , F , P and λ are as in Table 2

The results of models were the same based on either means or maximum/minimum values for the four morphological traits, and therefore only results from means are reported

* $P < 0.05$; *** $P < 0.001$; ns not significant

and habitat preference were reduced to zero, showing that there were no phylogenetic signals within subfamily. The only exception was culm height, which retained a significant λ value of 0.51 ($P < 0.001$ for $\lambda = 0$). Analysis of variance showed that subfamily provided a significant explanation for the variance in all of the traits except culm height, leaf width, water score and shade habitat (Table 2B). These results indicated that, for most traits, the overall phylogenetic signal arose predominantly from the divergence between the two subfamilies. However, phylogenetic differences within subfamily still remained for culm height (Table 2A).

Phylogenetic effects on trait correlations

Leaf width, guard cell length and 1,000-seed weight all showed a statistically significant association with culm height (Table 3; Fig. 2), but none of these relationships showed phylogenetic dependences (all P ns for $\lambda = 0$). There was no significant relationship between guard cell length and leaf width (Table 3; Fig. 2), and only weak phylogenetic dependence ($\lambda = 0.25$, $P < 0.001$ for $\lambda = 0$). Direct associations between plant size traits showed generally positive scaling relationships, and a clear divergence between the two subfamilies (Fig. 2).

All the models for morphological traits versus habitat traits showed strong evidence for phylogenetic signal in the residuals, with λ values ranging from 0.36 to 0.75 (all $P < 0.01$ for both $\lambda = 0$ and $\lambda = 1$). However, the analyses of variance found little evidence that habitat factors could explain variance in morphological traits (all $P > 0.05$ for the three factors), except in the case of habitat water for leaf width, and habitat shade for the 1,000-seed weight (Table 4A). Examination of the two subfamilies separately tended to result in lower λ values that were not significant from zero, except for culm height (Table 4B, C). These

analyses showed that the association between leaf width and water arose within Panicoideae, and the relationship between 1,000-seed weight and shade was within Chloridoideae (Table 4B, C).

When morphological traits were plotted against water score, there were no clear trends. However, if subfamilies were examined separately, it emerged that there were no relationships for genera in Chloridoideae, but loosely positive relationships for genera in Panicoideae. The reason was that, although the two subfamilies show different overall preferences for dry or wet habitats, both occurred across the whole range of habitats (Fig. 1). The difference was that Chloridoideae remained as shorter plants, with narrow leaves and smaller stomata across all habitats (Fig. 1; Table 4B), while Panicoideae tended to develop into large plants with wide leaves in conditions of high water availability (Fig. 1; Table 4C).

Photosynthetic type comparison

Phylogenetic models for morphological and habitat traits which tested subfamily and photosynthetic type as predictors successfully accounted for the phylogenetic signal (P ns for $\lambda = 0$), except in the case of culm height (Table 5). For the M1 models with subfamily as the first factor, most of the variance in traits was explained by subfamily (all $P < 0.05$). However, the M2 models with PT as the first factor showed nearly reversed results, emphasizing PT in explaining variance. Only for culm height was subfamily retained as the main source of variance in both models (Table 5). However, for the other morphological traits, a comparison of the F values between M1 and M2 showed that those for subfamily were higher than those for PT when they were the main explanatory factor (e.g., for leaf width, $F = 26.13$ for subfamily in M1, while $F = 9.27$ for PT in M2; Table 5). Therefore subfamily was a better explanation for variance in culm height, leaf width and 1,000-seed weight. On the same basis, photosynthetic type was a better explanation for the variance in guard cell length and water score. When these traits were separated by photosynthetic type, clear gradients were shown, from highest values in C_3 genera, followed by NADP-me and PCK genera, then NAD-me genera (Fig. 3).

Discussion

Phylogenetic niche conservatism in grass morphology and habitat

Our analyses found significant phylogenetic signals for the morphological traits and habitat preferences, supporting the

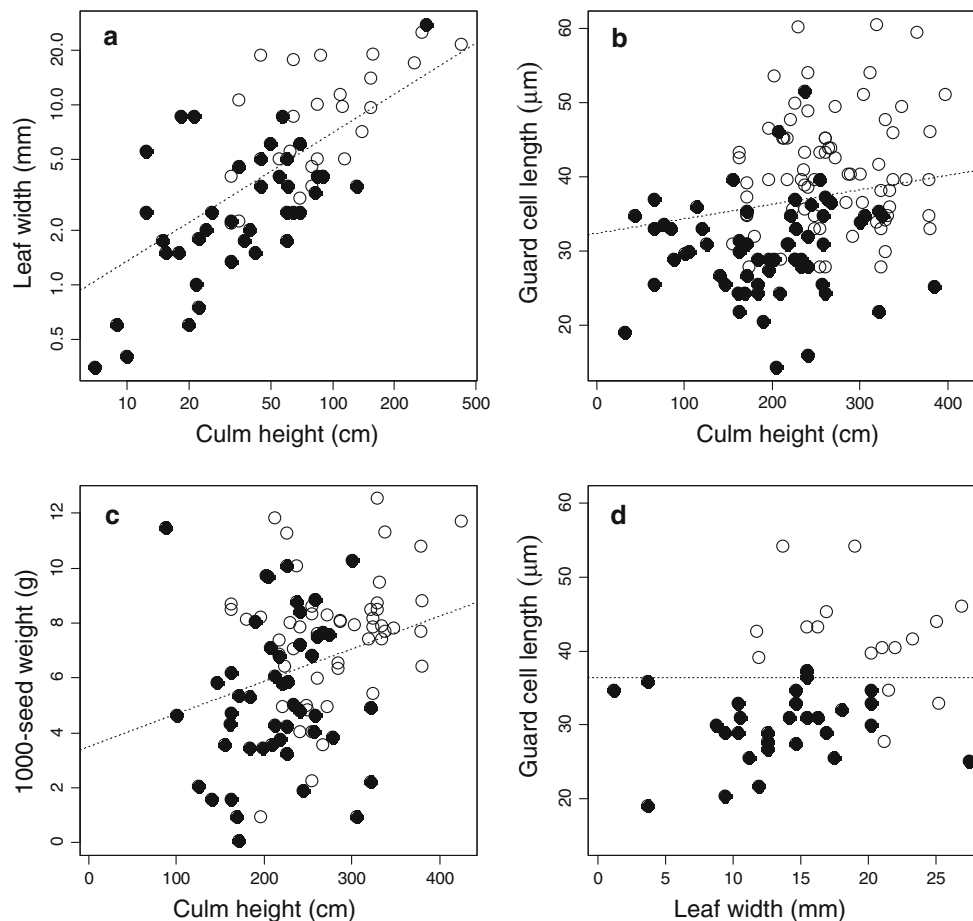


Fig. 2 Relationships of **a** leaf width, **b** guard cell length, **c** 1,000-seed weight with culm height, and **d** guard cell length with leaf width. *Lines* were fitted from phylogenetic linear models in Table 3 rather than direct linear regressions, and therefore indicate the phylogenetically

independent relationship between pairs of traits. Two subfamilies, Chloridoideae (*black circles*) and Panicoideae (*white circles*) are shown

hypothesis of PNC in C_4 grasses. Moderate phylogenetic signals in our results, relative to a drift model, indicated that morphological and ecological traits accumulate their divergences across multiple lineages, although were not very strongly conservative. Phylogenetic signals can be influenced by scale and convergent evolution (Losos 2008) and, as we noted in the “Introduction”, a weaker phylogenetic signal than predicted by a Brownian model is consistent with both traits lability (i.e., weak PNC) and PNC via strong stabilizing selection (Donoghue 2008). Although our results were consistent with both mechanisms, the general pattern seemed to be that most traits exhibited a great deal of variability (especially noting the log transformations; Fig. 1). Hence, it did not seem likely that stabilizing selection was the mechanism generating weak phylogenetic dependence.

Our findings added mechanistic detail to the long-standing observation that different subfamilies and tribes of grasses with the Kranz anatomy typical of C_4 species prefer specific climatic zones (Hartley 1950, 1958a, b; Hartley

and Slater 1960). For instance, the geographic distribution of species diversity and phylogenetic inference indicate an origin of Chloridoideae in xeric habitats of tropical or subtropical Africa during the Oligocene, and this lineage now occupies water limited habitats across the world (Hartley and Slater 1960; Osborne and Freckleton 2009; Bouček-Khelladi et al. 2010; Edwards and Smith 2010), indicating the maintenance of both habitat and morphological niches. Therefore, our data emphasized the importance of considering phylogeny in biogeographic modeling and the assessment of global change impacts on the geographical distributions of species (Edwards et al. 2007).

Another important implication of PNC is that adaptations to climatic changes might not easily be accomplished in all lineages (Donoghue 2008), because trait evolution in some phylogenetic groups will be more sensitive to global changes than others. In our study, most of the phylogenetic signal in traits was generated by a single deep divergence between the two subfamilies (Table 2B), which may be associated to some degree with the different origins of C_4

Table 4 F and λ values for phylogenetic linear models testing the relationships between morphological traits and habitat factors based on 300 phylogenetic trees

	n	Habitat factors (F^P)			λ	$P(\lambda = 0)$	$P(\lambda = 1)$
		Water (w)	Shade (sh)	Saline (sa)			
(A) Whole dataset							
Culm height $\sim w + sh + sa$	155	0.06 ^{ns}	0.90 ^{ns}	1.24 ^{ns}	0.75	***	***
Leaf width $\sim w + sh + sa$	59	5.71*	0.46 ^{ns}	0.18 ^{ns}	0.36	**	***
1,000-seed weight $\sim w + sh + sa$	90	2.67 ^{ns}	5.97*	1.16 ^{ns}	0.36	**	***
Guard cell length $\sim w + sh + sa$	121	0.60 ^{ns}	2.12 ^{ns}	1.74 ^{ns}	0.55	***	***
(B) Chloridoideae							
Culm height $\sim w + sh + sa$	77	0.53 ^{ns}	0.21 ^{ns}	2.66 ^{ns}	0.81	***	*
Leaf width $\sim w + sh + sa$	36	0.62 ^{ns}	0.08 ^{ns}	0.61 ^{ns}	0.27	ns	*
1,000-seed weight $\sim w + sh + sa$	44	2.10 ^{ns}	4.28*	0.12 ^{ns}	0.00	ns	***
Guard cell length $\sim w + sh + sa$	59	0.52 ^{ns}	2.08 ^{ns}	0.42 ^{ns}	0.00	ns	***
(C) Panicoideae							
Culm height $\sim w + sh + sa$	78	1.67 ^{ns}	0.25 ^{ns}	0.20 ^{ns}	0.49	***	*
Leaf width $\sim w + sh + sa$	23	9.72**	0.10 ^{ns}	0.68 ^{ns}	0.00	ns	**
1,000-seed weight $\sim w + sh + sa$	46	2.89 ^{ns}	2.59 ^{ns}	0.96 ^{ns}	0.00	ns	***
Guard cell length $\sim w + sh + sa$	62	0.13 ^{ns}	3.16 ^{ns}	1.76 ^{ns}	0.43	ns	***

Results are for (A) the whole dataset, (B) Chloridoideae only, and (C) Panicoideae only. F and P values are reported for three habitat factors (w water, sh shade, sa saline) in each model. Standard errors of 300 lambda values were not shown. The definitions of n , F , P and λ are as in Table 2
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; *ns* not significant

Table 5 Analysis of variance of the quantitative indices from subfamily and photosynthetic type without phylogenetic effects

	n	Models	Model comparison (F^P)		Lambda test		
			Subfamily	PT	λ	$P(\lambda = 0)$	$P(\lambda = 1)$
Culm height	140	M1	2.49*	1.09 ^{ns}	0.46	*	***
		M2	5.27*	1.17 ^{ns}			
Leaf width	45	M1	26.13***	0.59 ^{ns}	0.00	ns	***
		M2	1.20 ^{ns}	9.27***			
Guard cell length	105	M1	4.93***	1.61 ^{ns}	0.05	ns	***
		M2	2.31 ^{ns}	14.48***			
1,000-seed weight	84	M1	14.03**	0.55 ^{ns}	0.00	ns	***
		M2	0.15 ^{ns}	5.17***			
Water score	120	M1	1.5**	2.91 ^{ns}	0.37	ns	***
		M2	0.18	6.20**			

λ values and model comparisons were from phylogenetic linear models. Data of indices were natural logged. PT included C_3 , C_4 NAD-me, C_4 NADP-me and C_4 PCK, and only genera with certain PT were involved in the tests. Two models were: M1 $y \sim$ subfamily + PT; M2 $y \sim$ PT + subfamily, since direct interaction effects of two factors cannot be tested for the unbalanced dataset. All models were run repeatedly for 300 phylogenetic trees

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; *ns* not significant

photosynthesis in the two subfamilies. One of the earliest origins of C_4 photosynthesis occurred in Chloridoideae, and the C_4 members within this subfamily formed a monophyletic group (Christin et al. 2008). In contrast, C_4 photosynthesis has evolved as many as 15 times in Panicoideae, making these C_4 species polyphyletic (Edwards and Smith 2010). This evolutionary difference may explain the conser-

vatism within Chloridoideae, and the relatively large variations in morphological and habitat traits within Panicoideae (data not shown). Therefore, simply grouping the multiple, independently derived C_4 lineages into a single C_4 functional type may be inappropriate because it conceals important underlying variation in traits at the community or ecosystem level.

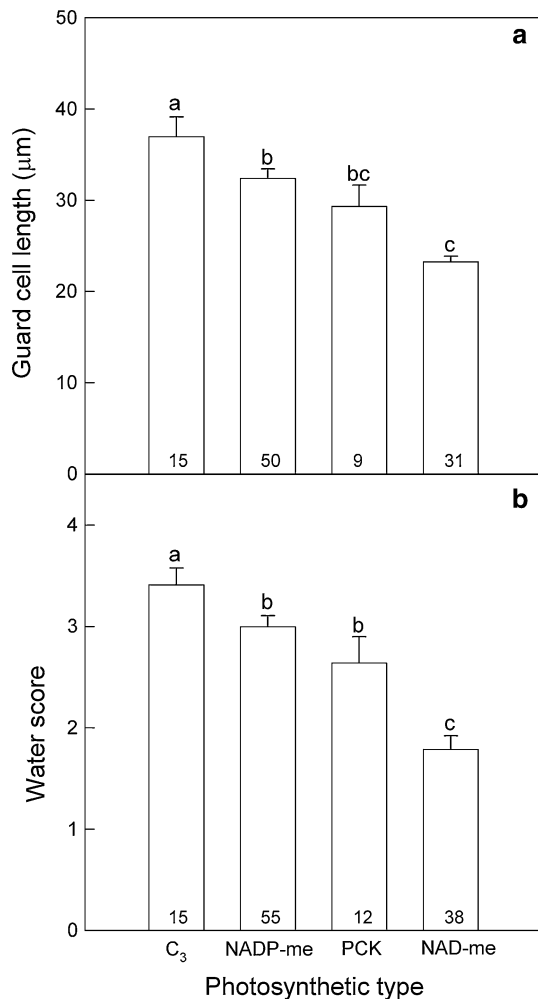


Fig. 3 **a** Guard cell length, and **b** water score for different photosynthetic types. Data are mean \pm SE. Sample sizes are reported at the bottom of each bar. Letters on the top of each bar are multiple comparison results (Tukey HSD)

Finally, we found evidence that morphological traits related to plant size, leaf width, and stomatal guard cell size all evolved in a manner that was broadly consistent with the drift model. Meanwhile, morphological traits also showed evolutionary covariance, confirming an allometric scaling effect that was independent of evolutionary background (Table 3), and which has been reported previously (Poorter and De Jong 1999).

From morphology to habitat and distribution

The associations between morphological and habitat traits in our data exhibited strong phylogenetic signals based on the divergence between subfamilies, suggesting that PNC in both inherited traits and habitat preferences determined the distributions of C₄ grasses. For example, genera of Chloridoideae had smaller plants and stomata than Panicoi-

deae, but we found no statistical evidence of adaptation to habitat characteristics within the former lineage. On the other hand, Chloridoideae also had a greater occurrence in drier and saline habitats, and were less likely to occur in shady environments, suggesting ecological sorting of these traits.

Although habitat water availability is an important factor in defining the environmental niche for plants, it cannot explain some observations. For instance, Eragrostideae exists typically in drier habitats, but many species also occur in wet, disturbed conditions, like some noxious weeds of irrigated croplands (e.g., *Eragrostis minor*). Therefore, we analyzed other dimensions of the ecological niche in our models. First, the different tolerance of shading indicated the potential importance of woody plant cover, especially for Chloridoideae. Woody plant cover affects growth and evaporative demand directly through shading, but is also associated indirectly with other factors such as seasonal aridity, fire occurrence, herbivore density and soil nutrient conditions (Sankaran et al. 2008). Second, our data supported the hypothesis that size-related traits play an important role in determining the ecological niche of grasses. Since competition is expected to be more intense along productivity gradients driven by increasing rainfall gradients, taller plants with bigger leaves will be advantaged. Therefore multiple factors have to be considered within the framework of PNC when investigating the ecological niche of C₄ grasses.

From photosynthetic type to phylogeny

Our results have a number of important implications for future work on the ecological significance of photosynthetic types. First, our data confirm that PNC exists in C₄ grasses and demonstrate that variation in the biology of grasses is explained better by lineage (subfamily) than by C₄ subtype, which is consistent with previous studies (e.g., Hattersley 1983; Taub 2000; Taylor et al. 2010). Second, this finding asserts that phylogeny should be integrated into the design of experiments comparing multiple species of C₄ grasses, because species that inherited their traits from a common ancestor cannot be considered independent replicates (Christin et al. 2009). Finally, lineage-specific differences in biogeography (Gibbs Russell 1988), climatic niche (Edwards and Smith 2010), plant invasion (Burns et al. 2011; Hill and Kotanen 2011), habitat and morphology (this study), and physiology (Edwards et al. 2007; Taylor et al. 2010) make it inappropriate to consider all C₄ grasses as a single functional group in the model simulation of global change impacts on the functioning and biogeography of plants. A growing body of data now supports this proposal, and would be sufficient to begin the necessary model parameterization.

Further progress in this area is likely to be made through improvements in data quality in three areas. First, although our genus level analysis has revealed large-scale macroevolutionary patterns in traits, it is less well suited for investigations of trait coevolution, where heterogeneity within genera blurs relationships. Species-level analyses are likely to sharpen our view of the evolutionary processes at work. Second, future work will benefit hugely from ongoing improvements in our understanding of grass phylogeny (Edwards et al. 2010). These will allow the relationships among subfamilies to be explored in more depth, and to better establish which lineages are monophyletic. Previous work with other plant taxa has shown that the consideration of larger taxonomic scales reveals greater phylogenetic conservatism (Cavender-Bares et al. 2006). And, third, coarse categorical or binary classification of habitat should ideally be replaced by more quantitative measures. Given a precisely mapped set of occurrences for each species, an ecological informatics approach (e.g., Edwards and Smith 2010) can deliver quantitative estimates of habitat characteristics such as vegetation productivity. However, for locally heterogeneous traits such as shading, quantitative field measurements may be the only way forward, bringing major logistic challenges for large samples of species.

Conclusions

This study has demonstrated moderate PNC in the morphological and habitat traits of C_4 grasses, indicating that it is important to consider the variation among different grass lineages, rather than treating them as one C_4 functional group. The presence of phylogenetic signals suggested that both plant morphology and habitat preference may be involved in determining the contrasting distribution patterns of C_4 grass lineages. Our analysis showed the phylogenetically independent correlated evolution of morphological traits, and offered limited evidence for phylogenetically dependent relationships between plant traits and habitat. We suggest that evolutionary lineages rather than photosynthetic type should be the central focus when studying the biodiversity, biogeography, habitat, morphology, and physiology of grasses under global change.

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