

Functional traits of grasses growing in open and shaded habitats

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Abstract The amount of light available for photosynthesis is a key environmental factor that shapes the form and function of plants. Several plant traits affect the manner in which different species fix carbon during vegetative growth. Under the hypothesis that grasses respond to environmental selective pressures, we analyzed the differences in certain leaf, culm, and regenerative traits of 283 native Uruguayan grasses growing in open (grassland) and shaded (forest) habitats. In order to differentiate the phylogenetic effects from the adaptive changes to current local conditions, we used phylogenetically controlled comparative analysis. We found that the divergence of grass species between grasslands and forests was accompanied by changes in leaf traits. Narrow and filiform blades (higher length/width ratio) were favored in species growing in grasslands, while wider and oval blades were favored in species growing in forests. The response of the leaf blades in forests was probably directed towards maximizing light interception, while in grasslands could be linked to the loss of water and heat. In contrast, we found that neither the culm nor the caryopsis length exhibited significant evolutionary changes associated with open or shaded habitats. Our results highlight the functional significance and adaptive value of the width and shape of the grass blades to the current environment.

Keywords Open and shaded habitats · Adaptive divergence · Phylogenetically independent contrasts (PICs) · Functional traits · Forest grasses · Grassland

Introduction

Functional traits are phenotypic traits that influence fitness through biochemical, physiological, morphological or developmental mechanisms (Donovan et al. 2011). A central goal of comparative plant ecology is to understand how functional traits vary among species and to what extent this variation has adaptive value (Reich et al. 2003; Poorter et al.

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2008). A functional trait can be considered adaptive if the phenotype occurring in a particular environment enhances fitness in that environment relative to alternative phenotypic states (Ackerly et al. 2000; Ackerly 2003).

The amount of light available for photosynthesis is a key environmental factor shaping the form and function of plants. Plants that thrive in the deep shade of the understory of a forest are expected to differ in several traits compared to those successful in open, well-lit habitats. Since photosynthesis is directly influenced by the amount of light leaves intercept, most comparative studies focused on the significance of several leaf traits of plants adapted to sunny or shady conditions, and how those traits influenced the plant's photosynthetic response to different light levels (Givnish 1988). For example, leaf mass per area, leaf erectness, nitrogen content, and photosynthetic capacity per area were reported to increase with increasing light availability. In contrast, extended leaf life span is usually associated with shaded habitats (Westoby et al. 2002; Wright et al. 2006; Milla and Reich 2007; Hallik et al. 2009).

Plant growth depends not only on the photosynthetic rate of individual leaves, but also on the geometry and dynamics of the canopy and the energy allocation patterns among all organs. In addition to leaves, non photosynthetic organs like stems, roots, and seeds also vary across species and habitats, affecting the way in which species fix carbon during growth (Westoby et al. 2002). For instance, plant height is a quantitative trait largely studied in comparative plant ecology, as being taller than neighbors confers a competitive advantage through prior access to light (Westoby et al. 2002; Moles et al. 2009). However, such competition involves a trade-off between photosynthetic gains and the energetic cost of supporting leaves and lifting water (Givnish 1982). Thus, it is likely that the density and height of shading competitors determine the net benefit of growing taller (Givnish 1982; Westoby et al. 2002).

A regenerative trait such as seed mass has been related to the light regime. Many studies showed that species established in closed or shaded environments usually have larger seeds than those established in open environments (Salisbury 1942; Baker 1972; Foster 1986; Mazer 1989; Metcalfe and Grubb 1995; Hewitt 1998). Also, seedlings from larger-seeded species perform better under deep shade than those from smaller-seeded species (Leishman and Westoby 1994; Kidson and Westoby 2000; Walters and Reich 2000). However, the enhanced survival ability is confined to the cotyledon phase and does not persist into later seedling life (Saverimuttu and Westoby 1996; Westoby 1998; Leishman et al. 2000; Walters and Reich 2000).

Variation in plant functional traits results from evolutionary and environmental drivers operating at different spatial and temporal scales (Reich et al. 2003). Although natural selection can lead to rapid changes in phenotypes and local adaptation, not all the characteristics possessed by an organism were necessarily selected under the environment in which they currently live. Throughout their evolutionary history, organisms have gained and lost traits. Thus, closely related species tend to be more similar than those more distantly related, partially because their features had less time to diverge. Furthermore, species with a shared evolutionary history tend to occupy the same type of environment as their ancestors (Harvey and Pagel 1991). Consequently, phenotypic and ecological similarities of closely related species or lineages may produce associations between environmental conditions and functional traits (Ackerly 2003), not necessarily having an adaptive significance. Conversely, differences between closely related species occupying different habitats provide strong evidence of adaptive divergence (Ackerly et al. 2000). In order to differentiate phylogenetic effects (or historical reasons) from adaptive changes to current local conditions, phylogenetically controlled comparative methods have been proposed.

The grass family (Poaceae) is a good study system to assess adaptive evolution to different environmental conditions. This is a very diverse family whose members are found on virtually every terrestrial habitat on earth (Kellogg 1998; Gibson 2009). Recently, a clear picture has been formed of the evolutionary history of the family (GPWG 2001; Kellogg 2001; Christin et al. 2008; Vicentini et al. 2008; Strömberg 2011; GPWGII 2012). The original grasses were plants of forest margins or deep shade, characteristics that are retained today in the earliest diverging lineages of the family and in the bamboos (Kellogg 2001). In such habitats, they persisted for many millions of years without much diversification. On the contrary, most of the grasses belong to lineages that extensively radiated once they acquired tolerance to drought and the capacity to grow and thrive in dry open habitats (Jacobs et al. 1999; Kellogg 2001). One of the most novel functional attributes of the grass family identified as an adaptation to drier conditions is the emergence of the C₄ metabolic pathway (Pagani et al. 1999; Edwards and Still 2008). The origin of the C₄ photosynthesis in grasses is one of the most successful ecological and evolutionary innovations in the history of the plants (Christin et al. 2008). The evolutionary selection for C₄ photosynthesis required open environments (Osborne and Freckleton 2009) and C₄ plants rarely colonize forest habitats where less light and lower temperatures prevail (Sage et al. 1999). The ecological success of C₄ species is associated with the expansion of biomes dominated by herbaceous plants, such as savannas and grasslands (Christin et al. 2008; Edwards and Smith 2010).

Grasses also show other variations in traits related to environmental conditions. In the humid tropics, grass leaves are often large, with ovate or oblong blades. In contrast, grass leaves in semiarid regions are often narrow and linear, becoming rolled or folded under drought conditions (Redmann 1985; Gibson 2009). Along a climatic gradient, Oyarzabal et al. (2008) found that leaf size, leaf area (absolute and specific), and plant height decreased, while leaf dry matter content increased in grasses growing in more arid regions. However, these studies do not allow differentiation between adaptive evolution and phylogenetic signal due to the lack of phylogenetic information. Studies that incorporated phylogenetic control in their analysis showed that some grass traits are evolutionarily related to particular environmental conditions. Villar et al. (1998) found that *Aegilops* species growing in habitats with higher annual rainfall assigned more biomass to shoots than to roots and had higher relative growth rates than species adapted to low-rainfall habitats. It has been also suggested that edaphic heterogeneity was central in directing the evolution of alternative persistence strategies and growth forms of *Ehrharta* species (Verboom et al. 2004).

Uruguay is part of the Rio de la Plata grasslands which range from the Flooding Pampa in Argentina to southern Brazil, covering more than 700,000 km² and constituting one of the largest natural temperate sub-humid grassland areas of the world (Soriano 1992). In Uruguay, natural grasslands occupy 71 % of the total surface of the country, while native forests cover 3.6 % (Censo General Agropecuario 2000). Despite the difference in coverage, the set of species of native grasses growing in both types of vegetation constitutes a suitable system to explore the evolutionary relationship between functional traits and environmental conditions.

In this work, we tested for habitat effects on five functional traits of 283 native Uruguayan grasses growing in open or shaded habitats, and investigated whether such effects are independent of phylogeny. Specifically, we used leaf, culm, and regenerative traits to test if the divergence of grass species towards different habitats (grassland vs. forest) was associated with particular shifts in their traits.

Methods

Species information

The list of the native grass species from Uruguay was obtained from the description of Rosengurtt et al. (1970), Zuloaga et al. (1994) and the database of grasses generated by Brazeiro et al. (2008). We followed the nomenclature of the Missouri Botanical Garden (Tropicos.org). To define the set of native grasses belonging to grasslands or forests, we gathered the habitat description of each species reported by Rosengurtt et al. (1970). When the species information was incomplete, we used the database of Brazeiro et al. (2008) which includes information from the herbarium of the Facultad de Agronomía (Universidad de la República, Uruguay).

The following grass traits were analyzed: culm length, blade length, blade width, blade length/width ratio, and caryopsis length. These traits were selected because of their ecological significance in a plant's strategy to cope with energy capture, and because we were able to gather information for the majority of the species considered. The traits values were obtained from the "World Grass Species" database (Clayton et al. 2002). When the data was missing in the database, we used the trait values reported by Rosengurtt et al. (1970). Midpoint values of the reported ranges were considered.

Analysis of grass traits

In order to establish the main patterns of association between the different grass traits and the habitat, Student's *t* tests were conducted without taking into account the phylogeny, and using trait values of taxa (the tips of the phylogenetic tree, TIPs). The trait data were transformed to meet the assumptions required by the *t* test analysis (Zar 1996). Grass species are more common in open habitats than in forests, not only in Uruguay but worldwide as well. Therefore, our estimates from unequal sample size may have been biased, but this bias merely created a conservative comparison by reducing the chances of finding significant differences between traits.

The analysis of traits that incorporated the phylogenetic information of the species was performed using the method of phylogenetically independent contrasts (PICs) developed by Pagel (1992). This method was designed for imperfectly resolved phylogenies and is a generalization of the method developed by Felsenstein (1985). The contrasts are calculated as the standardized differences in trait values between current and/or ancestral pairs of species along the phylogeny. In a fully resolved phylogenetic tree with N species, the number of possible contrasts is $N - 1$, while a phylogenetic tree that includes polytomies produces fewer contrasts (Pagel 1992). Although this method was developed to analyze the relationship between pairs of continuous variables, it allows the inclusion of a dichotomous discrete variable (Purvis and Rambaut 1995). In this case, the discrete variable is assumed to be independent and the continuous variable to be dependent. The contrasts must include one or more species of both categories of the discrete variable. Under the null hypothesis that the evolution in the dependent variable (e.g., culm length) is not related to the evolution of the discrete variable (e.g., open and shaded habitats), half of the contrasts in the dependent variable are expected to be positive and half negative, and the mean value will be zero. Hence, the relationship between a continuous trait and a dichotomous discrete variable can be assessed through a one sample *t* test on the mean of the contrasts of the continuous variable. In this way, a mean value significantly different from zero indicates an association between the variables (Purvis and Rambaut 1995).

The method described above was used to assess the relationship between morphological traits (culm length, blade length, blade width, blade length/width ratio and caryopsis length) and habitat (grassland vs. forest). The contrasts were generated using the Comparative Analysis by Independent Contrast software package (CAIC) developed by Purvis and Rambaut (1995). We assumed equal length in all branches of the phylogeny. Before analyzing the standardized contrasts, we corroborated that the phylogenetic correlations were completely removed (Garland et al. 1992). In accordance with the predictions of the model, the absolute value of the standardized contrast must be independent of the estimated value of the character at the node at which the contrast was taken. Consequently, in the cases where significant correlations between variables were found, the contrasts were recalculated using the log-transformation of the trait values (Purvis and Rambaut 1995; Freckleton 2000). All statistical tests were performed using the statistical software PAST, version 2.09 (Hammer et al. 2001).

Phylogenetic relationships

The most comprehensive phylogenetic hypothesis for the subfamilies of grasses was proposed by the Grass Phylogeny Working Group (GPWG 2001; GPWGII 2012), combining eight sets of molecular and morphological data. According to the GPWG, the Poaceae family includes 12 subfamilies. Using the GPWG (2001) combined tree as the backbone, we assembled published relationships within subfamilies and within tribes: Zhang (2000) (Bambusoideae subfamily), Ge et al. (2002), Guo and Ge (2005) (Ehrhartoideae subfamily), Cialdella et al. (2007) (Stipeae tribe), Quintanar et al. (2007) (Soreng et al. (2007) (Poeae tribe), Petersen and Seberg (2003) (Triticeae tribe), Hilu and Alice (2001) (Chloridoideae subfamily), Duvall et al. (2001), Giussani et al. (2001), Aliscioni et al. (2003), Kellogg et al. (2004), Bess et al. (2005) (Panicoidae subfamily) and Mathews et al. (2002) (Andropogoneae tribe). When phylogenetic information was unavailable, we used taxonomic relationships. Taxonomies are suboptimal compared with phylogenetic information, but better than assuming that all taxa in a group are equally related (Tullberg and Hunter 1996; Silvertown and Dodd 1997). We used the taxonomy proposed by the GPWG (2001), and the genera were assumed to be monophyletic groups. Finally, the compiled phylogenetic tree included all the native Uruguayan grasses with different levels of resolution. A number of polytomies at the level of species and genera remained unresolved.

Results

The Poaceae family in Uruguay

The native grass flora of Uruguay comprises 340 species of the approximately 11,000 species of the Poaceae family, and accounts for 9 of the 12 subfamilies recently recognized (Duvall et al. 2007; Sánchez-Ken et al. 2007; GPWGII 2012). The subfamily Pooideae and the PACMAD clade (an acronym for Panicoideae, Arundinoideae, Chloridoideae, Micrarioideae, Aristidoideae, Danthoioideae subfamilies) are the most highly represented groups, and are widely diversified in open habitats. The remainder subfamilies are smaller groups. The species of the subfamilies Pharoideae and Bambusoideae, with one and five species respectively, grow in shaded habitats under native forests (ancestral habitat of the Poaceae family, Kellogg 2001). The Ehrhartoideae is represented by 4 species, 3 of which

grow in hydrophytic habitats (the ancestral habitat of the subfamily, Kellogg 2009), and one grows in seasonally flooded grasslands (Fig. 1).

The native Uruguayan grasses are found in virtually all natural habitats of the area. However, the highest richness is observed in grasslands, which account for 74 % of the species (253 spp.) while forests concentrate near 9 % (30 spp.). The remainder species are distributed among hydrophytic habitats, disturbed places, and other habitats (Fig. 2). There is an evident segregation of the photosynthetic metabolism among the different habitats. In grasslands, there is a bias towards the C_4 photosynthetic pathway, while in forests, C_3 grasses are dominant ($\chi^2 = 9.39$, $df = 1$, $P < 0.002$).

Grasses of grasslands and forests—TIPs

Grasses growing in grasslands and forests showed significant differences in culm length ($t = 2.838$, $df = 276$, $P < 0.005$), blade width ($t = 9.693$, $df = 281$; $P < 0.001$), and blade shape ($t = 8.586$, $df = 278$, $P < 0.001$, Fig. 3). The species in grasslands showed shorter culms and narrower filiform blades (higher length/width ratio) than grasses growing in forests (Fig. 3a, c–d, respectively). The difference in culm length was preserved even when bamboos were removed from the analysis ($t = 2.133$, $df = 272$, $P < 0.05$). The leaf blade length and the caryopsis length did not differ significantly between habitats (Fig. 3b, e).

Within grasslands, C_3 and C_4 species exhibited significant differences ($P < 0.05$) in blade width, blade length/width ratio, and caryopsis length (Fig. 3c–e, respectively).

Habitat effects on the grass traits—PICs

Eighteen contrasts were generated in the phylogenetically independent contrast analysis (Fig. 4). The divergence of species towards different habitats (grassland or forest) was accompanied by changes in the width and shape of leaf blades. The analysis showed that in grasslands, grasses blades are narrower ($t = 5.912$, $df = 17$, $P < 0.001$) and more filiform ($t = 4.162$, $df = 17$, $P < 0.001$) than in shaded habitats (Fig. 5, Table 1). Such changes were independent of the photosynthetic metabolism as 13 of the 18 contrasts generated for

Fig. 1 Phylogeny of the grass family (Poaceae) according to GPWGII (2012). The subfamilies marked in *bold* are those represented in the flora of Uruguay. *Numbers in parentheses* indicate the number of native species relative to the total number of species of each subfamily. The *cross bars* on the branches indicate shift to open habitats (Kellogg 2001)

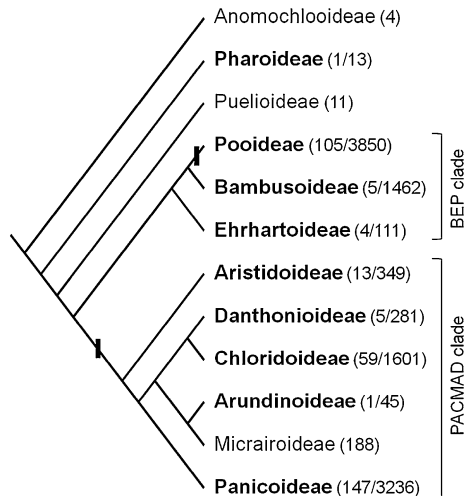
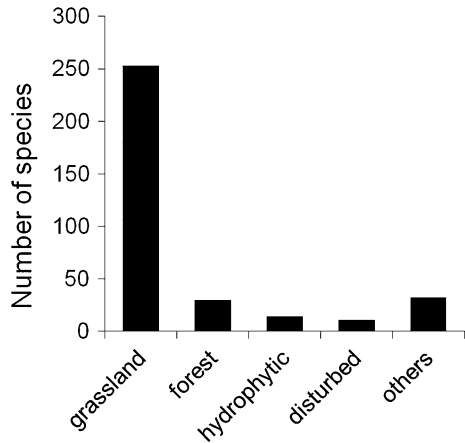


Fig. 2 Distribution of native Uruguayan grasses in the main habitats



the habitat comprised pairs of C_3 or C_4 taxa showing the same type of evolutionary change in the leaf blades: wider and oval blades in shaded conditions compared to the sister groups in grasslands.

No evolutionary effect of the habitat was found for the culm length, leaf blade length, and caryopsis length.

Discussion

The availability of habitats in the Uruguayan landscape underlies the distribution of species in the different clades of the phylogenetic tree. Most of the native grasses (97 %) belong to groups widely diversified in open habitats such as the Pooideae subfamily and the PAC-MAD clade. About 75 % of these species belong to grasslands and contribute to the high taxonomic diversity of this habitat. The remainder species of these clades grow in other exposed habitats, and some returned to the ancestral shaded habitat and grow in forests.

As we expected, native grasses responded to habitat pressure. The patterns exhibited by certain morphological traits were the result of evolutionary convergence. When phylogenetic information was incorporated in the analysis, we observed that forest species had wider and oval blades compared with the narrow and filiform blades of their sister groups growing in grasslands. In forests, the adaptive response of the leaf blade could be related to maximizing light interception, while in grasslands it could be linked to the loss of water and heat. In shaded habitats, the acquisition of broad oval leaves, increasing the surface area, enhances the amount of light intercepted. Although there is a balance between intercepted radiation and water loss, the risks of increasing the water loss by increasing the leaf surface may not be a major threat in shaded habitats (Parkhurst and Loucks 1972; Givnish and Vermeij 1976). In grasslands, plants are faced with high radiation loads and high temperature. The ability of a leaf to survive and function depends on its ability to maintain temperature within an acceptable range. Opening the stomata to allow cooling through latent heat exchange risks severe wilting and even death. But stomatal closure to restrict water loss will also reduce latent heat loss, potentially causing the leaf to heat to lethal temperatures (Gurevitch et al. 2006). The greater exposure to heat and water loss through transpiration can be offset by the acquisition of narrow and filiform shapes. The size and shape of the leaves, together with the wind speed determine the thickness of the

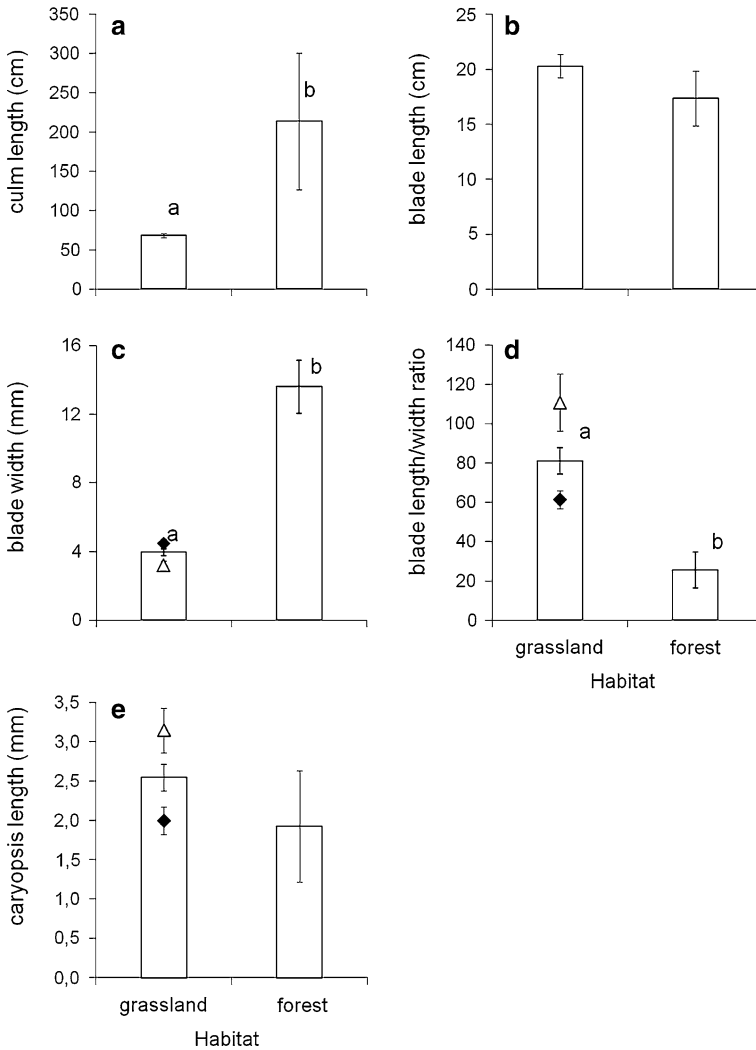


Fig. 3 Plant traits of native Uruguayan grasses growing in grasslands and forests **a** culm length, **b** blade length; **c** blade width; **d** blade length/width ratio; and **e** caryopsis length. Values are mean \pm SE. Letters above bars denote significant differences ($P < 0.05$). Within grasslands, C₃ (open triangle) and C₄ (filled square) species showed significant differences in **c**, **d** and **e** ($P < 0.05$)

boundary layer of the surrounding still air. Small and narrow leaves have a thinner boundary layer which could increase transpiration, but allows greater convective cooling, usually with a net reduction in water loss through evapotranspiration (Vogel 1968; Givnish 1979; Cunningham et al. 1999). Small leaves are likely to remain close to air temperature, even with closed stomata, as the slightest breeze will minimize their boundary layer resistance. Therefore, reducing leaf size helps to maintain favorable leaf temperatures and higher photosynthetic water-use efficiency under a combination of high solar radiation and low water availability (Parkhurst and Loucks 1972; Givnish and Vermeij 1976; Ackerly et al. 2002).

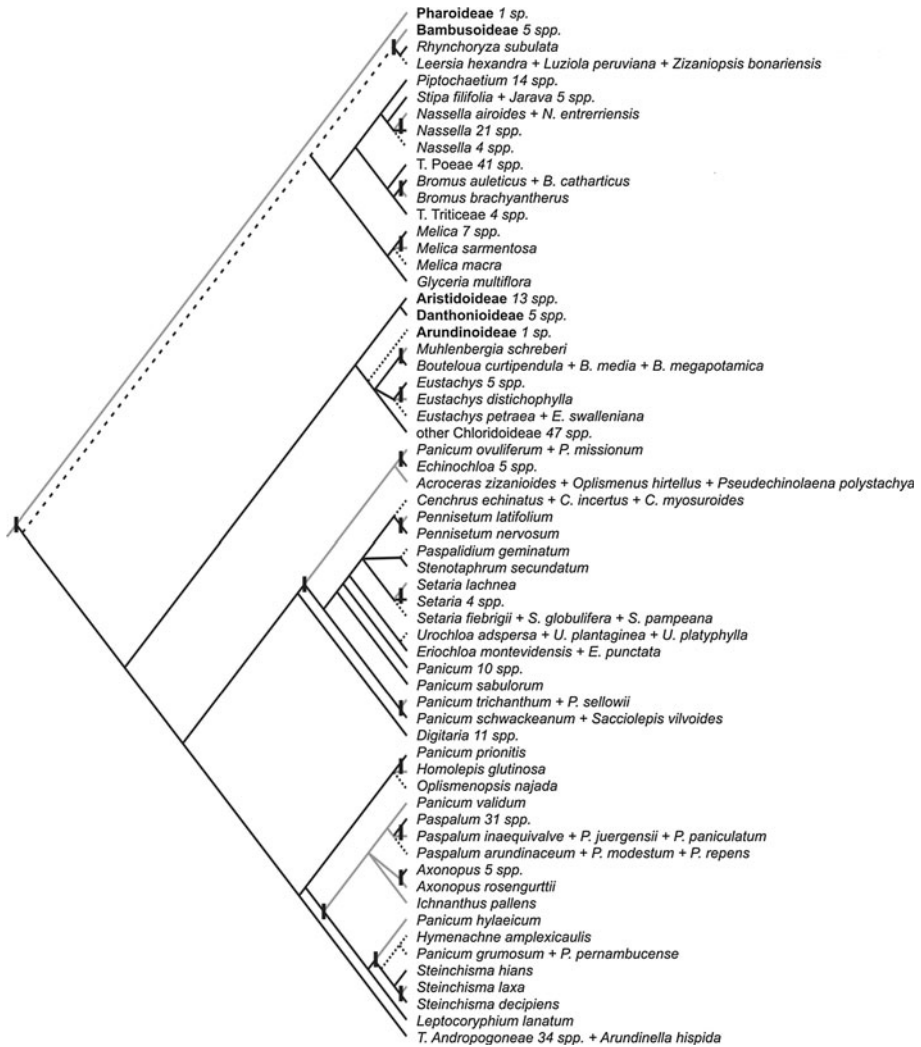


Fig. 4 Reconstruction of habitat on the phylogeny of 340 native Uruguayan grasses. The phylogenetic relationships follow GPWG (2001). Separate genera are shown only for the subfamilies with variation in habitat preference. Eighteen contrasts (cross bars) were created working from the terminal taxa to the basal branches of the phylogeny. Each contrast consists of sister groups carrying each of the states considered in the analysis, e.g. grassland and forest. *Black lines*, grassland; *grey lines*, forest; *dotted lines*, other habitats; *hatched lines*, equivocal

The C₄ photosynthetic pathway is common in grasses growing in open habitats such as grasslands. Higher levels of exposure to solar radiation and temperature occur in this habitat. Under such conditions, the evaporative demand and the probability of water stress increase, and C₄ plants are favored due to their greater water-use efficiency (Ehleringer and Monson 1993; Sage 2004). Despite the general view that C₄ photosynthesis is advantageous in more arid conditions, recent work has shown that C₄ NADP-me grass species are metabolically more sensitive to drought than their C₃ relatives and exhibit a slower

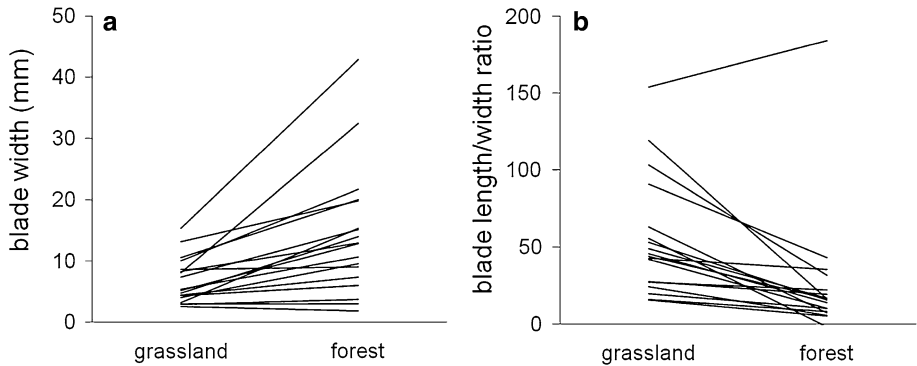


Fig. 5 Blade width (a), and blade length/width ratio (b) contrasts for 18 pairs of sister grasses (extant and/or ancestral) that diverged to different habitats, grassland and forest

Table 1 Relationships between grass traits and habitat according to the phylogenetically independent contrasts method of Pagel (1992)

Traits	Open habitat (grassland)–Shaded habitat (forest) (matrix of 283 spp.)			
	No. of spp. computed	No. of PICs	<i>P</i>	TIPs
Culm length	278	18	n.s.	<0.005
Blade length	280	18	n.s.	n.s.
Blade width	283	18	<0.001	<0.001
Blade length/width ratio	280	18	<0.001	<0.001
Caryopsis length	146	8	n.s.	n.s.

The significance of these relationships, treating each species as a data point (TIPs) is shown in the last column

recovery from drought (Ripley et al. 2010; Taylor et al. 2011). These results suggest that the significance of the C_4 photosynthetic and water-use efficiency will depend both on drought severity and rainfall frequency. Alternatively, C_3 grasses are favored in forests as they tolerate low light intensities, while most of the C_4 plants are intolerant to shade (Sage et al. 1999). However, photosynthetic metabolism did not affect the evolutionary changes in the leaf blades, at least in the direction of the change. Most of the contrasts generated for the habitat (grassland vs. forest) were uniform in photosynthetic metabolism, consisting of pairs of taxa that were either both C_3 or both C_4 . The divergence of species to different habitats was accompanied by the same adaptive change in the leaf blades for both C_3 and C_4 pairs; wider and oval blades in shaded conditions compared to its sister group in grassland.

Despite the difference found for the culm length on the TIPs (Fig. 3), the phylogenetically controlled analysis showed that changes in the habitat were not accompanied by particular changes in this trait. However, short stature is a well known characteristic of many grasses that are adapted to more arid conditions (Coughenour 1985; Milchunas et al. 1988). Plants under stronger radiation and where water is in short supply invest in root mass to improve water uptake and compensate for higher transpiration losses (Givnish 1987; Westoby et al. 2002). In contrast, competition for light in shaded habitats promotes

vertical growth, leading to taller statures that facilitate escaping from low-light environments (Coughenour 1985; Poorter 1999; Westoby et al. 2002). Nevertheless, plants grow taller until the expected photosynthetic gain is balanced by the structural cost to support tissues needed to ensure mechanical stability. In areas with dense plant covering, an increment in height confers an advantage over competitors and favors taller plants. But, if competing foliage is sparse, there is little photosynthetic advantage to an increment in leaf height (Givnish 1982, 1987). In this sense, Givnish (1982) found a close relationship between leaf height of forest herbs and the average herbaceous cover in temperate deciduous forests located in Virginia, USA. In Uruguay, forests are predominantly riparian, accompanying the numerous water courses that cross the country. The cover of the herbaceous stratum is low and is composed mostly by ferns and species of the families Poaceae, Fabaceae and Asteraceae (Brussa and Grela 2007). Therefore, it is likely that the low density of the herbaceous vegetation does not constitute a selective force towards taller plants.

Still, the effects of the habitat on the evolution of the culm length should not be excluded from our results. A posteriori estimation of the statistical power of PICs analyses indicated a low probability (power = 0.16) of detecting significant differences from the generated contrasts ($n = 18$). Comparative analyses using PICs lose statistical power when the number of generated contrasts is low. This problem is particularly true in comparisons between continuous and discrete variables, as there may be few contrasts for the discrete trait between related species. Further loss of power occurs with partially resolved phylogenies (Ackerly and Reich 1999).

According to the Salisbury hypothesis (1942), plants in shaded habitats have larger seeds than plants in open habitats. The adaptive significance of seed size is related to the seedling establishment conditions. Larger-seeded species perform better under light-limited conditions because the seedlings can grow at the expense of reserves, increasing their establishment success and competitive ability. Also, larger-seeded species tend to mobilize their metabolic resources over a longer period (Westoby 1998; Kidson and Westoby 2000; Leishman et al. 2000). This “reserve effect” (Westoby et al. 1996) allows species to hold a bigger percentage of seed reserves uncommitted to seedling structure, and available to support respiration or repair damage. However, studies designed to test this hypothesis including phylogenetic information showed different results. Within the British flora, Hodkinson et al. (1998) found a positive correlation between seed mass and shaded habitats, while Kelly (1996), reanalyzing the data from Salisbury (1974), did not observe such relationship. In the case of the native Uruguayan grasses, caryopsis size was not associated with the habitat according to the analysis with and without consideration of phylogeny, suggesting that this trait is phylogenetically conserved.

In summary, the results presented in this study show that the blade width and shape of grasses are traits that evolutionarily responded to the habitat. The agreement between TIPs and PICs analyses indicates that the relationship is independent of the phylogeny, and highlights the functional significance and adaptive value of these traits to the current environment. The incorporation of a phylogenetic framework is important in ecology not only because it highlights the historical context in which species have evolved, but also because it is a useful tool to identify the causal mechanisms behind observed patterns at the community level.

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