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Letter

C_4 photosynthesis evolved in warm climates but promoted migration to cooler ones

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Abstract

C_4 photosynthesis is considered an adaptation to warm climates, where its functional benefits are greatest and C_4 plants achieve their highest diversity and dominance. However, whether inherent physiological barriers impede the persistence of C_4 species in cool environments remains debated. Here, we use large grass phylogenetic and geographic distribution datasets to test whether (i) temperature influences the rate of C_4 origins, (ii) photosynthetic types affect the rate of migration among climatic zones, and (iii) C_4 evolution changes the breadth of the temperature niche. Our analyses show that C_4 photosynthesis in grasses originated in tropical climates, and that C_3 grasses were more likely to colonize cold climates. However, migration rates among tropical and temperate climates were higher in C_4 grasses. Therefore, while the origins of C_4 photosynthesis were concentrated in tropical climates, its physiological benefits across a broad temperature range expanded the niche into warmer and enabled diversification into cooler environments.

[150 words]

INTRODUCTION

Temperature varies significantly over the surface of the Earth and through time, and is considered the primary factor determining the global distributions of plant species (Woodward 1990; Larcher 2003). During evolution, plants have colonized almost all possible temperature niches (Kier *et al.* 2005; Araújo *et al.* 2013) via a variety of biochemical, physiological and growth adaptations to either cool or warm temperatures (Sakai & Larcher 1987; Larcher 2003). One particular dimension of the temperature niche is the capacity to gain net benefits from photosynthesis under the ambient temperature conditions. Net photosynthetic gains are damped at higher temperatures because CO_2 fixation by Rubisco is offset by competition with O_2 fixation, boosting the costly photorespiratory cycle (Ehleringer & Björkman 1977; Sage & Kubien 2007). Some plants have evolved CO_2-concentrating mechanisms that minimize photorespiration and, in land plants, one of the two successful mechanisms for doing this is C_4 photosynthesis (Ehleringer & Björkman 1977; Sage & Monson 1999; Still *et al.* 2003; Sage *et al.* 2012).

C_4 photosynthesis is a physiological process resulting from a series of biochemical and anatomical modifications over the ancestral C_3 photosynthetic type (Hatch 1987). Together, these concentrate CO_2 around Rubisco, thereby increasing its efficiency and reducing photorespiration (von Caemmerer & Furbank 2003; Sage *et al.* 2012). However, the extra biochemical reactions required for C_4 photosynthesis increase the energetic cost of carbon fixation, so that C_4 photosynthesis is predicted to outperform C_3 only when photorespiration is significant, especially under high temperatures (Ehleringer & Björkman 1977; Osborne & Beerling 2006). The improved performance of C_4 plants at high temperatures predicted from physiological measurements and theory is supported by global distribution patterns (Ehleringer & Björkman 1977; Ehleringer 1978; Griffith *et al.* 2015). Indeed, while trees are almost all C_3, open biomes are predominantly occupied by C_4 species in hot to warm climates, and cooler open biomes are dominated by C_3 species (Sage *et al.* 1999b; Edwards *et al.* 2010).

The majority of C_4 species belong to the grass family (Poaceae) (Sage *et al.* 1999a). At large scales, C_3 and C_4 grasses sort largely according to temperature (Ehleringer & Björkman 1977; Ehleringer 1978; Sage *et al.* 1999a; Osborne *et al.* 2014), and C_4 grasses dominate most open biomes in tropical and subtropical areas, where they achieve greater biomass and higher growth rate (Still *et al.* 2003; Edwards *et al.* 2010; Atkinson *et al.* 2016). Despite these general patterns, low temperatures do not completely exclude C_4 grasses. A number of C_4 grass species are found in alpine, steppe or high latitude habitats where they survive cold conditions during the growing season, with some species developing physiological cold acclimation to tolerate freezing (Long *et al.* 1975; Beale & Long 1995; Sage & Sage 2002; Márquez *et al.* 2006; Liu & Osborne 2008, 2013; Sage *et al.* 2010). These examples demonstrate that C_4 species can survive and compete with C_3 species in cold environments, at least under some circumstances.

The rarity of C_4 plants in cold climates might be explained by inherent physiological constraints on the amounts or activities of key photosynthetic proteins (Sage & Kubien 2007). However, it is also possible that the current geographical distributions reflect historical processes, with C_4 plants evolving in tropical and subtropical climates and inheriting traits that are poorly suited for cooler climates (Long 1999). Differentiating these scenarios requires large-scale comparisons of C_3 and C_4 lineages, while considering their evolutionary history. Past comparative work has shown that C_4 grasses emerged from tropical C_3 lineages (Edwards & Still 2008; Edwards & Smith 2010), and suggested that C_4 evolution in specific clades enabled a niche expansion into both cooler and warmer climates (Christin & Osborne 2014; Lundgren *et al.* 2015; Aagesen *et al.* 2016). However, systematic tests for an effect of photosynthetic types on transition rates among temperature biomes are lacking.

In this study, we use phylogenetic and geographic distribution data for 2,133 grass species (a fifth of all grass species), including 948 C_4 species (a fifth of all C_4 grass species) representing 18 independent C_4 lineages (most of the 24 C_4 groups), to assess the relationships between photosynthetic types and temperature niches. Using comparative analyses, we first test for an effect of temperature on evolutionary transitions between the C_3 and C_4 photosynthetic types as well as the influence of these photosynthetic types on the migration of plant lineages among climatic zones. We then evaluate quantitatively the effect of the photosynthetic type on temperature niche breadth. Our investigations shed new light on the interplay between physiology and evolutionary history in determining the sorting of plants across the ecological space.

METHODS

Climate dataset

All available geo-referenced occurrence records (~14.3M) for the grass family (Poaceae) were extracted from the Global Biodiversity Information Facility (GBIF) web portal (http://www.gbif.org, accessed 10th December 2015). Records were cleaned to filter out unreliable location data using the following steps. First, duplicate records from the same location were removed. Potentially incorrect geographical data were then excluded, including those with coordinates outside the map, where the country did not match the coordinates, within 20 km of the GBIF headquarters, where longitude and latitude were exactly the same because these may represent a typo or default value, or where the georeference was recorded to a precision fewer than three decimal places. The species names were then checked against the Kew grass synonymy database (Clayton *et al.* 2006) via the software package Taxonome (Kluyver & Osborne 2013), and records without a valid name were discarded. The species with less than ten occurrences were also excluded to increase accuracy of the temperature range estimated for each species. For each set of coordinates, we extracted the mean and minimum temperatures of the coldest month, and the mean temperature of the warmest month from WorldClim version 2, 30-arc seconds resolution data (Fick & Hijmans 2017). The median and 5th and 95th percentiles from each species were considered in further analyses, to investigate range limits while avoiding extreme outliers. The temperature range occupied by each species was estimated as the difference between the 5th percentile of the coldest month and 95th percentile of the warmest month (Quintero & Wiens 2013).

Phylogenetic tree

We used a published time-calibrated phylogenetic tree that includes 3,595 species of grasses, covering the whole family and using a time-calibration hypothesis based upon macrofossils and microfossils (Spriggs *et al.* 2014). Taxa without occurrence data after filtering were pruned from the tree, resulting in a dataset of 2,133 species with both phylogenetic and distribution data. Comparison of the proportions of C_3 and C_4 species in each climatic region between those available in GBIF and the subset included in the phylogenetic tree shows that the filtering by the phylogeny did not bias the dataset (Table S1 and Figure S1). Each analysis was first conducted on the whole grass family. However, C_4 origins only exist within one of the two major clades of grasses, the PACMAD clade (Grass Phylogeny Working Group II 2012). We therefore repeated the analyses considering only this clade of 1,197 species to check the robustness of results.

Modelling transition rates between photosynthetic and climatic types

The aims of this analysis were to test whether climatic regions influence the rates of transitions among photosynthetic types, and whether photosynthetic types in turn influence the rates of transitions among climatic types. The photosynthetic type of each species was assigned based on the literature (Osborne *et al.* 2014). Climate was categorized using the Köppen-Geiger classification, as the analysis required binary characters, and this is a well-established and widely known climate scheme (Peel *et al.* 2007). Specifically, we used three climatic types, based on temperature: tropical (mean temperature of the coldest month above 18 °C); temperate (mean temperature of the coldest month between 0 and 18 °C); and cold (mean temperature of the coldest month below 0 °C, which includes continental, polar and alpine climates). The temperate range was further divided into freezing and non-freezing conditions, since freezing represents a particular physiological challenge to plants (Sakai & Larcher 1987). Minimum temperature of the coldest month was used to identify regions that are exposed to freezing. Climate types were assigned based on species median values.

Transition rates among photosynthetic and climatic types were estimated for each pair of climate classes that are adjacent on the temperature gradient: (i) tropical vs temperate without freezing; (ii) temperate without freezing vs temperate with freezing; and (iii) temperate with freezing vs cold. For each independent combination of adjacent climates, Pagel's method (Pagel 1994, 1999; Pagel & Meade 2006) was used to model the eight possible evolutionary transitions between the four states (two adjacent climates multiplied by two photosynthetic types). The model was fitted using a maximum likelihood method to derive point estimates of log-likelihoods in the package BayesTraits (Pagel & Meade 2006).

By fixing some parameters, we tested four hypotheses for each pair of adjacent climates using likelihood ratio tests on nested models. First, we tested whether the rate of transitions from warmer to cooler climates (i.e. tropical to temperate without freezing, temperate without freezing to temperate with freezing, or temperate with freezing to cold climates) differs between C_3 and C_4 lineages (by contrasting rates of 1→2 and 5→6, 2→3 and 6→7, or 3→4 and 7→8, Fig. 1). Second, we tested whether the rate of reverse transitions from cooler to warmer climates differs between C_3 and C_4 lineages (by contrasting rates of 2→1 and 6→5, 3→2 and 7→6, or 4→3 and 8→7, Fig. 1). Third, we tested whether the rate of transitions from C_3 to C_4 states differs between warmer and cooler climates (by contrasting rates of 1→5 and 2→6, 2→6 and 3→7, or 3→7 and 4→8, Fig. 1). Finally, based upon previous work suggesting that reversions from C_4 to C_3 photosynthesis are unlikely (Christin *et al.* 2010; Grass Phylogeny Working Group II 2012), we tested whether transitions from C_4 to C_3 are possible in either of the two climates (by contrasting rates of 5→1, 6→2, 7→3, and 8→4 with a rate fixed to zero, Fig. 1).

Bias in the underlying species sampling could theoretically influence the results of these tests if either C_3 or C_4 species within a particular climate regime were under- or over-represented. The GBIF database has a known bias, with particular regions being well sampled (e.g. Europe, North America, Australia) and other regions being poorly covered, especially in the tropics (e.g. India, parts of Africa). However, this bias only creates problems for our analysis if C_3 or C_4 species are differentially sampled within tropical regions, and we can think of no reason why this should be the case. On the other hand, the phylogenetic tree is likely biased as taxa judged interesting for a variety of reasons would be preferentially sequenced. However, because the sampling of species from the phylogeny is filtered by the availability of GBIF, the final dataset is representative of GBIF without any bias from phylogeny (Table S1 and Figure S1).

To visualize the historical transitions between climatic types of C_3 and C_4 species, ancestral values were computed for climatic types using the ace function in the ape package in R (Pagel 1994; Paradis *et al.* 2004) and the most likelihood climate of ancestors was mapped for each node on the phylogenetic tree using the ggtree package in R (Yu *et al.* 2017).

Phylogenetic comparisons of temperature niches

Phylogenetic generalized least squares (PGLS) were used to confirm that the photosynthetic type influences thermal maxima and minima as well as the breadth of the temperature niche, as suggested previously with smaller datasets (Lundgren *et al.* 2015; Aagesen *et al.* 2016). Temperature was the independent variable, with photosynthetic type as the categorical predictor. Phylogeny was incorporated to control for phylogenetic dependence and to estimate the phylogenetic signal using Pagel's λ (Pagel 1999; Freckleton *et al.* 2002).

RESULTS

Climatic distributions of C_3 and C_4 species

The percentage of C_4 species decreases from tropical to cold climates (Table S2), with more than ten per cent of C_4 species available in GBIF occurring in freezing temperate or cold climates (Table S1), the latter in our dataset of 2,133 species being colonized by C_4 species from three different subfamilies (Table S2), including a number of perennial species. The predominance of C_3 species in cold climates mainly reflects the success of members of the Pooideae subfamily, although other groups are also represented (Table S2). Yet only members of Pooideae can inhabit areas where the mean temperature of the coldest month is lower than -10 °C (Fig. 2).

Rates of transition among photosynthetic types and climates

Models were used to test whether climate influences transitions among photosynthetic types. They supported the hypothesis that C_4 origins are more frequent in tropical than temperate climates (rates of $1 \rightarrow 5 > 2 \rightarrow 6$; $p < 0.001$; Fig. 1; Table 1). The ancestral state reconstructions confirmed that C_4 photosynthesis evolved from C_3 PACMAD ancestors in tropical climates, while the ancestor of Danthonioideae moved to temperate climates, with descendants that remained C_3 (Fig. 3). The rate of C_4 origins is not significantly different from zero in freezing temperate and cold climates (rates of $3 \rightarrow 7$, $4 \rightarrow 8 = 0$; $p > 0.05$; Fig. 1; Table 1), but it is greater than zero in non-freezing temperate climates (rate of $2 \rightarrow 6 \neq 0$; $p < 0.05$; Fig. 1; Table 1). Based on our models, the rate of transition from C_4 to C_3 types is not significantly different from zero under any climatic conditions (rates of $5 \rightarrow 1$, $6 \rightarrow 2$, $7 \rightarrow 3$, $8 \rightarrow 4 = 0$; $p > 0.05$; Fig. 1; Table 1), which is consistent with previous conclusions that the rate of reversal from C_4 to C_3 is extremely low or null in grasses (Christin *et al.* 2010; Grass Phylogeny Working Group II 2012).

The same models were used to test whether the photosynthetic type influences transitions between climatic zones. The rates of transitions between tropical and non-freezing temperate climates across the whole family and in the PACMAD clade are significantly higher in both directions in C_4 than C_3 taxa (rates of 5→6 > 1→2 and 6→5 > 2→1; $p < 0.001$; Fig. 1, S2; Table 1, S3). The ancestral state reconstructions indicate that these transitions occurred many times since the split of Chloridoideae, Panicoideae and Aristidoideae (Fig. 3). Moreover, C_4 photosynthesis increases the rate of transition from temperate climates with freezing to those without freezing (rates of 7→6 > 3→2; $p < 0.001$; Fig. 1; Table 1). The shift to occupy temperate climates without freezing during the winter therefore occurred more frequently in C_4 than C_3 taxa (Fig. 3). The rate of transition from temperate climates without freezing to climates with freezing was also higher in C_4 than C_3 types, but only in the analysis of the PACMAD clade (rates of 6→7 > 2→3; $p < 0.05$; Fig. S2, Table S3). The transition from non-freezing to freezing conditions was found commonly in C_4 PACMAD lineages, but the shift also occurred frequently in C_3 Pooideae (Fig. 3), leading to equal rates when considering the grass family as a whole (rates of 6→7 = 2→3; $p > 0.05$; Fig. 1; Table 1). However, transitions from temperate to cold climates are more frequent within C_3 than C_4 lineages (rates of 7→8 < 3→4; $p < 0.001$; Fig. 1, Table 1), while the rate of transitions in the opposite direction, from cold to temperate climates, is independent of the photosynthetic type, with both C_3 and C_4 grasses moving at the same rate (rates of 4→3 = 8→7; $p > 0.05$; Fig. 1; Table 1). Phylogenetic reconstructions suggest a few transitions to cold climates within C_4 groups, while the large Pooideae C_3 clade migrated early to cold climates and diversified there (Fig. 3).

Evolution of temperature niche breadth

In our study, temperature extremes and ranges were used to confirm that differences exist between C_3 and C_4 plants in the breadth of the temperature niche. The PGLS analyses indicate that C_4 evolution led to an expansion of the species-level temperature niche (Table 2). C_4 photosynthesis is specifically associated with increases in the upper bound of the species range toward higher temperatures during the warmest month (Table 2), mirroring previous conclusions with a smaller species sampling (Aagesen *et al.* 2016). By contrast, the lower bounds of the temperature ranges within species did not differ significantly between C_4 and C_3 groups, which indicates that C_4 evolution does not affect the lower range of the temperatures that are occupied (Table 2). The conclusions remained the same whether the comparison was made across all grasses or just the PACMAD clade, which includes all C_4 lineages (Table 2).

DISCUSSION

C_4 plants evolved in tropical climates, expanded to warmer and shifted to cooler environments

Our analyses of evolutionary transitions across the whole grass phylogeny provide general statistical support for the hypothesis that C_4 photosynthesis in grasses evolved in tropical climates (Figs. 1 and 3), confirming previous work (Sage 2004; Edwards & Still 2008; Edwards & Smith 2010). Hot climates, under the low-CO_2 atmosphere that prevailed for the last 30 million years (Pagani *et al.* 2005) exacerbated photorespiration, providing a selective pressure for novel photosynthetic physiologies that decrease the net cost of this process (Ehleringer & Björkman 1977; Osborne & Beerling 2006; Christin *et al.* 2008). Current models indicate that C_4 photosynthesis evolved via a series of intermediate stages, including photorespiratory bypasses and weak C_4 cycles, which progressively decreased the adverse effects of photorespiration (Sage 2004; Heckmann *et al.* 2013; Mallmann *et al.* 2014). While it has been questioned whether extant taxa with an intermediate physiology are similar to those that enabled C_4 evolution, with some arguing that they might instead result from hybridization events (Kadereit *et al.* 2017), these intermediates are concentrated in hotter climates (Lundgren & Christin 2017). If states that preceded C_4 evolution were similarly restricted to hotter climates, C_4 origins would consequently be concentrated in warm climates, as observed here (Fig. 1). However, because the physiological effects of C_4 photosynthesis are broader than those of these intermediates (Vogan & Sage 2011; Christin & Osborne 2014), the ecological consequences of C_4 evolution might not be limited to warm climates (Christin & Osborne 2014). Our analyses support this hypothesis.

Our modelling analysis shows for the first time that C_4 photosynthesis accelerated the migration of grass taxa between tropical and temperate climates in comparison with C_3 lineages (Fig. 1). Therefore, C_4 photosynthesis presents no inherent physiological barrier to the colonization of temperate environments. It has been hypothesised that cold acclimation in C_4 plants may be impeded by their leaf anatomy, which provides insufficient cellular volume to accumulate Rubisco protein (Sage & Kubien 2007), although this is debated (Long & Spence 2013). It has also been proposed that C_4 species should be excluded from low temperature regions by competition with C_3 plants, which have a higher photosynthetic efficiency than the C_4 type in cool environments, especially in low light conditions within dense leaf canopies or under cloudy skies (Ehleringer & Björkman 1977; Ehleringer 1978). However, modelling suggests that, under cloudless, high light conditions, the cost resulting from the extra C_4 reactions may be more than compensated at the canopy scale by light-saturated photosynthetic rates in sunlit leaves (Long 1999; Long & Spence 2013). In addition, the C_4 syndrome provides advantages besides carbon-fixation efficiency. These include greater nitrogen- and water-use efficiencies than the C_3 type (Long 1999), and increased net assimilation rates enable investment into different growth strategies (Atkinson *et al.* 2016). Together, these properties might contribute to the success of C_4 species across a range of temperature conditions.

However, our analysis also shows that C_4 species are overall less likely than C_3 ones to migrate into continental, polar or alpine climates (grouped as “cold climates” in our analysis). A colonization of cold climates has previously been inferred early during the history of C_3 Pooideae (Edwards & Smith 2010), the group that dominate cold and temperate climates (Fig. 3; Table S2). This suggests that adaptation in this group to survive under prolonged cold conditions has been enabled by traits that evolved early during their history and may not characterise other grass lineages (Sandve & Fjellheim 2010; Vigeland *et al.* 2013; Spriggs *et al.* 2014; McKeown *et al*., 2016). The early migration and adaptation to cold climates allowed the subsequent diversification of Pooideae in cold and temperate climates (Table S2, Fig. 3).

Biogeography affects the current distribution of C_4 plants

Evolutionary history, coupled with biogeographical pattern, explains the higher frequency of C_4 species in tropical or temperate climates (Table S2). C_4 origins happened predominantly in tropical climate regions (Fig. 1), allowing grasses to tolerate higher temperatures and expanding the temperature niche (Table 2), as shown previously (Christin & Osborne 2014; Lundgren *et al.* 2015; Aagesen *et al.* 2016; Bena *et al.* 2017). However, our analysis is the first to show that C_4 photosynthesis also increased the rate of transitions among climate types, with frequent migration into temperate climates without freezing (Fig. 1). The rate of C_4 plant migration into freezing temperate climates was also considerable, and was higher than that of close C_3 relatives within the PACMAD clade. When considered across the grass family as a whole it was comparable to the rates in Pooideae, indicating that C_4 lineages are physiologically capable of colonizing cold environments.

Geographical barriers could have played important roles in limiting the expansion of some C_4 groups into cold climates. Most tropical climate regions are geographically distant from cold climates (Donoghue 2008), presenting little opportunity for tropical plants to migrate into cooler environments (Edwards & Donoghue 2013). C_4 species of cold climates are therefore found mostly in high altitude habitats located at low latitudes, and only rarely at high latitudes (Long 1999; Sage & Monson 1999; Sage *et al.* 2010), but we argue that this pattern does not stem from physiological limitations, instead being the direct consequence of the increased rate of C_4 origins in tropical regions.

CONCLUSIONS

Using a large phylogeny for the grass phylogeny, we show for the first time that C_4 photosynthesis evolved primarily in tropical climates, and subsequently enhanced the rates of evolutionary transitions between tropical and temperate climates. When compared to close relatives, C_4 plants were furthermore more likely to colonize freezing environments. Our conclusions therefore contradict previous work based solely on geographical distributions and physiological theory. The macroevolutionary processes revealed in our large comparative study underpin the high ecological diversity and global expansion of C_4 species. Although there appear to be no physiological barriers to prevent C_4 plants from colonizing cooler environments, C_4 grass clades have still migrated less frequently from temperate to cold climate regions than members of some C_3 lineages (especially Pooideae). This pattern arises from the recent origins of C_4 photosynthesis in warm climates, in lineages with warm adapted traits, which contrasts with the ancient origin of cold adaptation in Pooideae. C_4 plants must therefore have both the time and the opportunities to acquire further traits needed to successfully colonize cold climates.

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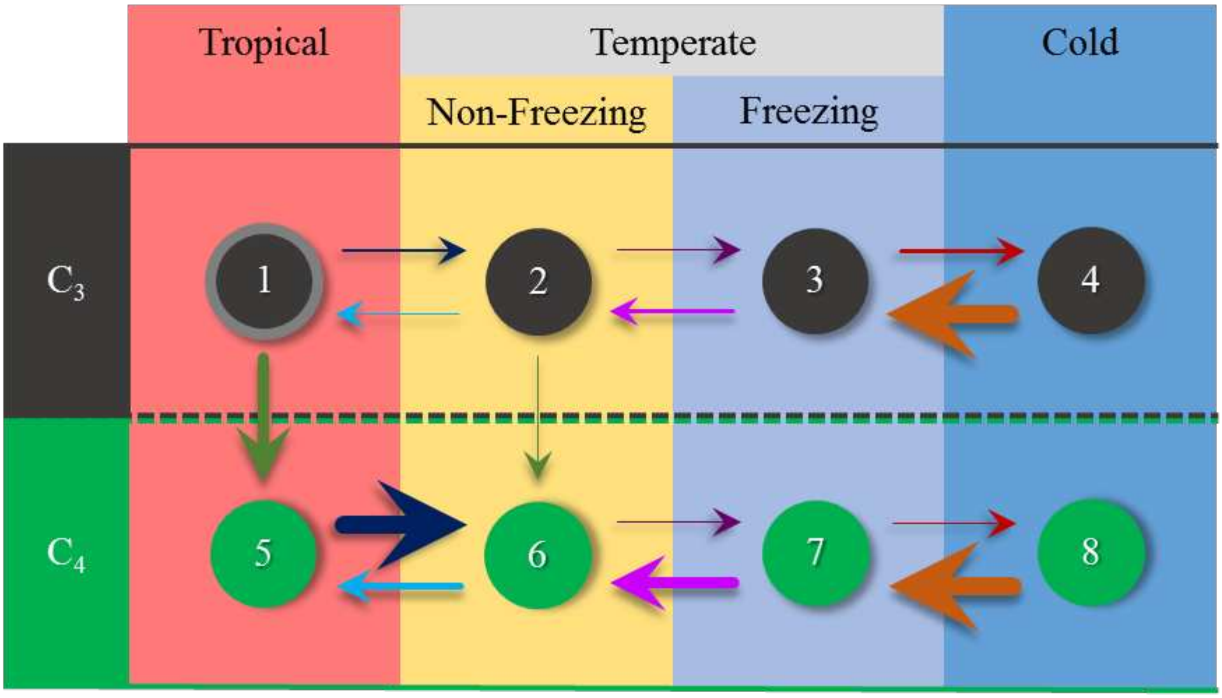

Figure 1 Model of coevolution of photosynthetic types and temperature niches. Sizes of arrows indicate transition rates among climate and photosynthetic types. The most likely ancestral condition is indicated by the grey outer circle.

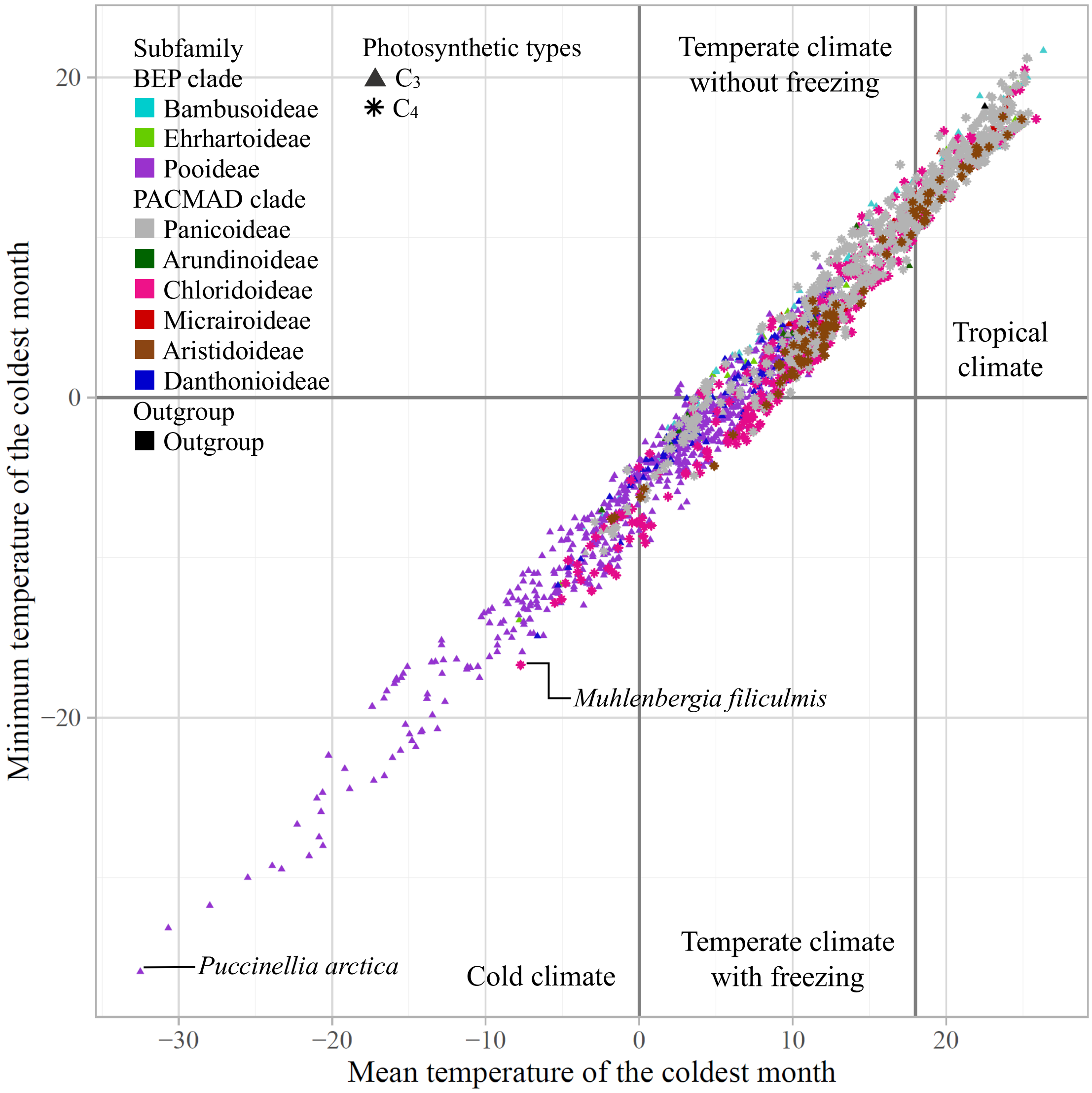

Figure 2 Climatic distributions of C_4 and C_3 species from various subfamilies indicated by temperature regimes. The thick grey lines indicate the boundaries between climatic types. The C_3 and C_4 species in the lowest temperature regimes are indicated.

Table 1 Rates of transitions determined from point estimates of models.

State	Rate	Estimated rates
Tropical vs. Temperate climates and C$_3$ vs. C$_4$ photosynthesis		
Transition from tropical to temperate climate		
C$_3$	Rate$_{1\to 2}$	0.02200
C$_4$	Rate$_{5\to 6}$	0.11663
C$_4$ – C$_3$	Rate$_{5\to 6}$ – Rate$_{1\to 2}$	0.09464***
Transition from temperate to tropical climate		
C$_3$	Rate$_{2\to 1}$	0.00004
C$_4$	Rate$_{6\to 5}$	0.04316
C$_4$ – C$_3$	Rate$_{6\to 5}$ – Rate$_{2\to 1}$	0.04313***
Transition from C$_3$ to C$_4$ photosynthesis		
Tropical	Rate$_{1\to 5}$	0.00749
Temperate	Rate$_{2\to 6}$	0.00016
Tropical – Temperate	Rate$_{1\to 5}$ – Rate$_{2\to 6}$	0.00734***
Temperate = 0	Rate$_{2\to 6}$ – 0	0.00016*
Transition from C$_4$ to C$_3$ photosynthesis		
Tropical	Rate$_{5\to 1}$	0
Temperate	Rate$_{6\to 2}$	0
Tropical = Temperate = 0	Rate$_{5\to 1}$ + Rate$_{6\to 2}$ – 0	0^{ns}
Temperate climates without freezing vs. with freezing and C$_3$ vs. C$_4$ photosynthesis		
Transition from temperate climate without freezing to with freezing		
C$_3$	Rate$_{2\to 3}$	0.01095
C$_4$	Rate$_{6\to 7}$	0.01356
C$_4$ – C$_3$	Rate$_{6\to 7}$ – Rate$_{2\to 3}$	0.00262^{ns}
Transition from temperate climates with freezing to without freezing		
C$_3$	Rate$_{3\to 2}$	0.03054
C$_4$	Rate$_{7\to 6}$	0.07954
C$_4$ – C$_3$	Rate$_{7\to 6}$ – Rate$_{3\to 2}$	0.04900***
Transition from C$_3$ to C$_4$ photosynthesis		
Non-freezing	Rate$_{2\to 6}$	0.00355
Freezing	Rate$_{3\to 7}$	0.00000
Non-freezing – Freezing	Rate$_{2\to 6}$ – Rate$_{3\to 7}$	0.00355***
Freezing = 0	Rate$_{3\to 7}$ – 0	0^{ns}
Transition from C$_4$ to C$_3$ photosynthesis		
Non-freezing	Rate$_{6\to 2}$	0
Freezing	Rate$_{7\to 3}$	0
Non-freezing = Freezing = 0	Rate$_{6\to 2}$ + Rate$_{7\to 3}$ – 0	0^{ns}
Temperate vs. Cold climates and C$_3$ vs. C$_4$ photosynthesis		
Transition from temperate to cold climates		
C$_3$	Rate$_{3\to 4}$	0.03118
C$_4$	Rate$_{7\to 8}$	0.00421
C$_4$ – C$_3$	Rate$_{7\to 8}$ – Rate$_{3\to 4}$	-0.02697***
Transition from cold to temperate climates		
C$_3$	Rate$_{4\to 3}$	0.13183
C$_4$	Rate$_{8\to 7}$	0.13628
C$_4$ – C$_3$	Rate$_{8\to 7}$ – Rate$_{4\to 3}$	0.00445^{ns}
Transition from C$_3$ to C$_4$ photosynthesis		
Temperate	Rate$_{3\to 7}$	0.00233
Cold	Rate$_{4\to 8}$	0.00000
Temperate – Cold	Rate$_{3\to 7}$ – Rate$_{4\to 8}$	0.00233*
Cold = 0	Rate$_{4\to 8}$ – 0	0^{ns}
Transition from C$_4$ to C$_3$ photosynthesis		
Temperate	Rate$_{7\to 3}$	0
Cold	Rate$_{8\to 4}$	0
Temperate = Cold = 0	Rate$_{7\to 3}$ + Rate$_{8\to 4}$ – 0	0^{ns}

Asterisk indicates the differences between rates of transitions, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ^{ns} indicates no differences between rates of transitions

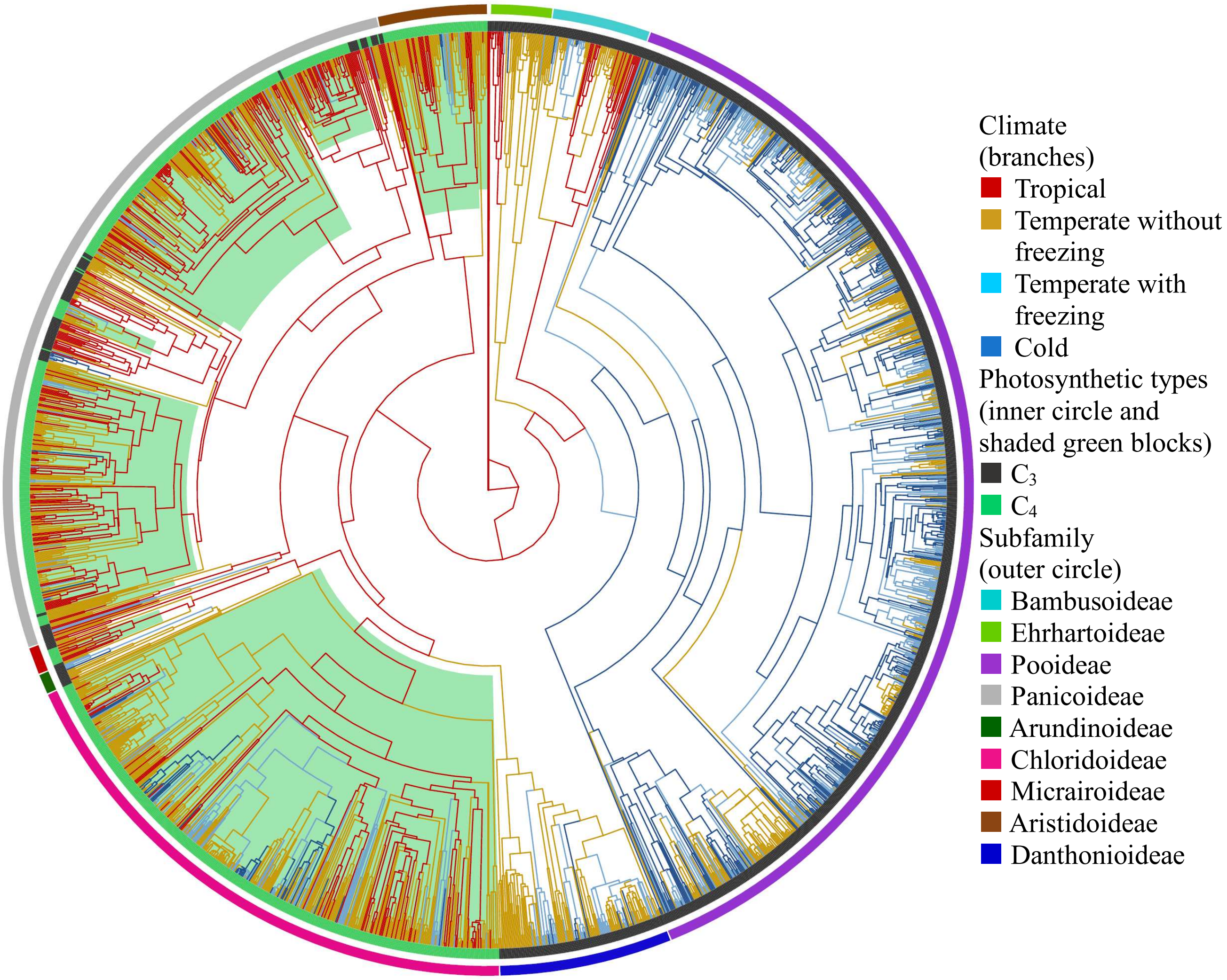

Figure 3 Maximum likelihood reconstruction of the transitions between climatic regions: tropical, temperate without freezing, temperate with freezing and cold climates. Photosynthetic types and subfamilies are indicated.

Table 2 Results from phylogenetic generalized least square regression testing for an association between photosynthetic pathway and climate, and making statistical comparisons between photosynthetic types.

Clade	Variable[1]	C_3	C_4	*p*-value[2]	λ
All grasses	MTCM max	17.0	17.7	0.5154	0.78
	MTCM min	9.0	8.0	0.5191	0.80
	MTWM max	26.0	27.8	0.0142*	0.69
	MTWM min	20.1	20.8	0.3995	0.81
	Range	15.3	18.3	0.0126*	0.79
PACMAD	MTCM max	17.7	18.3	0.5577	0.68
	MTCM min	9.6	8.4	0.3873	0.72
	MTWM max	26.7	28.4	0.0031**	0.64
	MTWM min	20.3	21.0	0.3748	0.75
	Range	16.2	19.4	0.0057**	0.68

[1] MTCM max = 95th mean temperature of the coldest month; MTCM min = 5th mean temperature of the coldest month; MTWM max = 95th mean temperature of the warmest month; MTWM min = 5th mean temperature of the warmest month; Range = difference between MTWM max and MTCM min. [2] * $p < 0.05$, ** $p < 0.01$.

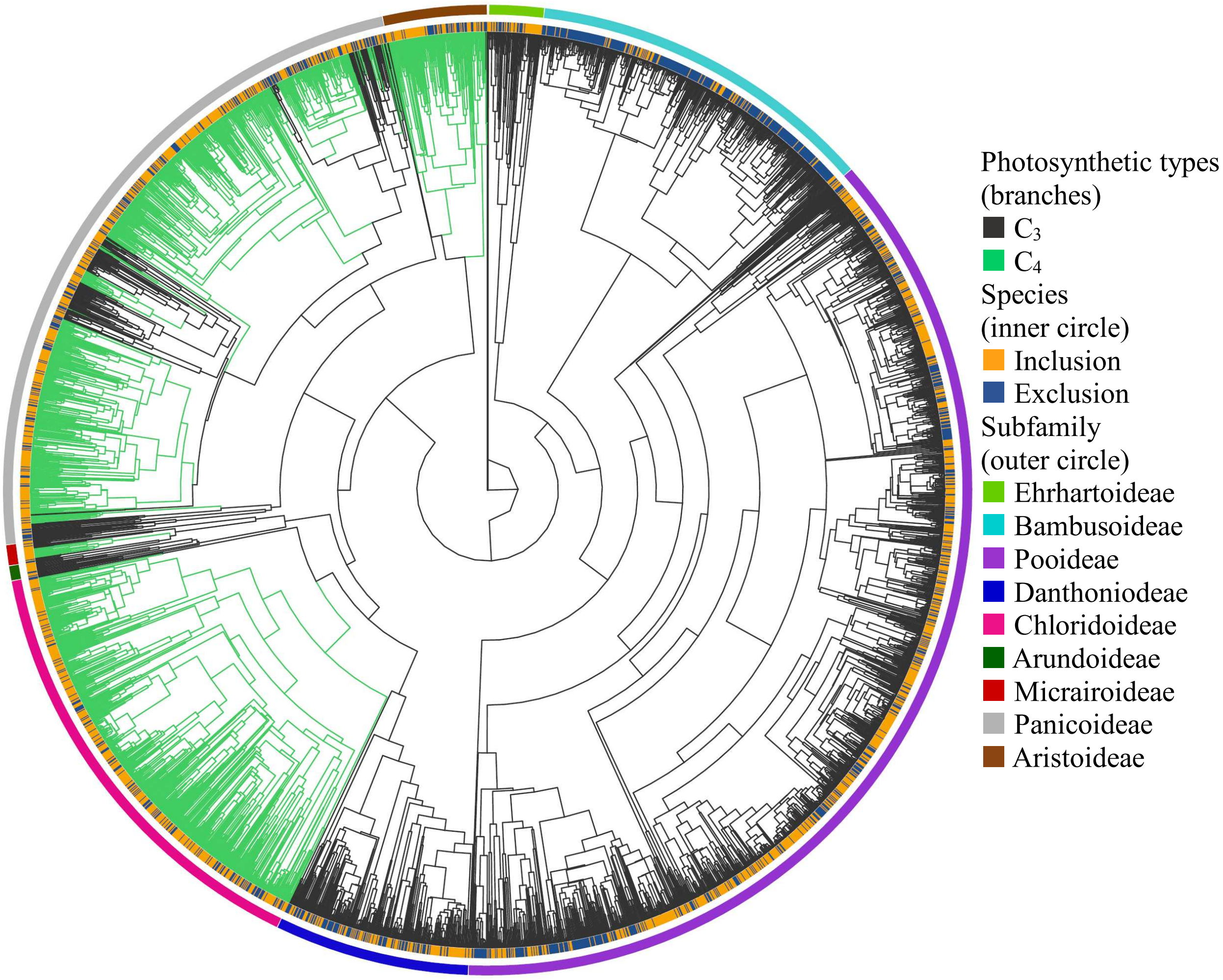

Figure S1 Phylogenetic tree showing the distribution of species excluded due to lacking occurrence data.

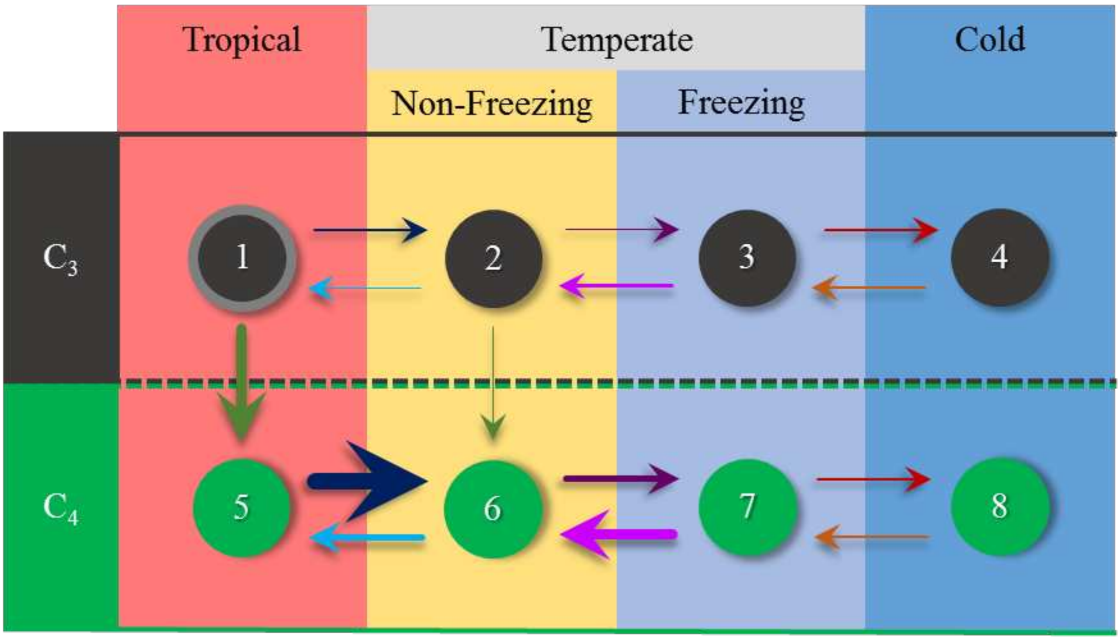

Figure S2 Model of coevolution of photosynthetic types and temperature niches in the PACMAD clade. Size of arrows indicate transition rates among climate and photosynthetic types. The most likely ancestral condition is indicated by the grey outer circle.

Table S1 Distribution of C_3 and C_4 photosynthesis among climatic types before and after fitting into the phylogenetic tree.

Group	Number of species				
	Tropical	Temperate		Cold	Total
		Non-freezing	Freezing		
Before fitting into phylogenetic tree					
C_3	210 (5%)	859 (21%)	623 (15%)	475 (11%)	2,167 (52%)
C_4	710 (17%)	1,097 (26%)	154 (4%)	60 (1%)	2,021 (48%)
Total	920	1,956	777	535	4,188
After fitting into phylogenetic tree					
C_3	83 (4%)	439 (21%)	370 (17%)	293 (14%)	1,185 (56%)
C_4	263 (12%)	542 (25%)	105 (5%)	38 (2%)	948 (44%)
Total	346	981	475	331	2,133

Table S2 Distribution of grass subfamilies and C_4 photosynthesis among climatic types

Group	Number of species (Number of C_4 species)			
	Tropical	Temperate		Cold
		Non-freezing	Freezing	
Bambusoideae	18 (0)	37 (0)	12 (0)	3 (0)
Ehrhartoideae	10 (0)	28 (0)	3 (0)	3 (0)
Pooideae	0 (0)	234 (0)	314 (0)	272 (0)
Panicoideae	227 (179)	288 (243)	41 (36)	11 (7)
Arundinoideae	1 (0)	9 (0)	4 (0)	1 (0)
Chloridoideae	57 (57)	244 (244)	64 (64)	29 (29)
Micrairoideae	10 (7)	10 (5)	0 (0)	0 (0)
Aristidoideae	21 (20)	50 (50)	5 (5)	2 (2)
Danthonioideae	0 (0)	81 (0)	32 (0)	10 (0)
Outgroup	2 (0)	0 (0)	0 (0)	0 (0)
Total	346 (263)	981 (542)	475 (105)	331 (38)

Table S3 Rates of transitions in the PACMAD clade determined from point estimates of models.

State	Rate	Estimated rates
Tropical vs. Temperate climates and C$_3$ vs. C$_4$ photosynthesis		
Transition from tropical to temperate climate		
C$_3$	Rate$_{1\to2}$	0.02355
C$_4$	Rate$_{5\to6}$	0.11088
C$_4$ – C$_3$	Rate$_{5\to6}$ – Rate$_{1\to2}$	0.08733***
Transition from temperate to tropical climate		
C$_3$	Rate$_{2\to1}$	0.00104
C$_4$	Rate$_{6\to5}$	0.03975
C$_4$ – C$_3$	Rate$_{6\to5}$ – Rate$_{2\to1}$	0.03872***
Transition from C$_3$ to C$_4$ photosynthesis		
Tropical	Rate$_{1\to5}$	0.01161
Temperate	Rate$_{2\to6}$	0.00100
Tropical – Temperate	Rate$_{1\to5}$ – Rate$_{2\to6}$	0.01061***
Temperate = 0	Rate$_{2\to6}$ – 0	0.00100**
Transition from C$_4$ to C$_3$ photosynthesis		
Tropical	Rate$_{5\to1}$	0
Temperate	Rate$_{6\to2}$	0
Tropical = Temperate = 0	Rate$_{5\to1}$ + Rate$_{6\to2}$ – 0	0^{ns}
Temperate climates without freezing vs. with freezing and C$_3$ vs. C$_4$ photosynthesis		
Transition from temperate climate without freezing to with freezing		
C$_3$	Rate$_{2\to3}$	0.00510
C$_4$	Rate$_{6\to7}$	0.01390
C$_4$ – C$_3$	Rate$_{6\to7}$ – Rate$_{2\to3}$	0.00881**
Transition from temperate climates with freezing to without freezing		
C$_3$	Rate$_{3\to2}$	0.04660
C$_4$	Rate$_{7\to6}$	0.08201
C$_4$ – C$_3$	Rate$_{7\to6}$ – Rate$_{3\to2}$	0.03541*
Transition from C$_3$ to C$_4$ photosynthesis		
Non-freezing	Rate$_{2\to6}$	0.00695
Freezing	Rate$_{3\to7}$	0.00000
Non-freezing – Freezing	Rate$_{2\to6}$ – Rate$_{3\to7}$	0.00695*
Freezing = 0	Rate$_{3\to7}$ – 0	0^{ns}
Transition from C$_4$ to C$_3$ photosynthesis		
Non-freezing	Rate$_{6\to2}$	0
Freezing	Rate$_{7\to3}$	0
Non-freezing = Freezing = 0	Rate$_{6\to2}$ + Rate$_{7\to3}$ – 0	0^{ns}
Temperate vs. Cold climates and C$_3$ vs. C$_4$ photosynthesis		
Transition from temperate to cold climates		
C$_3$	Rate$_{3\to4}$	0.01196
C$_4$	Rate$_{7\to8}$	0.00403
C$_4$ – C$_3$	Rate$_{7\to8}$ – Rate$_{3\to4}$	-0.00793^{ns}
Transition from cold to temperate climates		
C$_3$	Rate$_{4\to3}$	0.24525
C$_4$	Rate$_{8\to7}$	0.12936
C$_4$ – C$_3$	Rate$_{8\to7}$ – Rate$_{4\to3}$	-0.11590^{ns}
Transition from C$_3$ to C$_4$ photosynthesis		
Temperate	Rate$_{3\to7}$	0.00596
Cold	Rate$_{4\to8}$	0.00000
Temperate – Cold	Rate$_{3\to7}$ – Rate$_{4\to8}$	0.00596^{ns}
Cold = 0	Rate$_{4\to8}$ – 0	0^{ns}
Transition from C$_4$ to C$_3$ photosynthesis		
Temperate	Rate$_{7\to3}$	0
Cold	Rate$_{8\to4}$	0
Temperate = Cold = 0	Rate$_{7\to3}$ + Rate$_{8\to4}$ – 0	0^{ns}

Asterisk indicates the differences between rates of transitions, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ^{ns} indicates no differences between rates of transitions