

LETTER

C₄ photosynthesis evolved in warm climates but promoted migration to cooler ones

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Abstract

C₄ photosynthesis is considered an adaptation to warm climates, where its functional benefits are greatest and C₄ plants achieve their highest diversity and dominance. However, whether inherent physiological barriers impede the persistence of C₄ species in cool environments remains debated. Here, we use large grass phylogenetic and geographical distribution data sets to test whether (1) temperature influences the rate of C₄ origins, (2) photosynthetic types affect the rate of migration among climatic zones, and (3) C₄ evolution changes the breadth of the temperature niche. Our analyses show that C₄ photosynthesis in grasses originated in tropical climates, and that C₃ grasses were more likely to colonise cold climates. However, migration rates among tropical and temperate climates were higher in C₄ grasses. Therefore, while the origins of C₄ photosynthesis were concentrated in tropical climates, its physiological benefits across a broad temperature range expanded the niche into warmer climates and enabled diversification into cooler environments.

Keywords

Adaptation, C₄ photosynthesis, climate, evolution, phylogeny, temperature niche.

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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 colonised 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 increase net benefits from photosynthesis under the ambient temperature conditions. Net photosynthetic gains are damped at higher temperatures because CO₂ fixation by Rubisco is offset by competition with O₂ fixation, increasing the rate of photorespiration (Ehleringer & Björkman 1977; Sage & Kubien 2007). Some plants have evolved CO₂-concentrating mechanisms that minimise photorespiration, including C₄ photosynthesis, which has been very successful in land plants (Ehleringer & Björkman 1977; Sage & Monson 1999; Still *et al.* 2003; Sage *et al.* 2012).

C₄ photosynthesis is a physiological process resulting from a series of biochemical and anatomical modifications over the ancestral C₃ photosynthetic type (Hatch 1987). Together, these concentrate CO₂ around Rubisco, thereby increasing its efficiency and reducing photorespiration (von Caemmerer & Furbank 2003; Sage *et al.* 2012). However, the extra biochemical reactions required for C₄ photosynthesis increase the energetic cost of carbon fixation, so that C₄ photosynthesis is predicted to outperform C₃ only when photorespiration is significant, especially under high temperatures (Ehleringer & Björkman 1977; Osborne & Beerling 2006). The improved performance of C₄ 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 C₃ species, open biomes are predominantly occupied by C₄ species in hot to warm climates and cooler open biomes are dominated by C₃ species (Sage *et al.* 1999b; Edwards *et al.* 2010).

More than half of C₄ species belong to the grass family (Poaceae) (Sage *et al.* 1999a). At large scales, C₃ and C₄ grasses sort largely according to temperature (Ehleringer & Björkman 1977; Ehleringer 1978; Sage *et al.* 1999a; Osborne *et al.* 2014), and C₄ 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 C₄ grasses. Several C₄ 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 adaptations 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 C₄ species can survive and compete with C₃ species in cold environments, at least under some circumstances.

The rarity of C₄ plants in cold climates could 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 C₄ 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 C₃ and C₄ lineages, while considering their evolutionary history. Past comparative work has shown that C₄ grasses emerged from tropical C₃ 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 different climatic zones are lacking.

In this study, we use phylogenetic and geographical distribution data for 2133 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 ecological space.

METHODS

Climate data set

All available georeferenced occurrence records (~14.3 M) 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 10 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 3595 species of grasses, covering the whole family

and using a time-calibration hypothesis based on macrofossils and microfossils (Spriggs *et al.* 2014). Taxa without occurrence data after filtering were pruned from the tree, resulting in a data set of 2133 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 data set (Table S1 and Fig. 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 the 1197 species from this clade 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 categorised 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 °C 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. Climatic 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: (1) tropical vs. temperate without freezing; (2) temperate without freezing vs. temperate with freezing and (3) 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 C₃ and C₄ 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 C₃ to C₄ 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 C₄ to C₃ photosynthesis are unlikely (Christin *et al.* 2010; Grass Phylogeny Working Group II 2012), we tested whether transitions from C₄ to C₃ 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 C₃ or C₄ 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 C₃ or C₄ 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 data set is representative of GBIF without any bias from the phylogeny (Table S1 and Fig. S1).

To visualise the historical transitions between climatic types of C₃ and C₄ 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 likely 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 generalised 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 data sets (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 C₃ and C₄ species

The percentage of C₄ species decreases from tropical to cold climates (Table S2). More than 10% of C₄ species available in GBIF occur in freezing temperate or cold climates (Table S1). Our data set of 2133 species includes perennial C₄ species from three different subfamilies that colonized cold climates (Table S2), including a number of perennial species. The predominance of C₃ species in cold climates mainly reflects the success of members of the Pooideae subfamily, although other groups are also present (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 C₄ origins are more frequent in tropical than temperate climates (rates of 1→5 > 2→6; $P < 0.001$; Fig. 1; Table 1). The ancestral state reconstructions confirmed that C₄ photosynthesis evolved from C₃ PACMAD ancestors in tropical climates, while the ancestor of Danthonioideae moved to temperate climates, with descendants that remained C₃ (Fig. 3). The rate of C₄ origins is not significantly different from zero in freezing temperate and cold climates (rates of 3→7, 4→8 = 0; $P > 0.05$; Fig. 1; Table 1), but it is greater than zero in non-freezing temperate climates (rate of 2→6 $\neq 0$; $P < 0.05$; Fig. 1; Table 1). Based on our models, the rate of transition from C₄ to C₃ types is not significantly different from zero under any climatic conditions (rates of 5→1, 6→2, 7→3, 8→4 = 0; $P > 0.05$; Fig. 1; Table 1), which is consistent with previous conclusions that the rate of reversal from C₄ to C₃ 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 C₄ than C₃ 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 divergence of Chloridoideae, Panicoideae and Aristidoideae subfamilies (Fig. 3). Moreover, C₄ 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 C₄ than in C₃ taxa (Fig. 3). The rate of transition

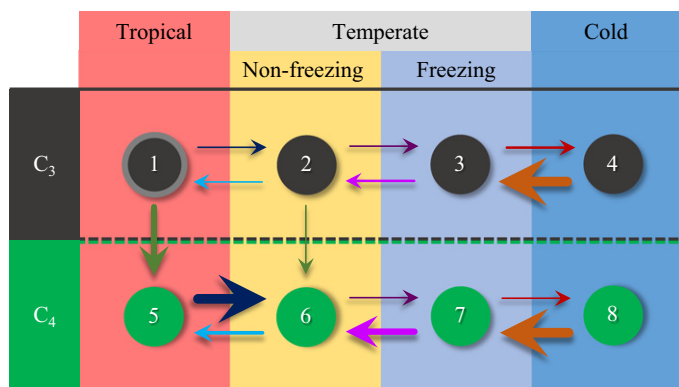


Figure 1 Model of coevolution of photosynthetic types and temperature niches. Sizes of arrows indicate transition rates among climate and photosynthetic types. The most likely ancestral condition is indicated by the grey outer circle.

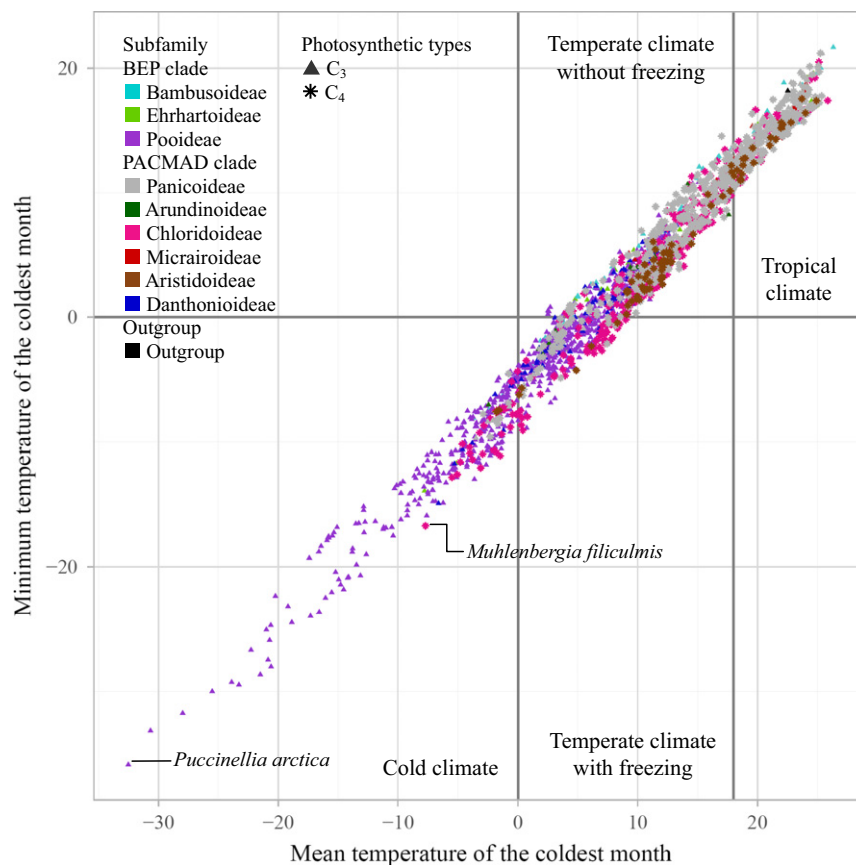


Figure 2 Climatic distributions of C_4 and C_3 species from various subfamilies indicated by temperature regimes. The thick grey lines indicate the boundaries between climatic types. The names of the C_3 and C_4 species reaching the lowest temperatures are indicated.

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 $6 \rightarrow 7 > 2 \rightarrow 3$; $P < 0.05$; 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 $6 \rightarrow 7 = 2 \rightarrow 3$; $P > 0.05$; Fig. 1; Table 1). However, transitions from temperate to cold climates are more frequent within C_3 than C_4 lineages (rates of $7 \rightarrow 8 < 3 \rightarrow 4$; $P < 0.001$; 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 $4 \rightarrow 3 = 8 \rightarrow 7$; $P > 0.05$; 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 towards higher temperatures during the warmest month (Table 2), mirroring previous conclusions with a smaller species sampling (Aagesen *et al.* 2016). By contrast, the lower bound 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

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

photosynthesis evolved via a series of intermediate stages, including photorespiratory bypasses and weak C₄ 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 C₄ evolution, with some arguing that they might instead result from hybridisation events (Kadereit *et al.* 2017), these intermediates are concentrated in hotter climates (Lundgren & Christin 2017). If states that preceded C₄ evolution were similarly restricted to hotter climates, C₄ origins would consequently be concentrated in warm climates, as observed here (Fig. 1). However, because the physiological effects of C₄ photosynthesis are broader than those of these intermediates (Vogan & Sage 2011; Christin & Osborne 2014), the ecological consequences of C₄ 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 C₄ photosynthesis accelerated the migration of grass taxa between tropical and temperate climates in comparison with C₃ lineages (Fig. 1). Therefore, C₄ photosynthesis presents no inherent physiological barrier to the colonisation of temperate environments. It has been hypothesised that cold acclimation in C₄ 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 C₄ species should be excluded from low temperature regions by competition with C₃ plants, which have a higher photosynthetic efficiency than the C₄ 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 C₄ 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 C₄ syndrome provides advantages besides carbon-fixation efficiency. These include greater nitrogen- and water-use efficiencies than the C₃ type (Long 1999), and increased net assimilation rates that enable investment into different growth strategies (Atkinson *et al.* 2016). Together, these properties might contribute to the success of C₄ species across a range of temperature conditions.

However, our analysis also shows that C₄ species are overall less likely than C₃ ones to migrate into continental, polar or alpine climates (grouped as ‘cold climates’ in our analysis). A colonisation of cold climates has previously been inferred early during the history of C₃ Pooideae (Edwards & Smith 2010), the group that dominates both 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 C₄ plants

Evolutionary history, coupled with biogeographical pattern, explains the higher frequency of C₄ species in tropical or

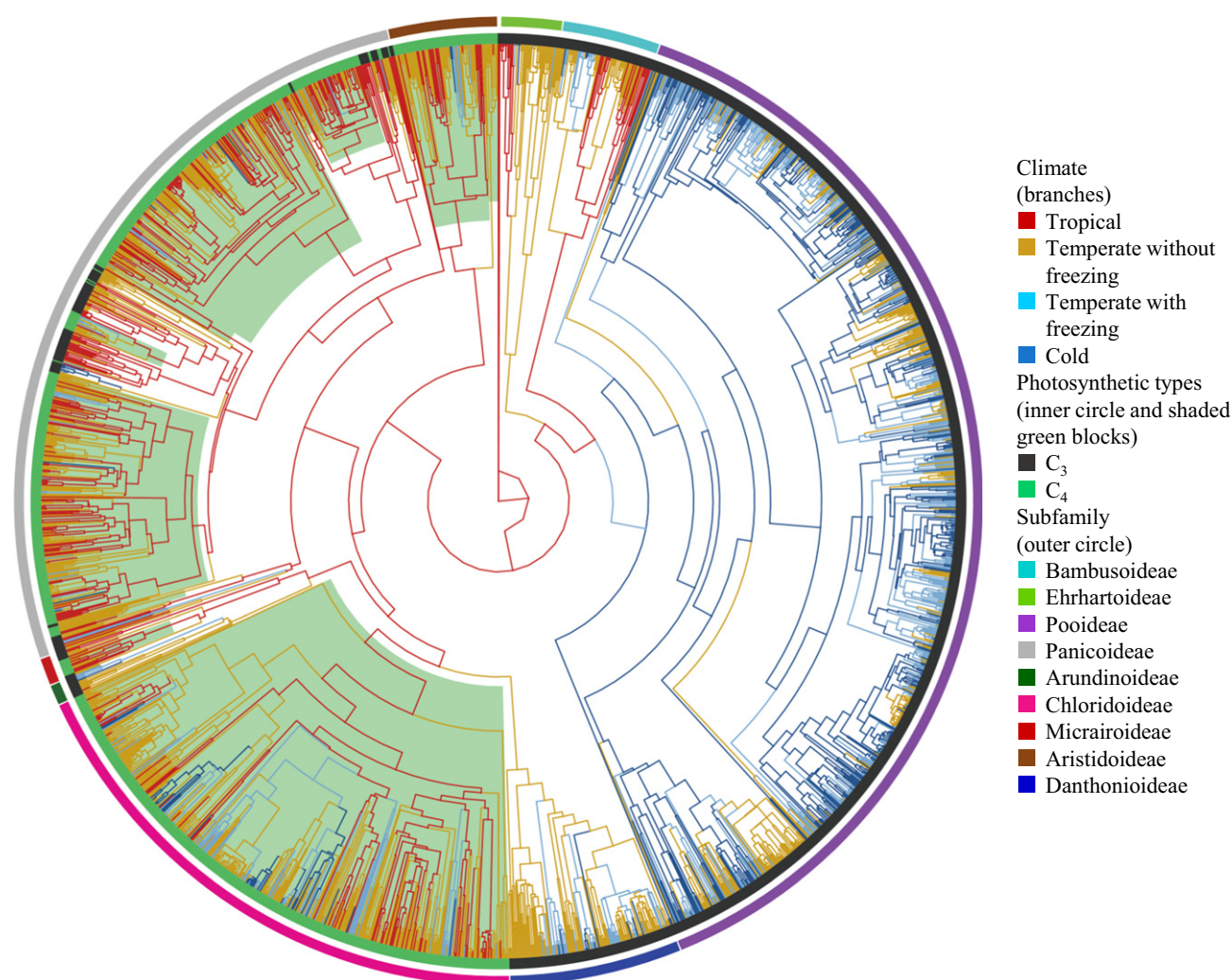


Figure 3 Maximum likelihood reconstruction of the transitions between climatic regions: tropical, temperate without freezing, temperate with freezing and cold climates. Photosynthetic types and subfamilies are indicated.

Table 2 Results from phylogenetic generalised 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	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

¹MTCM 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.

* $P < 0.05$, ** $P < 0.01$.

temperate climates (Table S2). C₄ 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 C₄ photosynthesis also increased the rate of transitions among climate types, with frequent migration into temperate climates without freezing (Fig. 1). The rate of C₄ plant migration into freezing temperate climates was also considerable, and was higher than that of close C₃ relatives within the PACMAD clade. When considered across the grass family as a whole, it was comparable to the rates in Pooideae, indicating that C₄ lineages are physiologically capable of colonising cold environments.

Geographical barriers could have played important roles in limiting the expansion of some C₄ 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). C₄ 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 C₄ origins in tropical regions.

CONCLUSIONS

Using a large phylogeny for the grass phylogeny, we show for the first time that C₄ photosynthesis evolved primarily in tropical climates, and subsequently enhanced the rates of evolutionary transitions between tropical and temperate climates. When compared to close relatives, C₄ plants were also more likely to colonise 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 C₄ species. Although there appear to be no physiological barriers to prevent C₄ plants from colonising cooler environments, C₄ grass clades have still migrated less frequently from temperate to cold climate regions than members of some C₃ lineages (especially Pooideae). This pattern arises from the recent origins of C₄ photosynthesis in warm climates, in lineages with warm adapted traits, which contrasts with the ancient origin of cold adaptation in Pooideae. C₄ plants must therefore have both the time and the opportunities to acquire further traits needed to successfully colonise cold climates.

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AUTHOR CONTRIBUTION

TW, CPO and PAC designed the study. TW generated and analysed the data. TW, CPO and PAC wrote the paper.

DATA ACCESSIBILITY STATEMENT

The primary data supporting these results is archived in Dryad <https://doi.org/10.5061/dryad.g8f18>.

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SUPPORTING INFORMATION

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