

Letters

A global database of C₄ photosynthesis in grasses

Introduction

C₃, C₄ or Crassulacean acid metabolism (CAM) photosynthetic pathways represent a fundamental axis of trait variation in plants, with importance at scales from genome to biome. Knowing the distribution of these pathways among wild species is a crucial first step in understanding the patterns and processes of photosynthetic evolution and its role in ecological processes at large scales (e.g. changes in the composition of biomes under global change). C₄ photosynthesis is most prevalent in the Poaceae (grasses), which account for about half of all C₄ species (Sage *et al.*, 1999a). Research on the evolution and ecology of these plants has undergone a renaissance during the last 7 yr, catalyzed by phylogenetic analyses showing multiple parallel C₄ origins (e.g. Christin *et al.*, 2007; Vicentini *et al.*, 2008; GPWG II, 2012), insights into the distribution of C₄ species and assembly of the C₄ grassland biome (Edwards & Still, 2008; Edwards & Smith, 2010; Edwards *et al.*, 2010), and efforts to introduce the C₄ pathway into rice (Hibberd *et al.*, 2008; von Caemmerer *et al.*, 2012). C₄ photosynthesis is an excellent model for investigating complex trait evolution, because of the broad knowledge base describing its biochemical basis, evolutionary history, and ecological interactions (Christin *et al.*, 2010).

Why do we need a C₄ database?

Investigations of the evolution and ecological significance of C₄ photosynthesis are increasingly turning to large-scale comparisons of C₃ and C₄ species. These are straightforward for well-characterized common or model species. However, when comparisons are extended to include large numbers of nonmodel species, two important challenges arise. First, there are > 62 000 published scientific names for grasses corresponding to over 11 000 accepted species (Clayton *et al.*, 2002b onwards), making an average of five synonyms for each accepted name. This leads to problems when linking data based on alternative names for the same species concept, and to redundancy in published data surveys, when values for synonyms are presented as independent data. Second, although there have been extensive previous surveys of the photosynthetic pathway spanning the diversity of wild species (Hattersley & Watson, 1992; Sage *et al.*, 1999a), the rarity of most species means that this work is incomplete, and the synonymy problem makes it difficult to identify the gaps in these data.

Accounting for synonymy and spelling variants/mistakes has become one of the central challenges for the emerging fields of ecological and evolutionary informatics, in which data are synthesized across different sources on increasingly larger scales (Jones *et al.*, 2006; Sidlauskas *et al.*, 2009). In one infamous example, a 22.5 million record database of plant species occurrences and traits for the Americas contained more scientific names than there are thought to be plant species on Earth (Whitfield, 2011). However, this taxonomic impediment to data synthesis has been progressively broken down by a combination of new methodological developments for name matching (Patterson *et al.*, 2010; Boyle *et al.*, 2013; Chamberlain & Szocs, 2013; Kluyver & Osborne, 2013), and the compilation of nomenclatural databases by botanic gardens and natural history museums (e.g. The Plant List, 2010). Here, we showcase how such resources may be used to assemble and index databases of discrete traits for large numbers of species.

Compilation and overview of the data

Our database of C₃ and C₄ photosynthetic types in grasses is based principally on published anatomical and stable carbon isotope evidence. We followed previous authors in assuming that all species within each genus shared the same photosynthetic pathway, unless the evidence suggested otherwise. However, we also measured $\delta^{13}\text{C}$ for 99 species that had not previously been surveyed, including 96 species of *Panicum* s.l., *Acostia gracilis*, *Lophopogon tridentatus* and *Theclachloa annua* (Supporting Information Table S1). We also obtained information on leaf anatomy and measured $\delta^{13}\text{C}$ to check previous unverified reports of a C₃ species (*Stipagrostis paradisea*) in an otherwise C₄ genus (Sage *et al.*, 1999a), and a C₄ subspecies (*Chaetobromus dregeanus* ssp. *involutratus*) in an otherwise C₃ subfamily (Danthonioideae; Watson & Dallwitz, 1992 onwards). In both cases, our data contradicted previous reports, showing that the photosynthetic pathway of these taxa matches that of their close relatives; *S. paradisea* is C₄ and *C. dregeanus* ssp. *involutratus* is C₃ (Table 1).

The photosynthetic pathways of *Taeniorhachis repens*, *Veldkampia sagaingensis* and 39 rare species of *Panicum* s.l. remain unclassified, because we were unable to take samples of type specimens from herbarium collections. Most of these species are endemics of Madagascar (26 species), and the remaining 13 species are endemics of a small number of countries in Africa and Southeast Asia, and oceanic islands (Table S2). This means that the database is complete for most countries of the world.

Our approach has been to map the photosynthetic pathway data onto accepted species names in the Poaceae taxonomy of Clayton & Renvoize (1986) and Clayton *et al.* (2002b onwards), which is the most comprehensive treatment of accepted names and synonymy for grasses (see Methods S1 for full methodology). Coupling our

Table 1 Photosynthetic type for genera previously reported to include a mixture of C₃, C₄ and C₃–C₄ intermediate species

Taxon	Type	References
1. <i>Alloteropsis</i>		
<i>A. angusta</i>	C ₄	Metcalfe (1960), Ellis (1974), Hattersley <i>et al.</i> (1977), Watson & Dallwitz (1992 onwards)
<i>A. cimicina</i>	C ₄	
<i>A. paniculata</i>	C ₄	
<i>A. papillosa</i>	C ₄	
<i>A. semialata</i> ssp. <i>semialata</i>	C ₄	
<i>A. semialata</i> ssp. <i>eckloniana</i>	C ₃	
2. <i>Aristida</i>		
<i>A. longifolia</i>	C ₃	Cerros-Tlatilpa & Columbus (2009)
Other <i>Aristida</i> species	C ₄	Watson & Dallwitz (1992 onwards)
3. <i>Chaetobromus involuocratus</i>		
ssp. <i>involuocratus</i> ¹	C ₃	This study; H. P. Linder (pers. comm.)
ssp. <i>sericeus</i>	C ₃	This study; H. P. Linder (pers. comm.)
ssp. <i>dregeanus</i>	C ₃	This study; H. P. Linder (pers. comm.)
4. <i>Dregeochloa</i>		
<i>D. calviniensis</i> ²	C ₃ –C ₄ (?)	Watson & Dallwitz (1992 onwards)
<i>D. pumila</i>	C ₃	
5. <i>Eragrostis</i> ³		
<i>Eragrostis walteri</i> ⁴	C ₃	Schulze <i>et al.</i> (1996); Ingram <i>et al.</i> (2011); Watson & Dallwitz (1992 onwards)
Other <i>Eragrostis</i> species	C ₄	
6. <i>Homolepis</i>		
<i>Homolepis aturensis</i> ⁵	C ₃ –C ₄ (?)	Christin <i>et al.</i> (2013); Watson & Dallwitz (1992 onwards)
Other <i>Homolepis</i> species	C ₃	
7. <i>Neurachne</i>		
<i>N. alopecuroides</i>	C ₃	Hattersley <i>et al.</i> (1982); Hattersley & Roksandic (1983); Christin <i>et al.</i> (2012)
<i>N. annularis</i>	C ₃	
<i>N. lanigera</i>	C ₃	
<i>N. minor</i>	C ₃ –C ₄	
<i>N. munroi</i>	C ₄	
<i>N. queenslandica</i>	C ₃	
<i>N. tenuifolia</i>	C ₃	
<i>Paraneurachne muelleri</i> ⁶	C ₄	
8. <i>Panicum</i> s.l. ³		
252 species	C ₃	Various sources, including this study (see Table S4 for full details)
169 species	C ₄	
<i>P. ruspolii</i>	C ₃ –C ₄ (?)	
9. <i>Steinchisma</i>		
<i>S. cupreum</i>	C ₃ –C ₄	Brown (1977), Morgan & Brown (1979, 1980); Renvoize (1987); Watson & Dallwitz (1992 onwards)
<i>S. decipiens</i>	C ₃ –C ₄	
<i>S. exiguiflorum</i>	C ₃ –C ₄	
<i>S. hians</i>	C ₃ –C ₄	
<i>S. laxum</i>	C ₃	
<i>S. spathellosum</i>	C ₃ –C ₄	
<i>S. stenophyllum</i>	C ₃ –C ₄	
10. <i>Stipagrostis</i>		
<i>S. paradisea</i> ⁷	C ₄	Renvoize (1986); this study
Other <i>Stipagrostis</i> species	C ₄	Watson & Dallwitz (1992 onwards)
11. <i>Streptostachys</i> s.l. ³		
<i>S. acuminata</i>	C ₄	Morrone & Zuloaga (1991); Watson & Dallwitz (1992 onwards); Filgueiras <i>et al.</i> (1993); De Olivera & Longhi-Wagner (2007); P-A. Christin (pers. comm.)
<i>S. asperifolia</i> ⁵	C ₃ –C ₄ (?)	
<i>S. lanciflora</i>	C ₃	
<i>S. macrantha</i>	C ₄	
<i>S. ramosa</i>	C ₄	
<i>S. rigidifolia</i>	C ₄	
<i>S. robusta</i>	C ₃	

Taxonomy follows Clayton *et al.* (2002b onwards).

¹Anatomical (H. P. Linder, pers. comm.) and $\delta^{13}\text{C}$ evidence (Supporting Information Tables S1, S3) conflicts with a previous report that this subspecies is C₄ (Watson & Dallwitz, 1992 onwards).

²Anatomical evidence shows that in *D. calviniensis* most mesophyll cells are no more than one cell distant from bundle sheath cells, making it potentially a C₃–C₄ intermediate.

³Genus known to be polyphyletic.

⁴Note that a recent phylogenetic treatment (Ingram *et al.*, 2011) places *E. walterii* outside the genus *Eragrostis*. However, its taxonomy has not yet been revised.

⁵Anatomical evidence showing a concentration of chloroplasts within large bundle sheath cells suggests that this species is potentially a C₃–C₄ intermediate (Christin *et al.*, 2013; P-A. Christin, pers. comm.).

⁶Phylogenetic analysis places the genus *Paraneurachne* nested within the genus *Neurachne* (Christin *et al.*, 2012).

⁷Anatomical (Renvoize, 1986) and $\delta^{13}\text{C}$ evidence (Tables S1, S3) conflicts with a previous report that this species is C₃ (Sage *et al.*, 1999a).

dataset with this synonymy allows users to return the photosynthetic type for all except 46 (corresponding to 41 accepted species) of the 62 678 published scientific names (accepted names and synonyms) for grasses (Clayton *et al.*, 2002b onwards). We have developed software tools to facilitate this task for users, which are detailed in the following.

The database covers 99.6% of the 11 087 grass species. It shows that 42% of these species use the C_4 photosynthetic pathway and 57% the C_3 pathway (Table S3; Notes S1). Six genera (*Alloteropsis*, *Aristida*, *Eragrostis*, *Neurachne*, *Panicum* s.l., and *Streptostachys* s.l.) contain both C_3 and C_4 species (Tables 1, S4). Seven C_3 – C_4 intermediate species (Table 1) are distributed between the genera *Neurachne* (one species) and *Steinchisma* (six species). Within the genus *Panicum* s.l., 169 species are C_4 , 250 are C_3 , and 41 remain unknown, with the photosynthetic type of *Panicum ruspolii* ambiguous on the basis of new $\delta^{13}C$ measurements (Tables S1, S2, S4; Notes S1). The latter species may be a previously unrecognized C_3 – C_4 intermediate, but further work is required to test this hypothesis. A number of further potential C_3 – C_4 intermediates have been identified on the basis of anatomical observations (Tables 1, S3), and also need to be investigated physiologically. These are *Dregeochloa calviniensis* (most mesophyll cells are no more than one cell distant from bundle sheath cells; Watson & Dallwitz, 1992 onwards), *Homolepis aturensis* and *Streptostachys asperifolia* (concentration of chloroplasts in large bundle sheath cells; Christin *et al.*, 2013; P-A. Christin, pers. comm.). In total there are therefore 11 putative C_3 – C_4 intermediates in the grasses.

Caveats

A number of caveats are important when collating and using large trait databases of this kind. The assumption that all species within each genus share the same photosynthetic pathway is reasonable in most cases. However, significant and interesting exceptions, such as the C_3 *Aristida* species in an otherwise C_4 genus (Cerros-Tlatilpa & Columbus, 2009), raise the possibility of errors at the species level. Misclassification is most likely in lineages where multiple evolutionary transitions between photosynthetic pathways have occurred, especially in Paniceae and Paspaleae (Morrone *et al.*, 2012). The polyphyly of many grass genera accentuates this problem, most acutely illustrated by *Eragrostis walteri*, which was previously considered to be a C_3 species within a wholly C_4 genus (Table 1). Recent phylogenetic work has demonstrated that this species is actually a member of the C_3 Arundinoideae lineage and misplaced within *Eragrostis* (Ingram *et al.*, 2011).

The polyphyly of grass genera means that Tables 1, S3 and S4 should be interpreted with caution. While they do catalogue the known distribution of C_4 photosynthesis among taxa, they do not necessarily provide information about its evolutionary history. However, ongoing phylogenetic work is steadily resolving the polyphyly issue, which is most acute in the genus *Panicum*. We have used the conservative circumscription of *Panicum* s.l. adopted in GrassBase (Clayton *et al.*, 2002b onwards) and recently carried over to the World Checklist of Poaceae (Clayton *et al.*, 2012 onwards) and The Plant List (The Plant List, 2010), because these

online resources provide the most comprehensive, global list of accepted names and synonyms, and are regularly updated in the light of new publications. Using the software tools detailed in the following, it is straightforward to link the C_3 / C_4 data listed for *Panicum* s.l. (see Table S4) to the new genus circumscriptions. The same applies to *Streptostachys* s.l. (Table 1).

How to access the database

Easy routes for users to access information are crucial determinants of the usefulness and usage of data. Our database may be accessed via three routes. The first is static, but the second and third will report updates to the database as we make them.

First, simple tables list photosynthetic pathway by accepted scientific name, and may be accessed in the Supporting Information (Tables S3, S4). These require the user to first prepare a list of accepted species names according to the taxonomy of Clayton *et al.* (2002b onwards) for the taxa of interest.

Second, the name-matching and data-linkage steps may be combined within the software package Taxonome (Kluyver & Osborne, 2013; <http://taxonome.bitbucket.org>; persistent URL <http://purl.org/NET/taxonome>). Taxonome links datasets using species names, handling both synonyms and spelling variants (including spelling mistakes). It deals rapidly with millions of names, and runs via either a simple Graphical User Interface (GUI) for basic functionality or python scripts for advanced users. A user first loads the Kew taxonomy and photosynthetic pathway database via a data file obtained from the Taxonome website. Custom lists comprising any published grass names may then be rapidly matched to this database, and outputted in CSV format.

Third, the photosynthetic pathway data are linked to the Kew taxonomy, together with morphological, phylogenetic, biogeographic and environmental data within the GrassPortal system (Osborne *et al.*, 2011; www.grassportal.org). GrassPortal enables users to easily assemble large-scale, synthetic data products based on multiple original sources, and is accessed via an intuitive and simple GUI. Using this system, users are able to assemble a list of all grass species present in a particular geographic area, linked to photosynthetic pathway, growth form, and environmental niche data.

Large-scale data synthesis

By carrying out technically challenging bioinformatic steps of data processing and linkage, services like GrassPortal open up new possibilities for a broad biological community to explore large-scale synthetic data products. For example, linkage of the photosynthetic pathway dataset with species occurrence data (Clayton *et al.*, 2002a) allows the distribution of C_4 grass species to be mapped at the global scale (Fig. 1). This map improves the global coverage compared with previous data compilations, especially for Africa, South America and Southeast Asia (Sage *et al.*, 1999b). It particularly highlights the prevalence of C_4 photosynthesis among African grasses (Fig. 1a), and the importance of central-east Africa, India and northern Australia as hotspots of C_4 grass species richness (Fig. 1b). The new dataset also facilitates large-scale

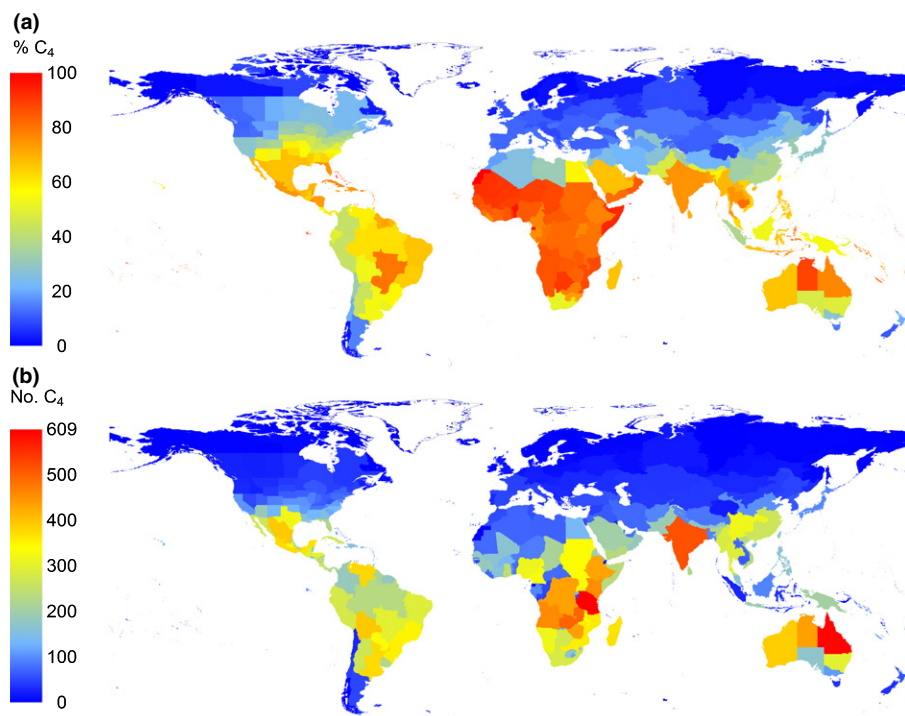


Fig. 1 Global map of C_4 grass species distributions. (a) Percentage of grass species within each mapping unit that uses the C_4 pathway; (b) the species richness of C_4 grasses in each mapping unit. The map shows species distributions at the Taxonomic Databases Working Group (TDWG) level 3 'botanical country' scale, a biodiversity information standard corresponding largely to political countries, but with large countries subdivided into smaller mapping units (Brummitt *et al.*, 2001).

macroevolutionary analyses. For example, the Grass Phylogeny Working Group II (2012) used our data in phylogenetic analyses to discover multiple new C_4 lineages, and to infer that evolutionary gains prevail over losses of this trait. Another recent study used our data in a macroevolutionary analysis to show an association between C_4 photosynthesis and salt tolerance in grasses (Bromham & Bennett, 2014).

The integration of our C_4 pathway data with information on geographical distributions, environmental niche, and phylogenetic relationships promises important novel insights into the ecological significance and evolution of this complex physiological and anatomical trait. More generally, it offers biologists an example of how functional trait data may be used in large-scale synthesis and analysis to advance our understanding of the ecological and evolutionary processes acting on organisms.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Methods S1 Full methodology used to compile and index the dataset.

Notes S1 Full list of literature sources used to compile the dataset presented in Tables S3 and S4.

Table S1 Herbarium specimens of grass species analyzed for stable carbon isotope ratio

Table S2 Uncharacterized species with countries of occurrence and synonyms (Clayton *et al.*, 2002a,b onwards)

Table S3 Full genus-level dataset with numbers of species for each, and references for evidence

Table S4 Full species-level dataset for the genus *Panicum* s.l., as circumscribed by Clayton *et al.* (2002b onwards), with the evidence and references used to ascribe photosynthetic type for each

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Key words: C₃ photosynthesis, C₄ photosynthesis, comparative plant ecology, ecological informatics, evolutionary biology, grasses, Poaceae.



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