REVIEW PAPER

The C$_4$ plant lineages of planet Earth

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Abstract

Using isotopic screens, phylogenetic assessments, and 45 years of physiological data, it is now possible to identify most of the evolutionary lineages expressing the C$_4$ photosynthetic pathway. Here, 62 recognizable lineages of C$_4$ photosynthesis are listed. Thirty-six lineages (60%) occur in the eudicots. Monocots account for 26 lineages, with a minimum of 18 lineages being present in the grass family and six in the sedge family. Species exhibiting the C$_3$–C$_4$ intermediate type of photosynthesis correspond to 21 lineages. Of these, 9 are not immediately associated with any C$_4$ lineage, indicating that they did not share common C$_3$–C$_4$ ancestors with C$_4$ species and are instead an independent line. The geographic centre of origin for 47 of the lineages could be estimated. These centres tend to cluster in areas corresponding to what are now arid to semi-arid regions of southwestern North America, south-central South America, central Asia, northeastern and southern Africa, and inland Australia. With 62 independent lineages, C$_4$ photosynthesis has to be considered one of the most convergent of the complex evolutionary phenomena on planet Earth, and is thus an outstanding system to study the mechanisms of evolutionary adaptation.

Key words: Angiosperms, C$_3$–C$_4$ photosynthesis, CO$_2$-concentrating mechanism, convergent evolution, photorespiration, phylogeny.

Introduction

The metabolic pathway of C$_4$ photosynthesis was first described in the mid-1960s (Hatch and Slack, 1966, 1967; Osmond, 1967; Hatch, 1999), although many of the traits associated with C$_4$ photosynthesis, such as Kranz anatomy, low CO$_2$ compensation points of photosynthesis, and dimorphic chloroplasts were described years earlier (Haberlandt, 1914; Rhoades and Carvalho, 1944; Hodge *et al.*, 1955; Moss, 1962). Once the C$_4$ pathway was identified in ~1966–1967 (Hatch and Slack, 1966, 1967; Osmond, 1967), the integrated picture of C$_4$ photosynthesis was quickly formulated from the distinct patterns of physiology, structure, and ecology that were associated with the group of plants known to have Kranz anatomy (Downton and Tregunna, 1968; Black *et al.*, 1969; Downton *et al.*, 1969; Hatch *et al.*, 1971). With this comprehensive understanding, plant biologists were able to survey the plant kingdom rapidly and by the mid-1970s identified most of the genera containing C$_4$ species (Smith and Epstein, 1971; Downton, 1975; Smith and Turner, 1975; Webster *et al.*, 1975; Sankhla *et al.*,

1975). Detailed surveys of individual families followed, providing comprehensive understanding of the distribution of the C$_4$ pathway in grasses (Brown, 1977; Raghavendra and Das, 1978; Hattersley and Watson, 1992; Watson and Dallwitz, 1992), sedges (Ueno and Koyama, 1987; Soros and Bruhl, 2000; Bruhl and Wilson, 2007), and various eudicot groups (Raghavendra and Das, 1978; Winter, 1981; Ziegler *et al.*, 1981; Pyankov and Vakrusheva, 1989; Batanouny *et al.*, 1991; Akhani *et al.*, 1997). In the edited volume *C$_4$ plant biology* (Sage and Monson, 1999), Sage *et al.* (1999) assembled this information into comprehensive lists of genera containing C$_4$ species, and Kellogg (1999) mapped many of the C$_4$ genera onto the phylogenetic trees available at the time. While these treatments synthesized understanding as of the late 1990s, they also identified significant gaps in the knowledge of the taxonomic distribution of C$_4$ photosynthesis in higher plants. For example, phylogenetic information was often sparse, and isotopic surveys were incomplete for most taxonomic groups. As a

result, the treatments in *C₄ plant biology* stimulated new research that has filled in many of these gaps. Of major significance since the publication of *C₄ plant biology* has been the generation of increasingly detailed phylogenies that resolve the relationships between C₃ and C₄ species within an evolutionary group (e.g. McKown *et al.*, 2005 for *Flaveria*; Giussani *et al.*, 2001, Aliscioni *et al.*, 2003, Christin *et al.*, 2008; Vicentini *et al.*, 2009 for grasses; Besnard *et al.*, 2009; Roalson *et al.*, 2010 for sedges; and Pyankov *et al.*, 2001; Kapralov *et al.*, 2006, Kadereit *et al.*, 2003, 2010; Kadereit and Freitag, 2011 for Chenopods). In addition, intensive isotopic surveys of both C₃ and C₄ species have been undertaken in families known to contain C₄ species, and the result has been detailed accounting of C₃ and C₄ species in a phylogenetic context in the Amaranthaceae (Sage *et al.*, 2007), Cleomeaceae (Marshall *et al.*, 2007; Voznesenskaya *et al.*, 2007; Feodorova *et al.*, 2010), Cyperaceae (Roalson *et al.*, 2010), Molluginaceae (Christin *et al.*, 2011b), and Portulacineae (Ocampo and Columbus, 2010; Vosnesenskaya *et al.*, 2010). This accumulation of phylogenetic information coupled with a better understanding of the taxonomic distribution of the C₄ pathway facilitates the formulation of evolutionary hypotheses regarding the number of C₄ origins and their ecological and geographic context. Here, this phylogenetic and biogeographic information is synthesized to present the most comprehensive listing of C₄ plant lineages known to date.

Methodology

The literature was screened for taxonomic surveys of photosynthetic types as well as phylogenetic information, and then the occurrence of C₄ photosynthesis was mapped onto recent phylogenies following the approach of Kellogg (1999) and Sage (2004). Where C₄ groups were separated in the phylogeny by nodes branching to C₃ taxa, they were inferred to be independent C₄ lineages, unless a scenario of fewer C₄ origins followed by reversal to the C₃ ancestral state was more parsimonious. The exception to this approach occurs in the Chenopodiaceae, where the biochemical and anatomical differences between the different clusters of C₄ taxa were also taken into account. If a C₄ group appeared to have arisen following a C₄ to C₃ reversion, it was only accepted as a distinct lineage if there were multiple intervening C₃ lineages suggesting that the C₄ line evolved from C₃ species. However, the direction of past photosynthetic transitions is still unknown for several groups, and the possibility of C₄ to C₃ reversion is an open question that is difficult to address based solely on species relationships (Christin *et al.*, 2010).

In some taxonomic groups (e.g. *Polycarpea* in the Caryophyllaceae), taxa that are known to contain C₄ species have not yet been included in any molecular phylogenetic study. Because they are the only known C₄ representatives of broader and presumably monophyletic taxonomic groups whose phylogenetic positions have been determined (APG III, 2009), they are reported as distinct C₄ lineages. This approach provides only a minimum estimate, as it cannot discern

whether C₄ species form more than one lineage within a genus unless there is a detailed, species level phylogeny and the photosynthetic pathways of the species are clearly known. Higher resolution phylogenies and surveys of photosynthetic pathways have been forthcoming in the past decade for many C₄-containing groups such as Aizoaceae (Hassan *et al.*, 2005), Amaranthaceae (Sage *et al.*, 2007), Cyperaceae (Bruhl and Wilson, 2007; Besnard *et al.*, 2009; Roalson *et al.*, 2010), *Flaveria* (Asteraceae; McKown *et al.*, 2005), Nyctaginaceae (Douglas and Manos, 2007), Chenopodiaceae (Pyankov *et al.*, 2001; Kadereit *et al.*, 2003, 2010; Kapralov *et al.*, 2006; Akhani *et al.*, 2007; Wen *et al.*, 2010; Kadereit and Freitag, 2011), Euphorbiaceae (Steinmann and Porter, 2002), Molluginaceae (Christin *et al.*, 2011b), and Poaceae (Giussani *et al.*, 2001; Aliscioni et al., 2003; Duvall *et al.*, 2003; Christin *et al.*, 2008; Vicentini *et al.*, 2008; Edwards and Smith, 2010). In most cases, these detailed phylogenies have revealed more complex evolutionary patterns than originally anticipated. In Amaranthaceae *sensu stricto* (*ss*) (excluding Chenopodiaceae),
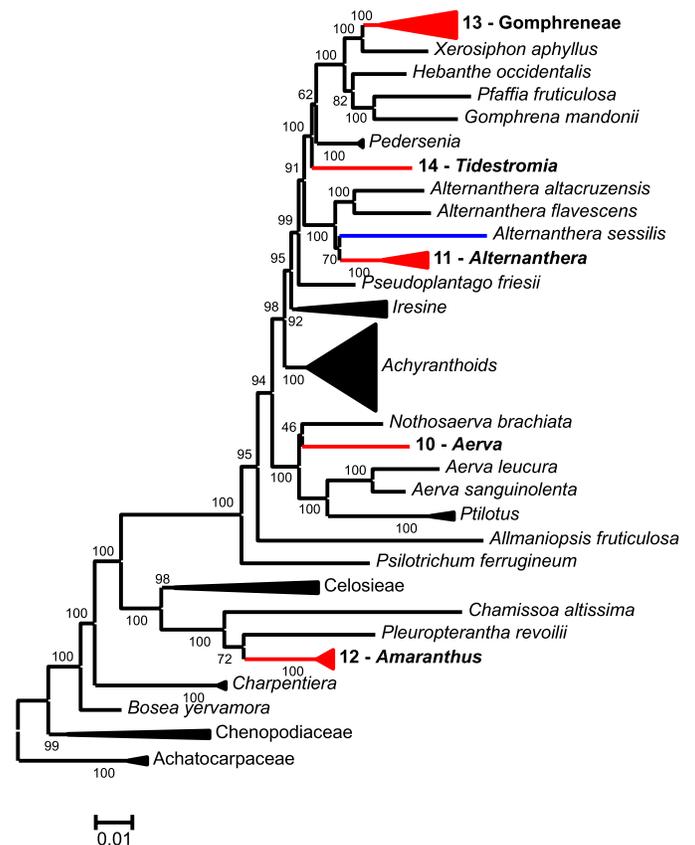
Fig. 1. The distribution of C₄ photosynthesis in the Amaranthaceae *sensu stricto*. The phylogeny was obtained through Bayesian inference on the *trnK–matK* data set of Sage *et al.* (2007). It is rooted on the Achatocarpaceae. Bayesian support values are indicated near branches. Clades that contain a single photosynthetic type for which names are available are compressed and coloured in red for C₄, blue for C₃–C₄, and black for C₃. Names of C₄ clades are in bold and numbers beside C₄ groups correspond to lineage number (Table 1). Photosynthetic types were determined previously by $\delta^{13}C$ assay (Sage *et al.*, 2007).

for example, two lineages were hypothesized by Kellogg (1999) based on poorly resolved phylogenies. Three lineages were suggested by Kadereit et al. (2003), although there was uncertainty in the relationship between C$_4$ species in the Gomphrenoids, *Alternanthera* and *Tidestromia*. Sage et al. (2007) provided detailed sampling of the isotopic ratios of most Amaranthaceae *ss* species, and with the inclusion of additional species in the region of the phylogeny containing the Gomphrenoids, *Alternanthera* and *Tidestromia*, they were able to resolve five distinct clades of C$_4$ photosynthesis (Fig. 1). A more problematic situation is present in the Chenopodiaceae *ss*. While recent phylogenetic work has clarified relationships within this family, patterns of C$_4$ evolution remain uncertain because the C$_3$ and C$_4$ pathways have not been clearly identified in some parts of the phylogeny. To clarify matters, the photosynthetic types were mapped on a phylogenetic tree for the Chenopodiaceae inferred from data accumulated in recent studies (Fig. 2). This approach indicates that 10 C$_4$ lineages are present in the Chenopodiaceae *ss*. More lineages may be present, as there is a possibility that the *Salsola kali* and *Halothamnus* groups may represent two independent C$_4$ lines. The situation is also unclear in Camphorosmeae, where anatomical variations could be inter-preted as the fingerprint of two different C$_4$ origins (Kadereit and Freitag, 2011). The distribution of photosynthetic types also indicates a C$_4$ to C$_3$ reversion and reacquisition of the C$_4$ pathway in the branches between the *S. kali* group and the *Haloxylon/Anabis* group (Fig. 2). Because *Haloxylon/Anabis* presumably acquired C$_4$ photosynthesis from ancestors with a fully expressed C$_3$ pathway, as indicated by the *Sympegma* and *Oreosalsola* nodes, it is treated as an independent lineage, regardless of whether there may have been ancestral C$_4$ species at deeper nodes in the phylogeny.

Species level resolution has also facilitated the identifica-tion of the centres of origin for many of the listed C$_4$ lineages. This can be accomplished by identifying closely related C$_3$ and C$_4$ species within a phylogeny, and any related C$_3$–C$_4$ intermediate species. By mapping the geo-graphic distribution of the sister groups and intermediate forms, the region where the C$_4$ lineage arose can in many cases be identified with a good degree of confidence, thereby facilitating evaluation of the environmental conditions that promoted the emergence of C$_4$ photosynthesis.

To visualize broadly the phylogenetic distribution of C$_4$ taxa, as many C$_4$ groups as possible were mapped onto a recently published phylogeny of 9412 angiosperms (Smith
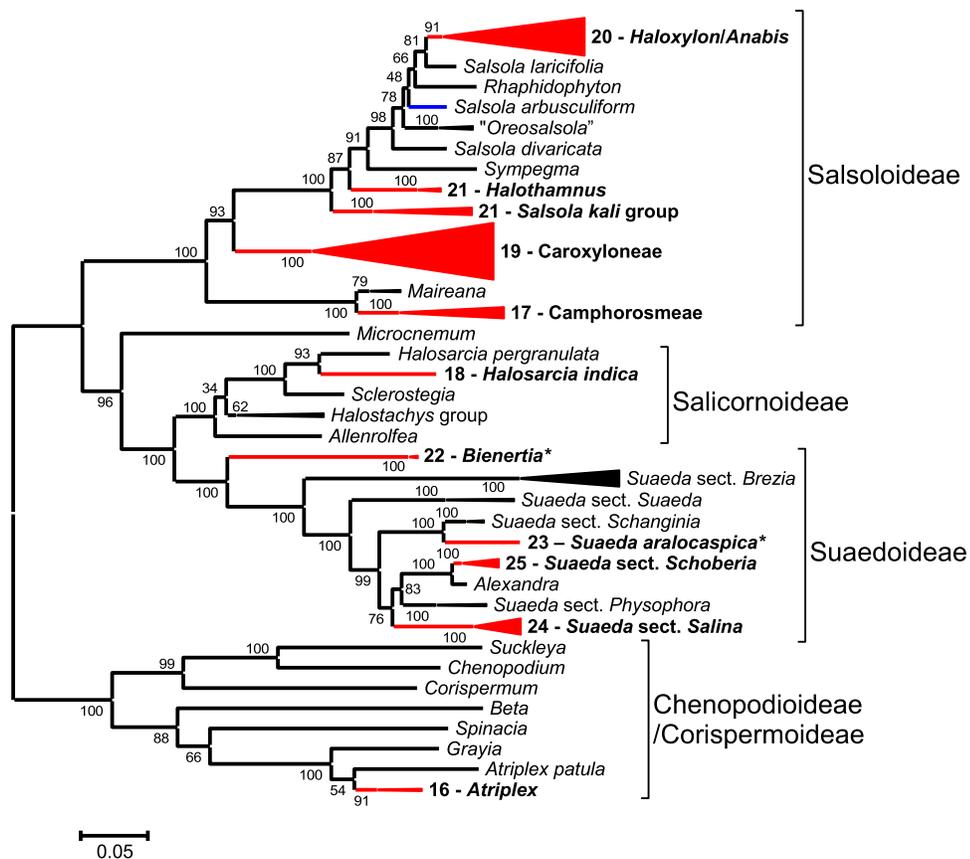
Fig. 2. The distribution of C$_4$ photosynthesis in the Chenopodiaceae *sensu stricto*. The phylogeny was obtained through Bayesian inferences on the nuclear internal transcribed spacer (ITS) and plastid *psbB–psbH* markers generated in previous studies (Kapralov et al., 2006; Akhani et al., 2007; Wen et al., 2010; see Christin et al., 2011a for details) and is rooted on the Chenopodioideae/Corispermoideae. Bayesian support values are indicated near branches. Clades that contain a single photosynthetic type are compressed and coloured in red for C$_4$, blue for C$_3$–C$_4$, and black for C$_3$. Names of C$_4$ clades are in bold and numbers beside C$_4$ groups correspond to lineage number (Table 1). Asterisks indicate single-celled C$_4$ taxa. Subfamilies are circumscribed on the right.

et al., 2009; Fig. 3). Because the phylogeny was built for other purposes and taxon sampling was therefore agnostic with respect to the photosynthetic pathway, it was felt that this presents a valid means to evaluate broad phylogenetic patterns of C4 evolution across angiosperms. It was possible to place 47 C4 lineages on the tree. In several cases (e.g. *Mollugo*, *Flaveria*, and *Cleome*), the phylogeny included only closely related C3 congeners, which were used as 'placeholders'. Futhermore, to place *Blepharis* (Acanthaceae), the close relative *Acanthus* (McDade *et al.*, 2005) was highlighted, and *Aptosimum* and *Peliostomum* (Scrophulariaceae) were used to represent *Anticharis* (Oxelman *et al.*, 2005).

Results

The lineages of C4 photosynthesis

Table 1 lists 62 distinct groups of C4 taxa in terrestrial and aquatic vascular plants. Some diatoms can also operate C4 metabolic cycles but are not discussed here (Reinfelder *et al.*, 2004). These 62 groups are treated as distinct evolutionary lineages, on the assumption that each lineage arose from ancestors that were fully functional C3 species. It is recognized that the evolutionary independence of some C4 lines could be debated if their common ancestors share well-developed traits associated with the C4 syndrome, notably Kranz anatomy. This could be the case in *Flaveria*, *Mollugo*, and Camphorosmeae, where multiple C4 species may derive from well-developed C3–C4 intermediates expressing Kranz-like anatomy (McKown *et al.*, 2005; Christin *et al.*, 2011b; Kadereit and Freitag, 2011). None of these potential lineages are included in Table 1 because of phylogenetic ambiguity and uncertainty regarding evolutionary independence.

Thirty-six of the 62 lineages occur in the eudicots, six in the sedges, and 18 in the grasses (Table 1). The aquatic monocot family Hydrocharitaceae has two C4 lineages, in the genera *Hydrilla* and *Egeria*. C4 photosynthesis in these two groups is distinct from that of the other 60 lineages
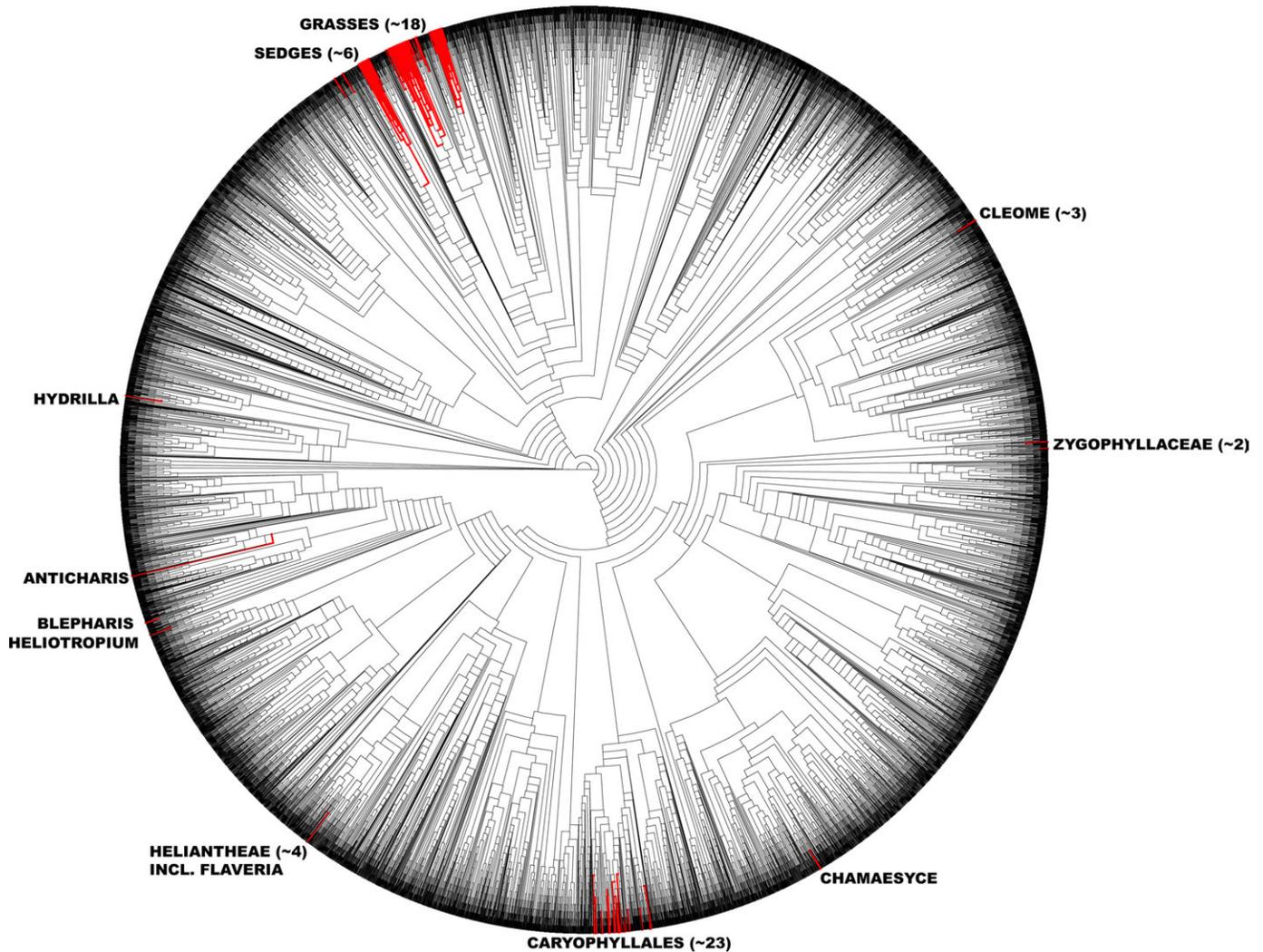
Fig. 3. The phylogenetic distribution of C4 lineages in the angiosperms, depicted on a phylogeny of 9412 angiosperms species that was pruned from the viridiplantae phylogeny of Smith et al. (2009). C4 lineages are indicated by red branches. Numbers beside named lineages refer to the estimate of the number of independent origins of C4 in that clade. Forty-seven of the 62 C4 lineages could be placed on the phylogeny; in several cases, C3 taxa were highlighted to represent the position of closely related C4 species (see text).

Table 1. The postulated lineages of C$_4$ taxa in higher plants

Centre of origin codes are AA, northeastern Africa and Arabia; Aus, Australia; CeA, central Asia; SA, southern Africa; SAM, South America; NAM, North America. Kranz types and biochemical types are from Muhaidat et al. (2007), Edwards and Vosnesenskya (2011), and the references noted in the last column. Grass lineage names follow those of Roalson (2011) and Christin et al. (2009). Centres of origin are estimated from the references indicated, and unpublished results from RFS and EJE.

No.	Order	Family	Lineage	Kranz type	Biochemical type	Centre of origin	Reference
Eudicots							
1	Unplaced	Boraginaceae	*Heliotropium* section *Orthostachys* (= *Euploca*)	Atriplicoid	NADP-ME	NAM	Frohlich (1978)
2	Asterales	Asteraceae/ Heliantheae	*Chrysanthellum/Isostigma*	Simplicifolioid, Isostigmoid, Glossocardioid	NADP-ME, NAD-ME	Unknown	Kellogg (1999)
3	Asterales	Asteraceae/ Heliantheae	*Flaveria* clade A	Atriplicoid	NADP-ME	NAM	Powell (1978); McKown et al. (2005)
4	Asterales	Asteraceae/ Heliantheae	*Flaveria brownii*[a] (*Flaveria* clade B)	Atriplicoid	NADP-ME	NAM	Powell (1978); McKown et al. (2005)
5	Asterales	Asteraceae/ Heliantheae	*Pectis*	Atriplicoid	NADP-ME	NAM	Kellogg (1999)
6	Brassicales	Cleomeaceae	*Cleome angustifolia*	Angustifolioid	NAD-ME	AA	Feodorova et al. (2010)
7	Brassicales	Cleomeaceae	*Cleome gynandra*	Atriplicoid	NAD-ME	SA	Feodorova et al. (2010)
8	Brassicales	Cleomeaceae	*Cleome oxalidea*	Unknown	Unknown	AUS	Feodorova et al. (2010)
9	Caryophyllales	Aizoaceae/ Sesuvioideae	*Sesuvium/ Trianthema/ Zaleya*	Atriplicoid	NADP-ME	AA, SA	Hassan et al. (2005)
10	Caryophyllales	Amaranthaceae	*Aerva*	Atriplicoid	NADP-ME	AA	Sage et al. (2007)
11	Caryophyllales	Amaranthaceae	*Alternanthera*	Atriplicoid	NADP-ME	SAM	Sage et al. (2007)
12	Caryophyllales	Amaranthaceae	*Amaranthus*	Atriplicoid	NAD-ME	New World	Sage et al. (2007)
13	Caryophyllales	Amaranthaceae	Gomphreneae	Atriplicoid	NADP-ME	SAM	Sage et al. (2007)
14	Caryophyllales	Amaranthaceae	*Tidestromia*	Atriplicoid	NADP-ME	NAM	Sage et al. (2007)
15	Caryophyllales	Caryophyllaceae	*Polycarpaea*	Atriplicoid	NADP-ME	Old World	Kellogg (1999)
16	Caryophyllales	Chenopodiaceae	*Atriplex*	Atriplicoid	NAD-ME	CeA	Kadereit et al. (2010)
17	Caryophyllales	Chenopodiaceae	Camphorosmeae	Kochioid	NADP-ME	CeA	Kadereit et al. (2003); Kadereit and Freitag (2011)
18	Caryophyllales	Chenopodiaceae	*Halosarcia indica*	Tecticornoid	NAD-ME	CeA	Kadereit et al. (2003); Kapralov et al. (2006)
19	Caryophyllales	Chenopodiaceae/ Salsoloideae	Caroxyloneae	Salsoloid	NAD-ME	CeA	Kadereit et al. (2003); Kapralov et al. (2006)
20	Caryophyllales	Chenopodiaceae/ Salsoloideae	*Haloxylon/Anabasis* (includes *Noaea*)	Salsaloid	NADP-ME	CeA	Kapralov et al. (2006); Wen et al. (2010)
21	Caryophyllales	Chenopodiaceae/ Salsoloideae	*Salsola kali* group (includes *Halothamnus*)	Salsaloid	NADP-ME	CeA	Kadereit et al. (2003)
22	Caryophyllales	Chenopodiaceae/ Suaedoideae	*Bienertia*	Single-celled (Bienertioid)	NAD-ME	CeA	Kapralov et al. (2006)
23	Caryophyllales	Chenopodiaceae/ Suaedoideae	*Suaeda aralocaspica* (=*Borszczowia*)	Single-celled (Borszczowioid)	NAD-ME	CeA	Kapralov et al. (2006)
24	Caryophyllales	Chenopodiaceae/ Suaedoideae	*Suaeda* sect. *Salsina*	Salsinoid	NAD-ME	CeA	Kapralov et al. (2006)
25	Caryophyllales	Chenopodiaceae/ Suaedoideae	*Suaeda* sect. *Schoberia*	Schoberioid	NAD-ME	CeA	Kapralov et al. (2006)
26	Caryophyllales	Gisekiaceae	*Gisekia*	Unknown	Unknown	Old World	APG III (2009)
27	Caryophyllales	Molluginaceae	*Mollugo cerviana/ M. fragilis*	Atriplicoid	NADP-ME	SA	Christin et al. (2011b)
28	Caryophyllales	Nyctaginaceae	*Allionia*	Atriplicoid	NADP-ME	NAM	Douglas and Manos (2007)
29	Caryophyllales	Nyctaginaceae	*Boerhavia*	Atriplicoid	NADP-ME	NAM	Douglas and Manos (2007)
30	Caryophyllales	Polygonaceae	*Calligonum*	Salsaloid	NAD-ME	CeA	Kellogg (1999)
31	Caryophyllales	Portulacaceae	*Portulaca*	Pilosioid, Portulacenoid	NADP-ME, NAD-ME	SAM	Ocampo and Columbus (2010); Voznesenskaya et al. (2010)
32	Lamiales	Acanthaceae	*Blepharis*	Atriplicoid	NADP-ME, NAD-ME	SA	Vollesen (2000); Akhani et al. (2008)
33	Lamiales	Scrophulariaceae	*Anticharis*	Atriplicoid	NAD-ME	SA	Kellogg (1999)

Table 1. *Continued*

No.	Order	Family	Lineage	Kranz type	Biochemical type	Centre of origin	Reference
34	Malpighiales	Euphorbiaceae	*Euphorbia* subgenus *Chamaesyce*	Atriplicoid	NADP-ME	NAM	Steinmann and Porter (2002); Sage *et al.* (2011)
35	Zygophyllales	Zygophyllaceae	*Tribulus/Kallstroemia*	Atriplicoid	NADP-ME	AA	Sheahan and Chase (1996)
36	Zygophyllales	Zygophyllaceae	*Zygophyllum simplex*	Kochiod	NAD-ME	AA	Sheahan and Chase (1996)
Monocots							
37	Alismatales	Hydrocharitaceae	*Hydrilla*	Single cell, non-Kranz	NADP-ME	Unknown	Bowes (2011); Roalson (2011)
38	Alismatales	Hydrocharitaceae	*Egeria*	Single cell, non-Kranz	NADP-ME	Unknown	Bowes (2011); Roalson (2011)
39	Poales	Cyperaceae	*Bulbostylis*	Fimbrystyloid	NADP-ME	Unknown	Besnard *et al.* (2009)
40	Poales	Cyperaceae	*Cypereae*	Chlorocyperoid	NADP-ME	Unknown	Besnard *et al.* (2009)
41	Poales	Cyperaceae	*Eleocharis* section *Tenuissimae ss*	Eleocharoid	NAD-ME	Unknown	Roalson *et al.* (2010); Roalson (2011)
42	Poales	Cyperaceae	*Eleocharis vivipara*	Fimbrystyloid	NAD-ME	NAM	Besnard *et al.* (2009); Roalson *et al.* (2010)
43	Poales	Cyperaceae	*Fimbrystylis*	Fimbrystyloid	NADP-ME	Unknown	Besnard *et al.* (2009)
44	Poales	Cyperaceae	*Rynchospora*	Rynchosporoid	NADP-ME	Unknown	Besnard *et al.* (2009)
45	Poales	Poaceae/ PACMAD clade	*Alloteropsis*	Neurachneoid	PCK	Africa	Christin *et al.* (2009)
46	Poales	Poaceae/ PACMAD clade	*Altoparidisum/ Arthropogon/ Mesosetum/Tatianyx*	Classical	NADP-ME	SAM	Christin *et al.* (2009)
47	Poales	Poaceae/ PACMAD clade	Andropogoneae	Classical	NADP-ME	Unknown	Christin *et al.* (2009)
48	Poales	Poaceae/ PACMAD clade	*Anthaenantia lanata* (=*Leptocoryphium*)	Classical	NADP-ME	SAM	Christin *et al.* (2009)
49	Poales	Poaceae/ PACMAD clade	*Aristida*	Aristidoid	NADP-ME	Unknown	Christin *et al.* (2009)
50	Poales	Poaceae/ PACMAD clade	*Axonopus/Ophiochloa*	Classical	NADP-ME	SAM	Christin *et al.* (2009)
51	Poales	Poaceae/ PACMAD clade	*Centropodia*	Classical	NADP-ME	Africa	Christin *et al.* (2009)
52	Poales	Poaceae/ PACMAD clade	Core Chloridoideae	Classical	NAD-ME, PCK	Old World	Christin *et al.* (2009)
53	Poales	Poaceae/ PACMAD clade	*Danthoniopsis/ Loudetia*	Arundinelloid	NADP-ME	Unknown	Christin *et al.* (2009)
54	Poales	Poaceae/ PACMAD clade	*Digitaria*	Classical	NADP-ME	Unknown	Christin *et al.* (2009)
55	Poales	Poaceae/ PACMAD clade	*Echinochloa*	Classical	NADP-ME	Unknown	Christin *et al.* (2009)
56	Poales	Poaceae/ PACMAD clade	*Eriachne/ Pheidochloa*	Eriachnoid	NADP-ME	Unknown	Christin *et al.* (2009); Roalson (2011)
57	Poales	Poaceae/ PACMAD clade	*Neurachne*	Neurachneoid	NADP-ME, PCK	AUS	Christin *et al.* (2009)
58	Poales	Poaceae/ PACMAD clade	*Panicum/Pennisetum/ Urochloa/Setaria*	Classical	NADP-ME, NAD-ME, PCK	Unknown	Christin *et al.* (2009)
59	Poales	Poaceae/ PACMAD clade	*Paspalum*	Classical	NADP-ME	SAM	Christin *et al.* (2009)
60	Poales	Poaceae/ PACMAD clade	*Sorengia* (ex *Panicum prionitis*)	Neurachneoid	NADP-ME	SAM	Christin *et al.* (2009)
61	Poales	Poaceae/ PACMAD clade	*Streptostachys*	Classical	NADP-ME	SAM	Christin *et al.* (2009)
62	Poales	Poaceae/ PACMAD clade	*Stipagrostis*	Stipagrostoid	NADP-ME	Old World	Christin *et al.* (2009)

[a] *Flaveria brownii* is physiologically a C_4-like intermediate, in that it expresses Rubisco in the mesophyll (Cheng *et al.*, 1988). It is treated here as an independent C_4 clade as it has a fully functional C_4 cycle, and photosynthetic gas exchange properties and resource use efficiencies that are equivalent to those of many C_4 species.

where plants have aerial photosynthetic structures. In *Hydrilla* and *Egeria*, the C$_4$ pathway operates in submersed leaves and concentrates CO$_2$ from the cytosol into an adjacent chloroplast of a single cell (Bowes, 2011). In all other known C$_4$ plants, the C$_4$ pathway concentrates CO$_2$ from a mesophyll-like compartment into a distinct inner tissue region (58 lineages) or concentrates CO$_2$ from an outer to an inner region of the same cell (in two Chenopodiaceae lineages, *Bienertia* and *Suaeda aralocaspica*; Edwards and Voznesenskaya, 2011). Clustering is evident in the distribution of the lineages in the angiosperm phylogeny (Fig. 3), with large numbers of lineages in the Poales (grass and sedge families) and Caryophyllales (which includes Aizoaceae, Amaranthaceae, Caryophyllaceae, Chenopodiaceae, Gisekiaceae, Molluginaceae, Nyctaginaceae, Polygonaceae, and Portulacaceae).

In the eudicots, there are ~1600 C$_4$ species (Sage *et al.*, 1999). The Amaranthaceae *sensu lato* (= Chenopodiaceae *ss* and Amaranthaceae *ss*; APG III, 2009) is the most prolific family, with 15 distinct lineages of C$_4$ taxa and ~750 C$_4$ species (Figs 1, 2; Sage *et al.*, 1999). Of these 15 lineages, the largest is *Atriplex*, with 200–300 C$_4$ species (Kadereit *et al.*, 2010). Two of the C$_4$ clades in the Salsoloideae (Caroxyloneae and *Haloxylon/Anabis*) have 100–140 species each, while the *S. kali* lineage contains a minimum of 23 species (Sage *et al.*, 1999; Akhani *et al.*, 2007). In the Amaranthaceae *ss*, the Gomphreneae have ~125 C$_4$ species and *Amaranthus* ~70 C$_4$ species (Sage *et al.*, 2007). The most species-rich C$_4$ eudicot lineage other than *Atriplex* is *Euphorbia* section *Chamaesyce* with about 250 species. Other large C$_4$ lineages include *Heliotropium* section *Orthostachys* (=*Euploca*) with ~120 C$_4$ species (Frohlich, 1978), and the *Calligonum*, *Pectis*, and *Portulaca* lineages with 80–100 C$_4$ species each (Sage *et al.*, 1999). Fourteen eudicot C$_4$ lineages contain <10 C$_4$ species: *Aerva*, *Allionia*, *Bienertia*, and *Mollugo* have two C$_4$ species each, while *Suaeda aralocaspica* (=*Borszczowia aralocaspica*), the three C$_4$ lineages in the Cleomeaceae, *Flaveria* clade B, *Halosarcia*, and *Zygophyllum* have one C$_4$ species each. *Anticharis*, *Flaveria* clade A, and *Gisekia* have 5–10 C$_4$ species each (Sage *et al.*, 1999; McKown *et al.*, 2005 for *Flaveria*).

In the sedges, there are ~1300 species (Bruhl and Wilson, 2007; Roalson, 2011). The largest C$_4$ sedge lineages are the Cypereae (eight genera with ~750 species), *Fimbrystylis* (300 species), and *Bulbostylis* (205 species). Fifteen C$_4$ species are known in the *Eleocharis* section *Tenuissimae ss*, while 21 C$_4$ species are known from *Rhynchospora*. In both *Eleocharis* and *Rhynchospora*, >90% of the species are C$_3$ plants (Bruhl and Wilson, 2007). The smallest C$_4$ sedge lineage is *Eleocharis vivipara* with a single C$_4$ species (Bruhl and Wilson, 2007; Roalson *et al.*, 2010; Roalson, 2011).

There are ~4600 C$_4$ grasses, all occurring in the PACMAD clade (Sage *et al.*, 1999). The largest C$_4$ lineage is the core Chloridoideae with 160–170 genera and 1500 species, followed by the Andropogoneae lineage with ~1100 species and then the *Panicum/Pennisetum/Urochloa/Setaria* clade with >500 species (Christen *et al.*, 2009; Roalson, 2011). Thirty-five to 350 species are in each of the lineages repre-

sented by *Altoparadisium*, *Aristida*, *Axonopus*, *Danthoniopsis*, *Digitaria*, *Echinochloa*, *Eriachne*, *Paspalum*, and *Stipagrostis*. C$_4$ grass lineages with a small (<10) number of species are *Alloteropsis* (5 species), *Anthaenantia* (2 species), *Centropodia* (4 species), *Neurachne* (2 species in two genera), *Sorengia* (>5 species), and *Streptostachys* (1 species). The estimates of C$_4$ grass numbers within numerous lineages will change, as many grass genera and species cannot be accurately placed in a lineage yet, due to limited phylogenetic information. This is especially true for *Panicum*, which contains several hundred C$_4$ taxa, but is highly polyphyletic (Aliscioni *et al.*, 2003). Summing the C$_4$ eudicot and monocot estimates, the total number of C$_4$ species on planet Earth is ~7500, which is the same as estimated by Sage *et al.* (1999).

About 43 of the 62 lineages contain species using the NADP-malic enzyme (NADP-ME) as their primary decarboxylase (Table 1; Gutierrez *et al.*, 1974; Edwards and Walker, 1983; Hattersley and Watson, 1992; Sage *et al.*, 1999; Muhaidat *et al.*, 2007; R Khoshravesh, H Akhani, and RF Sage, unpublished data). NAD-malic enzyme (ME) is used by species from 20 lineages. Most of the lineages with NAD-ME species are eudicots, as only two grass and two sedge lineages include species that are classified into this biochemical subtype. Only grasses appear to utilize PEP carboxykinase (PCK) as the primary decarboxylating enzyme; however, this enzyme may also be active as a secondary decarboxylase in the C$_4$ cycle of eudicots in the Sesuvioideae (Muhaidat *et al.*, 2007). In the grasses, four C$_4$ lineages include species that use PCK as the primary decarboxylating enzyme. Of these, two have species that are primarily PCK or NAD-ME, one has species that are PCK or NADP-ME, and one lineage (the *Panicum/Pennisetum/Urochloa/Setaria* clade) has species that are NADP-ME, NAD-ME, or PCK. In the eudicots, three lineages (*Blepharis*, *Chrysanthellum/Isostigma*, and *Portulaca*) contain species reported to utilize primarily more than one of the C$_4$ decarboxylating enzymes (Table 1).

Anatomical types are far more varied than biochemical subtypes. Some 22 Kranz anatomy types have been described, and numerous variations within a number of these subtypes are noted (Brown, 1977; Dengler and Nelson, 1999; Kadereit *et al.*, 2003; Edwards and Voznesenskaya, 2011). In the eudicots, the most common anatomical type is the Atriplicoid, which occurs in at least 20 of the 36 eudicot lineages (Table 1). The next most common Kranz type is the Salsaloid, occurring in three lineages in the Chenopodiaceae and one in *Calligonum* (Polygonaceae). In sedges and grasses, the variation in Kranz type is greater, with most lineages having evolved a unique version of C$_4$ anatomy. The classical type of Kranz anatomy is described for seven grass lineages; however, there can be important variations in the anatomies that are associated with biochemical subtypes (Dengler and Nelson, 1999). Among these variations, classical NADP-ME species have bundle sheath cells (BSCs) with centrifugally placed chloroplasts that are depleted in photosystem II (PSII) and grana stacks; in contrast, classical NAD-ME species have

Table 2. The postulated lineages of C_3–C_4 intermediate photosynthesis in higher plants

C_3–C_4 as defined here refers to photosynthetic modifications that include refixation of photorespiratory CO_2 in bundle sheath cells, and the engagement of a C_4 metabolic cycle. Compiled from Sage *et al.* (1999), Bauwe (2011), and the references listed below. The list does not include species which show C_3 and C_4 expression in different regions of the same plant (termed C_3/C_4 in Sage *et al.*, 1999) or C_3 and C_4 plants in different subspecies (termed $C_3 \times C_4$ in Sage *et al.*, 1999).

No.	Family	Lineage	Representative species	Species number	Ancestry of a C_4 lineage?	References
Eudicots						
1	Amaranthaceae	*Alternanthera*	*Alternanthera ficoides*, A. tenella	2	Yes	Rajendrudu *et al.* (1986)
2	Asteraceae I	*Flaveria sonorensis*	*Flaveria sonorensis*	1	No	McKown *et al.* (2005)
3	Asteraceae II	*Flaveria* clade A	*Flaveria ramossissima*	1	Yes	Monson *et al.* (1984); McKown *et al.* (2005)
4	Asteraceae III	*Flaveria* clade B	*Flaveria angustifolia* and 6 other species	7	Yes	Monson *et al.* (1984); McKown *et al.* (2005)
5	Asteraceae IV	*Parthenium*	*Parthenium hysterophorus*	1	No	Hedge and Patil (1981); Kellogg (1999)
6	Boraginaceae	*Heliotropium* section *Orthostachys* I	*Heliotropium convolvulaceum*, H. racemosum	2	Yes	Vogan *et al.* (2007); Muhaidat (2007)
7	Boraginaceae	*Heliotropium* section *Orthostachys* II	*Heliotopium greggii*, H. lagoense	2	Yes	Vogan *et al.* (2007); Frohlich, 1978
8	Brassicaceae	*Moricandia*	*Moricandia arvense*	5	No	Apel *et al.* (1978); Holaday *et al.* (1981); Kellogg (1999)
9	Brassicaceae	*Diplotaxis*	*Diplotaxis tenuifolia*	1	No	Apel *et al.* (1980); Kellogg (1999)
10	Chenopodiaceae	Camphorosmae	*Bassia sedoides* (=*Sedobassia*)	1	Yes	Kadereit and Freitag (2011)
11	Chenopodiaceae	Salsoleae ss	*Salsola arbusculiformis*	1	No	Voznesenskaya *et al.* (2001); Fig. 3
12	Cleomeaceae	*Cleome*	*Cleome paradoxa*	1	Yes	Feodorova *et al.* (2010)
13	Euphorbiaceae	*Chamaesyce*	*Chamaesyce acuta*, C. johnstonii	2	Yes	Sage *et al.* (2011)
14	Molluginaceae I	*Mollugo* I	*Mollugo nudicaulis*	2	Yes	Christin *et al.* (2011b)
15	Molluginaceae II	*Mollugo* II	*Mollugo verticillata*	1	No	Christin *et al.* (2011b)
16	Nyctaginaceae	*Bougainvillia*	*Bougainvillia* cv. Mary Palmer	1	No	Sabale and Bhosale (1984)
17	Portulaceae	*Portulaca*	*Portulaca cryptopetala*	1	Uncertain	Ocampo and Columbus (2010); Voznesenskaya *et al.* (2010)
Monocots						
18	Cyperaceae subgenus *Scirpidium*	*Eleocharis*	*Eleocharis acicularis*, E. pusilla, E. reverchonii	3	No	Bruhl and Perry (1995); Roalson *et al.* (2010); Keeley 1999); Sage *et al.* (1999)
19	Hydrocharitaceae	*Vallisneria*	*Vallisneria spirilis*	1	Unknown	Bowes (2011)
20	Poaceae 12	*Neurachne*	*Neurachne minor*	1	Yes	Hattersley *et al.* (1982, 1986); Christin *et al.* (2009)
21	Poaceae 13	Paniceae	*Steinchisma*	6	No	Duvall *et al.* (2003); Christin *et al.* (2009)

centripedal chloroplasts and an abundance of PSII and grana stacks (Dengler and Nelson, 1999; Edwards and Voznesenskaya, 2011).

Lineages of C_3–C_4 intermediacy

Twenty-one distinct clades have been identified that contain species with photosynthetic characteristics that are intermediate between C_3 and C_4 species (Table 2). Ten of the C_3–C_4 groups branch immediately sister to C_4 lineages, which is consistent with models proposing that C_3–C_4 intermediacy originated before C_4 photosynthesis and served as an ancestral stage (Monson *et al.*, 1984; Monson, 1999; Sage, 2004; Bauwe, 2011). Most C_3–C_4 intermediates

cluster in genera known to contain C_4 plants (Table 2), as is best demonstrated by *Flaveria* which has ~9 C_3–C_4 species (McKown *et al.*, 2005). Some of these intermediates, however, do not appear at sister nodes. Where C_3 species branch between the C_3–C_4 intermediate and a C_4 node, as occurs with the C_3–C_4 intermediate *Mollugo verticillata*, it appears that the C_3–C_4 line has independently arisen from different C_3 ancestors than the C_4 line (Christin *et al.*, 2011b). Where a C_3–C_4 species branches between two C_4 nodes, as occurs with the C_3–C_4 intermediate *Portulaca cryptopetala* (Ocampo and Columbus, 2010), a reversion from the C_4 condition is possible. Notably, about a quarter of the identified C_3–C_4 species occur in taxa that are not

closely related to any C$_4$ lineage. There are no C$_4$ species in the Brassicaceae, where two C$_3$–C$_4$ clades occur (*Moricandia* and *Diplotaxis*; Sage *et al.*, 1999), and three C$_3$–C$_4$ *Eleocharis* species occur in the *Eleocharis* subgenus *Scirpidium*, which lacks any C$_4$ species (Keeley, 1999; Roalson *et al.*, 2010). These patterns highlight the need to consider the C$_3$–C$_4$ condition as a distinct photosynthetic adaptation in its own right, and not just a transitional stage leading to the C$_4$ condition.

Geographic centres of origins

Geographic centres of origins for C$_4$ photosynthesis can be estimated for most eudicot lineages, and some of the sedge and grass lineages (Table 1). In the eudicots, lineages occur in one of six centres of origin, corresponding to regions of the Earth that are now warm, semi-arid, and arid (Fig. 4). Central Asia, North America, and a region corresponding to northeast Africa and southern Arabia produced the most C$_4$ eudicot lineages, with 4–11 each. Two centres corresponding to semi-arid regions of South Africa and South America each produced 4–5 C$_4$ eudicot lineages, while the driest continent, Australia, produced only one C$_4$ lineage in the eudicots that can be confirmed at this time.

Identifying the geographic origins of the C$_4$ monocots is more problematic due to their wide geographical distribution and greater uncertainty regarding the phylogenetic placement of the C$_4$ lineages. Nevertheless, South America appears to be a major hotspot for C$_4$ grass origins, including many of the transitions in the x=10 Paniceae clade (Table 1; Fig. 4). Two C$_4$ grass origins in Africa are apparent, in *Centropodia* and *Alloteropsis*. Only one C$_4$ grass clade (*Neurachne*) is known to have originated in Australia. *Eleocharis vivipara* is the only sedge lineage where a centre of origin (in Florida, USA) can be postulated at this time.

Discussion

The present survey identified 62 distinct lineages of C$_4$ taxa, containing ~7500 species in 19 families of angiosperms. This compares with 45 lineages listed by Sage (2004) and 31 listed by Kellogg (1999). The increase in the number of lineages is largely due to improved phylogenetic coverage of clades that include C$_4$ plants, and a more complete accounting of C$_3$ and C$_4$ occurrence in the species within these clades. As an example, where only three clades were resolved in the Amaranthaceae *ss* in 2003 (Kadereit *et al.*, 2003), five lineages were observed by Sage *et al.* (2007) following a thorough isotope analysis of the family and additional sampling for the phylogeny. Similarly, early molecular phylogenies suggested a minimum of four C$_4$ grass lineages (Kellogg, 1999), a number that has now increased to 18. The current list of C$_4$ groups is most probably incomplete, as relationships in some clades are still unresolved. Additional lineages are suspected in *Blepharis* (Aizoaceae), *Flaveria* and *Isostigma* (Asteraceae), *Heliotropium* section *Orthostachys* (Boraginaceae), *Eleocharis viridans* (Cyperaceae), Sesuvioideae (Aizoaceae), Camphorosmae (Chenopodiaceae), and Salsoloideae (Chenopodiaceae) (Hassan *et al.*, 2005; McKown *et al.*, 2005; Roalson *et al.*, 2010; Kadereit and Freitag, 2011; RFS, unpublished results).

C$_3$–C$_4$ intermediacy

C$_3$–C$_4$ intermediacy is a term originally used to describe plants with traits intermediate between C$_3$ and C$_4$ species, on the assumption that they might represent an evolutionary transition (Kennedy and Laetsch, 1974; Monson *et al.*, 1984). Currently, C$_3$–C$_4$ intermediacy mainly refers to plants with a photorespiratory CO$_2$-concentrating mechanism, where expression of the photorespiratory enzyme
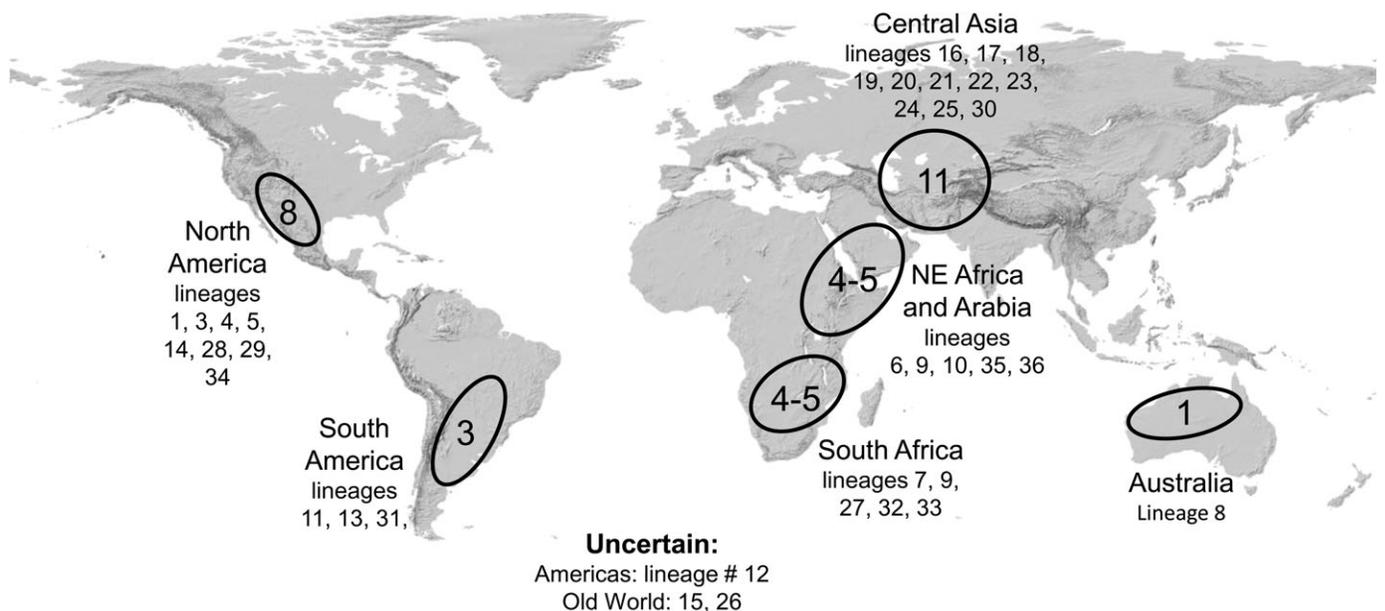
Fig. 4. Locations for the centres of origin for 35 of the 36 C$_4$ eudicot lineages listed in Table 1. Numbers shown correspond to lineages listed in Table 1. Unlisted lineages have an unknown centre of origin.

glycine decarboxylase (GDC) is localized to BSCs (Monson, 1999; Duvall *et al.*, 2003; Bauwe, 2011; Sage *et al.*, 2011). Localization of GDC to the bundle sheath forces all the glycine produced by photorespiration to move into the BSCs to complete the photorespiratory cycle. A product of the GDC reaction is CO_2, which accumulates in the BSCs, enhancing the efficiency of BSC Rubisco. Following the mutation leading to GDC localization, C_3–C_4 species evolve many C_4-like traits such as close vein spacing and enlarged BSCs to optimize the efficiency of photorespiratory CO_2 concentration (Sage, 2004). While these developments may facilitate C_4 evolution (Bauwe, 2011), they also confer fitness in their own right, as reflected by numerous C_3–C_4 lineages that are distinct from C_4 clades, and the ecological success of numerous C_3–C_4 species in warm to hot environments (Monson, 1999; Christin *et al.*, 2011b; Sage *et al.*, 2011).

Twenty-one distinct lineages of C_3–C_4 intermediate plants have been identified. The first C_3–C_4 intermediate described was *M. verticillata*, a widespread weedy species (Kennedy and Laetsch, 1974), followed in the late 1970s to mid-1980s by the identification of intermediate species in *Alternanthera*, *Bougainvillea*, *Diplotaxis*, *Flaveria*, *Moricandia*, *Neurachne*, Panicum *sensu lato* (=*Steinchisma*), and *Parthenium* (Brown and Brown, 1975; Morgan and Brown, 1979; Apel *et al.*, 1978, 1980; Apel and Maas, 1981; Hedge and Patil, 1981; Holaday *et al.*, 1981; Hattersley *et al.*, 1982, 1986; Ku *et al.*, 1983; Monson *et al.*, 1984; Sabale and Bhosale, 1984; Rajendrudu *et al.*, 1986). After this initial phase of discovery 25–35 years ago, the identification of new intermediates trailed off until recently, when phylogenies and isotopic screens helped identify additional intermediates. For example, the Euphorbiaceae phylogeny of Steinmann and Porter (2002) identified two species of *Chamaesyce* that are basal in this large C_4 group. One of these, *C. acuta*, is a C_3–C_4 intermediate (Sage *et al.*, 2011). Intermediates have also been found recently in *Cleome* and *Portulaca* (Voznesenskaya *et al.*, 2007, 2010).

Most of the known C_3–C_4 intermediates are in eudicots, while only two intermediate lineages are described in the grasses, and one each in the Hydrocharitaceae and sedges. This discrepancy may reflect greater species turnover in grasses and sedges, which led to a greater rate of extinction of C_3–C_4 taxa. Alternatively, the greater number of eudicot intermediates may reflect sampling bias. Most known C_3–C_4 intermediates are associated with C_4 eudicots, because investigators often focused on eudicot genera having both C_3 and C_4 species (Monson, 1999; Vogan *et al.*, 2007; Voznesenskaya *et al.*, 2007, 2010; Sage *et al.*, 2011). With a wider sampling and improved phylogenetic resolution of poorly studied groups, the tally of C_3–C_4 lineages should grow in the near future. Isotopic screens will pick up some intermediates with less negative $\delta^{13}C$ than typical C_3 plants (see, for example, Feodorova *et al.*, 2010 for *Cleome*); however, most C_3–C_4 intermediates have $\delta^{13}C$ values that cannot be differentiated from those of C_3 species, so such screens will be of limited value. To have a less negative $\delta^{13}C$ value than C_3 species, there must be significant engagement of a C_4 cycle (von

Caemmerer, 1992). In order to detect potential C_3–C_4 intermediates lacking a C_4 cycle, anatomical screens are a useful first step, but detailed physiological studies with live material will still be needed for confirmation.

The biogeography of C_4 evolution

For the eudicots, and the handful of monocots where the centre of C_4 origin can be estimated with confidence, there appear to be six geographic regions where the C_4 pathway evolved. All of these correspond to areas that are now semi-arid to arid, with summer precipitation from monsoon weather systems. By identifying the putative centres of origin for many of the C_4 lineages, we hope to facilitate follow-up studies that will evaluate the environmental selection factors responsible for the evolution of specific C_4 lineages. Such studies could examine the ecophysiology of the close C_3 and C_3–C_4 relatives of the C_4 lines currently present in the centres of origins. Alternatively, paleontology studies could correlate past environmental events with the appearance of a C_4 lineage in a given area. To date, the leading environmental hypothesis for C_4 evolution is that reduction in atmospheric CO_2 in the late Oligocene increased photorespiration in warm climates, thereby facilitating selection for CO_2-concentrating mechanisms such as C_4 photosynthesis (Sage, 2001, 2004; Christin *et al.*, 2008; Vicentini *et al.*, 2009; Edwards *et al.*, 2010; Osborne, 2011). However, C_4 photosynthesis repeatedly arose in the 25–30 million years since the late-Oligocene CO_2 reduction (Christin *et al.*, 2011a). In light of this, it is better to think of low CO_2 as a pre-condition, or environmental facilitator, which acted in concert with multiple selection factors. Other proposed drivers of C_4 evolution include increasing aridity, creation of high light habitats, increasing seasonality, fire, and large animal disturbance (Sage, 2001; Osborne and Freckleton, 2009; Edwards and Smith, 2010; Osborne, 2011). While a careful paleo-evaluation is beyond the scope of this study, it should be noted that global climates became cooler and drier in the past 40 million years, promoting the rise of arid-adapted vegetation types (Sage, 2001; Willis and McElwain, 2002). By the late Miocene (11–5 million years ago), warm, semi-arid, summer-wet climate zones were present in south-central North America, central Asia and Arabia, and northeastern Africa (Willis and McElwain, 2002). The mid-to-late Miocene corresponds to the mid-range of estimates for the divergence of many eudicot lines (Christin *et al.*, 2011a). The continent with the fewest and youngest C_4 lineages, Australia, developed the warm, dry conditions postulated to support C_4 evolution relatively late, only in the past 4–5 million years (Archer *et al.*, 1995).

If the current environments in the C_4 centres of origin are similar to those at the time when the C_4 lineages appeared, then the climate similarities between the centres of origin suggest the following environmental model for C_4 evolution. Each of the centres of origin experiences hot summers with peak air temperatures >40°C (Walter *et al.*, 1975). Summer humidity is often low, which in combination with the summer heat leads to low humidity and high

transpiration potential. Solar radiation is intense, causing high surface temperatures and substantial heating of plants near the ground. Due to the monsoon activity, however, soil moisture is episodically present, allowing for substantial photosynthetic activity during the summer. Because of the high temperature, and reduced stomatal conductance caused by low humidity, photorespiration must have been high in C$_3$ species, particularly in the low CO$_2$ conditions of the recent past. This could have favoured the rise of CO$_2$ scavenging systems such as C$_3$–C$_4$ intermediate types of photosynthesis, leading in turn to the more elaborate C$_4$ carbon-concentrating mechanisms.

Convergence in C$_4$ evolution

C$_4$ photosynthesis involves the coordinated changes to genes that affect leaf and stem anatomy, ultrastructure, energetics, metabolite transport, and the location, content, and regulation of many metabolic enzymes, leaving no doubt that it is a complex trait (Hibberd and Covshoff, 2010). Despite this complexity, it has evolved repeatedly in diverse groups of flowering plants and thus can be considered genuinely convergent, in the sense that it has independently emerged from deep within many of the major angiosperm clades (e.g. Asterids, Rosids, Caryophyllales, and Monocots; though not Eumagnoliads). At the same time, however, most origins are clearly clustered in two particular areas of the angiosperm tree. The Poales (~18000 C$_3$ and C$_4$ species) harbour fully one-third of C$_4$ lineages, and Caryophyllales (~11000 C$_3$ and C$_4$ species) roughly another third. Within both of these groups, there is additional clustering of C$_4$ clades. C$_4$ taxa are absent from most Poales families and the large BEP clade of grasses; however, it has evolved 18 or more times within the PACMAD clade (mostly in the Panicoideae subfamily). Similarly, the Amaranthaceae/Chenopodiaceae alliance encompasses most of the C$_4$ lineages in Caryophyllales, with 15 postulated origins. This extreme clustering of C$_4$ lineages, in conjunction with the lack of C$_4$ taxa across large regions of the angiosperm phylogeny, indicates that there are a limited number of C$_3$ plant lineages that possess an appropriate suite of characteristics that can facilitate the evolution of C$_4$ photosynthesis (Sage, 2001). These characteristics could be inherent within the anatomical structure and physiology of the species within a lineage, or may be related to a specific ecological setting. Anatomical characters in C$_3$ ancestors could include close vein spacing, enlarged BSCs, or low mesophyll to bundle sheath ratios (Sage, 2004; McKown et al., 2005; Sage et al., 2011). These anatomical traits could facilitate C$_4$ evolution by enabling the establishment of a two-tissue mechanism to refix photorespiratory CO$_2$, which is considered a major step in C$_4$ evolution (Monson, 1999). Physiological facilitators include increased organelle number in BSCs of C$_3$ ancestors, allowing for more photosynthetic activity (Brown and Hattersley, 1989). Cryptic enhancers promoting the evolvability of C$_4$ photosynthesis may be found in the genomes of shared ancestors of clustered C$_4$ groups. For example, a large reservoir of duplicated genes has been suggested as a genetic pre-condition for C$_4$ evolution (Monson, 2003; Bauwe, 2011). In addition, regulatory elements conferring tissue specificity may be common in C$_3$ groups from which the C$_4$ pathway arose. Ecologically, specialization for hot, dry, or saline landscapes could select for carbon conservation mechanisms such as refixation of photorespired CO$_2$. Thus, groups such as the C$_3$ Chenopodiaceae may be prone to evolve C$_4$ photosynthesis because they tend to occur in extreme habitats where photorespiration would be high and there would be strong and consistent selection pressure favouring carbon-concentrating mechanisms.

With 62 or more distinct origins, the C$_4$ pathway must be considered one of the most convergent of complex evolutionary phenomena in the living world. On the one hand, such frequent convergence suggests C$_4$ evolution is relatively easy, a point that has encouraged efforts to engineer the C$_4$ pathway into C$_3$ crops (Hibberd et al., 2008). Alternatively, this statement might be overly simplistic, as it implies that all C$_4$ origins had a similar starting point within a C$_3$ physiological setting, and evolution converged on a uniform C$_4$ pathway. As shown by the variation in decarboxylation types, leaf anatomy, and cellular ultrastructure, CO$_2$ concentration by C$_4$ photosynthesis can be accomplished in a variety of different ways. Uniformity between the different C$_4$ types is largely observed in the role of PEP carboxylase and the functional significance of the C$_4$ pathway (Kellogg, 1999). In this light, convergence on a common function has occurred 62 times, but less so in terms of the specific mechanisms by which CO$_2$ concentration is achieved.

In addition, there are differing degrees of convergence in terms of the magnitude of modifications to the ancestral C$_3$ state within the 62 C$_4$ lineages. Complete transitions from the full C$_3$ condition to the full C$_4$ condition unequivocally occurred independently in several clades. Examples include the C$_4$ clades in Acanthaceae (Blepharis), Boraginaceae, Euphorbiaceae, Scrophulariaceae, Zygophyllaceae, and Asteraceae. At the other extreme are situations where multiple C$_4$ lineages arose from common C$_3$–C$_4$ ancestors, such as two C$_4$ clades in Mollugo (Christin et al., 2011b). In these instances, the evolutionary transition from C$_3$–C$_4$ to C$_4$ would be relatively small, involving fewer changes than the complete C$_3$ to C$_4$ transition. Such cases might better be thought of as a parallel realization of the C$_4$ condition. The more ambiguous situation involves the independent evolution of C$_4$ lineages from C$_3$ ancestors expressing traits which might facilitate C$_4$ evolution, such as high vein density and gene duplication. An example of this occurs in Flaveria, where C$_4$ photosynthesis in clades A and B appears to have independently arisen from C$_3$ ancestors with high vein density (McKown and Dengler 2007). In such cases, it is difficult to pinpoint the true 'origin' of the pathway, as multiple lineages have independently built upon a shared ancestral set of key facilitating traits.

Regardless of the categorizations, it is clear that the many lineages of C$_4$ plants demonstrate the power of evolution repeatedly to resolve the critical environmental challenges

imposed by declining levels of atmospheric CO_2 and increasing aridity that occurred in recent geological time. By comparing the many C_4 groups, it will be possible to better understand how evolution was able to co-opt varying features present in C_3 ancestors to arrive at the C_4 solution to the photorespiratory challenge. In doing so, researchers should be able to identify many of the genetic elements responsible for the recurrent emergence of the C_4 pathway in higher plants.

Acknowledgements

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