

REVIEW PAPER

The C₄ plant lineages of planet Earth

Rowan F. Sage^{1,*}, Pascal-Antoine Christin² and Erika J. Edwards²

¹ Department of Ecology and Evolutionary Biology, The University of Toronto, 25 Willcocks Street, Toronto, Ontario M5S3B2 Canada

² Department of Ecology and Evolutionary Biology, Brown University, 80 Waterman St., Providence, RI 02912, USA

* To whom correspondence should be addressed. E-mail: R.Sage@utoronto.ca

Received 30 November 2010; Revised 1 February 2011; Accepted 2 February 2011

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 C₄ photosynthetic pathway. Here, 62 recognizable lineages of C₄ 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 C₃–C₄ intermediate type of photosynthesis correspond to 21 lineages. Of these, 9 are not immediately associated with any C₄ lineage, indicating that they did not share common C₃–C₄ ancestors with C₄ 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, C₄ 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, C₃–C₄ photosynthesis, CO₂-concentrating mechanism, convergent evolution, photorespiration, phylogeny.

Introduction

The metabolic pathway of C₄ photosynthesis was first described in the mid-1960s (Hatch and Slack, 1966, 1967; Osmond, 1967; Hatch, 1999), although many of the traits associated with C₄ photosynthesis, such as Kranz anatomy, low CO₂ 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 C₄ pathway was identified in ~1966–1967 (Hatch and Slack, 1966, 1967; Osmond, 1967), the integrated picture of C₄ 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 C₄ 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 C₄ 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; Akhiani *et al.*, 1997). In the edited volume *C₄ plant biology* (Sage and Monson, 1999), Sage *et al.* (1999) assembled this information into comprehensive lists of genera containing C₄ species, and Kellogg (1999) mapped many of the C₄ 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 C₄ 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 Portulacaceae (Ocampo and Columbus, 2010; Voznesenskaya 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),

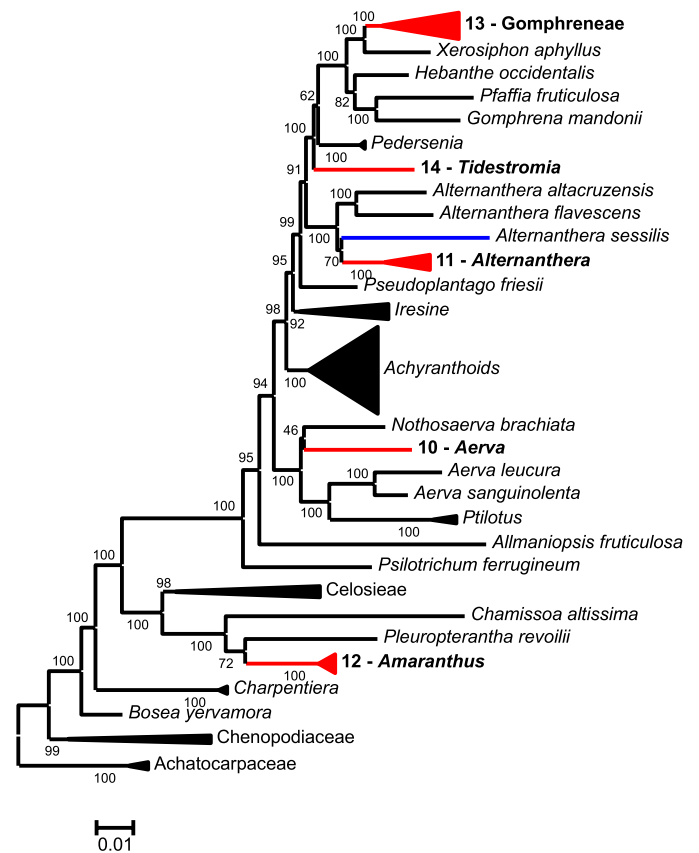


Fig. 1. The distribution of C₄ photosynthesis in the Amaranthaceae *sensu stricto*. The phylogeny was obtained through Bayesian inference on the *trnK-matK* data set of Sage et al. (2007). It is rooted on the Achatocarpaceae. Bayesian support values are indicated near branches. Clades that contain a 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 $\delta^{13}\text{C}$ 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 C₄ 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 C₄ 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 C₄ evolution remain uncertain because the C₃ and C₄ 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 C₄ lineages are present in the Chenopodiaceae *ss.* More lineages may be present, as there is a possibility that the *Salsola kali* and *Halothammus* groups may represent two independent C₄ lines. The situation is also unclear in Camphorosmeae, where anatomical variations could be inter-

preted as the fingerprint of two different C₄ origins (Kadereit and Freitag, 2011). The distribution of photosynthetic types also indicates a C₄ to C₃ reversion and reacquisition of the C₄ pathway in the branches between the *S. kali* group and the *Haloxylon/Anabis* group (Fig. 2). Because *Haloxylon/Anabis* presumably acquired C₄ photosynthesis from ancestors with a fully expressed C₃ pathway, as indicated by the *Sympegma* and *Oreosalsola* nodes, it is treated as an independent lineage, regardless of whether there may have been ancestral C₄ species at deeper nodes in the phylogeny.

Species level resolution has also facilitated the identification of the centres of origin for many of the listed C₄ lineages. This can be accomplished by identifying closely related C₃ and C₄ species within a phylogeny, and any related C₃–C₄ intermediate species. By mapping the geographic distribution of the sister groups and intermediate forms, the region where the C₄ 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 C₄ photosynthesis.

To visualize broadly the phylogenetic distribution of C₄ taxa, as many C₄ groups as possible were mapped onto a recently published phylogeny of 9412 angiosperms (Smith

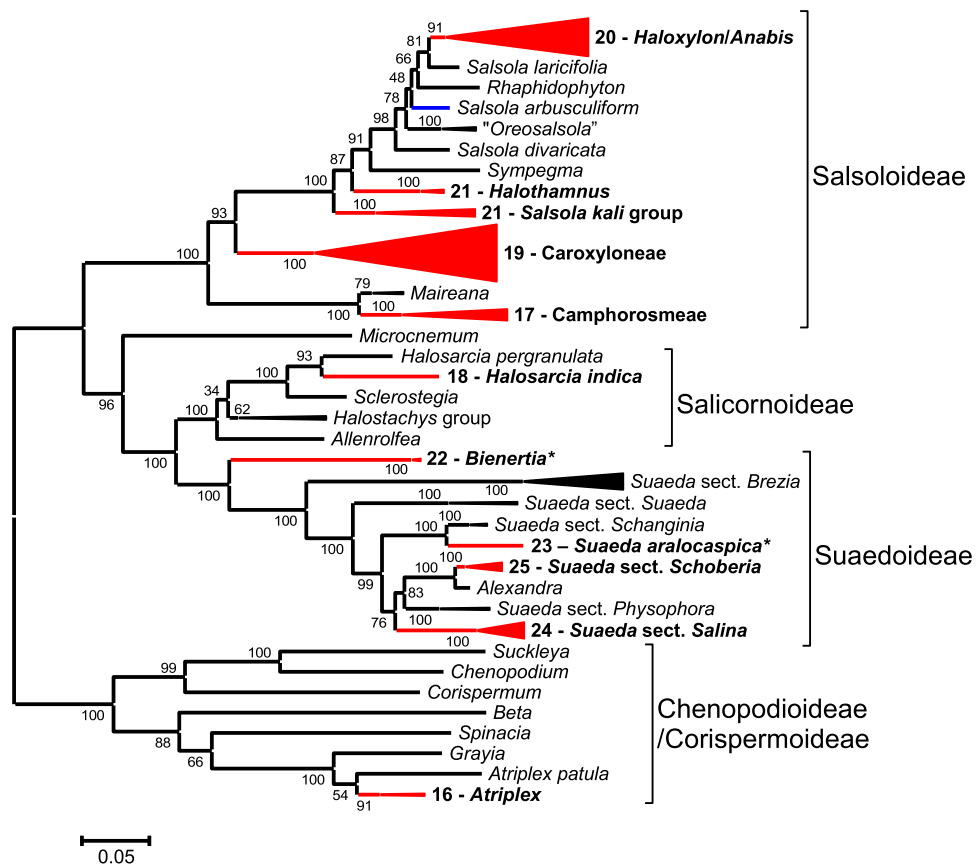


Fig. 2. The distribution of C₄ photosynthesis in the Chenopodiaceae *sensu stricto*. The phylogeny was obtained through Bayesian inferences on the nuclear internal transcribed spacer (ITS) and plastid *psbB–psbH* markers generated in previous studies (Kapralov *et al.*, 2006; Akhiani *et al.*, 2007; Wen *et al.*, 2010; see Christin *et al.*, 2011a for details) and is rooted on the Chenopodioideae/Corispermoidae. Bayesian support values are indicated near branches. Clades that contain a single photosynthetic type 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). Asterisks indicate single-celled C₄ 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’. Furthermore, 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

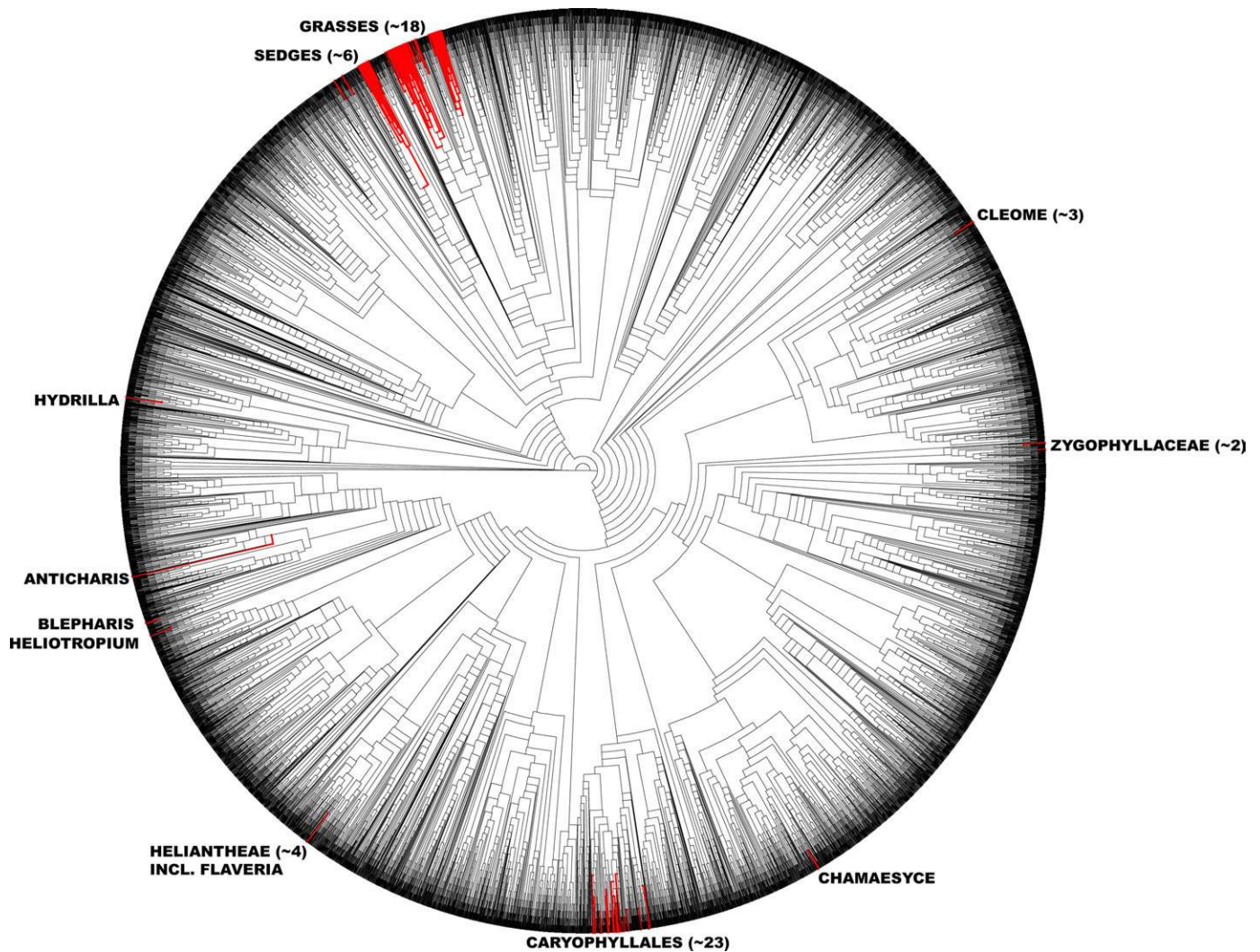


Fig. 3. The phylogenetic distribution of C₄ lineages in the angiosperms, depicted on a phylogeny of 9412 angiosperms species that was pruned from the viridiplantae phylogeny of Smith *et al.* (2009). C₄ lineages are indicated by red branches. Numbers beside named lineages refer to the estimate of the 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 C₄ 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 Vosnesenskaya (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	<i>Heliotropium</i> section <i>Orthostachys</i> (= <i>Euploca</i>)	Atriplicoid	NADP-ME	NAM	Frohlich (1978)
2	Asterales	Asteraceae/ Heliantheae	<i>Chrysanthellum/Isostigma</i>	Simplicifolioid, Isostigmoid, Glossocardoid	NADP-ME, NAD-ME	Unknown	Kellogg (1999)
3	Asterales	Asteraceae/ Heliantheae	<i>Flaveria</i> clade A	Atriplicoid	NADP-ME	NAM	Powell (1978); McKown <i>et al.</i> (2005)
4	Asterales	Asteraceae/ Heliantheae	<i>Flaveria brownii</i> ^f (<i>Flaveria</i> clade B)	Atriplicoid	NADP-ME	NAM	Powell (1978); McKown <i>et al.</i> (2005)
5	Asterales	Asteraceae/ Heliantheae	<i>Pectis</i>	Atriplicoid	NADP-ME	NAM	Kellogg (1999)
6	Brassicales	Cleomeaceae	<i>Cleome angustifolia</i>	Angustifolioid	NAD-ME	AA	Feodorova <i>et al.</i> (2010)
7	Brassicales	Cleomeaceae	<i>Cleome gynandra</i>	Atriplicoid	NAD-ME	SA	Feodorova <i>et al.</i> (2010)
8	Brassicales	Cleomeaceae	<i>Cleome oxalidea</i>	Unknown	Unknown	AUS	Feodorova <i>et al.</i> (2010)
9	Caryophyllales	Aizoaceae/ Sesuvioideae	<i>Sesuvium/ Trianthema/</i> <i>Zaleya</i>	Atriplicoid	NADP-ME	AA, SA	Hassan <i>et al.</i> (2005)
10	Caryophyllales	Amaranthaceae	<i>Aerva</i>	Atriplicoid	NADP-ME	AA	Sage <i>et al.</i> (2007)
11	Caryophyllales	Amaranthaceae	<i>Alternanthera</i>	Atriplicoid	NADP-ME	SAM	Sage <i>et al.</i> (2007)
12	Caryophyllales	Amaranthaceae	<i>Amaranthus</i>	Atriplicoid	NAD-ME	New World	Sage <i>et al.</i> (2007)
13	Caryophyllales	Amaranthaceae	Gomphreneae	Atriplicoid	NADP-ME	SAM	Sage <i>et al.</i> (2007)
14	Caryophyllales	Amaranthaceae	<i>Tidestromia</i>	Atriplicoid	NADP-ME	NAM	Sage <i>et al.</i> (2007)
15	Caryophyllales	Caryophyllaceae	<i>Polycarpaea</i>	Atriplicoid	NADP-ME	Old World	Kellogg (1999)
16	Caryophyllales	Chenopodiaceae	<i>Atriplex</i>	Atriplicoid	NAD-ME	CeA	Kadereit <i>et al.</i> (2010)
17	Caryophyllales	Chenopodiaceae	Camphorosmeae	Kochioid	NADP-ME	CeA	Kadereit <i>et al.</i> (2003); Kadereit and Freitag (2011)
18	Caryophyllales	Chenopodiaceae	<i>Halosarcia indica</i>	Tecticomoid	NAD-ME	CeA	Kadereit <i>et al.</i> (2003); Kapralov <i>et al.</i> (2006)
19	Caryophyllales	Chenopodiaceae/ Salsoloideae	Caroxyloneae	Salsoloid	NAD-ME	CeA	Kadereit <i>et al.</i> (2003); Kapralov <i>et al.</i> (2006)
20	Caryophyllales	Chenopodiaceae/ Salsoloideae	<i>Haloxylon/Anabasis</i> (includes <i>Noaea</i>)	Salsaloid	NADP-ME	CeA	Kapralov <i>et al.</i> (2006); Wen <i>et al.</i> (2010)
21	Caryophyllales	Chenopodiaceae/ Salsoloideae	<i>Salsola kali</i> group (includes <i>Halothamnus</i>)	Salsaloid	NADP-ME	CeA	Kadereit <i>et al.</i> (2003)
22	Caryophyllales	Chenopodiaceae/ Suaedoideae	<i>Bienertia</i>	Single-celled (Bienertioid)	NAD-ME	CeA	Kapralov <i>et al.</i> (2006)
23	Caryophyllales	Chenopodiaceae/ Suaedoideae	<i>Suaeda aralocaspica</i> (= <i>Borszczowia</i>)	Single-celled (Borszczowioid)	NAD-ME	CeA	Kapralov <i>et al.</i> (2006)
24	Caryophyllales	Chenopodiaceae/ Suaedoideae	<i>Suaeda</i> sect. <i>Salsina</i>	Salsinoid	NAD-ME	CeA	Kapralov <i>et al.</i> (2006)
25	Caryophyllales	Chenopodiaceae/ Suaedoideae	<i>Suaeda</i> sect. <i>Schoberia</i>	Schoberoid	NAD-ME	CeA	Kapralov <i>et al.</i> (2006)
26	Caryophyllales	Gisekiaceae	<i>Gisekia</i>	Unknown	Unknown	Old World	APG III (2009)
27	Caryophyllales	Molluginaceae	<i>Mollugo cerviana/</i> <i>M. fragilis</i>	Atriplicoid	NADP-ME	SA	Christin <i>et al.</i> (2011b)
28	Caryophyllales	Nyctaginaceae	<i>Allionia</i>	Atriplicoid	NADP-ME	NAM	Douglas and Manos (2007)
29	Caryophyllales	Nyctaginaceae	<i>Boerhavia</i>	Atriplicoid	NADP-ME	NAM	Douglas and Manos (2007)
30	Caryophyllales	Polygonaceae	<i>Calligonum</i>	Salsaloid	NAD-ME	CeA	Kellogg (1999)
31	Caryophyllales	Portulacaceae	<i>Portulaca</i>	Pilosoid, Portulacenoid	NADP-ME, NAD-ME	SAM	Ocampo and Columbus (2010); Voznesenskaya <i>et al.</i> (2010)
32	Lamiales	Acanthaceae	<i>Blepharis</i>	Atriplicoid	NADP-ME, NAD-ME	SA	Vollesen (2000); Akhani <i>et al.</i> (2008)
33	Lamiales	Scrophulariaceae	<i>Anticharis</i>	Atriplicoid	NAD-ME	SA	Kellogg (1999)

Table 1. *Continued*

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

^a *Flaveria brownii* is physiologically a C₄-like intermediate, in that it expresses Rubisco in the mesophyll (Cheng *et al.*, 1988). It is treated here as an independent C₄ clade as it has a fully functional C₄ cycle, and photosynthetic gas exchange properties and resource use efficiencies that are equivalent to those of many C₄ species.

where plants have aerial photosynthetic structures. In *Hydrilla* and *Egeria*, the C₄ pathway operates in submersed leaves and concentrates CO₂ from the cytosol into an adjacent chloroplast of a single cell (Bowes, 2011). In all other known C₄ plants, the C₄ pathway concentrates CO₂ from a mesophyll-like compartment into a distinct inner tissue region (58 lineages) or concentrates CO₂ 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 C₄ 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 C₄ taxa and ~750 C₄ species (Figs 1, 2; Sage *et al.*, 1999). Of these 15 lineages, the largest is *Atriplex*, with 200–300 C₄ species (Kadereit *et al.*, 2010). Two of the C₄ 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 C₄ species and *Amaranthus* ~70 C₄ species (Sage *et al.*, 2007). The most species-rich C₄ eudicot lineage other than *Atriplex* is *Euphorbia* section *Chamaesyce* with about 250 species. Other large C₄ lineages include *Heliotropium* section *Orthostachys* (= *Euploca*) with ~120 C₄ species (Frohlich, 1978), and the *Calligonum*, *Pectis*, and *Portulaca* lineages with 80–100 C₄ species each (Sage *et al.*, 1999). Fourteen eudicot C₄ lineages contain <10 C₄ species: *Aerva*, *Allionia*, *Bienertia*, and *Mollugo* have two C₄ species each, while *Suaeda aralocaspica* (= *Borszczowia aralocaspica*), the three C₄ lineages in the Cleomeaceae, *Flaveria* clade B, *Halosarcia*, and *Zygophyllum* have one C₄ species each. *Anticharis*, *Flaveria* clade A, and *Gisekia* have 5–10 C₄ 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 C₄ sedge lineages are the Cypereae (eight genera with ~750 species), *Fimbristylis* (300 species), and *Bulbostylis* (205 species). Fifteen C₄ species are known in the *Eleocharis* section *Tenuissimae ss*, while 21 C₄ species are known from *Rhynchospora*. In both *Eleocharis* and *Rhynchospora*, >90% of the species are C₃ plants (Bruhl and Wilson, 2007). The smallest C₄ sedge lineage is *Eleocharis vivipara* with a single C₄ species (Bruhl and Wilson, 2007; Roalson *et al.*, 2010; Roalson, 2011).

There are ~4600 C₄ grasses, all occurring in the PACMAD clade (Sage *et al.*, 1999). The largest C₄ 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*. C₄ 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 C₄ 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 C₄ taxa, but is highly polyphyletic (Aliscioni *et al.*, 2003). Summing the C₄ eudicot and monocot estimates, the total number of C₄ 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 C₄ cycle of eudicots in the Sesuvioideae (Muhaidat *et al.*, 2007). In the grasses, four C₄ 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 C₄ 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 C₄ 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 C₃–C₄ intermediate photosynthesis in higher plants

C₃–C₄ as defined here refers to photosynthetic modifications that include refixation of photorespiratory CO₂ in bundle sheath cells, and the engagement of a C₄ metabolic cycle. Compiled from Sage *et al.* (1999), Bauwe (2011), and the references listed below. The list does not include species which show C₃ and C₄ expression in different regions of the same plant (termed C₃/C₄ in Sage *et al.*, 1999) or C₃ and C₄ plants in different subspecies (termed C₃×C₄ in Sage *et al.*, 1999).

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

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

Lineages of C₃–C₄ intermediacy

Twenty-one distinct clades have been identified that contain species with photosynthetic characteristics that are intermediate between C₃ and C₄ species (Table 2). Ten of the C₃–C₄ groups branch immediately sister to C₄ lineages, which is consistent with models proposing that C₃–C₄ intermediacy originated before C₄ photosynthesis and served as an ancestral stage (Monson *et al.*, 1984; Monson, 1999; Sage, 2004; Bauwe, 2011). Most C₃–C₄ intermediates

cluster in genera known to contain C₄ plants (Table 2), as is best demonstrated by *Flaveria* which has ~9 C₃–C₄ species (McKown *et al.*, 2005). Some of these intermediates, however, do not appear at sister nodes. Where C₃ species branch between the C₃–C₄ intermediate and a C₄ node, as occurs with the C₃–C₄ intermediate *Mollugo verticillata*, it appears that the C₃–C₄ line has independently arisen from different C₃ ancestors than the C₄ line (Christin *et al.*, 2011b). Where a C₃–C₄ species branches between two C₄ nodes, as occurs with the C₃–C₄ intermediate *Portulaca cryptopetala* (Ocampo and Columbus, 2010), a reversion from the C₄ condition is possible. Notably, about a quarter of the identified C₃–C₄ species occur in taxa that are not

closely related to any C₄ lineage. There are no C₄ species in the Brassicaceae, where two C₃–C₄ clades occur (*Moricandia* and *Diploaxis*; Sage *et al.*, 1999), and three C₃–C₄ *Eleocharis* species occur in the *Eleocharis* subgenus *Scirpidium*, which lacks any C₄ species (Keeley, 1999; Roalson *et al.*, 2010). These patterns highlight the need to consider the C₃–C₄ condition as a distinct photosynthetic adaptation in its own right, and not just a transitional stage leading to the C₄ condition.

Geographic centres of origins

Geographic centres of origins for C₄ 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 C₄ eudicot lineages, with 4–11 each. Two centres corresponding to semi-arid regions of South Africa and South America each produced 4–5 C₄ eudicot lineages, while the driest continent, Australia, produced only one C₄ lineage in the eudicots that can be confirmed at this time.

Identifying the geographic origins of the C₄ monocots is more problematic due to their wide geographical distribution and greater uncertainty regarding the phylogenetic placement of the C₄ lineages. Nevertheless, South America appears to be a major hotspot for C₄ grass origins, including many of the transitions in the $x=10$ Paniceae clade (Table 1; Fig. 4). Two C₄ grass origins in Africa are apparent, in *Centropodia* and *Alloteropsis*. Only one C₄ 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 C₄ 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 C₄ plants, and a more complete accounting of C₃ and C₄ 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 C₄ grass lineages (Kellogg, 1999), a number that has now increased to 18. The current list of C₄ 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).

C₃–C₄ intermediacy

C₃–C₄ intermediacy is a term originally used to describe plants with traits intermediate between C₃ and C₄ species, on the assumption that they might represent an evolutionary transition (Kennedy and Laetsch, 1974; Monson *et al.*, 1984). Currently, C₃–C₄ intermediacy mainly refers to plants with a photorespiratory CO₂-concentrating mechanism, where expression of the photorespiratory enzyme

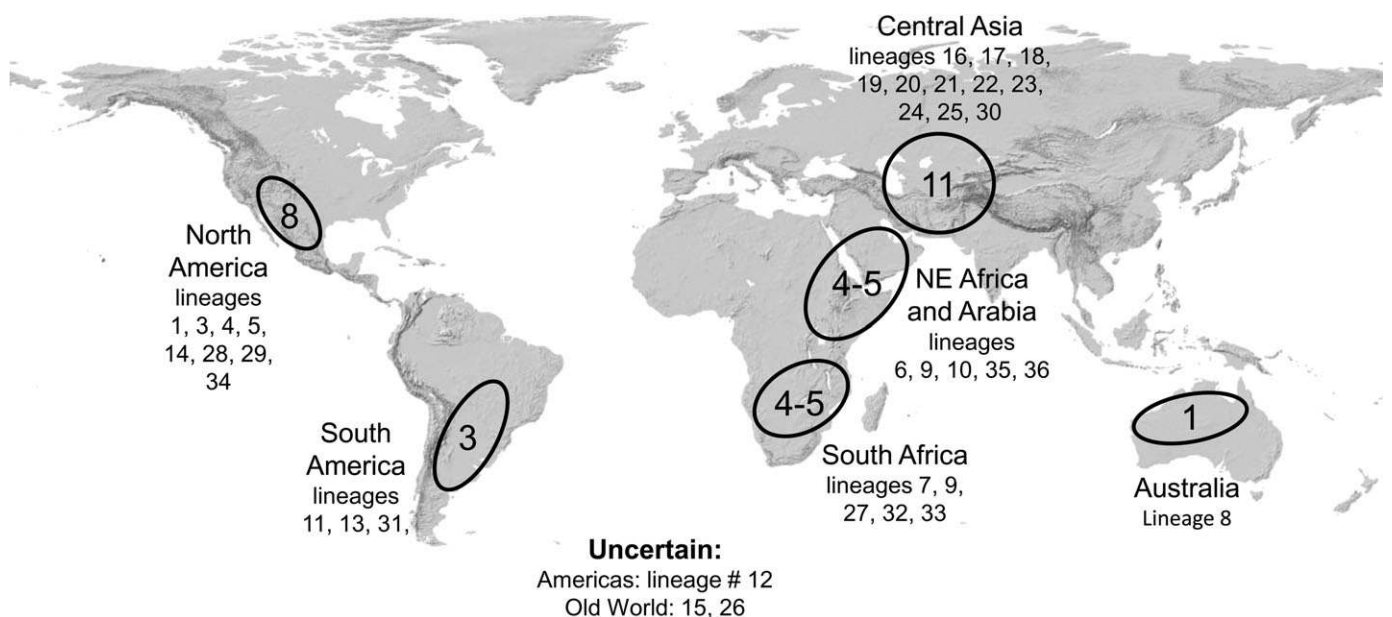


Fig. 4. Locations for the centres of origin for 35 of the 36 C₄ eudicot lineages listed in Table 1. Numbers shown correspond to lineages listed in Table 1. Unlisted lineages have an unknown centre of origin.

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 CO₂, which accumulates in the BSCs, enhancing the efficiency of BSC Rubisco. Following the mutation leading to GDC localization, C₃–C₄ species evolve many C₄-like traits such as close vein spacing and enlarged BSCs to optimize the efficiency of photorespiratory CO₂ concentration (Sage, 2004). While these developments may facilitate C₄ evolution (Bauwe, 2011), they also confer fitness in their own right, as reflected by numerous C₃–C₄ lineages that are distinct from C₄ clades, and the ecological success of numerous C₃–C₄ species in warm to hot environments (Monson, 1999; Christin *et al.*, 2011b; Sage *et al.*, 2011).

Twenty-one distinct lineages of C₃–C₄ intermediate plants have been identified. The first C₃–C₄ 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*, *Diploaxis*, *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 C₄ group. One of these, *C. acuta*, is a C₃–C₄ intermediate (Sage *et al.*, 2011). Intermediates have also been found recently in *Cleome* and *Portulaca* (Voznesenskaya *et al.*, 2007, 2010).

Most of the known C₃–C₄ 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 C₃–C₄ taxa. Alternatively, the greater number of eudicot intermediates may reflect sampling bias. Most known C₃–C₄ intermediates are associated with C₄ eudicots, because investigators often focused on eudicot genera having both C₃ and C₄ 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 C₃–C₄ lineages should grow in the near future. Isotopic screens will pick up some intermediates with less negative δ¹³C than typical C₃ plants (see, for example, Feodorova *et al.*, 2010 for *Cleome*); however, most C₃–C₄ intermediates have δ¹³C values that cannot be differentiated from those of C₃ species, so such screens will be of limited value. To have a less negative δ¹³C value than C₃ species, there must be significant engagement of a C₄ cycle (von

Caemmerer, 1992). In order to detect potential C₃–C₄ intermediates lacking a C₄ cycle, anatomical screens are a useful first step, but detailed physiological studies with live material will still be needed for confirmation.

The biogeography of C₄ evolution

For the eudicots, and the handful of monocots where the centre of C₄ origin can be estimated with confidence, there appear to be six geographic regions where the C₄ 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 C₄ lineages, we hope to facilitate follow-up studies that will evaluate the environmental selection factors responsible for the evolution of specific C₄ lineages. Such studies could examine the ecophysiology of the close C₃ and C₃–C₄ relatives of the C₄ lines currently present in the centres of origins. Alternatively, paleontology studies could correlate past environmental events with the appearance of a C₄ lineage in a given area. To date, the leading environmental hypothesis for C₄ evolution is that reduction in atmospheric CO₂ in the late Oligocene increased photorespiration in warm climates, thereby facilitating selection for CO₂-concentrating mechanisms such as C₄ photosynthesis (Sage, 2001, 2004; Christin *et al.*, 2008; Vicentini *et al.*, 2009; Edwards *et al.*, 2010; Osborne, 2011). However, C₄ photosynthesis repeatedly arose in the 25–30 million years since the late-Oligocene CO₂ reduction (Christin *et al.*, 2011a). In light of this, it is better to think of low CO₂ as a pre-condition, or environmental facilitator, which acted in concert with multiple selection factors. Other proposed drivers of C₄ 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 C₄ lineages, Australia, developed the warm, dry conditions postulated to support C₄ evolution relatively late, only in the past 4–5 million years (Archer *et al.*, 1995).

If the current environments in the C₄ centres of origin are similar to those at the time when the C₄ lineages appeared, then the climate similarities between the centres of origin suggest the following environmental model for C₄ 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 C₃ species, particularly in the low CO₂ conditions of the recent past. This could have favoured the rise of CO₂ scavenging systems such as C₃–C₄ intermediate types of photosynthesis, leading in turn to the more elaborate C₄ carbon-concentrating mechanisms.

Convergence in C₄ evolution

C₄ 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 C₃ and C₄ species) harbour fully one-third of C₄ lineages, and Caryophyllales (~11000 C₃ and C₄ species) roughly another third. Within both of these groups, there is additional clustering of C₄ clades. C₄ 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 C₄ lineages in Caryophyllales, with 15 postulated origins. This extreme clustering of C₄ lineages, in conjunction with the lack of C₄ taxa across large regions of the angiosperm phylogeny, indicates that there are a limited number of C₃ plant lineages that possess an appropriate suite of characteristics that can facilitate the evolution of C₄ 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 C₃ 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 C₄ evolution by enabling the establishment of a two-tissue mechanism to refix photorespiratory CO₂, which is considered a major step in C₄ evolution (Monson, 1999). Physiological facilitators include increased organelle number in BSCs of C₃ ancestors, allowing for more photosynthetic activity (Brown and Hattersley, 1989). Cryptic enhancers promoting the evolvability of C₄ photosynthesis may be found in the genomes of shared ancestors of clustered C₄ groups. For example, a

large reservoir of duplicated genes has been suggested as a genetic pre-condition for C₄ evolution (Monson, 2003; Bauwe, 2011). In addition, regulatory elements conferring tissue specificity may be common in C₃ groups from which the C₄ pathway arose. Ecologically, specialization for hot, dry, or saline landscapes could select for carbon conservation mechanisms such as refixation of photorespired CO₂. Thus, groups such as the C₃ Chenopodiaceae may be prone to evolve C₄ 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 C₄ 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 C₄ evolution is relatively easy, a point that has encouraged efforts to engineer the C₄ pathway into C₃ crops (Hibberd *et al.*, 2008). Alternatively, this statement might be overly simplistic, as it implies that all C₄ origins had a similar starting point within a C₃ physiological setting, and evolution converged on a uniform C₄ pathway. As shown by the variation in decarboxylation types, leaf anatomy, and cellular ultrastructure, CO₂ concentration by C₄ photosynthesis can be accomplished in a variety of different ways. Uniformity between the different C₄ types is largely observed in the role of PEP carboxylase and the functional significance of the C₄ 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 CO₂ concentration is achieved.

In addition, there are differing degrees of convergence in terms of the magnitude of modifications to the ancestral C₃ state within the 62 C₄ lineages. Complete transitions from the full C₃ condition to the full C₄ condition unequivocally occurred independently in several clades. Examples include the C₄ clades in Acanthaceae (*Blepharis*), Boraginaceae, Euphorbiaceae, Scrophulariaceae, Zygophyllaceae, and Asteraceae. At the other extreme are situations where multiple C₄ lineages arose from common C₃–C₄ ancestors, such as two C₄ clades in *Mollugo* (Christin *et al.*, 2011b). In these instances, the evolutionary transition from C₃–C₄ to C₄ would be relatively small, involving fewer changes than the complete C₃ to C₄ transition. Such cases might better be thought of as a parallel realization of the C₄ condition. The more ambiguous situation involves the independent evolution of C₄ lineages from C₃ ancestors expressing traits which might facilitate C₄ evolution, such as high vein density and gene duplication. An example of this occurs in *Flaveria*, where C₄ photosynthesis in clades A and B appears to have independently arisen from C₃ 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 C₄ plants demonstrate the power of evolution repeatedly to resolve the critical environmental challenges

imposed by declining levels of atmospheric CO₂ and increasing aridity that occurred in recent geological time. By comparing the many C₄ groups, it will be possible to better understand how evolution was able to co-opt varying features present in C₃ ancestors to arrive at the C₄ 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 C₄ pathway in higher plants.

Acknowledgements

This work was supported by a Discovery grant from the National Science and Engineering Research Council of Canada to RFS, and funds to RFS from the International Rice Research Institute programme for the Engineering of C₄ Rice, which is funded by the Bill and Melinda Gates foundation. Additional support was provided by the Swiss National Science Foundation grant PBLAP3-129423 to PAC, and the National Science Foundation grants DEB-1026611 and IOS 0843231 to EJE.

References

- Akhani H, Edwards G, Roalson EH.** 2007. Diversification of the old world Salsola s.l. (Chenopodiaceae): molecular phylogenetic analysis of nuclear and chloroplast datasets and a revised classification. *International Journal of Plant Sciences* **168**, 931–956.
- Akhani H, Ghasemkhani M, Chuong SDX, Edwards GE.** 2008. Occurrence and forms of Kranz anatomy in photosynthetic organs and characterization of NAD-ME subtype C₄ photosynthesis in *Blepharis ciliaris* (L.) B.L. Burt (Acanthaceae). *Journal of Experimental Botany* **59**, 1755–1765.
- Akhani H, Trimborn P, Ziegler H.** 1997. Photosynthetic pathways in Chenopodiaceae from Africa, Asia and Europe with their ecological, phytogeographical and taxonomical importance. *Plant Systematics and Evolution* **206**, 187–221.
- Aliscioni SS, Giussani LM, Zuloaga FO, Kellogg EA.** 2003. A molecular phylogeny of *Panicum* (Poaceae: Paniceae): tests of monophyly and phylogenetic placement within the Panicoideae. *American Journal of Botany* **90**, 796–821.
- Apel P, Hillmer S, Pfeffer M, Muhle K.** 1980. Carbon metabolism of *Diplotaxis tenuifolia* (L.) DC. (Brassicaceae). *Photosynthetica* **32**, 237–243.
- Apel P, Maas I.** 1981. Photosynthesis in species of *Flaveria* CO₂ compensation concentration, O₂ influence on photosynthetic gas-exchange and delta C¹³ values in species of *Flaveria* (Asteraceae). *Biochemie und Physiologie der Pflanzen* **176**, 396–399.
- Apel P, Ticha I, Peisker M.** 1978. CO₂ compensation concentrations in leaves of *Moricandia-arvensis* (L.) DC. at different insertion levels and O₂ concentrations. *Biochemie und Physiologie der Pflanzen* **172**, 547–552.
- APG III.** 2009. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society* **161**, 105–121.
- Archer M, Hand SJ, Godthelp H.** 1995. Tertiary environment and biotic change in Australia. In: Vrba ES, Denton GH, Partridge TC, Burckle LH, eds. *Paleoclimate and evolution with emphasis on human origins*. New Haven, CT: Yale University Press, 77–90.
- Batanouny KH, Stichler W, Ziegler H.** 1991. Photosynthetic pathways and ecological distribution of *Euphorbia* species. *Oecologia* **87**, 565–569.
- Bauwe H.** 2011. Photorespiration: the bridge to C₄ photosynthesis. In: Raghavendra AS, Sage RF, eds. *C₄ photosynthesis and related CO₂ concentrating mechanisms*. Dordrecht, The Netherlands: Springer, 81–108.
- Besnard G, Muasya AM, Russier F, Roalson EH, Salamin N, Christin PA.** 2009. Phylogenomics of C₄ photosynthesis in sedges (Cyperaceae): multiple appearances and genetic convergence. *Molecular Biology and Evolution* **26**, 1909–1919.
- Black CC, Chen TM, Brown RH.** 1969. Biochemical basis for plant competition. *Weed Science* **17**, 338–344.
- Bowes G.** 2011. Single-cell C₄ photoynthesis in aquatic plants. In: Raghavendra AS, Sage RF, eds. *C₄ photosynthesis and related CO₂ concentrating mechanisms*. Dordrecht, The Netherlands: Springer, 63–80.
- Brown RH, Hattersley PW.** 1989. Leaf anatomy of C₃–C₄ species as related to evolution of C₄ photosynthesis. *Plant Physiology* **91**, 1543–1550.
- Brown RH, Brown WV.** 1975. Photosynthetic characteristics of *Panicum milioides*, a species with reduced photorespiration. *Crop Science* **15**, 681–685.
- Brown WV.** 1977. The Kranz syndrome and its subtypes in grass systematic. *Memoirs of the Torrey Botanical Club* **23**, 1–97.
- Bruhl JJ, Perry S.** 1995. Photosynthetic pathway-related ultrastructure of C₃, C₄ and C₃-like C₃–C₄ intermediate sedges (Cyperaceae), with special reference to *Eleocharis*. *Australian Journal of Plant Physiology* **22**, 521–530.
- Bruhl JJ, Wilson KL.** 2007. Towards a comprehensive survey of C₃ and C₄ photosynthetic pathways in Cyperaceae. *Aliso* **23**, 99–148.
- Cheng SH, Moore BD, Edwards GE, Ku MSB.** 1988. Photosynthesis in *Flaveria brownii*, a C₄-like species. *Plant Physiology* **87**, 867–873.
- Christin PA, Besnard G, Samaritani E, Duvall MR, Hodkinson TR, Savolainen V, Salamin N.** 2008. Oligocene CO₂ decline promoted C₄ photosynthesis in grasses. *Current Biology* **18**, 37–43.
- Christin PA, Freckleton RP, Osborne CP.** 2010. Can phylogenetics identify C₄ origins and reversals? *Trends in Ecology & Evolution* **25**, 403–409.
- Christin PA, Osborne CP, Sage RF, Arakaki M, Edwards EJ.** 2011a. C₄ eudicots are not younger than C₄ monocots. *Journal of Experimental Botany* **62**, 3171–3181.
- Christin PA, Sage TL, Edwards EJ, Ogburn RM, Khoshravesh R, Sage RF.** 2011b. Complex evolutionary transitions and the significance of C₃–C₄ intermediate forms of photosynthesis in Molluginaceae. *Evolution* **65**, 643–660.
- Christin PA, Salamin N, Kellogg EA, Vicentini A, Besnard G.** 2009. Integrating phylogeny into studies of C₄ variation in the grasses. *Plant Physiology* **149**, 82–97.

- Dengler NG, Nelson T.** 1999. Leaf structure and development in C₄ plants. In: Sage RF, Monson RK, eds. *C₄ plant biology*. San Diego, CA: Academic Press, 133–172.
- Downton WJS.** 1975. Occurrence of C₄ photosynthesis among plants. *Photosynthetica* **9**, 96–105.
- Downton WJS, Bisalputra T, Tregunna EB.** 1969. Distribution and ultrastructure of chloroplasts in leaves differing in photosynthetic carbon metabolism. II. *Atriplex rosea* and *Atriplex hastata* (Chenopodiaceae). *Canadian Journal of Botany* **47**, 915–919.
- Downton WJS, Tregunna EB.** 1968. Carbon dioxide compensation—its relation to photosynthetic carboxylation reactions, systematics of the Gramineae and leaf anatomy. *Canadian Journal of Botany* **46**, 207–215.
- Douglas NA, Manos PS.** 2007. Molecular phylogeny of Nyctaginaceae: taxonomy, biogeography, and characters associated with radiation of xerophytic genera in North America. *American Journal of Botany* **94**, 856–872.
- Duval MR, Saar DE, Grayburn WS, Holbrook GP.** 2003. Complex transitions between C₃ and C₄ photosynthesis during the evolution of Paniceae: a phylogenetic case study emphasizing the position of *Steinchisma hians* (Poaceae), a C₃–C₄ intermediate. *International Journal of Plant Sciences* **164**, 949–958.
- Edwards EJ, Osborne CP, Strömberg CAE, Smith SA, the C₄ Grasses Consortium.** 2010. The origins of C₄ grasslands: integrating evolutionary and ecosystem science. *Science* **328**, 587–591.
- Edwards EJ, Smith SA.** 2010. Phylogenetic analyses reveal the shady history of C₄ grasses. *Proceedings of the National Academy of Sciences, USA* **107**, 2532–2537.
- Edwards GE, Voznesenskaya EV.** 2011. C₄ photosynthesis: Kranz forms and single-cell C₄ in terrestrial plants. In: Raghavendra AS, Sage RF, eds. *C₄ photosynthesis and related CO₂ concentrating mechanisms*. Dordrecht, The Netherlands: Springer, 29–61.
- Edwards GE, Walker DA.** 1983. *C₃, C₄: mechanisms, and cellular regulation, of photosynthesis*. Oxford: Blackwell Scientific.
- Feodorova TA, Voznesenskaya EV, Edwards GE, Roalson EH.** 2010. Biogeographic patterns of diversification and the origins of C₄ in Cleome (Cleomaceae). *Systematic Botany* **35**, 811–826.
- Frohlich MW.** 1978. Systematics of *Heliotropium* section *Orthostachys* in Mexico. *PhD thesis*. Cambridge, MA: Harvard University.
- Giussani LM, Cota-Sánchez JH, Zuloaga FO, Kellogg EA.** 2001. A molecular phylogeny of the grass subfamily Panicoideae (Poaceae) shows multiple origins of C₄ photosynthesis. *American Journal of Botany* **88**, 1993–2012.
- Gutierrez M, Gracen VE, Edwards GE.** 1974. Biochemical and cytological relationships in C₄ plants. *Planta* **119**, 279–300.
- Haberlandt G.** 1914. *Physiological plant anatomy*. London: Macmillan.
- Hassan NS, Thiede J, Liede-Schumann S.** 2005. Phylogenetic analysis of Sesuvioideae (Aizoaceae) inferred from nrDNA internal transcribed spacer (ITS) sequences and morphological data. *Plant Systematics and Evolution* **255**, 121–143.
- Hatch MD.** 1999. C₄ photosynthesis: a historical overview. In: Sage RF, Monson RK, eds. *C₄ plant biology*. San Diego: Academic Press, 17–46.
- Hatch MD, Osmond CB, Slatyer RO, eds.** 1971. *Photosynthesis and photorespiration*. New York: Wiley Interscience.
- Hatch MD, Slack CR.** 1966. Photosynthesis by sugar-cane leaves—a new carboxylation reaction and pathway of sugar formation. *Biochemical Journal* **101**, 103–111.
- Hatch MD, Slack CR.** 1967. Participation of phosphoenolpyruvate synthetase in photosynthetic CO₂ fixation of tropical grasses. *Archives of Biochemistry and Biophysics* **120**, 224–422.
- Hattersley PW, Watson L.** 1992. Diversification of photosynthesis. In: Chapman GP, ed. *Grass evolution and domestication*. Cambridge: Cambridge University Press, 38–116.
- Hattersley PW, Watson L, Johnston CR.** 1982. Remarkable leaf anatomical variations in *Neurachne* and its allies (Poaceae) in relation to C₃ and C₄ photosynthesis. *Botanical Journal of the Linnean Society* **84**, 265–272.
- Hattersley PW, Wong SC, Perry S, Rokсандic Z.** 1986. Comparative ultrastructure and gas-exchange characteristics of the C₃–C₄ intermediate *Neurachne minor*. *Plant, Cell and Environment* **9**, 217–233.
- Hedge BA, Patil TM.** 1981. *Parthenium hysterophorus* L., a C₃ plant with Kranz syndrome. *Photosynthetica* **15**, 1–4.
- Hibberd JM, Covshoff S.** 2010. The regulation of gene expression required for C₄ photosynthesis. *Annual Review of Plant Biology* **61**, 181–207.
- Hibberd JM, Sheehy JE, Langdale JA.** 2008. Using C₄ photosynthesis to increase the yield of rice—rationale and feasibility. *Current Opinion in Plant Biology* **11**, 228–231.
- Hodge AJ, McLean JD, Mercer FV.** 1955. Ultrastructure of the lamellae and grana in the chloroplasts of *Zea mays* L. *Journal of Biophysical and Biochemical Cytology* **25**, 605–614.
- Holaday AS, Shieh YJ, Lee KW, Chollet R.** 1981. Anatomical, ultrastructural and enzymic studies of leaves of *Moricandia arvensis*, a C₃–C₄ intermediate species. *Biochimica et Biophysica Acta* **637**, 334–341.
- Kadereit G, Borsch T, Weising K, Freitag H.** 2003. Phylogeny of Amaranthaceae and Chenopodiaceae and the evolution of C₄ photosynthesis. *International Journal of Plant Sciences* **164**, 959–986.
- Kadereit G, Freitag H.** 2011. Molecular phylogeny of Camphorosmeae (Camphorosmoideae, Chenopodiaceae): implications for biogeography, evolution of C₄ photosynthesis and taxonomy. *Taxon* (in press).
- Kadereit G, Mavrodiev EV, Zacharias EH, Sukhorukov AP.** 2010. Molecular phylogeny of Atripliceae (Chenopodioideae, Chenopodiaceae): implications for systematics, biogeography, flower and fruit evolution, and the origin of C₄ photosynthesis. *American Journal of Botany* **97**, 1664–1687.
- Kapralov MV, Akhani H, Voznesenskaya EV, Edwards G, Franceschi V, Roalson EH.** 2006. Phylogenetic relationships in the Salicornioideae/Suaedoideae/Salsoloideae s.l. (Chenopodiaceae) clade and a clarification of the phylogenetic position of *Bienertia* and

- Alexandra* using multiple DNA sequence datasets. *Systematic Botany* **31**, 571–585.
- Keeley JE.** 1999. Photosynthetic pathway diversity in a seasonal pool community. *Functional Ecology* **13**, 106–118.
- Kellogg EA.** 1999. Phylogenetic aspects of the evolution of C₄ photosynthesis. In: Sage RF, Monson RK, eds. *C₄ plant biology*. San Diego, CA: Academic Press, 411–444.
- Kennedy RA, Laetsch WM.** 1974. Plant species intermediate for C₃, C₄ photosynthesis. *Science* **184**, 1087–1089.
- Ku MSB, Monson RK, Littlejohn RO, Nakamoto H, Fisher DB, Edwards GE.** 1983. Photosynthetic characteristics of C₃–C₄ intermediate *Flaveria* species. I. Leaf anatomy, photosynthetic responses to O₂ and CO₂, and activities of key enzymes in the C₃ and C₄ pathways. *Plant Physiology* **71**, 944–948.
- Marshall DM, Muhaidat R, Brown NJ, Liu Z, Stanley S, Griffiths H, Sage RF, Hibberd JM.** 2007. *Cleome*, a genus closely related to *Arabidopsis*, contains species spanning a developmental progression from C₃ to C₄ photosynthesis. *The Plant Journal* **51**, 886–896.
- McDade LA, Daniel TF, Kiel CA, Volleson K.** 2005. Phylogenetic relationships among Acantheae (Acanthaceae): major lineages present contrasting patterns of molecular evolution and morphological differentiation. *Systematic Botany* **30**, 834–862.
- McKown AD, Moncalvo JM, Dengler NG.** 2005. Phylogeny of *Flaveria* (Asteraceae) and inference of C₄ photosynthesis evolution. *American Journal of Botany* **92**, 1911–1928.
- Monson RK.** 1999. The origins of C₄ genes and evolutionary pattern in the C₄ metabolic phenotype. In: Sage RF, Monson RK, eds. *C₄ plant biology*. San Diego, CA: Academic Press, 377–410.
- Monson RK.** 2003. Gene duplication, neofunctionalization, and the evolution of C₄ photosynthesis. *International Journal of Plant Sciences* **164**, S43–S54.
- Monson RK, Edwards GE, Ku MSB.** 1984. C₃–C₄ intermediate photosynthesis in plants. *Bioscience* **34**, 563–574.
- Morgan JA, Brown RH.** 1979. Photosynthesis in grass species differing in carbon-dioxide fixation pathways. 2. Search for species with intermediate gas-exchange and anatomical characteristics. *Plant Physiology* **64**, 257–262.
- Moss DN.** 1962. The limiting carbon dioxide concentration for photosynthesis. *Nature* **193**, 587.
- Muhaidat R, Sage RF, Dengler NG.** 2007. Diversity of Kranz anatomy and biochemistry in C₄ eudicots. *American Journal of Botany* **94**, 362–381.
- Muhaidat R.** 2007. Diversification of C₄ photosynthesis in the eudicots: anatomical, biochemical and physiological perspectives. PhD Thesis. Toronto, Canada: University of Toronto.
- Ocampo G, Columbus JT.** 2010. Molecular phylogenetics of suborder Cactineae (Caryophyllales), including insights into photosynthetic diversification and historical biogeography. *American Journal of Botany* **97**, 1827–1847.
- Osborne CP, Freckleton RP.** 2009. Ecological selection pressures for C₄ photosynthesis in the grasses. *Proceedings of the Royal Society B: Biological Sciences* **276**, 1753–1760.
- Osborne CP.** 2011. The geologic history of C₄ plants. In: Raghavendra AS, Sage RF, eds. *C₄ photosynthesis and related CO₂ concentrating mechanisms*. Dordrecht, The Netherlands: Springer, 339–357.
- Osmond CB.** 1967. Beta-carboxylation during photosynthesis in *Atriplex*. *Biochimica et Biophysica Acta* **141**, 197–199.
- Oxelman B, Kornhall P, Olmstead RG, Bremer B.** 2005. Further disintegration of Scrophlariaceae. *Taxon* **54**, 411–425.
- Powell AM.** 1978. Systematics of *Flaveria* (Flaveriinae Asteraceae). *Annals of the Missouri Botanical Garden* **65**, 590–636.
- Pyankov VI, Artyusheva EG, Edwards GE, Black CC, Soltis PS.** 2001. Phylogenetic analysis of tribe Salsoleae (Chenopodiaceae) based on ribosomal ITS sequences: implications for the evolution of photosynthesis types. *American Journal of Botany* **88**, 1189–1198.
- Pyankov VI, Vakhrusheva DV.** 1989. Pathways of primary CO₂ fixation in C₄ plants of the family Chenopodiaceae from the arid zone of central Asia. *Soviet Plant Physiology* **36**, 178–187.
- Raghavendra AS, Das VSR.** 1978. Occurrence of C₄ photosynthesis— supplementary list of C₄ plants reported during late 1974–mid 1977. *Photosynthetica* **12**, 200–208.
- Rajendrudu G, Prasad JSR, Das VSR.** 1986. C₃–C₄ intermediate species in *Alternanthera* (Amaranthaceae)—leaf anatomy, CO₂ compensation point, net CO₂ exchange and activities of photosynthetic enzymes. *Plant Physiology* **80**, 409–414.
- Reinfelder JR, Milligan AJ, Morel FM.** 2004. The role of the C₄ pathway in carbon accumulation and fixation in a marine diatom. *Plant Physiology* **135**, 2106–2011.
- Rhoades MM, Carvalho A.** 1944. The function and structure of the parenchyma sheath plastids of the maize leaf. *Bulletin of the Torrey Botanical Club* **7**, 335–346.
- Roalson EH.** 2011. Origins and transitions in photosynthetic pathway types in monocots: a review and reanalysis. In: Raghavendra AS, Sage RF, eds. *C₄ photosynthesis and related CO₂ concentrating mechanisms*. Dordrecht, The Netherlands: Springer, 319–338.
- Roalson EH, Hinchliff CE, Trevisan R, da Silva CRM.** 2010. Phylogenetic relationships in *Eleocharis* (Cyperaceae): C₄ photosynthetic origins and patterns of diversification in the spikerushes. *Systematic Botany* **35**, 257–271.
- Sabale AB, Bhosale LJ.** 1984. C₃–C₄ photosynthesis in *Bougainvillea* cv. Palmer, Mary. *Photosynthetica* **18**, 84–89.
- Sage RF.** 2001. Environmental and evolutionary preconditions for the origin and diversification of the C₄ photosynthetic syndrome. *Plant Biology* **3**, 202–213.
- Sage RF.** 2004. The evolution of C₄ photosynthesis. *New Phytologist* **161**, 341–370.
- Sage RF, Li M, Monson RK.** 1999. The taxonomic distribution of C₄ photosynthesis. In: Sage RF, Monson RK, eds. *C₄ plant biology*. San Diego, CA: Academic Press, 551–584.
- Sage RF, Monson RK, eds.** 1999. *C₄ plant biology*. San Diego, CA: Academic Press.
- Sage RF, Sage TL, Percy RW, Borsch T.** 2007. The taxonomic distribution of C₄ photosynthesis in Amaranthaceae *sensu stricto*. *American Journal of Botany* **94**, 1992–2003.

- Sage TL, Sage RF, Vogan PJ, Rahman B, Johnson D, Oakley J, Heckel MC.** 2011. The occurrence of C₂ photosynthesis in *Euphorbia* subgenus *Chamaesyce* (Euphorbiaceae). *Journal of Experimental Botany* **62**, 3183–3195.
- Sankhla N, Ziegler H, Vyas OP, Stichler W, Trimborn P.** 1975. Eco-physiological studies on Indian arid zone plants. *Oecologia* **21**, 123–129.
- Sheahan MC, Chase MW.** 1996. A phylogenetic analysis of Zygophyllaceae R. Br. based on morphological, anatomical and *rbcL* DNA sequence data. *Botanical Journal of the Linnean Society* **122**, 279–300.
- Smith BN, Epstein S.** 1971. Two categories of C¹³/C¹² ratios for higher plants. *Plant Physiology* **47**, 380–384.
- Smith BN, Turner BL.** 1975. Distribution of Kranz syndrome among Asteraceae. *American Journal of Botany* **62**, 541–545.
- Smith SA, Beaulieu J, Donoghue MJ.** 2009. Mega-phylogeny approach for comparative biology: an alternative to supertree and supermatrix approaches. *BMC Evolutionary Biology* **9**, 37.
- Soros CL, Bruhl JJ.** 2000. Multiple evolutionary origins of C₄ photosynthesis in the Cyperaceae. In: Wilson KL, Morrison DA, eds. *Monocots: systematics and evolution*. Collingwood, Victoria, Australia: CSIRO Publishing, 629–636.
- Steinmann VW, Porter JM.** 2002. Phylogenetic relationships in Euphorbiae (Euphorbiaceae) based on ITS and *ndhF* sequence data. *Annals of the Missouri Botanical Garden* **89**, 453–490.
- Ueno O, Koyama T.** 1987. Distribution and evolution of C₄ syndrome in *Rhynchospora* (Rhynchosporaceae-Cyperaceae). *Botanical Magazine-Tokyo* **100**, 63–85.
- Vicentini A, Barber JC, Aliscioni SS, Giussani LM, Kellogg EA.** 2008. The age of the grasses and clusters of origins of C₄ photosynthesis. *Global Change Biology* **14**, 2963–2977.
- Vogan PJ, Frohlich MW, Sage RF.** 2007. The functional significance of C₃–C₄ intermediate traits in *Heliotropium* L. (Boraginaceae): gas exchange perspectives. *Plant, Cell and Environment* **30**, 1337–1345.
- Vollesen K.** 2000. *Blepharis: a taxonomic revision*. Richmond, UK: Kew Publishing.
- Von Caemmerer S.** 1992. Carbon isotope discrimination in C₃–C₄ intermediates. *Plant, Cell and Environment* **15**, 1063–1072.
- Voznesenskaya EV, Artyusheva EG, Franceschi VR, Pyankov VI, Kiirats O, Ku MSB, Edwards GE.** 2001. *Salsola arbusculiformis*, a C₃–C₄ intermediate in Salsoleae (Chenopodiaceae). *Annals of Botany* **88**, 337–348.
- Voznesenskaya EV, Koteyeva NK, Chuong SDX, Ivanova AN, Barroca J, Craven LA, Edwards GE.** 2007. Physiological, anatomical and biochemical characterisation of photosynthetic types in genus *Cleome* (Cleomaceae). *Functional Plant Biology* **34**, 247–267.
- Voznesenskaya EV, Koteyeva NK, Edwards GE, Ocampo G.** 2010. Revealing diversity in structural and biochemical forms of C₄ photosynthesis and a C₃–C₄ intermediate in genus *Portulaca* L. (Portulacaceae). *Journal of Experimental Botany* **61**, 3647–3662.
- Walter H, Harnickell E, Mueller-Dombois D.** 1975. *Climate-diagram maps of the individual continents and ecological climate regions of the earth—supplement to the vegetation monographs*. Berlin: Springer-Verlag.
- Watson L, Dallwitz MJ, eds.** 1992. *The grass genera of the world*. Wallingford, UK: CAB International.
- Webster GL, Brown WV, Smith BN.** 1975. Systematics and photosynthetic carbon fixation pathways in Euphorbia. *Taxon* **24**, 27–33.
- Wen ZB, Zhang ML, Zhu GL, Sanderson SC.** 2010. Phylogeny of Salsoleae s.l. (Chenopodiaceae) based on DNA sequence data from ITS, *psbB*–*psbH*, and *rbcL*, with emphasis on taxa of northwestern China. *Plant Systematics and Evolution* **288**, 25–42.
- Willis KJ, McElwain JC.** 2002. *The evolution of plants*. Oxford: Oxford University Press.
- Winter K.** 1981. C₄ plants of high biomass in arid regions of Asia—occurrence of C₄ photosynthesis in chenopodiaceae and polygonaceae from the Middle East and USSR. *Oecologia* **48**, 100–106.
- Ziegler H, Batanouny KH, Sankhla N, Vyas OP, Stichler W.** 1981. The photosynthetic pathway types of some desert plants from India, Saudi Arabia, Egypt, and Iraq. *Oecologia* **48**, 93–99.