

# On the Evolutionary Origin of CAM Photosynthesis<sup>[OPEN]</sup>

Andrea Bräutigam\*, Urte Schlüter, Marion Eisenhut, and Udo Gowik

Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK), Network Analysis and Modeling, D-06466 Seeland, OT Gatersleben; Germany (A.B.); and Institute of Plant Biochemistry (U.S., M.E.) and Institute of Developmental and Molecular Plant Biology (U.G.), Cluster of Excellence on Plant Sciences, Heinrich Heine University, 40225 Düsseldorf, Germany

ORCID IDs: 0000-0002-5309-0527 (A.B.); 0000-0002-2743-8630 (M.E.).

Evolution of carbon concentrating mechanisms appears complex. In the case of  $C_4$  photosynthesis, an enabling mutation is thought to have formed an initial  $C_4$  cycle, which is then selected for flux, and, finally, high expression of photorespiratory genes is lost (for summary, see Bräutigam and Gowik, 2016). However, in the case of Crassulacean acid metabolism (CAM) photosynthesis, we suggest that evolution directly acts on a low flux pathway already in place for amino acid metabolism. We thus propose a true continuum from  $C_3$  to CAM plants.

## CAM PHOTOSYNTHESIS

Photosynthesis in arid and/or hot and/or high light conditions faces unique challenges as water loss limits stomatal opening and thus limits  $CO_2$  supply for photosynthesis. Two different evolutionary solutions to this dilemma are known in land plants: CAM and  $C_4$  photosynthesis (Lüttge, 1988; Sage et al., 2012; Borland et al., 2014; Hartwell et al., 2016). Both traits are add-ons to the classic photosynthetic pathways, which are frequently termed  $C_3$  photosynthesis. CAM is not limited to the Crassulaceae but prevalent in many taxa (Silvera et al., 2010);  $C_4$  photosynthesis is named for the first labeled product of carbon fixation, a  $C_4$  acid. Many crop plants growing in challenging environments carry either adaptation: the CAM plants *Agave tequilana* and pineapple are productive in challenging climates (Borland et al., 2014).  $C_4$  photosynthesis is prevalent among productive crop plants, including maize, sugarcane, sorghum, and millet (Hibberd et al., 2008). Therefore, both CAM and  $C_4$  photosynthesis have been considered for engineering  $C_3$  crop plants to withstand adverse conditions while maintaining high yield (Hibberd et al., 2008; Borland et al., 2014).

CAM plants fix  $CO_2$  via phosphoenolpyruvate carboxylase (PEPC) during the night when it is cooler and less water is lost. The resulting organic acids, canonically malic acid but also citric acid (Knauff and Arditti,

1969; Lüttge, 1988), are stored in the central vacuole and decarboxylated during the day to provide  $CO_2$  to Rubisco and the Calvin-Benson-Bassham cycle (Silvera et al., 2010). During the night, stored carbohydrates are partially exported and partially used for organic acid synthesis (Borland and Dodd, 2002), which leads to a large proportion of storage carbohydrate cycling. CAM photosynthesis may be facultative, that is, induced environmentally and reversibly (i.e. *Talinum triangulare* [Brilhaus et al., 2016], *Mesembryanthemum* [Winter and Holtum, 2014]), or obligatory (Silvera et al., 2010). CAM plants also display great variation in their  $CO_2$  fixation efficiency with some species only cycling, i.e. refixing nightly respiratory  $CO_2$ , and others reaching high fixation rates under ideal conditions (Silvera et al., 2010).

Both  $C_4$  photosynthesis and CAM have evolved independently multiple times from  $C_3$  ancestors.  $C_4$  species represent about 3% of flowering plant species (Sage et al., 2012), while CAM species represent about 6% (Silvera et al., 2010). The evolutionary path and the fact that it has been traversed multiple times independently are somewhat puzzling given that both pathways represent complex traits, which require multiple genes to change simultaneously. They require architectural adaptations—large storage vacuoles in obligatory CAM, Kranz anatomy, or highly specialized cell anatomy in  $C_4$ —and biochemical adaptations with at least a dozen gene products altered in abundance and regulation.

As the basis for the evolution of CAM metabolism, a priori changes in abundance of multiple transcripts, especially with regard to their circadian patterns, were proposed (Silvera et al., 2010).

## LESSONS FROM $C_4$ EVOLUTION

$C_4$  and CAM photosynthesis are similar in the sense that initial  $CO_2$  fixation and Rubisco reaction are separated, spatially in the case of  $C_4$  and temporally in the case of CAM. The enzymes for the carbon concentrating mechanism are similar, too, occasionally down to the isoform recruited to either pathway (Christin et al., 2014). We use the concepts emerging from the recent progress in the study of  $C_4$  photosynthesis evolution

\* Address correspondence to braeutigam@ipk-gatersleben.de.

<sup>[OPEN]</sup> Articles can be viewed without a subscription.

[www.plantphysiol.org/cgi/doi/10.1104/pp.17.00195](http://www.plantphysiol.org/cgi/doi/10.1104/pp.17.00195)

(Heckmann et al., 2013; Williams et al., 2013; Mallmann et al., 2014) and apply them to CAM evolution.

Kinetic modeling revealed that the evolutionary path to Kranz anatomy based  $C_4$  is smooth with a Mount Fuji pattern, which has neither troughs nor steps (Heckmann et al., 2013), and that the path recapitulates the succession of earlier conceptual, stepwise evolutionary models (Monson et al., 1984; Rawsthorne et al., 1988; Sage et al., 2012). All of these models consider the establishment of a photorespiratory Gly shuttle via spatial expression of a key photorespiratory gene, also termed  $C_2$  photosynthesis, as an essential initial event of  $C_4$  evolution. It initiates the division of labor between the mesophyll and bundle sheath cells and the establishment of metabolic fluxes typical for  $C_2$  photosynthesis. However, the models could not explain the molecular events leading from photorespiratory shuttle to  $C_4$  photosynthesis. Modeling the metabolism of  $C_3$ - $C_4$  intermediates with a combination of a kinetic model and flux balance analysis indicated that the introduction of the photorespiratory Gly shuttle already predicts the immediate existence of a  $C_4$  cycle with low flux, to balance the nitrogen metabolism of mesophyll and bundle sheath cells (Mallmann et al., 2014). From this enabling mutation onward, selective pressure for higher expression of  $C_4$  cycle genes rests with the limiting enzyme or transporter capacity until high expression for all is reached (Mallmann et al., 2014; Bräutigam and Gowik, 2016). In summary, an enabling mutation transforms the trait from complex (i.e. multiple genes change at once) to additive (i.e. each change in gene expression increases fitness).

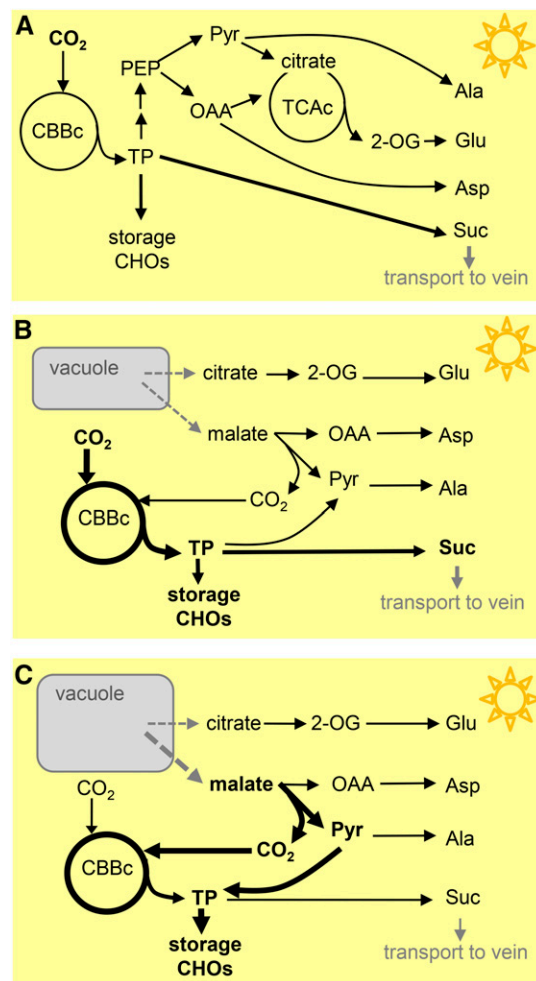
For CAM photosynthetic evolution, a model passing through intermediates with ever increasing cycle capacity has been proposed (Silvera et al., 2010). However, the possible starting point, the initial CAM event, has not been identified so far. To establish an efficient CAM cycle via natural selection on a limiting enzyme, transporter, or architectural adaptation, a basal “CAM cycle” has to be in place.

## CARBON FLUXES IN $C_3$ AND CAM PLANTS

We thus set out to identify whether metabolite fluxes similar to CAM fluxes are already in existence in  $C_3$  plants or generated by a simple mutation. The core features are daytime release of the  $CO_2$  for fixation by Rubisco and nightly production and storage of organic acids produced by PEPC.

### Use of Stored Organic Acids during the Day

Based on textbooks and depictions in repositories (i.e. <http://www.plantcyc.org/>), it is frequently assumed that amino acids are directly derived from photosynthesis during the day. In this concept, triosephosphates from the Calvin-Benson-Bassham cycle are converted to organic acids via cytosolic reactions and the TCA cycle (TCAc) and then transaminated to amino acids and derived products (Fig. 1A). However, flux analyses



**Figure 1.** Daytime metabolism of organic acids in  $C_3$  and CAM plants; arrow thickness denotes flux. A, Organic acids are directly derived from photosynthesis during the day. This model is obsolete for many  $C_3$  species due to the results of flux analyses. B, In many  $C_3$  plants, the use of organic acids is based on organic acids produced and stored during the night according to flux analyses. C, Daytime metabolism of organic acids in CAM plants. 2-OG, Oxoglutarate; CBBc, Calvin-Benson-Bassham cycle; CHOs, carbohydrates; OAA, oxaloacetate; Pyr, pyruvate; TP, triosephosphate.

using  $^{13}CO_2$ ,  $^{13}C$ -pyruvate, and  $^{13}C$ -Glc labeling have questioned this model for several  $C_3$  species from distant eudicot groups (rosids with Fabales and Brassicales, asterids with Asteraceae; Tcherkez et al., 2005, 2009, 2012; Gauthier et al., 2010; Szechowka et al., 2013).

Daytime pulse labeling with  $^{13}CO_2$  indicates that the organic acids malate, citrate, isocitrate, and fumarate remain largely unlabeled as do the derived amino acids (Szechowka et al., 2013). Similarly, Glu and Gln, both derived from the organic acid 2-oxoglutarate, are not labeled during a daytime  $^{13}CO_2$  pulse (Gauthier et al., 2010). Feeding with precursors such as  $^{13}C$ -pyruvate demonstrated that pyruvate entry into the TCAc is very low (Tcherkez et al., 2005, 2009). Taken together,

these observations refute the model depicted as Figure 1A (Tcherkez et al., 2012).

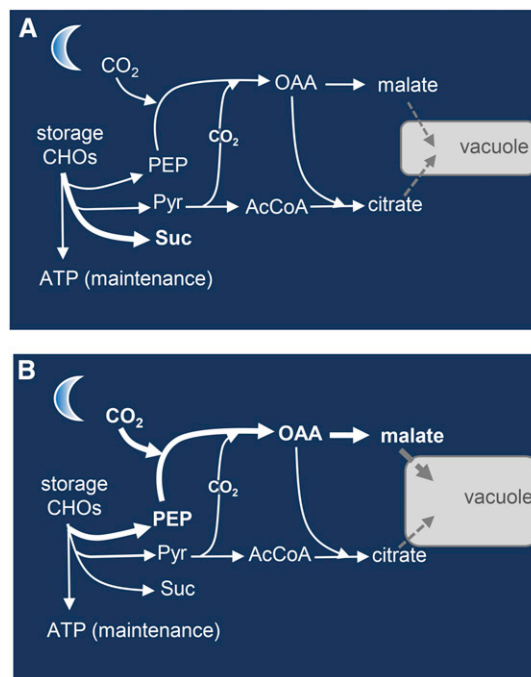
Observing label distribution in the night and the following day after giving a label pulse of  $^{13}\text{CO}_2$  on the previous day showed that, during the night and the following day,  $^{13}\text{C}$  is transferred to Glu and Gln (Gauthier et al., 2010). Under the current model (Tcherkez et al., 2012), daytime use of organic and amino acids is based on organic acids produced and stored during the night before and used during the following day (Fig. 1B). So there is no immediate connection of their production to the present daytime photosynthetic metabolism (Fig. 1B). Unlike, the pools of  $\text{C}_4$ ,  $\text{C}_5$ , and  $\text{C}_6$  organic acids and derived molecules, the pyruvate pool labels to about 50% (Szecowka et al., 2013). That is, half the pool is produced from photosynthetic products and half from reserves (Fig. 1B). In summary,  $\text{C}_3$  plants store organic acids at night to fuel daytime amino acid synthesis. Part of the stored organic acids is decarboxylated to pyruvate during the day.

If one considers the evolutionary changes required for daytime CAM photosynthesis, it becomes apparent that the framework of the CAM cycle actually is already in place in  $\text{C}_3$  species and that it carries flux. It is not a question of rewiring metabolism but of selecting for increased flux (Fig. 1C).

### Organic Acid Storage during the Night

Flux analysis has established that  $\text{C}_4$  and  $\text{C}_5$  organic acids used during the day are largely drawn from a store produced during the night (Gauthier et al., 2010; Szecowka et al., 2013), which in turn is dependent on storage carbohydrates synthesized on the previous day (Gauthier et al., 2010). PEPC for  $\text{C}_4$  acid synthesis and beyond requires activation by a kinase. In a  $\text{C}_3$  plant, a PEPC activating kinase is preferentially expressed during the night and PEPC phosphorylation indeed persists during the night (Aldous et al., 2014). The substrate of PEPC, phosphoenolpyruvate, is produced from stored carbohydrates (Gauthier et al., 2010). The resulting oxaloacetate can be reduced to malate and stored, or converted to citrate in the TCAc (Fig. 2A). The  $\text{CO}_2$  used during phosphoenolpyruvate carboxylation stems from pyruvate decarboxylation, from the TCAc, or from outside. If from interior sources, the resulting oxaloacetate would have an isotopic composition in the  $\text{C}_3$  range at the  $\text{C}_1$  position. If the  $\text{CO}_2$  stems from the outside, its isotopic composition at the  $\text{C}_1$  carbon would be in the  $\text{C}_4$  range. Analysis of Asp contained in proteins in *Nicotiana tabacum* demonstrated that half of the Asp  $\text{C}_1$  carbon is in the  $\text{C}_4$  range and thus derived from outside  $\text{CO}_2$  and not from  $\text{CO}_2$  prefixed by Rubisco (Melzer and O'Leary, 1987). Thus, nightly organic acid metabolism of  $\text{C}_3$  plants likely involves atmospheric  $\text{CO}_2$  fixation (Fig. 2A).

If one considers the evolutionary changes required for nighttime CAM photosynthesis, it becomes apparent that the framework of the CAM cycle actually is already in place in  $\text{C}_3$  species. Nighttime CAM metabolism does not require de novo fluxes but an increase in



**Figure 2.** Nighttime metabolism of organic acids in  $\text{C}_3$  (A) and CAM (B) plants. AcCoA, Acetyl coenzyme A; CHOs, carbohydrates; OAA, oxaloacetate; PEP, phosphoenolpyruvate; Pyr, pyruvate.

existing fluxes, including increased flux of  $\text{CO}_2$  from the outside (Fig. 2B).

### EARLY EVOLUTIONARY EVENTS OF CAM PHOTOSYNTHESIS

In summary, the temporally separated CAM cycle including the fixation of outside  $\text{CO}_2$  during the night, synthesis and storage of organic acids during the night, and use of organic acids including malate decarboxylation (Szecowka et al., 2013) during the day is already in place in  $\text{C}_3$  plants, but has never been called a CAM cycle (Tcherkez et al., 2012). A properly constrained diel stoichiometric  $\text{C}_3$  model is capable of predicting CAM photosynthesis (Cheung et al., 2014), underscoring that evolution of efficient CAM does not require rewiring or temporally changing flux capacity but only increasing existing flux capacity (Figs. 1C and 2B).

While stomatal opening patterns are completely reversed in strong CAM species (Abraham et al., 2016), initial evolution of weak CAM likely only requires incrementally increased flux and therefore incrementally increased stomatal opening during the night. At least in some CAM species, daytime stomata closure may simply be caused by water limitation (Winter and Holtum, 2014). A single mutation induces nightly stomatal opening while leaving daytime closure intact (Costa et al., 2015). Some known CAM plants remain capable of daytime stomatal opening if water is available to the transpiration stream (Winter and Holtum,

2014). Although architectural adaptations were not considered in the analysis, a rather small storage vacuole for organic acids is in place already in  $C_3$  plants and may come under selection if increased storage capacity is required.

This evolutionary scenario explains several previous observations about CAM and  $C_4$  plants. In many CAM species, citric acid coaccumulates with malic acid (Knauff and Arditti, 1969; Lüttge, 1988). The evolutionary scenario presented here explains citrate accumulation as an atavism of the evolutionary origin. The CAM trait can revert to  $C_3$  metabolism over evolutionary time (Crayn et al., 2004; Silvera et al., 2009). Unlike  $C_4$  evolution, which includes the loss of expression for multiple genes (Bräutigam and Gowik, 2016), weak CAM likely requires expression gain and no changes in temporal expression (Figs. 1B and 2A), which makes sliding back along the gradient of CAM toward  $C_3$  possible and likely. Indeed, many if not all CAM species retain the ability to photosynthesize in  $C_3$  mode (Winter and Holtum, 2014). In consequence, mutation of CAM genes in CAM plants is not lethal (Dever et al., 2015), while in  $C_4$  species, the  $C_4$  cycle is obligatory (Dever et al., 1995). CAM has a higher incidence in plant species (Silvera et al., 2010). The evolutionary scenario shows that, unlike during evolution of  $C_4$  photosynthesis, which requires the loss of expression of photorespiratory genes in a certain cell type, the pathway on which selective pressure for CAM can act does not require an enabling mutation to be present. CAM metabolism can be induced and shut off multiple times during a plant's life cycle (Taisma and Herrera, 1998). The continuum from  $C_3$  to CAM explains why seamless induction and recovery are possible in so-called facultative CAM plants but is unknown in  $C_4$ .

This evolutionary scenario predicts testable CAM features including but not limited to gene duplication are probably not required. CAM genes are orthologs to genes involved in amino acid assimilation, and selective pressures selected for higher expression rather than change of pattern. The evolutionary scenario also predicts that plants that do not possess nighttime organic acid storage (i.e. Leport et al., 1996) are unlikely to evolve toward CAM.

We propose to extend the currently accepted continuum of CAM evolution (cycling, weak, idling, strong; Silvera et al., 2010) to  $C_3$  species (amino acid metabolism in  $C_3$ , cycling, weak, idling, strong). The requirements for CAM listed by Silvera et al. (2010) are all present at the right time of the diurnal cycle and only need enhancement: nocturnal  $CO_2$  uptake, diel fluctuations of organic acids, associated transport activities, (enhanced) PEPC and malic enzyme expression, (enhanced) flow through glycolytic and gluconeogenic pathways, and a storage vacuole (Figs. 1B and Fig. 2A; derived from Tcherkez et al., 2005, 2009; Gauthier et al., 2010; Szecowka et al., 2013). We thus argue that CAM evolution, unlike  $C_4$ , is a true continuum from  $C_3$  to CAM. This bodes well for the engineering of CAM into  $C_3$  crops.

## ACKNOWLEDGMENTS

We thank two anonymous reviewers for their suggestions to improve the manuscript and A.P.M. Weber for pointing out that not all  $C_3$  species possess nighttime storage of organic acids.

Received February 8, 2017; accepted April 12, 2017; published April 17, 2017.

## LITERATURE CITED

- Abraham PE, Yin H, Borland AM, Weighill D, Lim SD, De Paoli HC, Engle N, Jones PC, Agh R, Weston DJ, et al (2016) Transcript, protein and metabolite temporal dynamics in the CAM plant Agave. *Nat Plants* 2: 16178
- Aldous SH, Weise SE, Sharkey TD, Waldera-Lupa DM, Stühler K, Mallmann J, Groth G, Gowik U, Westhoff P, Arsova B (2014) Evolution of the phosphoenolpyruvate carboxylase protein kinase family in  $C_3$  and  $C_4$  *Flaveria* spp. *Plant Physiol* 165: 1076–1091
- Borland AM, Dodd AN (2002) Carbohydrate partitioning in crassulacean acid metabolism plants: reconciling potential conflicts of interest. *Funct Plant Biol* 29: 707–716
- Borland AM, Hartwell J, Weston DJ, Schlauch KA, Tschaplinski TJ, Tuskan GA, Yang X, Cushman JC (2014) Engineering crassulacean acid metabolism to improve water-use efficiency. *Trends Plant Sci* 19: 327–338
- Bräutigam A, Gowik U (2016) Photorespiration connects  $C_3$  and  $C_4$  photosynthesis. *J Exp Bot* 67: 2953–2962
- Brillhaus D, Bräutigam A, Mettler-Altman T, Winter K, Weber APM (2016) Reversible burst of transcriptional changes during induction of crassulacean acid metabolism in *Talinum triangulare*. *Plant Physiol* 170: 102–122
- Cheung CYM, Poolman MG, Fell DA, Ratcliffe RG, Sweetlove LJ (2014) A diel flux balance model captures interactions between light and dark metabolism during day-night cycles in  $C_3$  and crassulacean acid metabolism leaves. *Plant Physiol* 165: 917–929
- Christin P-A, Arakaki M, Osborne CP, Bräutigam A, Sage RF, Hibberd JM, Kelly S, Covshoff S, Wong GK-S, et al (2014) Shared origins of a key enzyme during the evolution of  $C_4$  and CAM metabolism. *J Exp Bot* 65: 3609–3621
- Costa JM, Monnet F, Jannaud D, Leonhardt N, Ksas B, Reiter IM, Pantin F, Genty B (2015) Open all night long: the dark side of stomatal control. *Plant Physiol* 167: 289–294
- Crayn DM, Winter K, Smith JAC (2004) Multiple origins of crassulacean acid metabolism and the epiphytic habit in the Neotropical family Bromeliaceae. *Proc Natl Acad Sci USA* 101: 3703–3708
- Dever LV, Blackwell RD, Fullwood NJ, Lacuesta M, Leegood RC, Onek LA, Pearson M, Lea PJ (1995) The isolation and characterization of mutants of the  $C_4$  photosynthetic pathway. *J Exp Bot* 46: 1363–1376
- Dever LV, Boxall SF, Kneřová J, Hartwell J (2015) Transgenic perturbation of the decarboxylation phase of Crassulacean acid metabolism alters physiology and metabolism but has only a small effect on growth. *Plant Physiol* 167: 44–59
- Gauthier PPG, Bligny R, Gout E, Mahé A, Nogués S, Hodges M, Tcherkez GGB (2010) In folio isotopic tracing demonstrates that nitrogen assimilation into glutamate is mostly independent from current  $CO_2$  assimilation in illuminated leaves of *Brassica napus*. *New Phytol* 185: 988–999
- Hartwell J, Dever LV, Boxall SF (2016) Emerging model systems for functional genomics analysis of Crassulacean acid metabolism. *Curr Opin Plant Biol* 31: 100–108
- Heckmann D, Schulze S, Denton A, Gowik U, Westhoff P, Weber APM, Lercher MJ (2013) Predicting  $C_4$  photosynthesis evolution: modular, individually adaptive steps on a Mount Fuji fitness landscape. *Cell* 153: 1579–1588
- Hibberd JM, Sheehy JE, Langdale JA (2008) Using  $C_4$  photosynthesis to increase the yield of rice-rationale and feasibility. *Curr Opin Plant Biol* 11: 228–231
- Knauff RL, Arditti J (1969) Partial identification of dark  $^{14}CO_2$  fixation products in leaves of *Cattleya* (Orchideaceae). *New Phytol* 68: 657–661
- Leport L, Kandlbinder A, Baur B, Kaiser WM (1996) Diurnal modulation of phosphoenolpyruvate carboxylation in pea leaves and roots as related to tissue malate concentrations and to the nitrogen source. *Planta* 198: 495–501



- Lüttge U (1988) Day-night changes of citric acid levels in crassulacean acid metabolism – phenomenon and ecophysiological significance. *Plant Cell Environ* **11**: 445–451
- Mallmann J, Heckmann D, Bräutigam A, Lercher MJ, Weber APM, Westhoff P, Gowik U (2014) The role of photorespiration during the evolution of C<sub>4</sub> photosynthesis in the genus *Flaveria*. *eLife* **3**: e02478
- Melzer E, O’Leary MH (1987) Anapleurotic CO<sub>2</sub> fixation by phosphoenolpyruvate carboxylase in C<sub>3</sub> plants. *Plant Physiol* **84**: 58–60
- Monson RK, Edwards GE, Ku MSB (1984) C<sub>3</sub>-C<sub>4</sub> intermediate photosynthesis in plants. *BioScience* **34**: 563–574
- Rawsthorne S, Hylton CM, Smith AM, Woolhouse HW (1988) Distribution of photorespiratory enzymes between bundle-sheath and mesophyll cells in leaves of the C<sub>3</sub>-C<sub>4</sub> intermediate species *Moricandia arvensis* (L.) DC. *Planta* **176**: 527–532
- Sage RF, Sage TL, Kocacinar F (2012) Photorespiration and the evolution of C<sub>4</sub> photosynthesis. In SS Merchant, ed, *Annual Review of Plant Biology*, Vol 63. Annual Reviews, Palo Alto, CA, pp 19–47
- Silvera K, Neubig KM, Whitten WM, Williams NH, Winter K, Cushman JC (2010) Evolution along the crassulacean acid metabolism continuum. *Funct Plant Biol* **37**: 995–1010
- Silvera K, Santiago LS, Cushman JC, Winter K (2009) Crassulacean acid metabolism and epiphytism linked to adaptive radiations in the Orchidaceae. *Plant Physiol* **149**: 1838–1847
- Szecowka M, Heise R, Tohge T, Nunes-Nesi A, Vosloh D, Huege J, Feil R, Lunn J, Nikoloski Z, Stitt M, et al (2013) Metabolic fluxes in an illuminated *Arabidopsis* rosette. *Plant Cell* **25**: 694–714
- Taisma MA, Herrera A (1998) A relationship between fecundity, survival, and the operation of crassulacean acid metabolism in *Talinum triangulare*. *Can J Bot* **76**: 1908–1915
- Tcherkez G, Boex-Fontvieille E, Mahé A, Hodges M (2012) Respiratory carbon fluxes in leaves. *Curr Opin Plant Biol* **15**: 308–314
- Tcherkez G, Cornic G, Bligny R, Gout E, Ghashghaie J (2005) *In vivo* respiratory metabolism of illuminated leaves. *Plant Physiol* **138**: 1596–1606
- Tcherkez G, Mahé A, Gauthier P, Mauve C, Gout E, Bligny R, Cornic G, Hodges M (2009) *In folio* respiratory fluxomics revealed by <sup>13</sup>C isotopic labeling and H/D isotope effects highlight the noncyclic nature of the tricarboxylic acid “cycle” in illuminated leaves. *Plant Physiol* **151**: 620–630
- Williams BP, Johnston IG, Covshoff S, Hibberd JM (2013) Phenotypic landscape inference reveals multiple evolutionary paths to C<sub>4</sub> photosynthesis. *eLife* **2**: e00961
- Winter K, Holtum JAM (2014) Facultative crassulacean acid metabolism (CAM) plants: powerful tools for unravelling the functional elements of CAM photosynthesis. *J Exp Bot* **65**: 3425–3441