



New perspectives on crassulacean acid metabolism biology

Crassulacean acid metabolism (CAM) photosynthesis is one of the principle carbon-concentrating mechanisms in terrestrial plants. A primary feature of the CAM photosynthetic pathway revolves around the night-time uptake of CO₂ and its subsequent storage as organic acids for later daytime fixation into sugars. This unique, water-saving, and carbon-concentrating photosynthetic pathway is the major means by which land plants achieve superior levels of resource-use efficiency. As a result, CAM plants are increasingly recognized as among the world's most important climate-resilient crops for food, forage, fodder, fiber, and fuel, as well as being key drivers of ecosystem function in dry regions.

CAM photosynthesis stands out as among the most prolific examples of complex trait evolution in the biosphere, with >60 independent evolutionary origins, and occurrence found in >38 families encompassing >400 genera of vascular plant species (Smith and Winter, 1996; Silvera *et al.*, 2010; Winter *et al.*, 2015). Most of the origins are correlated with increasing aridity and declines in atmospheric CO₂ in the geological past, allowing examples of CAM evolution to serve as leading case studies for evolutionary responses to global climate change (Box 1). CAM also evolved with a series of co-adaptive traits such as stem or leaf succulence, water capture and storage strategies, thick cuticles and epicuticular wax deposition, low stomatal density, high stomatal responsiveness, and shallow rectifier-like roots, which allow plants to occupy harsh environments with limited or intermittent water availability (Niechayev *et al.*, 2019a). The high number of origins provides repeated examples of how CAM arose from C₃ photosynthesis ancestors, and thus can be exploited to provide new directions and genetic constructs for improving resource-use efficiency in ways that provide a wide range of societal benefits (Yang *et al.*, 2015; Lim *et al.*, 2019). Global warming is expected to result in increased surface drying and an expansion of drylands throughout the globe (Huang *et al.*, 2015; Dai *et al.*, 2018). However, some CAM species show positive growth responses to elevated CO₂ (Drennan and Nobel, 2000; Ceusters and Borland, 2010) and can tolerate high temperatures (Nobel *et al.*, 1986; Chetti and Nobel, 1988). Exploiting the efficiency potential of CAM photosynthesis is one of the most cost-effective and environmentally safe ways to simultaneously meet the

overlapping challenges of global food security, scarce water supplies, increasing bioenergy resources, and low-input carbon sequestration in the face of global climate change (Osmond *et al.*, 2008; Borland *et al.*, 2009; Cushman *et al.*, 2015; Davis *et al.*, 2015, 2017).

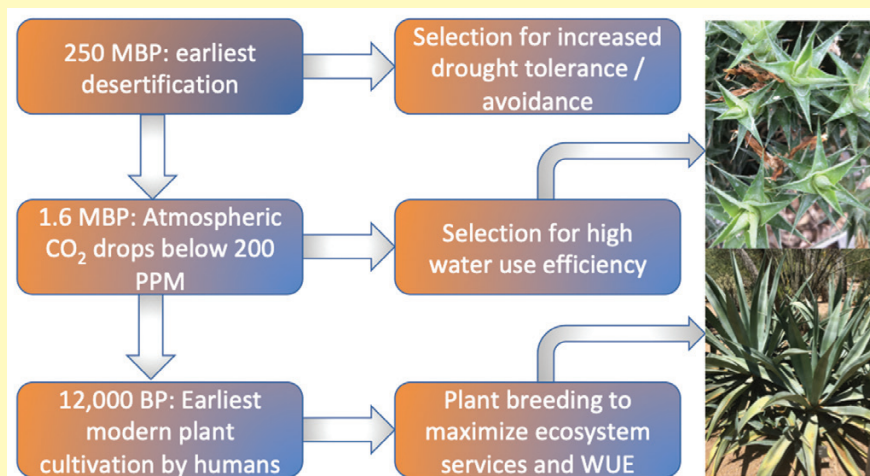
CAM plants are not only recognized as important crop plants (e.g. pineapple, *Agave*, *Opuntia*, *Aloe*, and vanilla) and valuable horticultural ornamentals (e.g. *Agave*, cactus, *Kalanchoe*, and orchids), they also include many of the most iconic and important foundation species of arid and semi-arid and epiphytic and atmospheric ecosystems. CAM metabolism is often, but not exclusively, associated with stem and leaf succulence: features that have evolved across a broad range of plant taxa in apparent coordination with the evolution of the CAM photosynthetic pathway among certain lineages (Arakaki *et al.*, 2011; Horn *et al.*, 2014). The co-evolution of succulence and subsequent internal water storage capacity and CAM metabolism has facilitated the radiation of many plant species into areas dominated by prolonged aridity and episodic drought (Box 1). Recent analysis within the Agavoideae (Asparagaceae) suggests that succulent leaf anatomy pre-dates the appearance of CAM (Heyduk *et al.*, 2016). However, to what extent amplified aridity from climate change is impacting the productivity and distribution of CAM plants across the globe remains an open question. CAM-related genes were among many gene families associated with adaptation to harsh or extreme environments (Wang *et al.*, 2019). Recent advances in functional genomics, stable isotope applications, and mechanistic niche modeling approaches are poised to shed light on the extent to which rapid environmental changes might alter the range and productivity of CAM plants and the resources that flow to dependent consumers and human populations.

Exploiting the productive potential of CAM for human enterprise while protecting CAM plants in naturally occurring environments requires the integration of a broad range of biological disciplines so that ecological and evolutionary discoveries can inform society in an era of global climate change. This special issue highlights this integration with reviews and original research reports that focus on cutting-edge topics that revolve around research on CAM, including the expression of CAM within phylogenetic contexts, opportunities surrounding the climate resilience of CAM plants, and advances in CAM genetics and genomics.

Box 1. The origins and evolution of the CAM photosynthetic pathway

CAM is known in 38 plant families and, due to its broad systematic distribution, it appears that CAM has arisen independently several times. CAM is estimated to exist in ~6% of all terrestrial plant taxa as well as 6% of all aquatic plant taxa (Keeley, 1998). The precise origins of the earliest CAM plants are not well known, in part because evidence for CAM is largely depauperate in the fossil record (Ehleringer and Monson, 1993). Over broad geographical scales, aridity appears to be the primary driver of terrestrial CAM and is perhaps best known in desert succulents that are regularly exposed to high daytime temperatures and low relative humidities. However, selection for CAM should also correspond to conditions of low available CO₂ due to the intensive carbon-concentrating mechanisms associated with the diurnal storage of organic acids during the CAM cycle. The presence of CAM in primitive aquatic plants such as those in the genus *Isoetes*, for example, probably evolved due to the low diffusion coefficient of CO₂ in water (Monson, 1989). Similarly, the evolution of CAM in terrestrial plant taxa probably accelerated in response to glacial episodes during the Pleistocene when reduced atmospheric CO₂ concentrations favored photosynthetic pathways such as CAM and C₄ (Ehleringer and Monson, 1993; Raven and Spicer, 1996; Keeley and Rundel, 2003).

More recently, cultivation of CAM plants for food, fiber, medicinal, and other uses may have enhanced diversification along a number of plant lineages. For example, plants within the genus *Agave* have been an important source of food and fiber for humans in Mesoamerica since at least 9000 years BP (Gentry, 1982). Intensive cultivation of *Agave* by pre-Columbian Native Americans has given rise to new species originating from North American ‘domestication centers’ selected for high water-use efficiency, sugar content, and productivity (Hodgeson et al., 2019). Modern agricultural practices are poised to follow the lead of pre-Columbian agronomists to further exploit the high water-use efficiency in CAM taxa to enhance crop yields relative to water allocation for food, fiber, and bioenergy production (Yang et al., 2015; Davis et al., 2019).



Timeline of the major geological/anthropological events that facilitated the evolution and diversification of CAM plants across the globe. Upper right photo: *Deuterocohnia brevifolia* (Griseb.), a perennial succulent-leaved shrub native to the Andes region of Argentina and Bolivia. Lower right photo: the widely cultivated *Agave tequilana* (Weber).

CAM expression within phylogenetic contexts

The classic model of CAM defined by inverted stomatal behavior and four phases of gas exchange and biochemical activity (Osmond, 1978) belies the diversity and complexity of CAM expression in nature. Many recent advancements in our understanding of CAM have come from studies at the margins of CAM expression, in the so-called ‘weak’, ‘facultative’, and ‘intermediate’ CAM plants. It is at the margins and across the spectrum of CAM types where the evolution, functional significance, and molecular and genomic features of CAM

are fruitfully explored, and where targets for introduction of CAM traits into C₃ and C₄ crops might be found and successfully engineered. That many CAM plants express CAM together with some C₃ or C₄ photosynthesis and this expression often varies over different stages of development, or facultatively when exposed to drought or salinity stress, challenges our notion of what CAM is, how it evolved, and even how it is defined. In this special issue, Winter (2019) presents a review of the complexity of CAM expression and establishes core terminology and definitions to help frame and articulate research directions and findings (Winter, 2019). Winter demonstrates, using continuous monitoring of daily CO₂ exchange and broad

comparisons of night-time malate accumulation, how the often-overlooked subtleties of CAM expression can be central to further advancements in the field, especially when considering weakly expressed (Winter *et al.*, 2019a) or stress-induced facultative CAM (including facultative CAM in C₃–C₄ intermediates; Winter *et al.*, 2019b). These are key points that should be embraced more broadly and, although the level of evidence required to diagnose the full range of CAM expression is technically demanding, the payoffs are demonstrably large.

Climate resilience of CAM plants: consequences and opportunities

The natural distribution of CAM species is dictated by their ability to persist in harsh climates. However, many CAM species, particularly those with stem succulence within the Old World Euphorbia and the New World Cactaceae, are threatened by human poaching activity and the unprecedented threat posed by ongoing global climate change. Hultine and colleagues review the challenges and opportunities associated with stable isotope analysis of the spines of large columnar cactus species, which serve as long-lived sentinels capable of documenting with exquisite accuracy changes in climatic conditions over tens to hundreds of years (Hultine *et al.*, 2019). Many CAM species serve as climate-resilient crops possessing immense agricultural value capable of meeting the food, feed, fiber, biofuel, and pharmaceutical needs of the future. As reviewed by Davis *et al.* (2019), although a small number of CAM species and their products are globally traded commodities, including *Agave tequilana*, *Ananas comosus* (pineapple), *Aloe* spp., *Vanilla* spp., and *Opuntia* spp., these species have been traditionally undervalued and very little investment in agricultural improvements have been made in them. However, recent advances in genomic resources for many of these CAM crops promises to facilitate genetic improvement through targeted, molecular-assisted breeding and genome editing approaches (Davis *et al.*, 2019). Among them, *Agave* spp. have received considerable attention as a bioenergy feedstock (Davis *et al.*, 2017). Although *Agave* spp. have been cultivated by pre-Columbian Native Americans for centuries (Box 1), the capacity to produce fiber and biofuels on an industrial scale in dryland regions has stimulated growing interest in *Agave* production (Davis *et al.*, 2019). To improve our understanding of the potential productivity of *Agave americana* in water-limited regions, a monthly environmental productivity index (EPI) was developed, which is based on light, temperature, and precipitation inputs (Niechayev *et al.*, 2019b). Such modeling efforts will be fundamentally important for estimating the geographical range and productivity potential of this and other CAM crops under current and future climatic conditions.

Advances in CAM genetics and genomics

The application of high-throughput ‘omics technologies continues to provide novel insights into the mechanistic basis of CAM function. Exploration of the early stages of CAM

induction by applying abscisic acid (ABA) to the leaves of the facultative CAM model species *Talinum triangulare* suggested new genes with possible functions in CAM including ABA signal transduction, amino acid metabolism, solute transport, protein degradation, and a set of putative transcriptional regulators involved in CAM induction (Maleckova *et al.*, 2019). Comparative transcriptome and preliminary metabolomic analyses of a 24 h time course comparing *Yucca* (Asparagaceae) species with C₃ photosynthesis (*Y. filamentosa*), C₃–CAM [*Y. gloriosa* (hybrid)], and CAM (*Y. aloifolia*) under both well-watered and drought-stressed conditions in a common garden setting revealed clear-cut differences among the two parents and the hybrid, yet all three shared some common changes in steady-state mRNA abundance. Such common expression patterns and resulting traits might have facilitated the convergent evolution of CAM within the Agavoideae (Heyduk *et al.*, 2019). In the context of CAM evolution, Yang *et al.* (2019) discuss possible pathway scenarios for CAM evolution, anatomical modifications associated with CAM, and potential amino acid and temporal reprogramming changes that might sustain CAM evolution informed by comparative genomic analyses. Taking a phylogenetic approach and using $\delta^{13}\text{C}$ tissue analysis of herbarium specimens, Li *et al.* (2019) report multiple independent origins of CAM within the highly diverse orchid genus *Dendrobium* in Australasia (Li *et al.*, 2019). A fine-scale understanding of the molecular genetic changes associated with CAM evolution will require a robust functional toolkit including the ability to readily transform favored model CAM species, RNAi, and genome editing using CRISPR/Cas (clustered regulatory interspaced short palindromic repeats/CRISPR-associated protein)-mediated genome editing approaches (Yang *et al.*, 2019). Like most other plants, CAM species can be subjected to genome editing. In one of the first ‘proof of concept’ demonstrations of CRISPR/Cas-mediated genome editing in a CAM species, Liu *et al.* (2019) showed that knocking out the PHOTOTROPIN 2 (PHOT2) gene in *Kalanchoë fedtschenkoi* reduced stomatal conductance and CO₂ fixation in the latter part of the photoperiod (Phase IV), whereas these behaviors were enhanced during the early evening and throughout the dark period (Phase I) of CAM, suggesting that blue-light signaling might be important for the proper functioning of the diel CAM cycle. To advance additional genome editing work, a genome-wide guide RNA (gRNA) database was developed for *K. fedtschenkoi* (Liu *et al.*, 2019).

Future directions

CAM photosynthesis was a leading topic in plant biology research in the two decades after its discovery in the 1960s. However, once the biochemical details of carbon flow in CAM photosynthetic pathways had been established, further progress in CAM research was dependent upon application of molecular genetic, phylogenetic, and isotopic approaches to investigate these metabolic adaptations. Recent advances in molecular phylogenetics, high-throughput sequencing, genome editing, and isotopic physiology have reinvigorated CAM research and contributed to major new initiatives in exploring CAM

evolution, the development of novel C₃ photosynthesis and CAM bioenergy crops, and the engineering of CAM pathways in non-CAM crop species for improved climate resilience. These initiatives are now producing exciting results that current and future CAM biologists will continue to build upon. Over the coming years, highly novel, transformative discoveries in CAM biology will have immediate significance towards commercial application and the conservation of natural ecosystems.

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