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PART OF A SPECIAL ISSUE ON CAM AT THE CROSSROADS

Crassulacean acid metabolism (CAM) at the crossroads: a special issue to honour 50 years of CAM research by Klaus Winter

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A spectacular feature of the botanical world is the succulent flora, which presents a diversity of life forms from the fascinating to the strange (Figs 1–3). Succulence describes fleshy tissue with high water content, yet as a character state is highly variable, ranging from slightly fleshy planar leaves to the grossly corpulent stems of cacti and aloes (Fig. 2; Grace, 2019; Peréz- Lopéz *et al*., 2023). The associated function also shows marked variation, ranging from water and salt storage, mechanical support and, in the majority of succulents, to the storage of organic acids used in crassulacean acid metabolism (CAM). Succulence is hypothesized to be essential for the function of the CAM photosynthetic pathway because it allows storage of large amounts of assimilated carbon in the form of malate, and assists the CAM process by trapping CO_2 released during the day (Males, 2017; Borland *et al*., 2018; Edwards, 2019). In the typical form of CAM photosynthesis, plants open stomata at night to fix inorganic carbon into malic acid using phosphoenolpyruvate (PEP) carboxylase (see figs 1 and 2 in Chomthong and Griffiths, 2023, in this issue for explanatory diagrams). The malic acid is then stored in large vacuoles. During the day, stomata close, and the stored malic acid is decarboxylated to raise CO_2 concentrations within leaves and stems to levels that suppress the wasteful process of photorespiration. Rubisco then refixes the released CO_2 that is trapped within the leaves by the closed stomata, and the fixation products are synthesized into sugars and starch by the same biochemistry used in C_3 photosynthesis. Because stomata are closed over much of the day, transpiration is low and thus CAM plants exhibit high water-use efficiencies (WUE) of photosynthesis. High WUE enables survival in dry locations, to include arid and semi-arid landscapes, and soil-less environments such as the epiphyte habitat on tree branches or the lithophyte habit on rock faces.

CAM is abundant and well dispersed across the world's flora occurring in ferns, gymnosperms, monocots and eudicots. As examined in this issue (Gilman *et al*., 2023), CAM photosynthesis independently evolved over 60 times across vascular plants, making it one of the best examples of evolutionary convergence on a complex trait in the living world. The repeated evolution of CAM has resulted in hundreds of genera and close to 18 000 species that exhibit some degree of CAM, with numerous species being recognized as the iconic plants of the world's arid landscapes and epiphytic habitats, for example as shown by the saguaro and prickly-pear cacti, agaves, aloes, epiphytic orchids, succulent euphorbs and Spanish moss (Figs 2 and 3). CAM is thus considered a key adaptation that helped establish the vegetation of drier habitats of the modern biosphere. For humans, CAM plants help support livelihoods in semi-arid climates, and for many more, the diverse forms of CAM vegetation bring beauty and inspiration in natural habitats and gardens, and as indoor succulents.

Since the elucidation of the CAM biochemical cycle, scholars have pondered its evolutionary origins and why it has evolved so frequently (Evans, 1971; Kluge and Ting, 1978; Ehleringer and Monson, 1993). With advances in phylogenomics, evolutionary research has accelerated, bringing new understanding to how, why and where CAM evolved, and when it evolved (Edwards, 2019, 2023; Heyduk, 2022; Sage *et al*., 2023). This research is exploiting the deep foundation of CAM knowledge built up over decades by physiologists, ecologists and anatomists, such that the diversity of CAM across the Earth is at least well appreciated, if not completely understood (Holtum, 2023*b*). On a separate front, exploiting CAM crops is now seen as a means of expanding agricultural production into non-arable lands, due to their tolerance of severe drought, anthropogenic degradation or enhanced salinity (Borland *et al*., 2009; Pereira *et al*., 2021). Moreover, many CAM plants are highly plastic, showing an ability to switch between C_3 and strong CAM photosynthetic modes (Dodd *et al*., 2002; Borland *et al*., 2011). In an agronomic context, this ability allows CAM plants to use a C_3 photosynthetic mode when water is abundant to realize high growth rates, and when drought intensifies, to rely on the CAM mode to survive (Borland *et al*., 2011). In a changing climate with greater frequencies of heat and drought stress, such flexibility could allow CAM crops to survive and maintain productivity in dry, marginal landscapes. In a world of increasing demand for agricultural products, CAM promises to make a significant contribution to global crop productivity, particularly if advances in molecular biology enable the introduction of the CAM pathway into existing C_3 and C_4 crops (Borland *et al*., 2014; Lim *et al*., 2019; Pereira *et al*., 2021).

As with many species across the Earth, CAM plants are also threatened by the multiple ways in which humans are altering

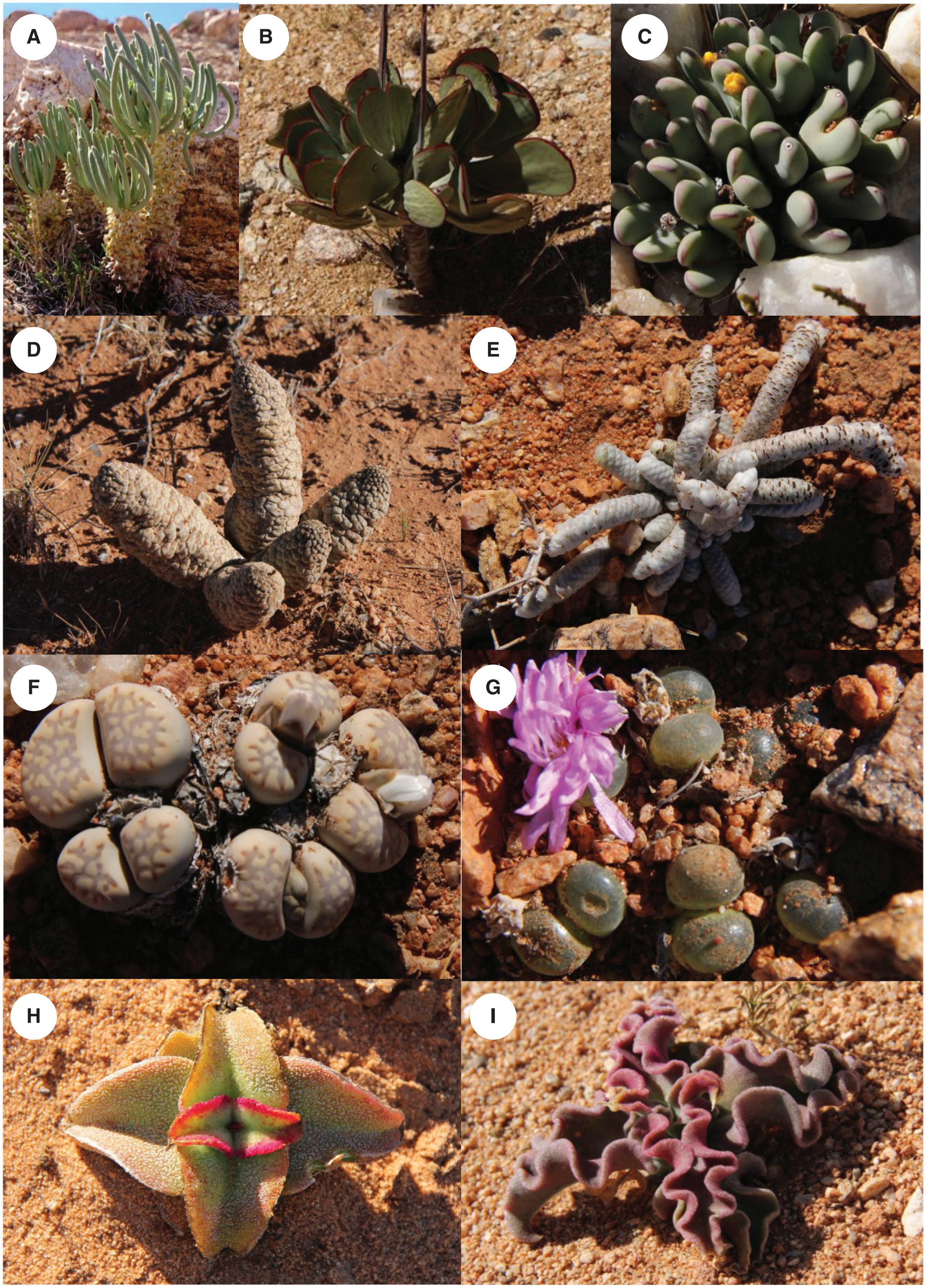

Fig. 1. A montage showing some of the morphological diversity of CAM succulents evident along roadsides of southern Africa in April 2016. (A) *Tylocodon wallichi* – Crassulaceae (photo courtesy of Matt Stata); (B) *Adromischus* spp., possibly *A. alstonii* – Crassulaceae; (C) *Conophytum bilobum* – Aizoaceae; (D) *Larryleachia* spp., possibly *L. marlothii* – Apocynaceae (barrel milkweed); (E) *Anacampseros papyracea* – Anacampserotaceae; (F) *Lithops* spp., possibly *L. julii* – Aizoaceae; (I) *Conophytum* spp., possibly *C. praesectum* – Aizoaceae; (I) *Mesembryanthemum* spp., possibly *M. crystallinum* – Aizoaceae; (J) *Mesembryanthemum*, possibly *M. guerichianum* – Aizoaceae. All photos by R. F. Sage except where noted.

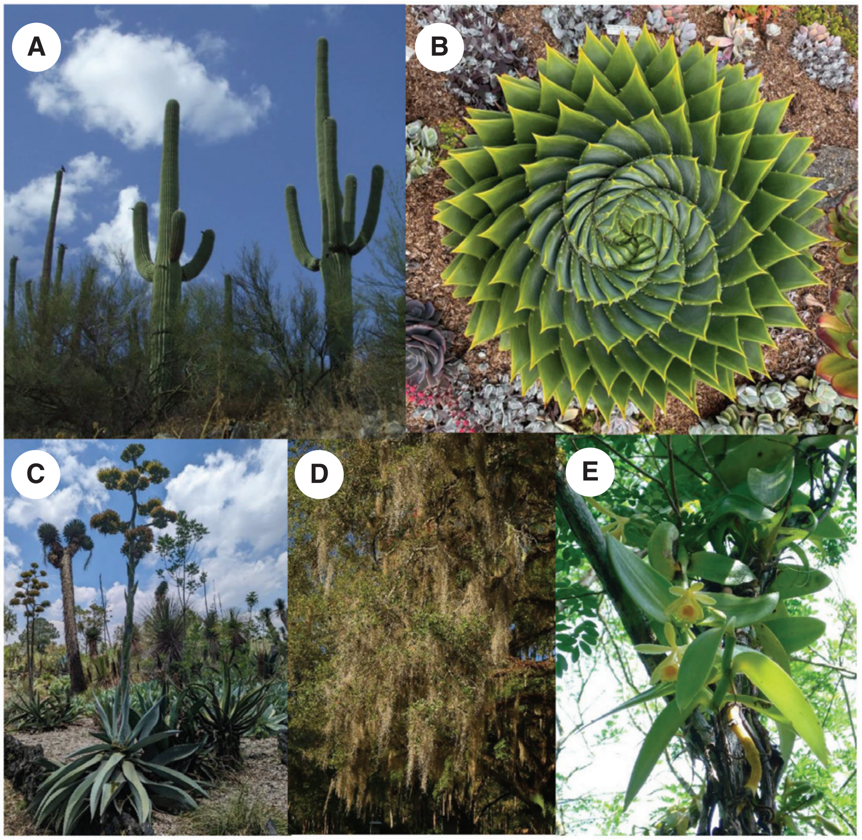

Fig. 2. Iconic CAM plants of the world. (A) Saguaro cactus (*Carnegiea gigantea*) near Tuscon, Arizona, USA; (B) spiral aloe (*Aloe polyphylla*, at Seascapes Succulent Nursery, Half Moon Bay, CA, USA) (photo taken with the permission of Janice Moody, owner of Seascapes Nursery); (C) *Agave* species at the UNAM Botanical Garden, Mexico City, Mexico (photo by K. Heyduk); (D) Spanish moss (*Tillandsia usneoides*, at Saint Marks, National Wildlife Refuge, FL, USA); (E) *Vanilla pomposa*, in cultivation in Panama (photo courtesy of Katia Silvera). Photos by R. F. Sage except where indicated otherwise.

the global environment (Sage and Stata, 2021; Hultine *et al.*, 2023). Epiphytic CAM species that comprise the majority of the global CAM flora are particularly endangered due to widespread loss of forested land in the tropics and subtropics (D'Antonio and Vitousek, 1992; Lewis *et al.*, 2015; Zotz *et al.*, 2023). Populations of arid-zone CAM plants such as the iconic cacti of the Americas are also being degraded by runaway fire cycles, climate extremes, infestation by invasive species and overharvesting for the horticultural trade (Hultine *et al.*, 2023). Cacti show some of the highest heat tolerance thresholds recorded in vascular plants (Downton *et al.*, 1984), but this ability could be both a blessing and a curse. Their high tolerance may pre-adapt them to increasing aridity as climates warm, but ironically, heat-tolerant organisms often exist near maximum limits of viability and for this reason are vulnerable to even modest climate change if it pushes them over their absolute viability thresholds (Somero, 2010).

Thus, CAM photosynthesis and the CAM flora can be viewed as approaching a crossroads. Likewise, CAM research is also at a crossroads, made possible by a new generation of tools and techniques. These developments promise rapid advances in the understanding of CAM origins in space and time, how CAM increases fitness of natural populations, and how humanity might exploit CAM in novel ways. At the same time, the CAM flora of the Earth is imperiled by rapid acceleration of anthropogenic activities that are changing the climate, the Earth's biogeochemistry and the cover of natural landscapes (Sage, 2020). Ironically, precisely when scientists and agronomists are in the best position to understand, appreciate and utilize CAM plants, much of the CAM flora might be lost to advancing global climate change.

With the realization that the CAM flora is facing great change, the editors of *Annals of Botany* decided that this is an ideal time to prepare a special issue entitled *CAM at the Crossroads*. In organizing the special issue, we sought the contributions of multiple generations of CAM researchers, including those at the end of distinguished careers, early career scientists and many in between. In particular, we wanted to highlight the contributions of Dr Klaus Winter, a leader whose research transcends multiple generations of CAM scholars. Since his first CAM publication (Winter and von Willert, 1972), Winter has maintained a leading research programme on CAM biology, first as a PhD student in Ulrich Lüttge's lab at the University of Darmstadt in Germany, then as a faculty member at the University of Würzburg. Since 1991, Winter has worked as a senior research scientist at the Smithsonian Tropical Research Institute in Panama City, Panama, allowing him direct access to a rich CAM flora in its natural element. His leadership in CAM goes well beyond his prolific publications, discoveries and data acquisition. The future of any field is dependent upon

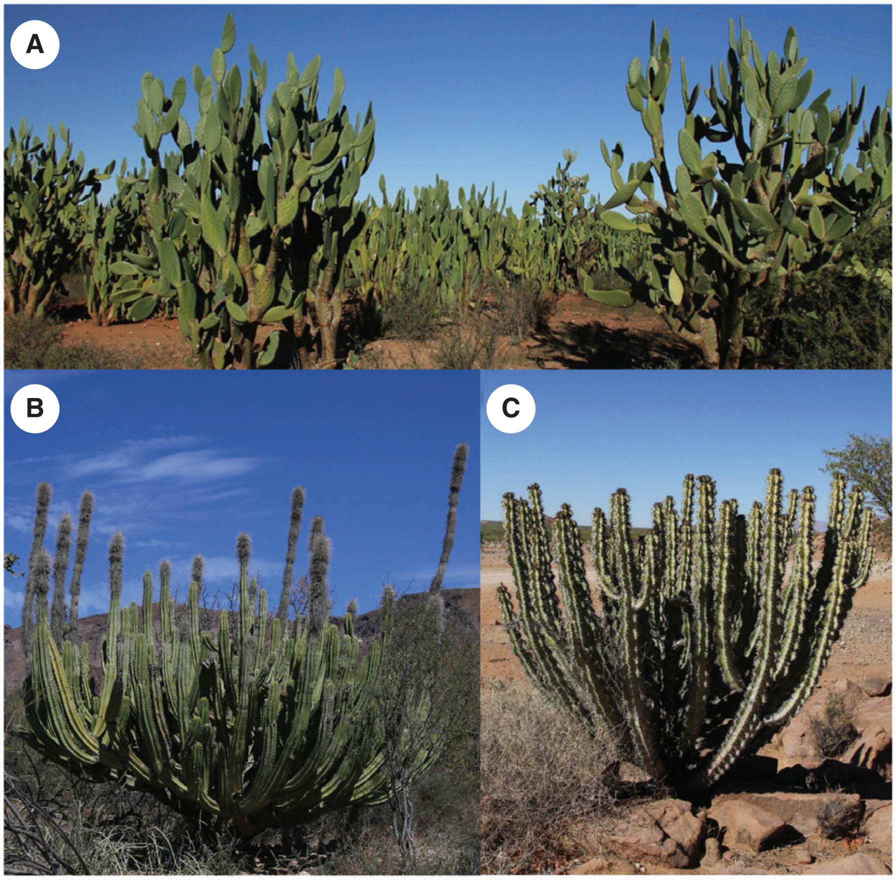

FIG. 3. Three iconic CAM species representing the potential for exploiting CAM in an agroforestry setting on semi-arid and often degraded lands. In A, invasive *Opuntia ficus-indica* infesting dry, marginal landscapes near Marysdale in central South Africa, demonstrating the potential of this genus to be a productive bioenergy crop on semi-arid landscapes with minimal agronomic inputs. In B, the cactus *Lophocereus schottii* (old man cactus) grows well on the harsh landscape of southern Baja California, Mexico. In C, a stem succulent *Euphorbia virosa* grows well in the arid landscape of the Namib desert in west-central Namibia. *Euphorbia* species are considered promising biofuel species because their latex can be refined into motor fuels. Panels B and C show remarkable convergent evolution onto a common form of stem succulence that is associated with CAM diversification in these two unrelated clades, both of which evolved from weakly succulent C_3 ancestors. Photos by R. F. Sage.

the engagement of colleagues in supporting and mentoring the next generation, in building networks of collegiality, and in serving as a source for conferences, reviews, books and perspectives that provide new directions while synthesizing the state of the art. Building the human capital in a discipline is as important to the advancement of a field as is the actual research output, and so it is essential to have inspiring leaders to provide the energy, resources and direction needed to sustain a field's growth and revitalization. Winter has been this leader for CAM biology, and because of his efforts, we can safely conclude that CAM research has a robust future. We thus dedicate this special issue of *CAM at the Crossroads* to his five decades of contributions. However, Dr Winter emphasized to us that he is not yet done and promises to maintain his high level of contributions for years to come.

The special issue consists of 23 review and original research papers, divided into four sections centred around the major themes that highlight the crossroads of CAM biology and Winter's five decades of CAM research. The issue begins with a biographical overview of Winter's research accomplishments by Professor Joseph Holtum, a close collaborator of many years (Holtum, 2023*a*). In this review, Holtum recalls many of the interactions and events that made for Winter's particularly rich scientific career. His discussion introduces the reader to many colleagues who worked with Winter over the decades, demonstrating again that great careers are commonly built by teamwork and collaboration. Holtum's review is followed by a perspective from Dr Winter himself, in which he provides a retrospective of his long career as a plant ecophysiologist, highlighting his initial discovery of facultative CAM, how he came to devote his professional life to CAM and some of the highlights of his years at the Smithsonian Tropical Research Institute (Winter, 2023). Winter's conclusion to the essay provides insights into the future of CAM research and reflects on the benefits and challenges of a life in science.

The second section emphasizes CAM diversity and its future prospects, beginning with an introduction to CAM and a perspective on the state of CAM research (Chomthong and Griffiths, 2023). The authors discuss promising developments for understanding CAM function, and conclude their overview with some intriguing observations on the future of the CAM flora, noting that CAM plants are expanding in areas such as the south-facing slopes of the European Alps, yet elsewhere are threatened by overharvesting for the horticultural trade. Holtum

(2023*b*) follows with a review on CAM diversity and global biogeographical patterns of major CAM lineages, which he calls the CAM diaspora. Species, life-form and habitat diversity are discussed for half a dozen major CAM clades, with insights into how climate, geography and evolution shaped the modern CAM flora. Gilman *et al.* (2023) follow with a major update to the phylogenetic distribution of CAM photosynthesis among the vascular plant lineages of the world, and list all known CAM genera, families and orders. This represents the first significant update to the CAM taxa of the world since Smith and Winter (1996) published their list in the influential book *Crassulacean Acid Metabolism: Biochemistry, Ecophysiology and Evolution* (Winter and Smith, 1996) Gilman and colleagues identified the presence of CAM activity in about 370 genera of vascular plants, representing 38 families, using various types of published experimental evidence for CAM, ranging from gas exchange, titratable acidity, carbon isotope discrimination and enzymatic measurements. By incorporating the phylogenetic distribution of these taxa, their assessment indicates a minimum of 66 evolutionary origins of CAM among the vascular plant flora. Their accounting also increases the estimated number of CAM species to about 7% of all vascular plants. The authors emphasize that this list is by no means complete, but represents a foundation that will facilitate the full identification of all CAM species and evolutionary origins in the coming decades.

Gilman *et al.* (2023) discuss at length the criteria required to be considered a CAM plant. In former times, terms such as obligate CAM and facultative CAM, and CAM cycling seemed to suffice, but with recent advances (many by Winter, Holtum and co-workers), CAM appears as a continuum of character states, from very weak, cryptic CAM, to obligate, very strong CAM with minimal contributions from direct assimilation of CO_2 entering the tissue via stomata (Winter *et al.*, 2015; Winter, 2019; Messerschmid *et al.*, 2021). Improved methodologies are now helping to identify many species with weaker CAM, leading to a realization that it might be better to consider CAM as an addendum to C_3 photosynthesis in such plants, rather than a distinct photosynthetic pathway as is the case with plants where most of the carbon is acquired by CAM.

Mok *et al.* (2023) follow with an examination of CAM potential in the South America desert shrub *Bulnesia retama*. This species has anomalously high carbon isotope ratios for C_3 plants, indicating the activity of C_4 or CAM photosynthesis. C_4 photosynthesis was quickly ruled out by gas exchange analyses. *Bulnesia retama*, however, lacks obvious succulence, has green stems and is drought deciduous – features which indicate it is a typical C_3 xerophytic shrub. These shrubs represent an important functional type that dominates many arid and sub-arid regions, yet none has been recognized as utilizing CAM (Smith *et al.*, 1997). In their assessment of photosynthesis in *B. retama*, Mok *et al.* determined it is a C_3 + CAM species, obtaining 10–25 % of its carbon at night via CAM in the stem cortical tissue. They hypothesize that while the C_3-mode of photosynthesis predominates over the growing season, its contributions greatly decline during drought, at which point the slow but steady contributions of CAM facilitates survival on particularly dry microsites in its arid habitat.

As Holtum (2023*b*) notes, global CAM diversity is the product of a long evolutionary history, but how threatened is the CAM flora by the accelerating forces of anthropogenic global change? Hultine *et al.* (2023) address CAM species in the future using the cacti as a case study. They review multiple threats from human-caused global change, including direct effects of rising CO_2, habitat loss from climate and land use change, and threats caused by invasive exotic plants. One of the most significant threats to CAM-dominated ecosystems is intensification of wildfire, due to a combination of climate change, invasive grasses and hotter, drier conditions. Exotic grasses are perhaps the most pernicious of these threats because they enable a synergistic interaction between the various causes of cactus mortality. The invasive grasses form a dense fuel layer which during the dry season increases fire frequency, spread rate and probabilities of ignition, while climate warming intensifies fires and increases the length of the burning season (Sage and Stata, 2021). Cacti and other succulents are intolerant of hot fires and populations of CAM succulents often crash following one or two fire events. Moreover, exotic grasses are also strong competitors that suppress cactus seedling establishment following a burn.

In the epiphytic domains of the rainforest, CAM plants are common, although not as widespread as previously thought (Zotz *et al.*, 2023). Altogether, epiphytic CAM species make up about two-thirds of the world's CAM flora, a point surprising to many given the iconic stature of terrestrial arid-climate CAM succulents in the minds of the broader audience. Widespread tropical deforestation and permanent conversion of forest lands to agriculture is directly reducing epiphytic habitat, particularly in regions of dry tropical forests where CAM epiphytes commonly dominate. Where forests do recover, epiphyte recovery can be much slower, although few studies have examined recovery in epiphytic communities. Climate change also poses a threat to the CAM epiphytes, although the situation can be complex given contrasting effects of warming, drier atmospheres and changing precipitation patterns. Bromeliads represent an important component of the CAM epiphytic flora in the Neotropics and, like most species, exhibit local adaptation to their climatic conditions. Males *et al.* (2023) model future climates of bromeliad habitat based on current distributions and ecophysiological characteristics, predicting that higher elevation species will lose habitat as favourable climate conditions migrate upwards. In many Caribbean islands, their model predicts that the elevation at which favourable habitat occurs will rise higher than the mountains, leaving little habitat space for upland CAM species.

The third section of the special issue emphasizes CAM evolution and begins with a perspective article by Edwards (2023) that explores continuous *versus* discrete models of CAM evolutionary trajectories. While many have speculated on how CAM has evolved, models that explain CAM evolution remain simplistic and poorly tested, unlike, for example, models of the evolution of C_4 photosynthesis (Heckman *et al.*, 2013). Edwards proposes that the research community might benefit from embracing models that describe discrete stages in CAM evolution, much the way that the C_4 community has embraced step-wise models of C_4 evolution. She presents two conceptual models of how a nascent CAM cycle might become established in C_3 plants. To evaluate such models, model clades of CAM evolution need to be well developed and phenotyped. Clades such as *Flaveria* and *Alloteropsis* have proven valuable for the advancement of our understanding of C_4 evolution but, as yet, CAM research lacks such a model evolutionary clade (Covshoff *et al*., 2014; Pereira *et al*., 2023). The large number of distinct CAM evolutionary lineages present many possibilities for model clade development, but few have been examined in more than preliminary detail. Two articles address whether the monocot clade Agavoideae and the eudicot clade *Clusia* could serve as phylogenetically robust models for CAM evolution (Heyduk *et al*., 2023; Lújan *et al*., 2023). Heyduk *et al.* (2023) discuss the history and future of CAM research in Agavoideae, while Luján *et al.* (2023) present a similar synthesis for *Clusia*. Each of these clades has multiple origins of CAM, providing a richness in terms of species and character states for addressing evolutionary hypotheses. Both Agavoideae and *Clusia* species are long-lived perennials, however, presenting logistical challenges for the rapid cycling of generations that facilitate evolutionary research (Covshoff *et al*., 2014). There remains a need to find tractable, rapid-cycling models and easily transformable systems in the CAM arena. The Montiaceae may be one such group, at least for C_3 + CAM evolution (Hancock *et al*., 2019), whereas herbaceous *Kalanchoë* species have proved useful in molecular studies of CAM function (Hartwell *et al*., 2016; Yang *et al*., 2017).

When CAM evolved is an important question that carries with it implications for how environmental change promoted the evolution of photosynthetic functional types, and how CAM plants might fare in a world where atmospheric CO_2 concentrations are increasing rapidly. Using a phylogenetic dating approach, Sage *et al.* (2023) inferred that CAM appeared in the past 30 million years in 45 of 47 clades examined, following declines in atmospheric CO_2 concentration. They hypothesize that low CO_2 promoted CAM evolution, probably in combination with other factors, namely tissue succulence and the spread of atmospheric aridification and associated droughts as climates changed. Recent experimental assessments of CAM performance under variable CO_2 indicate that low CO_2 exposure enhances the relative contribution of the CAM cycle to daily carbon gain (Sage *et al*., 2023). They conclude that elevated atmospheric CO_2 preferentially enhances the daily contribution from C_3 phases of photosynthesis, such that any selective advantage of CAM would be diminished. However, the phylogenetic evidence indicates that weak CAM plants persisted in particularly harsh sites in high CO_2 environments. At high CO_2 during extreme drought, CAM would be valuable in recycling respiratory carbon when stomata are completely closed. In the Crassulaceae (stonecrop family), the possibility of strong CAM evolving in high CO_2 is also supported. The name stonecrop refers to the common lithophyte life form of Crassulaceae species. As Sage *et al.* note, these plants are often specialized for rock outcrops where lack of soil could promote very low stomatal conductance and a constant CO_2 limitation in leaf tissue, even in high CO_2 atmospheres.

To understand CAM evolution, both *de novo* from C_3 ancestors and through diversification of CAM following its acquisition, the development of phylogenomic databases from most species in an evolutionary lineage is extremely useful. Because CAM lineages contain many species, due to often high diversification rates after CAM acquisition (Sage *et al*., 2023), this can be a daunting task, but feasible with modern high-throughput sequencing and computational capabilities. As an example of the potential of deep sequencing for evolutionary insights, Köhler *et al.* (2023) analysed the plastomes from 43 species of Opuntieae, a group under consideration as a novel bioenergy crop for marginal lands (Cushman *et al*., 2015). Within this single tribe, considerable variation in plastome size and structure was observed, possibly as a result of adaptation to a range of environments as the Opuntieae diversified. Understanding the significance of this variation in a fitness context could provide new avenues for enhancing the performance of cultivated *Opuntia* species, as well as understanding how evolution exploits novel traits to radiate into a new habitat.

One important question in CAM evolution is the relationship between CAM and succulence, and whether substantial succulence is a required state for strong CAM function (Edwards, 2019). Using a phylogenetic approach, Collobert *et al.* (2023) examine gain and losses of epiphytism and succulence in the epidendroid clade of Orchids. They identified three gains of epiphytism, one at 39 million years ago (Ma) and the other two at 11.5 and 7.1 Ma. Stem succulence appears ancestral to epiphytism in all epidendroid clades, leading the authors to suggest that it was a key innovation that enabled the clade to radiate into the epiphytic habitat. They also suggest CAM could be a key innovation that enabled epiphytism; however, this requires future evaluation of CAM expression across the epidendroids.

As with C_4 photosynthesis, CAM is recognized as being the evolutionary result of coordinated changes in physiology and structure (Sage, 2002; Sage, 2020), with succulence being one of a number of anatomical shifts. To provide a more comprehensive view of CAM structural evolution, Leverett *et al.* (2023*b*) examined changes in leaf venation and hydraulic conductance across a CAM gradient in *Clusia*. They observed that the contribution of the CAM mode is closely correlated with leaf vein density and suggested that low transpiration rates arising from CAM enabled a reduction in vein length per unit leaf area. This result indicates that to optimize CAM function in C_3 species bioengineered to express CAM, the vascular design would need to be re-engineered in addition to the engineering of biochemical and stomatal functions. One final perspective on CAM evolution is presented by Leverett *et al.* (2023*a*) who reconsider the function of tight packing of photosynthetic cells within CAM taxa, which has been proposed to reduce the diffusive conductance between the interior of the photosynthetic tissue and intercellular air spaces to slow escape of the released CO_2 following malate decarboxylation (Nelson and Sage, 2008). Leverett *et al.* (2023*a*) argue that fitness attributes associated with low diffusive conductance within photosynthetic cells are overstated, because the predominant conductance limitation in CAM leaves is from stomata and diffusion across the mesophyll cell membranes and wall, not within the intercellular spaces. Instead, they suggest that tight cell packing might be an evolutionary spandrel (after Gould and Lewontin, 1979) with little fitness role, or alternatively might arise for other reasons such as a need to maximize mesophyll cell volume for malate storage. Whether this hypothesis is eventually supported or not, it highlights the importance of following up suggestive ideas with rigorous experimental tests. The diffusion limitation hypothesis in CAM tissues has only limited direct corroboration, largely from Maxwell *et al.* (1997), with other support being circumstantial or correlative (Nelson *et al*., 2005; Nelson and Sage, 2008). Leverett *et al.* (2023*a*) might thus inspire evolutionary studies of CAM structure and function, ideally using taxa from distinct evolutionary lineages of CAM photosynthesis.

The fourth section of the special issue presents a diverse set of reviews and original research articles on the domestication of CAM species and mechanisms to understand the physiology and development of CAM tissues. Numerous CAM species are currently in cultivation, with a number of *Agave* species standing out as leading producers (along with pineapple, aloe and prickly pear cacti). As Davis and Ortiz-Cano (2023) discuss, *Agave* species have been cultivated for centuries by indigenous peoples in the Americas, who recognized the resilience of the plants in hot, dry climates. Unique features in the plants that can be linked to CAM partially explain their utility. Notably, the large vacuoles allow storage of a range of useful products (sugars and medicinal compounds), while dense fibre bundles that strengthen the succulent leaves provide reliable fibre sources for regional economies. These traits have provisioned people of hot, dry regions of the Americas for thousands of years, and provide promise for a wider range of products in future years as the crops are further improved. Hodgson *et al.* (2023) further our understanding of *Agave* domestication by examining how the Hohokam people in the American Southwest utilized wild *Agave* species and subsequently initiated *Agave* domestication. The authors identified at least six cryptic domesticated *Agave* species that now exist as remnant stands within ancient rock gardens built by farmers to catch and hold intermittent precipitation. The living plants are mostly clonal remnants of ancient populations that have not been farmed since before colonization by the Spanish. They once were, and promise to be again, excellent species for dryland cultivation.

To improve carbon gain in plants, mitochondrial respiration must be considered in addition to photosynthesis, because respiration determines the rate of carbon loss and thus influences net primary production as well as resource-use efficiency. Leverett and Borland (2023) evaluate respiratory flux in CAM plants (as nocturnal O_2 uptake), a subject they note has received much less attention than carbon assimilation. As a co-adaptive trait, respiratory C flux in CAM plants could differ from patterns in C_3 and C_4 plants because CAM species have distinctive anatomy, carbohydrate metabolism, enzyme complements and transport requirements that could increase maintenance and construction costs. Flux balance models predict CAM should enhance nocturnal respiration rates, a prediction they support with new data from a pair of *Clusia* species. Notably, induction of CAM in facultative plants increased nocturnal respiration rates, particularly during drought. Leverett and Borland conclude their study by asking five questions regarding distinct roles of nocturnal respiration in CAM plants, such as which components of CAM enhance nocturnal respiration? They further argue that effective bioengineering of CAM into C_3 crops will need to consider respiratory costs that are unique to CAM in order to optimize CAM function and carbon acquisition in the newly engineered plants.

One promising group for cultivating drylands are the *Opuntia* cacti whose fruits and cladodes already provide food for indigenous people in southwestern North America and for arid-land inhabitants throughout the world. *Opuntia* species are exceptional colonizers of degraded landscapes, such that they have become major invasive species outside of their native range (Fig. 3; Stange *et al.*, 1995). *Opuntia* plants promise to be excellent sources of carbohydrates for biofuel production, particularly on marginal land in semi-arid regions of the world (Cushman *et al.*, 2015; Neupane *et al.*, 2021). *Opuntia* is widely regarded as an obligate CAM genus, yet C_3 phases might help explain high levels of productivity of certain *Opuntia* species. To assess this, the development of CAM was explored in young seedlings and cladodes of prickly pear cactus (*Opuntia ficus-indica*; Niechayev *et al.*, 2023). In juvenile plants, the cladodes served as respiring sink tissues that transition directly to performing CAM once net positive CO_2 fixation is observed, confirming that CAM provides the primary means of photosynthetic carbon assimilation in these developing organs (Niechayev *et al.*, 2023). In another original research paper, a starch-deficient mutant of *Kalanchoë fedtchenkoi*, lacking phosphoglucomutase activity, showed reduced nocturnal CO_2 uptake. In the mutant, nocturnal stomatal opening was dependent upon starch degradation; however, during the day, starch biosynthesis did not appear to influence stomatal closure (Hurtado-Castano *et al.*, 2023). These results provide new insights into the controls over stomata aperture and WUE in CAM plants that will help target mechanisms for bioengineering the CAM pathway into C_3 species. Similarly, studies of C_3 + CAM hybrids can identify promising strategies for CAM biodesign. Yamaga-Hatakeyama *et al.* (2023) produced C_3 × CAM hybrids in *Cymbidium* orchids. C_3 × CAM hybrid studies are uncommon outside of *Yucca* (Heyduk *et al.*, 2020). Unlike *Yucca*, where early generation hybrids exhibited intermediate degrees of CAM, the F_1 hybrids between CAM fathers and C_3 mothers of *Cymbidium* hybrids resulted in plants that are largely C_3 in function, with weak intermediate traits. The authors concluded that *Cymbidium* is a promising genus in which to generate C_3 × CAM hybrids, thereby enabling the genetic dissection of CAM traits.

In the 23 papers presented in this special issue, readers will gain an appreciation of the latest developments in CAM diversity studies, CAM evolution, CAM domestication and CAM functional studies. Importantly, the special issue provides an opportunity for the reader to learn of the contributions of Klaus Winter, as an example of how a singular vision and thoughtful leadership can propel a field forward for over half-a-century and beyond. As we look to the future, we propose the following areas of CAM research deserve high priority: one area will be the identification and characterization of each distinct CAM lineage, which will help establish conservation priorities for CAM clades, further the understanding of CAM ecology in the diverse lineages and establish clear patterns of phylogenetic relationships. A second is the elucidation of how CAM evolved and why it occurred with high frequency. This undertaking will require the development of robust models of CAM evolution, the identification of model clades and mechanistic studies of how traits facilitate the assembly of the CAM character state through evolutionary time. Understanding CAM evolution will have wide significance beyond CAM, because CAM is an excellent system for studying complex trait and convergent evolution in general, in part because the many lineages allow for replicated comparative studies that evaluate hypotheses of CAM evolution. A third research priority is to explore ways to expand the cultivation and uses of agronomically important CAM crops to make more efficient use of often limiting freshwater resources for agricultural production systems and municipal needs. A fourth research priority is to engineer CAM into C_3 (or C_4) crops, and to genetically improve CAM in existing CAM species. These efforts, already initiated, could provide an important avenue for us to preserve or improve agricultural yields while also exploiting degraded and marginal landscapes. The engineering of CAM could prove to be a win–win strategy for humanity's efforts to both improve the environment while enhancing agricultural production in the face of the current global climate crisis. Fifth, new and better ways need to be identified to protect and conserve the CAM flora through, for example, preserving essential habitat, and where this is not possible, conserving CAM populations in safe locations, be they seedbanks, botanical gardens, private collections of succulent plants or other means. Thankfully, the many succulent and orchid societies, plus the botanical gardens of the world, represent a large human resource to assist conservation efforts. Lastly, renewal of the CAM research community with people from all over the world is imperative. This initiative is perhaps the best way to honour the legacy of Klaus Winter and his many colleagues who built the foundation of our current CAM understanding. Not only will the next generation of researchers pursue these and other priorities in CAM research, but also they will be leaders for the preservation of the CAM flora and promoting its value to people across the planet.

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