## Primer

Crassulacean acid metabolism

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Crassulacean acid metabolism (CAM) is an elaboration of the typical plant photosynthetic pathway that endows plants with remarkable water use efficiency. Through the use of a carbon-concentrating mechanism, plants that employ CAM are able to fix carbon while conserving water by minimizing transpiration during the hottest part of the day, or during stressful conditions. CAM is a common characteristic of arid zone plants and epiphytes, which lack readily available moisture in the soil, and is often accompanied by other traits associated with water limitation such as succulence, waxy cuticles, and stem-based photosynthesis. Paradoxically, certain regions of the world's arid lands lack any large CAM plants, and there are multiple lineages of aquatic plants that utilize CAM. CAM is found in almost every major lineage of land plants, and has evolved many dozens of times independently. This Primer will introduce the various CAM phenotypes and highlight how recent advances in molecular biology are deepening our knowledge of CAM evolution. Understanding the ecology and evolution of CAM will be crucial for predicting vegetation responses to climate change, particularly in drylands, which currently cover roughly 40% of Earth's land and are rapidly expanding. Furthermore, the diversity of relatively young CAM lineages suggests that unraveling the evolution of CAM may facilitate the introduction of CAM into existing crops — greatly improving the resilience of crops to drought stress.

With few exceptions, land plants are autotrophic — transforming atmospheric carbon dioxide (CO_2) and the sun's radiation into carbohydrates via photosynthesis. Carbon dioxide passively diffuses down a concentration gradient into the plant's leaves through stomata, the small pores that regulate gas exchange between the plant and atmosphere (Figure 1, left). This CO_2
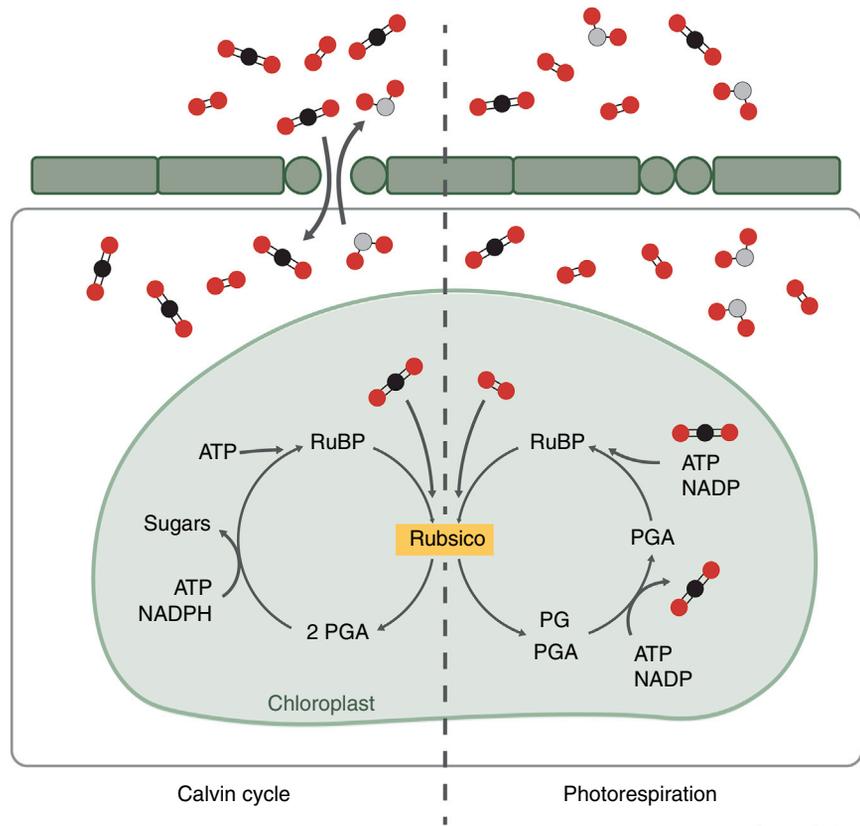

Figure 1. Calvin cycle and photorespiration in response to drought showing the enzyme Rubisco's carboxylase and oxygenase activities.
Left: when plants are not water limited, gas exchange occurs through open stomata in the epidermis (dark green) and CO_2 is fixed as part of the Calvin cycle. Right: stomata close in response to drought, and Rubisco begins to fix O_2 as CO_2 becomes scarce. For simplicity, not all photorespiratory reactions are shown, some of which take place outside of the chloroplast. ATP, adenosine triphosphate; PG, phosphoglycolate; PGA, 3-phosphoglycerate; RuBP, ribulose 1,5 bisphosphate.

then enters the chloroplasts where it is fixed by the enzyme Rubisco during the Calvin cycle to build two molecules of 3-phosphoglycerate (PGA) which, in turn, can be reduced to produce sugars. However, when water is limited, such as during times of drought, the stomata close to prevent water loss through evapotranspiration. While closing the stomata conserves water, it also begins to starve the plant of CO_2. As levels of CO_2 decrease in the leaf, Rubisco binds more frequently to O_2 than CO_2, and one of the resulting byproducts, phosphoglycolate (PG), must be further processed by the photorespiratory cycle (Figure 1, right). Photorespiration produces both PGA and CO_2 that can be used for photosynthesis, but at a large energetic cost. Furthermore, the rate of photorespiration increases

with temperature, which often accompanies water stress. Rates of photorespiration are ultimately governed by atmospheric CO_2 levels, which set an upper ceiling of internal CO_2 concentration that enables passive diffusion into the plant body. To combat prolonged bouts of photorespiration, plants have evolved mechanisms that decouple CO_2 uptake from CO_2 fixation by Rubisco — thereby shielding Rubisco from harsh atmospheric conditions. Plants have achieved this decoupling through spatial separation of CO_2 uptake and fixation, C_4 photosynthesis, and temporal separation, known as crassulacean acid metabolism (CAM), which is the focus of this primer. Plants that are able to use CAM, in any capacity, are referred to here as 'CAM plants'.
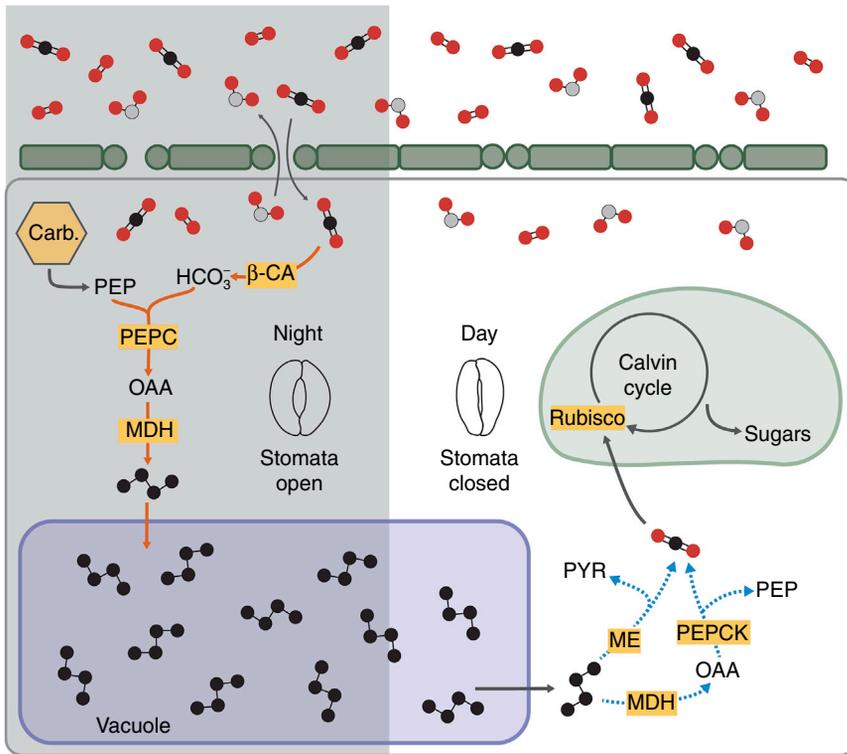
Figure 2. Crassulacean acid metabolism.
Major enzymes are highlighted in yellow. During the night, PEP is synthesized from carbohydrate pools (hexagon) and CO_2 is carboxylated (orange pathway) resulting in malate, which is stored in the vacuole. During the day, the stomata close, malate is exported and decarboxylated through two possible pathways (dotted blue lines) to release CO_2 that is then fixed by Rubisco as part of the Calvin cycle. β-CA, beta-carbonic anhydrase; HCO_3^-, bicarbonate; MDH, malate dehydrogenase; ME, malic enzyme; PEP, phosphoenolpyruvate; PEPC, PEP carboxylase; PEPCK, PEP carboxykinase; PYR, pyruvate; OAA, oxaloacetate.

Unlike C_3 and C_4 pathways, uptake of CO_2 during the CAM cycle occurs primarily at night. This often means that CAM plants open their stomata for gas exchange at night when the temperature is lower and the relative humidity is higher — significantly reducing the rate of water loss due to evapotranspiration. However, as will be discussed below (see CAM phenotypes), many CAM plants do not open their stomata at all during the night and instead utilize internally respired CO_2 as a carbon source. In either case, because the energy used in sugar production is generated by the light-dependent reactions of photosynthesis, this CO_2 does not immediately enter the Calvin cycle. Rather, it is converted to malate, a four-carbon compound, and transported into the vacuole where it accumulates overnight as malic acid (Figure 2). Then, during the day, as stomata close to limit water loss, malate exits the vacuole and is decarboxylated, boosting the concentration of CO_2 (hence 'carbon concentrating') — minimizing the ability of Rubisco to interact with O_2.

There has been increased interest in CAM photosynthesis across multiple biological disciplines as genome-scale data have become more accessible and global climate change predictions more dire. CAM species exhibit the greatest water use efficiency (WUE) of all land plants, which makes them attractive targets for sustainable agriculture, as arid lands are expected to dramatically expand across the planet. A holistic view of CAM, from its genetic underpinnings to its influence on ecosystem processes, is essential for understanding how such complex traits have repeatedly evolved. This Primer will introduce the various CAM phenotypes, present an overview of the

ecological, morphological, and genetic specialization of CAM lineages, and discuss the evolution of CAM.

CAM phenotypes
From the desert behemoth giant saguaro (*Carnegiea gigantea*) to the epiphytic dancing-lady orchids (*Oncindium* sp.), lineages vary immensely in the degree to which they use CAM photosynthesis. All CAM species also maintain a fully functional C_3 cycle in their photosynthetic cells, which affords a flexibility in proportions of dark and light CO_2 uptake. 'Strong', or 'obligate', CAM species uptake most of their CO_2 at night and engage a CAM cycle on a daily basis. Strong CAM plants include many long lived, highly-succulent desert species from varied lineages, such as the eponymous Crassulaceae, cacti (Cactaceae), aloes (Asphodeloideae), and agaves (Agavoideae), but also epiphytes from tropical rainforests including many orchids (Orchidaceae) and bromeliads (Bromeliaceae). On the other end of the CAM spectrum are what could be called 'C_3+CAM' plants, in that they fix most of their atmospheric CO_2 via C_3 photosynthesis, yet also have a functional CAM cycle. Many of these species are facultative, employing C_3 or C_4 photosynthesis under normal environmental conditions but expressing a CAM cycle under stress, most commonly in response to drought. In all species studied so far, this stress-induced physiology is reversible. C_3+CAM species occupy a wide range of environments (see Ecology of CAM plants, below), possess diverse life histories, and are often closely related to strong CAM species. For example, multiple clades within Bromeliaceae, Orchidaceae, Crassulaceae, and Euphorbiaceae contain the full diversity of CAM phenotypes. As will be discussed below (see Morphology of CAM plants), C_3+CAM species are often morphologically indistinguishable from their C_3 relatives, and so we are likely largely underestimating the number of evolutionary origins and the phylogenetic diversity of this relatively cryptic phenotype.

Most CAM plants lie near these two extremes — either CAM is used as the primary photosynthetic metabolism, or CAM is used rarely and

intermittently in response to stress. This distribution of CAM phenotypes is typified by carbon stable isotope surveys of C_3+CAM and strong CAM plants. Because CAM plants first fix CO_2 with phosphoenolpyruvate carboxylase (PEPC, Figure 2), which does not discriminate against the heavier carbon isotope as strongly as Rubisco, CAM plants have less negative carbon isotope ratios (usually expressed as parts per thousand, ‰) than C_3 plants. Strong CAM species are typically less negative than -18‰, while facultative species are indistinguishable in their isotopic signatures from C_3 plants, which are usually more negative than -20‰ (Figure 3). However, some lineages show intermediate isotopic values that suggest that these plants either do both daytime and nocturnal CO_2 uptake or use CAM for significant portions of their lives (Figure 3). Characterizing the phenotype of C_3+CAM plants is non-trivial because they usually have C_3-like isotopic values, exhibit C_3 physiology, and do not display obvious CAM-like morphology, such as high degrees of succulence. Phenotyping these plants requires drought experiments and measurements of diel patterns of gas exchange, nocturnal acid accumulation, and/or transcription of CAM-related genes (e.g., PEPC) with RNA-sequencing or quantitative PCR.

Ecology of CAM plants

The ecology of most CAM plants can be roughly divided into three, somewhat counterintuitive, combinations of habit and environment: the terrestrial flora of the world's deserts and semi-arid regions, the epiphytes of the world's tropical and subtropical forests, and aquatic plants. In the new world, deserts are dominated by CAM cacti and agaves; their old world desert counterparts include euphorbs (Euphorbiaceae), aloes, and ice plants (Aizoaceae). The extreme aridity of these environments forces plants to dramatically limit gas exchange, and many species can survive weeks or months without precipitation. This decreased photosynthetic rate ultimately causes many desert lineages to be slow-growing and long-lived. For taxa living in arid regions, CAM photosynthesis
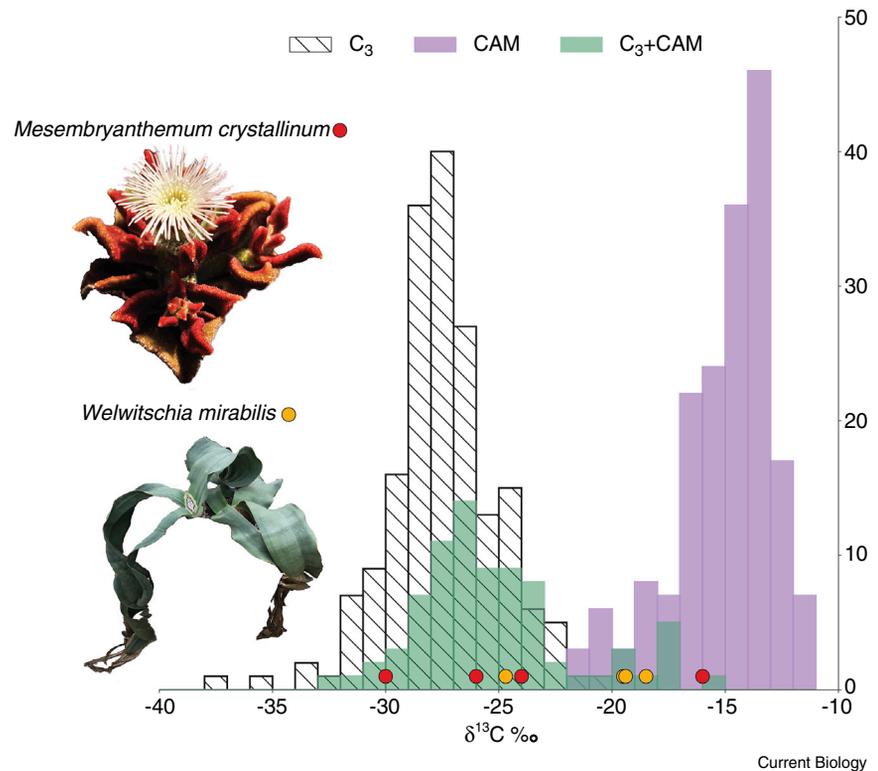

Figure 3. Distribution of carbon isotope ratios from 385 species from 19 clades of lycophytes, ferns, gnetophytes, monocots, and eudicots.
C_3+CAM taxa primarily exhibit C_3-like ratios, but some, for example *Mesembryanthemum crystallinum* (red points) and *Welwitschia mirabilis* (orange points), show a range of isotopic values that span both C_3 and CAM values. Photo credit: *M. crystallinum*, Hans Bernhard, Wikimedia Commons; *W. mirabilis*, Ian S. Gilman.

is one of many traits that help preserve water. Changes in the angle of leaves, or even the loss of leaves entirely, help reduce the radiation incident on plants, which in turn reduces their internal temperature, photorespiration rate, and leaf CO_2 efflux. Dense hairs, thick cuticles, and epicuticular wax also help reduce the effects of radiation and can disrupt boundary layer effects to reduce evapotranspiration when stomata are open. Many species, such as cacti and agaves, can respond rapidly to rainfall by increasing laterally-spreading surface roots ('rain roots'). These roots temporarily increase the plant's ability to capture rainfall but die shortly after to prevent water from leaking back out into the soil as soil water potential declines.

Although they inhabit many regions with moderate to high levels of precipitation, epiphytic orchids and bromeliads may constitute nearly 50% of all CAM species. With no access to

soil, these plants regularly encounter water stress despite inhabiting some of the wettest places on earth, such as the rainforests of Panama and Madagascar. Like arid-zone species, CAM is only one tool epiphytic lineages employ to cope with low water availability — for example, many epiphytes have evolved novel strategies for collecting water. Multiple bromeliad lineages have evolved a 'tank' habit that collects rainwater, while others, like *Tillandsia* sp., have lost roots entirely and instead use dense hairs on their leaves to soak up fog and mist. Orchids have evolved a similar water-absorbing structure called velamen that is made up of multiple layers of dead cells surrounding their aerial roots. When dry, velamen is white or grey and protects the roots from excess radiation and water loss due to transpiration. However, when wetted, velamen becomes translucent and allows photosynthesis to take place in

the roots, which are often also capable of CAM.

Unlike epiphytes or desert inhabitants, water is not limiting for CAM species in aquatic environments. Aquatic CAM species are less studied than their terrestrial counterparts, but multiple sources of evidence show that aquatic CAM has evolved in diverse lineages, including lycophytes, monocots, and eudicots — presumably in response to CO_2 deprivation. Most species inhabit oligotrophic lakes or shallow, often ephemeral, pools that experience dramatic fluctuations in CO_2 availability. In shallow pools, phytoplankton and other C_3 vegetation consume dissolved CO_2 during the day, but their respired CO_2 at night acts as a 'CO$_2$ pump' that provides a niche for CAM species. Dissolved CO_2 levels are lower and less adherent to diel patterns in oligotrophic environments, and root CO_2 uptake from the sediment makes up a large portion of the carbon budget for CAM species in these habitats.

Despite the apparent differences in the ecology of C_3 and CAM plants, research using global climate data has shown that C_3 and CAM species occupy a largely overlapping climate envelope. Both the 'portullugo' clade (~2,200 species including cacti) and multiple clades of orchids show little to no differentiation in temperature, precipitation, and elevational variables between photosynthetic types. In the Agavoideae, climatic spaces of C_3 and CAM species do appear to be distinct, but some C_3 species such as Joshua tree (*Yucca brevifolia*) co-occur with CAM species in the Mojave Desert. Some have argued that large CAM succulents are only able to inhabit arid regions that have predictable rainfall patterns, which may explain why some of the world's largest arid regions, like Australia and the deserts of central Asia, are lacking a significant CAM presence. We'd argue that the lack of large CAM succulents in these regions remains an open question, and likely requires consideration of biotic interactions, biogeographic patterns in the distribution of lineages with the evolutionary propensity to evolve succulence and CAM, and other kinds of environmental factors such as fire regime and freezing temperatures.

Finally, the predicted climate space of CAM plants depends on how C_3+CAM plants are coded in analyses, if they are recognized at all.

Morphology of CAM plants

In addition to the gross morphological characteristics discussed above, CAM species usually show a suite of anatomical features that increase the efficiency of CAM photosynthesis. Perhaps most notably, CAM plants tend to show increases in succulence — generally defined as the amount of water that a tissue or cell can lose while maintaining physiological functions. For this reason, many CAM plants are considered drought avoiding rather than drought resistant because their succulence buffers against cellular dehydration. CAM plants often have specialized water storage cells called hydrenchyma that ameliorate the effects of water loss due to transpiration and drought. Hydrenchyma can be present in the leaves or stem and act as water reservoirs, built to transfer water to photosynthetic tissue layers as they begin to dehydrate. Increased volume of hydrenchyma is often associated with strong CAM and may be considered part of a 'syndrome' of traits that allow for continued carbon fixation as the dry season commences. Cellular succulence may increase in the photosynthetic tissue layers in addition to the hydrenchyma, where increases in vacuole size contribute to increased storage of malate overnight. In turn, larger vacuoles drive larger cell sizes in mesophyll, and greater leaf thickness in general.

Modifications to the size and organization of cells also affects the movement of CO_2 through the photosynthetic tissues of CAM plants. Larger cell sizes typically have lower surface area to volume ratios, which decrease the surface area available for diffusion of CO_2 into and out of mesophyll cells. Additionally, CAM species tend to have a smaller volume of intercellular airspace, and therefore tighter cell packing than C_3 or C_4 species. Combined, larger cells packed more tightly together result in increased resistance to CO_2 movement through the photosynthetic tissue. Although reduced CO_2 conductance

lowers CO_2 influx, PEPC is less sensitive to CO_2 concentrations than Rubisco. Furthermore, the reduction in CO_2 efflux during the day, as malate is decarboxylated, is helpful in reducing loss of CO_2 back to the atmosphere due to an inverted concentration gradient.

Aquatic CAM species often show a contrasting set of morphological features compared with terrestrial species. Because water stress is typically unrelated to CAM in these plants, they do not exhibit succulence. Instead, these plants rely on highly permeable tissues and large intercellular air spaces to promote the diffusion of dissolved CO_2 into photosynthetic tissues. Oligotrophic species also have greater root:shoot mass and volume ratios for increased root CO_2 uptake. This suite of traits is typical of *Isoetes* (Isoetaceae) species — the 'Isoetid' growth form — and found in other aquatic lineages where CAM has evolved *in situ* (e.g., *Vallisneria* (Hydrocharitaceae), *Littorella* (Plantaginaceae)). However, *Crassula aquatica* (Crassulaceae), which is hypothesized to have evolved from a terrestrial CAM ancestor, shows many morphological characteristics of typical terrestrial CAM species.

CAM '-omics'

In step with most biological sciences, the study of CAM photosynthesis is experiencing an '-omics' revolution — particularly transcriptomics, but to an increasing extent genomics, proteomics, and metabolomics. Since the 1940s we have known that CAM plants can use two sources of carbon to produce carboxylation substrates (e.g., PEP): water-soluble sugars and starches. Similarly, there are two major metabolic pathways to decarboxylate malate: via a malic enzyme or PEP carboxykinase (PEPCK; Figure 2). CAM plants vary in the degree to which they use each carbon source and pathway, but typically favor one combination. Metabolomic studies focused on sugars and starches are beginning to reveal the carbon budgets of CAM plants and how they regenerate key photosynthetic intermediates. Because (de)carboxylation, and CAM in general, show strong diurnal

patterning, studying photosynthetic gene expression through diel cycles with RNA sequencing is now commonplace, and has revealed new aspects of CAM metabolic pathways. Whole transcriptomes from diverse lineages have shown that many key photosynthetic genes (Figure 2) have been duplicated one or more times in CAM species. For example, most CAM lineages examined thus far possess a recently duplicated copy of PEPC, the protein that has emerged as the workhorse for research into the expression and evolution of CAM photosynthesis. In general, CAM plants maintain a 'C$_3$ copy' of PEPC for use in the tricarboxylic acid cycle and a 'CAM copy' for nocturnal CO$_2$ fixation. In facultative species, the C$_3$ copy is used under normal environmental conditions and the CAM copy of PEPC is expressed only when CAM is induced by drought stress.

The first genome of a CAM plant (*Phalaenopsis equestris*, Orchidaceae) was published in 2014, and has been followed by a variety of others including pineapple (*Ananas comosus,* Bromeliaceae), the giant saguaro (*Carnegiea gigantea*), and a diversity of Crassulaceae species. Coupled with many available transcriptomes, the first comparative studies at a genomic scale have emerged. Comparing CAM species found in multiple, diverse lineages, genes and gene networks demonstrate convergent shifts in their pattern of expression throughout a 24-hour diel cycle. Similarly, there is evidence for convergent evolution of particular amino acid substitutions in protein-coding regions that are linked to CAM function and are under positive selection across independent CAM origins. PEPC was one of several genes found to be evolving convergently in pineapple, *Kalanchoe*, and *Phalaenopsis*, and the amino acid substitution in *Kalanchoe* was further found to have functional implications. Following a duplication event, the copy of PEPC used in CAM photosynthesis obtained an aspartic acid residue where C$_3$ species typically possess arginine, lysine, or histidine. This single change significantly increased the activity of the CAM PEPC without the need for phosphorylation, a typically limiting factor in PEPC activity.

Evolution of CAM plants

It is clear that CAM is associated with shifts in ecology, morphology, physiology, and changes to protein sequences and gene regulation, but how each of these components evolved to eventually build a canonical CAM syndrome is still largely unknown. Despite the apparent complexity of CAM photosynthesis, it has evolved in dozens of distantly related lineages, mostly within the last 20 million years. In contrast to the evolution of C$_4$ photosynthesis, there has been little evidence for a general stepping-stone or ratchet-like accumulation of CAM traits leading to first C$_3$+CAM and later strong CAM. C$_3$+CAM lineages appear to be much more numerous and sometimes quite old, suggesting that they exhibit evolutionarily stable phenotypes. A rigorous understanding of CAM evolution has been complicated by comparative studies that use distantly related taxa, as well as the difficulty of assaying intermediate CAM species. Comparing distantly related lineages reduces the precision to which we can assign evolutionary changes to particular branches of a phylogeny, and leaving out or failing to recognize intermediate CAM taxa exacerbates this problem and can make for incorrect comparisons. For example, careful studies in the Agavoideae have shown that two genera that had previously been hypothesized to be C$_3$ were capable of CAM photosynthesis, requiring a reassessment of where CAM originated in Agavoideae and which taxa to use in comparative research.

Despite these hurdles, a number of trends in CAM evolution are coalescing from comparative studies across the entire plant tree of life. The evolution of C$_3$+CAM appears to ubiquitously precede the evolution of strong CAM. In the facultative C$_3$+CAM plant *Sedum album* (Crassulaceae), it has recently been shown that many photosynthetic genes possess novel *cis*-regulatory elements that contribute to a massive rewiring of the majority of diel-cycling genes during CAM. Therefore, duplication events and changes in the regulation of existing photosynthetic genes are emerging as the earliest steps in CAM

evolution. When, if at all, selection acts to refine these genes has been more difficult to assess. Although a number of photosynthetic genes were found to be under selection in the portullugo, the distribution of selection coefficients in these genes was not elevated relative to non-photosynthetic genes. As new tools and resources have become available, the molecular evolution of CAM has become clearer, but the timing and importance of morphological changes have remained hard to place in the evolutionary trajectory of CAM. Many CAM plants are readily identified by their extreme succulence, and indeed, in the Orchidaceae and Bromeliaceae, strong CAM species have significantly thicker and more succulent leaves than both C$_3$ and C$_3$+CAM species. In contrast, pronounced leaf succulence appears to have evolved prior to strong CAM activity in Agavoideae. It is possible that morphological traits may arise through selection for increased CAM activity that involve increased vacuolar storage of malate, thereby increasing succulence and drought avoidance. But selection for drought avoidance could just as easily act to increase plant water storage and boost the efficiency of CAM indirectly. Morphology is a necessary element in the transition to strong CAM, but the relative timing of shifts and the degree to which they are the result of ecological or physiological pressures is still unknown and likely varies between clades.

Conclusions

Although CAM was discovered centuries ago, we are only now beginning to understand its evolutionary origins. This is in part due to the phenomenal ecological and phenotypic diversity of CAM species that span the entire evolutionary breadth of vascular plants, from lycophytes to angiosperms. It is also due to the difficulty of recognizing cryptic CAM species that may represent transitional forms in the evolution of CAM and the natural variation that can exist between individuals. However, these hurdles for basic research may represent strengths of CAM for applied sciences. The diversity of plant clades with CAM suggests that CAM may be

more readily evolved within selective breeding programs or via genetic engineering in a wider variety of crops than C_4. To fully understand the evolution of CAM and harness its benefits we'll need to continue to integrate diverse research programs, from ecology and physiology to plant systematics and genomics.

FURTHER READING

Borland, A.M., Hartwell, J., Weston, D.J., Schlauch, K.A., Tschaplinski, T.J., Tuskan, G.A., Yang, X., Cushman, J.C., et al. (2014). Engineering crassulacean acid metabolism to improve water-use efficiency. Trends. Plant Sci. 19, 327–338.

Dodd, A.N., Borland, A.M., Haslam, R.P., Griffiths, H., and Maxwell, K. (2002). Crassulacean acid metabolism: plastic, fantastic. J. Exp. Bot. 53, 569–580.

Edwards, E.J. (2019). Evolutionary trajectories, accessibility and other metaphors: the case of C_4 and CAM photosynthesis. New Phytol. 223, 1742–1755.

Griffiths, H., and Males, J. (2017). Succulent plants. Curr. Biol. 27, R890–R896.

Heyduk, K., McKain, M.R., Lalani, F., and Leebens-Mack, J. (2016). Evolution of a CAM anatomy predates the origins of Crassulacean acid metabolism in the Agavoideae (Asparagaceae). Mol. Phylogenet. Evol. 105, 102–113.

Heyduk, K., Moreno-Villena, J.J., Gilman, I.S., Christin, P.-A., and Edwards, E.J. (2019). The genetics of convergent evolution: insights from plant photosynthesis. Nat. Rev. Genet. 313, 1–9.

Keeley, J.E. (1998). CAM photosynthesis in submerged aquatic plants. Bot. Rev. 64, 121–175.

Nelson, E.A., Sage, T.L., and Sage, R.F. (2005). Functional leaf anatomy of plants with crassulacean acid metabolism. Funct. Plant Biol. 32, 409–419.

Ogburn, R.M., and Edwards, E.J. (2013). Repeated origin of three-dimensional leaf venation releases constraints on the evolution of succulence in plants. Curr. Biol. 23, 722–726.

Osmond, C.B. (1978). Crassulacean acid metabolism: a curiosity in context. Annu. Rev. Plant Physiol. 29, 379–414.

Silvera, K., Neubig, K.M., Whitten, W.M., Williams, N.H., Winter, K., and Cushman, J.C. (2010). Evolution along the crassulacean acid metabolism continuum. Funct. Plant Biol. 37, 995–1016.

Wai, C.M., Weise, S.E., Ozersky, P., Mockler, T.C., Michael, T.P., and VanBuren, R. (2019). Time of day and network reprogramming during drought induced CAM photosynthesis in Sedum album. PLoS Genet. 15, e1008209.

Winter, K., Holtum, J.A.M., and Smith, J.A.C. (2015). Crassulacean acid metabolism: A continuous or discrete trait? New Phytol. 208, 73–78.

Winter, K. (2019). Ecophysiology of strong and facultative CAM photosynthesis. J. Exp. Bot. 2, 16178–16214.

Yang, X., Hu, R., Yin, H., Jenkins, J., Shu, S., Tang, H., Liu, D., Weighill, D.A., Yim, W.C., Ha, J., et al. (2017). The Kalanchoë genome provides insights into convergent evolution and building blocks of crassulacean acid metabolism. Nat. Commun. 8, 1–15.

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Inbreeding risk and maternal support have opposite effects on female chimpanzee dispersal

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Sex-biased dispersal of individuals away from their natal group or area before breeding is a prevalent pattern among animals. Debate has been ongoing for more than 40 years over the importance of inbreeding avoidance in driving this phenomenon, as well the relative importance to each sex of intra-sexual competition (over food or mates) or intra-sexual cooperation in determining the extent to which each sex disperses in different taxa [1–2]. Clues to ultimate explanations of dispersal patterns come from examination of the proximate causes of dispersal, especially in cases in which dispersal is conditional [3]. Here we examine the factors involved in the dispersal of female chimpanzees in Gombe National Park, Tanzania (n = 31), a population in which not all females disperse. We find that females are more likely to disperse when they have more maternal brothers in the community and are less likely to disperse when their mother is present and high-ranking. This indicates a compromise between the opposing benefits of dispersal to avoid inbreeding and the benefits of remaining and receiving their mother's support.

Chimpanzees live in permanent, fission–fusion social groups in which females, especially in the eastern subspecies (*Pan troglodytes schweinfurthii*), are less social than males and concentrate their time in smaller overlapping core areas within a community territory that is cooperatively defended by males [4]. In a reversal of the typical mammalian pattern, males remain in their natal group whereas most females transfer to other groups before breeding [5]. Male philopatry has been linked to the advantages of kin cooperation

in resource defense [5]. Dispersal eliminates the risk to females of breeding with close relatives such as brothers and fathers. Mating between co-resident relatives does occur and, at Gombe, three of four offspring of parent–offspring or sibling pairs died before maturity [6], suggesting inbreeding depression. However, dispersal incurs costs. Compared to females that remain in natal groups, dispersing females experience heightened female–female aggression and stress [7], and give birth 2.5 years later [8]. Dispersing females leave not only familiar resources but also close female kin. In many mammals, females benefit from the presence of female kin through social support, and strong social bonds correlate with reproductive success [9]. Chimpanzee mothers and daughters maintain strong bonds even as daughters mature; for non-dispersing females this bond persists throughout adulthood, with daughters frequently sharing their mother's core area [4]. High rank correlates with reproductive success, probably in part because high-ranking females occupy high-quality core areas [5]. Whereas young females generally enter the dominance hierarchy at the bottom, natal females with a living mother often jump the queue and spend more of their lives at high rank [10]. Thus, for some females there are strong advantages to philopatry.

Here we test the hypothesis that the probability of female dispersal is influenced in opposite ways by the risk of close inbreeding and the benefits of maternal presence and support in the natal community. We also consider the possibility that females will be more likely to disperse if the number of unrelated males in the community is small or when resources are limited or decreasing, as measured by female density and the percentage of leaves and pith in the diet (Table S1).

Model selection using AICc selection criterion (GLMM, see Supplemental Information) identified a single best-fit model containing the terms 'Weaned maternal brothers' and 'Maternal presence/rank' (Table S2). Females with more maternal brothers present in the community at maturity were more likely to disperse and females with a high-ranking mother were less likely to disperse (Figure 1). The number of